MONOGRAPH ON CALCIUM SEQUESTRANTS #58

Monograph on **Calcium Sequestrants** #58 5/4/73

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CALCIUM SEQUESTRANTS

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CALCIUM SEQUESTRANTS

Summary

From very early times, calcium has occupied an important role in the materia medica, chiefly through empirical application; powdered sea shells, corals, limestone and even the bones of the dead, have been used to treat a variety of ills. The primary function of calcium is in the formation of bones and teeth, but it is also involved in the physiology of muscle contraction, cell membrane permeability, the heart beat, coagulation of the blood and the regulation of the body acid-alkaline balance. Among the earliest diseases recognized as representing a calcium deficiency were rickets and tetany. Calcium therapy has long been applied in these cases. The pharmacology of calcium, has, however, been neglected and only recently (1940's) have toxicological studies been performed. Calcium chloride and gluconate are the chief forms of calcium used in therapy, and what little information relative to the toxicity of calcium salts is available concerns largely these two compounds. The gluconate particularly enjoys growing use in therapy and may be found in anti-acids, analgesics, vitamin and mineral products, as well as in tablet form or sterile solutions for parenteral injection. The chloride is widely used as an anti-allergen and a diuretic.

As food additives, calcium salts are found among those chelating agents known as "sequestrants" which are applied to food stuffs to maintain their integrity or stability. They serve also as "firming agents" and as buffers. Sequestrants currently in use are not regarded as constituting a health hazard; among the sequestrants used in food processing are listed calcium acetate, calcium citrate, calcium gluconate and calcium phytate. A typical application of calcium salts as sequestrants is observed in processing eggs: prefreezing sterilization of whole eggs produces undesirable coagulation which may be reduced by adding calcium salts (sequestrants) after sterilization to inhibit oxidation and other deteriorating changes which occur during freezing.

It is not the purpose of this monograph to consider the results of calcium deficiency, nor do any of the studies reviewed indicate problems in the present use of calcium salts as food additives. There have been problems in the medical application of calcium chloride and calcium gluconate in therapy, but such instances have not been frequent. Durlocher et al. (215) reported three cases of very young infants treated for tetany with calcium chloride, 4, 3 and 2 grams given orally with water; the first two died, the third eventually recovered. The authors suggested that gavage with calcium chloride in treatment of newborn infants, suffering from hypocalcemic tetany, is contraindicated. Hall (357) described a case of neonatal tetany in a week-old infant treated with 2 grams of calcium chloride in 12 cc H_2O, given by gastric intubation. The convulsions ceased, but vomiting and diarrhea were almost continuous; necrotic tissue, presumably from the infant's stomach, was found, and while the child was discharged after three months of treatment, there

were indications of a constricted fundus of the stomach due to calcium deposits.

Abscess formation and one fatality were reported by Lamm (527) in three cases of infants injected intramuscularly in the buttock with 10 cc of 10% calcium gluconate. Strong objection was voiced by the author to textbook and drug literature statements that the gluconate could be safely used intramuscularly, intravenously and orally, even in infants. Severova and Teplukhin (867) reported several cases of anaphylactic shock due to sensitization of patients to intravenous $CaCl_2$ therapy; a 30-year old women treated for allergic eczematous otitis of one ear, with daily 10 ml of 10% $CaCl_2$ doses died. The authors caution doctors to observe more care and discrimination in drug selection for intravenous treatments.

A rather curious relationship between physical stress and the administration of calcium acetate was reported by Prioreschi and Selye (757). Combinations of restraints and single doses of about 20 mg/kg calcium acetate were administered to rats; heart, stomach and kidney lesions were noted, as well as fatalities. Neither treatment alone, stress or acetate, produced pathological changes; the combination was markedly effective and the authors credited stress and "adaptive hormones" as fundamental to the observed cardiopathy.

Reserpine, one of the Rauwolfia alkaloids, increases muscle and nerve excitability which may be due or related to lowering of the calcium concentration. Carrier et al. (144), using mice and combinations of calcium chloride doses and reserpine injections, found that pretreatment with reserpine increased fatalities from $CaCl_2$ and that larger doses of $CaCl_2$ produced different kinds of death from those treated with lower doses of $CaCl_2$. Mice given 650 mg/kg $CaCl_2$ died slowly and quietly; 1000 mg/kg $CaCl_2$ produced excited, rapidly moving animals that died quickly. The authors thought that this difference in dying was significant and related to tissue loss of calcium, affecting both peripheral and central nervous system; they recommended caution in the use of the Rauwolfia alkaloids in combination with calcium therapy.

Sharpless et al. (872) reviewed experimental studies indicating that calcium is goitrogenic, and performed experiments with rats which were given vitamin D and various calcium salts (chloride, lactate, carbonate and phosphate) added to the diet or drinking water. In the first experiment, vitamin D was omitted and the results were negative as regards an increase in the size of the thyroid. A second experiment, with vitamin D and calcium carbonate and chloride added to the diet, showed only the chloride plus vitamin D was goitrogenic. Excess of calcium chloride may have resulted in loss of iodine with a subsequent increase in the thyroid gland.

The relative toxicities of calcium gluconate, calcium gluconate-iodate and calcium chloride, orally administered to rats, was tested by Smith (896). The three salts were given in doses of 0.4 g Ca/kg daily; the chloride and gluconate-iodate were also given at 1 and 2 g Ca/kg/day. All animals on 2 g Ca-level died after a single dose; at 1 g Ca (as $CaCl_2$) all died after one or two doses, but of those on gluconate-

iodate, several survived. The chloride appeared definitely more toxic than either the gluconate or gluconate-iodate.

Calcium chloride, calcium lactate and ammonium chloride were given by gastric intubation to rabbits in a comparative study by Durlacher et al. (215). Doses of $CaCl_2$ ranging from 5-20%, or about 0.75-2.5 g/kg produced edema, mucosal necrosis and ulceration, and death in high doses; the largest possible doses of the lactate (limited solubility) were ineffective; the ammonium chloride was fatal at doses in excess of 1.5 g/kg body weight.

Calcium chloride, gastrically intubated in rabbits, at dose levels of 2.2-2.5 g/kg, produced marked acidosis, gross abnormalities and death within 5 days in an experiment by Govan and Parkes (324). Previous investigations with ammonium chloride had produced similar results; the authors were interested in eliminating the ammonium ion as the cause of death in rabbits. Analysis of the blood in the test animals showed, however, ammonia - which was absent in the controls. The implication was that ammonia had been spontaneously formed from adenosine phosphate in the test animals.

Thyroid hyperplasia (goiter) was induced in six dogs by intubation of 500 cc of 2% $CaCl_2$ for 4 days, followed by 0.05% $CaCl_2$ in the drinking water. Four of six dogs died after 6, 7, 12 and 26 days of treatment; two were sacrificed after 8 and 113 days. Mahorner (591) discussed at considerable length various ideas regarding causes of goiter; he concluded from his experiments with dogs that $CaCl_2$ was not directly the cause in producing colloid goiter, but did alter the microscopic appearance of the thyroid gland.

Smith (896) injected intravenously large doses (0.205 M) of three calcium salts, gluconate, gluconate-iodate and chloride into dogs. Pretreatment blood samples were analyzed for calcium and serum calcium determined after death. Electrocardiograms were taken at frequent intervals. Larger doses of gluconate or gluconate-iodate were required to produce death intravenously in dogs than of the chloride. No differences were observed in the two gluconates.

The LD_{50} doses of calcium kinate gluconate (CKG), gluconate and chloride were determined in mice by Coulston et al. (171). Intravenously injected, the LD_{50} for CKG was 1050 ! 57 mg/kg; for calcium gluconate, 950 ! 83 mg/kg; and for calcium chloride, 215 ! 14 mg/kg. The LD_{50} was based on a 24-hour fatality.

The same investigators administered CKG and calcium gluconate to dogs to determine tolerance levels; the CKG was better tolerated (measured at onset of vomiting) at a mean maximum dose of 19.7 ! 2.6 mg Ca/kg body weight than was the gluconate at 13.2 ! 1.7 mg Ca/kg.

Hoffman and Blinick (397) studied the metabolic effects of therapeutic oral doses of calcium citrate carbonate or lactate given to female hospital patients in their 21st to 31st weeks of pregnancy. The dosage of each calcium salt was 450 mg/day of elemental Ca. Calcium, phosphorus and serum protein of blood samples taken every 3 weeks were determined; no differences were noted in the mean

calcium-phosphorus levels with the different salts. The incidence of leg cramps diminished during treatment, most of all in those patients receiving calcium citrate.

Baldacci (34) in an early paper (1936) reviewed the literature on calcium salts used in medical therapy; the chloride and gluconate experiments of the author are noted here. Rabbits were intravenously administered $CaCl_2$ or the gluconate, and the minimum lethal doses determined: 0.0080 g/kg for $CaCl_2$; and 0.0130 g/kg for the gluconate. The author concluded that the lethality of the calcium salts given to rabbits was due to the "depressive effect of the calcium ion on the nervous centers". A second paper by Baldacci (35) is included in this monograph for a more complete picture of his interesting studies.

An investigator, Alha (11), noting the affinity of calcium chloride for alcohol (ethyl) in industrial applications, thought that alcoholism might be successfully treated with the chloride. $CaCl_2$ was given to volunteers, orally and intravenously, following measured doses of alcohol. Serum calcium levels and blood alcohol values were not affected; some slight reduction in the symptoms of intoxication were observed and attributed to influence of $CaCl_2$ on "neural functions".

An interesting, detailed review of the medical uses of calcium in general and in $CaCl_2$ in particular was presented by Blum in 1933 (84). He cited many works from the early 1900's, and considered $CaCl_2$ as occupying "an important place in the therapeutic arsenal".

Lieberman (564) administered calcium gluconate to thirty patients by oral and intramuscular routes before and after meals and concluded that the gluconate is the most convenient calcium salt for subcutaneous intramuscular or oral therapy and may be given orally to adults in doses of 3-4 grams daily after meals. The blood calcium concentration is maximum within an hour after subcutaneous or intramuscular injection and within four hours after oral administration. Calcium therapy is dangerous when given intravenously due to the possibility of intravascular clotting and death. The blood calcium level can be quantitatively measured by the urinary calcium.

Infants, 6 months to 3 years of age, with infantile tetany and with rickets were given intravenous calcium gluconate (plus sedative and $CaCl_2$ in tetany cases; intramuscular colloidal calcium and vitamin D in rickets) in a study by Habib and Ibrahim (346). Pretreatment serum calcium, sodium and potassium were determined and compared with treated levels; the serum potassium in tetany was below normal but was restored by the gluconate treatment; the serum sodium in tetany, and both sodium and potassium levels in rickets were as those of normal infants and were not affected by the gluconate treatment.

Naef (667) reported an unusual case of a rare disease, idiopathic hypoparathyroidism successfully treated with oral doses of 12 gm calcium gluconate and 150,000 units of vitamin D_2 daily. Alopecia, convulsions, cataract, impaired mental functions, spasticity, pain and inability to communicate were the symptoms of the patient, a 30-year-old woman. The patient responded well to treatment and recovered completely, making it a notable case.

Rothlin (801) has presented a very comprehensive discussion of calcium therapy in man, with emphasis on the uses of calcium gluconate which he regarded as the most suitable calcium salt for oral, intravenous and particularly intramuscular administration for treating calcium-related disorders.

The treatment of chlorcalcinomas (tumors from intravenous injections of calcium chloride) with sodium sulfate was described by Bulgatch (122). Injections of very small doses of $CaCl_2$ (5-6 drops of 10% $CaCl_2$ solution) used in therapy caused necrosis of tissues and tumors at the site of injection. Experiments with rabbits and dogs demonstrated that the simultaneous injection of Na_2SO_4 greatly reduced the tumorogenic effect of the $CaCl_2$.

Schreire and Schnepf (848) experimented with normal and rachitic rats to determine the influence of various sodium salts on calcium absorption. The detection and measurement of Ca was facilitated by the use of radioactive Ca^{45} incorporated in $CaCl_2$. A constant dose of 51.1 mg $Ca^{45}Cl_2$ was given orally in combination with sodium citrate, phosphate or tartrate; the citrate was particularly effective in increasing the retention and resorption of Ca^{45} in rats.

Yoshimoto et al. (1031) made a comparative study of the absorption of various calcium salts in rabbits (dog and man are briefly included without detail). $CaCl_2$, $CaCO_3$, Ca-gluconate, Ca-citrate, Ca-succinate and pearl calc (phosphate) were given intravenously or orally, and blood serum calcium levels were determined before and after treatment. Significant rises in serum calcium were observed in the $CaCl_2$ and $CaCO_3$ treatments, but most apparently in those receiving pearl calc. The other salts were relatively ineffective, the gluconate least of all.

In vitro studies of calcium absorption by blood and organ colloids were made using sheep blood and organs, and calcium chloride and gluconate solutions, by Scaglioni and Girino (829). All the sheep organs showed absorption of Ca, from the greatest degree in lungs, to the least degree in liver; the gluconate was more absorbed than the chloride. No measurable absorption by sheep muscle was observed.

Calcium chloride was demonstrated to behave as an acid substance in an early study by Gamble et al. (283), who administered orally daily doses of 1.5-2.0 g $CaCl_2$ to infants (5 and 4 months old, one normal, the other with symptoms of tetany). Urine, stool and blood serum studies showed a much larger excretion of Ca than Cl in the stools (60% of the calcium dose, 4% of the chloride).

Gerloczy and Berencsy (301, 302) demonstrated that $CaCl_2$ when given to patients as a solution in sodium chloride, orally - raises the blood serum calcium level, or is better absorbed, than when given as an aqueous solution. They also observed that the pH of the patients' system had significant effect on absorption of Ca; pretreatment with alkalizing $NaHCO_3$ resulted in a greater serum calcium level than did pretreatment with NH_4Cl.

Numerous methods of measuring the absorption of calcium in humans using the radioisotopes Ca^{45} and Ca^{47} in $CaCl_2$ were described by Mautalen (626). It was shown that inorganic $CaCl_2$ is 30-60%

absorbed, compared to half that much from milk. A diet lower than 400 mg Ca/day is poor in calcium; more than 1500 mg Ca/day is very rich.

McDonald et al. (631) hypothesized that patients with renal failure have an impaired ability to absorb calcium; this was tested by giving calcium citrate in water at levels of about 2 g daily for two to three weeks to six patients with chronic renal failure. Fecal, urine and blood samples were analyzed for plasma calcium, phosphate and creatinine. The increase in serum calcium, the phosphorus balance and urinary excretion, were consistent with an increased calcium absorption in the gut comparable to that of normal subjects. The hypothesis was not supported by the experimental evidence.

Male patients identified as idiopathic renal stone formers (hyper-calciuric) were treated with oral calcium citrate, 100 mg/kg, and tested for serum and urine calcium, by Peacock et al. (721). A significantly greater use in the hypercalciuric patients, when compared with non-hypercalciuric hospital staff members serving as controls, was observed, indicating that the renal failure is due to faulty calcium absorption. Oral calcium loading is recommended by the authors as a better diagnostic technique for identifying idiopathic hyper-calciuria than is the standard 24-hour urine collection on a free diet.

Hayami et al. (369) fed rats calcium gluconate (water-soluble) and calcium carbonate (water-insoluble) at levels of 2.25 g and 0.5 g, to determine a relationship between solubility of the calcium salt in the diet and its nutritional value. The results in general favor the carbonate as being better utilized than the gluconate, but not significantly; the solubility of the calcium does not appear to determine its nutritional value. In a later experiment, Hayami et al. (370) studied the absorption of the same two calcium salts added to the diet of four aged women, with much the same result. The calcium carbonate was 50% more utilized than the gluconate.

The absorption of calcium gluconate and lactate added to the diets of eight male patients, at levels of about 1600 mg Ca per day, was observed by Spenser et al. (909). Parallel studies, using single oral doses of Ca^{47} as the chloride were performed. Urinary excretion of the lactate was consistently higher than that of the gluconate; the reverse was true of fecal excretion. The blood plasma levels were higher in the lactate tests than for the gluconate. The apparent greater absorption of the lactate is not consistent with the observations of other investigators; the reasons are "conjectural" (authors).

Comparative absorption of calcium gluconate and the carbonate was investigated by Patton (719) in nine college women who were given milk, gluconate and carbonate supplements for a total of about 600 mg Ca daily in the diet. The basal metabolism of the subjects significantly affected the retention of calcium; retention was inversely related to the basal metabolic rate of the subject. An earlier study by Patton and Sutton (719) showed no significant differences in the calcium utilization from the lactate, gluconate, sulfate or carbonate; whichever salt was given first, in a series of alternate salts administered, was the one most utilized.

Large doses of calcium gluconate or lactate given three healthy

individuals, in an effort to determine "how much" should be given, and "when", were used by Lieberman (561) in various routines. The salts were first administered orally in water at levels of 20 g of gluconate and 10 g of lactate; the abdominal distress, vomiting and diarrhea which resulted led to reducing the doses to half that. The gluconate was better tolerated than the lactate, and both salts were better received after meals than on empty stomachs.

Staub (914) presented an excellent review of the application of the radioisotope of calcium, Ca^{45} in investigations of the absorption and excretion of calcium. In an experiment performed by the author, Ca^{45}-gluconate was given orally, intravenously and rectally to human subjects at a dose level of 0.5-1.0 g Ca as gluconate. A positive Ca balance was established with oral and intravenous administration; no Ca absorption occurred from rectal administration.

Waldroup et al. (996) experimented with chicks to demonstrate the influence of calcium and vitamin D_3 on the utilization of phosphorus from calcium phytate (described as phytic acid calcium-magnesium salt, 12% calcium). The phytate, dicalcium phosphate and vitamin D_3 were added to the diet of the chicks at various levels and the calcium utilization was determined by removing and ashing the right tibia of the sacrificed birds. The phytate phosphorus utilization was closely related to the calcium, phosphorus and vitamin D_3 levels, being greatest at low levels and reduced at higher Ca:P ratios. Increased vitamin D_3 levels improved both the phytate and the phosphate phosphorus utilization.

Similar findings to those above for chicks were reported by Krieger et al. (512) in rats given either calcium phytate or calcium carbonate added to the diet at levels of about 0.428 g/100 g of ration. Both calcium salts were equally utilized and both were more effectively absorbed with vitamin D added to the diet.

Danbara (181) studied the relative absorptions of five calcium salts in rats fed a diet supplemented with radioactive Ca^{45} incorporated in each salt. The deposition of Ca^{45} in the rat femur showed Ca-lactate and carbonate as most utilized by the animals, with the phosphate and phytate absorbed half as much, and the oxalate scarcely at all. The author recommended using the lactate or carbonate in Ca-enriched foods.

An early study by Briggs (109) discusses the use of acetates in treating nephritis, which is characterized by acidosis. Sodium bicarbonate or other alkali is given as the usual treatment. Calcium acetate was given orally to several subjects, one the author himself, and analyses of urine and feces for phosphorus, sodium, potassium, calcium and magnesium were made. The acetate reduced urine phosphoric acid excretion; decreased ammonia synthesis in the kidney, and reduced alkali retention with subsequent relief from edema.

The potential hazards of strontium-90 in our nuclear age led Malkura and Puchkova (598) to study the effects of calcium on the deposition of strontium-90 in the bone. Calcium, as the nearest chemical analogue of strontium, will compete with strontium, when both are present in the gastrointestinal tract, in absorption and incorporation in the skeleton. Rats were given diet supplements of

calcium chloride, phosphate, oxalate and the citrate and daily doses of 1 ml radioactive strontium nitrate (0.4 microcuries intensity). Excreted calcium and skeletal Sr^{90} were determined. The amount of strontium-90 deposited in the skeleton was inversely related to the calcium absorption; strontium-90 content was lowest in rats receiving calcium chloride-phosphate combined diet.

The oldest paper reviewed in preparing this monograph was by Haldane et al. (353), who reported the uses and effects of calcium chloride. Haldane served as the experimental subject in a study lasting 13 days, during which (for three days) he drank a solution of 85 g $CaCl_2$ in water. Intense diarrhea, followed by constipation and general discomfort, was experienced. Loss of body salts and water were observed and note was made of $CaCl_2$ being used to treat "dropsy, edema and exudations".

The amount of dietary calcium used to replace the endogenous losses of calcium from the body was studied by Steggerda and Mitchell (916), with Steggerda serving as the experimental subject. A low calcium diet, supplemented by graded additions of milk solids or calcium gluconate, was followed for 168 days. Food samples, urine and feces were analyzed periodically for calcium. Calcium metabolic equilibrium required about 542 mg Ca from 40 grams of milk powder, or 534 mg Ca from the gluconate. On the basis of the subjects' weight, Ca equilibrium required 9.2 mg Ca/kg body weight.

The nutritional role of phytin was studied by Gillis et al. (309) in chicks and turkey poults, using radiophosphorus, P^{32}-labelled calcium phytate, and for comparison, disodium phosphata, also with P^{32}. The diets were either vitamin D-deficient or vitamin D-supplemented. The phytate in capsules and the phosphate in solution were inserted into the crop; blood samples were taken and tibiae removed, after sacrifice of the birds, and ashed; both blood and ash were counted for radioactivity. The phytate was utilized by the chick only about one-tenth as effectively as was the phosphate; a similar experiment with turkey poults showed a negligible use (less than 2%) of the phytate.

Makino and Tanaka (593) studied the cytological effects of several dehydrating agents, one of which was $CaCl_2$, on ascites sarcomas in rats. Animals with transplanted MTK-sarcomas were injected peritoneally with 0.25 M solution of $CaCl_2$, and periodic samples of the ascites were examined microscopically. Mitotic cells were damaged, half of them within 60-70 minutes after injection with $CaCl_2$. Three to four hours later, numerous mitotic cells were observed, probably stem cells resistant to the $CaCl_2$, receiving the growth of the tumor. Doses of $CaCl_2$ of 0.5 M and 0.75 M damaged the tumor considerably, but were also lethal to the animals, killing them within several hours.

The uses of calcium chloride as a diuretic were reviewed by Segall and White (857) who then described several hospital cases. The usual dose of $CaCl_2$ was 2.5 g in 50-100 cc of water, given 6 times daily; in experiments with normal individuals, doses of 5-8 g were given daily. The cases described included normal, cardiac failure, tubercular polyserositis and hepatic cirrhosis. No deleterious effects of large doses of $CaCl_2$ were observed; doses of 10-15 g/day of $CaCl_2$ were well tolerated.

Tainter and Throndson (933) tested calcium gluconate, orally administered to four human subjects in doses of 13.5 g/day for 14-21 days to determine its efficacy in shortening blood coagulation time. A capillary tube was filled with blood from a pricked ear lobe and the tube periodically broken off at the top until a filament of fibrin was formed, denoting clotting. One subject showed a marked decrease in clotting-time; the remaining three, no change. Addition of vitamin D to the gluconate dose was also ineffective. The authors recommended abandonment of the clinical practice of oral Ca administration to shorten clotting time.

The relationships of calcium and lipids, particularly cholesterol was investigated in hospital patients by Kocian and Pechar (490) who observed an inverse relationship between calcium concentration, from daily doses of 16.8 g Ca-gluconate given orally, and the serum cholesterol level. It was suggested that the calcium in the intestine bonds with food and bile cholesterols, preventing their absorption.

Two experiments designed to demonstrate sensitization to the parathyroid hormone in rats by oral doses of calcium acetate, sodium phosphate and corn oil or paraffin oil were performed by Selye et al. (863). Subcutaneous doses of 50 U.S.P. units of parathyroid extract (later reduced to 30 U.S.P. units) were administered twice daily, and the sensitizing substances intubated into the stomach of the animal. After 3 days (or 8 days in one instance) the rats were sacrificed and autopsied. Cardiac lesions and nephrocalcinosis were noted, as was osteitis fibrosa. All three substances, calcium acetate, the phosphate and corn oil given orally sensitized the animals to parathyroid hormone.

Lorenzini (580) selected aged rest home patients for a study of the effects of intravenous $CaCl_2$ (10 cc of 10% $CaCl_2$) as related to age and various pathogenic medical phenomena. Calcemic curves were determined and vitamin D_2 given intramuscularly; serum calcium concentration was again measured. An hypercalcemic reaction followed the $CaCl_2$ injections, particularly in subjects with corneal xanthomatosis, after vitamin D_2 treatment.

In response to a medical controversy of the times regarding simultaneous application of calcium and digitalis in therapy, LaBarre and Heerswynghels (523) experimented with cats injected intravenously with these drugs (calcium as the gluconate, and digitalis) singly or in combination. It was concluded by the investigators that a synergistic or additive effect did characterize the use of calcium gluconate and digitalis; the gluconate alone produced circulatory abnormalities such as brachycardia and cardiac arrest, but with digitalis the effects were more immediate and quickly fatal.

A treatment of the withdrawal symptoms of drug addiction in humans was described by Williams (1012). Calcium gluconate was given intravenously, 10 cc of 10-20% solution, five times daily for four days, gradually tapering-off the dose. Several cases of heroin addiction successfully treated by gluconate are outlined; the chief merit of the treatment lay in the absence of pain during withdrawal.

CALCIUM ACETATE

Chemical Information

I. Nomenclature

A. Common Name

Calcium Acetate (Technical product called brown (or gray) acetate of lime)

B. Chemical Name

Calcium Acetate

C. Trade Name

None

D. Chemical Abstracts Services Unique Registry Number

000062544

II. Empirical Formula

$Ca(C_2H_3O_2)_2$

III. Structural Formula

Not applicable

IV. Molecular Weight

158.17

V. Specifications

Food Chemicals Codex	
Assay	Not less than 99.0% of $Ca(C_2H_3O_2)_2$ calculated on the anhydrous basis
Limits of impurities	
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Chloride	Not more than 500 ppm (0.05%)
Heavy metals (as Pb)	Not more than 25 ppm (0.0025%)
Lead	Not more than 10 ppm (0.001%)
Sulfate	Not more than 0.1%
Water	Not more than 7%

VI. Description

A. General Characteristics

Technical product known as "brown (or gray) acetate of lime"; pure form a fine, white, bulky, odorless powder. The hydrate appears as needles, granules or powder; the dihydrate forms long transparent needles.

B. Physical Properties

The anhydride is very hygroscopic, decomposes above 160 degrees to acetone and $CaCO_3$; soluble H_2O, slightly soluble in methanol; practically insoluble in ethanol, acetone or benzene.

The hydrate is somewhat deliquescent; soluble in H_2O; slightly soluble in alcohol. The dihydrate deliquesces to the hydrate in air.

C. Stability

Store in well-closed containers

VII. Analytical Methods

Food Chemicals Codex

A 1 in 10 solution gives positive tests:

Calcium: Insoluble oxalate salts are formed when solutions of calcium salts are treated in the following manner: Using 2 drops of methyl red T.S. as indicator, neutralize a solution of a calcium salt (1 in 20) with ammonia T.S., then add diluted hydrochloric acid T.S., dropwise, until the solution is acid. A white precipitate of calcium oxalate forms upon the addition of ammonium oxalate T.S. This precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

Calcium salts moistened with hydrochloric acid impart a transient yellowish red color to a nonluminous flame.

Acetate: Acetic acid or acetates, when warmed with sulfuric acid and alcohol, form ethyl acetate, recognizable by its characteristic odor. With neutral solutions of acetates, ferric chloride T.S. produces a deep red color which is destroyed by the addition of a mineral acid.

VIII. Occurrence

Calcium acetate has numerous commercial uses. It is used in foods as a sequestrant.

Calcium acetate is manufactured by reacting commercial grade lime

slurried in water with technical grade acetic acid, producing a 15-25% solution of calcium acetate. The solution is adjusted slightly basic, digested hot to precipitate out iron, heavy metals and magnesium impurities.

The solution is then carbon treated to remove color impurities, filtered and fed continuously to spray drier unit.

CALCIUM CHLORIDE

Chemical Information

I. Nomenclature

A. Common Name

Calcium Chloride

B. Chemical Name

Calcium Chloride

C. Trade Name

None

D. Chemical Abstracts Services Unique Registry Number

0100350408

II. Empirical Formula

$CaCl_2$
$CaCl_2 \cdot 2H_2O$
$CaCl_2 \cdot 6H_2O$

III. Structural Formula

Not applicable

IV. Molecular Weight

110.99 ($CaCl_2$)

V. Specifications

Food Chemicals Codex ($CaCl_2 \cdot 2H_2O$)	
Assay	Not less than 99.0% and not more than 107.0% $CaCl_2 \cdot 2H_2O$
Limits of impurities	
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Fluoride	Not more than 40 ppm (0.004%)
Heavy metals (as Pb)	Not more than 20 ppm (0.002%)
Lead	Not more than 10 ppm (0.001%)
Magnesium and alkali salts	Not more than 1%

VI. Description

A. General Characteristics

The anhydride forms cubic crystals, granules or fused masses. The dihydrate forms granules, flakes or powder; the hexahydrate forms trigonal crystals.

B. Physical Properties

The anhydride is very hygroscopic, mp greater than 72 degrees, bp greater than 1600 degrees; freely soluble H_2O and alcohol. The hexahydrate deliquesces, mp 30 degrees; loses all H_2O at 200 degrees; extremely soluble H_2O and alcohol.

The dihydrate, in general use as a food sequestrant, is deliquescent, one gram dissolves in 1.2 ml H_2O at 25 degrees, in 0.7 ml H_2O at boiling point, in 10 ml alcohol at 25 degrees, and in 2 ml boiling alcohol. The pH of 1-in-20 solution is between 4.5 and 8.5.

C. Stability

Store in tight containers

VII. Analytical Methods

Food Chemicals Codex

A 1 in 10 solution gives positive tests:

Calcium: Insoluble oxalate salts are formed when solutions of calcium salts are treated in the following manner: Using 2 drops of methyl red T.S. as indicator, neutralize a solution of a calcium salt (1 in 20) with ammonia T.S., then added diluted hydrochloric acid T.S., dropwise, until the solution is acid. A white precipitate of calcium oxalate forms upon the addition of ammonium oxalate T.S. This precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

Calcium salts moistened with hydrochloric acid impart a transient yellowish red color to a nonluminous flame.

Chloride: Solutions of chlorides yield with silver nitrate T.S. a white, curdy precipitate which is insoluble in nitric acid but soluble in a slight excess of ammonia T.S. Chlorine, recognizable by its distinctive odor, is evolved when solutions of chloride are warmed with potassium permanganate and diluted sulfuric acid T.S.

VIII. Occurrence

Calcium chloride is obtained as a by-product in the Solvay process

and from natural salt brines. It has many commercial uses. It is used in medicine as a diuretic and antiallergen; in foods as a sequestrant and firming agent (an example of which is its use in the preparation of glaceed orange peel).

CALCIUM CITRATE

Chemical Information

I. Nomenclature

A. Common Name

Calcium Citrate

B. Chemical Name

Calcium Citrate

C. Trade Name

None

D. Chemical Abstracts Services Unique Registry Number

000813945

II. Empirical Formula

$Ca(C_6H_5O_7)_2$ (anhydride)
$Ca(C_6H_5O_7)_2 \cdot 4H_2O$ (tetrahydrate)

III. Structural Formula

Not applicable

IV. Molecular Weight

498.44 (anhydride)
570.51 (tetrahydrate)

V. Specifications

Food Chemicals Codes (tetrahydrate)	
Assay	Not less than 97.5% $Ca_3(C_6H_5O_7)_2$ after drying
Loss on drying	Between 10 and 13.3%
Limits of impurities	
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Fluoride	Not more than 30 ppm (0.003%)
Heavy metals (as Pb)	Not more than 20 ppm (0.002%)
Lead	Not more than 10 ppm (0.001%)

VI. Description

A. General Characteristics

The commonly used form, the tetrahydrate, is a fine, white, odorless powder.

B. Physical Properties

Loses most H_2O at 100 degrees, all at 120 degrees; soluble in 1050 parts cold H_2O, somewhat more soluble hot H_2O; insoluble in alcohol.

C. Stability

Store in well-closed containers

VII. Analytical Methods

Food Chemicals Codex

A. Dissolve 500 mg in 10 ml of water and 2.5 ml of diluted nitric acid T.S., add 1 ml of mercuric sulfate T.S., heat to boiling and then add potassium permanganate T.S. A white precipitate is formed.

B. Ignite 500 mg completely at as low a temperature as possible, cool, and dissolve the residue in 10 ml of water and 1 ml of glacial acetic acid. Filter and add 10 ml of ammonium oxalate T.S. to the filtrate. A voluminous white precipitate appears which is soluble in hydrochloric acid.

Calcium: Insoluble oxalate salts are formed when solutions of calcium salts are treated in the following manner: Using 2 drops of methyl red T.S. as indicator, neutralize a solution of a calcium salt (1 in 20) with ammonia T.S., then added diluted hydrochloric acid T.S., dropwise, until the solution is acid. A white precipitate of calcium oxalate forms upon the addition of ammonium oxalate T.S. The precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

Calcium salts moistened with hydrochloric acid impart a transient yellowish red color to a nonluminous flame.

Citrate: When a few mg of a citrate are added to a mixture of 15 ml of pyridine and 5 ml of acetic anhydride, a carmine red color is produced.

VIII. Occurrence

Calcium citrate is used in foods as a sequestrant buffer and firming agent.

CALCIUM DIACETATE

Chemical Information

No information available

CALCIUM GLUCONATE

Chemical Information

I. Nomenclature

A. Common Name

Calcium Gluconate

B. Chemical Name

Calcium Gluconate

C. Trade Names

1. Calciofon
2. Calglucon
3. Ebucin
4. Glucal
5. Glucobiogen

D. Chemical Abstracts Services Unique Registry Number

000299285

II. Empirical Formula

$C_{12}H_{22}CaO_{14}$

III. Structural Formula

$(CH_2OH(CHOH)_4{:}COO)2Ca$

IV. Molecular Weight

430.38

V. Specifications

Food Chemicals Codex	
Assay	Not less than 98.0% and not more than equivalent of 102.0% of $C_{12}H_{22}CaO_{14}$ after drying
Limits of impurities	
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Heavy metals (as Pb)	Not more than 20 ppm (0.002%)
Lead	Not more than 10 ppm (0.001%)
Loss on drying	Not more than 3%
Sucrose and reducing sugars	Passes test

VI. Description

A. General Characteristics

White, crystalline granules or powder, odorless and tasteless.

B. Physical Properties

Solutions are neutral to litmus; one gram dissolves slowly in about 30 ml H_2O at 25 degrees, and in about 5 ml boiling H_2O; insoluble in alcohol and in many other organic solvents. Loses some H_2O on drying, but also decomposes.

C. Stability

Calcium gluconate is stable in air but should be stored in tight containers.

VII. Analytical Methods

Calcium: Insoluble oxalate salts are formed when solutions of calcium salts are treated in the following manner: Using 2 drops of methyl red T.S. as indicator, neutralize a solution of a calcium salt (1 in 20) with ammonia T.S., then added diluted hydrochloric acid T.S., dropwise, until the solution is acid. A white precipitate of calcium oxalate forms upon the addition of ammonium oxalate T.S. The precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

Calcium salts moistened with hydrochloric acid impart a transient yellowish red color to a nonluminous flame. A saturated solution gives a positive test.

Gluconate: Heat a mixture of 100 mg of the sample with 500 mg of potassium bisulfate. Pungent vapors of acrolein are evolved.

(Applicable to prepns whose aq. solns are neutral and which do not contain salts of other optically active hydroxy acids.)

Weigh two 0.5 g portions Ca gluconate or two 1 g portions powd. tablets contg 50% or less of the salt. If chocolate or fatty base is present, wash samples several times on hardened filter with absolute ether and warm residue until ether is driven off.

Transfer each portion to sep. 25 ml vol. flasks add 15 ml H_2O, and warm until Ca salt dissolves (Samples contg cocoa will have undissolved residue.) Cool mixt. to room temp.

To one flask (No. 1) add 3.5 g finely pulverized uranyl acetate, stopper, and shake in machine 1 hr. (If agitation is not vigorous enough, greater than 1 hr of shaking may be required.) Let other flask (No. 2) stand. If sample contains chocolate, add little alumina cream, 29.021 (b), to each flask. Cool to 20 degrees· dil. contents of flask No. 1 to vol. with uranyl acetate soln (10 g shaken with 95 ml H_2O until satd and then filtered), and flask No. 2 with H_2O. Filter, and polarize each soln in 200 mm tube, using 50 mm tube contg 1.8% $K_2Cr_3O_7$

soln as light filter. If soln is too dark to read in 200 mm tube, make reading in 100 mm tube and multiply result by 2. If A = rotation in oS of Soln No. 2 and B = rotation of Soln No. 1, with 1 g sample % $Ca(C_6H_{11}O_7)_2$=4.34 X (B-A), and with 0.5 g sample % $(Ca(C_6H_{11}O_7)_2$=8.52 X (B-A).

VIII. Occurrence

Calcium gluconate is manufactured by reacting gluconic acid (biosynthesized from glucose by a strain of Acetobacter suboxydans) with hydrated lime or calcium carbonate. The gluconate solution is treated with activated carbon, filtered, crystallized by cooling (677).

CALCIUM PHYTATE

Chemical Information

Phytin and salts found in cereals (corn source of phytate (309).

No additional information available.

Biological Data

I. Acute Toxicity

Substance	Animal	Sex and No.		Route	Dosage Mg/kg Body Wt.	Measurement	Ref.
Calcium Acetate	Rat	-		Oral	4.28 (3.86-4.75)	LD_{50}	898
Calcium Acetate	Rat	Female	10	Gast. Intub.	20	LD_{60}	757
Calcium Acetate	Rat	Female	10	Gast. Intub.	20 + restraint (1 hr)	LD_{10}	757
Calcium Acetate	Rat	Female	10	Gast. Intub.	20 + restraint (5 hr)	LD_{90}	757
Calcium Chloride	Mouse		10	i.v.	215 ± 14	LD_{50}	171
Calcium Chloride	Mouse		50	i.p.	600 - 650	LD_{50}	144
Calcium Chloride	Rat	-		Oral	4000	LD_{50}	677
Calcium Chloride	Rat		112	i. musc.	25	LD_{50}	98
Calcium Chloride	Rabbit	-		Oral	1384	LD_{50}	677
Calcium Chloride	Rabbit		10	i.v.	2-6 ml 10% sol.	auricular fibrillation	395
Calcium Chloride	Rabbit		7	Gast. Intub.	2200-2500	LD_{75}	324
Calcium Chloride	Rabbit		15	Gavage	1500	5 died, 5 dys 2 comatose	215
Calcium Chloride	Dog		1	Oral	1300	died, 7 dys	591
Calcium Chloride	Dog		1	Oral	980	died, 12 dys	591
Calcium Chloride	Man	-		Oral	30 g (estim.)	LD_{100}	677
Calcium Chloride	Man	Infant	2	Gavage	4 g/25 ml H_2O 3 g/18 ml H_2O	LD_{100}	215
Calcium Chloride	Man	Infant	1	Oral	2 g/H_2O + 2 later	very sick	215
Calcium Chloride	Man		1	i.v.	10 ml, 10% daily	fatal	867
Calcium Chloride	Man		2	i.v.	-	anaphylactic shock	867
Calcium Chloride	Man	Infant	1	Oral	2 g/12 ml H_2O	very sick	357
Calcium Gluconate	Mouse		10	i.v.	950 ± 83	LD_{50}	171
Calcium Gluconate	Man	Infant	1	i. musc.	10 ml 10% sol.	fatal	527
Calcium Gluconate	Man	Infant	2	i. musc.	10 ml, 10% sol.	near-fatal	527

A. Calcium acetate

None

B. Calcium chloride

Boyd and Seymour (98) in a paper primarily devoted to studies of ethylenediamine dihydrochloride included an experiment using albino rats, guinea pigs and rabbits given aqueous calcium chloride by gastric intubation at dose-levels of 0.05, 0.25 and 1.0 g/kg body weight. No evidence of toxic reactions was observed in the 150 animals of each kind tested.

Intramuscular injections of calcium chloride in 112 albino rats indicated an LD_{50} of 25 mgm/kg.

Hoff and Nahum (395) undertook a series of three experiments: (1) to determine the mechanism of calcium syncope; (2) to analyze the cardiac arhythmias which appear after calcium injection: and (3) to devise a method for their prevention.

Ten rabbits (not otherwise described) were used in each experiment; the first group of unanesthetized animals was given 2-6 cc of 10% calcium chloride solution intravenously at the rate of 1 cc/30 sec; the second group, unanesthetized, was given 25-50 mgm of atropine sulfate intravenously and the chloride as in the first group; the third group, anesthetized with 60 mgm sodium amytal/kg, also received the same calcium chloride treatment.

Serial electrocardiograms taken during all thirty experiments showed changes in the nature of the ventricular complex so uniform and characteristic as to constitute specific calcium effects; neither atropine nor sodium amytal modified this picture. Low doses of calcium chloride showed the heart rate, showed A-V delay and auricular fibrillation; larger doses produced extrasystoles, Luciani periods and cardiac arrest. Sodium amytal in narcotic doses suppressed all cardiac irregularities due to calcium chloride and prevented cardiac arrest.

An accepted form of treatment for neonatal tetany has been to administer oral doses of calcium chloride, as much as 6-8 gm daily. Hall (357) cited a case in which a week-old infant was convulsed and vomited, and upon hospitalization was given by stomach tube 2 grams of calcium chloride in 12 cc of water (16.6% solution) on the assumption that the convulsions were due to tetany. The convulsions ceased but vomiting and diarrhea were almost continuous. A week later, a piece of necrotic tissue was discovered on the bed by the baby's head and pathological examination suggested that the tissue was probably from the stomach lining of the baby. Thirteen days after admission (to the hospital), X-rays showed a coating of calcium outlining part of the stomach wall. The infant's condition improved in succeeding weeks, the calcium slowly disappearing. After three months the child was discharged in good condition. At eight months, x-ray showed a constant constriction of the fundus of the stomach where the calcium deposit had been most prominent. It was suggested that hydrolysis of the calcium chloride forms some hydrochloric acid which may penetrate tissues, inducing necrosis and subsequent deposition of calcium. Calcium chloride, even in small doses, may be harmful in the upper intestinal tracts of new-born infants

and may produce secondary calcium deposits in necrotic tissues of the esophagus, stomach and jejunum.

Durlacher et al. (215) reported three cases of infants treated with calcium chloride, two by gavage (4 g $CaCl_2$ in 25 cc H_2O, and 3 g $CaCl_2$ in 18 cc H_2O) and one orally (2 g $CaCl_2$ in water). The latter child vomited and was then given 2 g $CaCl_2$ at a later feeding which was retained. The first two infants died, the third was discharged from the hospital with a diagnosis of having suffered from hypocalcemic tetany. Gastrointestinal lesions and other abnormalities which occurred in the two fatalities led the author to suggest that administration by gavage of calcium chloride to new-born infants is contraindicated.

Severova and Teplukhin (867) reported several cases of anaphylactic shock due to apparent sensitization of patients receiving intravenous calcium chloride during therapy. The death of a 30-year-old woman in therapy was closely examined for causes of death and the conclusion was reached that daily injections of 10 ml of a 10% solution of calcium chloride in treatment of an allergic eczematous otitis of one ear were responsible. The authors caution doctors to exercise more care and discrimination in assigning various drugs for intravenous treatments because of the possibility of specific or paraspecific sensitization and subsequent allergic reactions.

C. Calcium citrate

None

D. Calcium diacetate

None

E. Calcium gluconate

Lamm (527) reported three cases of abscess formation and one death in infants following injection of calcium gluconate intramuscularly. In the two nonfatal cases, 10 cc of 10% calcium gluconate was injected into the muscles of the right buttock; in the fatality, 10 cc was injected into both buttocks. An autopsy of the infant, scarcely more than three weeks old at death, was detailed in the report which concluded with anatomic diagnoses of "abscess of buttocks, sepsis, atelectosis of both lungs, bilateral hemmorrhagic pneumonia and acute purulent basilor meningitis." The author objected to the widely published sources which indicate that calcium gluconate may be used with safety intramuscularly, intravenously or orally. The author suggested eliminating from book and drug literature the use of calcium gluconate for intramuscular injection in infants, where therapy should be by oral or intravenous means.

II. Short Term Studies

A. Calcium acetate

Rats

Prioreschi and Selye (757) produced gastritis and cardiorenal lesions by combined stress and calcium acetate intubated gastrically in 60 female Sprague-Dawley rats of weights ranging from 90-110 g. The animals were divided into 6 equal groups and given various treatments including stress (forced restraint, fastened to a board, prone. for 24 hours), calcium acetate and combinations of treatments. The acetate was administered as 3 ml of 2 or 4 m M solutions, divided or single doses (approximately 200 mg/kg body weight). Groups 1 through 3 were kept 24 hours and Groups 4 - 6 kept for 8 hours upon which all survivors were sacrificed. Hearts, kidneys and stomachs were examined for lesions then prepared for demonstrating Ca deposits. Severity of lesions was graded as 0 = no lesions, 1 = barely detectable lesions, 2 = moderate lesions, and 3 = most severe lesions; mean grades with standard errors, treatment and mortalities are shown in the following table:

TABLE.—*Production of Cardio-Renal Lesions by Calcium Acetate and Stress*

Group	Treatment	Myocardial lesions	Coronary calcification	Nephrocalcinosis	Gastric lesions	Mortality per cent
1	Calcium acetate 2mM × 2	0	0	0	0·	
2	Restraint 24 hr.	0	0	0	1·1 ± 0·20	0
3	Calcium acetate 2mM × 2 + restraint 24 hr.	2·4 ± 0·30	2·0 ± 0·45	1·7 ± 0·30	2·8 ± 0·17	60
4	Calcium acetate 4mM × 1	2·0 ± 0·43	1·0 ± 0·45	1·5 ± 0·25	3·0 ± 0	10
5	Restraint 5 hr.	0	0	0	0·4 ± 0·17	0
6	Calcium acetate 4mM × 1 + restraint 5 hr.	2·0 ± 0·45	1·7 ± 0·27	1·2 ± 0·38	3·0 ± 0	90

The role of stress in this experiment producing cardiopathy seemed fundamental; restraint was necessary for production of pathological changes in the calcium acetate treated rats, which showed little effect when treated with the acetate alone. The cardiopathy herein observed seemed to be pluricausal, depending not upon a single pathogen, but upon the creation of complex pathogenic situations in which stress and "adaptive hormones" play important roles.

B. Calcium chloride

Mice

The effects of reserpine administration on calcium homeostasis were tested in mice by Carrier et al. (144). The possibility that the increased nerve and muscle excitability following administration of reserpine may be due to the lowering of the calcium content of the extracellular fluid bathing these tissues suggested the following experiments.

Albino mice of both sexes, weighing 30 g each, were used in the first of two experiments to determine the lethal effects of intraperitoneally injected $CaCl_2$ at various concentrations. Control mice were injected as follows: 20 received 400, 20 received 500, 25 received 600, 25 received 650, and 25 received 700 mg/kg $CaCl_2$. Fifty-three mice were given 0.1 mg/kg reserpine 4 hours before administering in about half of them 400 mg/kg $CaCl_2$, and the remainder, 500 mg/kg $CaCl_2$. Another group (omitted by authors in discussion) received 0.25 mg/kg reserpine and 400 and 500 mg/kg $CaCl_2$. The results are shown in the table below:

Increased toxicity of $CaCl_2$ after reserpine in 30g mice

Group	Calcium chloride (mg/kg ip)	Survival (after 4 hours)
Control	400	20/20*
Control	500	16/20
Control	600	12/25
Control	650	10/25
Control	700	4/25
Reserpine (0.1 mg/kg, 4 hr)†	400	12/26
Reserpine (0.1 mg/kg, 4 hr)	500	2/27
Reserpine (0.25 mg/kg, 4 hr)	400	11/21
	500	0/20

* Ratio of survivors to total group.
† Reserpine administered intraperitoneally at this concentration and time before experiment.

The second experiment used younger, 20 g mice of which 25 received 650 mg/kg $CaCl_2$ and 27 mice received 1000 mg/kg $CaCl_2$, serving as controls; 26 mice were given a dose of 1.25 mg/kg reserpine and 25 given 2.5 mg/kg reserpine intraperitoneally 24 hours before being divided into 4 groups and administered 650 or 1000 mg/kg $CaCl_2$. Survival times were observed and the results are shown below:

Effect of reserpine on the time until death of 20g mice after intraperitoneal injections of $CaCl_2$

Group	Calcium chloride (mg/kg, ip)	Time until death (up to 2 hrs)	Survival (after 2 hrs
Control	650	94 ± 14 min.*	12/25¶
Control	1,000	37 ± 10 min.†	13/27
Reserpine (1.25 mg/kg, 24 hr)§	650	43 ± 17 min.‡	5/12
Reserpine (1.25 mg/kg, 24 hr)	1,000	10 ± 20 min.	3/14
Reserpine (2.5 mg/kg, 24 hr)	650	56 ± 20 min.	4/12
Reserpine (2.5 mg/kg, 24 hr)	1,000	13 ± 1 min.	3/13

* Mean value with standard error of the mean.
† Times at 1,000 mg/kg significantly different from times at 650 mg/kg for all groups ($P<0.05$)
‡ Times of experimental groups significantly different from corresponding control ($P<0.05$).
§ Reserpine administered intraperitoneally at this concentration and time before experiment.
¶ Ratio of survivors to total in group.

Those mice receiving the higher dose of 1000 mg/kg $CaCl_2$ in controls and pretreated with reserpine died in a different fashion from those receiving the lower, 650 mg/kg $CaCl_2$. The latter appeared sedated, moving slowly and dying quietly; those given the higher dose were highly excited, moving about rapidly and dying suddenly.

The Rauwolfia alkaloids, of which reserpine is an important member, must be used carefully as regards side-effects; the observation just noted of the differences in dying of the mice at different dose-levels is significant. Depression versus excitability appears to be related to the tissue loss of calcium, affecting the central nervous system as well as the peripheral nerves.

Rats

Sharpless et al. (872) reviewed goiter surveys and experimental studies suggesting that calcium is a goitrogenic agent. The influence of vitamin D, by its action on absorption, together with four calcium salts (chloride, lactate, carbonate and phosphate) orally administered in the diet, was studied by using rats, male and female, 4 to 5 weeks old. The basal diet counted, in percentage composition, of soybean flour, 75.0; dried brewer's yeast 3.0; calcium carbonate 0.5; sodium chloride 1.0; butterfat, 5.0; sucrose 15.5; and potassium iodide, 4 micrograms. In the experiment with calcium in the drinking water, 1% calcium chloride was added. The results of these diets in thyroid enlargement show insignificant or no effect of calcium salts as shown in the following table:

TABLE

Effect of calcium salts on weight and iodine content of the thyroid.

DIET	NO. OF RATS	THYROID WEIGHT		THYROID IODINE	
		Ave. total	Per 100 gm. body wt.	Total per gland	Dry basis
		mg.	*mg.*	*μg.*	%
Basal	212	22	17 ± 0.21	1.5	.026
1% $CaCl_2$ in drinking water	24	21	17 ± 0.7	1.7	.025
2% $CaCl_2$ in basal diet	71	23	17 ± 0.47	1.6	.029
6% calcium lactate	15	18	18 ± 0.8	1.2	.026
2% calcium carbonate	23	25	17 ± 0.97	1.2	.020
2% $Ca_3(PO_4)_2$	11	20	19 ± 0.8	1.8	.030
Rolled oats 50.0% Corn meal 50.0%	12	12	14 ± 0.5	3.3	.11
2% $CaCl_2$ in drinking water	16	16	12 ± 0.5	2.1	.06

Only those animals which lived for the full 12 weeks of the experiment are recorded in the table. Microscopic examination of thyroid/parathyroid glands showed no real differences. High calcium intake does not affect the size of the thyroid.

Experiments were undertaken to show the influence of vitamin D (as viosterol), in large and small amounts, together with calcium carbonate and calcium chloride in the diets. The results, shown in the following table, indicate that only the chloride has significant goitrogenic effect when vitamin D is present:

TABLE

Effect of viosterol and calcium on weight and iodine content of the thyroid.

BASAL DIET SUPPLEMENT	NO. OF ANIMALS	BODY WEIGHT		THYROID WEIGHT		IODINE IN GLAND	
		Initial	Final	Ave. total	Per 100 gm. body wt.	Ave. total	Dry basis
		gm.	*gm.*	*mg.*	*mg.*	*μg.*	%
Basal —40 μg. I_2 per kg.	36	65	160	53	33 ± 1.1	0.7	.007
15 drops viosterol per kg.	29	68	131	44	34 ± 1.4	0.9	.009
2% viosterol	39	60	147	50	34 ± 1.1	0.6	.006
2% $CaCO_3$	17	73	145	55	37 ± 2.1	0.7	.007
2% $CaCO_3$ + 2% viosterol	17	70	134	49	37 ± 1.2	0.6	.006
Basal	82	65	139	23	17 ± .40	2.2	.038
I_2 + 2% viosterol	28	61	133	24	18 ± .72	2.3	.043
I_2 + 2% $CaCl_2$	70	65	128	23	18 ± .47	2.0	.038
I_2 + 2% $CaCl_2$ + 15 drops viosterol	55	67	141	28	20 ± .52	2.0	.033
I_2 + 2% $CaCl_2$ + 2% viosterol	27	63	134	29	22 ± 1.0	1.62	.029
I_2 + 2% $CaCO_3$	23	72	145	25	17 ± .97	2.2	.039
I_2 + 2% $CaCO_3$ + 2% viosterol	24	69	128	21	16 ± .63	2.0	.040

The mechanism of the action which limits the calcium chloride vitamin D goitrogenicity was not clearly shown in these experiments, but it was suggested that the chloride causes some loss of iodine which is followed by an increase in thyroid weight when an excess of calcium is absorbed.

The action of three calcium salts (gluconate-idonate, gluconate, and chloride) administered by stomach tube to rats was studied by Smith (896). The gluconate-idonate and the chloride were given in solution and the gluconate in a heavy suspension, all in amounts equivalent to 0.4 g-Ca/kg of body weight, daily. The gluconate-idonate and chloride were also tested at 1 and 2 g Ca/kg/day. Ten animals were in each group except the one receiving 1 g Ca/kg as the gluconate-idonate, which contained 20 rats.

All animals at the 2 g/kg level died after a single dose. At the level of 1 g/kg Ca as the chloride, death occurred after one or two doses, but as the gluconate-idonate, 4 animals survived the entire period of 70 days. At the dose level of 0.4 g/kg, 5 of the original 10 rats survived the entire period, while all receiving the gluconate-idonate or gluconate survived.

Examination of heart, kidney and liver tissues from animals given the gluconate-idonate at 1 g/kg/day for seventy days, showed no histological

abnormalities attributable to the drug. Similarly, neither gluconate (for 70 days) nor chloride (for 65 days) at the dose level of 0.4 g/kg/day produced any apparent changes. Calcium chloride, orally administered to rats is definitely more toxic than calcium gluconate-idonate or calcium gluconate.

Rabbits

Govan and Parkes (324) in an earlier study had observed that administration of ammonium chloride to rabbits caused acidosis and death, probably due to uremia resulting from widespread renal tubule necrosis. In the present study, calcium chloride was selected for a similar study, in an effort to eliminate the ammonium ion as a possible factor causing death. Twelve rabbits (not otherwise identified) were fed a diet of most bran and oats; urine was collected and estimates made of urea, chlorides, phosphates, ammonia and pH. Blood samples every second day were taken for analysis. After one week of this treatment calcium chloride (10% solution) was iutubated gastrically at doses of 1.5 g/kg (2 animals), 1.8 g/kg (2 animals), 2.2 g/kg (2 animals) and 2.5 g/kg (6 animals). Blood and urine examinations were performed as before.

The reactions of the animals to calcium chloride formed two distinct groups: group I, 5 animals (4 receiving 1.5-1.8 g/kg, 1 receiving 2.2 g/kg) and group II, 7 animals (6 receiving 2.5 g/kg and 1 receiving 2.2 g/kg). All animals in group I were sacrificed on the 5th day of the experiment and only minor abnormalities were observed. The results are shown in the table below:

TABLE

	Blood urea, mg./100 ml.	CO_2 c.p., vol./100 ml.	pH.	Cl', mg./100 ml.	Serum Ca, mg./100 ml.	Serum P, mg./100 ml.	Cholesterol, mg./100 ml.	Lipoids, mg./100 ml.	Ammonia, mg./100 ml.
Before . .	42·1	40	7·19	594	12·0	3·76	43·6	0·14	0
After . .	57·7	13·5	6·8	742	13·3	4·25	70	0·13	0

In group II, 5 animals died on the 5th day; the remaining two were comatose and were killed. Marked acidosis was present and gross abnormalities appeared in the second group. The results are shown below:

TABLE

	Blood urea, mg./100 ml.	CO_2 c.p., vol./100 ml.	pH.	Cl', mg./100 ml.	Serum Ca, mg./100 ml.	Serum P, mg./100 ml.	Cholesterol, mg./100 ml.	Lipoids, mg./100 ml.	Ammonia, mg./100 ml.
Normals . .	42·1	40	7·19	594·6	12·0	3·76	43·6	0·14	0
After $CaCl_2$.	92	16 .	7·00	752·6	16·2	8·1	116·0	0·6	5·35

The appearance of blood ammonia after 2.5 g of $CaCl_2$ indicated the possibility of spontaneous formation of ammonia from adenosine phosphate. The renal damage found in ammonium chloride experiments was absent in the present study.

Durlacher et al. (215), following observations of human toxicity of calcium chloride, performed experiments with rabbits to demonstrate similar effects of three compounds: anhydruis calcium chloride, calcium lactate and ammonium chloride.

Forty-two rabbits, from 2 days to 6 weeks of age were used (no further identification given). The animals were fasted for 48 hours and permitted to eat 6 hours after gavage. The $CaCl_2$ was given in 5, 7.5, 10, 15, and 20% aqueous solutions; the lactate in 5 and 10% aqueous solution; ammonium chloride in 5, 10, and 20% solution; 3 rabbits were given combined lactate and ammonium chloride. Two rabbits were simply intubated with the catheter alone to indicate whether or not mechanical irritation was a possible cause of trauma (it caused no lesions). The results are shown in the following table:

EFFECTS OF CALCIUM SALTS BY GAVAGE

TABLE

EFFECTS OF CERTAIN SALTS ADMINISTERED TO RABBITS BY GAVAGE

Rabbit	*Weight (gm.)*	*Salt*	*Dose (gm./kg.)*	*Concentration (%)*	*Manner of death*	*Edema*	*Mucosal necrosis*	*Submucosal necrosis*
1	60	—			S	—	—	—
2	80	—			S	—	—	—
3	215	$CaCl_2$	0.75	20	S	+++	++	—
4	180	$CaCl_2$	1.50	20	D	+++	+++	++
5	275	$CaCl_2$	1.50	20	S	+++	++	—
6	900	$CaCl_2$	1.50	20	S***	+++	+++	+++
7	1000	$CaCl_2$	1.50	20	D	+++	+++	+++
8	80	$CaCl_2$	0.68	15	S	—	—	—
9	80	$CaCl_2$	1.25	15	S	+++	++	—
10	60	$CaCl_2$	1.25	15	D	+++	+++	—
11	60	$CaCl_2$	1.25	15	S	+++	++	++
12	80	$CaCl_2$	2.50	15	S	+++	+++	+++
13	160	$CaCl_2$	1.50	15	S	+++	+++	+++
14	375	$CaCl_2$	1.50	15	S*	+	—	—
15	780	$CaCl_2$	0.75	15	S	+	—	—
16	810	$CaCl_2$	1.50	15	S	+	—	—
17	920	$CaCl_2$	1.50	15	S	+	—	—
18	150	$CaCl_2$	1.50	10	D	+++	+++	—
19	330	$CaCl_2$	1.50	10	S	++	—	—
20	775	$CaCl_2$	0.75	10	S	—	—	—
21	810	$CaCl_2$	1.50	10	S	+	—	—
22	1000	$CaCl_2$	1.50	10	S	—	—	—
23	60	$CaCl_2$	1.25	7.5	S	—	—	—
24	760	$CaCl_2$	0.75	5	S	—	—	—
25	110	$CaCl_2$	1.50	5	D	++	++	+
26	810	$CaCl_2$	1.50	5	S	—	—	—
27	1100	$CaCl_2$	1.50	5	S	—	—	—
28	120	Ca lact.	4.0	10	S	—	—	—
29	275	Ca lact.	6.0	10	S	—	—	—
30	340	Ca lact.	6.0	10	S*	—	—	—
31	650	Ca lact.	2.0	10	S	—	—	—
32	700	Ca lact.	2.0	10	S	—	—	—
33	645	Ca lact.	4.0	5	S	—	—	—
34	200	NH_4Cl	0.5	20	S	—	—	—
35	342	NH_4Cl	1.5	20	D**	—	—	—
36	315	NH_4Cl	0.5	10	S	—	—	—
37	320	NH_4Cl	1.0	10	S	—	—	—
38	320	NH_4Cl	0.5	5	S	—	—	—
39	344	NH_4Cl	1.0	5	S	—	—	—
40	280	NH_4Cl	2.0	10	D**	—	—	—
		Ca lact.	2.0	10				
41	210	NH_4Cl	4.0	10	D**	—	—	—
		Ca lact.	4.0	10				
42	240	NH_4Cl	1.0	5	S	—	—	—
		Ca lact.	2.0	10				

S — Sacrificed after 48 hours.
D — Died before 48 hours.
* — Sacrificed after 96 hours.
** — Died of acidosis after 1 hour.
*** — Perforation of stomach wall found at autopsy.

All animals receiving 20% $CaCl_2$ showed severe gastric damage consisting of mucosal necrosis and ulceration, submucosal edema, cellular infiltration and angionecrosis. In one instance, the stomach was perforated. The intestine was free of lesions. In those animals receiving smaller doses (5 to 15%) lesions were not found in the older rabbits, but severe ulcers appeared in the unweaned young.

The lactate was ineffective in producing abnormalities at the largest doses possible (limited solubility restricted solutions to 10% or less). The ammonium chloride was fatal at doses greater than 1.5 g/kg.

Dogs

Mahorner (591) performed a series of experiments designed to produce goiter in animals, one experiment of which subjected six dogs to oral (by intubation) administration of calcium chloride. (The animals are not otherwise described except as to weight). A preliminary operation removed small samples of the thyroid gland of each dog for microscopic study. At the beginning of the experiment 4 dogs were given 2% $CaCl_2$ which they refused to drink, so after 5 days, the solution (500 cc) was intubated twice daily. Continuous vomiting by all the animals for 4 days, and the subsequent death of one, led to administering 0.05% $CaCl_2$ in the drinking water during the remainder of the experiment; the 2 other dogs had received this treatment from the start. Four of the six dogs died after 6, 7, 12 and 96 days; the remaining two were sacrificed after drinking the calcium water for 8 and 113 days, respectively. At necropsy the thyroid glands were removed, examined, and prepared for histological studies. Blood calcium had been determined prior to the experiment for each dog, and weekly determinations were made during the experiment. The results are shown in the following table:

TABLE —*Results with a High Calcium Intake*

Dog No.	Initial Weight, Kg.	Method	Duration of Experiment, Days	Clinical Appearance	Comparison of First and Second Sections of Thyroid Gland
68	3.8	Calcium chloride 0.05%	6	Died	Hyperplasia (?)
65	7.8	Calcium chloride 2%, 500 cc. daily	7	Sick; died	Smaller acini; less colloid
66	10.2	Calcium chloride 2%, 500 cc. daily	12	Sick; died	No change
69	6.6	Calcium chloride 0.05%	80	Healthy	Definite hyperplasia
64	10.3	Calcium chloride 2%, 500 cc. daily for 8 days and 0.05% for 88 days	96	Sick; died	Hyperplasia (?); post-mortem degeneration
67	10.1	Calcium chloride 2% for 8 days and 0.05% for 105 days	113	Healthy	Definite hyperplasia

TABLE —*Blood Calcium Determinations on Dogs Drinking Calcium Water*

Dog No.	Preliminary Estimation of Blood Calcium, Mg. High	Low	Date of Starting Experiment	Subsequent Calcium Determinations, Mg.
68	10.12	10.06	July 17	July 23, death
65	10.78	10.62	June 14	June 15, 10.52; June 20, 11.01; June 22, death
66	11.19	10.92	June 14	June 15, 11.48; June 20, 11.21; June 26, death
69	11.42	11.42	July 17	July 27, 10.98; August 2, 11.14; August 9, 12.78; August 16, 11.48; August 23, 12.49; August 30, 14.20; September 6, 11.32; September 13, 12.03; September 20, 10.92; September 27, 11.46; October 4, 12.33; October 5, killed
64	10.90	10.51	June 14	June 15, 11.58; June 21, 11.96; June 27, 11.14; July 3, 11.14; July 10, 11.14; July 27, 10.51; August 2, 11.14; August 9, 12.78; August 16, 9.64; August 23, 9.49; August 30, 10.12; September 6, 8.82; September 13, 8.69; September 17, necropsy
67	11.33	10.90	June 14	June 15, 11.2; June 20, 14.61; June 27, 11.28; July 3, 11.28; July 10, 11.14; July 27, 10.51; August 2, 11.14; August 9, 12.78; August 16, 11.64; August 23, 12.25; August 30, 11.70; September 6, 10.17; September 13, 11.23; September 20, 10.92; September 27, 10.26; October 4, 10.64; October 5, killed

Gross measurements showed no appreciable difference in the thyroid glands between preliminary observations and necropsy; microscopically, however, marked changes throughout the glands had occurred. Hyperactivity was indicated by the loss of colloid from the vesicles; columnar epithelium was enlarged; the colloid appeared to have degenerated into bubbles. It appeared that the calcium chloride was not the cause of colloid goiter, but did change the histologic appearance of the gland.

A comparative study of the effect of intravenous injection of large doses of calcium gluconate-idonate, gluconate, and chloride on dogs was made by Smith (896). Eighteen adult dogs received subcutaneously morphine sulfate, 10 mg/kg body weight, 30 minutes before the intravenous treatment with the calcium salts. The salt solution (0.205 M) were injected into the femoral vein through a cannula at varying rates. Blood samples were secured prior to cannulation from the femoral vein and at death by direct cardiac puncture. Serum calcium was determined and electrocardiograms were taken at frequent intervals.

Appreciably larger quantities of calcium as the gluconate-idonate or the gluconate were required to produce death intravenously than were needed when given as the chloride. Intravenous injection of 0.205 M solutions continued to the point of cardiac arrest (usually with ventricular fibrillation) showed no differences between the gluconate-idonate and gluconate.

C. Calcium citrate

None

D. Calcium diacetate

None

E. Calcium gluconate

Mice, Dogs

A number of toxicological studies are reported by Coulston et al. (171) in an evaluation of the toxicity of calcium kinate gluconate (CKG) in comparison with that of calcium gluconate and calcium chloride. The CKG is a highly soluble complex of calcium kinate and calcium gluconate in an approximate molar ratio of 2:1.

Three groups of 10 mice each were injected intravenously with the three calcium compounds to determine LD_{50}. The CKG and gluconate were of nearly equal toxicity, but both were less toxic than the chloride. The LD_{50} for 24 hours and for seven days were the same.

TABLE

ACUTE TOXICITY IN MICE INJECTED INTRAVENOUSLY WITH CALCIUM SALTS

Medication	I.v. 24-hr[a] LD_{50} (mg/kg ± S.E.)	
	As Compound	As Ca
Calcium kinate gluconate	1050 ± 57	83 ± 4
Calcium gluconate	950 ± 83	86 ± 8
Calcium chloride	215 ± 14	78 ± 5

[a] Same LD_{50} at 7 days.

Two groups of six of unanesthetized dogs were injected intravenously with CKG and gluconate; a week later a crossover was done, the alternate substances were injected. The rate of injection was at 3 mg Ca/kg/minute, or twice that recommended in therapy. Maximum tolerance of calcium was determined at the moment when vomiting occurred, and the total milligrams of calcium injected was noted. The CKG was better tolerated than calcium gluconate: the mean maximum tolerated dose of CKG was 19.7 ! 2.6 mg Ca/kg, and for the gluconate, 13.2 ! 1.7 mg Ca/kg.

Other experiments were performed but were irrelevant to this section of the monograph.

F. Calcium phytate

None

III. Long Term Studies

None

IV. Special Studies

Therapy

Hoffman and Blinick (397) studied the metabolic influence of phosphorus-free calcium supplements in 42 female hospital patients in their 21st to 31st weeks of pregnancy. Six equal groups of 7 each were formed, and control values of serum calcium were determined. For successive 3-week periods, each group was given a supplement of either calcium citrate, carbonate or lactate, 6 capsules daily, for a total dose of 450 mg/day of elemental calcium. At the end of each 3-week period, calcium, phosphorus and serum protein were determined, the presence or absence of leg cramps noted, and another of the salts prescribed. For a number of reasons, only 36 of the 42 patients successfully completed the 9-week series.

No significant differences in the mean calcium-phosphorus levels were found in the three salts used. The incidence of leg cramps was smallest with calcium-citrate; the overall incidence of cramps during treatment diminished through the second 3-week period of treatment, leveling off during the last three weeks.

A comprehensive review of the role of calcium in human physiology has been presented by Blum (84). A general review of the results of investigators in the early decades preceding the author's presentation (1933) was followed by a detailed history of the medical uses of calcium chloride: a therapeutic agent in "states of spasmophilia", anxiety, asthma, neurasthenic syndrome, vomiting in pregnancy, tuberculosis, diarrhesis or constipation (dose-dependent), and diuresis. $CaCl_2$ has been used in myocardia and as an antixenic (antiinfection); other applications are noted in hemorrhage, in albuminurias and in colloidoclasis.

Baldacci (35), noting the wide use of calcium medication in therapy,

reviewed the literature on calcium salts and undertook a series of experiments on four calcium salts, two of which - the chloride and the gluconate - are of interest to this monograph. The author found little information in the literature concerning the toxicology of these compounds that was based on good experimental procedures. Knowledge of toxicity is as indispensible as that of the pharmacological and therapeutic effects of drugs. Using methods developed by a co-worker of the author in the determination of toxicity of injected drugs, Baldacci performed a series of experiments using rabbits to determine the "distant minimum lethal dose" of four calcium salts. The expression "distant minimum lethal dose" implies the use of increasingly larger doses of the salt from a point at which no animals die at some time following administration of the drug, to that time when death is observed from that and succeeding doses given. Sixty-two rabbits, in all, were used. Solutions of the calcium salts were 0.5 N, and were injected into the marginal vein of the rabbits ear at a rate of 0.5 cc/kg body weight/ minute and the animal observed until dead or recovered. Nineteen rabbits were used in the $CaCl_2$ experiment which resulted in a distant, minimum lethal dose of 0.0080 g/kg; in the gluconate studies; the dose was 0.0130 g/kg. The works of other investigators are discussed by the author, and his own conclusions are that death in his experiments can be attributed to the depressive dffect of the calcium cation on the nervous centers.

A second study by Baldacci (35) is included in this monograph to complete the story of his studies on calcium; his work appears to be of considerable merit.

Three test subjects showing expressive clinical symptoms of alcohol intoxication were selected for a series of studies to determine whether therapeutic doses of calcium chloride would influence the degree of intoxication or blood alcohol content. Alha (11) notes the affinity of calcium chloride for alcohol in industrial uses for isolation and quantitative determinations of alcohol and speculates whether the chloride might be of therapeutic value in treating alcoholism. The three volunteers were given measured doses of alcohol and $CaCl_2$, 2 g in 10% solution orally administered, or 10 ml of 10% solution intravenously in 6 minutes. Blood samples were taken periodically and serum calcium and blood alcohol determined. The amount of alcohol given was measured from 30.6% alcohol in water at about 1 g/kg body weight. Seven experiments in all were performed, with results generally negative or inconclusive. Serum calcium levels were unaffected by alcohol and blood alcohol values were not influenced by $CaCl_2$. There was some, slight reduction of the symptoms of intoxication by the $CaCl_2$, possibly due to influence on renal functions.

The pharmacology of calcium gluconate has been extensively studied by Lieberman (564) who reported results of experiments on dogs and humans by various methods of administering the gluconate. The intravenous studies in dogs were described elsewhere; the human experiments involved some thirty industrials and are also detailed in a separate report. Of these humans, ten received 10 gm orally of the gluconate on an empty stomach; ten received 10 gm after a standard breakfast; five had 20 cc of 10% solution of the gluconate injected deep in the buttocks with no food; and five received similar intramuscular injections following a standard breakfast. Lieberman concludes with regard to the use of calcium gluconate:

1. Calcium gluconate is taken as a most convenient representative calcium salt which can be administered subcutaneously in repeated and adequate amounts without causing irritation or necrosis; three or four grams a day after meals appears to be the right dose orally given to adults.

2. Blood calcium reaches maximum elevation within an hour after subcutaneous or intramuscular injection and within four hours after oral administration.

3. Intravenous calcium therapy is dangerous due to possible sudden intravascular clotting and death.

4. Urinary calcium appears to be a qualitative index of the state of blood calcium level. Hyper- or hypocalcemia can be avoided by maintaining a urinary value of about 10 to 20 mg/hour.

5. Suggestive digitalis-like effects of calcium were observed and require further study.

Rothlin presented in 1930 (801), a detailed and comprehensive discussion of earlier investigations - and some of his own - into the therapeutic value of calcium gluconate, concluding that the gluconate may be safely used in medical therapy by oral, intravenous and intramuscular administration. The route chosen for giving calcium should be determined by the medical exigencies of the patient: oral administration is indicated, when prolonged effect is desired as in metabolic Ca disorders involving demineralization, particularly when accompanying parathyroid hormone treatments, or in tetany or rickets; parenteral application, with rapid effects, is called for as a specific pharmaceutical in hemorrhage, asthma, rhinitis, intestinal tuberculosis, shock effects, etc.; intramuscular administration of calcium should be applied similarly to oral uses in cases of hypercalcemia or demineralization. Calcium gluconate was recommended as the most suitable calcium salt for intramuscular applications.

Naef (667) reported an interesting case of a rare disease, idiopathic hypoparathyroidism, successfully treated by oral administration of 12 gm calcium gluconate and 150,000 units of vitamin D_2 (calciferol) daily. The symptoms of this disease are alopecia, convulsions, cataract and impairment of mental functions. Body spasticity and inability to communicate, and considerable pain characterized the patient, a 30-year-old woman. After about a month of the gluconate plus calciferol therapy, aluminum hydroxide gel, 15 ml three times a day, and an increased level of calciferol (200,000 units) were added to the treatment. The patient's response to therapy was rapid, and steady improvement during the following several weeks led to the disappearance of tetany, a noticeable regrowth of hair, and increased mental activity. Complete clinical recovery made this case noteworthy.

The relationships of serum calcium, phosphorus and potassium in infantile tetany were studied by Habib and Ibrahim (346). Twenty-six infants (6 months to 3 years of age) suffering from infantile tetany; 21 infants with rickets; and 32 normal infants were observed in the study. Preliminary blood samples were taken and serum sodium and potassium concentrations determined. The treatment of the infants with tetany

consisted of intravenous calcium gluconate together with calcium chloride and a sedative by mouth, (dosage not given by author). In the rickitic infants the gluconate injection was accompanied in four cases by intramuscular colloidal calcium and vitamin D. No sex differences were observed in the results of treatment. Serum potassium concentration in cases of tetany was statistically lower than in normal infants; the calcium gluconate treatment and sedatives produced a rise in serum potassium concentration to normal levels even though no potassium salts were given. The serum sodium in tetany cases and both sodium and potassium levels in rickets did not differ from those levels in normal children.

Carcinogenicity

The hazards of the use of $CaCl_2$ in therapy were observed by Bulgatch (122) in 1929. He described "an extremely unpleasant complication" arising from intravenous or subcutaneous Ca-therapy in the form of tumors or chlorcalcinomas. Sharp pains, inflamation and gangrene characterized the clinical chlorcalcinomas observed by the author who became interested in the existing treatments for such tumors. The injection of very small amounts (5-6 drops) of a dilute $CaCl_2$ solution (10%) produced chlorcalcinomas or necrosis of tissues at the site of subcutaneous administration; the degree of damage appeared directly related to the quantity of $CaCl_2$ injected. Various investigators had suggested the simultaneous injection of a 25% solution of sodium sulfate with that of the $CaCl_2$ to minimize the tumorogenic effects of the $CaCl_2$. The relationships of timing and quantities used of the Na_2SO_4-$CaCl_2$ administered were studied by Bulgatch in rabbits and dogs. The following table indicates the experimental results:

No.	Name of Animal	Weight	Quantity of Subcutaneous Injection: Right	Left	Results: Right	Left
1	[illegible]	1.100g	0.[illegible] soln. $CaCl_2$	0.[illegible] soln. [illegible]	Surface necrosis of localized size	No deviation from norm
2	[illegible]	[illegible]g	0.[illegible] soln. $CaCl_2$	[illegible]	Necrosis of the size of a [illegible]	"
3	[illegible]	1.[illegible]g	0.[illegible] soln. $CaCl_2$	[illegible]	Necrosis the size of a [illegible]	"
4	[illegible]	1.[illegible]g	0.[illegible] soln. $CaCl_2$	[illegible]	Necrosis the size of a [illegible]	"
5	[illegible]	[illegible]g	[illegible] soln. [illegible]	[illegible]	"	"
6	[illegible]	[illegible]g	1.0 soln. $CaCl_2$	[illegible]	Necrosis the size of a [illegible]	Superficial necrosis the size of a [illegible]
7	[illegible]	[illegible]g	1.[illegible] soln. [illegible]	[illegible]	"	Necrosis the size of a [illegible]
8	[illegible]	[illegible]g	[illegible]	[illegible]	[illegible]	Necrosis the size of a [illegible]
9	[illegible]	[illegible]g	[illegible]	[illegible]	Necrosis the size of a [illegible]	Necrosis the size of a [illegible]

Note: [illegible] (D. M. [illegible]), [illegible] the first [illegible] dogs.

The most favorable results were obtained by aspirating the $CaCl_2$ solution and injecting the Na_2SO_4 in 3 or 4 multiple doses. The degree of necrosis occurred by the $CaCl_2$ seemed inversely related to the time elapsed before administering the sodium sulfate; half an hour after the chloride injection is too late for effective use of the sulfate.

I. Breakdown

None

II. Absorption - Distribution

The optimum conditions for the utilization of calcium from food were studied by Schreier and Schnepf (848) using rats, normal and rachitic, and radioactive Ca^{45} in the chloride, $Ca^{45}Cl_2$. In all tests, 51.1 mg $Ca^{45}Cl_2$ were gastrically intubated, followed by a series of sodium salts, sodium citrate, phosphate or tartate mixed in water or milk. After 18 hours the animals were sacrificed and the Ca^{45} content determined for blood serum, femur, tibia and fibula, teeth, gastrointestinal tract, urine and feces.

In the first test series, 12 normal rats (not otherwise described), were divided into 4 groups of 3 each. In addition to all groups receiving 51.1 mg $Ca^{45}Cl_2$, group I served as control; group II was given 4 mg of trisodium citrate; Group III, 10 mg trisodium citrate; and group IV, 4 mg trisodium citrate plus 20 mg Na_2HPO_4. A second group of 12 normal rats were similarly treated, but group II received 13 mg trisodium citrate; group III received 20 mg Na_2HPO_4; and group IV, 13 mg trisodium citrate plus 20 mg Na_2HPO_4. A third group of 12 rats, rachitic, were treated as in the second group. The third tabular results are shown below:

Tabelle 1

Versuchs-Gruppe (1)	Salze (2)	Körpergewicht (3) (g)	Ca^{45} in % der zugeführten Dosis: Rumpf (4)	Serum (6) (1 ml)	Knochen (7)	Intestinum (8)	Urin (9)
I	$Ca^{45}Cl_2$	91,0	50,0	0,4	9,5	1,2	2,0
		84,5	50,7	0,3	9,9	1,1	1,8
		86,5	51,6	0,4	9,6	1,2	1,9
II	$Ca^{45}Cl_2$	78,0	56,8	0,3	10,8	1,1	1,4
	+Na-Citr.	91,0	55,2	0,4	11,8	1,3	1,7
	(4 mg)	84,0	53,7	0,3	11,1	1,1	1,8
III	$Ca^{45}Cl_2$	102,0	54,2	0,6	10,2	0,9	1,5
	+Na-Citr.	120,0	57,0	0,6	10,5	0,9	1,8
	(10 mg)	110,5	55,9	0,4	11,3	0,9	1,9
IV	$Ca^{45}Cl_2$	88,5	52,3	0,4	9,5	1,3	2,0
	+Na-Citr.	68,0	51,4	0,4	9,3	1,2	2,0
	$+Na_2HPO_4$	104,5	53,7	0,5	9,4	1,4	2,0

Tabelle 2

Vers.-Gruppe (1)	Salze (2)	Körpergewicht (3) (g)	Ca⁴⁵ in % der zugeführten Dosis (4) Resorption	Serum (6) (1 ml)	Knochen (7)	Incisoren (8)	Urin (9)
I	$Ca^{45}Cl_2$	65,0	50,3	0,4	9,2	1,2	1,7
		74,0	50,0	0,5	9,0	1,1	1,8
		74,0	49,7	0,4	9,3	0,9	1,5
II	$Ca^{45}Cl_2$	52,4	54,6	0,3	9,9	1,5	1,1
	+Na-Citr.	54,2	52,2	0,4	9,7	1,2	1,3
		57,6	55,4	0,4	10,9	1,3	1,1
III	$Ca^{45}Cl_2$	88,5	40,9	0,5	7,3	0,9	0,9
	$+Na_2HPO_4$	80,8	41,7	0,4	6,9	1,0	1,1
		75,7	43,9	0,4	7,7	1,2	1,0
IV	$Ca^{45}Cl_2$	70,3	52,8	0,3	8,9	1,2	1,1
	+Na-Citr.	75,5	53,9	0,3	8,4	0,9	1,2
	$+Na_2HPO_4$	55,6	51,8	0,3	8,3	1,1	0,9

Tabelle 3 (5)

Vers.-Gruppe (1)	Salze (2)	Körpergewicht (3) (g)	Ca⁴⁵ in % der zugeführten Dosis (4) Resorption	Serum (6) (1 ml)	Knochen (7)	Incisoren (8)	Urin (9)
I	$Ca^{45}Cl_2$	54,8	37,4	0,4	5,1	0,8	2,7
		65,4	41,0	0,4	6,2	0,8	2,5
		50,7	36,1	0,4	5,0	0,8	2,6
II	$Ca^{45}Cl_2$	60,3	45,9	0,5	7,7	1,0	2,6
	+Na-Citr.	57,3	47,5	0,5	7,5	1,0	3,3
		51,2	44,7	0,5	9,2	0,9	2,9
III	$Ca^{45}Cl_2$	41,0	40,8	0,3	3,5	0,7	3,4
	$+Na_2HPO_4$	52,0	37,0	0,5	4,0	0,7	3,9
		56,8	44,9	0,5	4,4	0,7	3,2
IV	$Ca^{45}Cl_2$	63,3	49,3	0,5	7,3	0,9	4,1
	+Na-Citr.	64,2	48,2	0,5	6,9	1,0	5,2
	$+Na_2HPO_4$	56,8	47,9	0,5	7,4	0,9	5,3

1. test group; 2. salts; 3. body weight (grams);
4. Resorption; 5. Ca^{45} in % of the dose administered;
6. serum (1 ml), 7. bones; 8. incisors; 9. urine

Ten rats were tested with $Ca^{45}Cl_2$ and $Ca^{45}Cl_2$ plus sodium tartrate; the chloride was incorporated into teeth and bones to a greater degree when the tartrate was given.

Resorption and retention of Ca^{45} from various mixtures with Ca^{45}-labelled citrate and phosphate and a mixture of Ca^{45}-citrate plus KH_2PO_4 plus citric acid showed that the presence of the calcium citrate increased the resorption and retention of the Ca^{45}-labelled phosphate. Sodium citrate, in doses of up to 4 mg given the rat also increases the retention and resorption of Ca^{45}.

A lengthy and detailed discussion closed this study by Schreier and Schnepf (848).

Comparative studies of the absorption of various calcium salts in rabbits (dog and man) were made by Yoshimoto et al. (1031). The kind and number of rabbits used in separate experiments were not indicated, other than "a domestic male" weighing 2-3 kg. The calcium salts used were $CaCl_2$, $CaCO_3$, pearl calc ($CaHPO_4 \cdot 2H_2O$), Ca-citrate, Ca-gluconate, and Ca-succinate. Administration of the salts was by intravenous injection and oral supplements mixed with the bran mash basic diet. After treatment blood samples were withdrawn from the heart periodically and analyzed for serum calcium levels. The serum calcium levels in an untreated control rabbit are shown in Fig. 1. The results of 0.5 g $CaCl_2$ intravenously given appear in Fig. 2; a similar dose given to man is shown in Fig. 3. In Fig. 4, the results of an oral dose of 0.5 g and 1.0 g $CaCl_2$ in rabbits are shown; a similar treatment of a dog is shown in Fig. 5. The results of oral doses of the remaining calcium salts are shown in Figs. 6, 7, 8, 9 and 10.

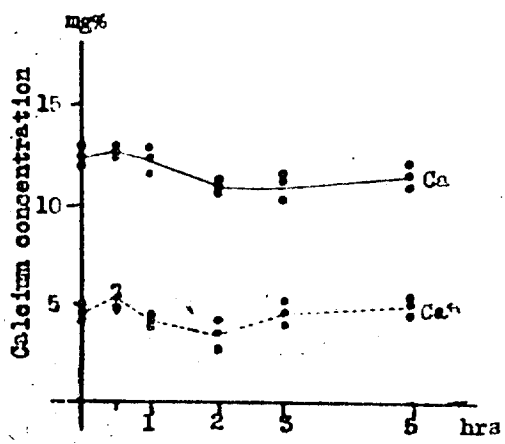

Fig. 1 Serum-calcium levels in untreated rabbit.

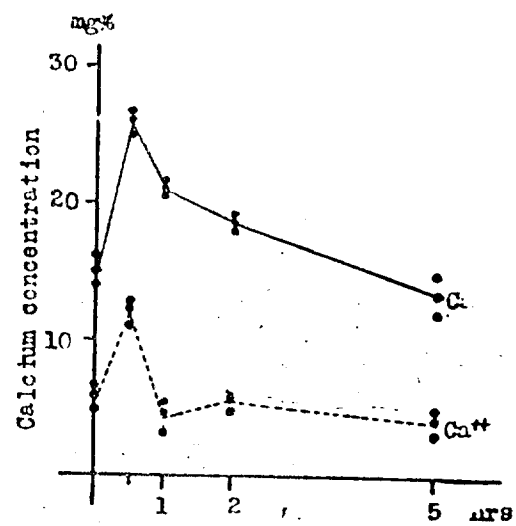

Fig. 2 Serum-calcium levels in rabbit after the intravenous administration of 500 mg $CaCl_2$.

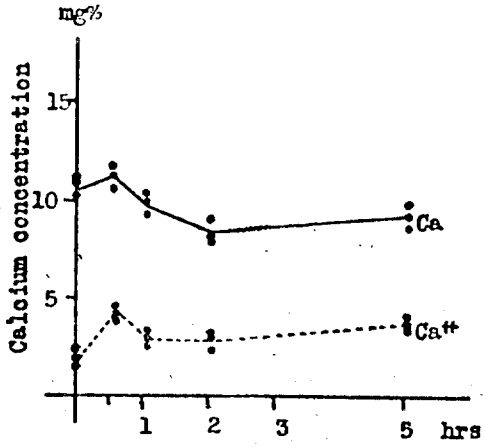

Fig. 3 Serum-calcium levels in man after the intravenous administration of 500 mg $CaCl_2$.

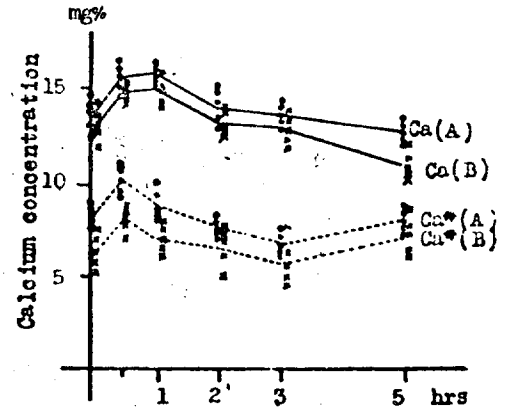

Fig. 4 Serum-calcium levels in rabbit after the oral administration of 0.5 gm(A) and 1.0 gm(B) of $CaCl_2$.

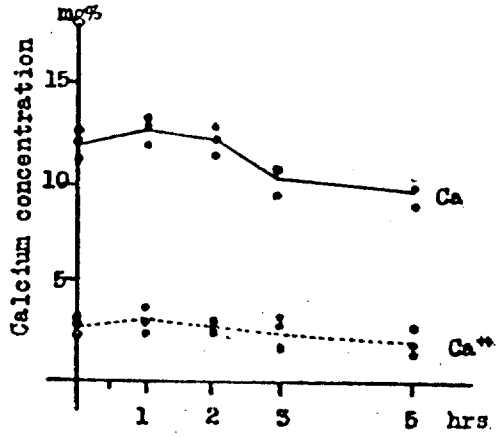

Fig. 5 Serum-calcium levels in dog after the oral administration of 1 gm $CaCl_2$.

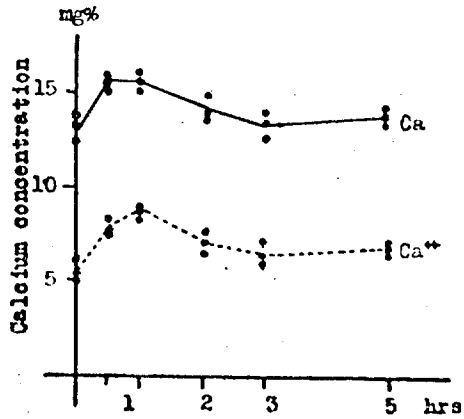

Fig. 6 Serum-calcium levels in rabbit after the oral administration of 0.4 gm $CaCO_3$.

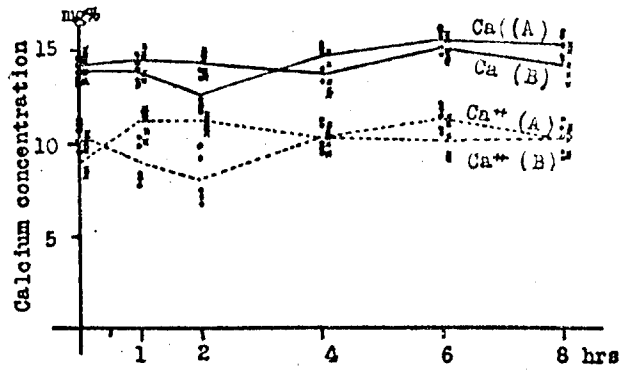

Fig. 7 Serum-calcium levels in rabbit after th oral administration of 0.8 gm(A) and 1.6 gm(B) Pearl Calc.

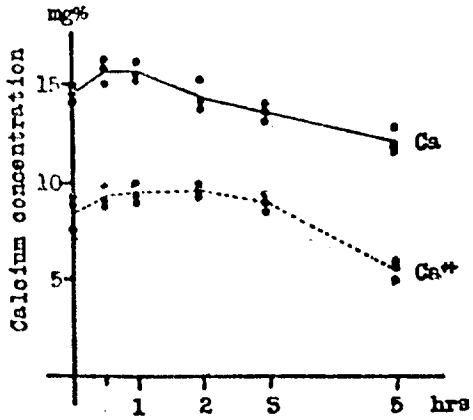

Fig. 8 Serum-calcium levels in rabbit after the oral administration of 0.45 gm Calcium glutamate.

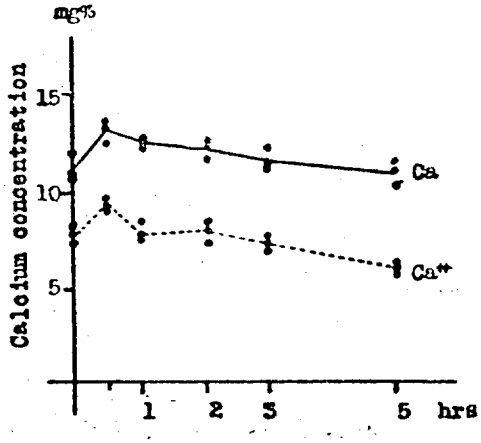

Fig. 9 Serum-calcium levels in rabbit after the oral administration of 0.81 gm Calcium succinate.

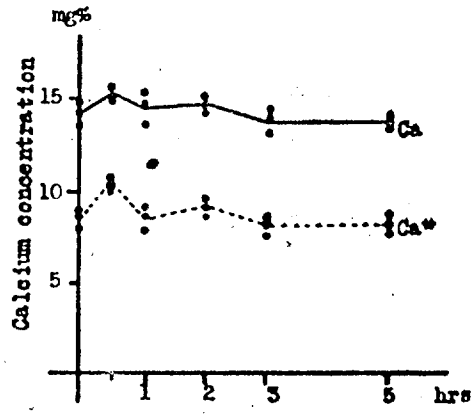

Fig. 10 Serum-calcium levels in rabbit after the oral administration of 0.5 gm Calcium citrate.

In the rabbits, a significant rise in serum calcium followed the administration of $CaCl_2$ and $CaCO_3$; the most obvious, continuous rise in serum calcium was observed in the pearl calc (calcium phosphate). The remaining salts were relatively ineffective, in the order of the gluconate least effective, followed by citrate and succinate.

It was considered by Scaglioni and Girino (829) to be of "great scientific and practical importance to determine how much of the calcium in several salts used in therapy is absorbed by the blood and organ colloids. A number of experiments, all "in vitro" were performed using calcium chloride and calcium gluconate solutions added to Ringer saline. Ultrafiltration with filters of collodion was the method used in quantitative determination of Ca. The experiments on blood were done by adding the calcium chloride and gluconate solution to Ringer solution, whole defibrinated mutton blood, mutton serum and red corpuscles. The calcium salts were adsorbed by the colloids of the blood and its components in very small amounts, or not at all, as in the red cells; the gluconate appeared to be more adsorbed than the chloride.

Various sheep organs, liver, spleen, kidney, lungs, brain and muscles were finely ground and studied, as was the blood. It was found that the colloids of all the organs adsorbed Ca, the lungs most and in decreasing order, spleen, kidneys, brain and liver least. Again, the gluconate was more adsorbed than the chloride.

No measurable adsorption of Ca was observed in the muscles of the sheep.

A study of the influence of calcium chloride on the acid-base metabolism in two infants (5 and 4 months old) was presented by Gamble et al. (283). Preliminary data from analysis of stools, urine and blood serum were obtained from a normal infant and from one with marked symptoms of tetany. The acid effect from the ingestion of calcium chloride possibly assumes a more complete absorption of the chloride ion (Cl^-) than of the calcium ion (Ca^{++}). During the period of treatment both the normal infant and the one with tetany received daily doses of calcium chloride (2 gm and 1.5 gm). Measurements of calcium and chloride ions in the stools showed a much larger excretion of calcium than of chloride; 60% of the added calcium was excreted in the stools, but only 4% of the added chloride. Thus, the calcium chloride behaved as an acid substance.

Gerloczy and Berencsy (301) have discussed in two early papers experiments relating to the absorption of $CaCl_2$, given orally in man. Other investigators had established that calcium taken orally raises the blood level of Ca, particularly when administered in the presence of NaCl. In the first of their own experiments, Gerloczy and Berencsy gave patients a 10% solution of $CaCl_2$ (0.15 g $CaCl_2$/kg body weight of patient) for a total of 8-10 g, after which blood samples were analyzed for serum Ca. In the same individual, the aqueous $CaCl_2$ solution was later replaced by $CaCl_2$ dissolved in physiological NaCl solution, the dose of Ca remaining the same. The blood serum calcium levels in the saline-$CaCl_2$ experiments were significantly higher than in the aqueous-$CaCl_2$ case; the average increase amounted to 140% more calcium absorbed in the NaCl solution than in water. The following tables show the results: Table 1, blood calcium values of aqueous $CaCl_2$; Table 2, values for NaCl-$CaCl_2$; and Table 3, comparison of average values of first and second tables.

TABLE I. Blood calcium values after peroral administration of $CaCl_2$.

Nr.	1 Geschlecht	2 Körpergewicht in kg	$CaCl_2$ – 10% ccm	$CaCl_2$ – 10% Ca g	Serum-Calcium: 3 Ausgangswert	4 Erhöhung nach ½ Std.	4 Erhöhung nach 1 Std.	4 Erhöhung nach 7 Std.	5 Maximaler Unterschied	6 in % zum Ausgangswert
14	♂	80	120	2,64	11.40	12.40	11.90	11,60	1,0	8,8
15	♂	80	120	2,64	10.50	12.40	12.20	10.90	1.9	18.1
16	♀	70	100	2,20	11.21	11.83	11.74	10.85	0.62	5.5
17	♀	52	78	1,72	10,72	12.19	11.74	10.50	1,47	13.7

Key:
1.= sex 2.= body weight in kg 3.= initial value 4.= increase after
5.= maximum difference 6.= in % to initial value
Std.= hour

Table 2.

Nr.	Geschlecht	Körpergewicht in g	10% $CaCl_2$	Serum-Calcium: Ausgangswert	Erhöhung nach ½ Std.	Erhöhung nach 1 Std.	Erhöhung nach 7 Std.	Maximaler Unterschied	%
1	♀	51	76 ccm 1.71 g Ca	11,02	12.21	11,90	10.88	1.19	10,8
			mit 0,9% NaCl	11,13	12.28	13,13	12,10	2,00	18,0
2	♂	60	90 ccm 1,98 g Ca	11,70	13.20	12,90	11,80	1,50	12,8
			mit 0,9% NaCl	13,90	18.00	15.70	14,90	4,10	30,0
3	♀	47	70 ccm 1,54 g Ca	11,60	13,50	13.20	11,70	1,90	16,4
			mit 0.9% NaCl	12,00	17.10	15.20	11,80	5.10	42,5
4	♂	55	82 ccm 1,8 g Ca	11,20	12,50	12.90	11,60	1,70	15,2
			mit 0.9% NaCl	11,00	14.50	13,60	12,60	3,50	31,8

for key see previous table

Table 3. Comparison of the average values of the results of the experiments.

Anwendungsart	Ausgangswert	Serum-Calcium: Erhöhung nach ½ Std.	Erhöhung nach 1 Std.	Erhöhung nach 7 Std.	Differenz: in mg	in % des Anfangsgehalts an Ca des Blutes	Mehr Ca resorbiert: in mg	das sind um x% mehr als nur nach wässeriger $CaCl_2$-Lösung
0,9% NaCl + 10% $CaCl_2$	11,92	15,23	14,40	12,90	3,31	27,8	1,95	143
10% $CaCl_2$	11,17	12,53	12,31	11,22	1,36	12,1	—	—

Key:
1.= manner of application 2.= initial value 3.= increase after
4.= difference
a) in % of the blood's initial content of Ca

In the second paper of Gerloczy and Berencsy (302), the "reaction environment" for the administration of $CaCl_2$ or the influence of $NaHCO_3$ and NH_4Cl on calcium absorption was presented; it was essentially a study of the influence of pH on calcium absorption. Before administering the oral doses of $CaCl_2$, the patient was given either 200 g $NaHCO_3$ or 24 g NH_4Cl; the blood-ph was determined before and after treatment. The increases in calcium levels in the blood of the patient receiving the alkalzing $NaHCO_3$ were significantly greater than in those patients pretreated with NH_4Cl. The $CaCl_2$ was given in both aqueous and NaCl solutions before treatment with sodium bicarbonate or ammonium chloride. The following tables show the results of the second series of experiments: Table 1 shows effects of changing pH on aqueous $CaCl_2$ serum calcium levels; Table 2 shows results when NaCl-$CaCl_2$ is given; and Table 3 presents average values of Tables 1 and 2 compared.

TABLE 1.

	Nr.	Geschlecht (1)	Körpergewicht in kg (2)	10% $CaCl_2$ ccm	10% $CaCl_2$ Ca g	Serum-Calcium: Ausgangswert (3)	Erhöhung nach (4) ½ Std.	1 Std.	7 Std.	Maximaler Unterschied (5)	%
Bei NH_4Cl	9.	♀	85	125	2,75	11,80	13,10	13,10	11,90	1,30	11,—
	10.	♀	80	120	2,75	10,10	12,10	10,80	10,20	2,—	20,—
	11.	♀	55	80	1,76	11,—	11,90	12,10	11,20	1,10	10,—
	12.	♂	70	100	2,2	10,78	11,92	11,65	10,52	1,14	10,5
Bei $NaHCO_3$	28.	♀	55	83	1,86	10,50	12,60	13,40	10,50	2,90	26.7
	29.	♂	75	107	2,35	11,60	15,50	15,40	11,40	3,90	33,60
	38.	♂	70	100	2,2	11,—	13,02	13,21	11,02	2,21	20.1
	39.	♀	52½	80	1,76	9,96	12,23	11,19	10,58	2,27	22,8

Key:
1.= sex 2.= body weight in g 3.= initial value
4.= increase after 5.= maximum difference

Table 2.

	Nr.	Geschlecht (1)	Körpergewicht in kg (2)	10% $CaCl_2$ in 0,9% NaCl: ccm	Ca g	Serum-Calcium: Ausgangswert (3)	Erhöhung nach (4) ½ Std.	1 Std.	7 Std.	Maximaler Unterschied (5)	%
Bei NH_4Cl	5.	♀	61	90	2,0	12,50	14,50	12,50	11,50	2,—	16,—
	6.	♂	72	100	2,2	11,—	12,80	11,90	10,80	1,80	16,4
Bei $NaHCO_3$	21.	♀	60	90	2,0	10,70	13,—	11,70	10,80	2,30	21,5
	25.	♀	51	75	1,55	12,—	13,60	12,20	11,90	1,60	13,30

For key see table 1.

Table 3.
Average values of the blood calcium concentration in the various absorption influences.

Anwendungsart	Ausgangswert	Serum-Calcium: Erhöhung nach ½ Std.	1 Std.	7 Std.	Differenz: in mg	in % des Anfangsgehalts an Ca des Blutes	± Ca resorbiert: in mg	das sind um x % mehr oder weniger als nur nach wässeriger $CaCl_2$-Lösung
10% $CaCl_2$	11,17	12,53	12,31	11,22	1,36	12,1	0	0
0,9% NaCl + 10% $CaCl_2$	11,92	15,23	14,40	12,90	3,31	27,8	+1,95	+143
NaCl+$CaCl_2$ neben NH_4Cl	11,35	13,02	11,72	11,—	1,67	14,7	+0,31	+22.7
$CaCl_2$ neben HN_4Cl	10,93	12,05	11.86	10.96	1.12	10,2	—0,24	—18,3
NaCl+$CaCl_2$ neben $NaHCO_3$	11,35	13,30	11,95	11,35	1,95	17,2	+0,59	+43,8
$CaCl_2$ neben $NaHCO_3$	10,76	13,33	13,30	10,87	2,57	23,9	+1,21	+88

Key:
1.= method of use 2.= initial value 3.= increase after
4.= difference

Various methods of measuring the absorption of calcium in humans using Ca^{45} and Ca^{47}-labelled $CaCl_2$ were described by Mautaken (626). Distinguishing between calcium ingested with foods and that which has not been absorbed and comes from digestive juices and secretions, or endogenous calcium, has been difficult. Age, diet, vitamin D and physical activity are all factors which determine Ca absorption in man. The applications of the radioisotopes, Ca^{45} and Ca^{47} were detailed in a series of discussions, examples of which were: methods for determining material calcium absorption in man; measurement of the fecal excretion in oral dose of Ca*; double isotope relation in blood and urine; body counters, etc. Mautalen concluded that when $CaCl_2$ is administered, 30-60% of the dose is absorbed; from milk, however, scarcely half that much is absorbed. Diets with less than 400 mg Ca/day are considered poor in calcium; those with more than 1500 mg Ca/day are very rich in calcium.

A study of six patients with chronic renal failure and two subjects convalescing from duodenal ulcer was made by McDonald et al. (631) in a test of the hypothesis that patients with renal failure have an impaired ability to absorb calcium. After a preliminary period of 7-10 days of normal diet (with respect to calcium and phosphorus intake), a series of 5 or 6-day periods with various high levels of calcium intake were undergone, the calcium administered as calcium citrate suspended in distilled water and given in four equal doses. The average amount of calcium as the citrate administered was 2112 mg/day or 2210 mg/day (in one patient and one subject) for a period of 15-20 days duration. Fecal collections were made, urine collected and blood samples taken for the determination of plasma calcium, phosphate and creatinine. The rise in calcium absorption from the gut in normal subjects and in patients with chronic renal failure was about the same, and the plasma calcium balance, phosphorus balance and urinary calcium excretion were consistent with an increase in calcium absorption having taken place. The table below gives the average results of the experiment.

TABLE

The average results in each individual obtained in the calcium and phosphate balances before and during the period of high calcium intake in six patients with chronic renal failure (No. 1-6) and two normal subjects (A and B). The urinary excretion of calcium after the high calcium intake is also shown

Patient No.	Period	Calcium mg./day					Phosphate mg./day				
		Intake	Fæces	Absorption	Urine	Balance	Intake	Fæces	Absorption	Urine	Balance
1	Before	454	666	−212	59	−271	817	423	+394	310	+84
	During	2650	2333	+317	64	+253	817	463	+354	197	+157
	After				59						
2	Before	289	408	−119	12	−131	597	349	+248	216	+32
	During	2400	2165	+235	14	+221	650	477	+173	52	+121
	After				11						
3	Before	1070	840	+230	31	+199	1263	719	+544	257	+287
	During	3146	2598	+548	45	+503	1265	890	+375	139	+236
	After				29						
4	Before	552	690	−138	52	−190	723	371	+352	573	−221
	During	2653	2601	+52	64	−12	722	411	+311	264	+47
	After				47						
5	Before	1110	1236	−126	54	−180	1470	840	+630	490	+140
	During	3260	3197	+63	29	+34	1470	929	+541	242	+299
	After				25						
6	Before	855	768	+87	58	+29	1124	376	+748	808	−60
	During	2920	2533	+387	156	+231	1124	474	+650	545	+104
	After				51						
Subject											
A	Before	1213	1033	+180	295	−115	1644	748	+896	813	+83
	During	3438	2934	+504	419	+85	1640	847	+793	682	+111
	After				224						
B	Before	1302	1123	+179	270	−91	1775	878	+897	960	−63
	During	3471	3068	+403	340	+63	1775	999	+776	726	+50
	After				246						

The results did not support the hypothesis being tested, that the low absorption of calcium in chronic renal failure was due to impaired gut absorption of calcium.

From a series of studies on the utilization of calcium in the diet by Japanese investigators, Hayami et al. (369), using rats, have suggested that the solubility of the calcium salt used in human diet does not determine its nutritional value. Calcium gluconate (water-soluble), 2.25 g and calcium carbonate (water-soluble), 0.5 g, were added to a standard diet of the rats, with the results shown in the following tables (no details of the experiments were given):

Table I. First experiment

Group	No. of rats	Sex	Body weight (g)			Length of Bone (cm)		Length of Body (cm)	Diet	
			Initial	Final	Increase	Femur	Tibia		Total Amount of Food Intake	Utilization of Food (%)
Ca-Gluconate	344	♂	38	99	61	2.36	2.83	16.5	194.5	31.36
	345	♂	36	103	67	2.38	2.84	17.4	234	28.63
	346	♂	34	99	65	2.34	2.82	16.3	218	29.81
	347	♀	36	85	49	2.23	2.72	15.8	196	25.00
	348	♀	32	92	60	2.30	2.79	15.8	217	27.65
	Average				60.2	2.32	2.80			28.49
Ca-Carbonate	349	♂	37	96	59	2.29	2.79	15.8	186	31.72
	350	♂	36	124	88	2.47	2.90	17.5	253.5	34.71
	351	♀	36	101	65	2.30	2.80	16.3	204	31.86
	352	♀	35	106	71	2.30	2.80	16.8	224.5	31.63
	Average				70.8	2.34	2.82			32.48

Table II. First Experiment

Group	No. of Rats	Sex	Calcium Intake			Retention and Utilization of Ca			
			Food intake (g)	Ca content (%)	Amount of Ca (g)	Beginning of Exp.	End of Exp.	Retention (g)	Utilization (%)
Ca-Gluconate	344	♂	194.5	Ca	0.7391	0.2387	0.8047	0.5669	76.58
	345	♂	231	0.369	0.8832	0.2258	0.8532	0.6274	70.56
	346	♂	218	P	0.8264	0.2135	0.8435	0.6300	76.05
	347	♀	196	0.547	0.7448	0.2457	0.7388	0.4931	66.21
	348	♀	217		0.8346	0.2585	[illegible]	[illegible]	61.94
	Average								72.27
Ca-Carbonate	349	♂	186	Ca	0.6863	0.2319	0.8395	0.6076	88.53
	350	♂	253.5	0.369	0.9354	0.2283	0.9755	0.7477	79.93
	351	♀	204	P	0.7528	0.2457	0.8447	0.5989	79.56
	352	♀	224.5	0.525	0.8284	[illegible]	0.8[illegible]	0.6732	77.40
	Average								81.36

Table III. First Experiment

Group	No. of Rats	Sex	Femur and Tibia: Defatted dry bone (g)	Femur and Tibia: Ash (g)	Femur and Tibia: Ash / Defatted dry bone (%)	Femur and Tibia: Rickets Coefficient	Femur and Tibia: Ca-content of Ash (%)	Carcase (Except Femurs and Tibias): Ash (g)	Carcase (Except Femurs and Tibias): Ca-content of Ash (%)
Ca-Gluconate	344	♂	0.2763	0.1426	51.61	1.06	38.35	2.4508	28.37
	345	♂	0.2848	0.1479	51.93	1.08	37.67	2.5865	28.68
	346	♂	0.2815	0.1488	52.85	1.12	38.25	2.4905	29.30
	347	♀	0.2651	0.1358	51.22	1.05	37.80	2.2759	27.96
	348	♀	0.2745	0.1499	54.61	1.20	38.00	2.4429	28.37
				0.1450	52.44	1.10			
Ca-Carbonate	349	♂	0.2715	0.1406	51.78	1.07	38.29	2.3707	30.87
	350	♂	0.3401	0.1733	50.95	1.03	39.54	2.9619	28.24
	351	♀	0.2638	0.1377	52.19	1.09	38.44	2.4578	30.06
	352	♀	0.2630	0.1430	53.34	1.14	39.50	2.5639	30.01
				0.1480	52.07	1.08			

Table IV. Second Experiment (Average)

Group	No. of Rats	Body Weight (g): Initial	Body Weight (g): Increase	Length of Bone (cm): Femur	Length of Bone (cm): Tibia	Length of Body (cm)	Diet: Total Amount of Food Intake (g)	Diet: Utilization of Food (%)	Diet: Amount of Ca (g)
Ca-Gluconate	♂ 2 ♀ 1	42.8	62.2	2.40	2.94	16.6	224.5	27.65	0.8531
Ca-Carbonate	♂ 2 ♀ 1	39.3	78.7	2.49	2.97	17.1	261.3	30.03	0.9545

Table V. Second Experiment (Average)

Group	Femur and Tibia: Defat. dry bone (g)	Femur and Tibia: Ash (g)	Femur and Tibia: Ash / Def. Dry Bone (%)	Femur and Tibia: Ca-cont. (%)	Carcase: Ash (g)	Carcase: Ca-content of Ash (%)	Retention and Utilization of Ca: Beginning of Exp. (g)	Retention and Utilization of Ca: End of Exp. (g)	Retention and Utilization of Ca: Retention (g)	Retention and Utilization of Ca: Utilization (%)
Ca-Gluc.	0.3257	0.1624	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.6463	[illegible]
Ca-Carb.	0.3240	0.1724	51.78	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Idiopathic hypercalciuria was the subject of a study by Peacock et al. (721) who observed nine male idiopathic renal stone formers with persistent hypercalciuria on a free diet; their ages ranged from 24 to 54 years. Controls were nine healthy males, staff members of the hospital, aged 17 to 40 years, none of whom was hypercalciuric. The patients were first studied in a non-fasting state, with blood and urine samples analyzed for calcium and creatinine. A day of total starvation, with only tapwater given, yielded urine and blood samples, again analyzed. A similar treatment, followed by an oral dose of calcium citrate, 100 mg/kg, and with subsequent blood and urine samples analyzed was given. This large dose of calcium citrate produced a significant rise in serum and urine calcium in both hypercalciuric and control groups, with a significantly greater rise in the hypercalciuric patients. The results of the experiments tended to show that idiopathic hypercalciuria is absorptive in origin, and that oral calcium loading may constitute a better diagnostic test of this condition than the standard 24-hour urine collection on a free diet.

Staub (914) has presented a very comprehensive and excellent review of work done on calcium metabolism (absorption and excretion) particularly that utilizing the radioactive form, Ca^{45}, by investigators since 1940. An experiment using Ca^{45} given to human patients as calcium gluconate was described; the gluconate was given as 0.5 g or 1.0 g in aqueous solution, (20 ml), orally, intravenously or rectally. Urine and feces were collected and analyzed for Ca^{45}. Given rectally, 94-99% of the calcium is found in the feces at 1-2 hours, none in the urine; given intravenously, 4-15% Ca appears in the feces and 10-20% in the urine; given orally, 64-83% Ca is found in the feces and 0.6-6.5% in the urine. Both oral and intravenous calcium produced a positive Ca balance with about 80% retention after intravenous administration, and about 20-25% retention following oral administration. Rectal application produced no retention of Ca.

The comparative absorption of calcium gluconate and lactate was studied under controlled dietary conditions in 8 male patients, all in good physical condition despite minor chronic abnormalities, by Spenser et al. (909). All patients received the same constant, low calcium diet containing an average of 200 mg Ca and 820 mg phosphorus per day. Supplementation with calcium gluconate raised the Ca intake to about 1657 mg Ca/day; the intake with calcium lactate averaged 1672 mg/day. Patients received the basal low calcium diet for 30 to 60 days before supplementation; the duration of the experiments averaged 45 days for the gluconate and 28 days for the lactate. A list of patients and type of study performed is shown on the following page.

TABLE

List of patients studied

Patient	Age	Diagnosis	Study	
			Type	Days
1	61	Pulmonary calcifications	calcium gluconate	66
			calcium lactate	30
2	51	Osteoporosis, mild	calcium gluconate	36
			calcium lactate	38
3	51	Osteoarthritis	calcium gluconate	40
			calcium lactate	14
4	40	Peripheral neuropathy	calcium gluconate	50
			calcium lactate	24
5	43	Peripheral neuropathy	calcium gluconate	24
			calcium lactate	32
6	47	Osteoporosis	calcium gluconate	48
			calcium lactate	38
7	42	Osteoporosis	calcium gluconate	48
			calcium lactate	18
8	48	Cortisone osteoporosis	calcium gluconate [1]	30
			calcium lactate [1]	30
			calcium gluconate [1]	30
			calcium gluconate [2]	42
			calcium lactate [2]	32
			calcium gluconate [3]	54
			calcium lactate [3]	24

[1] During high protein-high calcium intake.
[2] During normal protein-high calcium intake.
[3] During high protein-medium calcium intake.

Body weight, fluid intake, urine output and excretions of creatinine, calcium and phosphorus were determined daily.

Metabolic balances of calcium, phosphorus and nitrogen were determined on aliquots of 6-day urine and stool collections, as well as on the diet during each 6-day period. The following table shows the results:

TABLE

Calcium balances of patients receiving calcium gluconate and calcium lactate supplements

Patient	Study days	Study	Calcium					Phosphorus		Nitrogen	
			Intake	Urine	Stool	Balance	Change in balance	Intake	Balance	Intake	Bala
			mg/day	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/*
1	66	Calcium gluconate	1894	163	1727	+ 4		874	+ 82	14,893	+17
	30	Calcium lactate	1864	180	1558	+126	+122	938	+ 93	14,876	+16
2	36	Calcium gluconate	2022	45	1757	+220		924	+225	15,552	+40
	38	Calcium lactate	2107	43	1792	+272	+ 52	892	+161	15,035	+42
3	40	Calcium gluconate	1813	64	1568	+181		882	+183	14,838	+30
	14	Calcium lactate	1800	67	1455	+278	+ 97	816	+156	13,826	+29
4	50	Calcium gluconate	1065	76	892	+ 97		835	+198	14,852	+37
	24	Calcium lactate	1113	69	863	+181	+ 84	859	+169	14,468	+33
5 [1]	24	Calcium gluconate	853	111	808	− 66		802	+ 64	11,346	+14
	32	Calcium lactate	796	108	598	+ 90	+156	794	+132	12,761	+19
6	48	Calcium gluconate	2201	321	1650	+230		929	+ 83	15,177	+29
	30	Calcium lactate	2260	376	1575	+309	+ 79	949	+153	15,396	+26
7	48	Calcium gluconate	1752	140	1500	+112		780	+ 93	13,421	+17
	18	Calcium lactate	1766	246	1351	+169	+ 57	837	+131	13,326	+12

[1] The lower nitrogen and calcium intake of patient 5 was due to adjustments in food intake (see text).

Radioisotope studies were also carried out simultaneously with the foregoing experiments, consisting of a single oral dose of Ca^{47} as the chloride. Plasma, urinary and stool radioassays were made. The average urinary calcium excretion per 6-day period ranged from 260 mg/day to 236 mg/day, with the lactate showing consistently higher rates of excretion. The average fecal excretion of calcium was consistently lower by about 100 mg/day for the lactate compared to the gluconate. Similarly, the plasma levels of Ca^{47} were higher during the lactate intake than during the gluconate intake.

The results of this study, showing the lactate as being more readily absorbed than the gluconate, were not consistent with the findings of other workers; the reasons remain "conjectural".

Studies of the comparative utilization of two calcium sources added to the diet of four aged women by Hayami et al. (370) showed that calcium carbonate was 50% more utilized than calcium gluconate in the diet. Two levels of calcium supplement in skim milk were given the four subjects: one experiment of 7.6 mg/kg body weight (310 mg total); and 14.2 mg/kg (579 mg total) in a second experiment. In the first case, the absorptions of calcium ranged from 59% to 4.4%; in the second case, 100 mg Ca was absorbed - 59.2 and 58.8% of the carbonate and 50.2 and 17.9% of the gluconate.

These results are similar to those found by Hayami et al. in an earlier paper on rat studies of the absorption of the same two calcium salts (369). Feces and urine analyses of the four women in this experiment produced the results shown in the table below:

Table Calcium Balance (mg/day)

Studium	Subjects		S.S.	M.K.	S.A.	T.O.
	Age Body weight		80 40.0kg	80 40.5kg	60 45.0kg	68 38.0kg
I	Intake		308	304	308	318
	Excretion	Feces	208	124	249	304
		Urine	120	183	92	43
		Total	328	303	341	347
	Balance		−20	+ 1	−33	−29
II	Intake	Diet	298	293	298	309
		Ca. Salt	243	316	243	316
		Total	541	609	541	625
	Excretion	Feces	264	299	361	491
		Urine	159	156	76	108
		Total	423	455	437	599
	Balance		−118	+154	+104	+26
Absorption rate of Ca. in I Stad. %			32.5	59.2	19.2	4.4
Utilization of Ca. Salts in II Stad. %			59.2	50.2	58.8	17.9

Comparative absorption of calcium from three sources, milk, calcium gluconate and calcium carbonate was studied by Patton (718) in nine college women in three groups of 3 each over a period of seven weeks. Basal metabolic rates were determined and urine samples collected and analyzed for calcium. During the first week all subjects were maintained on a basal diet containing a daily average of 307 mg of calcium. During the remaining six weeks, one group received milk supplement; a second group, calcium gluconate; and the third group received calcium carbonate. All supplements added approximately 300 mg of calcium to the diet. The results are shown in the following table:

TABLE

Calcium balances, in milligrams, of 9 college women on a basal diet and during 6 periods of supplementation with milk, calcium gluconate, and calcium carbonate

SUBJECT	PERIOD OF BASAL DIET[1]	PERIOD OF SUPPLEMENTATION[1]					
		1	2	3	4	5	6
			Milk				
J. M.	— 42	57	— 10	67	— 13	19	72
M. E. W.	— 59	41	— 66	34	— 106	— 76	2
L. Y.	— 113	30	— 90	— 37	— 90	— 21	60
			Calcium gluconate				
O. D.	— 9	47	39	— 2	30	13	72
P. R.	— 26	— 83	— 33	— 26	— 71	— 4	— 13
R. S.	— 48	— 7	— 52	49	— [illegible]	— 44	87
			Calcium carbonate				
E. H.	— 25	62	— 29	23	— 31	25	70
R. J.	— 78	44	— 87	— 4	— 29	— 22	11
D. S.	— 34	26	— 44	20	— 71	94	0

[1] All periods were of 7 days duration.

The basal metabolic rate of the subjects significantly affected the retention of calcium; retention was inversely related to basal metabolic rate. In an earlier study by Patton and Sulton (719), calcium lactate, gluconate, sulfate, and carbonate utilization in similar circumstances showed no significant differences in these four calcium salts as sources of calcium in the diet. The order in which the salts were administered was a significant factor; given in successive, alternate doses, the first salt given was utilized to a greater degree, regardless of which salt it was.

In answer to the questions about a given drug of "how much" and "when" it should be given, Lieberman (561) submitted three healthy individuals to a series of experiments with calcium lactate and calcium gluconate to determine blood calcium changes. Various routines were followed, alternating the lactate and gluconate, recording symptoms of distress, and collecting hourly urine samples. Though only about 10% of the calcium ingested was excreted in the urine, it was felt that urinary calcium would serve as a suitable index of blood calcium changes.

The salts were given in water, 20 grams of gluconate or 10 grams of lactate at the beginning, which produced abdominal distress, vomiting and diarrhea, so that the doses of each salt were halved. Given both. on empty stomachs and after meals, the salts as given indicated that 10 grams of calcium gluconate and 5 grams of the lactate were the maximal doses, not only from the viewpoint of comfort, but in that larger amounts actually decreased the calcium absorbed.

Overdosage created a violent diarrhea, causing such rapid passage through the bowels of the salts that proper absorption could not occur. The gluconate was better tolerated than the lactate in terms of disagreeable symptoms produced by ingestion. It also appeared highly desirable to give the salts after a meal; the distress would be minimal and the absorption of calcium maximal.

Waldroup et al. (986) have reported on experiments with chicks to demonstrate the influence of calcium and vitamin D_3 on the utilization of phosphorus from calcium phytate. Day-old broiler chicks were sexed, debeaked, wing banded and intraocularly vaccinated for Newcastle disease and infectious bronchitis. Three groups of 5 male and 5 female chicks were used in each of two experiments. The basic diet of chiefly degerminated corn meal and dehulled soybean meal was analyzed for phosphorus (0.30%). The first experiment compared calcium phytate and dicalcium phosphate at various levels of vitamin D_3 and calcium phytate supplements. The phytate was described as phytic acid calcium - magnesium salt, 12% calcium, a white, odorless powder insoluble in water. At 21 days of age the chicks were weighed and two each, male and female from each group, were sacrificed. The right tibia was removed, cleaned and bone ash determined. The second experiment was similar, using two phosphorus sources (calcium phytate vs dicalcium phosphate), two supplemented levels of phosphorus, two calcium levels and two vitamin D_3 levels.

The results demonstrated a close relationship between calcium and phosphorus levels and vitamin D_3 level on utilization of phosphorus from calcium phytate; lower levels tended to improve availability of the phytate phosphorus but optimum levels of higher Ca:P ratios lowered the availability of phytate phosphorus. Increase in vitamin D_3 levels improved the utilization of phosphorus from both the phytate and phosphate.

Among a series of experiments performed by Krieger et al. (512) was one designed to test the availability of the calcium in calcium phytate fed to rats. An optimum diet, lacking only calcium was the basal form, augmented by either calcium carbonate or calcium phytate to the extent of 0.428 gm/100 gm of ration. Eight groups of six rats each received various diets of calcium carbonate or phytate and vitamin D. The utilization of the phytate and the carbonate was the same; the presence of vitamin D improved both utilizations equally well. A tabular presentation of the experimental results is shown on the following page.

TABLE

Comparative study of the availability of calcium in calcium phytate and calcium carbonate

ADDITIONS TO THE BASAL RATION [1]		FOOD CONS.	CHANGE IN WT.	WEIGHT OF BONE	WEIGHT OF ASH	BONE ASH
		grams	*grams*	*grams*	*grams*	%
XIV	0.107 gm. (1/4 opt.) Ca as Ca phytate plus D[2]	728	130	0.2588	0.1314	50.8
XVI	0.107 gm. (1/4 opt.) Ca as $CaCO_3$ plus D	692	127	0.2630	0.1290	49.1
XV	Same as ration XIV except no vitamin D	535	76	0.2005	0.0856	42.5
XVII	Same as ration XVI except no vitamin D	548	58	0.1588	0.0691	43.5
XVIII	0.054 gm. (1/8 opt.) Ca as Ca phytate plus D	663	102	0.2102	0.0878	40.9
XX	0.054 gm. (1/8 opt.) Ca as $CaCO_3$ plus D	582	76	0.1776	0.0730	41.5
XIX	Same as ration XVIII except no vitamin D	502	46	0.1576	0.0579	36.8
XXI	Same as ration XX except no vitamin D	493	53	0.1582	0.0582	35.9

[1] Each ration was fed to six rats for 12 weeks. The additions represent those made for 100 gm. of ration.

[2] 4.5 U.S.P. units vitamin D (viosterol) per 100 gm.

Danbara (181) noted that insufficient data had been collected concerning relative availabilities of Ca in calcium salt food additives consumed by the Japanese farmer. Calcium carbonate was the most used form of Ca in food enrichment but had the disadvantage of forming CO_2 upon decomposition, with further subsequent effects on other ingredients, such as vitamins. Danbara selected rats for experimentation with several calcium salts added to the basal diet. Twenty-five rats, 7 weeks old, weighing about 150 g, 14 males and 11 females, were divided into 5 groups; each group received a different calcium salt which incorporated the radioisotope Ca^{45}. Group 1 received $Ca^{45}CO_3$ which served as a control reference; Group 2 was given $Ca^{45}HPO_4$; Group 3, Ca^{45}-phytate; Group 4, Ca^{45}-lactate; and Group 5, Ca^{45}-oxalate. An average of 10-15 g of the Ca^{45}-salt added to the diet was consumed by each animal; the Ca^{45} intake amounted to about 12 microcuries/animal. The basal diet lasted for one week; the Ca^{45}-salt-supplemented diet for 3 days, after which the animals were sacrificed. One femur from each rat was analyzed for Ca^{45} content. No sex differences was observed. The degree with which each salt was absorbed ranked the lactate and carbonate highest, followed by the phosphate, phytate and oxalate in that order. The author discussed the experimental data and concluded that calcium lactate or carbonate were most suited for preparation of Ca-enriched foods.

III. Metabolism and Excretion

An early paper by Briggs (109) described the use of various acetates in the therapy of nephritis, one characteristic of which is an acidosis, typically overcome by administering sodium bicarbonate or other alkali. Phosphoric acid retention in nephritis is an important factor in the

acidosis and accompanying edema which result. Two subjects, one the author, were given calcium acetate in separate experiments. In the first, 60 ml of 25% calcium acetate was given in divided doses over several days time, followed by magnesium and potassium acetates and calcium chloride solutions similarly administered. In the second experiment, calcium acetate was given over a period of four days time in 20% solution, 25 ml/day for a total of about 1.14 g of calcium. In both experiments, feces and urine were collected and analyzed, for phosphorus in the first case, and for sodium, potassium, calcium, magnesium and phosphorus in the second case. Chlorine and sulfur were separately determined, and urine ammonia measured.

The results of these experiments showed that calcium acetate, orally administered, diverted considerably the amount of excreted phosphoric acid from the urine to the feces; less ammonia is synthesized by the kidney; and alkali retention is reduced, with subsequent relief from edema.

Malkura and Puchkova (598) have studied the effect of calcium on the deposition of strontium-90 in the bones of rats; the hazards of strontium-90 in the environment of man in a nuclear age are of some concern. Calcium, as the nearest chemical analogue of strontium, is able to reduce the absorption of strontium within limits, if the two elements enter the gastrointestinal tract simultaneously. The absorption of calcium depends upon the anion with which it is associated; as the chloride, phosphate, citrate and protein complexes the calcium of milk and meat foodstuffs are readily absorbed in the intestine. In vegetables and cereals the calcium is chiefly in the form of oxalates and phytates and is poorly absorbed.

Wistar strain rats weighing 160-180 g were divided into four test groups of 20 animals each and fed basic diets containing only 3 mg Ca plus 23-25 mg daily of a different calcium salt, sufficient to satisfy the physiological requirements of the animals. The calcium metabolism of all groups was stabilized during a 4-week period on an optimum calcium diet preliminary to the experiment. The experiment itself lasted 14 days; the calcium salts supplemented the diet of each group (chloride, phosphate, oxalate and citrate) and are shown in the table below. Groups I through III received daily doses of 1 ml strontium nitrate (0.4 microcuries); Group IV received by gastric intubation 10 ml of milk containing 0.006 microcuries of strontium-90 from nitrate fed to the cows.

Content of Ca and Sr^{90} in the diets of rats

Group of animals	Source of calcium	Amount of calcium, mg/day	Amount of strontium-90 ingested per rat per day, μc	Diet
1st	$CaCl_2$ $CaHPO_4$	12 11	0.4	Na-milk 20 ml, sugar 5 mg
2nd	CaC_2O_4	25	0.4	Starch 4 g, hulled millet 5 g
3rd	Ca citrate $CaCl_2$ $CaHPO_4$	15 5 5	0.4	Gelatine 0.02 g, cod liver oil 0.2 g, yeast 0.2 g
4th	Natural milk	23	0.006	

Three rats from each group were kept in metabolic cages throughout the experiment and excreta collected daily for radiometric assay and calcium determination. Thigh and skin bones were removed after sacrifice of the animals, and strontium-90 determinations made. The results are shown in the following table:

Ca content of the diet and excreta, and Sr^{90} content of the skeleton of experimental rats

Group of animals	Source of calcium	Amount of calcium in the diet, mg	Calcium content in excreta		Sr^{90} content in the skeleton, % of the administered dose
			urine, mg/24 hr	feces, mg/24 hr	
1st	$CaCl_2$ $CaHPO_4$	23	0.66 ± 0.08	5.4 ± 0.62	12 ± 5.0
2nd	CaC_2O_4 Ca citrate	25	0.42 ± 0.12	14.5 ± 0.40	42 ± 1.0
3rd	$CaCl_2$ $CaHPO_4$	25	0.78 ± 0.06	9.0 ± 0.42	30 ± 2.0
4th	Natural milk	23	0.72 ± 0.06	10 ± 0.5	35 ± 3.0

The absorption of calcium was poorest in the group receiving the oxalate-citrate diet and greatest in chloride-phosphate diet; the deposition of strontium-90 was inversely related to calcium absorption, and the lowest skeletal content of Sr^{90} was seen in the groups receiving the chloride-phosphate diet.

A paper of some historical interest provides a review and discussion of the use and effects of calcium chloride by Haldane et al. (353). A single human, the author, served in an experiment lasting 13 days during which for three days he drank 85 g $CaCl_2$ in water. The solution produced no nausea, but there was intense diarrhea, then constipation and general discomfort. Acidosis was the chief effect and was explained as the result of formation of calcium carbonate in the intestine which was then secreted. Water and salts are lost, and mention was made of the use of $CaCl_2$ in treatment of "dropsy, edema and exudations".

A study designed to demonstrate the relationship of the replacement of endogenous losses of calcium from the body and the amount of dietary calcium used in this manner, was made by Steggerda and Mitchell (916), with Steggerda serving as the experimental subject. Calcium in milk solids and calcium gluconate were sources of calcium in the experiment which extended over a period of 168 days. Throughout the experiment the subject subsisted on a low-calcium diet, with determination of daily loss of calcium in urine and feces. Graded additions of calcium as skim milk powder or as calcium gluconate were added to the diet and the calcium balance determined. Collection of food samples, urine and feces over 4 days duration were analyzed for calcium; forty-three such 4-day periods comprised the experiment.

The basal diet provided an average of 195 mg of calcium daily; average daily balances for three basal periods (no calcium supplement) were -99, -121 and -109 mg/day. The daily calcium excreted on this low-

calcium diet averaged 304 mg, of which 68% was excreted by way of the intestinal tract.

An approximate calcium metabolic equilibrium was maintained by about 40 grams of skim milk powder (542 mg calcium) daily, or more than adequately maintained by 639 mg of calcium gluconate. It appeared that 534 mg of the gluconate would have established calcium equilibrium. On the basis of body weight of the subject, equilibrium required 9.2 mg calcium/kg of body weight, about three-fourths of which were equally well supplied by the milk solid or the gluconate. The calcium in both forms was only about 20% utilized, however.

The nutritional role of phytin, widespread in cereals and plants, was studied by Gillis et al (309) using chicks and turkey poults fed P^{32}-labelled calcium phytate and disodium phosphate in comparative metabolic studies.

In the experiments with chicks, day-old white Leghorn male chicks were divided into two groups of 20 each. One group received a vitamin D-deficient diet, the other a diet supplemented by 50 International Chick Units of vitamin D/100 gm of diet. After five weeks of this diet, 8 chicks from each group were isolated, four chicks of each group received radioactive calcium phytate for two days; the remaining four from each group received radioactive inorganic phosphorus (phosphate). The radioactivity of the phosphorus sources was determined in a windowless gasflow counter: phytate, 5.10×10^7 cpm/gm; disodium phosphate, 6.59×10^6 cpm/ml. The phytate was administered in gelatin capsules inserted into the chick's crop; the phosphate was pipetted into the crop. Two days after dosing with the radioactive phosphorus, blood samples were taken from wing veins, and prepared for counting; after sacrifice, tibia were removed and ashed; excretia, collected during experimentation, were also ashed, and ashes counted for radioactivity. The results are shown in the following table:

TABLE

Results of chick study; radioactivity measurements made after dosing with equivalent quantities of isotope carrier

DIETARY HISTORY	P^{32} CARRIER [1]	RADIOACTIVITY OF TOTAL PLASMA	RADIOACTIVITY OF 100 MG TIBIA ASH	% DOSE RETAINED/ 100 MG TIBIA ASH	% DOSE EXCRETED
		av. counts/ min.	*av. counts/ min.*[2]		
Vitamin D-deficient	Ca phytate	3.2×10^3	6.0×10^3	.025	76.6
Vitamin D-deficient	$Na_2HP^{32}O_4$	1.1×10^5	2.95×10^5	1.180	23.2
Vitamin D-adequate	Ca phytate	9.6×10^3	2.20×10^4	0.09	56.5
Vitamin D-adequate	$Na_2HP^{32}O_4$	9.6×10^4	2.57×10^5	1.040	18.5

[1] A total of 4 doses of isotope carrier were administered to all chicks over a two-day period. The initial dose was administered 48 hours and the final dose approximately 18 hours before the chicks were sacrificed.

[2] Corrected to a standard body weight of 200 gm.

Day-old Beltsville White turkey poults were similarly treated with results shown in the table below:

TABLE

Results of turkey poult study; radioactivity measurements made 48 hours after a single dosing with equivalent quantities of isotope carrier

DIETARY HISTORY	P^{32} CARRIER	RADIOACTIVITY OF TOTAL PLASMA	RADIOACTIVITY OF 100 MG TIBIA ASH	% DOSE RETAINED/ 100 MG TIBIA ASH	% DOSE EXCRETED
		av. counts/ min.	*av. counts/ min.*[1]		
Vitamin D-deficient	Ca phytate	7.14×10^2	4.36×10^3	0.058	47.7
Vitamin D-deficient	$Na_2HP^{32}O_4$	2.00×10^4	3.66×10^5	4.27	26.3
Vitamin D-adequate	Ca phytate	1.23×10^5	6.56×10^3	0.087	30.3
Vitamin D-adequate	$Na_2HP^{32}O_4$	2.11×10^4	4.41×10^5	5.16	6.7

[1] Corrected to a standard body weight of 135 gm.

The normal young chicks utilized P^{32}-labelled calcium phytate only about one-tenth as effectively as the phosphate; the young turkey poults made negligible use (less than 2%) of the phytate, compared with the disodium phosphate. In the vitamin D-deficient birds no significant use of the phytate phosphorus was observed; the chick's utilization of the phosphate was not, however, impaired by the absence of vitamin D, as it was in the turkey poults.

IV. Effects on Enzymes and Other Biological Parameters

Makino and Tanaka (593) studied the cytological effects of a number of dehydrating agents upon ascites sarcomas in the rat; one agent of which was calcium chloride. The experiments were performed with white rats (not otherwise described) in which MTK-sarcomas (Toshida sarcoma) were transplanted. Six days later, five or six tumor-bearing animals were intraperitoneally injected with 2 cc of 0.25 M solution of $CaCl_2$; a like number of rats bearing the tumor were untreated as controls. Within 15 to 20 minutes after injection, smear preparations from a droplet of the ascites obtained by abdominal puncture, showed a large number of damaged tumor cells. The damage was largely found among actively dividing or mitotic cells; the resting stages seemed less affected. The changes were first observed in the ascites cytoplasm, followed by pycnotic disintegration of the naked nuclei or a thickening of the chromosomes. Within 30 minutes, more than a third of the tumor cells appeared damaged; 40-50 minutes following injection of the $CaCl_2$, half the cells were damaged; at 60-70 minutes, most tumor cells had degenerated. Scattered among the damaged cells were numbers of small tumor cells which appeared unaffected by the calcium chloride and which divided rapidly. Three to four hours after injection, those numerous dividing figures were very conspicuous and indicated a tumor with renewed growth. The authors concluded that these resistant cells were the stem cells. Higher dosages of $CaCl_2$, such as 0.5 M and 0.75 M, damaged the tumor to a greater degree than the lower dosage, but were lethal to the animals, killing within several hours.

A brief review of the uses of calcium chloride as a diuretic was presented by Segall and White (857) who then described its use in a number of hospital cases. The chloride was usually administered orally 6 times a day in doses of 2.5 gm diluted in 50-100 cc of water; in the experiments with normal individuals, doses of 5 and 8 gm were given daily. The cases discussed are headed as follows: diuresis in normal persons during calcium chlorid administration (2 cases); effect of calcium chlorid in cases of cardiac failure (15 cases); effect of calcium chlorid in a case of tubercular polyserositis; effect of calcium chloride in a case of hepatic cirrhosis with ascites; and effect of calcium chlorid administration on the electrocardiogram (in 11 cases).

The effect of large, oral doses of $CaCl_2$ observed in the foregoing cases showed no deleterious results in the patients' therapy, but marked diuresis was obtained with calcium chloride where other diuretics had failed. Doses of 10-15 gm/day of calcium chloride were well tolerated by the patients studied.

The effect of oral administration of calcium and its role in the coagulation of blood has been studied by Tainer and Throndson (933). Four human subjects were given calcium gluconate for from 14 to 21 days in doses of 13.5 gm/day at meal time and blood coagulation time observed. The capillary tube method was used; the ear lobe is pricked, capillary tube filled and held warmly in the hand, and every 10-15 seconds a short piece of the tube is broken off until a filament of fibrin is formed, denoting clotting.

In only one subject was a marked decrease in clotting-time observed and the authors concluded that large therapeutic doses of the gluconate could not be relied upon to reduce coagulation time. The experiment was repeated, but with the addition of 1 cc viosterol (vitamin D) accompanying the calcium gluconate dosage. This, too, proved ineffective for practical applications, and the authors recommended (from additional experiments, also) abandonment of the clinical practice of oral calcium administration to shorten clotting time.

Kocian and Pechar (490) reviewed investigations of the relationships of calcium and lipids, noting particularly reports that the incidence of cardiovascular diseases throughout the world appears inversely related to the calcium content of the water. In a test of this relationship, the authors selected 26 patients, most of whom suffered from malabsorption, giving them oral doses of 16.8 g calcium gluconate daily (1.5 g Ca) for three weeks. Vitamin D and a Kaolin placebo were given to some patients. Blood samples were taken and calcium concentration determined spectrophotometrically; cholesterol and phospholipids were also determined. The serum cholesterol decreased in proportion to the initial level and when the calcium was replaced in the treatment by the placebo, the cholesterol level returned to the original pretreatment value. Intravenous Ca-gluconate did not produce the cholesterol-lowering effect, and the authors assume that the calcium salt in the intestine bonds with cholesterol from food and prevents its absorption, and similarly prevents resorption of cholesterol that was secreted in the bile.

V. Drug Interaction

Selye et al. (863) studied the effects of oral doses of calcium acetate, sodium phosphate and corn oil in the sensitization to parathyroid hormone in rats. Eighty female Holtzman rats ranging in weight, initially - from 95-106 gm, (were divided into eight groups of ten each; six groups were the subject of one experiment and two groups of a second experiment. In the first experiment, all animals received subcutaneously a dose of 50 U.S.P. units of parathyroid extract, twice daily; the remaining treatment, intubated into the stomach, consisted variously of Na_2HPO_4, calcium acetate and corn oil. The treatments lasted three days after which the animals were sacrificed (if not expiring earlier) and autopsied. Cardiac lesions and nephrocalcinosis were recorded, with results shown in the following table:

TABLE DIETARY SENSITIZATION FOR PARATHYROID HORMONE
(First experiment)

Group	Treatment*	Cardiac lesions (Scale: 0-3)	Nephrocalcinosis		Mortality (%)
			Degree (Scale: 0-3)	Type	
1	None	0	0.6 ± 0.20	Cortical	0
2	Na_2HPO_4	0	0.8 ± 0.20	Corticomedullary	0
3	Ca-acetate	1.0 ± 0.30	1.8 ± 0.20	Cortical	0
4	Corn oil	0	1.0 ± 0.23	Cortical	0
5	Corn oil + Na_2HPO_4	0.3 ± 0.10	2.0 ± 0.15	Corticomedullary	30
6	Corn oil + Ca-acetate	2.4 ± 0.33	2.4 ± 0.33	Cortical	90

* In addition to the treatments listed in this column, the rats of all groups received 50 U.S.P. units of *parathyroid horomone* twice daily, as indicated in the text.

In the second experiment with acetate and corn oil in addition to parathyroid hormone, the dose of the hormone was reduced to 30 U.S.P. units to prolong survival of the animals; the experiment was terminated on the eighth day. Cardiac lesions, nephrocalcinosis and osteitis fibrosa were recorded and the results shown in the following table:

TABLE DIETARY SENSITIZATION FOR PARATHYROID HORMONE
(Second experiment)

Group	Treatment	Cardiac lesions (Scale: 0-3)	Nephrocalcinosis		Osteitis fibrosa (Scale: 0-3)	Mortality (%)
			Degree (Scale: 0-3)	Type		
1	Paraffin oil + Ca-acetate + Parathyroid hormone	0.5 ± 0.15	0.4 ± 0.17	Cortical	1.2 ± 0.20	20
2	Corn oil + Ca-acetate + Parathyroid hormone	2.5 ± 0.20	2.7 ± 0.20	Cortical	2.8 ± 0.23	80

The first group in the second experiment received paraffin oil serving as a control replacing the corn oil given to the second group. The lack of pronounced effects of the corn oil in the first experiment was overcome in experiment number two.

All three substances tested, the calcium acetate, the phosphate and the lipid (corn oil) administered orally proved effective in sensitizing the animals to the parathyroid hormone. The selective sensitivity of various organ systems to the potential pathogenicity of substances like those studied in these experiments appeared to the authors to be largely dependent upon topical and systemic conditioning factors.

The interrelationships of calcium, vitamin D, age of the subject human, and various pathogenic medical phenomena were studied by Lorenzini (580) in a number of rest home patients. Forty-one males were selected, 20 without gerontoxon serving as controls and 21 with apparent "senile arches" or corneal xanthomatosis. Blood samples taken during a fasting state, and following intravenous injection of $CaCl_2$ (10 ml of 10% $CaCl_2$, equal to 0.182 g Ca) were taken at 3, 30 and 90 minutes, and calcemic curves determined. Three intramuscular injections of vitamin D_2 for three consecutive days were given and calcium curves again determined, the calcium expressed in mg Ca/100 ml blood serum. In all cases, the intravenous injection of $CaCl_2$ was followed by a hypercalcemic reaction, almost immediately observed and persisting for half an hour. The calcemic values then resumed the initial values. After vitamin D_2 treatment, especially in subjects with corneal xanthomatosis, the hypercalcemic values were even higher than without the vitamin.

A review of the pharmacodynamics of calcium and digitalis when administered therapeutically in combination was presented by LaBarre and Heerswynghels (523). Some controversy has followed the simultaneous application of calcium and digitalis in that a synergistic effect appears to exist; the authors of this paper attempted, through experimentation on cats, to resolve the inconsistency of opinion regarding the prescription of calcium treatment in a digitalized subject.

Cats, 2 kg weight, were anesthetized, placed under artificial respiration, the thorax opened and the pericardium incised to expose the heart. Clamps were fixed to ventricle and auricle and the rhythmic contractions recorded. Calcium gluconate, 10 ml of 10% solution, was given intravenously alone or with half a lethal dose of isotonic digitalis followed 15 minutes later by a second similar dose of gluconate. No symptoms of digitalis intoxication appeared 15 minutes after injection with the drug. Cats, previously digitalized show, however, a particular sensitivity to the calcium gluconate, the animals frequently dying shortly after the gluconate is injected.

Using an electrocardiographic technique, further studies of the effects of varying rates of injection of the gluconate in digitalized cats were made. Administered at the rate of 1 ml/15 sec., the 10% calcium gluconate proved fatal after a dose equal to 22 ml/kg body weight; injected more rapidly, at 1 ml/3 sec., death occurred at doses of about 15 ml/kg. The authors discussed their results and those of other investigators and concluded that digitalis and calcium exert very

additive effects that can lead to serious cardiovascular difficulties. Calcium gluconate administered alone produced brachycardia, decreased R-wave amplitude, progressive inversion of the T-wave, ventricular fibrillation and cardiac arrest. In digitalzed animals, the effects were more immediate and quickly fatal. It was recommended by the authors that the combination of calcium and digitalis in medicinal therapy be avoided, especially in patients already showing EEG symptoms of digitalis poisoning.

The problem of drug addiction, the withdrawal symptoms and methods used to treat withdrawal, were described by Williams (1012), who has used successfully intravenous calcium gluconate, 10 cc of 10-20% solution, five times daily for four days, four times daily for three days and periodically thereafter to control the anxiety attending withdrawal. Two cases of drug addition (heroin) were described in which calcium gluconate was used in therapy; the chief merit of the gluconate lay in the absence of pain during withdrawal with this therapy.

VI. Consumer Exposure Information

Little specific information regarding details of the use of the calcium salts discussed in this monograph is available. The naturally occurring metals in foodstuffs such as magnesium, iron, copper, zinc, manganese and cobalt are found chiefly as complexes; sequestrants react with those metals not so bound in large organic complexes to form chelates which result in greater stability in foodstuffs processed for human use. Fats and oils particularly are unstable and when oxidized become rancid and lose their flavor. Sequestrants, together with antioxidants are widely used to stabilize edible fats and oils. Food color, flavor and texture may be improved by the use of sequestrants.

The following tables were compiled from data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on frequency of eating and from the USDA data on mean portion size of foods in each food category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of each substance.

Table 2 reports the usage of calcium salts in foods, and table 3 the usage levels in infant formula products and baby foods. Table 11 reports the annual poundage data and table 13 reports the possible daily intake per food category and total dietary based on food consumption by total sample.

TABLE 2 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
CALCIUM ACETATE NAS 0035 FEMA 2228	06	CHEESE(R)	*	.00434	.01730
	19	SWEET SAUCE(R)	*	.00680	.15000
	20	GELATIN PUD(R)	*	.19044	.20363
	23	BEV TYPE I(R)	*	.00000	.00000
	24	BEV TYPE II(R)	*	.00002	.00008
CALCIUM CHLORIDE NAS 0039	01	BAKED GOODS(R)	6	.07131	.07131
	05	MILK PRODS(R)	*	.01000	.01400
	06	CHEESE(R)	8	.06811	.08678
	08	PROCSD FRUT(R)	*	.12000	.20000
	10	MEAT PRODS(R)	*	.02498	.02740
	13	FISH PRODS(R)	*	.01500	.01500
	14	PROCSD VEGS(R)	17	.05439	.12293
	15	CONDM RELSH(R)	*	.06339	.09672
	18	JAM JELLY(R)	*	.01702	.02002
	19	SWEET SAUCE(R)	*	.00750	.01000
	23	BEV TYPE I(R)	*	.00583	.00583
	24	BEV TYPE II(R)	*	.00089	.01885
	26	RECONST VEG(R)	*	1.50000	2.00000
	27	GRAVIES(R)	*	.07289	.10482
	28	IMIT DAIRY(R)	*	.07839	.07839
	34	INS COF TEA(R)	*	.00320	.32000
CALCIUM CITRATE NAS 0040	20	GELATIN PUD(R)	*	.03000	.03000
CALCIUM GLUCONATE NAS 0041	01	BAKED GOODS(R)	*	1.59136	1.59136
	20	GELATIN PUD(R)	*	2.34430	3.08283
	28	IMIT DAIRY(R)	*	.40000	.40000
	33	SUGAR SUBS(R)	*	.00900	.00900

TABLE 3 -- USUAL LEVELS REPORTED FOR GAS APPENDIX A SUBSTANCES (GROUP II) USED IN INFANT FORMULA PRODUCTS & BABY FOODS(1)

GAS SURVEY NO.	SUBSTANCE NAME	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
0039	CALCIUM CARBONATE	03	FORMULAS(8)	*	.19600	.22464
0048	CALCIUM CHLORIDE	03	FORMULAS(8)	*	.01290	[illegible]
0049	CALCIUM CITRATE	03	FORMULAS(8)	*	.09720	[illegible]

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/03/72

TABLE II, PART A -- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SEE KEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FDA	POUNDAGE REPORTED TO FDA -- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FDA
CALCIUM SULFATE [illegible]	5/ 6	52,103	52,128	52,741	*	3	52,744
CALCIUM CHLORIDE [illegible]	41/ 50	4,087,350	15,060,656	15,245,796			15,245,796
CALCIUM CITRATE [illegible]	5/ 5	4,450	17,869	17,869			17,869
[illegible]	5/ 7	51,000	309,695	315,186			315,186

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH B
CALCIUM ACETATE NAS 0035	06	CHEESE(R)	*	0-5 MO.	**********	.004340	**********
				6-11 MO.	.117180	.422900	.467100
				12-23 MO.	.339620	.963400	1.349400
				2-65+ YR.	.407960	1.024240	1.626200
CALCIUM ACETATE NAS 0035	19	SWEET SAUCE(R)	*	0-5 MO.	.020400	.027200	.450000
				6-11 MO.	.061200	.210800	1.550000
				12-23 MO.	.176800	.516800	3.900000
				2-65+ YR.	.462400	1.217200	10.200000
CALCIUM ACETATE NAS 0035	20	GELATIN PUD(R)	*	0-5 MO.	3.800800	5.141980	4.672000
				6-11 MO.	24.376320	73.899720	26.064040
				12-23 MO.	26.280720	63.997940	28.190140
				2-65+ YR.	38.849760	99.981000	41.140020
CALCIUM ACETATE NAS 0035	23	BEV TYPE I(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000000	.000000	.000000
				12-23 MO.	.000000	.000000	.000000
				2-65+ YR.	.000000	.000000	.000000
CALCIUM ACETATE NAS 0035	24	BEV TYPE II(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	**********	.000070	**********
				12-23 MO.	**********	.000040	**********
				2-65+ YR.	.006500	.016800	.026000
CALCIUM ACETATE NAS 0035	ALL CATEGORIES ********** ********** **********		7	0-5 MO.	3.829200	5.173420	4.922000
				6-11 MO.	24.554700	74.522520	27.081740
				12-23 MO.	26.796040	65.469160	33.350340
				2-65+ YR.	39.726620	102.241320	53.392720

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
CALCIUM CHLORIDE NAS 0039	04	BAKED GOODS(R)	6	0-5 MO.	2.424540	3.200950	2.424540
				6-11 MO.	18.112740	36.938580	18.112740
				12-23 MO.	38.863950	64.036380	38.863950
				2-65+ YR.	97.837320	145.329760	97.837320
CALCIUM CHLORIDE NAS 0039	05	MILK PRODS(R)	*	0-5 MO.	.540000	.400000	.796000
				6-11 MO.	6.240000	30.010000	8.720000
				12-23 MO.	5.450000	17.440000	7.630000
				2-65+ YR.	3.950000	12.060000	5.530000
CALCIUM CHLORIDE NAS 0039	06	CHEESE(R)	8	0-5 MO.	**********	.068110	**********
				6-11 MO.	1.838970	6.606670	2.343060
				12-23 MO.	5.312580	15.120420	6.766840
				2-65+ YR.	6.402340	16.073960	8.157320
CALCIUM CHLORIDE NAS 0039	08	PROCSD FRUT(R)	*	0-5 MO.	5.640000	15.120000	9.400000
				6-11 MO.	62.160000	154.800000	103.[illegible]000
				12-23 MO.	120.720000	239.640000	201.200000
				2-65+ YR.	141.960000	300.720000	236.600000
CALCIUM CHLORIDE NAS 0039	10	MEAT PRODS(R)	*	0-5 MO.	.274780	.724420	.301400
				6-11 MO.	5.170360	13.[illegible]	5.[illegible]
				12-23 MO.	7.543960	12.964620	8.274800
				2-65+ YR.	19.584320	32.493980	21.481600
CALCIUM CHLORIDE NAS 0039	13	FISH PRODS(R)	*	0-5 MO.	.015000	.045000	.015000
				6-11 MO.	.195000	.735000	.195000
				12-23 MO.	.810000	2.025000	.[illegible]
				2-65+ YR.	1.860000	4.635000	1.[illegible]
CALCIUM CHLORIDE NAS 0039	14	PROCSD VEGS(R)	17	0-5 MO.	.761460	2.284380	1.721020
				6-11 MO.	13.053600	30.458400	29.503200
				12-23 MO.	21.212100	35.516670	47.942700
				2-65+ YR.	46.231500	77.886480	104.400000
CALCIUM CHLORIDE NAS 0039	15	CONDM RELSH(R)	*	0-5 MO.	**********	.063390	**********
				6-11 MO.	.507120	1.394580	.773760
				12-23 MO.	1.774920	4.817640	2.709160
				2-65+ YR.	5.578320	13.438680	8.511360
CALCIUM CHLORIDE NAS 0039	18	JAM JELLY(R)	*	0-5 MO.	**********	.051060	**********
				6-11 MO.	1.140340	3.795460	1.341840
				12-23 MO.	.510600	1.906240	.600600
				2-65+ YR.	.970140	3.012540	1.141140

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
CALCIUM CHLORIDE NAS 0039	19 SWEET SAUCE(R)	*	0-5 MO.	.022500	.030000	.030000
			6-11 MO.	.067500	.232500	.090000
			12-23 MO.	.195000	.570000	.260000
			2-65+ YR.	.510000	1.642500	[illegible]
CALCIUM CHLORIDE NAS 0039	23 BEV TYPE I(R)	*	0-5 MO.	.139920	[illegible]	[illegible]
			6-11 MO.	1.323410	4.529910	1.323410
			12-23 MO.	3.159860	9.473750	3.159860
			2-65+ YR.	6.063200	16.189910	6.063200
CALCIUM CHLORIDE NAS 0039	24 BEV TYPE II(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	**********	.000000	**********
			12-23 MO.	**********	.001780	**********
			2-65+ YR.	.289250	.840160	6.126250
CALCIUM CHLORIDE NAS 0039	26 RECONST VEG(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	.000000	.000000	.000000
			12-23 MO.	.000000	.000000	.000000
			2-65+ YR.	3.000000	9.000000	4.000000
CALCIUM CHLORIDE NAS 0039	27 GRAVIFS(R)	*	0-5 MO.	.072890	.218670	.104820
			6-11 MO.	1.020460	2.842710	1.467600
			12-23 MO.	2.624060	7.423760	3.773500
			2-65+ YR.	6.049870	15.526370	8.730060
CALCIUM CHLORIDE NAS 0039	28 IMIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	1.097460	1.802970	1.097460
			12-23 MO.	.627120	2.665280	.627120
			2-65+ YR.	.705510	1.175850	.705510
CALCIUM CHLORIDE NAS 0039	34 INS COF TEA(R)	*	0-5 MO.	.006400	.105600	.640000
			6-11 MO.	.169600	.419200	16.960000
			12-23 MO.	.198400	.716800	19.840000
			2-65+ YR.	3.875200	8.300800	387.520000
CALCIUM CHLORIDE NAS 0039	33 FORMULAS(B)	*	0-5 MO.	42.264630	77.428500	161.639550
			6-11 MO.	8.611560	41.030810	32.934600
			12-23 MO.	2.769800	.780590	10.593000
CALCIUM CHLORIDE NAS 0039	ALL CATEGORIES ********** ********** **********	48	0-5 MO.	52.162120	99.957960	177.172250
			6-11 MO.	120.708620	329.536520	224.149850
			12-23 MO.	211.772330	415.109920	353.052550
			2-65+ YR.	344.866970	658.030210	899.404260

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH B
CALCIUM CITRATE NAS 0040	20 GELATIN PUD(R)	*	0-5 MO.	.600000	.810000	.600000
			6-11 MO.	3.840000	11.640000	3.840000
			12-23 MO.	4.140000	10.080000	4.140000
			2-65+ YR.	6.120000	15.750000	6.120000
CALCIUM CITRATE NAS 0040	83 FORMULAS(B)	*	0-5 MO.	328.650300	602.085000	332.812980
			6-11 MO.	66.963600	319.056100	67.911760
			12-23 MO.	21.538000	6.069800	21.810900
CALCIUM CITRATE NAS 0040	ALL CATEGORIES **********	4	0-5 MO.	329.250300	602.085000	333.412980
			6-11 MO.	70.803600	330.696100	71.811760
			12-23 MO.	25.678000	16.149000	25.950800
	**********		2-65+ YR.	6.120000	15.750000	6.120000
CALCIUM GLUCONATE NAS 0041	01 BAKED GOODS(R)	*	0-5 MO.	54.106240	71.611200	54.106240
			6-11 MO.	404.205440	824.324400	404.205440
			12-23 MO.	867.291200	1429.041280	867.291200
			2-65+ YR.	2183.345920	3243.191680	2183.345920
CALCIUM GLUCONATE NAS 0041	20 GELATIN PUD(R)	*	0-5 MO.	46.885000	63.296100	61.766600
			6-11 MO.	300.070400	900.988400	394.602240
			12-23 MO.	323.513400	787.604000	425.420840
			2-65+ YR.	478.237200	1230.757500	628.897320
CALCIUM GLUCONATE NAS 0041	23 IMIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	5.600000	9.200000	5.600000
			12-23 MO.	3.200000	13.600000	3.200000
			2-65+ YR.	3.600000	6.000000	3.600000
CALCIUM GLUCONATE NAS 0041	33 SUGAR SUBS(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	.000900	.001800	.000900
			12-23 MO.	**********	.001800	**********
			2-65+ YR.	.007200	.007200	.007200
CALCIUM GLUCONATE NAS 0041	ALL CATEGORIES **********	7	0-5 MO.	100.992240	134.907300	115.768840
			6-11 MO.	709.676740	1743.114680	804.408880
			12-23 MO.	1194.004600	2230.327830	1295.921740
	**********		2-65+ YR.	2665.190320	4479.556380	2815.870440

CALCIUM SEQUESTERANTS

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CALCIUM CHLORIDE AND ALCOHOL

EFFECT OF THERAPEUTIC DOSES OF CALCIUM CHLORIDE ON THE CLINICAL STAGE OF ALCOHOLIC INTOXICATION AND ON BLOOD ALCOHOL[1]

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Graham (4) observed in 1828 that calcium chloride forms molecular compounds with alcohols. Later on the structure of the molecular compound formed by anhydrous calcium chloride and ethyl alcohol was expressed by such formulae as $CaCl_2 \cdot 3C_2H_5OH$ (5) and $2CaCl_2 \cdot 7C_2H_5OH$ (9). But no compound formed by this molecular compound and water, with a constant water content, has so far been found.

This quality of calcium chloride was soon made use of industrially to isolate alcohol, and to determine it quantitatively in mixtures. Ant' Vuorinen (1) described a method based on it by which he established the alcohol content of liqueurs and essences.

Knowing this chemical quality of calcium chloride we may ask ourselves whether it has any importance in living organism. The purpose of the present study is to find out whether calcium chloride, when given in therapeutic doses, has any effect on the blood alcohol content and on intoxication at a clinical stage, produced by test drinks and, on the other hand, whether alcohol affects the serum calcium values.

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It is well known that calcium also regulates the functioning of the nervous system, and it has been used in the treatment of states of nervous over-excitement (2, 10).

The present study was carried out in connection with a larger series of studies on the relation of blood alcohol and the clinical stage of intoxication. A group of 22 prisoners in the Central Prison at Helsinki volunteered as test subjects. From among them three were chosen as showing expressive clinical symptoms of intoxication in experiments in which alcohol was given in the ratio of 1 g per kg of body weight. Before the investigation, which began in January this year, the test subjects had taken no alcohol for several months.

Test subject No 1, a chauffeur aged 24, was 168.5 cm tall and weighed 66—67 kg in the course of the experiments. The subject was of athletic build, of usual bone structure, scanty fatty tissue, and schizoid by psychic habitus. He had taken regularly large amounts of alcohol and also narcotics previous to imprisonment some years ago.

Test subject No 2, a smelter aged 24, 182 cm tall, weighing 80.5 kg. His body was pyknic, bone structure usual, fatty tissue moderate, psychic habitus cyclothyme. Previous to imprisonment, about 1½ years ago, he had taken large amounts of alcohol periodically.

Test subject No 3, an electric works operator aged 23, 173 cm tall, weighing 66.5—67.5 kg, of leptosomatic build, slender framework, scanty fatty tissue, schizothymic by psychic habitus. Before imprisonment about a year ago he had taken alcohol in moderation.

The experiments were performed in the mornings at about one week's intervals, starting usually at 8—9 a.m., and the test subjects came to the experiments after fasting 12 hours. During the experiment they were allowed to smoke freely.

In tests carried out on rabbits (2), when calcium compounds were given by the mouth in great quantities, a clear increase could be observed in the serum calcium, and a maximum in about 1—2 hours after the intake, and after about 6—8 hours the serum calcium had been restored to normal. But when the calcium compounds were given intravenously, the maximum occurred, as is natural immediately after the injection. The serum calcium decreased then very rapidly and after about 2 hours it differed very slightly from the control. This is a proof of the rapid elimination of calcium chloride from the blood, its diffusion into the tissue fluids, and of its simultaneous excretion into the urine or into the bowels

The maximum concentration of the blood alcohol, on the other hand, occurs about $\frac{1}{2}$—$1\frac{1}{2}$ hours after the end of the intake, if the alcohol has been taken in a comparatively short time (11).

In the present study these facts were applied with the view of dosing the calcium chloride so that the maxima of the serum calcium and of blood alcohol would occur simultaneously.

The maximum peroral dose of anhydrous calcium chloride is about 1 g (8). The maximum dose for intravenous injections is usually said to be 1 g (10 ml 10% solution). In the experiments 2 g $CaCl_2$ sicc. in 10 per cent solution (Kaisaniemi Pharmacy, Helsinki) was given per os and 10 ml 10 per cent solution (Orion) injected intravenously in 5 minutes. In one preliminary experiment Subject 1 was given 20 ml. Before the intake of calcium chloride or alcohol a blood sample was taken in all experiments to control the serum calcium and blood alcohol, and then blood samples during the whole experiment, usually after $\frac{1}{2}$, 1, 2 and 3 hours from the beginning of the experiment.

The serum calcium was determined according to the modified Kramer-Tisdall (6) method as the mean value of two parallel determinations. In the literature the error of the method is considered to be $\pm$ 5 per cent (7). In the preliminary determinations the limit values usually deviated from each other by about 4 per cent. In one series of determinations, for instance, the following 6 values were obtained:

Mg per cent Ca: 9.12, 8.74, 9.03, 8.74, 8.93, 9.12.

A measured amount of 30.6 per cent alcohol solution mixed with water, 1 g per kg of body weight, was given in all the alcohol experiments. The test subjects ingested the alcohol in a very short time, practically in one draught. The samples for the blood alcohol were taken with a syringe needle from the vein into tubes prepared with oxalate and fluoride, and two Widmark's capillaries were filled out of each tube by slanting the tube. The alcohol determination was made according to Widmark's micromethod (11). The results of the parallel determinations, of which the mean value was taken, did not differ more than 0.05 per mil from each other in any of the cases.

Immediately after the blood samples a clinical examination of the stage of intoxication was performed according to the suggestion of the Blood Alcohol Committee (12) (see Table 3).

The pulse was counted within ½ min. on a subject standing. The smell of alcohol was of course not expressed quantitatively. Symptoms describing motor and psychological functions were expressed, as in the examination carried out by the Blood Alcohol Committee, in relative figures as follows:

0 = sure performance
1 = slightly uncertain performance
2 = uncertain performance
3 = wavering or completely uncertain performance.

Since any comparison between the degrees of these functions must be very subjective, the numerical value of certain types of performance was defined beforehand.

The numerical value 0 represents a sure performance. Yet it cannot be called normal, as many persons can show more or less uncertainty even physiologically, for instance in the walking test, in the Romberg test, and above all in the subtractio ntest. Moreover, even the same person may show slight daily deviations depending on his state of mind, due to sleepless night etc. A control examination was therefore carried out in all alcohol experiments before the taking of alcohol to find out the normal functions of the subjects.

The colour of the face was defined as follows: ± = pale, — = «usual», + — = congestion, + + + = strong congestion.

DETAILS OF THE EXPERIMENTS

Experiment 1. — Physiological serum calcium at various times.

Experiment 2. — Serum calcium, blood alcohol and the clinical symptoms of intoxication when alcohol was ingested, at various times.

Experiment 3. — Serum calcium when calcium chloride was given per os, at various times.

Experiment 4. — Alcohol one hour after calcium chloride had been given per os. Serum calcium, blood alcohol, and the clinical symptoms of intoxication, at various times.

Experiment 5. — As in experiment 4, except that alcohol was given immediately after the intake of calcium chloride.

Experiment 6. — Calcium chloride intravenously. The first serum calcium determination 20 minutes after completion of injection; the others after 1, 2 and 3 hours.

Experiment 7. — First alcohol, then calcium chloride intravenously after 35—40 minutes. Serum calcium determinations as in previous experiment, blood alcohol and the clinical examination as in experiments 2, 4 and 5.

RESULTS AND CONCLUSIONS

Table 1 shows the serum calcium determinations, Table 2 the blood alcohol determinations and Table 3 the clinical examinations of the stage of intoxication. Table 3 shows also the clinical control examinations which were performed at the beginning of all the alcohol experiments, combined into one set of statistics for each subject, as they did not differ from one another (except for a slight difference in the pulse frequency — the average value of which appears in the Table).

I. SERUM CALCIUM

1. Experiment 1 gave the following limit values of the determinations made at various times:

Test subject	1:	8.74	and	9.41	mg	per cent
» »	2:	9.50	and	10.11	»	» »
» »	3:	9.89	and	10.27	»	» »

Test subject 1 and 2 showed some physiological change in the serum calcium at various times during the same morning, as the limit values deviated from each other more than $\pm$ 5% considered to be the common error in this method.

2. In the control tests made at the beginning of all experiments the following limit values were obtained:

Test subject	1:	8.37	and	9.02	mg	per cent
» »	2:	8.65	and	9.50	»	» »
» »	3:	9.02	and	10.27	»	» »

This shows that daily changes occurred in the serum calcium.

The existence of these changes makes it difficult to draw any conclusions about the possible changes of serum calcium in calcium chloride-alcohol experiments.

3. In experiments 3, 4 and 5 an evident though slight increase of a similar type was observed in the serum calcium of all test sub-

TABLE 1

SERUM CALCIUM AT VARIOUS TIMES, EXPRESSED IN MG PER CENT

Experiment	Subject N:o 1						
	8.00	9.00	9.20	9.30	10.00	11.00	12.00
Exp. 1. Physiological serum calcium		8.74		8.74	9.31	9.22	9.41
» 2. Alcohol at 9 o'clock		8.37		8.74	8.56	8.83	8.65
» 3. $CaCl_2$ per os at 9		9.01		9.37	9.65	9.83	9.65
» 4. $CaCl_2$ per os at 8 and alcohol at 9	8.65			9.58	9.58	9.67	9.58
» 5. $CaCl_2$ per os at 9 and alcohol directly afterwards		8.46		9.20	9.38	9.29	9.94
» 6. $CaCl_2$ intravenously at 9		9.02	11.35		10.70	10.42	
» 7. Alcohol at 8.20 and $CaCl_2$ intravenously at 8.55–9.00	8.84		10.23		9.67	9.58	[illegible]

Experiment	Subject N:o 2						
	8.00	9.00	9.20	9.30	10.00	11.00	12.00
Exp. 1. Physiological serum calcium		9.50		10.11	9.30	9.75	[illegible]
» 2. Alcohol at 9 o'clock		9.11		8.83	8.92	9.38	[illegible]
» 3. $CaCl_2$ per os at 9		8.65		8.92	9.46	9.65	[illegible]
» 4. $CaCl_2$ per os at 8 and alcohol at 9	8.93			9.39	9.39	9.67	[illegible]
» 5. $CaCl_2$ per os at 9 and alcohol directly afterwards		9.02		9.11	9.57	9.57	[illegible]
» 6. $CaCl_2$ intravenously at 9		8.93	9.67		9.49	9.58	
» 7. Alcohol at 8.20 and $CaCl_2$ intravenously at 8.55–9.00	9.11		9.95		9.86	9.77	[illegible]

Experiment	Subject N:o 3						
	8.00	9.00	9.20	9.30	10.00	11.00	12.00
Exp. 1. Physiological serum calcium		10.26		9.89	9.93	10.18	[illegible]
» 2. Alcohol at 9 o'clock		9.29		9.48	9.48	9.48	[illegible]
» 3. $CaCl_2$ per os at 9		9.19		9.37	9.56	9.46	[illegible]
» 4. $CaCl_2$ per os at 8 and alcohol at 9	9.49			10.23	10.14	10.23	[illegible]
» 5. $CaCl_2$ per os at 9 and alcohol directly afterwards		9.29		10.03	10.12	9.57	[illegible]
» 6. $CaCl_2$ intravenously at 9		9.02	10.14		9.77	9.49	
» 7. Alcohol at 8.20 and $CaCl_2$ intravenously at 8.55–9.00	9.86		10.97		11.25	10.97	[illegible]

TABLE 2

BLOOD ALCOHOL, EXPRESSED PER MIL. ½, 1, 2 AND 3 HOURS AFTER THE INTAKE OF ALCOHOL. CONTR. = BEFORE INTAKE

Experiment	Subject No 1					Subject No 2					Subject No 3				
	Contr.	½	1	2	3	Contr.	½	1	2	3	Contr.	½	1	2	3
Exp. 2. Alcohol at 9 o'clock	0.03	0.75	0.98	1.09	0.95	0.00	1.07	1.22	1.19	1.06	0.00	0.37	0.66	1.03	0.99
» 4. $CaCl_2$ per os at 8 and alcohol at 9		0.54	1.07	1.19	1.06		0.31	1.07	1.13	1.07		0.38	0.57	1.05	0.96
» 5. $CaCl_2$ per os at 9 and alcohol directly afterwards		0.80	1.31	1.15	1.04		0.50	0.82	1.14	1.03		0.30	0.64	1.13	1.09
» 7. Alcohol at 8.20 and $CaCl_2$ intravenously at 8.55–9.00		1.03	1.29	1.19	1.08		0.92	1.24	1.21	1.02		0.42	0.77	1.16	0.99

TABLE 3

CLINICAL SYMPTOMS OF INTOXICATION ½, 1, 2 AND 3 HOURS AFTER THE INTAKE OF ALCOHOL. EXPERIMENTS: SEE PREVIOUS TABLES. CONTR. = BEFORE INTAKE

Symptom	Subject N:o 1																
	Contr.	Exp 2				Exp. 4				Exp. 5				Exp. 7			
		½	1	2	3	½	1	2	3	½	1	2	3	½	1	2	3
Smell of alcohol in breath	–	+	+	+	+	+	+	+	+								
Pulse frequency/min.	72	100	104	96	92	78	82	90	92	76	96	88	94	88	76	76	86
Colour of the face	+	+	+	+	+	+	+	+	+	+ +	+ +	+ +	+	+ +	+	+	±
Walking test, *eyes* open ..	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
» » » closed	0	1	2	0	0	0	2	1	1	1	2	1	0	2	2	0	0
Turning.....	0	0	1	0	0	0	2	1	0	0	0	0	0	1	2	0	0
Romberg, eyes open	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
» » closed	1	1	2	1	1	1	2	2	2	1	1	0	0	1	2	1	0
Finger-finger test	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0
Match test	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Speech and behaviour	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Subtraction test	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Relative sum of clinical symptoms	1	3	8	2	1	1	7	4	3	2	4	1	0	4	9	2	0

Table 3 cont.

Symptom	Subject No 2																
	Contr.	Exp. 2				Exp. 4				Exp. 5				Exp. 7			
		½	1	2	3	½	1	2	3	½	1	2	3	½	1	2	3
Smell of alcohol in breath	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pulse frequency/min	77	92	78	88	68	80	78	76	72	80	82	80	72	100	90	88	80
Colour of the face	+	++	+	+	++	+++	+++	+++	++	++	++	++	++	++	+++	++	++
Walking test, eyes open ..	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
» » » closed..	0	2	2	0	0	0	1	0	0	0	1	1	1	1	1	1	1
Turning.....	0	1	1	1	0	0	0	1	0	0	1	1	1	0	1	0	0
Romberg, eyes open	0	1	2	1	0	0	1	1	0	0	0	0	0	1	2	1	0
» » closed	1	3	3	2	2	1	1	2	2	1	1	2	1	2	2	2	1
Finger-finger test	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Match test	0	1	2	2	1	0	0	0	0	0	0	0	0	1	2	0	0
Speech and behaviour	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Subtraction test	0	2	2	1	0	0	0	2	1	0	1	0	0	2	1	1	
Relative sum of clinical symptoms	1	12	14	7	3	1	4	6	3	1	5	4	3	8	10	5	2

Table 3 cont.

Symptom	Subject No 3																
	Contr.	Exp. 2				Exp. 4				Exp. 5				Exp. 7			
		½	1	2	3	½	1	2	3	½	1	2	3	½	1	2	3
Smell of alcohol in breath	—	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	—
Pulse frequency min.	90	108	102	96	100	108	104	116	116	106	98	100	106	106	92	86	88
Colour of the face	+	++	++	++	+	++	++	++	++	++	++	++	++	++	++	++	++
Walking test, eyes open ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
» » » closed..	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0
Turning.....	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Romberg, eyes open	0	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	0
» » closed	1	2	2	2	2	1	1	2	1	1	1	1	1	1	1	1	1
Finger-finger test	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0
Match test	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Speech and behaviour	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Subtraction test	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Relative sum of clinical symptoms	1	3	4	3	3	2	3	6	3	1	3	2	1	3	5	2	1

jects. This shows that when calcium chloride was taken per os, the alcohol could not be proved to have any effect on the serum calcium values.

4. Slight but evident increases of the same type were observed in the serum calcium in test subjects 2 and 3 in experiments 6 and 7 and in test subject 1 in experiment 7. In experiment 6 test subject 1 showed an obvious increase, when injected a double amount of calcium chloride 20 minutes after the injection, but even this was relatively slight. This proves that calcium chloride, even when injected, is very quickly eliminated from the blood, and the alcohol did not seem to affect the serum calcium values in any way.

II. BLOOD ALCOHOL

The blood alcohol curves of each test subject in all alcohol experiments were very similar type. Calcium chloride, given per os or intravenously, did not seem to have any effect on the blood alcohol values.

III. CLINICAL STAGE OF INTOXICATION

To make the differences between the symptoms clear, the numerical values obtained in different experiments at various times were added together and the relative sums thus obtained were compared (Table 3). The smell of alcohol in breath, the pulse and the congestion of face were not included in the sums, since these symptoms cannot be considered to indicate anything about the degree of the intoxicated.

When alcohol was taken immediately after the dose per os of calcium chloride, the symptoms were found to be slighter than when alcohol was taken without calcium chloride. Calcium chloride, given intravenously, had no effect on the symptoms of the stage of intoxication one hour after the ingestion of alcohol and 20 minutes after the calcium chloride injection, but 2 and 3 hours after the ingestion of alcohol the symptoms of intoxication were milder than when alcohol was taken without giving calcium chloride. The possible reducing effect of calcium chloride on the symptoms of intoxication had in this case been delayed.

According to this study calcium chloride, given in therapeutic doses, cannot be considered to show any peculiar characteristics due to the molecular compound formed by it and ethyl alcohol.

The reducing effect, if any, of calcium chloride on the clinical symptoms of intoxication should be studied by methods allowing less scope for possible errors due to the examiner's subjectivity, to the compensation and influence of practice of test subjects in successive experiments (3). It would also be interesting to find out in what manner other calcium salts, such as gluconate and lactate, affect these symptoms.

SUMMARY

Calcium chloride was given per os and administered intravenously in therapeutic doses to three test subjects, to whom 1 g per kg of body weight of alcohol was given simultaneously. Serum calcium and blood alcohol determinations and clinical examinations of the stage of intoxication were made at various times after the ingestion of alcohol. The following conclusions could be made:

1. No effect of alcohol on the serum calcium values could be established, nor did calcium chloride affect the blood alcohol values.

2. Calcium chloride possibly had a reducing effect on the symptoms of intoxication. This may be due to the effect of calcium chloride on neural functions.

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COMPARATIVE RESEARCH ON SEVERAL CALCIUM SALTS USED IN THERAPY

I. Distant minimum lethal dose of calcium chloride, calcium lactate, calcium gluconate, calcium pyruvate, administered intravenously (*)

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The introduction of calcium medication in therapy can be traced to a very distant date, and in fact, it preceded the pharmacological and physiological study of calcium.

Success has in part justified the empirical method. The materia medica of antiquity abounds in medicines based on calcium: principally used were the bones of the dead, shells, corals, all of which contain an abundant amount of calcium carbonate. These preparations, fallen out of use today, were prepared with the calcium salts that the pharmaceutical industry prepares in numerous forms, and which are tested on the most assorted diseases. Among the salts most generally adopted in therapy are calcium chloride, calcium lactate, calcium pyruvate and calcium gluconate, on which numerous clinical, but few pharmacological, experiments have been conducted with that scientific rigor and precision which are necessary in comparative research.

Therefore, I undertook a series of comparative experiments on these four calcium salts. First ofall, I believed it opportune to determine with an exact method the toxicity of each of them because, as we shall see, the less precise experiments led to ideas and opinions which did not correspond to the reality of the facts. The notes that can be collected from the literature concerning this question are very scarce and insufficient, the least lethal doses reported having been determined by means of experimental procedures which do not allow for the exact observation of the symptomatology and comparison of the respective toxicities.

The question is therefore worthy of a more precise study. Knowledge of toxicity is in fact as indispensible as that of the pharmacological and therapeutical effects, when one wants to offer a precise judgment on the value of several remedies which are similar for all the salts and have the same therapeutic indications. Advisable would be the use in practice of that medication which

(*)A preventive communication was made of this work. See Bulletin of the Italian Society of Biology, 1936, p.310.

will unite a low toxic effect with another activity.

I report succinctly the few notes which I was able to find on the argument in the vast literature available on calcium salts.

Delogu (1), in some experiments on the compared toxicity of calcium in animals, found that rabbits injected intravenously with a solution of calcium chloride would die, at a rate relative to the speed of the injection, upon the use of doses ranging from 0.26 g per kg (high speed of the injection =cc. 12.5 per kg and first minute) to 0.78 g per kg (low speed of injection =cc. 2.79 per kg and first minute). From the experiments of Delogu it is revealed that the average of the immediate minimum lethal doses is equal to 0.50 g per kg of body weight.

Rothlin (2), studying intravenous toxicity of the four calcium salts, among them chloride and gluconate, found that the average of the lethal doses in rabbits was 0.20 g per kg of the animal for chloride and 1.70 g for gluconate.

Liebermann (3), in a study on the intercedent relationships between the effect of calcium gluconate and the scillaren B on the cardiac activity and on the blood pressure, injected into the veins of dogs a solution of calcium gluconate at 10% with the speed of 4 cc per kg and first minute, and observed that, in the experimental conditions which he had preselected, the lethal dose was equal to 0.185 g ± 57 of calcium per kg of body weight.

Morton F., Mason and Harry Resnik (4) attempted to determine the toxicity of calcium chloride, calcium lactate, and calcium gluconate injected into the cisterna of dogs.

They found that doses of 0.25 mg of these salts, administered via this route, did not compromise the life of the animal; doses of 0.40 mg led, in 90% of the cases, to death in 5 or 6 seconds from respiratory paralysis. Of the salts examined, the least toxic, in respect to the immediate effects, turned out to be, according to these authors, calcium lactate.

Actually, from these notes no certain conclusion concerning toxicity can be drawn, since the experiments were conducted using methods so imprecise that they really do not allow for any reliable comparisons. The figures which I have reported all represent in fact an immediate minimum lethal dose which, perhaps, is the least suitable when one wishes to establish comparisons between the toxicity of several medicines.

According to Simon (5), in fact, when one wishes to determine the minimum lethal intravenous dose in the higher animal, it is necessary to distinguish between the immediate minimum lethal dose and the distant minimum lethal dose. By immediate minimum lethal dose is meant the smallest dose which, injected into the veins of the animals, immediately kills them. It was believed that regular curves could be obtained, from which the immediate minimum lethal dose could at once be calculated. In fact Simon (6) described particular curves for some pharmaceuticals, in which, starting from a minimal speed (very high lethal dose), increasing the speed a bit each successive time, an optimum speed could be reached, which yielded the maximum toxicity (the lowest point of

the curve). Starting from this value, with the increase of the speed, the lethal dose increased and the curve rose. That is to say, that according to the first experiment of Simon, for every medicine there existed a single speed which allowed for the certain determination of the immediate minimum lethal dose. Simon attributed the slight toxicity of the medicine with the minimal speed to the fact that the introduction of very small doses of the medicine at regular intervals gave the organism a chance to eliminate it; he interpreted the speed suitable for attaining minimal toxicity as that which allowed the medicine to become better anchored in the tissue cells and to exercise there the most complete influence: he explained the decreased toxicity which is obtained when the speed of the injection is increased with the fact that there was being introduced into the organism a quantity of the medicine greater than that which could in a unity of time reach the cells of the tissues to exercise there its toxic influence, so that a dose which actually was not able to act in its entirety appeared to be toxic. Later research of Simon (7) and other authors (8) has demonstrated, detailing a great deal in the establishing of the curves, that instead of obtaining that ideal curve described by Simon, there were obtained rather others, in which, relative to the speed of the injection, the minima are several and not only one, and one can obtain several minima with identical doses, but with various speeds. These curves assume an importance of the first order as regards the pharmacological study of the immediate effect of the medicine, and often succeed in giving us a broad view of the extremely complex mechanism through which the medicines injected into the veins cause death. If we wish to avail ourselves of them in order to compare the toxicity of various medicines, they cannot of course lead to a resolution of the problem. Therefore, these experiments, useful from a purely pharmacological point of view, are incapable of telling us the truth when we wish to arrive at the values of their comparative minimum lethal doses.

For this reason, Simon proposes, and adopted in his Institute, a method for water-soluble medicines of determining the distant toxicity via the intravenous injection in the higher animal, which consists of the intravenous injection into many animals, at a constant and of itself harmless speed, of increasing amounts of solution, always of the same concentration for any given medicine, and picking the smallest dose that kills the animal at a certain distance from the injection. This is then the distant minimum lethal dose. After administration of the medicine, the animals were kept under observation so that the symptomatology could be determined exactly. One then proceeded to the autopsy of those that died, and to the histological examination of the organs in order to determine, when possible, the cause of death. Following this method, many experiments have already been performed in the laboratory of Simon and a considerable amount of results has already been achieved.

Using this method, I determined the intravenously injected minimum lethal dose of calcium chloride, calcium lactate, calcium gluconate and calcium pyruvate.

EXPERIMENTS

Conducted on 62 healthy rabbits, all coming from the same litter. The animals were kept in the laboratory for some days before being subjected to the experiment. The solutions to be injected were prepared recently from salts whose purity was controlled. For every salt I used solutions of 0.5 N obtained by weighing the exact quantity of salt added to the solution in distilled water and in a tared balloon. For calcium gluconate, since the solubility of this substance at atmospheric temperature is 3%, the salt was mixed in cold water, then immediately distributed in a phial and stabilized with permanence in an autoclave for an hour and a half. The solutions thus obtained were injected into the marginal vein of the ear of rabbits at a speed of 0.5 cc per kg of body weight and first minute. This speed was of itself absolutely harmless. The injection finished, the animal was placed in the cage, fed, and placed under intensive observation, so that the symptomology could be watched until the animal died or had apparently recovered.

The results of the experiments are shown in tables I,II,III,IV.

CRITICAL EXAMINATION OF THE EXPERIMENTS

The examination of table I shows that the distant minimum lethal doses for the salts I studied are equal respectively to 0.0070 g per kg for calcium lactate, 0.0080 g for calcium chloride, 0.0130 g for calcium gluconate, and 0.0180 g for calcium pyruvate.

The results of direct observation, like those of the necroscopic and histological observations, are, for the animals injected with the calcium salts I studied, basically equal, since the differences consist only of the fact that some of the salts reveal a more evident influence than the others on certain functions and organs. Therefore, I will briefly summarize here the symptomatological picture presented by the animals treated with calcium lactate, calcium chloride, calcium gluconate and calcium pyruvate, clarifying the possible differences which were brought to my attention.

a) We begin by examining the effects of the non-lethal doses. These, especially if larger in comparison with the therapeutic doses, considerably modify the thermoregulation, the respiratory function, the circulatory function, the diuresis, and the diameter of the pupil of the eye.

The animals bear the injection well and show no signs of intolerance, and I never observed any phenomena of excitation; on the other hand, a depressive action, more evident as the doses increased, was always ascertainable.

During the injection and immediately after the injection, for a period varying from one and one half hours to two hours, there is always a miosis which reaches its maximum intensity one hour after the beginning of the experiment, and recedes little by little until the pupil resumes its normal diameter. I cannot

Table I

CALCIUM CHLORIDE

Solution 0.5 N

1. Numero dell'esperienza	2. Data	3. Peso del coniglio in gr.	Gr. eq. per Kg.	4. Esito dell'esperienza	5. Osservazioni
1	21 III 1936	1800	0,0025	a) sopravvive	A. Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	8 VII 1935	1200	0,0040	»	
3	21 III 1936	1950	0,0050	»	
4	11 V »	1220	0,0060	»	
5	19 VII 1935	2000	0,0060	»	
6	12 VII »	1840	0,0070	»	
7	16 VII »	1450	0,0075	»	
8	16 XI »	1500	0,0075	»	
9	8 I 1936	1850	0,0075	»	
10	20 XI »	1430	0,0080	b) muore dopo ore 21	
11	8 V »	1150	0,0080	» » 22	
12	25 VII 1935	1650	0,0080	» » 44	
13	24 VII »	1450	0,0085	» » 19	
14	23 VII »	1480	0,0090	» » 24	
15	29 VII »	1420	0,0090	» » 26	
16	21 X »	1600	0,0090	» » 7	
17	23 VII »	1700	0,0095	» » 32	
18	17 VII »	1500	0,0100	» » 23	
19	18 VII »	1600	0,0125	» » 6	

Distant minimum lethal dose = 0.0080 g per kg

Key:
1.= Number of experiment 2.=date 3.= weight of rabbit in g.
4.= outcome of experiment 5.= observations
a) survives
b) dies after...hours

A. No convulsive phenomena were ever noted in the experiments. In the animals that died, death occurred after cardiac arrest, followed immediately by respiratory arrest.

Table II

CALCIUM LACTATE

Solution 0.5 N

1. Numero dell'esperienza	2. Data	3. Peso del coniglio in gr.	Gr. eq. per Kg.	4. Esito dell'esperienza	5. Osservazioni
					A.
1	25 III 1936	1700	0,0025	a) sopravvive	Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	26 III »	1800	0,0050	»	
3	9 XI 1935	1250	0,0060	»	
4	14 I 1936	1180	0,0060	»	
5	10 XI 1935	1400	0,0060	»	
6	21 II 1936	1250	0,0065	»	
7	25 II »	1100	0,0065	»	
8	7 XI 1935	1330	0,0070	b) muore dopo ore 19	
9	27 II »	1470	0,0070	» » 49	
10	30 VII »	1700	0,0080	» » 2.30	
11	30 VII »	1600	0,0080	» » 2.40	
12	31 VII »	1900	0,0080	» » l'iniez.	
13	7 XI »	1430	0,0080	» » »	
14	29 VII »	1600	0,0090	» » 7	
15	29 VII »	1530	0,0095	» » 4.30	
16	20 VII »	1670	0,0100	» » 8	

Distant minimum lethal dose = 0.0070 g per kg

For key see table I.

Table III

CALCIUM GLUCONATE

Solution 0.5 N

1. Numero dell'esperienza	2. Data	3. Peso del coniglio in gr.	4. Gr. eq. per Kg.	Esito dell'esperienza	5. Osservazioni
				a)	A.
1	25 III 1936	1350	0,0025	sopravvive	Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	25 III »	1560	0,0050	»	
3	5 II »	1450	0,0050	»	
4	11 XII 1935	1400	0,0080	»	
5	2 I 1936	1750	0,0100	»	
6	6 V »	1120	0,0100	»	
7	3 I »	1400	0,0110	»	
8	4 I »	1600	0,0125	»	
				b)	
9	3 II »	1420	0,0130	muore dopo ore 10	
10	29 IV »	1050	0,0130	» » 24	
11	30 IV »	1150	0,0130	» » 24	
12	21 I »	1550	0,0135	» » 5	
13	17 I »	1900	0,0135	» » l'iniez.	
14	16 I »	1640	0,0150	muore dopo ore 15	

Distant minimum lethal dose = 0.0130 g per kg

For key see table I.

CALCIUM PYRUVATE

Solution 0.5 N

1. Numero dell'esperienza	2. Data	3. Peso del coniglio in gr.	Gr. eq. per Kg.	4. Esito dell'esperienza	5. Osservazioni
1	27 III 1936	1750	0,0025	a) sopravvive	A. Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	27 III »	1700	0,0050	»	
3	21 XII 1935	1300	0,0080	»	
4	24 XII »	1620	0,0090	»	
5	27 XII »	1400	0,0100	»	
6	28 IV 1936	1150	0,0100	»	
7	8 I »	1800	0,0125	»	
8	11 I »	1570	0,0150	»	
9	14 I »	1500	0,0175	»	
10	28 I »	2000	0,0180	b) muore dopo ore 82	
11	14 IV »	1350	0,0180	» » 15	
12	23 I »	1900	0,0190	» » 48	
13	13 I »	1800	0,0200	» » 19	

Distant minimum lethal dose = 0.0180 g per kg

For key see table I.

whether the miosis is succeeded by a mydriasis, since the observations were made under varying conditions of light.

All the doses of the salts with which I experimented initially produce a drop in the temperature, which is greater in proportion with the increase of the dose; in the case of equimolecular doses, of the four salts, calcium gluconate and calcium chloride were shown to be most active from this point of view. As a rule, hypothermia is followed by hyperthermia; upon administration of doses approaching the lethal, such hyperthermia is no longer demonstrable.

The respiratory function is modified by all the salts in the sense that, as a rule, the rate of respiration decreases, sometimes notably, sometimes to a minor degree, but always evidently. Only in the case of the smallest doses -- 0.0025 g and 0.0050 g per kg -- was it impossible to demonstrate a decrease of this rate for calcium gluconate and calcium pyruvate. Bradipnea was generally followed by polypnea. When the injected dose is sufficiently high, breathing becomes particularly difficult: the animal puts its auxiliary muscles into play, and very loud rattling and hissing can be heard.

As regards the pulse, all the salts produce modifications in its rate and sometimes in its rhythm; as a rule, bradycardia develops, followed in general by a tachycardia. In this regard as well, calcium gluconate and calcium pyruvate at doses of 0.0025 and 0.0050 g were shown to have an indifferent effect on the circulatory function. I always noted a certain parallelism between the modifications of the heart and the respiration and those of the pupil: that is, they all reach their maximum intensity about one hour after the injection.

The diuresis is also considerably modified, but not in the same manner, by the various salts examined. While calcium chloride, calcium lactate and clacium gluconate produce a decrease (more evident as the doses are increased) of the volume of urine emitted in the 24 hours following the injection, calcium pyruvate, also administered in doses approaching the lethal, always allows an abundant elimination of urine. The larger doses often provoke albuminuria, most significant when calcium lactate and calcium chloride are administered, but always temporary. Glycosuria was never observed.

The animals recover quickly, so that on the day following the injection, no particular symptomatology is found upon direct examination.

b) The findings made on the animals treated with lethal doses of the four calcium salts are the following: the animals never show signs of intolerance, nor do any phenomena of motor excitement appear. The modifications of the pupil, those in the respiratory apparatus, the thermoregulatory modifications, and those of the diuresis, are qualitatively equal to those that can be observed when high doses of calcium are injected.

The drop in temperature, always notable, is particularly remarkable when calcium gluconate is injected.

The diuresis is more strongly inhibited by calcium chloride and calcium lactate; with this latter salt, complete anuria can sometimes be verified. Albuminuria, most significantly induced by calcium lactate, always accompanied the administration of

lethal doses of the salts I studied.

The general depressive action exercised by the lethal doses of the studied salts is remarkable. Freed from their cages, the animals struggle to resume their normal prone position and behave as though in a state of torpor. If they are stimulated until they are forced to move, their walk is troubled and uncertain. The musculature appears hypotonic. Placed in their cages, the animals constantly refuse the food which is offered them, and seem to be indifferent to their environment. With the advancement of the effect of the poison, the muscular hypotonia becomes more manifest: most intensely hypotonic are the flexor muscles in the limbs, so that the animals assumes a characteristic position -- the head droops inertly from its own weight, and the paws are stretched out on the floor on which the animal lies. The reflexes, though a little delayed, are present, and sensibility to pain is retained.

A progressive weakening of all the vital functions leads to the death of the animal, occurring calmly. In general, the heart stops first, while some respiratory movement may still persist.

With these lethal doses, however, we can notice a difference between the animals injected with calcium chloride, calcium lactate, and calcium pyruvate on the one hand, and those treated with calcium gluconate on the other. In the case of the former, the modifications in the thermoregulation, the pulse and the respiration, after having touched upon a miximum of intensity, generally recede, so that it seems that the animals are turning toward a recovery of their health; the rate of respiration and pulse increase again, until they reach and sometimes surpass the physiological value; the temperature, after having reached a maximum of descent, begins once again to rise, though remaining much below the normal value. In other words, it seems that the animal's powers of defensesare succeeding in prevailing over the violence of the poison. After a period varying from 38 to 72 hours, there occurs once again a weakening of all the vital functions. The breath becomes superficial, the pulse weak, and infrequent; the temperature descends progressively. The animal turns thus toward death, which arrives calmly.

However, in the rabbits that were injected with calcium gluconate, this short-lived resumption of the vital functions could not be observed. Calcium gluconate either leads rapidly and inexorably to death, or, whenever the animal succeeds in reacting against the violence of the poison, the return to normality proceeds to complete recovery of health.

<u>Autopsy and histological examination.</u> -- The autopsy, undertaken immediately after the death of the animals, revealed: strong congestion of the thoracic and abdominal organs; distended blood vessels full of liquid blood; subpleural and subperitoneal hemorraghic suffusions. Frequently, in the animals that died a certain distance from the injection, subperitoneal hematomies occupying the space located between the rectum and the bladder could be detected.

I was unable to find any differences in the effects of the four calcium salts.

c) Histological examination.-- The patches were withdrawn immediately after the death of the animal and treated with opportunely selected methods. Cuttings were executed with the congealing microtome, coloration with hematoxylin eosin, hematoxylin and Sudan III for the examination of fats. For the common preparations, the anchorage was done using the method of Zenker, the impaction in paraffin, the coloration in hematoxylin-eosin; for the connective tissues I used the coloration method of Van Gieson and the trichromic method of Mallori; for the examination of the fibrin I followed the method of Weigert; the adrenals were fixed in potassium bichromate, impacted in paraffin and colored with the method of Wiesel; the brain was fixed in alcohol, impacted in paraffin and colored with hematoxylin-eosin.

The results of the examinations are described below.

Encephalus. -- Cuttings in the bulb and cerebellum: vascular congestion; nothing of note in the parenchymal tissue.

Thymus. -- Strongly congested both in the connective vessels between the lobules and in the capillaries that penetrate the parenchyma; no hemorrhages.

Thyroid. -- Of normal aspect, also as regards vascularization.

Adrenal glands. -- Are revealed to be of normal aspect, both in the cortical and medullary sections. The various layers of the cortex are well-defined and very rich in lipoids. There is evident, as in the other organs, vascular congestion. Nothing of note in the central vein.

Heart. -- Nothing of note concerning the endocardial membrane and the epicardial serum. The aspect of the cardiac muscle is shown to be just about the same in all the levels, and shows very strong congestion of the blood vessels, which have nearly uniformly dilated walls and the membrane full of fresh, well-conserved blood. Therefore, one has the impression of a dilation of both the major and minor vessels and of the capillaries. Regressive phenomena in the cardiac muscle cell are absent.

Lung. -- Extensive vascular congestion both in the large blood vessels and in the alveolar capillaries. Nothing abnormal contained in the alveoles and in the interior of the bronchi. In the preparations prepared with the common methods of coloration, the wall of the arterial vessels appears to be slightly . This can be referred to the hypertrophy of the tunica muscularis, the colorations of Van Gieson and Mallori having demonstrated the absence of phenomena of true vasal sclerosis.

Liver. -- Imposing vasal congestion both of the large and fine capillary vessels. The epithelial elements of the organ demonstrate no findings worthy of note. The coloration with the method of Weigert for the demonstration of the fibrin yielded a negative outcome.

Kidneys. -- Vascular congestion is evident in this organ as well. With common coloration degenrative processes in the parenchymal elements can sometimes be perceived; in the case of coloration by means of Sudan III, however, no drops of fat were ever observed in the epithelial elements, for which the regressive phenomena, taken into consideration the normal colorability of the nucleus as well, can be limited to the degeneration of albumin.

Spleen. -- Congested pulp: the follicles reveal normal aspect and disposition, with slight variations in the different animals.

Intestine. -- Vascular congestion and dilation.

Preparing the preparations of the zones infiltrated with blood (see macroscopic examination) in a manner so as to involve the various organs surrounded by the same hemorraghing, it was ascertained that the vessels of the connective retro-peritoneal area, like those of the area corresponding to the retro-bladder space are intensely dilated and full of blood; in there walls can be noted long lesions from which the blood overflows in a considerable amount, showing itself in some points to be well-conserved, in others more or less altered and in the process of coagulating. On the other hand, nothing of note was remarked in the bladder wall, in that of the rectum, and in general in the contexture of the organs surrounded by blood.

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The results of the experiments must be considered in relation with the distant minimum lethal dose and the cause of death.

From the tables presented above, we see that the scale of toxicity for the four substances is as follows:

Salt	Distant minimum lethal dose in g per kg
Calcium lactate	0.0070
" chloride	0.0080
" gluconate	0.0130
" pyruvate	0.0180

These results confirm those of Rothlin (2) only in that they also demonstrate a greater toxicity for calcium chloride as compared to calcium gluconate, but they also demonstrate that calcium gluconate is much more toxic than was shown in the experiments of that author.

Besides this, they demonstrate that calcium lactate is the most toxic of the four salts studied, and that it is followed, in order, by calcium chloride, calcium gluconate and calcium pyruvate, which last is without doubt the least dangerous. Assuming that =1 is the distant minimum lethal dose of calcium lactate, the other distant minimum lethal doses assume the values shown below:

Calcium lactate	=1
" chloride	=1.14
" gluconate	=1.85
" pyruvate	=2.57

As regards the toxicity of calcium gluconate compared to that of calcium chloride, my experiments, though generally confirming Rothlin's affirmation that calcium gluconate is less toxic than calcium chloride, also show that it is much more toxic than this author finds. In fact, from my experiments, taking =1 as the distant minimum lethal dose of calcium chloride, the corresponding dose of calcium gluconate turns out to be =1.62; Rothlin, on the other hand, finds in his research, making =1 the minimum lethal dose of calcium chloride, that that of calcium gluconate rises to 4.25. Thus, while I show that the distant minimum lethal dose of calcium gluconate is barely one and one half times that of calcium chloride, he finds that it is more than four times greater, that is, that calcium gluconate is more than four times less toxic than calcium chloride.

The discrepancy is immediately explained when one observes that in his experiments Rothlin used different speeds of injection for the two salts, while it is necessary that the speed be absolutely constant.

As concerns calcium lactate, Morton F., Mason and Harry Resnik (4) have observed that this salt, injected into the spine of dogs, is less toxic than calcium chloride or calcium gluconate. This contradiction with what I found cannot be explained easily, as the authors do not explain the considerations on which their assertions are based, limiting themselves to saying that the suspension of lactate resulted, in respect to the immediate effects, in being less toxic than the solutions of calcium chloride and calcium gluconate with an equal metal content. On the other hand, the authors also do not specify the exact quantity of the various salts which is necessary to provoke death.

If we now examine the mechanism through which the calcium salts introduced into the veins induce death, we see that the literature can illuminate very little for us on this point, since the authors are anything but concordant in this regard.

Dastre (9) observed that in the case of intravenous injections of calcium chloride, the animals die as a result of extensive intravasal congestions and therefore attributes to the salt a particular toxic effect which was negated by all the authors who later worked on the same question. They never observed the finding described by this author.

Delogu (1), injecting animals with calcium chloride intravenously, observed that death occurred with convulsive actions when the speed of the injection was increased, while a clear depressive effect leading to the death of the animals was constantly apparent.

Rothlin (2) was able to note that during the injection of calcium salts, the respiration at first accelerated and became superficial, then becoming slower and deeper. The animal is restless; violent clonicotonic cramps with opisthotonus follow, as well as cessation of breathing and esophthalmus. If the dose is not lethal, the animals recover quickly; if the dose is lethal, then they die upon the first attack of cramps or soon after. As

a rule, a quick death occurs during a convulsive attack. I believe that in this case death can be attributed above all to the excessive speed of the injection, which agrees with the observation of Delogu.

Liebermann (3), injecting calcium gluconate into the veins of dogs of whose carotid pressure he had made a manometric tracing, was able to observe that death arrived gradually with a progressive abatement of the pressure. In another work on the modifications in the blood pressure in dogs injected with equimolecular doses of calcium lactate and calcium gluconate, the same author able to observe sometimes the unforeseen death of the animal, which he interpreted as due to premortal intravasal coagulation.

Morton F., Mason and Harry Resnik (4), introducing calcium lactate, calcium chloride and calcium gluconate into the cisterna of dogs, maintained that death occurred from respiratory paralysis.

From the notes that I have reported it can be seen that the greatest uncertainty exists when it must be decided through what mechanism the calcium introduced into the veins of the animals produces death.

From the experiments which I conducted systematically on 62 animals we can exclude the possibility that calcium, injected slowly into the veins, cause convulsions; these phenomena, as Delogu showed, can be attributed to the speed of the injection.

Intravascular coagulation also can be excluded as a cause of death. The autopsy performed immediately after the death of the animal always revealed liquid blood in the vessels, and the examination of the fibrin was always negative.

The histological examination of the preparations of the organs of the animals that died following the injection yields no possible explanation of the mechanism of death. In fact, the alterations consist essentially of an enormous vascular congestion, sometimes with the rupture of small vessels of some areas.

Interesting on the other hand is the narrow analogy, the near identity of the symptoms I found upon injecting high doses of calcium at a slow speed and those observed by various authors who brought calcium directly into contact with the nervous elements in various ways.

Sabbatani (10), in his classic research on calcium, shows that this cation describes a moderating effect on the tissues. Upon the local application of a calcium salt on the cortex, the medulla, the nerves and the muscles, he always observed phenomena of depression, while, on the other hand, the application of a decalcifying reagent, trisodium citrate, always produced phenomena of excitation, which he interpreted as due to the fact that said substance forms complex ions with calcium and therefore reduces the calcium in the tissue to the state of an ion, chemically and biologically active.

Roncoroni (11) confirmed the results in his Institute: the application of salts precipitating calcium onto the cerebral cortex provoked motor action such as myoclonia and epilepsy and Ferrari (12), in his turn, observed the same depressive phenomena by injecting calcium into the carotid.

By means of the direct application of calcium to the infundi-

bulum of female cats, Demole noted that the animals fell into a more or less profound sleep (13).

L. Stern and J. Chvoles (14) introducing a solution of calcium gluconate into the cerebral ventricles of dogs, found that such an injection produced in general a temporary excitation, which was followed in a few minutes by a complete loss of the muscular tonus which terminated after fifteen minutes in a profound narcosis with loss of reflexes. This state persisted for some hours, after which the animal recovered little by little. Very often however, the animal declined and ended by dying. The blood pressure would begin to drop after a few minutes and around the fifteenth to twentieth minute it was usually very low. The pulse would become slower and weaker, the breath slower and superficial. Equal doses injected into the circulus had no effect. As a consequence of these results, the authors conclude that calcium has a direct effect on the centers situated in the ventricle walls.

Bun-ichi Hasama (15), injecting 2 mmg of calcium chloride into the vicinity of the cineritious tubercle of the female cat, observed that the animals passed into a state of somnolence; the pulse and the respirationdiminished in rate; a decrease in temperature was also produced.

The results of these experiments are truly interesting because they demonstrate that the application of small amounts of calcium to encephalic centers is characterized by a symptomatology which recalls that which I observed upon the injection of high doses of calcium salts at low speed via the intravenous route.

In summary, the results of my experiments lead me to exclude the possibility that calcium salts administered intravenously at a slow speed of injection produce death through intravascular coagulation, and that the death occurs by means of convulsions. A rapid drop of the temperature, a slowing down and progressive weakening of the pulse and the respiration, an intense vascular congestion, form the symptomatological picture presented by the animals intoxicated with calcium; a symptomatological picture that can be summarized with the comprehensive term of collapse. This collapse, on the basis of the negative results of the anatomo-histological examination, can only be attributed to an intense, depressing effect of calcium on the nervous centers.

CONCLUSIONS

1 -- The distant minimum lethal intravenous dose in the rabbit is, according to Simon: 0.0070 g per kg of body weight for calcium lactate; 0.0080 g per kg for calcium chloride; 0.0130 g per kg for calcium gluconate; 0.0180 g per kg for calcium pyruvate.

2 -- These experiments, besides providing us with data which lend themselves to precise comparisons, are of value to the therapist in that they rectify opinions which were not based on sure experiments. And while up to now it has been maintained that

calcium gluconate is more than four times less toxic than calcium chloride, my reaearch shows that the difference in toxicity is much less, little more than one and a half times. Thus it can be easily understood how the abuse of this salt in therapy, founded upon its presumed slight toxicity, was able often to produce unexpected toxic phenomena.

The disturbances observed upon the administration of calcium lactate, which is the most toxic of the four salts, can also be understood now.

3 -- The cause of death in the case of all four of the salts studied is always a depressive action of the calcium cation on the nervous centers; without doubt, this effect is opposed by the anion to a degree which the actual experiments cannot establish.

SUMMARY

The distant minimum lethal intravenous dose in the rabbit is, according to Simon: 0.0070 g per kg of body weight for calcium lactate; 0.0080 g per kg for calcium chloride; 0.0130 g per kg for calcium gluconate; 0.0180 g per kg for calcium pyruvate. The toxicity of calcium gluconate is shown by these experiments to be much greater than what was hitherto believed, and thus explains the toxic phenomena observed in the therapeutic use of this salt. The cause of death can be attributed to the depressive effect of the calcium cation on the nervous centers.

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COMPARATIVE RESEARCH ON SEVERAL CALCIUM SALTS USED IN THERAPY

II. Intravenous distant minimum lethal dose of calcium camphosulfonate

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In the comparative research undertaken on the calcium salts most used in therapy, I limited myself in an earlier work to the study of calcium lactate, calcium pyruvate, calcium gluconate and calcium chloride. Recently, calcium camphosulfonate has benn put into use, and has found much favor in medical circles.

Therefore, I believed it to be opportune to extend my research to this new salt as well, and above all, I wished to determine the distant minimum lethal dose by means of the method of Simon (1).

In order to obtain exactly comparable results, I maintained the experimental conditions which I had observed in the preceding experiments with clacium lactate, calcium chloride, calcium pyruvate and calcium gluconate (2). Thus I injected pure calcium camphosulfonate in a solution of $\frac{N}{2}$ into the marginal vein of the rabbit ear, at a constant and of itself harmless speed of injection.

The results of the experiments are recorded on table I.

The animals treated with the reported doses of calcium camphosulphate showed a symptomatological picture identical to that observed in the rabbits that had been injected with the other calcium salts studied. Therefore, in this report I am dealing only with the most salient characteristics, and draw your attention to the earlier work for the finer details and the interpretation of the symptoms and of the cause of death.

The rabbit which was administered a non-lethal dose of calcium camphosulfonate, appears to be very bewildered, his senses deafened, for several hours after the injection. It lies in a corner of the cage, its fur tangled and ruffled, refuses all the food offered it, is indifferent to its environment, and shows evident signs of muscular weakness.

Immediately after the injection, the rate of respiration decreases. In the following hours, when the animal has passed the crisis of acute intoxication of the calcium, there is generally

(1) Simon I. --Archive of biological sciences, XII (1928), 479.
(2) Baldacci U. -- Archive of experimental pharmacology and related sciences, LXII (1936), 91.

an increase in the rate.

The pulse becomes slower, but remains rhythmic and valid.

The thermoregulatory function is notably altered: in fact, the camphosulfonate, like all the soluble calcium salts, exercises a hypothermial effect. In a rabbit injected intravenously with 0.0075 g per kg, a drop in the temperature to 10 degrees below the physiological value was noted.

Table I

Calcium camphosulfonate (solution $\frac{N}{2}$)

(speed of injection = 0.5 cc per kg and m')

1. Numero dell'esperienza	2. Data	3. Peso del coniglio in gr.	Gr. eq. per Kg.	4. ESITO
1	2-11-38	1850	0,0050	vive a)
2	3-11-38	1650	0,0075	vive
3	16-11-38	1520	0,0075	vive
4	17-11-38	1620	0,0090	vive b)
5	18-11-33	1780	0,0100	muore dopo 10 ore
6	12-11-33	1500	0,0100	muore dopo 8 ore

Key:
1.= number of experiment 2.- date 3.= weight of rabbit in g
4.= outcome a) lives b) dies after...hours

The curve of the rectal temperature is the surest prognostic sign for judging whether the administered dose will kill the animal or not; upon injection of non-lethal doses the temperature decreases, touches upon a minimum and then rises again toward the physiological value, and coinciding with this increase in the temperature is a return to the normal state of all the functions of the organism; when, however, the administered dose is lethal, the decrease in temperature is progressive until the death of the animal.

A dose of 0.0100 g reveals a typical picture of calcium intoxication: the walk is at first uncertain and troubled, later becoming altogether impossible due to an ever-increasing muscular weakness, which compels the animal to lie on its stomach, the limbs extended and spread apart, and the head also resting on the plane on which the animal lies, since the muscles are incapable of supporting it; the breath is profoundly altered, infrequent in the first hours following the injection, and becoming,

a few hours before death, notably more frequent and superficial, interspersed with long respiratory pauses, and finally terminates in a complete apnea that may precede the arrest of cardiac function. The pulse, as a rule infrequent, becomes, in this same preagonic state, arhythmic and reveals a progressive weakening; the diuresis appears to be inhibited and the urine reveals evident traces of albumin, while glucose is always absent. The feces are diarrheic.

The autopsy performed immediately after the death of the animal and the histological examination of the organs yielded results identical to those encountered in the rabbits dead from lethal doses of the other calcium salts I had studied earlier.

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If we now compare the distant minimum lethal intravenous dose of calcium camphosulfonate with those of calcium lactate, calcium chloride, calcium gluconate and calcium pyruvate as reported here below, it is seen that this dose is found between the minimum lethal doses of calcium chloride and calcium gluconate.

calcium	lactate	0.0070 g per kg
"	chloride	0.0080
"	camphosulfonate	0.0100
"	gluconate	0.0130
"	pyruvate	0.0180

Making the distant minimum lethal dose of calcium lactate equal to 1, the lethal doses of the other salts assume the respective values of 1.14, 1.72, 1.85, 2.57, which reveals that calcium camphosulfonate is 0.7 times less toxic than lactate, and 0.8 times less toxic than chloride, and is, on the other hand, 1.3 times more toxic than gluconate and 1.8 times more toxic than pyruvate.

Experiments still in progress will be able to establish whether there corresponds, to a lesser toxicity, a lesser, equal or greater pharmacological activity of each of the salts.

SUMMARY

The author determines the distant minimum lethal dose of calcium camphosulfonate in the rabbit and finds that it is 0.0100 g per kg. He establishes a comparison with the distant minimum lethal doses of calcium lactate, calcium chloride, calcium gluconate and calcium pyruvate, which had been determined in a preceding work.

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THE BIOLOGICAL AND METABOLIC ROLE OF CALCIUM: Therapeutic Indications of Calcium Chloride

by
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The variable role of calcium and the variety of its biological functions assign it an important place in physiology and therapeutics. It is also indispensable to specify this biological role before explaining its applications in cure, and to choose from among its numerous chemical combinations the form which is the most favorable in the therapeutic effects.

Calcium is present in the organism in several chemical forms, of which many are still unknown to us. Nonetheless, it has been possible to ascertain, in a certain manner, the mineral forms consisting above all of calcium carbonate and calcium phosphate, and the organic forms, the type being represented by the proteinates.

The major portion of calcium (97 to 98%) is relatively fixed, since it forms an integral part of the skeleton, where it plays a passive role, though being susceptible to mobilization in the case of calcium deficiency or in the course of certain pathological conditions.

The mobile calcium circulates in the state of free ions, colloidal bonds, and undissolved salts.

It is this active calcium which intervens in cellular nutrition, in the activity of ferments, in the equilibrium of liquids, in the stabilization of colloids, and as regulator of muscular motor conduction and cerebral excitation.

The calcium penetrates into the organism with the food and with drinks. The daily calcic ration, varying with age, is 0.20 g to 0.50 g. The ordinary diet of the adult offers, without milk, at least 0.15 g calcium per day. A diet with milk furnishes up to 7 or 8 g; 59% is eliminated in fecal matter and 41% in the urine, though this proportion varies with the nature of the food.

Its physiological action is similar to that of strontium and barium; on the other hand, it isopposite to that of potassium and sodium because of a mechanism which Busquet considered physiological. It is a stimulator of the sympathetic (Daniclopolou), while the sodium ion is rather a factor of stimulation of the motor phenomena of daily life. According to Herzfeld, the equilibrium of the autonomous nervous sytem depends on a certain concentration of calcium ions. If this decreases, the excitability of the vagus increases; if it increases, it is the sympathetic which becomes more excitable. For a good balance of the nervous functions, the relation $\frac{Ca}{K}$ should be equal, in the plasma, to 2. Thus it is that in the case of experimental tetany induced by thyroidectomy in the dog, it moves from 3.8 to 5.2,

to 16 and even to 23.

A similar equilibrium would seem to be necessary between the sum of the sodium and potassium and that of the calcium and magnesium: $\frac{Na + K}{Ca + Mg}$ is normally between 29 and 31. In parathyroidectomized animals, Winckler found figures from 40.2 to 52.8.

Even in very feeble doses of $\frac{L}{75,000}$, as Busquet and Pachon have shown, calcium can exercise an inhibiting effect on the vagus.

In normal doses, it has a positive inotropic function; it accelerates the cardiac contraction.

In very strong doses, it lowers arterial tension.

Hedon and Fleig showed that it stimulates intestinal peristalsis.

According to Vimmer, it stimulates the nutrition, as the increase in excretion of nitrogen, phosphorus, sulfur and chlorine proves.

It acts on muscular contraction. According to Loeb, the excitability of the muscles is proportional to the relation $\frac{Na + K}{Ca + Mg}$ in the blood. The calcium intervenes as a moderator. MacCallum, withholding it by dialysis, provoked an extreme superexcitability of the muscular system. In 1910 Silvestri, in 1921 Pic and Bonnamour ascertained that calcium can impede the convulsions which provoke strychnine.

Its action on the balance of fluids is not less important.

It was long ago that one recognized its role in the formation of blood clots and in certain clinical manifestations: hives, serous phenomena, erythemas, which Netter believed could be attributed to a decrease in the coagulability of the blood.

In 1908 Porges and Pribram, in 1911 Bonnamour, Imbert and Jourdan ascertained its diuretic effects, in 1920 Leon Blum, after L-F. Meyer and Magnus Levy, thought that the Na ion should be imputed with the hydropigenous power of NaCl, first revealed in 1900 by the works of Widal, Lemierre, Achard and Paisseau. Leon Blum believed to be able to attribute a dechlorurant action to the Ca ions through their substitution, in the interstitial liquid, for the Na ions. The experiments of Achard show that this interpretation raises numerous objections. The works of Carofeano and Labin have shown that even when calcium chloride provokes a diuresis, it is not demonstrated that the Na ions which are excreted are excreted otherwise than in association with the Cl ions. This is confirmed by the works of Mathieu, Achard, Rathery, Widal, Lemierre, and Andre Weill. Further it is seen that the level of the calcemia is of a remarkable fixity: in order to maintain it, a regulative mechanism much studied by Hamburger and Brinkmann seizes the water everywhere that it is found. Thus the interstitial diuretic action of calcium chloride, which is the most absorbable of the calcium salts, is more simply explained.

It is also thought that calcium activates the inactive trypsin in the absence of enterokinase. This is what is shown by the experiments of Delezenne, Pozerzki, Leon Binet and Pierre Brocq.

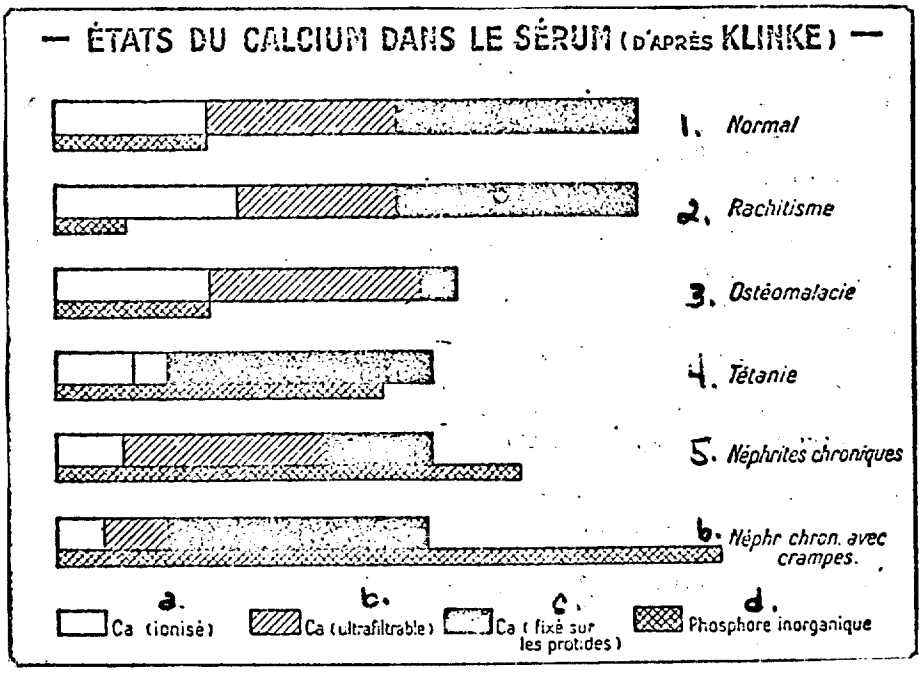
In the same way its stimulating action on the functioning of the liver and the kidneys no longer seems doubtful.

The physiological effects, also numerous and varied, allow us to see the importance of calcium therapy and the extent of its domain.

But it is not always easy to make the organism accept all the calcium introduced into it by way of the digestive or parenteral routes. Furthermore, the enormous doses which have been proposed by certain authors are eliminated, to a large extent, by the intestine, without having penetrated into the general circulation.

In effect, the absorption of the calcium salts is narrowly related to the acidity of the gastric juice and the content of the first portions of the duodenum, whose pH has a value between 4 and 6.5. Under the influence of hydrochloric acid, the phosphates and carbonates are transformed into soluble calcium chloride, which is also easily dialyzable. In youth, pepsin plays an important role in digesting the proteic calcium complexes, and in liberating the calcium which they contain. Finally, there is no doubt that the bile is capable of dissolving notable quantities of calcium. Nonetheless, Denis and Minot have indicated that they were not able to raise the calcemia by having patients inject 6 grams of calcium lactate for 10 successive days. If one injects calcium hypophosphite intravenously, the Ca ions stop increasing at the end of ten minutes, after the time necessary to set into action the regulator mechanism of the calcemia: in the case of chloride, the increase lasts about one half-hour. At the same time, the phosphates are more abundant in the urine and the feces. The phosphaturia which is observed is in reality a calciuria due to the fact that the insoluble bicalcic phosphate is transformed into monocalcic phosphate which can be eliminated by the kidneys. The excess is eliminated by the intestine and more by the colon than the small intestine. Finally, if these two emunctories are not sufficient to maintain the level of calcemia, a part of the calcium, as was shown by Heberyi, is put into reserve in the tissues and the skeleton. It is enough to say that it is not easy to create a balance of the calcium ingested, since a portion fixed temporarily in the organism, elsewhere than in the blood, escapes chemical analysis. In this way we see explained the contradictory results obtained by those who, basing their theories on the calcemia alone, hoped to arrive at pathogenic consideration of the role of calcium or its variations under the influence of certain extirpations of organs. In effect, the calcium level of the blood is remarkably fixed: it varies only within very narrow limits -- between 105 and 120 milligrams per liter of plasma -- while the normal level found by Hengfeld, Lubowski and Guillaumin varies in the area of 100-110 milligrams. On the other hand, as shown in the table below, which was created by Klinke and has already been reproduced in the text of Lichtwitz, it seems probable that the pathological phenomena are much more related to the variations in the percentage of the different states of mobile calcium than to the calcemia itself, which -- and we emphasize this point -- only varies a very little.

FORMS OF CALCIUM IN THE SERUM (according to Klinke)



Key:

1.= normal 2.= rickets 3.= osteomalacia 4.= tetany
5.= chronic nephritis 6.= chronic nephritis with cramps

a.= ionized b.- ultrafiltrable c.- fixed in the proteids
d.= inorganic phosphorus

As this table shows, calcium is found in the blood in three forms: free calcium ions, undissociated salts such as phosphates, bicarbonates, lactate; and colloidal complexes where the calcium is in combination with the proteids.

The ions and the salts are ultrafiltrable. The calcium-albumin complexes do not dialyze. Very approximately, as will be shown, by indicating the techniques of analysis used by other authors, it can be estimated that the non-dialyzable calcium forms 80%, the free ions 10%, and the calcium in the form of salts another 10%.

The determination of the free calcium ions is not directly possible in the experiment: one can only calculate them by relying upon physico-chemical theories. Also, the figures obtained differ according to the theories adopted by the authors.

Some, like Dona and Takahashi, only considered the equilibrium of dissociation and solubility of the calcium carbonates in applying the formula $\frac{(Ca^{++})(HCO^{-})}{H^{+}}$ = K(180). They found a constant equal to 350, which corresponds, by application of the law, to a tenor of 25 milligrams ionized calcium per liter.

The calculation of Erik J. Warburg applies the theory of activity of Nernst and Bjerrum to the action of the ions on the product

of solubility of calcium carbonate and the constants of dissociation. The product $CO^3H \times Ca = K$ is called the product of solubility, K being a constant which the product can not exceed without there being precipitation of Ca. By proceeding as he did, Warburg, who worked at 38°, found a figure of 18 milligrams.

Other authors, Hasting, Murray and Sendroy, found the figure of 15.6 mg by adopting the same principles of calculation, but taking into consideration the conditions of dissolution of the calcium carbonates.

But the amount of free calcium ions does not depend only on H^+ and HCO^{3-}, but also on the phosphorus ions, as Behrendt, H. Kugelmas, and Shol, Holt, La Mer and Crown, have pointed out. Thus anew element, which takes into consideration the equilibrium of calcium carbonate and phosphate, enters into the calculations. In these conditions, the figure found is 4.5 mg. Still, two hypotheses exist, one believing that calcium phosphate decreases the solubility of the carbonate, the other that the phosphate adsorbs the calcium. In the first case, there is a precipitation; in the second it is necessary to make the physical forces of adsorption intervene. Ingenious experiments have been conducted in order to choose between these two points of view. None appeared demonstrative or definitive to me. The results obtained in vivo and in vitro do not agree; however, one can retain from them that, practically, the injection of large quantities of calcium salts results in the formation of precipitates which can causes paralyses, which supports the theories of Lumiere concerning floculation and its phenomena.

Neuhausen and Marshall used the electrometric method in their investigations; they found that the ionized calcium does not exceed 10% of the total calcium.

We should stop at this figure and consider that it is very approximate and susceptible to revision.

This ionized calcium is the most active fraction of the total calcium. For the old adage "Medicamenta non acta nisi soluta" there has been substituted the concept that in many cases the bodies only act if they are ionized.

This ionized calcium has been compared to the oxygen dissolved in the plasma; the organism rapidly takes from it the calcium which it needs.

The second form of calcium, composed of the ultrafiltrable but not ionized salts, consists of combinations which are fairly easily ruptured; in these combinations the calcium is represented above all by the calcium carbonates and phosphates stabilized, in their dissolution, by the presence of albumin. There is "reciprocal adsorption" between the base and the solution in such a manner that the CO^{3-} passes into the precipitate and the PO^{4--} into the solution. This portion has been compared to the oxygen reserve of the hemoglobin which progressively restores the losses undergone by the free ions in circulation.

The third fraction of the serum calcium is represented by the non-dialyzable calcium, the amount of which is 40 to 50 mg per liter. This is the figure found by Dena and Takahashi by dialysis of compensation. Meysenberg had found 60 . The difference

between the two results is explained by the fact that if the dialysis is prolonged, part of the non-dialyzable calcium nonetheless finally filters. This non-ultrafiltrable fraction is composed of an albumin-calcium complex. This depends narrowly upon the isoelectric point of the albumin of the serum, seroglobuline, globuline, fibrinogen, and the complexes which they can form, each of them having a different isoelectric point, varying between pH 5 and pH 8 (Wles). In the same way, the unequal distribution of calcium between the plasma and the globules can be attributed to the fact that the isoelectric point of the hemoglobin is found in a more acid zone. It depends first of all upon the constants of concentration and dissociation of the hemoglobin and those of the plasma albumins, considered colloidal electrolytes, and secondly upon the volume of the dissolution. This is equal to the volume of the liquid, with a deduction of that of the colloids.

We can now conclude from these investigations, all these analyses and all these experiments, that it is quite dangerous for clinicians to examine new pathogenies by basing themselves on figures concerning which the agreement of different authors is far from being complete, and that very often, the errors which can be made in these analyses are of an order of gravity which is greater than the normal variations of the proportion of the different forms of mobile calcium.

Nonetheless, it would seem to be well established that for certain diseases there exist important modifications of the calcemia such as we saw in the table reproduced above. But once again, and this will be the conclusion of this rapid study of the biological role of calcium, it is not so much the total calcemia which intervenes, since it is remarkably fixed, as the ponderous relations which exist between the different states of circulating calcium; and these are difficult to specify analytically.

This is what Mathieu-Pierre Weil has already expressed: "The measure of the calcemia does not allow us to judge the state of organic calcification or decalcification. The blood is not the constant reflection of the disturbances which occur in the tissues. It is an error to think, as it is often commonly admitted, that it can be. If a blood sample incontestably represents a biopsy, it is only the biopsy of one particular tissue, and it is more than dangerous to try to conclude, on the basis of its condition, that of the other tissues."

If a judicious therapeutics could one day give us the means of acting in an elective manner on the mechanism which regulates the relation of these fractions of blood calcium, we will have realized an important advance cert. One would be capable of acting in a favorable manner on a number of ailments or humoral alterations which are related to the disturbances occurring in the division of the different forms of calcium.

Numerous medicines are used to calcify or decalcify the organism. Among the first are calcium chloride, which is the most directly absorbable; we will review its diverse indications, which are narrowly bound to that which we will reveal concerning the biological role of calcium.

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Crystallized calcium chloride ($CaCl_2 + 6H_2O$) is presented in the form of colorless prisms of a saline bitter taste; they are very deliquescent and can be dissolved in an amount of cold water which is one-fourth their weight, and are also very soluble in alcohol.

This calcium chloride is easily absorbed and rapidly eliminated. It is lethal for the guinea pig at a dose of 0.70 g per kg of the animal's body weight.

The hypercalcemic syndrome which, according to Collip, is set into motion when the calcemic level reaches about 150 mg per liter of serum is characterized by: somnolence, vomiting, diarrhea, muscular atonia, increase in blood viscosity, and collapse.

In weak doses, it is well tolerated by the digestive tube. In ahigh dose it can cause nausea, vomiting and diarrhea.

Its penetration into the blood provokes a slight acidosis as shown by the formula:

$$CaCl_2 + 2NaHCO_3CaCO_3 + 2NaCl + H_2O + CO_2$$

where it would appear that one molecule of $CaCl_2$ introduces two molecules of Cl which are absorbed more rapidly than the calcium ions.

The action of calcium chloride and the calcium salts in general may be rendered more effective by the application of ultra-violet rays, light, and also certain vegetable oils irradiated by the quartz lamp. The mechanism of this action seems to be exercised by the intermediary of the sympathetic system and the endocrine glands. The parathyroids in particular, have an effect on the metabolism of calcium comparable to that of the isles of Langerhans in the utilization of the hydrats of carbon.

On the other hand, Professor Serono of Rome recommends the application of calcium therapy by means of a diet rich in cholesterin and organic phosphates, in which cheeses, milk, eggs and brains are important. He also recommends hypodermic injections of organic phosphatides and the absorption of ethers of cholesterin and thyroid extracts.

Since the calcium intervenes in all the vital functions where, as we have indicated, it has a major role, the physician will often have occasion to resort to the use of calcium chloride. It occupies an important place in the therapeutic arsenal. We will study its indications in following the classification of the indications which we have adopted in our teaching of therapy.

SEDATIVE MEDICATION

Calcium chloride is indicated in states of spasmophilia, where the muscles respond to nervous excitations with spasms. The works of Rohmer and Woringer, Debre, Lene, Turpin, Parrot, MacCallum and Voetglin have confirmed the ideas expressed by Netter in 1907, who was inspired by the works of Loeb and Quest. The mechanism of hypocalcemia in the spasmophilic terrain is not exactly know, but it is certain that the ionized calcium acts in its triple role of moderating and antitoxic agent. Certain

authors have proposed the hypothesis that it renders the cells impermeable to toxic products. Whatever it may be, it must, in this case, be used in very high doses. Marquezy recommends 4 to 6 grams in the food, the salt being strongly diluted and administered, in urgent cases, every hour for 6 hours. This should be continued for ten to twelve days, in the intervals between crisis, since its effect is very quickly exhausted.

Cornelius has recommended it in the treatment of states of anxiety. In a dose of 1 to 4 g it produces a decrease of paroxysms and even complete cure in the less serious forms.

Kylin reported its favorable effects on certain vagotonic states: asthma, nervous instability with neurasthenic syndrome. It acts favorably in cases of pure vagotonia.

Levy-Solal and Dalsace recommend its use in preeclamptics and for vomiting of pregnancy.

RESTORATIVE MEDICATION

It is above all in the case of tuberculosis that calcium medication proves its restorative value.

It was quite long ago that the favorable influence of calcium on tuberculosis was established. Do we not find the horn of the stag and crayfish eyes in the pharmacopeias of old? The observations of Renon concerning the lime-burners of Vermenton, those of Fischberg on the plasterers of Paris, the statistics of Behla concerning the laborers working with lime, plaster and cement, the declarations of Dr. Rousse of Bagneres-de-Bigorre (1864) on the effects of the calcium waters of the valley of Campon, have shown the preventive action of calcium in the development of tuberculosis.

The chalk serves not only to compensate for the losses arising from decalcification, but also to encyst the pulmonary or ganglionic tubercular foci into a sort of chalky shell. It is known, in effect, that in the guinea pig, where the tubercular lesions show no tendency to calcification, the progress of tuberculosis is particularly rapid and serious. (Pehu and Dufourt) It is an excellent complement to the usual hygieno-dietetic cure, but acts above all on the tuberculosis of the serous, and in those of the articulations, as is shown by the works of Pruvost, Halbron, Duhamel and Levassor, Clavelin and A. Sicard. In 1913, Pirquet already wrote that calcium chloride is the salt which yields the best results in tubercular children.

Blair-Bell, Kastle and Healy administer it in cases of danger of post-partum uterine hemorrhage, departing from the idea that primitive uterine inertia can be due to a hypocalcemia. This is also the opinion of Louvel, who has emphasized its preventive action in the hemorrhages of delivery. Each pregnancy, it is said, costs one tooth. The administration of calcium chloride can compensate for a negative calcium balance.

It is indicated in the painful loosening of symphyses, and in incomplete forms of osteomalacia.

In cases of rickets, its indication may be shown, but under the condition of being completed by irradiations or the administration of products activated by ultra-violet rays, for in all the cases which will be enumerated, the calcium introduced into the organism only acts insofar as the sympathetic nervous system

has been stimulated by the rays or by vitamin D.

Loeper and Lemaire recommend it as a recalcifiant in the case of enteritis.

In osteomalacia of famine, described by Cramer and Schiff, it yielded good results.

The same is true in cases of the osteopsathyrosis of Lobstein and the osteocia of Ferrier.

ANTICATARRHAL MEDICATION

Vedel and Puech treated diarrhea by intravenous injections of calcium chloride at a dose of 2 centimeters cubed of a solution at 50%.

A. Aymes and A. Raybaud obtained good results in subjects intolerant of mercury cyanide by associating cyanide and calcium chloride in the same preparation.

Faroy considers it an excellent antidiarrhetic in all the forms of enteritis and in acute defacations of chronic colitis.

However, in this case, it must be prescribed in less elevated doses, for in high doses it is laxative and even purgative.

In inflammatory pleurisies some good results have been noted.

DIURETIC MEDICATION

Borges and Pribram in 1908, and later Moncany, Bonnamour, Imbert, Levin and Leon Blum emphasized the diuretic effects of calcium chloride. The interpretation of the mechanism is not yet definitively determined. In opposition to the hypothesis of Blum are the experiments of Achard and the works of Carofeano and Labin; Ambard and Schmidt think that the diuresis is the result of a surcharge of albumina in HCl. The HCl increases the osmotic pressure and thus favors an aqueous diuresis and exaggerates the renal secretion of chlorine. Whatever its mode of action may be, it is certain that calcium chloride is diuretic, but the too high doses which have been recommended must be renounced.

CARDIO-VASCULAR MEDICATION

$CaCl_2$ has an incontestable effect on the cardiac rhythm, though it can not be specified whether the effect of the medicine is worked on the myocardial or on the intracardiac nervous ganglia. The properties of calcium, which reinforces the contraction of the heart, increases its excitability and decreases its conductability, are analogous to those of digitalis. They justify the opinion of the authors who have concluded the similarity of action of the two medications and the empirical use which certain medicines make of them by associating the tonic action of calcium chloride with digitalis in the treatment of pneumonia (E. Frommel, of Geneva). Joltrois recommends calcium chloride for nervous tachycardies and patients who no longer react to digitalis. The optimum dose would be, in this case, 3 grams

per day. Singer and Danielopolou advocate the association of calcium chloride and cardiotonics. It would reactivate the action of digitalis and strophanthus, and would permit the attainment of rapid and important effects in those cases where the cardiotonics have become ineffective.

Marchal says that it is indicated in myocardia, and Calunni uses it in periods of dilatation of the heart.

ANTIVIRIC MEDICATION

Professor Metalnikov of the Pasteur Institute, has shown that it was possible to increase the force of resistance of the organism cells to diverse infections, by the preliminary introduction of stimulating substances. Thus the guinea pigs injected with weak doses of solutions of diverse chemical substances ($CaCl_2$, $MgCl_2$), then a day later with a lethal dose of choleric vibrios, most often survive, while the control animals die. Among these stimulents, calcium chloride appears to be one of the best. It excites phagocytosis and the mobilization of the cells of defense. It would seem thus to be indicated in the therapy of infections.

HEMAPOIIC MEDICATION

Withetto this has been called alterating medication. It is related to the blood, the lymph, all the fluids in general, normal or pathological.

1. Hemostatic action. -- Because of its action on the coagulation of the blood, calcium is an excellent medication in the treatment of hemorrhages, when these are related to the rupture of vessels as in stomach ulcers, hemoptysis, intestinal tumors, or operational aftermaths, or to modifications in the crasis of the blood. This property of the calcium salts was indicated, in 1903, by Carnot.

In cases of tubercular hemoptyses, calcium chloride, combined with opium and incense, has the double advantage of acting on the coagulation of the blood to increase and recalcify the organism. Sembuc and Ha-van-sue use it in hypodermic injections, in 1 to 4 injections of 2 centimeters cubed of a 25% solution. But the level seemed dangerous to us. The solution is caustic, can cause eschars and provoke vivid general reactions. It was once again Carnot and Blamoutier who were the first to advocate intravenous injections and to indicate its good effects. They used a solution of 5%. Since then, $CaCl_2$ has been prescribed for all varieties of hemorrhages.

At a dose of 3 to 4 grams, Mathieu, Loener and Farov use it in cases of digestive hemorrhages caused by rupture of esophagal or gastric varices, in catrorrhages of gastritis of ulcerous or cancerous origin, in hemorrhaginarous diseases which occur in the intestine. Its action is double: locally, it favors the formation of a clot on the level of the points which are bleeding; generally, it increases the power of coagulation of the total

blood mass. In cases of intestinal hemorrhages, Gutmann recommends a drop-by-drop administration of glucosed serum containing 4 g calcium chloride and a gelatinous broth, containing $CaCl_2$. Weil-Halle advocates, for moloena of the newborn, a gelatine solution of 10% which he alternates with a solution of calcium chloride of the same concentration, which he administers every hour by a spoonful in coffee.

Michel Bechet and Montaigner prescribe it in injections at 5% after operations in order to assist hemostasis, prevent pulmonary complications, facilitate the diuresis and impede the hematomas which are sometimes the consequence of local anesthesia. Vieeville resorts to the same precaution administered orally, but before and after administrations into the nose, the throat and the larynx. Toubert, Dubard, Bord, Blechmann, Viela, Thibaudet, Feldstein and Geland.

Guillemin indicates its advantages in the menorrhagias of menopause by associating it with the fluid extract of witchhazel.

Roger and Garnot speak of its good effects in hemorrhagic nephritis. The investigations of Vincent and Donter have demonstrated, clinically and experimentally, the protective action of calcium chloride vis-a-vis hemoglobinuric seizures.

In the case of hemoglobinuric bilious fever, Rieux recommends administering 3 to 5 g calcium chloride from the first hour.

In the case of purpura, it is always used at a dose of 2 to 5 grams per day.

In the case of thrombocytopenia, it can be of service. Roskam recommends it in a dose of 2 to 4 grams per day or 5 or 6 at most. The treatment is worked for a week and is then interrupted for 3 or 4 days.

In the case of hemophilia, Garnot and Plamoutier have obtained good results with intravenous weekly injections of 20 centimeters cubed of a solution of calcium chloride at 5%. These injections can be replaced by the daily oral administration of 2 to 3 grams $CaCl_2$, for periods of 4 to 5 days, separated by interruptions of equal duration. Simpson indicates the case of a hemophiliac observed for the first time at the age of 2 years, and who has had no hemorrhages for 6 years, absorbing 4 grams $CaCl_2$ per day in periods of 4 days, with 4 days of rest.

2. Antialbuminuric action. -- In 1905, Wright and Ross advocated calcium chloride in functional albuminurias, which they attributed to a defect in the coagulation of the blood. Thus it has been prescribed in cases of orthostatic, digestive, and cyclic albuminuria.

In this case, Krauss proposes a daily dose of 1 gram to 1.5 grams.

Netter, Renon, Teissier, Gade, Chuminis, Lereboullet express the opinion that calcium chloride is the medication of choice of most varieties of albuminuria.

In cases of nephritis of war, which were so frequent in Germany, Hulse administered 11 to 18 g calcium chloride per day, Schultz 10 grams.

3. Antiseric action. -- In serio phenomena, calcium chloride deserves first place. It is a medication which is at once preventive and curative. Weil-Halle and Chabanier report than an intravenous

injection of 1 cm^3 5 of a 50% solution ended seric phenomena more than once. Netter administers 1 g calcium chloride per day, to all the subjects injected, in a notion which he administers during the days following the injection. Ramond advocates it at a dose of 2 to 4 grams per day for a period of 8 to 10 days. Other authors, such as Besredka, Danielopolou, begin by administering it even before the injection has been given, in order to diminish " the state of general amphotonia". Danielopolou gives 1 g $CaCl_2$ immediately after an injection of serum, and repeats the dose 2 to 3 times in the day. In a child of 3 years, the doses are 1.5 g, and of 6 years 2.5 the first day.

Gewin, of Amsterdam, came across four cases of rash in 100 children subjected to $CaCl_2$, while in 100 children who did not receive the chloride, there were 2% cases of rash. By elevating the dose to 0.50 g from 10 centimeters cubed of the injected serum, there was neither fever nor rash in 30 diphtherics thus treated.

Netter reunited three series of children observed in 1906, 1907 and 1909, and observed 3% of rashes in 598 subjects treated with chloride, and 16.8% in 648 not having received it or receiving it in isufficient amounts.

4. Anticlasic action. -- Calcium chloride is a good medication in cases of manifestations of colloidoclasis. It is probable that in this case it acts as a electrolytic stabilizer of the colloids, which it prevents from floculating. In other words, it consolidates the fluids. What we have said of its metabolism and its intervention in the equilibrium of the ions of plasma can explain this anticlasic action. It should also be tested in cases of asthma, hay-fever, hives, migraine, certain eczemas, in strophulus infantum and itching. It may cause some happy surprises; the pathogeny of these phenomena is so disparate that one must neglect to resort to a medication such as calcium chloride, whose effects on the fluids are as numerous as they are varied. In short, it must be used each time that one supposes, on the basis of a syndrome, the presence of blood, humoral or vago-sympathetic mechanism.

These are the therapeutic indications of calcium chloride. They are numerous, as the biological role of calcium suggested.

In the future, when the regulatory mechanism of calcium on the fluids and the blood has been better grasped, and the means of acting in a more elective way on on the electrolytes which enter into the composition of the above has been found, the calcium which exists in the organism in quite different forms will, I believe, assume an important place in the therapeutic arsenal. It is a medication which will last and which will still, for a long time, exercise the knowledge of physiologists and physico-chemists.

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ETHYLENEDIAMINE DIHYDROCHLORIDE OR "CHLOR-ETHAMINE"

II. UNTOWARD AND TOXIC REACTIONS

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In the previous paper by Boyd and Dorrance,[1] the effect of ethylenediamine dihydrochloride or chlor-ethamine upon the pH of urine of albino rats, guinea-pigs and rabbits was compared with that of ammonium chloride, calcium chloride, mandelic acid and sodium acid phosphate. The therapeutic usefulness of a drug depends not only upon its pharmacological and therapeutic properties but also upon any toxicological or untoward reactions which may occur, especially with doses of the order of the therapeutic dose. Hence a further study has been made of the toxic and untoward reactions of chlor-ethamine as compared with those of the other four acidifiers mentioned.

I. Ammonium Chloride

Ammonium chloride was given by stomach tube and dissolved in water to approximately 150 albino rats, 150 guinea-pigs and 150 rabbits, the animals in each species being divided into three groups receiving respectively 0.05, 0.25 and 1.0 grams per kilo body weight of the drug. This range of doses has been previously[1] shown to include the optimal therapeutic dose with respect to acidification of urine. No untoward effects were noted from any of these doses in any species. The same doses were given to 41 cats also without evidence of toxic reactions. The LD 50 of ammonium chloride was determined in 46 albino rats. To overcome the possibility of incomplete or variable intestinal absorption of ammonium chloride, the LD 50 was determined by giving the various doses of ammonium chloride intramuscularly. As shown in Table I, the LD 50 was found to be 30 mgm. per kilo body weight given by this route.

II. Calcium Chloride

Similar experiments were performed upon approximately the same numbers of albino rats, guinea-pigs and rabbits given calcium chloride dissolved in water and in the same doses by stomach tube. No abnormal reactions were seen in any of the animals. The LD 50 of calcium chloride given intramuscularly to 112 adult albino rats was found to be 25 mgm. per kilo body weight, as shown in Table I.

TABLE I.

The LD 50 of chlor-ethamine given intramuscularly to albino rats compared with that of four other urinary acidifiers.

DRUG	*NUMBER OF ANIMALS*	*LD 50 (Mgm. PER KILO)*
Ammonium Chloride	46	30
Calcium Chloride	112	25
Chlor-Ethamine	60	150
Mandelic Acid	50	300
Sodium Acid Phosphate	44	250

III. Chlor-Ethamine

Chlor-ethamine was dissolved in water and given by stomach tube to albino rats, guinea-pigs and rabbits in the same numbers and in the same manner as described for the previous two drugs. The dose of 0.25 grams per kilo body weight given by stomach tube produced a mild to moderate diarrhea in all three species of animals, and a similar effect was noted with the 1.0 gram per kilo dose. A peculiar effect noted was irritability produced by the 1.0 gram per kilo dose, especially in rabbits. While rabbits are ordinarily docile animals, when given this dose of chlor-ethamne most of them raced about their cages and actually fought with each other. The dose required to produce this irritability was higher than that necessary for acidification of urine and hence this reaction may be considered as a toxic reaction to an excessive dose of the drug. Nevertheless it was an interesting reaction and it was decided to investigate it further.

Irritability and increased activity produced by large doses of chlor-ethamine were investigated in albino rats placed in activity wheels so that any increased activity could be adjudicated by increased numbers of revolutions of the wheels and hence increased

physical work done. Healthy, adult albino rats were divided into four groups, a control group given no drug and three other groups given by stomach tube respectively 0.05, 0.25 and 1.0 grams per kilo body weight of chlor-ethamine. The animals were then placed in activity wheels and readings of the number of revolutions of the wheels were made at the end of 4 hours and 24 hours after giving the drug. The number of ergs. of work required to turn the activity wheels was determined and the work done by each rat was then expressed as millions of ergs per rat per hour. These data were averaged for each of four groups of 32 albino rats each and the means are given in Table II. The quantitative experiment in albino rats confirmed the qualitative observations in that it was found that a dose of 1 gram per kilo body weight of chlor-ethamine approximately doubled the physical activity of the animals and this effect lasted for at least 24 hours.

TABLE II.

The effect of increasing doses of chlor-ethamine upon the work done by albino rats in activity wheels.

DOSE OF DRUG (Grams per kilo)	*NUMBER OF ANIMALS*	*WORK DONE (Mean million ergs per hour)*	
		First 4 Hours	*Subsequent 20 Hours*
0	32	4.3	5.4
0.05	32	3.3	7.2
0.25	32	4.4	6.9
1.0	32	9.4	10.7

In further studies upon the toxicology of chlor-ethamine, it was found that the LD 50 of the drug given intramuscularly was 150 mgm. per kilo body weight. In this respect the drug was found to be one fifth to one sixth as lethal as ammonium chloride and calcium chloride.

Chlor-ethamine was also given to 20 dogs in the three doses used on the other animals, and given by stomach tube. The untoward reactions were similar in dogs to those seen in albino rats, guinea-pigs and rabbits with the addition that vomiting occurred in many animals given the 0.25 and over gram per kilo dose. Since albino rats, guinea-pigs and rabbits seldom if ever vomit, the vomiting produced in dogs may be taken as a further manifestation of the gastro-intestinal disturbance which produced diarrhea in

the other animals. To ascertain if the other acidifiers produced vomiting in dogs, the 0.25 gram per kilo dose of ammonium chloride, calcium chloride, mandelic acid and sodium acid phsphate was given in rotation to a group of 10 dogs. Vomiting did not occur in any animal given the other acidifiers. In conclusion, it would appear that chlor-ethamine is a relatively safe urinary acidifier, in the sense that the lethal dose is high, but that if used in doses much greater than the therapeutic dose it may give rise to such untoward reactions as a gastro-intestinal upset and excitement. These results were consistently obtained in animals and would likely occur in man but a definite conclusion as to the effects in man must await published clinical reports.

IV. Mandelic Acid

The toxicology of mandelic acid is well known and the experiments performed in the present investigation, for purposes of comparison with chlor-ethamine, will be described briefly. The drug was given by stomach tube in doses of from 0.05 to 1.0 grams per kilo body weight to some 100 albino rats, 100 guinea-pigs and 100 rabbits. The only untoward reaction was diarrhea which occurred with the largest dose of 1.0 grams per kilo body weight in almost all animals. The LD 50 of mandelic acid given intramuscularly to albino rats was found to be greater than 300 mgm. per kilo body weight, as shown in Table I.

V. Sodium Acid Phosphate

This drug has been used for many years and its toxicology is also well understood. For comparative purposes, it was given in the same range of doses to approximately 200 of the same three species of animals as mentioned before. Diarrhea occurred with doses of 0.25 grams per kilo body weight given by stomach tube to albino rats, guinea-pigs and rabbits, and also, of course, with larger doses. The LD 50 of sodium acid phosphate given intramuscularly to albino rats was found to be 250 mgm. per kilo, as shown in Table I.

SUMMARY

The untoward and toxic reactions of ethylenediamine dihydrochloride or chlor-ethamine were compared with those of four other urinary acidifiers, namely ammonium chloride, calcium chloride, mandelic acid and sodium acid phosphate. Over 2,000 animals were used, including albino rats, guinea-pigs, rabbits, cats and

dogs. It was found that the LD 50 of chlor-ethamine in albino rats was a good deal higher than that of ammonium chloride or calcium chloride and comparable to that of mandelic acid and sodium acid phosphate. In oral doses not much greater than that required for effective acidification of urine, chlor-ethamine produced vomiting in dogs and diarrhea in all animals, the latter also occurring with corresponding doses of mandelic acid and sodium acid phosphate. A reaction peculiar to chlor-ethamine when given in toxic doses was excitement, irritation and increased physical activity. It was concluded that, compared with other commonly used urinary acidifiers, the lethal dose of chlor-ethamine is relatively high but that if the drug is used in doses much greater than the therapeutic, undesired toxic reactions may occur in animals, some of these toxic reactions being similar to those seen following corresponding large doses of some other urinary acidifiers.

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SOME METABOLIC ASPECTS OF CALCIUM THERAPY*

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It was shown by Marriott and Howland[1] that retention of phosphoric acid was an important factor in the acidosis which develops in nephritis. It was also pointed out by the same authors that although the acidosis could be overcome by administration of alkali, such as sodium bicarbonate, little else was accomplished. There was no reduction of serum phosphate. They also suggested the possibility of calcium therapy as a means of eliminating phosphates by the bowel.

More recent work on calcium therapy has been especially concerned with the problem of edema in nephrosis, and it appears[2] that calcium chlorid or other salts, such as ammonium chlorid, which leave a residue of mineral acid, will, by bringing the hydrogen ion concentration of the tissues nearer to their iso-electric points, cause the elimination of sodium and edematous fluid. However, the questions of phosphate elimination and other effects of calcium on mineral metabolism have not been so extensively investigated.

This paper deals, first, with the effect of various alkalis and also of calcium chlorid on phosphate elimination in urine and feces, and, second, with the effect of calcium acetate on mineral metabolism.

PROTOCOLS

EXPERIMENT 1.—*Effect of alkalis and of calcium chlorid on elimination of phosphorus in urine and feces.*

F. M., an instructor in the department of medicine, incidentally having a typical case of nephrosis with edema, restricted his diet on twelve consecutive days to measured amounts of prepared foodstuffs. During the fifth and sixth days he took in addition, in divided doses, a total of 60 cc. of a 25 per cent calcium acetate solution; during the seventh and eighth days he took similarly 60 cc. of a 25 per cent magnesium acetate solution; during the ninth and tenth days, 60 cc. of a 25 per cent potassium acetate solution, and during the last two days, 60 cc. of a 25 per cent calcium chlorid solution. Twenty-four hour specimens of urine and feces were collected for each of the last ten days of the experiment. The daily urines were terminated with the passing of the morning urine on arising; a single bowel evacuation occurred each day about an hour later. Total phosphorus was determined on each of the daily specimens, but the results (Table 1) are grouped into five periods of two days each.[3]

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* From the department of medicine, St. Louis University School of Medicine.

1. Marriott, W. M., and Howland, J.: Phosphate Retention, Arch. Int. Med. **18**:708 (Nov.) 1916.

2. Keith, N. M.; Barrier, C. W., and Wheelan, M.: Treatment of Nephritis and Edema with Calcium, J. A. M. A. **83**:666 (Aug. 30) 1924.

3. In both experiments excreta on the first two days were discarded as preliminary.

EXPERIMENT 2.—*Effect of calcium on mineral metabolism.*

A measured diet of known inorganic composition was taken by the subject (A. P. B.) on ten consecutive days. On each of the last four days a measured amount of calcium acetate solution was taken by mouth. Urines and feces were collected to represent the twenty-four hour periods and determinations made on each of the various inorganic elements. Results of these are given in Table 2.

The diet consisted of:

25 Gm. of peanut butter
25 Gm. of condensed milk (sweetened)
20 Gm. of deviled ham
140 Gm. of bananas
147 Gm. of graham crackers
20 Gm. of jelly
5 Gm. of prepared coffee (George Washington)
20 Gm. of sucrose
2,000 Gm. of tap water

The food value of this diet is approximately protein, 28 Gm.; fat, 36 Gm., and carbohydrate, 204 Gm., with a heat value of about 1,250 calories. Although less than maintenance, this diet satisfied the appetite during the first two days and was adhered to throughout the experiment. The inorganic composition of the diet as determined from analysis was: chlorin, 0.950 Gm.; sulphur, 0.3136 Gm.; phosphorus, 0.450 Gm.; sodium, 1.707 Gm.; potassium, 0.772 Gm.; calcium, 0.2937 Gm., and magnesium, 0.2626 Gm.

Collection of twenty-four hour urines was terminated with the morning urine passed on arising. In this experiment the urines were preserved with chloroform. The daily feces, as in the previous experiment, also consisted of a single specimen passed within a half hour after breakfast. This breakfast consisted only of four graham crackers, 20 Gm. of jelly, and coffee with sugar but not milk, and contained a relatively small amount of the daily inorganic intake.

On the last four days calcium acetate was taken as follows: 10 cc. of a 20 per cent solution a half hour before the noon meal; 10 cc. again a half hour before the evening meal, and 5 cc. at bedtime. The amount of calcium taken daily in this way was approximately 1.14 Gm.

METHODS OF ANALYSIS

Urines were measured, diluted with distilled water to 1,500 cc., and mixed. Feces were passed through a sieve and diluted to 500 cc. and mixed. Measured or weighed samples of urine, feces and food were dried and charred in a platinum dish, the charred mass extracted with water, and the residue ignited. This ash was dissolved in dilute hydrochloric acid, added to the water extract, and the whole evaporated to a convenient volume, from which aliquots were taken for the determinations of sodium, potassium, calcium, magnesium and phosphorus. Chlorin and sulphur were determined on separate samples. Calcium, magnesium, potassium and phosphorus were determined essentially as reported in a previous paper;[4] however, in this study the samples taken for analysis contained approximately ten times as much of the inorganic element concerned as in the previous work which was done on blood plasma. Sodium was determined by the volumetric method of Kramer.[5]

4. Briggs, A. P.: J. Biol. Chem. **57**:351 (Sept.) 1923.
5. Kramer, B., and Gittleman, I.: J. Biol. Chem. **62**:353, 1924.

Chlorin was done by the wet ash method of Van Slyke,[6] and sulphur by the method of Benedict.[7]

It is commonly stated[8] that alkali causes more phosphates to be eliminated in the feces and less in the urine. In their study on the mineral metabolism of infants Shohl and Sato[9] found a slight change in that direction with the administration of sodium bicarbonate. The

TABLE 1.—*Excretion of Phosphorus During Two-Day Periods on Various Salts (Milligrams Phosphorus)*

	Period				
	Control	Calcium Acetate Solution	Magnesium Acetate Solution	Potassium Acetate Solution	Calcium Chlorid Solution
Urine phosphorus	976	494	935	905	690
Feces phosphorus	623	810	585	625	650
Total	1,599	1,304	1,510	1,530	1,340

TABLE 2.—*Minerals Excreted in Urine and Feces (Milligrams per Twenty-four Hours) in Experiment 2*

	Sodium	Potassium	Calcium	Magnesium	Chlorin	Phosphorus	Sulphur
Urine control period	2,990	870	241	149	1,320	484	520
	1,900	1,120	189	168	1,020	484	400
	1,900	1,220	147	152	1,020	455	394
	1,600	1,420	126	156	790	470	206
Total	8,390	4,630	703	625	4,150	1,893	1,520
Urine calcium acetate period	1,700	1,120	315	196	945	395	437
	2,300	1,280	294	173	826	234	350
	1,400	1,280	284	156	826	250	330
	1,300	1,280	294	180	915	220	384
Total	5,700	4,960	1,187	705	3,512	1,099	1,501
Feces control period	370	180	415	369	20	470	150
	130	100	161	68	16	117	111
	130	110	77	75	11	154	148
	230	180	168	78	11	263	63
Total	860	570	821	690	58	1,004	472
Feces calcium acetate period	200	100	455	123	12	360	82
	400	180	1,245	246	15	625	143
	400	123	989	151	12	360	76
	370	88	1,110	109	16	264	96
Total	1,370	491	3,790	629	55	1,609	397

results shown in Table 1 indicate that potassium or magnesium acetate have little influence on the partition of phosphorus, whereas either calcium acetate or calcium chlorid lead to the diversion of considerable amounts of phosphorus from the urine to the feces. This brings to mind the experiments of Fiske and Sokhey[10] in which the administra-

6. Van Slyke, D. D.: J. Biol. Chem. **58**:523 (Dec.) 1923.
7. Benedict, S. R.: J. Biol. Chem. **6**:363, 1909.
8. Shohl, A. T.: Physiological Rev. **3**:529 (Oct.) 1923.
9. Shohl, A. T., and Sato, A.: J. Biol. Chem. **58**:248 (Nov.) 1923.
10. Fiske, C. H., and Sokhey, S. S.: J. Biol. Chem. **63**:309 (March) 1925.

tion of small amounts of mineral acid by various pathways led to no change in phosphate excretion.

Values for the minerals excreted in the second experiment are given in Table 2. Balances for each are shown in Table 3. Since the diet in this experiment was below maintenance, the deficiency was probably met at the expense of skeletal muscle.[11] This probably accounts for the negative balances of potassium, magnesium, phosphorus and sulphur.

Sodium and chlorin were in approximate equilibrium during the greater part of the experiment.

TABLE 3.—*Balance of the Various Minerals in Experiment 2*

	Sodium	Potassium	Calcium	Magnesium	Chlorin	Phosphorus	Sulphur
Control Period							
Urine	8.330	4.630	703	623	4.150	1.893	1.520
Feces	880	570	821	690	58	1.004	472
Total	9.250	5.200	524	1.314	4.208	2.897	1.992
Intake	6.828	3.088	1.175	1.040	3.790	1.800	1.254
Balance	−2.422	−2.112	−349	−274	−418	−1.097	−738
Calcium Acetate Period							
Urine	5.700	4.960	1.187	705	3.512	1.090	1.501
Feces	1.370	491	3.700	629	55	1.609	397
Total	7.070	5.451	4.977	1.334	3.567	2.708	1.898
Intake	6.828	3.088	5.715	1.040	3.790	1.800	1.254
Balance	−242	−2.363	+738	−294	+223	−908	−644

TABLE 4.—*Daily Excretion of Ammonia in Experiment 2*

	Day							
	3	4	5	6	7*	8	9	10
Cubic centimeters of tenth normal ammonium hydroxid	190	173	173	173	121	104	104	86

* Calcium acetate was taken on the last four days.

With the ingestion of calcium acetate, the small negative calcium balance was changed to a positive one. Presumably depleted calcium reserves were being restored at this time, and the coincident decrease in the negative phosphorus balance indicates the storage of some calcium phosphate.

In spite of a definite decrease in total phosphorus elimination during the calcium acetate period, there is a conspicuous increase in the phosphorus excreted with the feces. In a clinical report to follow it will be shown that the elevated plasma phosphate in nephritis can be brought down to normal by the administration of calcium acetate.

The excretion of sodium potassium and magnesium is not appreciably influenced by calcium acetate, which, together with other recent

11. Gamble, J. L.; Ross, G. S., and Tisdall, F. F.: J. Biol. Chem. **57**:640 (Oct.) 1923.

work,[12] supports the theory that the increased sodium elimination following the administration of calcium chlorid is due, not to an antagonism between cations, but to the residue of hydrochloric acid from excretion of calcium by the bowel.

As previously stated, it seems probable that the positive balance for calcium with the administration of calcium acetate is due to the storage of calcium carbonate and phosphate in solid tissues. Certainly there appears to be nothing brought out in this study to suggest that administration of calcium acetate to a nephritic patient would lead to retention of alkali with edema.

Whether various other salts are excreted in the urine passively or not, the ammonia appears to be synthesized by the kidney[13] as an important agent in neutrality regulation and base conservation;[11] hence the increase of ammonia found in the urine following the administration of calcium chlorid which leaves a residue of hydrochloric acid. On the other hand, the administration of an organic salt of calcium, such as calcium acetate, should tend to decrease the formation of ammonia, since the organic radicle would be oxidized and eliminated by the lungs and the calcium component would divert a part of the phosphoric acid from the urine to the feces. Ammonia was determined in the urines in Experiment 2. The results shown in Table 4 support the contention that a certain amount of the phosphoric acid load has been taken by the intestine from the kidney.

SUMMARY

Calcium acetate given by mouth causes the diversion of a considerable amount of the excreted phosphoric acid from the urine to the feces.

The relief from this phosphoric acid load necessitates the synthesis of a smaller amount of ammonia by the kidney.

The administration of calcium acetate affords a means of correcting the phosphoric acid retention in nephritis free from the disadvantages of alkali retention and resulting edema which is observed with sodium bicarbonate therapy.

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12. Keith (Footnote 2). Atchley, D. W.; Loeb, R. F., and Benedict, E. M.: Physicochemical Studies of Calcium Chlorid Diuresis, J. A. M. A. **80**:1643 (June 2) 1923.

13. Nash, T. P., and Benedict, S. R.: J. Biol. Chem. **48**:463 (Oct.) 1921.

Vopr. tuberk. 7:1258-1263 (1928)

Treatment of chlorcalcinoma with sodium sulfate [1]

by

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During the introduction of intravenous Ca-therapy an extremely unpleasant complication was sometimes observed- chlorcalcinoma. Recently such an instance was found when solutions of $CaCl_2$ were subcutaneously injected into cellular tissue. A long succession of authors has discussed this complication: M. G. Serdyukov, S. A. Gol'dberg, V. L. Eynis, F. P. Chekalov, N. M. Kuznetsov, Kozlova and Lyakhovich, Dietel, and others. I personally have observed several tumors (chlorcalcinoma) in cellular tissue subcutaneously injected with $CaCl_2$ of which two tumors were subject to gangrene. Clinical chlorcalcinoma produced sharp pains in the region of the injection, sometimes inflammation in the forearm, hyperemia, and an irruption of swellings. Furthermore, in relation to the quantity and concentration of the $CaCl_2$ solution injected subcutaneously, and the resistance of the tissue, tumors under the effect of compresses and massages were reabsorbed within 2-3 days during concentration of the swelling, forming a demarcation ridge of a round shape, inside of which tissue became gangrenous. The gangrenous part within 2-3 weeks tore away, after which the defect healed with a scar. In general, the duration of the course of chlorcalcinoma is 4-5 weeks, during which the sick person experiences pain, particularly sharp during the first week.

Tyczka and Sznajderman write about three occurences of the disease n. mediani on account of injection of a solution of $CaCl_2$ paravenous in extent. The inflammatory process of the above-mentioned extent from cellular tissue passing over the nerve sheath is called perineuritis circumscr. Clinically in all cases there were vasoimpelled causal pains and trophic disruptions. Duration of these symptoms is significant-- up to six months. Tamarin writes of an analogous case.

In this manner we see that chlorcalcinoma appears not only irrespective of the complications for the sick, but may even be found on the arms of the experimenting doctors; if connected with the data of two cases of extensive use of Ca-therapy, not only during hemorrhage but also during some forms of tubercular lungs, the conclusion may be drawn that the question about treatment of chlorcalcinoma has great practical significance.

On the question of the treatment of chlorcalcinoma hints can be found in the literature which, regrettably, do not give the answer as it is necessary to treat the above-mentioned complication. V. A.

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[1] Demonstrated (from experiments with animals) in the Clinical Conferences of the Tuberculosis Clinic of the Dnepropetrovsk Medical Institute, August 8, 1928.

Chukanov recommends energetic massage of the ulnar fold during the subcutaneous injection of weak solutions of $CaCl_2$ (1-2% soln.) and the injection of a physiological solution in the place of chlorcalcinoma with subsequent massage during the subcutaneous injection of a concentrated solution of $CaCl_2$ (10% soln.). An analogous method of treatment is also recommended by Dietel. Such treatment, in the opinion of the author, excludes the appearance of lengthy, painful tumors and necroses. In the following cases, an injected physiological solution relieves the concentration of the $CaCl_2$ solution. If, for example, 1 cc 10% soln. of $CaCl_2$ was injected subcutaneously and we then injected 10 cc physiological solution such as that recommended by Dietel in the region of the injection, then in the results the concentration of the $CaCl_2$ solution is less than 1%, that is we obtain in subcutaneous cellular tissue a solution of such concentration, during which necrosis is possible (a case, cited by M. G. Serdyukov). Therefore, the data on this method are neccessarily calculated to be inconclusive.

N. M. Kuznetsov suggests that during the subcutaneous injection of solutions of $CaCl_2$ the surrounding tissue and tumorous places be injected with a 25% soln. of sodium sulfate subcutaneously, first filtered when possible. From the reactions of the $CaCl_2$ and sodium sulfate solutions ($CaCl_2 + Na_2SO_4 \rightarrow 2NaCl + CaSO_4$) giving common table salt which is easily absorbed, and calcium sulfate which does not possess a cauterizing effect. Out of the absence of clinical observations, Kuznetsov's article presents a purely theoretical interest and is of no practical value.

Kozlova and Lyakhovich use a practical statement of Kuznetsov's method in 20 cases with excellent results. In the transition to an analysis of their work, it is necessary to note that they factually did not substantiate the specificity of the reaction of sodium sulfate in relation to the treatment of chlorcalcinoma. The point is that during such types of complications, it is difficult to take into consideration the amount of subcutaneously injected $CaCl_2$ solution and still more difficult to predict what will be the course of the chlorcalcinoma- if it will be tumors or necrosis. For some of the subjects necrosis came from a small quantity of $CaCl_2$ solution; at the same time, other subjects received only tumors from large quantities, which within several days were reabsorbed. It is very possible that the 20 patients of the quoted authors without injections of sodium sulfate would have been subject to only the inflammatory tumors, which within several days would have been reabsorbed.

For the two above-mentioned works, a third part is necessary- experimental work, which would document the specificity of the sodium sulfate solution during the treatment of chlorcalcinoma. It is necessary to study what quantities of $CaCl_2$ solution can cause necrosis and either whether or not the $CaCl_2$ solution burns quickly, or the possible safe length of contact with the tissues. The last two questions are of great practical significance, first in their relationship to that question of in which cases it is necessary to inject a solution of sodium sulfate, and second, whether or not it is significant to aspirate the subcutaneously injected solution of $CaCl_2$,

and within how long after the subcutaneous injection of the $CaCl_2$ solution can the specific reaction of the injection of the sodium sulfate solution still be shown. The three above questions interested me; therefore I worked upon them experimentally with animals.

The objects of my observations were rabbits and dogs. I treated them with a 10% $CaCl_2$ soln. and a 25% sodium sulfate soln. The operating area was shaved thoroughly and washed with water and alcohol. The injections were placed under the skin with sterile needles and hypodermic syringes. Each of the first 7 animals received 2 injections: from one side of the thoracic cavity, only $CaCl_2$ (control), and from the other side of the thoracic cavity, $CaCl_2$ with an immediately following injection of sodium sulfate (treatment). In these 7 animals I studied the specificity of the sodium sulfate solution and the relationship to the treatment of chlorcalcinoma, and during which precise quantities of $CaCl_2$ solution injected subcutaneously necrosis originates. The last two animals I gave 4 injection, one from each side (a solution of $CaCl_2$, and then after 5-10-15-30 minutes injected sodium sulfate). From these 2 animals I learned when burns are caused, and within what time after the subcutaneous injection of the $CaCl_2$ solution it is still significant to inject the sodium sulfate solution. The results obtained are apparent from the accompanying table.

Leading up to the results of my observations (see accompanying table), I calculated the necessary experimental animals divided into 2 groups: 1) the first 5 rabbits and dogs, and 2) the eighth and ninth rabbits. If we trace the record of the first group of animals and turn our attention to the first side injected, it is necessary to note that $CaCl_2$ coming in contact with the tissues of the animals possesses properties causing necrosis, as a succession of authors turned their attention to that, and a majority of us were obliged to observe during the execution of Ca-therapy. Analyzing further data of the righthand side, we see that necrosis took possession of sections dissimilar in size and depth. In the first rabbit necrosis was superficial, of localized dimensions (0.25 cc $CaCl_2$ solution was injected); in the second rabbit, necrosis was also superficial, of the size of a grain of wheat (exactly the same quantity of $CaCl_2$ solution injected); in the third rabbit, necrosis was superficial, of the size of a ten-kopek coin (0.5 cc $CaCl_2$ solution was injected); in the fourth and fifth rabbits, necrosis was of the size of a 20-kopek coin, and deeper (0.75 cc $CaCl_2$ solution was injected); but in the dogs, necrosis was of the size of a silver ruble with implication of the skin, subcutaneous cellular tissue to the muscular layer (injected with 1.0 and 1.5 cc $CaCl_2$ solution). In this manner we see that with an increase in the quantity of $CaCl_2$ solution injected subcutaneously, we obtain greater necrosis, in terms of magnitude and depth. In general, the factual conclusions can be formulated: the area and depth of necrosis located in the first case is in direct relation to the quantity of $CaCl_2$ solution injected subcutaneously. I turn attention to the first 2 rabbits (1 and 2), which were injected subcutaneously with 0.25 cc $CaCl_2$ solution- and in the results there was also necrosis, from which we can draw the conclusion that the above mentioned quantity of $CaCl_2$ solution (0.25 cc or 5-6 drops) is already sufficient to cause necrosis. Tracing the left-hand side of

TABLE I.

No.	Name of Animal	Weight	Quantity of Subcutaneous Injection		Results	
			Right	Left	Right	Left
1	Rabbit	1.100g	0.25 soln. $CaCl_2$	0.25 soln. $CaCl_2$ + 0.5 soln. Na_2SO_4	Surface necrosis of localized size	No deviation from norm
2	Rabbit	950g	0.25 soln. $CaCl_2$	0.25 soln. $CaCl_2$ + 0.5 soln. Na_2SO_4	Necrosis of the size of a grain of wheat	"
3	Rabbit	1.580g	0.5 soln. $CaCl_2$	0.5 soln. $CaCl_2$ + 1.0 soln. Na_2SO_4	Necrosis the size of a 10 kopeck piece	"
4	Rabbit	1.620g	0.75 soln. $CaCl_2$	0.75 soln. $CaCl_2$ + 2.0 soln. Na_2SO_4	Necrosis the size of a 20 kopeck piece	"
5	Rabbit	1.775g	0.75 soln. $CaCl_2$	0.75 soln. $CaCl_2$ + 2.0 Soln. Na_2SO_4	"	"
6	Dog[1]	2.000g	1.0 soln. $CaCl_2$	1.0 soln. $CaCl_2$ + 2.0 soln. Na_2SO_4	Necrosis the size of a silver ruble with implication of subcutaneous tissue	Superficial necrosis the size of a ½ kopeck piece
7	Dog	2.800g	1.5 soln. $CaCl_2$	1.5 soln. $CaCl_2$ + 3.0 soln. Na_2SO_4	"	Necrosis the size of a 50 kopeck coin
8	Rabbit	1.600g	0.75 soln. $CaCl_2$+2.0 soln. Na_2SO_4 (after 5 min)	0.75 soln. $CaCl_2$ + 2.0 soln. Na_2SO_4 (After 10 min)	Surface necrosis the size of a millet grain	Necrosis the size of a bean, deeper
9	Rabbit	1.800g	0.75 soln. $CaCl_2$+2.0 soln. Na_2SO_4 (after 15 min)	0.75 soln. $CaCl_2$ + 2.0 soln. Na_2SO_4 (after 30 min)	Necrosis the size of a 10 kopeck piece	Necrosis the size of a 20 kopeck piece

[1] Note: Because a dose higher than 1.5 10% soln. of $CaCl_2$ appears to be a toxic dose for rabbits (D. M. Lavrov), I continued my study of the first

the above-mentioned 7 animals, we may conclude that sodium sulfate in the tissues of animals (at body t°) is sufficient to enter into a chemical reaction with $CaCl_2$ and prevent the formation of lengthy painful tumors and necroses. On the right side, the complete effect obtained only for the first five animals (rabbits), since in the dogs, as is apparent from the table, necroses formed on both sides, but nevertheless in the last ones the sodium sulfate showed its specific effect. In the first dog, on the left, tumors were less than on the right, and necrosis was more superficial and occupied an area of the size of a ½-kopeck piece, and healed with a scar more quickly than on the right, where the necrosis occupied an area greater than the size of a silver ruble. We observed analogous figures for the second dog, on which the left side necrosis occupied an area exactly half the area of the necrosis of the right side. The incomplete therapeutic effect in dogs is linked to the fact that the region of chlorcalcinoma was not sufficiently injected with sodium sulfate for the reason that after the first injection the dogs were very restless, and that therefore I had to hurriedly finish the operation. The dogs were injected in the area of chlorcalcinoma with a two-fold quantity of sodium sulfate (but not a 3-4 multiple, as was planned), which was not uniformly divided among the area of chlorcalcinoma and the surrounding regions of tissue, as in the case of the rabbits. A solution of sodium sulfate was simply injected in the central part of the chlorcalcinoma as per the above principle. Owing to an incomplete effect, obtained for dogs, we found it possible to turn our attention to that which is important- the practical significance of the question on the relation of the techniques of sodium sulfate injection. The fact is that in agreement with the chemical expression "uniformity of composition", for conversion, say 1 cc of a 10% $CaCl_2$ solution in NaCl by means of a 25% soln. Na_2SO_4, necessary to make use of approximately 0.5 of the remainder of the solution, because that is in in vitro but in vivo a much greater quantity is necessary according to the idea of the principle. In consideration of the indifference of the sodium sulfate solution for the tissue, in order to obtain the complete therapeutic effect, it is necessary to inject 3-4 multiple quantities of sodium sulfate, injecting the area of chlorcalcinoma evenly.

Passing to the calculation of the results, received in two groups of experimental animals, it is necessary to fix attention on the size of the necroses in relation to the intervals of time, within which sodium sulfate was injected. The eighth rabbit's right side was injected with sodium sulfate within 5 minutes after the injection of $CaCl_2$, producing necrosis the size of a grain of millet. The same rabbit's left side was injected with sodium sulfate within 10 minutes after the injection of $CaCl_2$ producing necrosis the size of a bean. The ninth rabbit's right side was injected with sodium sulfate within 15 minutes, producing necrosis the size of a 50 kopeck piece; the same rabbit's left side was injected with sodium sulfate within 30 minutes producing necrosis the size of a 20 kopeck piece. The last results stir interest in the fact that they substantiate the development of necrosis of the tissues not instantaneously, but greater or less continous touching of the $CaCl_2$ solution with the tissues, from which we conclude to produce aspiration of the subcutaneously injected $CaCl_2$ solution and then, as soon as possible, to inject sodium sulfate.

Turn your attention to the ninth rabbit whose left side was injected with sodium sulfate within 30 minutes after the subcutaneous injection of $CaCl_2$; necrosis was produced in this rabbit of exactly the same size as the necrosis produced in 4 or 5 rabbits on the right side, that is, on the untreated side; that is why I gave the injection because after 30 minutes the $CaCl_2$ solution caused complete destruction of the tissue, because after the above-mentioned interval of time (30 minutes) after the subcutaneous injection of the $CaCl_2$ solution there was already no point in injecting sodium sulfate. By injecting the same sodium sulfate not immediately but within the first 15 minutes after the subcutaneous injection of the $CaCl_2$ solution, it is possible to obtain the effect if not completely then partially as is apparent from the first three manipulations performed on the last two animals.

Conclusions

1. An insignificant amount, such as 0.25 cc (5-6 drops) of 10% soln. $CaCl_2$ is already sufficient to cause necrosis of tissue.

2. The area and depth of necrosis of the tissues is found in the simple relation to the quantity of the given $CaCl_2$ solution injected subcutaneously.

3. Sodium sulfate possesses qualtities at body t^o entering into chemical combination with $CaCl_2$ and appearing as the specific medium during the treatment of chlorcalcinoma, preventing the appearance of lengthy, painful necrosis and tumors.

4. For the obtaining of the complete therapeutic effect, it is necessary to inject sodium sulfate in 3-4 multiple quantities (in relation to the cases) according to the relation to the subcutaneously injected $CaCl_2$ solution.

5. Solutions of $CaCl_2$ cause burning not instantaneously but from greater or lesser contact with the tissues.

6. Aspiration of the $CaCl_2$ solution injected subcutaneously seems to be desirable.

7. The area and depth of necrosis is found in the inverse relation to the time of application of sodium sulfate.

8. Application of sodium sulfate during a half-hour after the subcutaneous injection of $CaCl_2$ solution does not give any effect.

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ALTERATION OF CALCIUM MEDIATED EFFECTS IN MICE BY RESERPINE

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Reserpine; calcium; tetany; citrate

ABSTRACT

The effects of reserpine administration on calcium homeostasis were tested in mice. It was found that reserpine pretreatment increased the lethal effects of $CaCl_2$. Fifty percent of control mice died when injected with 600 mg/kg $CaCl_2$; those pretreated with either 0.1 or 0.25 mg/kg reserpine were more sensitive and 50% died when they received 400 mg/kg $CaCl_2$. Reserpine administration resulted in a significant ($P < 0.02$) decrease in the time between the $CaCl_2$ injection and death. Reserpine (1.25 or 2.5 mg/kg) potentiated the ability of sodium citrate to cause tetany. The required dose for controls was 0.74 mg/kg, while after reserpine it was 0.5 mg/kg. These results suggest that reserpine does alter in some manner calcium homeostasis. Based on this and previous work, it is suggested that this phenomenon may be the basis for reserpine's hypotensive and tranquilizing properties.

INTRODUCTION

During the course of several studies concerning the effects of reserpine (Carrier and Holland, 1965; Carrier and Shibata, 1967; Carrier, et al, 1967) it was observed that some animals pretreated with reserpine were hypersensitive to external stimuli. These animals appeared to be suffering from the latent form of tetany. This was evidenced by the fact that muscle twitching could be seen when tactile stimuli were applied to the skin in areas of the ears or limbs.

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Tetany results from a lowering of the ionized calcium of the extracellular spaces, including the blood serum (MacCallum and Voegtlin, 1909). This low calcium results in increased sensitivity of nervous synapses and muscle end plates (Kuffler, 1944; Kuffler, 1945; Brink, 1954). These changes are probably the result of an increased permeability of the cell membranes to sodium associated with an increase in threshold potential, a decrease in resting membrane potential, and an increase in excitability of nerve and muscle cells (Norcenti, 1968).

Carrier and Shibata (1967) showed that reserpine lowers the calcium content of blood vessel tissue; however, nothing was said of the serum or extracellular calcium. Wolfe (1968) has observed an increase in the calcium content of the submaxillary gland after reserpine. These observations suggest that there is a profound alteration in calcium homeostasis following the administration of reserpine. It is thus possible that reserpine causes a generalized increase in nerve and muscle excitability by lowering the calcium content of the extracellular fluid bathing such tissues. The increase in sensitivity of the vascular system is well known (Trendelenburg, 1963) and recently it has been reported that heart muscle is supersensitive to the calcium ion after reserpine (Westfall and Fleming, 1968).

In view of the foregoing it was felt that an investigation into the various aspects of calcium homeostasis as affected by reserpine would prove both fruitful and beneficial due to the widespread use of reserpine today in hypertension. Initially a study was done to confirm our observations that reserpine pretreated animals had latent tetany. The subject of this report is the result of this study wherein the effects of sodium citrate (known to cause tetany) and of calcium chloride were used to assess the degree of involvement of reserpine in calcium mediated phenomenon in vivo.

MATERIALS AND METHODS

In these studies a total of 474 albino mice of both sexes weighing either 20 or 30 g each were used. They were divided into several experimental and control groups as follows:

The lethal effects of $CaCl_2$

These experiments were performed in two ways. One consisted of injecting $CaCl_2$ into 30 g mice at various concentrations and observing how many survived after 3 hours. Control mice were injected as follows: 20 received 400, 20 received 500, 25 received 600, 25 received 650, and 25 received 700 mg/kg $CaCl_2$. Fifty-three mice were given 0.1 mg/kg reserpine 4 hours before administration of the $CaCl_2$. These two experimental groups were then divided into approximately equal portions, one half of each receiving 400 mg/kg $CaCl_2$ and the others 500 mg/kg $CaCl_2$. As the

higher concentration used here was almost 100% lethal no other concentrations were tested. The second part of these experiments consisted of administering to 20 g control mice 650 (25 mice) and 1,000 (27 mice) mg/kg $CaCl_2$ and observing the time required for death to occur up to 2 hours (after which time death rates were too variable) and the number of survivors at this time. Twenty-six mice were given 1.25 mg/kg reserpine and 25 were given 2.5 mg/kg reserpine 24 hours before they were subdivided into 4 equal groups and given the same quantities of $CaCl_2$ as the control mice.

The effect of reserpine on sodium citrate induced tetany

Mice were divided into several control groups of 45 mice each. Each control group received a measured amount of sodium citrate and the development of tetany was observed. As there was no sign of tetany at doses less than 0.50 mg/kg, this dose and that just producing tetany in 100% of the animals were the only two concentrations recorded. Seventy-two mice were given reserpine 24 hours before sodium citrate, 36 received 1.25 mg/kg and 36 received 2.5 mg/kg (two of these latter mice died before completion of the experiment). These two groups were then subdivided into 4 equal groups. Several other mice (not recorded) were tested after reserpine to establish the dose of sodium citrate which would produce tetany in 100% of the mice. Only this group and the dose which just failed to produce any signs of tetany were recorded for presentation.

All $CaCl_2$ solutions used were made up in distilled water at such concentrations to allow injections to be 0.1 ml in total volume. All solutions were administered intraperitoneally. Sodium citrate was prepared in a 10% solution by weight in distilled water. In each control group several mice (>5) were given intraperitoneal injections of 0.1 or 0.2 ml of saline with no apparent effect on the results.

RESULTS

Effects of reserpine

All mice receiving reserpine were heavily sedated from 1 to 10 hours after which they all appeared normal in activity.

The lethal effects of $CaCl_2$

The results of these experiments are summarized in Tables 1 and 2. Pretreatment of the mice with reserpine increased their sensitivity to the lethal effects of $CaCl_2$. In Table 1 it can be seen that 400 mg/kg $CaCl_2$ was ineffective during the first four hours and 600 killed approximately 50% of the mice. After either 0.1 or 0.25 mg/kg reserpine, 400 mg/kg $CaCl_2$ killed half of the mice tested, 500 being lethal to almost 100%. There was no apparent dose dependence on reserpine. When time to death was recorded for the first 2 hours after the calcium salt, there was a significant decrease in time from a mean of 94 ± 14 minutes at 650 mg/kg to 37 ± 10 minutes at 1,000 mg/kg ($P < 0.05$). After reserpine there were further decreases in time indi-

TABLE 1

Increased toxicity of $CaCl_2$ after reserpine in 30g mice

Group	Calcium chloride (mg/kg ip)	Survival (after 4 hours)
Control	400	20/20*
Control	500	16/20
Control	600	12/25
Control	650	10/25
Control	700	4/25
Reserpine (0.1 mg/kg, 4 hr)†	400	12/26
Reserpine (0.1 mg/kg, 4 hr)	500	2/27
Reserpine (0.25 mg/kg, 4 hr)	400	11/21
	500	0/20

* Ratio of survivors to total group.
† Reserpine administered intraperitoneally at this concentration and time before experiment.

cating reserpine increased the animals sensitivity to the $CaCl_2$. All decreases were significant ($P < 0.02$). Again there appeared to be no dependence on the dose of reserpine used. The older mice (30g) seemed to be more sensitive than the smaller animals (data in Table 1 versus that in Table 2). However, this difference could also have resulted from the fact that the larger mice were purchased at a different time and were kept in the laboratory about 3 months before they were tested. Thus, laboratory conditions just prior to the two series of experiments were not identical.

While performing the experiments outlined in Table 2 it was observed that at the higher dose of $CaCl_2$ (1,000 mg/kg), both control and reserpine-treated mice died in a different fashion than those re

TABLE 2

Effect of reserpine on the time until death of 20g mice after intraperitoneal injections of $CaCl_2$

Group	Calcium chloride (mg/kg, ip)	Time until death (up to 2 hrs)	Survival (after 2 hrs
Control	650	94 ± 14 min.*	12/25¶
Control	1,000	37 ± 10 min.†	13/27
Reserpine (1.25 mg/kg, 24 hr)§	650	43 ± 17 min.‡	5/12
Reserpine (1.25 mg/kg, 24 hr)	1,000	10 ± 20 min.	3/14
Reserpine (2.5 mg/kg, 24 hr)	650	56 ± 20 min.	4/12
Reserpine (2.5 mg/kg, 24 hr)	1,000	13 ± 1 min.	3/13

* Mean value with standard error of the mean.
† Times at 1,000 mg/kg significantly different from times at 650 mg/kg for all groups ($P<0.05$).
‡ Times of experimental groups significantly different from corresponding control ($P<0.02$).
§ Reserpine administered intraperitoneally at this concentration and time before experiment.
¶ Ratio of survivors to total in group.

TABLE 3

Effect of reserpine on the sodium citrate induced tetany in 20g mice

Group	Sodium citrate (mg/kg, ip)	Animals showing tetany	Survival (after 24 hrs)
Control	0.75	46/46*	40/46
Control	0.50	0/45	45/45
Reserpine (1.25 mg/kg, 24 hr)†	0.50	18/18	16/18
Reserpine (1.25 mg/kg, 24 hr)	0.40	0/18	18/18
Reserpine (2.5 mg/kg, 24 hr)	0.50	17/18‡	16/17
Reserpine (2.5 mg/kg, 24 hr)	0.40	0/17	17/17

* Ratio of survivors to total group.
† Reserpine given at this concentration and time before the experiment.
‡ One animal in this group died immediately.

ceiving the lower dose (650 mg/kg). The mice receiving the lower dose became sedated in appearance, ceased to move around and died quietly of some peripheral failure. All lost their righting ability just before they died. When the higher dose was given, those mice that died in the first 2 hours, especially, died in an excited state. After injection they would move around more rapidly than normally observed. All of a sudden they would rise to their hind legs and fall over dead after a second or so.

The effect of reserpine on sodium citrate tetany

As can be seen in Table 3, after reserpine either 1.25 or 2.5 mg/kg 24 hours before the experiment, it required less sodium citrate to induce tetany in 100% of the mice tested. The dose needed for 100% of the mice to show signs of tetany was 0.74 mg/kg before reserpine administration, but this decreased to 0.5 mg/kg after reserpine.

DISCUSSION

The universal use, and as a consequence, abuse, of drugs today demands that the clinician as well as the basic investigator have an understanding of those effects of drugs which are termed side-effects, especially since, in many drugs, side-effects can more than offset the therapeutic benefit derived from the drug. One such group of agents, which, if not administered with a full view to all of their actions, could prove more detrimental than beneficial, are the *Rauwolfia* alkaloids (Moser and Goldman, 1967; Zaimis, 1966; Lee, 1966; Page, 1966; Thienes and Haley, 1964).

The *Rauwolfia* alkaloids, one of the more important of which is reserpine, in addition to their well-known clinical manifestations, of

which psychic depression is probably the most dramatic (Moser and Goldman, 1967), have many lesser known effects. These include potentially beneficial as well as detrimental actions. Reserpine has been shown to cause histological changes (Zaimis, 1966). However, on the other hand, reserpine has been shown to inhibit the atherosclerotic process (Deming, 1966; Carrier, et al, 1968; Whittington-Coleman, et al. 1968) and to be beneficial in the treatment of dissecting aneurysms (Wheat, et al, 1965; Simpson, et al, 1968).

Another of the side-effects of the *Rauwolfia* alkaloids, especially reserpine, is a Parkinsonian-like rigidity. Reportedly this is only rarely seen (Moser and Goldman, 1967) though it has been mentioned by other authors (Page, 1966; Thienes and Haley, 1964). This phenomenon may have some basis in the results of the present study.

In the present study, reserpine was shown to potentiate the ability of sodium citrate to cause tetany. It also altered the effects of intraperitoneally administered $CaCl_2$. It increased the sensitivity of the mice to the lethal effects of $CaCl_2$ and also decreased the time until death of mice dying during the first 2 hours after the calcium salt was given. In addition to these changes, it was observed that the two groups of mice which received the highest calcium dose (1,000 mg/kg, Table 2) died in a manner different from all other groups. At the higher $CaCl_2$ dose, some reflex activity, possibly mediated by the central nervous system, caused the mice to die in an apparent excited state. At the lower calcium dose, death seemed to be the result of a generalized depression.

Carrier and Shibata (1967) showed that reserpine caused a loss of vascular tissue calcium. If this loss also occurs in other tissues, such as skeletal muscle, the effects of reserpine to potentiate citrate tetany can be understood. Both agents probably act synergistically.

In the case of the calcium chloride effects, some other mode of action must be sought. The lowering of the extracellular calcium is known to result in an increase in nerve and muscle excitability (Brink, 1954). At the low dose of reserpine the calcium losses from tissue and extracellular space apparently had little influence on the actions of the calcium salt. Reserpine is known to potentiate other cardiac depressants such as the barbiturates (Richards and Taylor, 1956). When the reserpine dose administered was increased, there was an increase in the length of the sedation but no change in sensitivity to the $CaCl_2$.

At a high dose of calcium chloride (1,000 mg/kg) and high dose of reserpine, a new phenomenon appeared, one in which excitability, rather than depression, was observed. Recently, it has been shown that reserpine can sensitize the heart (Westfall and Fleming, 1968) and

blood vessels (Pegram and Carrier, 1968) to calcium. That is, after a calcium loss of the tissue mediated by reserpine occurs, the tissue becomes more reactive to stimuli at lower calcium doses. Thus, if calcium losses also occur in the nervous tissue after reserpine, one would expect to see a generalized state of nervous excitement as seen in the present experiments. Schuman (1966) has reported a loss of calcium concomitantly with a loss of norepinephrine from adrenal medullary granules after reserpine treatment. A similar loss of norepinephrine occurs from peripheral nerve ending granules. It may be assumed that the calcium loss doses also occur here, At the high doses of reserpine and calcium the central nervous system, as well as the peripheral, was probably affected by the two drugs, while in the lower-dose experiments, activity, at least of the calcium, was only peripheral. However, as there was no increase in death rate or number of deaths, we must assume death was the result of a mechanism common to both situations, probably cardiac arrest.

From these considerations, the most important fact to emerge is that reserpine certainly alters calcium homeostasis. Such alterations probably reflect reserpine's ability to affect membrane function. It is suggested that these effects, rather than being side-effects, are the underlying mechanisms mediating reserpine's hypotensive and tranquilizing properties.

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TOXICOLOGY AND APPLIED PHARMACOLOGY 4, 492-503 (1962)

Comparison of Parenterally Administered Calcium Kinate Gluconate with Calcium Gluconate and Calcium Chloride

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The homeostatic nature of the human body requires that there be a continuous intake of calcium. A sufficient amount of calcium is obtained from a normal diet, and the dietary sources of calcium need to be supplemented by extra dietary administration only in abnormal circumstances. The use of calcium therapy in clinical conditions is not always associated with actual deficiency states. Evidence has been presented in the past that parenterally administered calcium aids in the control of edema (Morris and Rogen, 1940; Lecomte, 1950), allergy (Rudolph, 1936; Sullivan, 1941), urticaria (Chambers and Bernton, 1944; Parker, 1950), lead poisoning (Shields and Mitchell, 1941), and intestinal, renal, and biliary colic (Bauer *et al.*, 1931).

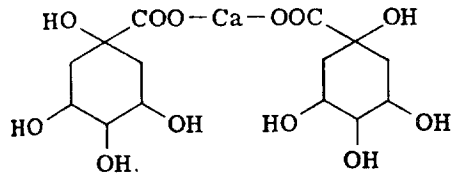
Excessively large quantities of calcium salts have been cited as possessing an anticoagulant effect (Loomis and Seegars, 1944). The addition of small quantities of calcium to normal blood is generally not considered to have a significant effect.

The studies reported here are concerned with the experimental evaluation of the toxicity of calcium kinate gluconate in comparison with that of calcium gluconate and calcium chloride.

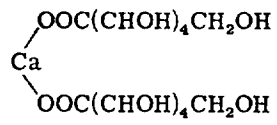
MATERIALS AND METHODS

Calcium kinate gluconate (CKG) prepared by Albro and Buck (195[illegible]) is a highly soluble complex of calcium kinate and calcium gluconate in an approximate molar ratio of 2:1. The complex yields a higher concentration of calcium than is obtainable from saturated solutions of either

component alone. The calcium kinate gluconate solution used in this experiment was water clear and contained 50 mg of elemental calcium per milliliter.



Calcium kinate



Calcium gluconate

Calcium gluconate (CG), 10% solution, USP (E. R. Squibb and Sons and the Upjohn Co.), the generally accepted parenteral formulation of calcium, and calcium chloride ($CaCl_2$) were used as standards for comparison in most instances during these studies. The various experimental procedures are described in detail in this section.

Acute Intravenous Toxicity

Mice in groups of ten were injected intravenously with calcium kinate gluconate (CKG), calcium gluconate (CG), or calcium chloride ($CaCl_2$). The LD_{50} was calculated according to the logarithm dose-probit method of Miller and Tainter (1944).

Acute Intravenous Tolerance

Two groups of six unanesthetized dogs were injected intravenously with calcium kinate gluconate or calcium gluconate. One week later a crossover experiment was done in which each dog was injected with the alternate solution. The preparations were injected into the saphenous vein at a rate of 3 mg Ca per kilogram per minute. This rate of injection was approximately twice that which is recommended for therapeutic purposes. Maximum calcium tolerance was determined at the moment vomiting occurred and the total milligrams of calcium of the injected solution per kilogram body weight was noted.

Antagonism to Magnesium Toxicity[1]

The comparative availability of calcium ion in calcium kinate gluconate, calcium gluconate, and calcium chloride preparations was determined by the antagonism of calcium ion to the toxicity of magnesium ion. Solutions

[1] The authors wish to thank Dr. James O. Hoppe for this study.

of the three calcium preparations with a constant 5:6 ratio of calcium ions to magnesium ions (as magnesium sulfate) were injected intravenously into mice. The injected quantity of magnesium sulfate was sufficient to kill ten out of ten mice. Survival of the mice injected with an antidotal dose of calcium was used as a measure of the availability of calcium ions.

Cardiovascular Effects[2]

The effects of calcium kinate gluconate on the heart and on the blood pressure were evaluated in dogs in comparison with calcium gluconate. Four dogs were anesthetized intravenously with approximately 15 mg/kg of sodium thiopental followed by 250 g/kg of sodium barbital. Femoral blood pressure was recorded by means of a P23A pressure transducer (Statham) supplying a Grass balance demodulator unit and an encephalograph. Lead I and II electrocardiograms were obtained simultaneously with the above recordings. CKG or CG were injected intravenously in doses ranging from 1.35 mg Ca to 7.5 mg Ca per kilogram at a rate of 7.5 mg Ca per kilogram per minute. The electrocardiograms and blood pressures were recorded prior to the injections and at 10, 20, 30, 60, 90, 120, 180, and 240 minutes after the injections. A crossover experiment was done on the same day so that each dog received both solutions.

Intramuscular Tolerance

This study was done to determine the irritation of calcium kinate gluconate when injected intramuscularly at various concentrations in the rabbit. Calcium gluconate was injected and studied as a standard preparation. Twelve adult albino rabbits were divided into six groups of two rabbits each and were injected as shown in the tabulation.

Group	Drug	Ml injected	Mg Ca injected
I	CKG	1.0	5.0
II	CKG	1.0	10.0
III	CKG	1.0	15.0
IV	CKG	1.0	25.0
V	CKG	1.0	50.0
VI	CG	1.0	10.0

Each rabbit was injected with the test solution into three different muscles on the first, sixth, and seventh days. On the eighth day, the

[2] The authors are thankful to Dr. Leonard Grumbach for this study.

rabbits were sacrificed with intravenous sodium thiopental and the injection sites of 1, 2, and 7 days' duration were examined grossly. The tissues of the injection sites were fixed in Zenker-formalin, stained by the hematoxylin and eosin method, and examined microscopically.

Chronic Intravenous Tolerance

Fifteen dogs were divided into five groups of three dogs each. Calcium kinate gluconate was administered intravenously to two groups at 2 or 10 mg Ca per kilogram, calcium gluconate to one group at 10 mg Ca per kilogram, and sodium kinate to two groups at 75 or 375 mg/kg. A sixth group of two dogs received physiological saline as a control of the experimental procedure. Sodium kinate was administered as a control of the effects of kinic acid, one of the constituents of the calcium kinate gluconate complex. All preparations were injected three times weekly for 6 months.

The dogs were observed carefully, during and after medications, for changes in general appearance and behavior. Body weights and rectal temperatures were determined weekly. Catheterized urine specimens were examined three times during the experiment to determine the effects of the drugs on the color, odor, turbidity, and the chemical and microscopic properties of the urine. At the end of the experiment, the control and medicated dogs were sacrificed with intravenous sodium thiopental and the thoracic and abdominal viscera were examined grossly. Sections of tissues were fixed in Zenker-formalin, stained by the hematoxylin and eosin method, and examined microscopically.

Hematologic Studies

Hematologic studies were done on the same 17 dogs used for the chronic 6-month intravenous tolerance study. The dosage regimen of these dogs is described in the previous section.

Another experiment was done with monkeys. Six rhesus monkeys (*Macaca mulatta*) were divided into three equal groups. CKG or CG at 7.5 mg Ca/kg or saline in an equivalent volume to the CG dosage were injected three times intravenously on alternate days to one group each.

Total red and white blood cell counts, differential counts, hematocrits, and hemoglobin concentrations were determined before medication and approximately once a month thereafter on dogs, and before medication and at 1 and 4 days after the last injection on monkeys. Platelet counts were made, by the Rees-Eckert method, from the blood of dogs twice

during the fourth and fifth months, and of monkeys following each injection. Clotting time of blood from dogs receiving the high doses of calcium and from the control dogs was determined by the capillary tube method three times during the fourth and fifth months; clotting time of blood from monkeys was determined after each injection. Serum calcium levels were determined at monthly intervals on dogs by the Cramer-Tisdall method, and following each injection on monkeys by the colorimeter method of Kibrick *et al.* (1951).

RESULTS

Acute Intravenous Toxicity

Calcium kinate gluconate and calcium gluconate had approximately the same acute intravenous toxicity in terms of 24-hour LD_{50} and on the basis of total compound administered, but they were less toxic than calcium chloride. On basis of calcium content, no significant difference in the acute intravenous toxicity was observed among the three compounds. The 7-day LD_{50} was the same as that at 24 hours. These data are presented in Table 1.

TABLE 1

ACUTE TOXICITY IN MICE INJECTED INTRAVENOUSLY WITH CALCIUM SALTS

Medication	I.v. 24-hr[a] LD_{50} (mg/kg ± S.E.)	
	As Compound	As Ca
Calcium kinate gluconate	1050 ± 57	83 ± 4
Calcium gluconate	950 ± 83	86 ± 8
Calcium chloride	215 ± 14	78 ± 5

[a] Same LD_{50} at 7 days.

Acute Intravenous Tolerance

Calcium kinate gluconate was better tolerated than calcium gluconate in terms of total Ca administered when injected intravenously into dogs. The mean maximum tolerated dose of CKG as determined by occurrence of vomiting was 19.7 ± 2.6 mg Ca per kilogram and that of CG was 13.2 ± 1.7 mg Ca per kilogram.

Antagonism to Magnesium Toxicity

Injections of magnesium sulfate ($MgSO_4$) alone at a dosage of 48 mg/kg produced death in all mice. The death rate was reduced sharply when one of the three calcium salts was added at 40 mg Ca per kilogram to the injections of $MgSO_4$. No definite difference in effectiveness was

observed among the three calcium preparations. As a control, a volume of saline equal to that of the calcium solution was added to the $MgSO_4$ solution; no decrease in toxicity was observed, a result indicating that the marked reduction in mortality of the magnesium was not due to dilution of the magnesium solution by the calcium solution: this reduction was caused by the addition of the calcium salts. The results of this experiment are presented in Table 2.

TABLE 2
ANTAGONISM OF CALCIUM SALTS TO MAGNESIUM TOXICITY IN MICE

Medication	Dose mg/kg i.v. as		Mortality
	Ca	Mg	No. dead/no. injected
Calcium kinate gluconate	40	—	1 / 10
Calcium gluconate	40	—	1 / 10
Calcium chloride	40	—	1 / 10
Magnesium sulfate ($MgSO_4$)	—	48	20 / 20
Calcium kinate gluconate + $MgSO_4$	40	48	2 / 20
Calcium gluconate + $MgSO_4$	40	48	3 / 20
Calcium chloride + $MgSO_4$	40	48	2 / 20
Saline + $MgSO_4$	—	—	10 / 10

Cardiovascular Effects

Calcium kinate gluconate or calcium kinate produced no significant changes in the electrocardiograms, heart rates, or blood pressures in dogs when injected intravenously at doses up to 7.5 mg Ca per kilogram.

Intramuscular Tolerance

There was no apparent evidence of pain in any of the rabbits injected intramuscularly with calcium kinate gluconate or with calcium gluconate. The skin at the sites of injections was normal in appearance. The general gross and microscopic observations differed primarily in the severity of the lesions produced by the calcium preparation rather than in the type of inflammation.

CKG was well tolerated when injected at 5 or 10 mg Ca per dose, as was CG at 10 mg Ca per dose. Minimal tissue damage attributable to the medication at these dosages was observed at 24 and 48 hours, and healing was well advanced by the seventh day.

Intramuscular injections of CKG at 15 or 25 mg Ca per dose were well tolerated, but moderate tissue damage was observed at 24 and 48 hours. Although the nature of the tissue reaction was similar following these

two doses, the 25 mg Ca per dose resulted in larger areas of inflammatory reaction. Healing was advanced by the seventh day.

Intramuscular injection of CKG at 50 mg Ca per dose was not as well tolerated as the more dilute solutions. Moderate to severe tissue damage was observed at 24 and 48 hours and the rate of healing was slower.

Chronic Intravenous Tolerance

The dogs injected intravenously with calcium kinate gluconate or calcium gluconate three times weekly for 6 months were normal in appearance and behavior throughout the experiment. The body weight gain of the medicated and control dogs was normal. No symptoms of hypercalcemia occurred and no evidence of irritation was observed in any of the sites of repeated intravenous injections at any time. The rectal body temperature of the medicated dogs was within normal limits throughout the experiment.

No significant change attributable to medication occurred at any time in color, odor, turbidity, or the chemical or microscopic properties of urine of the dogs. No significant macroscopic or microscopic lesions attributable to medication were observed in any of the dogs autopsied at the termination of the experiment.

Hematologic Studies

Total red and white blood cell counts, differential counts, hematocrits and the hemoglobin concentrations of dogs or monkeys injected with calcium kinate gluconate or calcium kinate were normal as compared to control dogs or monkeys.

Clotting time in *dogs* injected with the calcium solutions at 10 mg Ca per kilogram or with sodium kinate at 375 mg/kg for 3–4 months was faster than in the controls. Usually, this was observed within 15 minutes after each injection, and the clotting time returned to the premedication time within 45 minutes. Determinations made at 60 minutes and at 1 and 2 days following the last injection were within normal limits. A decrease in clotting time was noted also in both groups of *monkeys* medicated with CKG or CG. The decrease was observed within 15 minutes after each injection and reached a maximum by 30 minutes. The clotting time returned to normal at 90 minutes following injections of CG whereas after CKG the clotting time returned to normal at 150 minutes or more. Four days after the last injection the clotting time of monkeys was normal. The clotting time values are presented in Table

TABLE 3

CLOTTING TIME IN DOGS AND MONKEYS AFTER INTRAVENOUS INJECTIONS OF CALCIUM SALTS

		Clotting time								
			Time after medication							
Medication[c]	Species	Pre-med.	5′	15′	30′	60′	90′	150′	24 Hr	48 Hr
Saline control	Dog[a]	2′15″	—	2′57″	2′30″	2′57″	—	—	2′30″	2′50″
CKG 10 mg Ca/kg	Dog	2′55″	—	2′45″	2′01″	2′53″	—	—	2′48″	2′57″
CK 10 mg Ca/kg	Dog	2′20″	—	1′51″	1′40″	2′18″	—	—	2′27″	2′27″
Na kinate 375 mg/kg	Dog	2′30″	—	2′21″	1′53″	2′41″	—	—	2′43″	2′48″
Saline control	Monkey[b]	1′23″	1′18″	1′18″	1′18″	—	1′18″	—	—	—
CKG 7.5 mg Ca/kg	Monkey	1′27″	1′18″	0′51″	0′41″	—	0′53″	1′08″	—	—
CG 7.5 mg Ca/kg	Monkey	1′31″	1′15″	0′53″	0′41″	—	1′23″	—	—	—

[a] Averages of 3 dogs in each medicated group and 2 in the controls at 3–4 months of medication.

[b] Averages of 2 monkeys in each group following 3 injections on alternate days.

[c] CKG = calcium kinate gluconate; CG = calcium gluconate.

A slight increase in platelet counts was observed in dogs and monkeys injected with CKG and in dogs injected with sodium kinate within 15 or 30 minutes after the injections. No definite increase was noted in dogs or monkeys injected with CG. The platelet counts are presented in Table 4.

TABLE 4
PLATELET COUNTS OF DOGS AND MONKEYS AFTER INTRAVENOUS INJECTIONS OF CALCIUM SALTS

Medication[c]	Species	Platelet count (1000/mm³)						
		Pre-med.	Time after medication					
			5′	15′	30′	60′	90′	24 Hr
Saline control	Dog[a]	400	—	—	354	454	—	346
CKG 10 mg Ca/kg	Dog	389	—	—	475	476	—	429
CK 10 mg Ca/kg	Dog	422	—	—	435	380	—	366
Na kinate 375 mg/kg	Dog	379	—	—	435	448	—	38[illegible]
Saline control	Monkey[b]	296	287	301	300	—	305	—
CKG 7.5 mg Ca/kg	Monkey	292	361	407	429	—	375	—
CG 7.5 mg Ca/kg	Monkey	412	407	445	405	—	387	—

[a] Averages of 3 dogs in each medicated group and 2 in the controls at [illegible] months of medication.

[b] Averages of 2 monkeys in each group following three injections on alternate days.

[c] CKG = calcium kinate gluconate; CG = calcium gluconate.

A significant increase in the serum calcium level was observed in the dogs injected with CKG or CG when compared to the controls. The maximum increase was reached within 15 minutes following the injection and was still slightly elevated 3 hours later. Similarly, a significant increase was observed in the monkeys injected with CKG or CG when compared to the controls. This increase reached a maximum within [illegible] minutes following the injections and returned to an approximately normal level within 90 minutes. Serum calcium levels are presented in Table [illegible]

Clinical Studies

Detailed clinical reports (Nobel, 1959) on over 200 patients treated with calcium kinate gluconate have been analyzed. These reports indicated excellent tolerance of CKG. Besides the transient flushing or feeling of warmth experienced by many patients receiving parenteral calcium, the only reported untoward reaction was a temporary dizziness felt by [illegible] asthmatic patients receiving an intravenous solution containing dextro[illegible]

Benadryl, ACTH, and CKG. The number of treated patients included 125 cases of dermatoses, 18 cases of asthma, 4 cases of lead poisoning, and 60 cases in a state of calcium deficiency.

TABLE 5

SERUM CALCIUM VALUES AFTER INTRAVENOUS INJECTIONS OF CALCIUM SALTS

Medication[c]	Species	Serum calcium (mg/100 ml) at minutes after injection							
		Pre-med.	5′	15′	30′	60′	90′	120′	180′
Saline control	Dog[a]	10.3	—	10.4	—	10.5	—	10.7	10.7
CKG 10 mg Ca/kg	Dog	10.3	—	14.4	—	13.2	—	12.7	12.0
CG 10 mg Ca/kg	Dog	10.7	—	16.1	—	14.0	—	13.4	12.2
Na kinate 375 mg/kg	Dog	10.7	—	10.9	—	10.5	—	10.7	10.5
Saline control	Monkey[b]	11.8	11.3	11.5	11.6	—	11.8	—	—
CKG 7.5 mg Ca/kg	Monkey	10.8	14.2	13.5	13.0	—	11.7	—	—
CG 7.5 mg Ca/kg	Monkey	12.1	15.0	14.3	13.4	—	12.5	—	—

[a] Averages of 3 dogs in each medicated group and 2 in the controls from 6 determinations at monthly intervals following chronic administration.

[b] Averages of 2 monkeys in each group following three injections on alternate days.

[c] CKG = calcium kinate gluconate; CG = calcium gluconate.

DISCUSSION

The pharmacologic activity of calcium kinate gluconate was similar to that of calcium gluconate and was apparently due to the calcium ion. No significant difference was observed among CKG, CG, or $CaCl_2$ in protecting mice against a lethal dose of magnesium when the doses were calculated in terms of their calcium ion content. The acute intravenous toxicity of these compounds also appeared to be directly related to the calcium ion when calculated in terms of the calcium content of these preparations. On a molecular basis, CKG was the least toxic of the three compounds. No chronic toxicity was produced by either CKG or CG.

The degrees of intramuscular irritation were similar for CKG and CG when administered at an equal calcium concentration. If the CKG concentration was decreased, the degree of intramuscular irritation decreased proportionally. CKG was well tolerated with calcium concentrations up to 25 mg/ml.

The specific effect of the calcium ions was less evident in acute intravenous tolerance studies in dogs where CKG was better tolerated than

CG when administered under identical conditions and at the same rate of calcium injection. It is possible that the anionic moiety of CKG increases the tolerance of the recipient to calcium.

Another possible difference between CKG and CG was observed in hematologic studies where slightly increased platelet counts were noted in dogs and monkeys following injections of CKG but not of CG. However, both Ca preparations shortened blood clotting time. These effects of CKG and CG could not be attributed only to high serum calcium levels since injections with sodium kinate into dogs also resulted in a shorter blood clotting time with a possible slight increase in platelet counts. A similar observation with kinic acid has been reported by Noda and Kurakake (1939): kinic acid injected intravenously into rabbits reduced blood coagulation time. The effect of CKG on blood clotting time and platelet counts may be attributable to the presence of Ca ions and kinic acid.

CKG is of interest in the treatment of lead poisoning. In recent years chelating agents have been successfully used to tie up heavy metals. CKG in these cases may be used similarly in view of its chelating properties of the kinic acid moiety, superimposed on the mobilizing effect of calcium.

SUMMARY

The pharmacologic and toxic properties of calcium kinate gluconate, calcium gluconate, and calcium chloride have been studied in mice, dogs, and monkeys. The pharmacologic properties appeared to be based on the calcium concentration except for acute intravenous tolerance where calcium kinate gluconate was better tolerated than calcium gluconate. Calcium kinate gluconate and calcium gluconate injections produced transient decrease in blood clotting time, but increased platelet counts were observed only following calcium kinate gluconate injections. The calcium kinate gluconate effect on blood probably was attributable to the presence of calcium and kinic acid. No toxic properties of calcium kinate gluconate and calcium gluconate were observed in a 6-month tolerance study in dogs. Good clinical tolerance of calcium kinate gluconate was reported on over 200 cases.

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INTRODUCTION

In order to increase the calcium intake by farmers, the provision of Ca-enriched foods is imperative. The significance of miso (bean paste) and pickled vegatables in this respect has already been discussed /1-2/. Calcium carbonate ($CaCO_3$) has been regarded as most suitable for the preparation of Ca-enriched food. However, as is expected without profound analysis, $CaCO_3$ contained in a fermented food elevates pH as it is decomposed during the manufacturing process, and the liberated CO_2 may exert undesirable effects on other ingredients (e.g., vitamins). Thus, with many advantages of $CaCO_3$ as a Ca-enriching agent, there seem to be harmful effects as well. At the present, the availability of Ca from various Ca-enriching agents as compared to that from $CaCO_3$ has merely been estimated on the basis of experimental data. In an attempt to encourage increased intake of calcium-enriched food by farmers, the choice of Ca agent is an important decision and requires thorough investigation.

In order to study the availability of Ca in Ca salt-added foods, the author conducted an animal experiment using radioactive Ca-Ca^{45} compounds.

EXPERIMENTAL SECTION

Animals: Twenty-five 7 weeks old rats weighing approximately 150 g, of which 14 were male and 11 female. They were divided into 5 groups.

Feed: basal feed (maintenance feed) to which various Ca^{45} salts were added. The composition and analytical values of the feeds are shown in Table 1.

Ca^{45} salts: The Ca^{45} salts added include $Ca^{45}CO_3$ (control), Ca^{45} HPO_4, Ca^{45}-phytate, Ca^{45}-lactate, and Ca^{45}-oxalate. These calcium salts were prepared as follows:

1. $Ca^{45}CO_3$: $(NH_4)_2CO_3$, alkalized with ammonium, was added to $Ca^{45}Cl_2$, and the precipitates were filtered out, washed with ammonia water, and dried at 110°C.

2. $Ca^{45}HPO_4$: Five parts of $NaHPO_4$ was added to phosphoric acid-acidified $Ca^{45}Cl_2$, and the mixture was stirred. The crystals that precipitated was separated by filtration, washed with distilled water, and dried.

3. Ca^{45}-phytate: Commercial Ca-phytate (a product of Tokyo Kasei Co.) was dissolved in 0.5% HCl, and alcohol was added in order to induce precipitation and the purification process was repeated twice. Then, NaOH was added. Na-phytate thus obtained was dissolved in water, and $Ca^{45}Cl_2$ was added. Ca^{45}-phytate was permitted to precipitate from alcohol, filtered out, and dried.

4. Ca^{45}-lactate: Special-grade lactic acid was combined with an equal amount of $Ca^{45}CO_3$, and the mixture was heated over water bath. The solution was concentrated by heating until CO_2 was no longer produced. The mixture was left standing overnight, and solids were separated, dried, and pulverized. No crystalline substance could be obtained.

5. Ca^{45}-oxalate: While heating $Ca^{45}Cl_2$ solution over water bath, a hot aqueous solution of saturated ammonium oxalate was added gradually. The mixture was left standing overnight, and the precipitates were filtered out, washed with water, and dried.

TABLE 1. FEED-COMPOSITION

		%	蛋白質 g/100g	脂肪 g/100g	カロリー Cal/100g	Ca mg/100g	P mg/100g
a 基礎飼料	b 砕麦	85	8.5	1.6	278	29	326
	c 魚粉	10	4.2	1.5	31	39	200
	d 野菜	k 若干	–	–	–		–
	e 食塩	2	–	–	–		–
	f 肝油	3	–	3.0	27	–	–
	g 添加 Ca^{45} 塩					300	230 ($CaHPO_4$)
			12.7	6.1	336		357 (Ca-Phyt)
m 実験飼料 No.	l Ca^{45} 塩						Ca/P
1	$Ca^{45}CO$	12.7	6.1	336	368	526	0.69
2	$Ca^{45}HPO_4$	12.7	6.1	336	368	756	0.48
3	Ca^{45}-Phyt	12.7	6.1	336	368	893	0.41
4	Ca^{45}Lact	12.7	6.1	336	368	526	0.69
5	Ca^{45}Oxal	12.7	6.1	336	368	526	0.69

Keys: a, basal feed; b, crushed wheat; c, fish powder; d, vegetable; e, common salt; f, liver oil; g, Ca^{45} salt; h, protein; i, fat; j, calorie; k, little; l, Ca^{45} salt; m, experimental feed.

The Ca^{45} salts thus obtained were combined with a non-radioactive Ca salt as the carrier at a final volume of 15 - 30 g. The radioactivity of each salt was approximately 300 uc/g. The salts were added to basal feed at 3 g/kg (Table 1).

Rats were raised individually in metal mesh cages and received basal fedd for the first week and the test feed containing a Ca^{45} salt for 3 days, after which they were sacrificed for anatomical observation. The Ca^{45} in the femur (one side only) was measured, and the mode of Ca deposition was studied.

TABLE 2. THE BODY WEIGHT, WEIGHT OF FEMUR, AND CALCIUM CONTENT AND AMOUNT OF Ca^{45} DEPOSITED IN THE FEMUR

a 飼料 No.	b 添加Ca^{45}塩	c 体重 g	d 大腿骨 mg	
			e 重量(生)	f Ca 含量
1	$Ca^{45}CO_3$	134.0± 9.68	318.4±18.49	62.2±4.30
2	$Ca^{45}HPO_4$	147.8±12.47	344.8±21.93	69.0±2.58
3	Ca^{45}-Phyt	136.3±16.56	330.4±38.27	66.0±8.60
4	Ca^{45}-Lact	140.0±14.41	319.2±27.95	64.4±6.02
5	Ca^{45}-Oxal	133.1±18.71	294.8±44.72	64.6±4.30

Average of five ± standard deviation

Keys: a, feed; b, Ca^{45} salt added; c, body weight; d, femur; e, weight; f, Ca content.

(TABLE 2 - continued)

Ca^{45} DEPOSITED IN THE FEMUR

(% · · · Ca^{45} CPM in femur/Ca^{45} CPM taken)

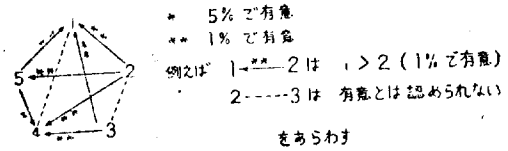
a 飼 料 No.	1	2	3	4	5
b 添加 Ca^{45} 塩	$Ca^{45}CO_3$	$Ca^{45}HPO_4$	Ca^{45}-Phyt	Ca^{45}-Lact	Ca^{45}-Oxal
	0.82	0.74	0.70	1.20	0.06
	0.66	0.44	0.40	1.00	0.15
	0.91	0.61	0.35	0.93	0.04
	0.73	0.56	0.43	0.81	0.20
	1.14	0.37	0.51	0.86	0.11
c 平 均	0.85	0.54	0.48	0.96	0.11

Keys: a, feed; b, Ca^{45} salt added; c, average

EXPERIMENTAL RESULTS

Each rat consumed an average of 10 - 15 g of Ca^{45} salt containing feed, the Ca^{45} intake being approximately 10 uc/animal. Due to the characteristic taste of Ca salt, the feed intake varied according to the individual rat. During the experiment, all the rats appeared normal and maintained good physical condition. The amounts of Ca^{45} deposited in the femur, measured after the experiment, are tabulated. The body weight, the weight of the femoral bone, and the Ca content of the rats showed no significant variation or difference by sex. The deposition of Ca^{45} salts in the femoral bone is illustrated in the following figure. Ca^{45} lactate and $Ca^{45}CO_3$

COMPARISON AMONG GROUPS



Keys: a, significant by 5%
b, significant by 1%
c, 1 2 (significant by 1%)
d, not regarded as significant.
e, For instance.

showed the highest rate of femoral deposition, followed in rank by $Ca^{45}PHO_4$ and Ca^{45} phytate, and Ca^{45} oxalate in the order of higher rate. These results are generally in agreement with the data published by other investigators, but it must be noted that the poor availability of $CaHPO_4$ has never been mentioned before.

DISCUSSION

There are many papers on the digestion and absorption of various Ca salts and their deposition in the femurs. However, these experiments used the Ca salts as the only source of Ca. In other words, the Ca salt under test was the only Ca contained in the feed. Such experimental condition would naturally permits pure comparison of the availability of various Ca salts. However, the Ca salt contained in food generally includes many components. When a Ca agent is added for the Ca-enrichment of the food, the utilization of the additive is influenced by the Ca salt already contained

in the food. Thus, the availability of the Ca from a Ca agent must be examined in relation to the Ca already in the food in order to achieve meaningful analysis. In the present experiment, designed with this aspect into consideration, Ca lactate and Ca carbonate equally showed the highest availability, the rate being more than twice that of Ca phosphate (precipitated) and Ca phytate. In terms of solubility, there is no difference between precipitated Ca phosphate, calcium carbonate and calcium lactate, all being readily dissolved within a range of the HCl concentration of the gastric juice. The difference in availability is most likely due to the excess P. According to Sherman /4/, the absorption of Ca is not interfered by the Ca/P to this degree, but as compared to the optimum rate, the condition may still affect the availability. The poor availability of Ca phytate has already been reported /5/, but when added to the basal feed, it was utilized as much as Ca phosphate. At least over 10% of the Ca in the experimental fedd is derived from the Ca in the crushed wheat in the basal fedd, which takes the form of phytate. Thus, the Ca of this feed should be regarded as calcium phytate. On the other hand, the Ca in fish powder is derived from fish bone, and a greater proportion of it is in the form of calcium phosphate. The Ca in fish powder amounts to approximately 1% of the total Ca contained in the experimental feed, therefore, the Ca in Feed No. 2 is mostly Ca phosphate (precipitated Ca phosphate and Ca phosphate). Thus, the Ca phytate itself is readily soluble in gastric HCl, and phytin can be decomposed to some degree. From this analysis, the utilization and absorption of Ca is deemed to have a close relationship with Ca/P ratio. Ca oxalate has been known as poorly digestable, and this was confirmed in this experiment. Although Ca oxalate is sparingly soluble, it can be dissolved in gastric HCl. The poor utilization may be due to the fact that the liberated oxalic acid enters the intestine without being decomposed and is again bonded with Ca. As was noted by other investigators who administered calcium oxalate as the only source of Ca, the combined administration with other Ca salts did not improve the deposition of the compound in the femoral bone.

CONCLUSION

When rats were raised with feeds containing various Ca-enriching agents, Ca lactate and Ca carbonate showed the highest availability as determined by the amount of deposited in the femur. The rate of Ca phosphate and Ca phytate was 50% lower, and the deposition of Ca oxalate was extremely low. In terms of the utilization of Ca, Calcium lactate or Calcium carbonate is considered to be the most suitable compound for the preparation of Ca-enriched foods.

SUMMARY

1) The utilization of Ca agents *in vivo* was studied.

2) Rats were raised with experimental feeds prepared by adding Ca^{45}-labelled Ca salts ($Ca^{45}CO_3$, $Ca^{45}HPO_4$, Ca^{45}-phytate, Ca^{45}-lactate, and Ca^{45}-oxalate) to normal maintenance feed.

3) The animals were killed after receiving labelled feed for 3 days, and the Ca contained in the femur was measured. The availability of calcium salts under test was estimated in terms of the proportion of deposit Ca^{45} for Ca^{45} intake.

4) Ca lactate and $CaCO_3$ showed the highest availability. $CaHPO_4$ and Ca phytate were utilized to a lesser degree. Ca oxalate showed the lowest

rate of utilization and is seemingly of no practical value.

The author wishes to express deep gratitude to Drs. Kawakami and Nagasawa of this institute for their advice and cooperation.

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THE EFFECTS OF CALCIUM CHLORIDE AND OF CALCIUM LACTATE ADMINISTERED BY GAVAGE

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Post-mortem examination of two infants who had received calcium chloride by gavage while under treatment on the private service of the New Haven Hospital revealed extensive damage to the mucosa of the stomach and upper intestinal tract. These findings stimulated investigation to determine whether similar lesions could be produced experimentally. Although it is frequently stated in the literature that oral administration of calcium chloride is irritating and may cause vomiting,[6, 8] no description of the lesions produced has been encountered. Numerous clinical experiences similar to those cited in the third case described here confirm the impression that the doses of calcium chloride usually administered in tetany neonatorum are excessive and perhaps that calcium chloride is always a dangerous drug during the early months of life.

Case I

White female. Age 36 days. Autopsy by Dr. J. J. McGillicuddy. No. 5391.

Clinical history:

This 2900-gram infant was born spontaneously after a normal pregnancy. The first two days of life were not remarkable. On the morning of the third day, tremors (especially of the right arm and leg) were noted. The Chvostek, Trousseau, and peroneal signs were absent, and the physical examination was otherwise normal. Four grams of calcium chloride in 25 cc. of water (16 per cent solution) were administered by gavage. Blood-stained vomitus appeared two hours later, followed shortly thereafter by blood in the stool. Shock occurred in 4½ hours, and peritonitis was suspected. An x-ray revealed free fluid but no air in the peritoneal cavity. On the following day, exploratory laparotomy confirmed the presence of considerable clear

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fluid in the abdominal cavity, but no changes of the abdominal viscera were noted. Examination of the fluid revealed no cells or organisms by smear and culture. The postoperative course was characterized by disruption of the wound, peritonitis, and the development of a fecal fistula. Terminally the child vomited repeatedly and died on the thirty-sixth day of life. The clinical diagnosis was "Intestinal obstruction, cause unknown."

Autopsy findings:

The body was emaciated and skin turgor was diminished. An organizing suppurative peritonitis with metastatic abscesses in the lungs, subcutaneous tissues, and viscera was found. The serosa of the stomach was dull and covered by fibrinous adhesions. The mucosa was replaced by opaque, yellow, calcareous plaques, which were brittle and firmly attached to the submucosa. Plaques of similar consistency were seen in the small intestine, particularly in the duodenum and upper jejunum. Microscopic examination revealed ulceration and necrosis of the mucosa and submucosa of stomach and small intestines. The stomach wall was composed of plaques of hyaline scar tissue, here and there infiltrated by small round cells. Many of these scars contained granular material which stained black in the von Kossa preparation indicating the presence of calcium. Organizing and calcified thrombi occluded the arterial vessels of the submucosa (Fig. 1), and there were similar lesions in the small intestine. Focal necrosis and calcification were present in the media of many large arteries (Fig. 2).

Case II

White male. Age 7 days. Autopsy by Dr. S. H. Durlacher. No. 5452.

Clinical history:

The baby, born by low forceps, weighed 3060 grams. The first two days of the infant's life were uneventful. On the evening of the second day shaking of the arms and legs as well as tremors of the eyelids were noted. The physical examination revealed hyperactive reflexes and positive plantar and Chvostek responses bilaterally. The serum calcium was 6.8 mg. %. A gavage containing 3 grams of calcium chloride in 18 cc. of water (16.6 per cent solution) was administered, and one gram of calcium chloride was added to each four-hour feeding thereafter. On the following day the infant vomited the feedings and respiratory difficulty was observed. This was attributed to aspiration. Signs of collapse ensued, and despite transfusions and other supportive measures, death occurred on the seventh day of life. The clinical diagnosis was "Aspiration pneumonia; Icterus neonatorum; Tetany neonatorum."

Autopsy findings:

The clinical diagnosis of aspiration pneumonia was confirmed at autopsy. The other striking findings were confined to the stomach, which was slightly

dilated and contained approximately 200 cc. of curdled milk. The serosa was smooth and glistening. The mucosa along the lesser curvature was pale and intact, but that of the greater curvature, especially in the fundic portion, was bright red and ulcerated. The bases of these ulcers were filled by white opaque, firm, friable material which had the consistency of an egg-shell. Microscopically, sections of the damaged portion of the stomach showed necrosis and disappearance of the mucosa with edema, polymorphonuclear cellular infiltration, and necrosis of the blood vessel walls in the submucosa and muscularis (Figs. 3 and 4).

Case III

White female. Newborn. N.H.H. No. B19725.

A full-term infant was born spontaneously September 5, 1941, in good condition. During the pregnancy the mother drank only one pint of milk a day, and developed extensive dental caries. The baby, breast fed for two days, was then placed on formula. When seven days old, a clonic convulsion which lasted six minutes occurred. A similar convulsion followed soon thereafter. Physical examination was essentially negative; Chvostek and Trousseau signs could not be elicited. The serum calcium was 6.2 mg.%. On the eighth day, through a misunderstanding of the ruling that medication should not be added to infants' milk mixtures, the infant was given two grams of calcium chloride in water before the 10 A.M. and 2 P.M. feedings. Both feedings were promptly vomited, and the vomitus was blood streaked. She took and retained one-half of the 6 P.M. feeding, which contained 2 grams of calcium chloride. Saline clysis, transfusion, and hykinone were given because of the hematemesis. The baby did well on the regular formula without added calcium, and saline clyses for the next two days. No convulsions occurred. On the eleventh day of life, 0.5 cc. of 50 per cent calcium chloride were added to each feeding, and continued for two days without incident. Discharge diagnosis was hypocalcemic tetany. The infant has gained weight since discharge and has shown no subsequent gastro-intestinal symptomatology.

The above histories suggest that calcium chloride produces gastro-intestinal lesions. In the first case, the long interval before death may make interpretation somewhat difficult but the other two cases leave little room for doubt. It should be emphasized that these three infants received some of the calcium chloride in aqueous solution at a time when the stomach was empty. Doses of the drug administered in milk mixtures were better tolerated. The following experimental work shows the effect of similar and smaller doses of calcium chloride in rabbits.

Experimental

Methods: Forty-two rabbits, from two days to six weeks of age, were used. The animals were fasted for 48 hours before gavage and permitted to eat six hours after gavage. A soft rubber catheter (French No. 8) was employed and the animals were sacrificed 48 hours after treatment, except as noted in table 1. Anhydrous calcium chloride was administered in 5, 7.5, 10, 15, and 20 per cent aqueous solutions; calcium lactate in 5 and 10 per cent aqueous solutions; ammonium chloride in 5, 10, and 20 per cent aqueous solutions; and three rabbits were treated with combined solutions of ammonium chloride and calcium lactate as shown in table 1. Two rabbits were intubated and the catheter manipulated roughly to determine whether this procedure alone would cause trauma. Gross and microscopic study of the stomach and intestines was performed in all instances.

Results: The results of these experiments are outlined in table 1. Rough intubation caused no lesions. All animals, independent of weight and age, receiving 20 per cent solution of calcium chloride had severe gastric damage, which consisted of mucosal necrosis and ulceration (Fig. 5), submucosal edema, cellular infiltration (Fig. 6), and angionecrosis. Necrosis and exudation occasionally extended through all coats and fibrinous exudate was present on the serosal surface. Perforation of the stomach of one of the larger rabbits occurred. The lesions were most marked along the greater curvature of the stomach, especially in the region of the fundus. No lesions were produced in the small or large intestine. With doses of 15 per cent calcium chloride, lesions were not found in the older rabbits but severe ulcers, similar to those described above, occurred in the unweaned animals receiving over 0.75 gm. per kg. Similar results were obtained with the 10 and 5 per cent solutions.

Calcium lactate in doses of 6 gm. per kg. produced no changes, even in the most immature animals. Concentrations higher than 10 per cent could not be given because of the limited solubility of the drug.

Animals receiving ammonium chloride in 20 per cent aqueous solution showed no morphological changes in the viscera. Those receiving doses greater than 1.5 gm. per kg. had convulsions and died within one hour after the administration. Those animals receiving both calcium lactate and ammonium chloride were likewise free of anatomical lesions.

The above experiments show that calcium chloride is quite irritating to the stomach of rabbits. When the weaker concentrations

TABLE 1

EFFECTS OF CERTAIN SALTS ADMINISTERED TO RABBITS BY GAVAGE

Rabbit	*Weight (gm.)*	*Salt*	*Dose (gm./kg.)*	*Concentration (%)*	*Manner of death*	*Edema*	*Mucosal necrosis*	*Submucosal necrosis*
1	60	—			S	—	—	—
2	80	—			S	—	—	—
3	215	$CaCl_2$	0.75	20	S	+++	++	—
4	180	$CaCl_2$	1.50	20	D	+++	+++	++
5	275	$CaCl_2$	1.50	20	S	+++	++	—
6	900	$CaCl_2$	1.50	20	S***	+++	+++	+++
7	1000	$CaCl_2$	1.50	20	D	+++	+++	+++
8	80	$CaCl_2$	0.68	15	S	—	—	—
9	80	$CaCl_2$	1.25	15	S	+++	++	—
10	60	$CaCl_2$	1.25	15	D	+++	+++	—
11	60	$CaCl_2$	1.25	15	S	+++	++	++
12	80	$CaCl_2$	2.50	15	S	+++	+++	+++
13	160	$CaCl_2$	1.50	15	S	+++	+++	+++
14	375	$CaCl_2$	1.50	15	S*	+	—	—
15	780	$CaCl_2$	0.75	15	S	+	—	—
16	810	$CaCl_2$	1.50	15	S	+	—	—
17	920	$CaCl_2$	1.50	15	S	+	—	—
18	150	$CaCl_2$	1.50	10	D	+++	+++	—
19	330	$CaCl_2$	1.50	10	S	++	—	—
20	775	$CaCl_2$	0.75	10	S	—	—	—
21	810	$CaCl_2$	1.50	10	S	+	—	—
22	1000	$CaCl_2$	1.50	10	S	—	—	—
23	60	$CaCl_2$	1.25	7.5	S	—	—	—
24	760	$CaCl_2$	0.75	5	S	—	—	—
25	110	$CaCl_2$	1.50	5	D	++	++	+
26	810	$CaCl_2$	1.50	5	S	—	—	—
27	1100	$CaCl_2$	1.50	5	S	—	—	—
28	120	Ca lact.	4.0	10	S	—	—	—
29	275	Ca lact.	6.0	10	S	—	—	—
30	340	Ca lact.	6.0	10	S*	—	—	—
31	650	Ca lact.	2.0	10	S	—	—	—
32	700	Ca lact.	2.0	10	S	—	—	—
33	645	Ca lact.	4.0	5	S	—	—	—
34	200	NH_4Cl	0.5	20	S	—	—	—
35	342	NH_4Cl	1.5	20	D**	—	—	—
36	315	NH_4Cl	0.5	10	S	—	—	—
37	320	NH_4Cl	1.0	10	S	—	—	—
38	320	NH_4Cl	0.5	5	S	—	—	—
39	344	NH_4Cl	1.0	5	S	—	—	—
40	280	NH_4Cl	2.0	10	D**	—	—	—
		Ca lact.	2.0	10				
41	210	NH_4Cl	4.0	10	D**	—	—	—
		Ca lact.	4.0	10				
42	240	NH_4Cl	1.0	5	S	—	—	—
		Ca lact.	2.0	10				

S — Sacrificed after 48 hours.
D — Died before 48 hours.
* — Sacrificed after 96 hours.
** — Died of acidosis after 1 hour.
*** — Perforation of stomach wall found at autopsy.

TABLE 2

CHANGES IN SERUM CALCIUM, PHOSPHORUS, AND TOTAL PROTEIN FOLLOWING THE ADMINISTRATION OF CALCIUM LACTATE TO NEWBORN INFANTS

Infant	*Weight (gm.)*	*Ca.* (mg.%)*	*Ca.† (mg.%)*	*Difference (mg.%)*	*Phos.* (mg.%)*	*Phos.† (mg.%)*	*Difference (mg.%)*	*Total protein* (gm.%)*	*Total protein† (gm.%)*	*Difference (gm.%)*	*Rise in Ca accounted for by rise in protein*
1	3045	10.1	11.6	1.5	6.2	5.2	−1.0	5.6	5.8	0.2	0.15
2	3175	11.7	12.4	0.7	6.8	5.7	−1.1	5.7	5.7	0.0	0.0
3	3105	9.4	12.4	3.0	7.6			5.9	6.8	0.7	0.5
4	3725	9.6	11.4	1.8	8.6	7.9	−0.7	5.7	6.4	0.7	0.5
5	3450	9.8	12.5	2.7	7.5	5.0	−2.5	5.5	5.7	0.2	0.15
6	5960	10.0	13.2	3.2	5.3	5.0	−0.3	5.7	6.3	0.6	0.45

* Values before 10 A. M. feeding.

† Values before 2 P. M. feeding, 4 hours after receiving 60 cc. of 10 per cent calcium lactate by gavage.

are administered the lesions are produced only in very young rabbits. Amounts of calcium lactate of equivalent calcium content produced no lesions, but the solubility of the calcium lactate is so low that concentrations equivalent only to 5 per cent $CaCl_2$ could be tested.

The effect of calcium lactate on the serum calcium in infants

The following work was undertaken in order to determine whether calcium lactate is effective in raising the concentration of calcium in the serum.

Methods: Six infants were studied; five were newborn, the sixth was four months old. Ten cubic centimeters of venous blood were withdrawn from the normal neonatal infants during the hour preceding their regular 10 A.M. feeding. Immediately following this they were gavaged with 60 cc. of a 10 per cent calcium lactate solution. The regular morning feeding was allowed. Preceding the 2 P.M. feeding, the second blood sample was taken. Blood samples were taken at similar intervals from the four-month-old baby. Blood serum was analyzed for calcium by the Clark-Collip modification of the Kramer-Tisdal method.[4] Serum phosphorus was determined by the Fiske-Subbarow colorimetric method, using a photoelectric colorimeter.[5] Total proteins were determined by the method of Barbour and Hamilton.[1]

Results: Three of the babies had mild diarrhea during the four-hour period following gavage. No vomiting occurred. Significant rise in the serum calcium (see table 2) occurred in all six cases. Initial calcium levels ranged from 9.4 to 11.7 mg.%. Levels taken four hours after gavage ranged from 11.4 to 13.2 mg.%. The largest increase occurred in the four-month-old baby. The levels of phosphorus were found to drop as the calcium level rose. This drop varied from 0.3 to 2.5 mg.%, or from 6 to 33 per cent of the original level. The serum proteins showed very small increases in concentration, varying from 0 to 0.7 gm. %, or from 0 to 12 per cent of the original value. The last column of table 2 represents the rise in serum calcium which might be due to the concomitant rise in proteins, calculated according to the equation $Ca = 0.75\,P + 6.8$. This equation uses the mean value of those found by five authors, as reported by McLean and Hastings.[11] It can be seen in Chart I that the average rise in serum calcium is not accompanied by a proportional rise in serum protein. The rise in protein could account for only a very small fraction of the calcium rise, the remaining rise being due solely to unbound calcium. The graph charts this rela-

tionship on the scale indicated by the above formula: i.e., 0.75 gm. of protein equals 1 mg. of calcium, protein equals 0 when calcium equals 6.8.

Discussion

It appears that present pediatric practice employs excessive and dangerous doses of calcium chloride for the newborn infant. For the symptomatic treatment of tetany, many pediatric text-books[3, 7, 9, 12] recommend doses of calcium chloride ranging from 2 to 4 gm., followed by repeated smaller doses administered in 10 per cent solution or even higher concentrations. This dangerous practice probably arose from the fact that these doses were originally used for older infants suffering from rachitic tetany. Under these circumstances the material was relatively harmless. Improved knowledge and practice of infant nutrition has reduced the incidence of rachitic tetany, and at present tetany of the newborn is the commonest type treated in New Haven. A dose of calcium chloride not particularly excessive for an older child apparently is dangerous when administered to a newborn infant. The production of ulcers of the stomach in newborn rabbits with a 5 per cent solution of calcium chloride indicates the danger of this salt to the mucosa of the stomach of unweaned animals. Calcium chloride added to milk undoubtedly is less irritating than are aqueous solutions. Nevertheless, our experience indicates that calcium chloride, in any vehicle, is probably dangerous to newborn babies.

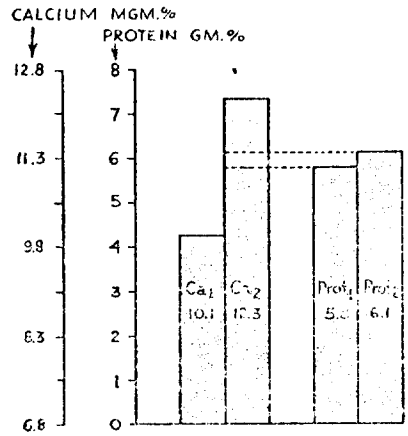

CHART 1

The chart shows the average concentrations of calcium and protein in serum before and four hours after administration of calcium lactate. The scale of the protein is equivalent to the calcium bound to serum protein and permits direct comparison of the rise in calcium due to rise in serum protein concentration. The chart illustrates that administration of calcium lactate produced a rise in unbound calcium.

It is noteworthy that other calcium salts are ineffective when given by mouth in the treatment of tetany of infants. This lack of therapeutic result is not explained by the lower calcium content, which is roughly one-third and one-fourth, respectively, for the

lactate and gluconate. The absence of a rise in serum calcium in cases of tetany is hard to reconcile with the easily demonstrated rise in serum calcium following oral administration of calcium lactate in normal newborn babies, adults,[2,10] and experimental animals.[8] In five recent trials, calcium gluconate (5 to 10 cc. of a 10 per cent solution) given intravenously has immediately relieved convulsions, but in two cases signs of tetany and low serum calcium have persisted for several days. The method of treatment of tetany of the newborn needs re-examination in order to find a method as effective as oral calcium chloride without its potential dangers.

Summary and conclusions

Necrosis and ulceration of the stomach, and in one instance of the small intestine, occurred in two infants who died after receiving calcium chloride by gavage. Similar lesions were reproduced in rabbits with comparable and with smaller doses of calcium chloride. Equivalent and larger doses of calcium lactate produced no anatomical changes in rabbits. The latter salt administered to six newborn infants was effective in raising the serum calcium.

Calcium chloride, as commonly recommended, is damaging to the stomach and intestines. Its administration by gavage to newborn infants is contraindicated.

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STUDIES OF TETANY

I. THE EFFECT OF CALCIUM CHLORID INGESTION ON THE ACID-BASE METABOLISM OF INFANTS *

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It has for sometime been a matter of observation in this clinic, and no doubt elsewhere, that infants receiving calcium chlorid for relief of the symptoms of tetany will occasionally develop hyperpnea. In these instances a marked lowering of the plasma bicarbonate may be demonstrated.

It is possible to explain an acid effect from the ingestion of calcium chlorid by assuming a more complete absorption of the chlorid ion (Cl^-) than of the calcium ion (Ca^{++}) from the gastro-intestinal tract. The probability that a large part of the calcium will form insoluble phosphates and soaps, and, in consequence, escape absorption affords ground for this surmise. If this is the case the uncovered chlorid ion must be provided before absorption with other base derived from the carbonates and dibasic phosphates of the food. The effect on acid-base metabolism of an excess of the chlorid ion over the calcium ion absorbed should be the same as would be produced by placing in the food an equivalent amount of hydrochloric acid, except to such extent as the calcium ion released from the chlorid ion interferes with the absorption of other inorganic acids and thereby offsets the increased absorption of the chlorid ion. The only other accessible inorganic acid in the food is phosphoric. A lowering of absorption of this acid by the calcium ion from ingested calcium chlorid will doubtless, to some extent, occur, owing to an increased formation of insoluble phosphates. Since, however, much of the calcium from ingested calcium chlorid may reasonably be assumed to be carried into the stools by organic acid products of digestion, a large part of an excess of the chlorid ion over the calcium ion absorbed would not be compensated for; interference with the absorption of these organic acids being of no moment from the point of view of acid-base equilibriums within the body.

The efficacy of calcium chlorid administration in relieving the symptoms of tetany being well established, definite information regarding

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the manner and extent of its acid effect is desirable. The experiments to be described in this paper were undertaken with the purpose of obtaining data on this point.

PLAN OF STUDY

The plan of study consisted in obtaining certain data from analyses of stools, urine and blood serum during a fore period, and then repeating the measurements during a period in which calcium chlorid was administered. The same diet, a usual one, was continued throughout. The data obtained from the fore period were used as a basis by means of which alterations in acid-base values during the calcium chlorid period could be noted and approximately measured. Two infants were studied in this way; Infant W., 5 months old, weighing 5.9 kilograms, regarded for the purposes of this study as a normal infant, and Infant H., 4 months old, weighing 4.3 kilograms and presenting, before calcium chlorid was given, marked symptoms of tetany. Infant W. was given each day 1,000 c.c. of 10:20 milk, with 5 per cent. added cane sugar. Infant H. received each day 700 c.c. of a 12:20 mixture, with 5 per cent. of added cane sugar. During the calcium chlorid periods, 2 gm. of calcium chlorid each day were added to the milk of the normal infant, and the infant with tetany was given 1.5 gm. each day.[1] The urine and stool collection periods were of three days' duration, except the fore period in the case of the infant with tetany, which, because of the active symptoms manifested, had to be abbreviated to twenty-four hours. Information as to the cause of the acid effect from calcium chlorid ingestion was sought in the data obtained from the stools. The size of this effect was estimated by means of measurements made in the urine. Bicarbonate and chlorid concentrations in the plasma were determined with the purpose of obtaining, if possible, indication of the direct cause for the lowering of the former. The effect of calcium chlorid ingestion on the excretion of fixed alkali in the urine was also directly measured. The findings are given below in the order just indicated. The methods used in obtaining them were these: Sodium, potassium, calcium and magnesium were determined in blood serum, urine and stools by the methods of Kramer and Tisdall.[2] The bicarbonate of the plasma was measured by the method of Van Slyke.[3] For chlorids in the plasma a combination of Van Slyke's plan of precipitating the protein with picric acid and Volhard's thiocyanate

1. Holt and Howland (Diseases of Infancy and Childhood, New York, D. Appleton and Co., 1917) recommended from 30 to 60 grains (2 to 4 gm.) of calcium chlorid daily for control of the symptoms of tetany. The degree of the acid affect following the administration of calcium chlorid in usual doses may, therefore, be taken to be at least as large as was exhibited by these two infants.

2. Kramer, B.: J. Biol. Chem. **41**:263 (Feb.) 1920. Kramer, B., and Tisdall, F. F.: J. Biol. Chem. **47**:475 (Aug.) 1921. Tisdall, F. F., and Kramer, B.: J. Biol. Chem. **48**: 1 (Sept.) 1921.

3. Van Slyke, D. D.: J. Biol. Chem. **30**:347 (June) 1917.

titration was used.[4] The procedure was essentially the same as that recently published by Myers and Short.[5] The p_H and titratable acidity of the urine were determined by the methods of Palmer and Henderson.[6] Ammonia was measured in the urine by the method of Folin and Macallum [7] and urine nitrogen by the usual Kjeldahl method.

THE CAUSE OF THE ACID EFFECT FOLLOWING CALCIUM CHLORID INGESTION AS INDICATED BY DATA OBTAINED FROM STOOL ANALYSES

The measurements of calcium and of chlorid found in the stools are given in Table 1. Since in this study we are concerned with the calcium ion (Ca^{++}) as the base and with the acid value of the chlorid ion (Cl^{-}), the amounts found have been expressed as cubic centimeters of tenth normal acid or base; e. g., the values given for calcium indicate the number of cubic centimeters of a tenth normal solution of base

TABLE 1.—*Measurements of Calcium and of Chlorid in Stools: Values for Each Twenty-Four Hours.*

	Infant W., Normal		Infant H., Tetany	
	Ca C.c. N/10	Cl- C.c. N/10	Ca C.c. N/10	Cl- C.c. N/10
Added to milk, $CaCl_2$ period	365	365	270	270
Found in stools, $CaCl_2$ period	492	31	418	94
Fore period	272	17	166	9
From added $CaCl_2$	220	14	252	85
Per cent. from added $CaCl_2$	60	4	93	32

Estimated Cl metabolized without Ca.....Infant W. 207 — 14 = 193 c.c. N/10
From added $CaCl_2$.....Infant H. 252 — 85 = 167 c.c. N/10

which could be obtained from the amounts of calcium found. That there is actually a very much larger excretion of calcium than of chlorid from ingested calcium chlorid is shown in the table. Estimated by subtracting the amount of calcium found in the stools during the fore period from the amount found when calcium chlorid was given, 60 per cent. of the added calcium was excreted in the stools in the case of the normal infant. In contrast, estimated in the same way, only 4 per cent. of the added chlorid escaped absorption. With the 220 c.c. of tenth normal calcium from added calcium chlorid there appears in the stools but 14 c.c. tenth normal chlorid. It is, therefore, evident that other base than calcium was used in the metabolism of 220 — 14 = 206 c.c. tenth normal of the 365 c.c. tenth normal chlorid given. It cannot be assumed that 206 c.c. of tenth normal of the

4. The method used was an unpublished one devised by Dr. Dana W. Atchley.
5. Myers, V. C., and Short, J. J.: J. Biol. Chem. **44**:47, 1920.
6. Palmer, W. W., and Henderson, L. J.: Arch. Int. Med. **12**:153, 1913.
7. Folin, O., and Macallum, A. B.: J. Biol. Chem. **11**:523, 1912.

added chlorid was absorbed without calcium. Doubtless a part of the calcium represented by this value was absorbed and then excreted through the wall of the lower intestine. Since, however, this part does not carry chlorid with it into the stools, it may correctly be included in the calcium, measuring the extent to which absorbed chlorid is left to bind other base in the body fluids. The value thus found, 206 c.c. of tenth normal, it will be seen is more than half of the administered chlorid (365 c.c. of tenth normal). In the case of the infant with tetany (Infant H.), the ingestion of calcium chlorid resulted in the production of loose stools and in consequence a much larger proportion of the ingested calcium and also of chlorid appeared in the stools (Table 1). The estimated chlorid conveyed through the body fluids and excreted without calcium was, in this instance, $252 - 85 = 167$ c.c. tenth normal, again more than half of the chlorid (270 c.c. tenth normal) placed in the milk.

ESTIMATION OF THE ACID VALUE OF INGESTED CALCIUM CHLORID BY MEANS OF DATA OBTAINED FROM THE URINE

If the excess of the chlorid ion over the calcium ion absorbed were the only consequence of the addition of calcium chlorid to the food, the just estimated values for this excess could be taken as measurements of the acid effect of the calcium chlorid ingested. They would indicate that the calcium chlorid administered to these infants was, from the point of view of acid-base metabolism, an acid substance to the extent of more than half of its equivalence as hydrochloric acid. It is hardly likely, however, that other values for acid and base absorbed would be unaffected by calcium chlorid ingestion. The probability, for instance, that the absorption of phosphoric acid would be lowered as a consequence of an increased formation of insoluble calcium phosphates in the intestine has been mentioned. A measurement of the actual extent of the acid effect of calcium chlorid is best obtained by noting in the urine the extension of those factors whose purpose it is to cover an increase in the metabolism of acid as compared with that of fixed alkali.[8] Henderson[9] and Henderson and

8. The term "fixed alkali" is used to distinguish the bases which are contained preformed in the food; viz., sodium, potassium, calcium and magnesium, from the base ammonia which is derived from the metabolism of protein. Although ammonia is derived from organic substances, it may not, since it does not contain carbon, be correctly called an organic base. This fact makes it necessary to use the terms fixed alkali and ammonia, rather than inorganic and organic base, in describing the metabolism of the base. The differentiation of ammonia from fixed alkali is important from the point of view of acid-base metabolism, for the reason that ammonia is biologically a toxic ion and cannot be used to appreciable extent as a base within the body, and in consequence the base concentrations of the body fluids must be composed practically entirely of fixed alkali.

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Palmer [10] have shown that the extent to which acids are permitted to carry fixed alkali into the urine is controlled by (1) a regulation of the acidity of the urine, and (2) a regulated production ammonia. By the operation of these two factors of acid excretion, the base concentration of the plasma, which must be practically entirely fixed alkali, is sustained at a constant level. The necessity for this regulatory function of the kidney is indicated by the fact that even though the diet is a usual one, the quantity of acid substances claiming excretion in the urine is much larger than the intake of fixed alkali. It has been shown that this regulatory mechanism is widely extensible and will manage the excretion of large additions to the acid excretion without lowering of the base concentration in the plasma (Gamble, Ross and Tisdall [11]). Henderson [9] and Henderson and Palmer [10] have shown that a measurement of the titratable acidity of the urine plus the ammonia excretion measures the excess of acid excretion over fixed alkali excretion. The sum of these two measurements they have designated "total acid," i. e., that part of

TABLE 2.—*Increase in Ammonia in the Urine Following Calcium Chlorid Ingestion*

	Ammonia in Urine			
	Fore Period, Average 24 Hours, C.c. N/10	$CaCl_2$ Period		
		1st Day, C.c. N/10	2d Day, C.c. N/10	3d Day, C.c. N/10
Infant W. (normal)	116	194	275	306
Infant H. (tetany)	53	104	141	180

the acid excretion which has been accomplished without an expenditure of fixed alkali. Addition to the food of a substance increasing the discrepancy between the amount of acid claiming excretion and the amount of fixed alkali available for binding it in the urine should cause an equivalent extension of this "total acid" value in the urine. By the use of this principle we have undertaken to measure the acid effect of ingested calcium chlorid, using the values found for titratable acidity of the urine and ammonia excretion during the fore period as a basis from which to measure the extension of these values when calcium chlorid was given.

Ammonia, much the larger of the two factors conserving inorganic base, was determined in the urines day by day. The values found (Table 2) indicate very clearly that ingested calcium chlorid is, from the point of view of acid-base metabolism, an acid substance. Ammonia being derived at the expense of the neutral end-product of protein

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metabolism, urea, an extension of the ammonia factor can be well shown by noting the increasing proportion of the total urine nitrogen which is ammonia nitrogen, when, as in this case, the metabolism of protein remains a stationary value. This often used index of the degree of an acid effect, the "ammonia coefficient," is given in Table 3. These data are from the normal infant (W.) and will satisfactorily indicate that in terms of the "ammonia coefficient" the acid effect following calcium chlorid ingestion may be designated as large.

In Table 4 are given the measurements of urinary acidity and ammonia excretion and the sum of these measurements, "total acid," found for the fore and calcium chlorid periods. Subtracting the values

TABLE 3.—*Increase in "Ammonia Coefficient" Following Calcium Chlorid Ingestion in the Case of Infant W. (Normal)*

	Fore Period, Average 24 Hours	$CaCl_2$ Period		
		1st Day	2d Day	3d Day
Total nitrogen, gm.	1.61	1.67	1.72	1.69
Ammonia nitrogen, gm.	0.16	0.27	0.39	0.43
Percentage ratio of ammonia nitrogen to total nitrogen	10	16	22	25

TABLE 4.—*Extension of the Factors Regulating Fixed Alkali Excretion Following Calcium Chlorid Ingestion: Values for Each Twenty-Four Hours*

Subject		pH	Values for Each 24 Hours		
			Titratable Acidity, C.c. N/10	Ammonia, C.c. N/10	Total Acid, C.c. N/10
Infant W. (normal)	$CaCl_2$ period	5.5	67	258	325
	Fore period	6.2	58	116	174
	Increase		9	142	151
Infant H. (tetany)	$CaCl_2$ period	5.3	54	143	197
	Fore period	5.5	45	53	98
	Increase		9	90	99

for the fore period from those found when calcium chlorid was given, measurements of the extension of these values and of their sum are obtained. Provided the kidney has accurately carried out its regulatory function, the increase in "total acid" should measure the increase in the excess of acid claiming excretion over available fixed alkali caused by adding calcium chlorid to the food; i. e., the acid effect following ingestion of this neutral salt. As may be seen in the table, the acid value of the ingested calcium chlorid was found to be 151 c.c. tenth normal, and 99 c.c. tenth normal, for the normal infant and the infant with tetany, respectively. These values are considerably less than those obtained for the excess of chlorid over calcium metabolized obtained from the stool analyses (Table 1); namely, 193 c.c. tenth normal and 167 c.c. tenth normal. Nevertheless, they indicate that a

very considerable part of the chlorid given as calcium chlorid was metabolized in the same way as would have been the case if placed in the food as hydrochloric acid. The acid equivalence of the calcium chlorid given may be estimated as $\frac{151}{365} = 0.41$ in the case of the normal infant, and for the infant with tetany as $\frac{99}{270} = 0.37$. In other words, calcium chlorid was, from the point of view of the acid-base metabolism of these infants, an acid substance to the extent of about 40 per cent. of its equivalence as hydrochloric acid.

THE DIRECT CAUSE OF THE LOWERING OF THE SERUM BICARBONATE FOLLOWING CALCIUM CHLORID INGESTION AS INDICATED BY DATA OBTAINED FROM THE BLOOD SERUM

In obtaining the foregoing estimate of the acid value of ingested calcium chlorid, the premise is used that the regulatory function of the kidney has been accurately operative in sustaining the normal total base (fixed alkali) concentration of the serum. Direct test of the

TABLE 5.—*Measurements of Fixed Alkali in Blood Serum from Infant H, Before and After Calcium Chlorid Ingestion*

	Before $CaCl_2$ Period, C.c. N/10 per 100 C.c.	At End of $CaCl_2$ Period, C.c. N/10 per 100 C.c.
Sodium	139.0	138.2
Potassium	6.1	5.6
Calcium	2.6	4.0
Magnesium	1.5	0.9
Total base	149.2	148.7

accuracy of the regulation of fixed alkali excretion may be made by measuring the total concentration of fixed alkali in the blood serum. This measurement involves separate determinations of the four bases, sodium, potassium, calcium and magnesium. The recently devised methods of Kramer and Tisdall makes this a feasible project. The four measurements were made in the case of Infant H. before and after the administration of calcium chlorid, and the data obtained are given in Table 5, the values being expressed as cubic centimeters tenth normal base per hundred cubic centimeters of blood serum. The sum of the four values before and after calcium chlorid administration are approximately the same, their agreement being well within the limits of error (taken to be $\pm$ 4 per cent.) in obtaining a measurement of the total base in this way.

The significance of these data is that the metabolism of extra acid from calcium chlorid has not disturbed the concentration of the total base in the blood serum. It is, therefore, not possible to explain a lowering of bicarbonate concentration in the serum, following calcium chlorid ingestion, as a consequence of decrease in the concentration of the base. There was found, nevertheless, a marked lowering of the

bicarbonate concentration in the serum in the case of both the normal infant and the infant with tetany following administration of calcium chlorid. The extent of this lowering is seen in Table 6; 24 per cent. and 21 per cent., respectively, in the case of the normal infant and of the infant with tetany. Since it is impossible to explain this decrease in bound carbonic acid as a consequence of base loss from the serum, there remains the possibility that an increase in the concentration of acid substance (or substances) has replaced a part of the bound carbonic acid. The acid value which might be expected to rise in the blood serum following calcium chlorid ingestion is obviously the chlorid ion concentration. Measurements of the chlorid concentration in serum samples from Infant H. (tetany) before and after giving

TABLE 6.—*Measurements of Bicarbonate Concentration in Serum Before and After Calcium Chlorid Ingestion*

	Infant W. (normal), per Cent. of Bound Carbon Dioxid by Volume	Infant H. (tetany), per Cent. of Bound Carbon Dioxid by Volume
Before $CaCl_2$	54.1	50.4
After $CaCl_2$	40.9	40.0
Decrease	13.2	10.4
Percentage of decrease	24	21

TABLE 7.—*Comparison of Chlorid Increase with Bicarbonate Reduction in Serum from Infant H., Before and After Calcium Chlorid Ingestion*

	Cl^-,* C.c. N/10 per 100 C.c. Serum	HCO_3^-, C.c. N/10 per 100 C.c. Serum
Before $CaCl_2$	106	23
After $CaCl_2$	112	18
	+6	−5

* Chlorids as grams NaCl per thousand cubic centimeters: Before $CaCl_2$, 6.13 gm.; after $CaCl_2$, 6.53 gm.

calcium chlorid showed a very considerable rise in the chlorid concentration of the serum at the end of the calcium chlorid period; from 6.13 to 6.53 gm. as sodium chlorid per liter. The increase in bound hydrochloric acid was, moreover, approximately equivalent to the decrease in bound carbonic acid. The lowering of bicarbonate in the case of this infant is thus adequately explained by a rise in chlorid concentration, and assumption of a loss of the base from the serum is, therefore, not required. The data demonstrating this point are given in Table 7.

In order further to illustrate the manner in which increase of chlorid in the serum may be expected to diminish the concentration of bound carbonic acid, assuming that the total base remains stationary, the diagrams in Figure 1 were prepared. The left side of

each of the diagrams represents the total base concentration, expressed as cubic centimeters tenth normal base per hundred cubic centimeters of serum, as determined by the four measurements given in Table 5. On the right side of the diagram, the values for the acids which bind the base in the plasma are indicated. Beginning at the top, and using the measurements given in Table 7, the concentrations of bicarbonate (HCO_3^-) and then of chlorid (Cl^-) are laid off. The sum of the usual concentrations of three other acids known to be carried in the plasma, phosphoric, sulphuric and certain organic acids, is indicated by the small block below the measurement of the chlorid (Cl^-) concentration.[12] After laying off the directly measured values for bicarbonate (HCO_3^-) and chlorid (Cl^-) and an assumed average value for the other three acids, there remains a portion of the base in the plasma in excess of the sum of these values. It is a reasonable surmise that this remainder of the total base is bound by the plasma proteins. At the reaction of blood plasma the proteins may correctly be regarded as acid substances. The diagrams indicate fairly completely the character of the acid-base composition of the plasma. It may be noted that four of the acids in the plasma, hydrochloric, phosphoric, sulphuric and the organic acids, are being conveyed for excretion in the urine and that one of them, hydrochloric acid, is carried at a comparatively huge concentration covering nearly three fifths of the total base of the plasma. This fact is striking when it is remembered that, diet being a usual one, a twenty-four hour urine specimen contains approximately the same amount of each of these four acids. The other two acid values, bicarbonate (HCO_3^-) and protein, do not under normal circumstances enter the urine. Remaining in the plasma they have to do with stabilizing the reaction of the blood. As regards the character of the base in blood plasma, the fact that it is practically entirely fixed alkali may be illustrated by stating that the concentration of ammonia in blood plasma would be approximately represented by the thickness of the line at the top of the diagram. In contrast to this minute concentration in the

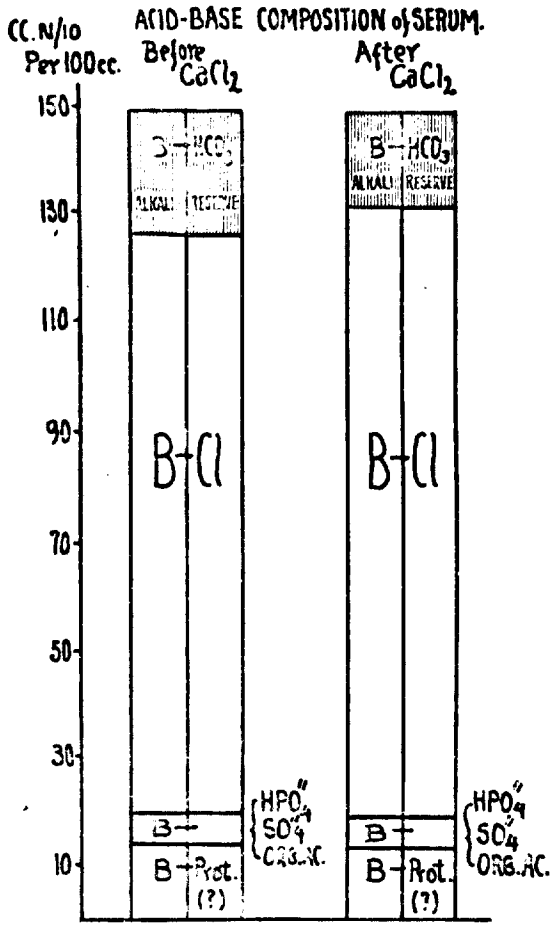
12. The base bound in the plasma by the usual concentrations of the normal phosphate ion ($HPO^=$), of the sulphate ion ($SO_4^=$) and organic acids together is here taken as 6 c.c. tenth normal per hundred cubic centimeters of plasma. From published data the base bound as phosphate may be taken as 2.3 c.c. tenth normal per hundred cubic centimeters, and as sulphate as 1.7 c.c. tenth normal per hundred cubic centimeters. The concentration of the organic acids normally present in the plasma has not been accurately measured. Attempts at such a measurement in this laboratory by one of the authors (Ross), although unsatisfactory from the point of view of a sufficient accuracy, at least indicate that this concentration is a very small one. On this premise it has been assigned 2 c.c. tenth normal per hundred cubic centimeters. It may also be noted here that the base equivalence of the normal phosphate (HPO_4) per molecule indicated in the accompanying chart as 2 is actually, according to Sörensen's data, 1.8 at the usual reaction of blood plasma.

plasma is the fact that under usual conditions, as regards diet, from one third to one half of the base in urine is found to be ammonia. There is thus indicated the fact that the sum of the acid substances derived from a usual diet and claiming excretion in the urine is much in excess of the intake of fixed alkali, and the necessity for a regulated use of ammonia in the excretion of these acid substances in order that the base in the plasma may be sustained is apparent. The manner of the effect of an increase in the concentration of chlorid (Cl^-) on the bicarbonate (HCO_3^-) of the plasma may now, with the help of these diagrams, be briefly indicated. This effect is a consequence of the peculiar character of the concentration of bound carbonic acid, which may be described by stating that the block on the right side of the diagram indicating bound carbonic acid is automatically adjustable and will change in size following alteration of any of the other values indicated in the diagram. This adjustability is due to the fact that carbonic acid is an extremely weak acid, and also that in the plasma there is an approximately sustained level of free carbonic acid. In consequence, loss of the base will be at the expense of bicarbonate; increase of a stronger acid will obtain base from bicarbonate, and, on the other hand, if, by any means, the amount of base available for use as bicarbonate is increased it will at once be bound by the free carbonic acid.[13]

In this instance, as is illustrated in the diagram, the reduction of bicarbonate is a consequence of an increase in chlorid (Cl^-), the base remaining stationary. The fact that total base was found to remain stationary is a point of considerable physiologic significance. Obviously, a decrease in bicarbonate could be compensated for by an increase in the base concentration. Apparently, however, the mechanism regulating fixed alkali excretion serves primarily the purpose of maintaining a constant level of the base in the serum and does not raise this level in behalf of a diminished bicarbonate. In other words, the regulatory function of the kidney is not, as is often inferred, directly in support of the "alkali reserve." The maintenance of a constant level of total base is thus indicated as a matter of greater importance than maintenance of the bicarbonate concentration at its usual size. The necessity for a

13. In this discussion the adjustability of the acid side of the accompanying chart to the base level is attributed entirely to the elasticity of the bicarbonate concentration. This is practically the case. It should be mentioned, however, that the phosphate and proteinate values (in terms of base bound) are not to be regarded as immovable in the presence of strong acids claiming base. Secondary phosphate and the proteinates being salts of weak acids will tend to supply the base at the expense of a shift in reaction due to release of acid phosphate and protein. In the case of bicarbonate the tendency to shift of reaction is rapidly offset by removal of carbonic acid by way of the lungs. It is owing to this fact chiefly that bicarbonate supplies nearly all of the base for neutralization purposes; i. e., it practically constitutes the "alkali reserve."

constancy of the total base concentration is understandable from the fact that, owing to the adjustability of the value for bound carbonic acid, it is the size of the base concentration which determines the total salt content of the plasma. The osmotic pressure of the plasma may, therefore, be said to be closely dependent on the mechanism sustaining



Acid-base composition of blood serum from infant H. before, and the third day following, calcium chlorid administration. Constructed from measurements of base given in Table [illegible], of chlorin and bicarbonate in Table 7, to illustrate reduction of the alkali reserve caused by increase in the chlorid (Cl^-) concentration.

the base concentration. It is difficult to imagine means of extensive compensation for the effect on osmotic pressure of alterations in the base concentration. The relatively less necessity for a constancy of bicarbonate concentration is emphasized by because of the known

extensibility of the other factors concerned in neutrality regulation (circulatory and respiratory) in compensation for a lowered alkali reserve.

THE SIGNIFICANCE OF AN INCREASED EXCRETION OF FIXED ALKALI IN THE URINE FOLLOWING CALCIUM CHLORID ADMINISTRATION

The measurements of the total base in the blood serum from Infant H. given above constitute evidence that the excretion of the extra acid when calcium chlorid was given was managed without appreciable depletion of the base concentration of the body fluids. In making this statement the premise is used that a normal concentration of the base in the serum may be taken to indicate that the concentrations

TABLE 8.—*Excretion of Inorganic Base Before and Following Calcium Chlorid Ingestion: Values for Each Twenty-Four Hours*

Base	Infant W.		Infant H.	
	Fore Period. C.c. N/10	$CaCl_2$ Period. C.c. N/10	Fore Period. C.c. N/10	$CaCl_2$ Period. C.c. N/10
Urine				
[illegible]	[illegible]	84.8	14.7	99.1
[illegible]	[illegible]	110.0	110.0	96.4
[illegible]	1.2	[illegible]	2.5	5.0
Calcium	2.8	17.5	4.0	4.5
Total	162.2	215.7	131.2	205.0
Increase	.....	53.5	.....	73.8
Stools				
[illegible]	16.1	20.0	4.4	43.5
[illegible]	[illegible]	62.0	19.7	[illegible]
[illegible]	[illegible]	46.7	22.5	[illegible]
Calcium	[illegible]	[illegible]	166.0	418.0
Total	[illegible]	[illegible]	212.6	546.0
Increase	[illegible]	270.1	.....	[illegible]
[illegible]	[illegible]	[illegible]	345	751
[illegible]	[illegible]	[illegible]	...	468
[illegible]	[illegible]	[illegible]	...	[illegible]

of base [illegible] water cannot be greatly alter[illegible] inference from the base [illegible] the urine from both [illegible] excretion during the [illegible] only in the instance [illegible] calcium which was [illegible] this increased base [illegible] table also includes [illegible] during the [illegible] of sodium [illegible]

volume of body water remained the same during the two experimental periods, such an inference would be unavoidable. It is a nearly certain premise, however, that the ingested calcium chlorid would exert a diuretic effect, with consequent decrease in the volume of body water. A reduction of body water would provide a quantity of inorganic base for use in the process of acid excretion without disturbance of the base concentrations in the remaining body fluids. With the evidence from Infant H. of a correctly sustained base level in the serum, it would seem reasonable to regard the increased base excretion as probably entirely a consequence of the diuretic effect of calcium chlorid. As indication that there was probably a considerable loss of body water, the increase in volume of urine during the calcium chlorid periods may be noted. During the fore period of Infant W., the average twenty-four hour volume of urine was 420 c.c., and during the calcium chlorid period this value was found to be 510 c.c. Infant H. voided only 270 c.c. the day before calcium chlorid administration. The average twenty-four hour urine volume for the three following days was 390 c.c. This large increase in the volume of urine is especially significant since it occurred in the presence of a considerable loss of water in the stools.[14] In definite agreement with the postulation of a loss of body water during the periods of calcium chlorid administration, losses in body weight were in both instances measured, Infant W. losing 90 gm., and Infant H., 300 gm.

As an objection to the inference of a loss of body water in explanation of the increased excretion of sodium, potassium and magnesium, it might be argued that the increased excretion of these bases was offset by an increased retention of the added base calcium. It may, indeed, be derived from the data in Table 8 that in the case of Infant W. the increased retention of calcium was larger than the combined increase in excretion of the three other bases. This increase in calcium retention may be estimated by subtracting the increase in excretion from the calcium added to the milk, thus, from Table 8, 510 — 275 = 235 c.c. tenth normal increase in excretion of calcium when calcium chlorid was given. Subtracting this value from the 365 c.c. tenth normal calcium added to the intake during this period, 130 c.c. tenth normal calcium is indicated as the increase in calcium retention following calcium chlorid administration. The increased excretion of the other three bases was 327 — 238 = 89 c.c. tenth normal and was, therefore, more covered by the

14. The stools of Infant H. (tetany) were, during the fore period, small in amount, formed and dry. During the calcium chlorid period they became definitely diarrheal in character and contained 140 c.c. of water each twenty-four hours. The stools of Infant W. (normal) contained rather more than a usual amount of water, during both the fore and the experimental periods, their water content for each twenty-four hours being 70 c.c. and 110 c.c., respectively, during the two periods.

increased retention of calcium. It would be almost certainly incorrect, however, to assume that the retained calcium replaced in the body water the lost sodium, potassium and magnesium. Calcium being held at a constant and relatively very small concentration, it is scarcely likely that in this normal infant this level would be raised. In all probability the extra calcium retained was added to the calcium deposits of the body. In the case of Infant H., owing largely to the loose stools, the increase in the excretion of sodium, potassium and magnesium (155 c.c. tenth normal) was greatly in excess of the slight increase in calcium retention (17 c.c. tenth normal). Insomuch as sodium, potassium and magnesium, in contrast to calcium, are presumably present in the body entirely in solution in the body water, it seems reasonable to infer a reduction of the volume of body water from an increased excretion of these bases, provided evidence can be found, as in this instance, indicating that base concentrations in the body fluids have been correctly sustained. We are thus led to conclude that the acid effect of calcium chlorid; i. e., the increase in the excess of acid over fixed alkali claiming excretion, is probably entirely covered (and completely measured) by the increase in the titratable acidity of the urine, together with the increased ammonia excretion, and that the extra base found in the urine is a consequence of reduction of the volume of body water and does not represent depletion of base concentration within the body. The indicated ability of the kidney to sustain a constant base concentration in the body fluids under circumstances causing both a large alteration in the excess of acid over base claiming excretion and a considerable change in the volume of body water is worth admiring.

The importance of taking into account in any study of the metabolism of inorganic substances the possibility of alterations of the volume of body water is, we believe, worth emphasizing. Particularly in the case of sodium, potassium, chlorid and phosphate,[15] because of the high concentrations at which these ions are held in the body fluids, a marked alteration of the amounts eliminated will be a necessary consequence of relatively slight changes in the volume of body water. That fluctuation of this value is not an improbability is indicated by the irregularities of the day to day weight curve of a normal infant. In the case of malnourished infants these irregularities are often of such size as must very greatly effect the amounts of inorganic substances eliminated. That

15. The conspicuous [illegible] of the chlorin concentration may be seen in the accompanying chart. The [illegible] larger concentration of sodium may be indicated by the statement that it constitutes 92 per cent. of the base in blood plasma. Katz (Arch. f. d. ges. Physiol. 63:13, 1896) measurements of the inorganic constituents of [illegible] muscle demonstrate that potassium is the chief base in [illegible] considerable concentration of phosphates in [illegible]

this factor will largely determine the excretion of inorganic substances, especially those mentioned above, following ingestion of unusual amounts of various salts, would seem to be altogether probable. It is our impression that many of the data obtained to represent the metabolism of the minerals have been incorrectly interpreted from failure to take into account the possibility of alterations of the volume of body water. Particularly do we wish to emphasize the incorrectness of inferring an alteration of concentrations within the body from the finding of an increase or decrease in elimination.

CONCLUSIONS

The following conclusions as regards the effect of calcium chlorid ingestion on acid-base metabolism are offered with the reservation that they rest upon data obtained from but two infants.

Ingested calcium chlorid in consequence of the relatively much greater absorption of the chlorid ion (Cl^-) than the calcium ion (Ca^{++}) behaves as does an acid substance. The effect on acid-base metabolism of the ingestion of 1 gm. of calcium chlorid may be described as of the same extent as would be produced by placing in the milk 75 c.c of tenth normal hydrochloric acid. The considerable increase in the excess of acid over fixed alkali claiming excretion in the urine is apparently accurately compensated for by an increase in urinary acidity and of ammonia excretion, with the result that the normal base concentration of the blood plasma is sustained. The lowering of bicarbonate following calcium chlorid ingestion is, therefore, not a consequence of depletion of the plasma base. It is found to be due to a rise in chlorid (Cl^-) concentration, displacing an equivalence of bound carbonic acid.

Following calcium chlorid ingestion there occurs a moderately large increase in the excretion of fixed alkali, especially of sodium and potassium in the urine. This is correctly to be regarded as a consequence of a reduction of the volume of body water, and not as indicating withdrawal of base from body fluids.

The total base concentration of the serum is sustained at a constant level by a remarkably accurate regulation of fixed alkali excretion in the urine. This regulation is successfully accomplished in the presence both of a large increase in the excess of acid over base claiming excretion and of a considerable change in the volume of body water. It is to be noted, moreover, that the normal total base concentration is not altered in behalf of a lowered bicarbonate.

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(Report from the II. Intern Clinic of the Royal Hungarian Pazmany Peter University in Budapest
Director: Prof. Dr. Ladislaus von Ketly)

THE ABSORPTION OF CALCIUM CHLORIDE ADMINISTERED PERORALLY IN A PHYSIOLOGICAL SODIUM CHLORIDE SOLUTION

Report I.

by
Dr. Ceza Gerloczy and Dr. Gabor Berencsy

(Entered on July 4, 1930.)

As early as 1886, Gumilewski, from the Haidenhain Institute, found that water absorption can be increased by the administration of sodium chloride; substances dissolved in water (sugar, peptone) are also absorbed better, if some sodium chloride is present in the solution as well.

It seems that the experiments recently carried out by Lasch, in which the physiological sodium chloride solution promotes the absorption of calcium, can be interpreted in the same manner.

Lasch, employing a precise method, proved that, of the $CaCl_2$ introduced into the isolated, surviving small intestine of the guinea pig, under perfectly equal experimental conditions, about 230% more calcium disappears when the liquid introduced into the surviving intestine contains sodium chloride in a relatively high concentration (0.9%), then when no NaCl is found in the solution. This striking difference interested us in testing these relations in the human as well. In the case of "in situ" experiments, the ratios are much more developed, so that it is not entirely possible to pursue the absorption ratios of the orally introduced solution in every detail. On the other hand, the physiological preconditions undoubtedly create a more favorable situation for the evaluation of the results.

Through determination of the blood calcium, one can probably observe the absorption ratios of the calcium introduced into the system quite accurately. In short-term comparative experiments, the control of the simultaneous Ca-elimination can be disregarded, all the more because in the case of such experiments the comparison of the absolute values is of less importance than that of the values relative to the control experiments. That the perorally administered Ca raises the blood level can, despite many objections, be considered as good as proven today. Of the literary contributions applying to this matter, those of Lasch and Neumayer and Astanin are the definitive ones.

Under normal conditions, the calcium entering into the gastro-intestinal tract can raise the serum calcium level consider-

ably after 30-60 minutes. This effect fades after about seven hours. The contradictions which have hitherto existed can be explained by the smallness of the doses used, the not always successful technique of the regular control of the Ca-absorption, and primarily by the varying solubility of the individual Ca-salts. It was revealed by Irwing's experiments, that the solution buffered with acetate creates the most favorable conditions for absorption; the neutral chloride produces less favorable conditions, and the calcium buffered with lactate the least favorable. According to Jansen, calcium bicarbonate works best, then calcium chloride, calcium sulphate, calcium hydrophosphate, and calcium lactate, while calcium acetate causes no Ca-increase.

In our own experiments, we used Merck's calcium chloride in a concentration of 10%, and introduced it into the system in such a quantity that to 1 kg of the body weight of the individual there corresponded 0.15 g calcium chloride. We had the patient drink these 8-10 g of calcium chloride (in a 10% solution) in the morning, on an empty stomach. Apart from its somewhat unpleasantly biting taste, this liquid was rather easily consumed. The withdrawals of blood were made from the cubital vein at half-hour intervals; the last blood sample was taken after 7 hours. We determined the serum calcium content according to the procedure of Kramer-Tisdall. For the experiments we selected individuals whose condition or ailment could create no disturbing influence in respect to the permeability ratios. We took special consideration of menstruation, possible pregnancy, as of every disease or ailment connected with the endocrine system in general, and did not employ such cases in our experiments.

TABLE I. Blood calcium values after peroral administration of $CaCl_2$.

Nr.	Geschlecht	Körpergewicht in kg	$CaCl_2$ – 10%		Serum-Calcium					
			ccm	Ca g	Ausgangswert	Erhöhung nach ½ Std.	Erhöhung nach 1 Std.	Erhöhung nach 7 Std.	Maximaler Unterschied	in % zum Ausgangswert
14	♀	80	120	2,64	11.40	12.40	11.90	11,60	1,0	8,8
15	♀	80	120	2,64	10.50	12.40	12.20	10,90	1,9	18,1
16	♀	70	100	2,20	11,21	11,83	11,74	10,85	0,62	5,5
17	♀	52	78	1,72	10,72	12,19	11.74	10.50	1,47	13,7

Key:
1.= sex 2.= body weight in kg 3. initial value 4.= increase after
5.= maximum difference 6.= in % to initial value
Std.= hour

In further experiments it had to be decided what increase the serum calcium level reveals in the same individual, when the calcium chloride is introduced in a physiological sodium chloride solution.

The experiment was carried out using the above described procedure. After completion of the experiment with the aqueous calcium chloride solution, we determined the serum calcium value on several rest days as well, and only established further experi-

ments when the serum calcium concentration definitely reached its initial value. On the following morning, we administered 10% calcium chloride dissolved in a physiological sodium chloride solution in a quantity corresponding to the above procedures. The patient ingested the solution immediately.

The following table shows the increase of the serum calcium level resulting from the intake of aqueous and salt-containing calcium chloride in a comparative manner in one and the same patient. (Because of lack of space, only some of the equivalent results have been used.)

Table 2.

Nr.	Geschlecht	Körpergewicht in g	10% $CaCl_2$	Serum-Calcium: Ausgangswert	Erhöhung nach ½ Std.	Erhöhung nach 1 Std.	Erhöhung nach 7 Std.	Maximaler Unterschied	%
1	♀	51	76 ccm 1,71 g Ca mit 0,9% NaCl	11,02	12,21	11,90	10,88	1,19	10,8
				11,13	12,28	13,13	12,10	2,00	18,0
2	♂	60	90 ccm 1,98 g Ca mit 0,9% NaCl	11,70	13,20	12,90	11,80	1,50	12,8
				13,90	18,00	15,70	14,90	4,10	30,0
3	♀	47	70 ccm 1,54 g Ca mit 0,9% NaCl	11,60	13,50	13,20	11,70	1,90	16,4
				12,00	17,10	15,20	11,80	5,10	42,5
4	♂	55	82 ccm 1,8 g Ca mit 0,9% NaCl	11,20	12,50	12,90	11,60	1,70	15,2
				11,00	14,50	13,60	12,60	3,50	31,8

For key see table 1.

In all the cases experimented upon, the calcium chloride administered in equal quantities in a physiological salt solution yielded a significantly higher blood calcium level than the calcium introduced in an aqueous solution. The duration of absorption from the aqueous and saline solutions was approximately equal, and at its maximum was the same amount of time. The greatest deviation was observed in the cases designated under Nr. 3: 17.10 mg %, which corresponds to an increase of the original calcium value (12.0) by 42.5%. In case nr. 2 it was notable that the initial value itself was higher than usual, and that the reaction had not yet diminished after 7 hours (the patient suffered from chronic neurectopia). Apparently the elimination was also inhibited. The average increase-plus in contrast to the aqueous calcium value amounted to 3.31 mg%: i.e. around 1.40% more calcium was absorbed when NaCl was administered simultaneously.

Table 3. Comparison of the average values of the results of the experiments.

Anwendungsart	Ausgangswert	Serum-Calcium: Erhöhung nach ½ Std.	Erhöhung nach 1 Std.	Erhöhung nach 7 Std.	Differenz in mg	Differenz in % des Anfangsgehalts an Ca des Blutes	Mehr Ca resorbiert in mg	das sind um x% mehr als nur nach wässeriger $CaCl_2$-Lösung
0,9% NaCl + 10% $CaCl_2$	11,92	15,23	14,40	12,90	3,31	27,8	1,95	143
10% $CaCl_2$	11,17	12,53	12,31	11,22	1,36	12,1	—	—

Key:
1. - manner of application 2. - initial value 3. - increase after
4. - difference
a) in % of the blood's initial content of Ca

Key cont'd:
5. - more Ca absorbed
a) x% more than only after aqueous $CaCl_2$ solution

On the basis of the foregoing, it can be assumed as proven that the calcium chloride administered in a physiological sodium chloride solution increases the blood calcium level to a more significant degree than the aqueous solution.

SUMMARY

1. The calcium chloride in 10% concentration, administered perorally in a total amount of 0.15 g per kg of body weight raises the blood calcium value within ½-1 hour at an average of 1.36 mg% (0.62-1.90); the increase is equalized after about 7 hours.

2. Under the same experimental conditions, the 10% calcium chloride dissolved in a physiological sodium chloride solution raises the blood calcium level to a much more significant degree. In certain cases, this increase can amount to more than 170% (143% on the average) of the control value increases.

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THE INFLUENCE OF THE PERORAL ADMINISTRATION OF $NaHCO_3$ AND NH_4Cl ON THE $CaCl_2$-ABSORPTION RATIOS

Report II

by
Dr. Geza Gerloczy and Dr. Gabor Berencsy

(Entered on July 4, 1930)

In our first report we attempted to prove that the $CaCl_2$ introduced in an NaCl-solution of relatively high concentration causes, under the same experimental conditions, a much greater blood calcium increase than the aqueous $CaCl_2$-solution.

In our further experiments we wanted to decide in what way the perorally administered $CaCl_2$ influences the blood calcium concentration, when the reaction environment is changed because of alkali administration or the use of ammonium chloride.

The potassium contractor grows with the rising pH (Gellhorn). Loeb and Nichols, in their experiments related to the calcium dialysis, pointed out the importance of the pH. Nakashima investigated the absorption, from equimolecular solutions, of K-, Na-Ca-, NH_4-chloride in a dog with Vella-fistula, and ascertained that the alkalescence of the blood supported the absorption, while its acidity decreased the absorption. This phenomenon is probably based on the swelling and permeability alteration of the cells, although one must also reckon with the alteration of the ionisation of the calcium, for both the absorption and the elimination are most dependent on the H-ion concentration. Loeb proved this in relation to the calcium diffusion in the serum. In consequence of all this, it seemed interesting to make the calcium absorption in cases of acidification and alkalization of the organism the subject of an experiment. Such an experiment promised most interest from a clinical point of view, in regard to the Kraus-Zondek K-Ca-vegetative nervous system theory, the Grant-Goldmann hyperventilation tetany, or the Porges-Adlersberg neurotic respiration tetany question.

Although the alkalization or acidification of the organism provoke above all a more intensive balance, which keeps the blood and tissue reaction within certain narrow limits, it is not to be denied that with continuing administration of alkalis the blood pH, and evidently also the tissue reaction will deviate toward the alkaline direction temporarily, and after administration of acid ammonium salts or ammonium chloride, toward the acid direction (Balint, Haldane, Porges, Adlersberg, Leindorfer,

Markovici).

For our experiments we selected patients who suffered only from slight complaints, with the exception of metabolism or ulcer ailments and those with endocrine diseases; therefore those in whom the reaction environment could be seen as labile or deviating were excluded from the experiments.

Experimental method. -- In one and the same individual we determined above all the rise of the blood calcium level after administration of 10% calcium chloride solution (15 cg to every kg of body weight). We further determined the blood-pH according to the Hollo-Weiss method. On 3 successive days, at hourly intervals, we administered, between 8 o'clock in the morning and 8 o'clock in the evening, a total of 200 g $NaHCO_3$, or for 3 days 8 g, or a total of 24 g ammonium chloride in an aqueous solution. On the morning of the fourth day the pH determination was made again on an empty stomach, whereupon the patient took the prescribed amount of calcium chloride in a 10% solution. In this way we examined 20 cases. (In view of the equivalent results, only a few are reported on the following table.)

TABLE 1.

	Nr.	Geschlecht	Körpergewicht in kg	10% $CaCl_2$ ccm	10% $CaCl_2$ Ca g	Serum-Calcium: Ausgangswert	Erhöhung nach ½ Std.	Erhöhung nach 1 Std.	Erhöhung nach 7 Std.	Maximaler Unterschied	%
Bei NH_4Cl	9.	♀	85	125	2,75	11,80	13,10	13,10	11,90	1,30	11,—
	10.	♀	80	120	2,75	10,10	12,10	10,80	10,20	2,—	20,—
	11.	♀	55	80	1,76	11,—	11,90	12,10	11,20	1,10	10,—
	12.	♂	70	100	2,2	10,78	11,92	11,65	10,52	1,14	10,5
Bei $NHCO_3$	28.	♀	55	83	1,86	10,50	12,60	13,40	10,50	2,90	26,7
	29.	♂	75	107	2,35	11,60	15,50	15,40	11,40	3,90	33,60
	38.	♀	70	100	2,2	11,—	13,02	13,21	11,02	2,21	20,1
	39.	♀	52½	80	1,76	9,96	12,23	11,19	10,58	2,27	22,8

Key:
1.= sex 2.= body weight in g 3.= initial value
4.= increase after 5.= maximum difference

After alkalization, the pH values varied from 7.61-7.65: after ammonium chloride, on the other hand, they varied between 7.51 and 7.53. As is shown in the table, there was a higher serum calcium concentration with 10% $CaCl_2$ after 200 g $NaHCO_3$ in the ½ and 1 hour values in every case, than in those cases treated with ammonium chloride. The increase of the calcium level which can be ascertained in alkalized individuals is a significant one, which far exceeds both the Ca-values of the controls and the Ca-values of the cases treated with ammonium chloride. Also, in the majority of the cases treated with ammonium chloride we found lower values in comparison with the values obtained in the preceding control experiments. In other words: the rise of the blood calcium level after peroral administration of $CaCl_2$ assumes a middle position between the blood-pH-calcium values modified by intake of sodium bicarbonate or ammonium chloride. The calcium chloride administered with sodium hydrocarbonate raised the blood calcium concentration much more

significantly and consistently, than it lowered it when administered with ammonium chloride. The alkalization yielded, in comparison with the control experiments, on the average a 80-100% higher calcium concentration in the serum.

The patients tolerated these relatively large doses of calcium both in cases of alkalization and acidification very well. In one case (anacidity) the patient complained of dizziness and discomfort a few minutes after the intake of calcium chloride in combination with ammonium chloride; shortly thereafter there appeared intense salivation, with occasional burping. This condition began to improve after 5 or 6 hours, and after a meagre defecation ceased altogether. In this case, the blood calcium value amounted, after a half hour, to 0.22%. (It should be remarked that in this case, for the purpose of other experiments, larger doses of calcium had already been administered a few days before.) In this case the elimination of the calcium was also inhibited, since after 7 hours a calcium concentration exceeding the initial value by 18% could still be observed. Individual variations can thus cause essential deviations, which must always be taken into consideration in the inferring of consequences. Such individual changes relating to the calcium sensitivity also appear in animals of the same species. We have information on this in the reports of Lasch, Neumayer, and Astanin. These authors worked with relatively large calcium doses, which the greater part of the experimental animals tolerated well -- others died in the midst of intense toxic phenomena (cats -- hypercalcemia). In the lethal cases, the blood calcium level was still rather high after 7 hours.

Those clinical experiments according to which in basedowians (Leicher) circulatory debility, arterial hypotonia (Jansen) a generally lower than normal blood calcium value can be found, agree with our experimental results relating to an acid pH. Dresel assumes a relatively low calcium concentration in cases of sympathicotonia, a rather high one in cases of vagotonia. In sympathicotonic individuals the acid reaction of the tissues ionizes the calcium flowing in the blood, and it enters the cells, so that the blood calcium level sinks. The acidification therapy of tetany also seems to exercise its effect in this manner. In vagotonic individuals, the calcium is rather in complex compounds and is less ionized due to the alkalescence of the cells (Freudenberg). It is nonetheless beyond doubt that the ascertainments attributable to such a relation could not be called generally valid for a long time, since in many cases even the assertions made in the literature are very contradictory; though, however the question may seem, we can at least draw from our experiments the fact that in the same individual, the serum calcium concentration ½-1 hour after peroral administration of $CaCl_2$ after preceding large doses of alkali or in the case of a higher blood pH is greater than in the case of orally administered ammonium chlorate or a lower pH. The curve of the control values lies between the two, and in fact closer to the values of the acid blood.

We further sought to obtain a judgment on how the blood calcium level reacts when we administer the calcium chloride in a physiological sodium chloride solution after the treatment with $NaHCO_3$ or ammonium chloride. The following table shows the results of these experiments.

Table 2.

	Nr.	Geschlecht	Körpergewicht in kg	10% $CaCl_2$ in 0,9% NaCl		Serum-Calcium					
						Ausgangswert	Erhöhung nach			Maximaler Unterschied	%
				ccm	Ca g		1/2 Std.	1 Std.	7 Std.		
Bei NH_4Cl	5.	♀	61	90	2,0	12,50	14,50	12,50	11,50	2,—	16,—
	6.	♂	72	100	2,2	11,—	12,80	11,90	10,80	1,80	16,4
Bei $NaHCO_3$	21.	♀	60	90	2,0	10,70	13,—	11,70	10,80	2,30	21,5
	25.	♀	51	75	1,55	12,—	13,60	12,20	11,90	1,60	13,30

For key see table 1.

Acidification and alkalization hindered the calcium absorption-increasing effect of the sodium chloride solution in the same way, although the obtained values were also higher, as in the case of the administration of the salt-free calcium chloride used in the control experiments. In the latter, the average increase of the blood calcium was 1.36 mg%; after calcium dissolved in salt it was 3.31 mg%; with a lower pH without sodium chloride 1.12 mg%, with saltl.67 mg%; with a higher pH without salt 2.57mg%, with salt 1.95 mg%.

If we compare the results mentioned in our former report with those obtained by use of $NaHCO_3$ and NH_4Cl, the following sequence of Ca-absorption is formed:

$$NaCl > NaHCO_3 > \begin{matrix} NaHCO_3 \\ NaCl \end{matrix} > \text{normal} > \begin{matrix} NH_4Cl \\ NaCl \end{matrix} > NH_4Cl$$

Table 3.
Average values of the blood calcium concentration in the various absorption influences.

Anwendungsart	Ausgangswert	Serum-Calcium			Differenz		± Ca resorbiert	
		Erhöhung nach			in mg	in % des Anfangsgehalts an Ca des Blutes	in mg	das sind um x % mehr oder weniger als nur nach wässeriger $CaCl_2$-Lösung
		1/2 Std.	1 Std.	7 Std.				
10% $CaCl_2$	11,17	12,53	12,31	11,22	1,36	12,1	0	0
0,9% NaCl + 10% $CaCl_2$	11,92	15,23	14,40	12,90	3,31	27,8	+1,95	+143
NaCl+$CaCl_2$ neben NH_4Cl	11,35	13,02	11,72	11,—	1,67	14,7	+0,31	+22,7
.$CaCl_2$ neben HN_4Cl	10,93	12,05	11,86	10,90	1,12	10,2	—0,24	—18,3
NaCl+$CaCl_2$ neben $NaHCO_3$	11,35	13,30	11,95	11,35	1,95	17,2	+0,59	+43,8
$CaCl_2$ neben $NaHCO_3$	10,76	13,33	13,30	10,87	2,57	23,9	+1,21	+88

Key:
1.= method of use 2.= initial value 3.= increase after
4.= difference

4 a).- in % of the initial Ca content of the blood
5.- + Ca absorbed
a)- x% more or less than after only the aqueous $CaCl_2$ solution.

SUMMARY

1. In the case of continuing alkali administration (200 g $NaHCO_3$ within 3 days), the $CaCl_2$ (in 10% solution) introduced in a quantity of 0.15 g per kg of body weight raises the blood calcium value an average of 88% in comparison with the $CaCl_2$ administered under completely identical conditions, but without alkalization.

2. After 24 g ammonium chloride taken in within 3x24 hours, the $CaCl_2$ introduced in the above quantities raises the blood calcium concentration on the average 1.83% less than that administered without ammonium salt.

3. The $CaCl_2$ given with physiological NaCl reveals 43.3% in the case of alkalization, 22.7% with ammonium salt, thus a higher Ca-concentration than the control experiments.

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COMPARATIVE METABOLISM OF PHYTATE AND INORGANIC P^{32} BY CHICKS AND POULTS

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The nutritional role of phytin has interested biochemists for many years due to its widespread occurrence in cereals and other foods and feedstuffs of plant origin. The possible adverse effect of phytin on utilization of other minerals has been widely discussed (Harris, '55). More recently the availability of phosphorus from this source for growth and bone formation has received attention. Students of this problem seem to be agreed that there is much less efficient utilization of phytate than of conventional sources of inorganic phosphate (Lowe et al., '39; Krieger et al., '40; Krieger and Steenbock, '40; Heuser et al., '43; McGinnis et al., '44; Singsen et al., '47; Singsen, '48; Gillis et al., '49, '53). Avian species have been shown to be particularly inefficient users of phytin.

Since radioactive phosphorus has become generally available to investigators, an additional, and possibly more precise, method is at hand to study the manner in which phytate phosphorus is metabolized by animals. In particular, it is easier to study the immediate fate of administered phosphorus in the digestive tract and its metabolic dispersal in the blood and other tissues.

The only report on the use of radioactive phytin which has come to our attention is that by Singsen, Matterson and Kozeff ('50) who described its administration to turkey poults. After feeding a diet containing 1% labeled calcium

phytate for a period of two weeks, they found that P^{32} had entered the bones, indicating at least a limited utilization. Its retention was increased by supplying vitamin D. No comparisons were made with inorganic radioactive sources of P^{32}. The amount of radioactivity administered and the percentage of the dose retained were not detailed, although, presumably, 1% labeled phytate in the diet constituted a high level of dosage.

The work reported herein was carried out for the purpose of comparing P^{32}-labeled phytate with P^{32}-labeled inorganic orthophosphate as sources of phosphorus for chicks and poults. The influence of vitamin D on the utilization of both types of phosphorus was also investigated. *In vitro* studies were also made in an effort to determine whether or not chemical exchange occurs between phytin phosphorus and phosphorus from an inorganic source.

EXPERIMENTAL

Preparation of P^{32}-labeled calcium phytate

Phytate was isolated as the calcium salt from kernels of corn which carried P^{32}. The corn was kindly prepared for our use by Dr. J. D. Sayre of the Ohio Experiment Station, who accomplished the radioactive labeling by injecting a solution of P^{32} directly into the corn stalks as the grain approached maturity. The phytate was isolated from the mature, dry grain by the general method described by Singsen et al. ('50), but modified to increase the extraction time with 0.5 N HCl from three hours to 18 hours. One kilogram of corn yielded about 15 gm of P^{32}-labeled calcium phytate. The phytate was recrystallized from hot 20% acetic acid just prior to use in the feeding experiments to remove traces of inorganic phosphorus which may result from breakdown of phytin. The samples of radioactive phytate used in the separate studies with chicks and poults were prepared at different times from different batches of corn.

For comparison with phytate, disodium phosphate appeared to be the most practical source of P^{32}-labeled inorganic orthophosphate readily obtainable. Since it was administered as a solution in tracer quantities, solubility was not considered to be a factor in these studies.

Radioactivity measurements. The calcium phytate, obtained as described above, as well as the solution of radioactive disodium phosphate used in these studies were assayed for radioactivity. The phytin was dissolved in acetic acid and diluted for counting. Aliquots were evaporated to dryness on copper planchets and counted, in triplicate, in a windowless gasflow counter. The radioactivity in the materials fed experimentally was as follows: In the chick experiment, calcium phytate — 5.10×10^7 counts/min./gm; disodium phosphate solution — 6.59×10^6 counts/min./ml. In the turkey poult experiment: calcium phytate — 7.53×10^7 counts/min./gm; disodium phosphate solution — 4.275×10^6 counts/min./ml.

All samples of blood, bone ash and excreta collected in the experiments were also counted in triplicate in the windowless gas-flow counter. The bone ash and excreta were first dissolved in acid, diluted, plated and dried on copper planchets. The expressions for radioactivity found in the tissues and excreta and recorded in tables 2 and 3 have been corrected for decay of P^{32}.

Experiments with chicks. One-day-old White Leghorn male chicks were divided into two groups of 20 each. One group was given a vitamin D-deficient chick diet (table 1) while the other group received the same diet plus a supplement of 50 International Chick Units of vitamin D per 100 gm of diet. After 5 weeks on this dietary regime, 8 chicks of approximately equal weight were selected from each dietary group and placed in wire metabolism cages. Four chicks from the vitamin D-deficient group and 4 from the normal group received doses of radioactive calcium phytate during a two-day period. The remaining 4 normal and 4 vitamin D-depleted chicks received doses of radioactive inorganic phosphorus at the same time. The 8 chicks selected to receive inorganic

phosphate were given 4 separate doses of 1 ml each of a solution of $Na_2HP^{32}O_4$ carrying 6.59×10^6 counts/min./ml at the time the first dose was administered. Two doses were administered approximately 6 hours apart on the first day and the dosing was repeated twice on the second day at the same time interval. The 8 chicks selected to receive radioactive calcium phytate were given 4 separate doses of 127 mg

TABLE 1

Basal diets used for chicks and poults

INGREDIENT	VIT. D-DEFICIENT CHICK DIET	VIT. D-DEFICIENT POULT DIET
	%	%
Yellow corn meal	67.5	30.95
Soybean meal	25.0	50.0
Crude casein	5.0	
Corn gluten meal		10.0
Alfalfa meal		3.0
Dried brewers' yeast		2.0
Methionine		0.1
Salt	0.5	0.5
Tricalcium phosphate	2.0	
Dicalcium phosphate		2.25
Limestone		1.10
	per lb.	*per lb.*
$MnSO_4 \cdot H_2O$	100 mg	120 mg
Vitamin B_{12}	5 μg	10 μg
Riboflavin	1.5 mg	3 mg
Niacin	10 mg	10 mg
Calcium pantothenate	3 mg	5 mg
Choline chloride	454 mg	454 mg

each of this material (6.46×10^6 counts/min. at the time of the first dose). The $Na_2HP^{32}O_4$ solution was placed in the chicks' crops by means of a pipet. The calcium phytate was weighed into gelatin capsules and inserted directly into the chicks' crops. During the two-day dosing period the chicks continued to receive the same diets as previously, ad libitum.

Forty-eight hours after administering the initial dose of radioactive phosphorus from the two sources, blood samples were collected from the wing vein of each chick. The blood

was heparinized, centrifuged, and aliquots of plasma were plated and dried for radioactivity counting as described above. After collection of the blood samples the chicks were sacrificed and both tibiae removed. All excreta were collected during the experimental period. The tibiae from each chick were freed from adhering flesh and ashed. The ash was prepared for counting by dissolving in hydrochloric acid and diluting suitable aliquots with distilled water. The excreta were similarly ashed, dissolved in hydrochloric acid and diluted with distilled water.

TABLE 2

Results of chick study; radioactivity measurements made after dosing with equivalent quantities of isotope carrier

DIETARY HISTORY	P^{32} CARRIER [1]	RADIOACTIVITY OF TOTAL PLASMA	RADIOACTIVITY OF 100 MG TIBIA ASH	% DOSE RETAINED/ 100 MG TIBIA ASH	% DOSE EXCRETED
		av. counts/ min.	*av. counts/ min.*[2]		
Vitamin D-deficient	Ca phytate	3.2×10^3	6.0×10^3	.025	76.6
Vitamin D-deficient	$Na_2HP^{32}O_4$	1.1×10^5	2.95×10^5	1.180	23.2
Vitamin D-adequate	Ca phytate	9.6×10^3	2.20×10^4	0.09	56.5
Vitamin D-adequate	$Na_2HP^{32}O_4$	9.6×10^4	2.57×10^5	1.040	18.5

[1] A total of 4 doses of isotope carrier were administered to all chicks over a two-day period. The initial dose was administered 48 hours and the final dose approximately 18 hours before the chicks were sacrificed.

[2] Corrected to a standard body weight of 200 gm.

The radioactivity of the blood is shown in table 2, expressed as counts/min./total plasma. Since the normal chicks were heavier than the vitamin D-deficient chicks and would, therefore, contain more blood, the radioactivity in the normal chicks would necessarily occupy a greater volume. The quantity of total plasma was arrived at by assuming the blood to be 9% of the body weight and the plasma to comprise 70% of the blood. The radioactivity of the blood plasma of normal chicks which received inorganic P^{32} was 10 times greater than that of normal chicks which received a com-

parable quantity of phytin P^{32}. The vitamin D-deficient chicks showed an even greater disparity in the utilization of phytin and inorganic phosphorus. The radioactivity of the plasma of the vitamin D-deficient chicks was approximately 34 times as great after administration of inorganic P^{32} as was found following the administration of phytin P^{32}. It is of interest to note that the plasma of the vitamin D-deficient chicks contained slightly more inorganic P^{32} than the plasma of vitamin D-adequate chicks. This indicates no impairment in the utilization of the readily available inorganic phosphate due to vitamin D-deficiency. This is in marked contrast to the profound effect which vitamin D has been observed to have on the absorption of calcium (Keane, Collins and Gillis, '56).

The corrected counts/min./100 mg of tibia ash as well as the percentage dose retained per 100 mg of tibia ash are also presented in table 2. Since the normal chicks were heavier than the vitamin D-deficient chicks, these values have also been corrected to a standard body weight of 200 gm. These data show that chicks receiving the normal vitamin D-adequate diet retained 11 times as much radioactivity per unit of tibia ash following administration of inorganic phosphorus as they retained from phytin phosphorus. Chicks which received the vitamin D-deficient diet retained 47 times as much radioactivity per unit weight of tibia ash when the source of phosphorus was sodium phosphate rather than calcium phytate. The data on tibia ash also reflects the fact that the vitamin D-deficient chicks utilized inorganic phosphate as efficiently as chicks having received an adequate amount of vitamin D.

The percentage of the dose of P^{32} excreted per chick is also presented in table 2. The data on excretion in this experiment are of limited value since the chicks were sacrificed before the digestive tract would normally have voided all of the unabsorbed and re-excreted phosphorus. It might also be expected that the soluble sodium phosphate would be more rapidly absorbed and partially eliminated via the kidneys, than would the phytate phosphorus. Consequently, at the time

the chicks were sacrificed the non-utilized fraction of sodium phosphate would be more completely present in the excreta than the non-utilized phytin phosphorus. The data in table 2 are of qualitative interest since they show that excretion of P^{32} is much greater immediately after ingestion of phytin than after ingestion of sodium phosphate.

Experiment with turkey poults. One-day-old Beltsville White turkey poults of mixed sexes were divided into two groups of 20 each. One group was fed the vitamin D-deficient poult ration shown in table 1 while the other group received a supplement of 180 International Chick Units of vitamin D in 100 gm of the same diet. After 11 days on this experimental regime, the poults in the first group exhibited definite symptoms of vitamin D-deficiency. At this time 12 poults of approximately the same weight were selected from each group and placed in wire metabolism cages. After placing the poults in metabolism cages, feed was withheld for a period of three hours prior to administration of the isotope although water was supplied ad libitum. Six normal and 6 vitamin D-deficient poults were then each given a 2 ml solution of $Na_2HP^{32}O_4$ containing a total of 8.55×10^6 counts/min. At the same time 6 normal and 6 vitamin D-deficient poults were each given an equivalent dose of isotope from P^{32}-labeled calcium phytate. The isotope carriers were placed in the crops of the poults in exactly the same manner as described above for chicks. After dosing the poults were allowed the same diets as previously ad libitum.

Forty-eight hours after administration of the radioisotope blood samples were collected and plasma obtained by the same procedure as in the experiment with chicks. The poults were sacrificed and both tibiae removed and prepared for ashing. All excreta were collected during the experimental period. The blood, bone ash and excreta were handled and counted in the same manner as described in the chick experiment.

The results obtained are presented in table 3. The radioactivity of the blood is again expressed on the basis of

counts/min./total plasma. The radioactivity of the blood of normal poults which received inorganic phosphorus was 17 times greater than that of normal poults which received the phytin phosphorus. In the case of poults which were deficient in vitamin D, there was 28 times as much radioactivity in the blood plasma of those which had received inorganic phosphorus as there was in the plasma of those which had received phytin phosphorus.

The corrected counts/min./100 mg of tibia ash as well as the percentage dose retained per 100 mg of tibia ash are presented in table 3. Since the normal poults were heavier

TABLE 3

Results of turkey poult study; radioactivity measurements made 48 hours after a single dosing with equivalent quantities of isotope carrier

DIETARY HISTORY	P^{32} CARRIER	RADIOACTIVITY OF TOTAL PLASMA	RADIOACTIVITY OF 100 MG TIBIA ASH	% DOSE RETAINED/ 100 MG TIBIA ASH	% DOSE EXCRETED
		av. counts/ min.	*av. counts/ min.*[1]		
Vitamin D-deficient	Ca phytate	7.14×10^2	4.36×10^3	0.058	47.7
Vitamin D-deficient	$Na_2HP^{32}O_4$	2.00×10^4	3.66×10^5	4.27	26.3
Vitamin D-adequate	Ca phytate	1.23×10^3	6.56×10^3	0.087	30.3
Vitamin D-adequate	$Na_2HP^{32}O_4$	2.11×10^4	4.41×10^5	5.16	6.7

[1] Corrected to a standard body weight of 135 gm.

than the vitamin D-deficient poults all values were corrected to a standard poult weight of 125 gm. Since the percentage of phosphorus in a given quantity of the bone ash of the poults was the same, for all practical purposes, regardless of the state of vitamin D nutrition, a given weight of ash from the different groups, in the [illegible] the same amount of phosphorus. [illegible] values [illegible], therefore, refer to specific activity, [illegible] per unit weight of phosphorus. The data [illegible] table 3 show that normal poults retained 59 times as much activity following the administration of P^{32} in disodium phosphate as [illegible]

from the administration of P^{32} in calcium phytate. The vitamin D-deficient poults made even less use of phytin. They retained 74 times as much radioactivity in the bone ash following administration of disodium phosphate as they retained following administration of calcium phytate.

The percentage of the dose of radioactive phosphorus excreted by the poults in the various groups is shown in table 3. It was found that normal poults excreted, within 48 hours, about 4.5 times as much P^{32} from phytin as from disodium phosphate. Poults deficient in vitamin D also excreted a larger proportion of the phytin phosphorus than the inorganic phosphorus. However, as compared with the normal poults their excretion of inorganic phosphorus was also much higher. Due to this high output of inorganic phosphorus the disparity between excretion of phytin and inorganic phosphorus was not so great for vitamin D-deficient poults in this study. It appears that, at least in this experiment, vitamin D-deficiency interfered to some extent with the utilization of inorganic phosphorus by the poult.

Chemical exchange studies, in vitro. Since it has been known for some time that simple chemical exchange occurs between certain inorganic phosphates, it was of interest to determine whether exchange may occur between inorganic phosphorus and the phytin molecule. Singsen et al. ('50), have leaned heavily toward the exchange theory to explain the apparent utilization of phytin phosphorus by poults. If such a reaction takes place in the digestive tract, appearance in the tissues of P^{32} from phytin may not be wholly ascribed to net utilization of this source of phosphorus. Conversely, the administration of P^{32}-labeled inorganic phosphate along with a diet containing substantial amounts of phytin might yield deceptively low values for the uptake of inorganic P^{32} due to its exchange with the phytate molecule.

In a preliminary experiment to explore the possibilities of exchange, 140 mg of non-radioactive calcium phytate were dissolved in 8 ml of 25% acetic acid. Two milliliters of solution containing $Na_2HP^{32}O_4$ were added and the solution al-

lowed to stand at room temperature, with occasional stirring, for 24 hours. The calcium phytate was reprecipitated by heating the solution in a water bath and filtering while hot. The precipitate was washed once with hot 8% acetic acid, three times with water and three times with 95% ethanol. The precipitate was dried by passing a stream of air through it for one hour and then placing overnight in a vacuum desiccator. When completely dry the weight was found to be 56 mg. It was then dissolved in 4 ml of 25% acetic acid, diluted 1:10 with distilled water and aliquots plated out for radioactivity counting. The results are shown in table 4.

TABLE 4

Results of in vitro studies on the possibility of phosphate exchange between calcium phytate and inorganic phosphate

NON-LABELED PHYTATE USED	RADIOACTIVITY ADDED AS $Na_2HP^{32}O_4$	Ca PHYTATE RECOVERED	RADIOACTIVITY AFTER EXCHANGE	% APPARENT EXCHANGE
	counts/min./mg phytate	*mg*	*counts/min./mg phytate*	
(Experiment 1)				
140 mg	1.53×10^4	56	6.57×10^3	43
(Experiment 2. First recrystallization)				
1141 mg	1.03×10^3	542	3.4×10^2	33
	(Second recrystallization)			
		161	3.6×10^2	35

In a second experiment a similar plan was followed except that the calcium phytate was recrystallized to a constant specific activity in order to be certain that all inorganic phosphorus was removed. One thousand one hundred and fourteen milligrams of non-radioactive calcium phytate were dissolved in 50 ml of 25% acetic acid. Three milliliters of $Na_2HP^{32}O_4$ were added and the solution allowed to stand at room temperature for 24 hours. The calcium phytate was then reprecipitated, washed and dried as previously described. The weight was found to be 542 mg. It was then dissolved in 30 ml of 25% acetic acid, a portion was diluted 1:10 with

25% acetic acid and radioactivity measurements made. The calcium phytate was found to contain 3.4×10^2 counts/min./mg.

The remainder of the solution was again treated to precipitate the calcium phytate which was washed and dried as described above. The weight was found to be 161 mg. This material was dissolved in 20 ml of 25% acetic acid and aliquots plated out for radioactivity measurement. The calcium phytate was found to contain 3.6×10^2 counts/min./mg. This was considered a constant specific activity within experimental error.

The data obtained in the exchange studies are presented in table 4 and indicate that P^{32} from $Na_2HP^{32}O_4$ exchanged quite readily with phosphorus from calcium phytate *in vitro*. We do not wish to draw the inference that these observations can be extrapolated to the surviving animal organism. Precise biological experiments would be required to establish the quantitative aspects, if any, of the exchange mechanism *in vivo*. In a qualitative way, however, the data do strongly suggest the probability of phosphate exchange between the phytin molecule and inorganic sources.

DISCUSSION

In the work detailed in this report the primary interest was a comparison of the effectiveness of phytate versus soluble inorganic orthophosphate as a dietary source of phosphorus for chicks and poults. In view of the effect of vitamin D on phytin utilization the comparison was made with both normal and vitamin D-deficient birds. Criteria of utilization were the concentrations of the radioisotope in blood and bones 48 hours after an initial dose followed by three succeeding doses in chicks, and 48 hours after a single dose in the case of poults. Data on excretion of the isotope were obtained to supplement the other findings, although, due to time limitations, these are of limited value.

Previous feeding experiments with phytin have been handicapped by high mortality in young chicks and poults when phytin was the only source of dietary phosphorus. Due to

the high mortality it has not been posible to make reliable comparisons between phytin and other materials as the sole source of phosphorus. A feeding experiment of this nature requires a minimum of two or three weeks time when growth and calcification are the measures of biological utilization. Short-term experiments with isotope dosing should provide more reliable data of a directly comparative nature.

In the work described in this report there was a much higher P^{32} content in blood and bones from chicks and poults given radioactive disodium phosphate than in birds given radioactive calcium phytate. This held true regardless of the previous history of the birds with respect to vitamin D nutrition. These data confirm earlier feeding experiments with unlabeled phytate which showed qualitatively that the young chick cannot make effective use of this source of phosphorus for growth and bone formation (Gillis et al., '49).

In drawing conclusions from the present work concerning the utilization of phytin and inorganic P^{32}, the data on retention of P^{32} in bones constitute probably the most useful and reliable statistic. The bone ash data indicate that chicks which received adequate vitamin D utilized calcium phytate approximately 10% as effectively as they utilized disodium phosphate. This value is corroborated by the values for retention in the blood plasma also. This rate of utilization also agrees well with the survival time of chicks fed phytin as the sole source of supplementary phosphorus in a low-P diet compared with that of chicks fed low levels of inorganic phosphorus (Gillis et al., '49). It is suggested, therefore, that on the basis of all available evidence, phytin phosphorus is utilized approximately 10% as effectively as inorganic orthophosphate by normal chicks under average dietary conditions.

Normal turkey poults made much poorer use of phytin than did chicks. It is well established that the requirement of poults for phosphorus is higher than that of the chick (Bird et al., '54). The specific activity of P^{32} in the bone ash indicates that less than 2% as much radioactivity was retained by

poults for calcification from phytin as from disodium phosphate. On the basis of these data it is suggested that calcium phytate should be considered valueless, for practical purposes, as a source of phosphorus for young turkey poults.

The effect of vitamin D on utilization of phytin phosphorus in these experiments confirms and extends the observations previously reported. In both chicks and poults vitamin D-deficiency resulted in negligible utilization of phytin phosphorus as measured by retention of P^{32} in bone ash. The phytin P^{32} retained by the deficient birds was in the order of 1 to 2% of that retained from disodium phosphate by deficient birds.

The utilization of inorganic phosphate was not affected significantly by vitamin D deficiency in the chick. In fact, both blood and bone ash showed slightly more inorganic radioactivity in the vitamin D-deficient chick than in the normal chicks. On the other hand, the turkey poults' utilization of inorganic phosphate seemed to be slightly, though significantly ($P < 0.05$) impaired by a deficiency of vitamin D. The vitamin D requirement, as well as the phosphorus requirement, of the poult is higher than for the chick. It is our opinion that the primary function of vitamin D is in calcium absorption (Keane, Collins, and Gillis, '56). In this case calcium absorption in the poults may have been interferred with to such an extent that retention of phosphorus was indirectly affected, although the effect was small.

CONCLUSIONS

Normal young chicks utilized P^{32}-labeled calcium phytate only about one-tenth as effectively as inorganic orthophosphate, $Na_2HP^{32}O_4$. Normal young turkey poults made only negligible (less than 2%) use of calcium phytate compared with a disodium phosphate.

In the presence of a deficiency of vitamin D, neither chicks nor poults made significant use of phytate phosphorus. However, the chicks' utilization of the inorganic source of isotope was not impaired by vitamin D deficiency. In poults, vitamin

D deficiency had a slight, but apparently significant, adverse effect on utilization of inorganic phosphorus.

In vitro studies indicated that radioactive inorganic phosphate may exchange with phosphate in the phytin molecule.

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ACUTE EXPERIMENTAL CALCIUM CHLORIDE ACIDOSIS.

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In a previous publication (Govan and Parkes, 1946) it was shown that the administration of ammonium chloride to rabbits caused acidosis and ultimately death of the animals. Widespread necrosis of the renal tubules occurred, suggesting that death might be due to uraemia. In certain respects, however, the biochemical changes were atypical, and did not fit into the syndrome usually shown in acute experimental nephrosis. In almost all cases of experimental nephrosis recorded there has been a marked reduction in the output of fluid and a retention of urea. This has been reported in the case of poisoning by uranium (Frothingham, Fitz, Folin and Denis, 1931), tartrates (Underhill, Wells and Goldschmidt, 1913), oxalates (Dunn, Haworth and Jones, 1924), uric acid (Dunn and Polson, 1926), and by mercury (McNider, 1918). After administration of ammonium chloride the blood urea rose, but the highest recording was 173 mg. per cent, and the urinary output of fluid and nitrogen remained high. These results resemble those found by Folin, Karsner and Denis (1912) in animals poisoned by chromates and cantharidin. It was suggested at the time that the relatively low blood urea values obtained after ammonium chloride might be due to the maintenance of fluid output, since Dunn and Jones (1925) have shown that intra-peritoneal injections of sodium chloride increased the fluid output and decreased the blood urea in rabbits rendered nephritic by oxalates. It is interesting to note, however, that Underhill, Wells and Goldschmidt found that the urea output was nil, although fluid was being excreted by animals poisoned by tartrates.

That there is some connection between acidosis and renal damage is suggested by the reports of diminished plasma CO_2 combining power after administration of proved nephritic agents, such as uranium (Goto, 1917), cantharidin, arsenic, diphtheria, chromates (Goto, 1918*a*, 1918*b*) and mercury (McNider, 1918; Ogilvie, 1932). Goto (1918*a*, 1918*b*) found retention of chlorides and urea as well as acidosis in these cases, and the administration of sodium bicarbonate by mouth lessened the severity of the changes.

It seemed worth while therefore to pursue the study of severe acute acidosis with the hope of eliciting further information regarding the cause of death of these animals and the relationship between acidosis and kidney function. In the experiments to be described it was decided to use calcium chloride, since in our previous work it was impossible to eliminate the ammonium ion as a possible factor in the cause of death.

METHODS.

Experimental.

Preliminary experimentation showed that doses of ammonium chloride and calcium chloride containing equivalent amounts of chlorine produced widely varying results. It required a relatively greater dose of calcium chloride to produce changes similar to those obtained with ammonium chloride. With the ammonium salt toxic changes resulting in death of the rabbit within four days were produced with a dose of 0·75 g. per kg., but it required a dose in excess of 2 g. per kg. of calcium chloride to do this.

Twelve rabbits were used in the experiment. These were housed in urine cages. Food consisted of moist bran and oats fed in bird-cage feeding boxes. Urines were collected daily for the estimation of urea, chlorides, phosphates, ammonia and pH. Routine tests for albumen were carried out. The animals were maintained on this simple diet for a week, blood being withdrawn every second day for analysis.

At the end of the week calcium chloride in a 10 per cent solution was administered daily by stomach-tube. Two animals received 1·5 g. per kg., two received 1·8 g. per kg., two received 2·2 g. per kg. and six received 2·5 g. per kg. Blood and urine examinations were carried out as above.

Chemical.

Urinary and blood urea values were estimated by the hypobromite and urease methods respectively. The usual Volhard technique was employed for chlorides. Kuttner and Lichtenstein's (1930) method was used for the estimation of phosphates. Ammonia levels were found by the aeration method. The apparatus used was that described by Van Slyke and Cullen (1916). It consisted of three corked Pyrex boiling tubes connected in series by glass and rubber tubing so that air could be sucked through them by means of a water pump attached to Tube 3, the inlet being at Tube 1. Tube 1 contained 5 per cent sulphuric acid in order that traces of ammonia in the atmosphere could be removed as the air bubbled through the acid before reaching Tube 2 containing the blood and reagents. Tube 3 contained 25 c.c. of 0·02 N hydrochloric acid. Blood was then collected under liquid paraffin. Five ml. of blood were placed in Tube 2. One drop of caprylic alcohol and 4 or 5 g. of potassium carbonate were added to the tube, which was corked and then shaken gently to mix the contents. A slow current of air was then drawn through the tubes for 2 or 3 minutes, followed by a rapid current for 15 minutes. At the end of this time the excess acid in Tube 3 was titrated with 0·02 N sodium hydroxide, and the necessary calculations made from the titration figure. Blank determinations on the reagents were made on occasion to eliminate the possibility of ammonia being present as an impurity. Serum calcium was determined, using the method of Kramer and Tisdall (1921) as modified by Tisdall (1923).

Histological.

Formol saline was used as a routine fixative for paraffin and frozen sections. Representative specimens for the demonstration of glycogen and phosphatase were fixed in alcohol. Sections were stained by haematoxylin and eosin, Masson's trichrome method, Dunn's aniline blue Orange G method, Gomori's method for alkaline phosphatase, Best's carmine stain for glycogen and Sudan IV.

RESULTS.

During the first week prior to calcium chloride administration the animals appeared to be healthy. Urinary volume was high at the beginning. In six animals it fell slightly during the first two or three days of the new diet, but rose or remained steady thereafter. Urea output was roughly parallel to water excretion, although the curve was always much flatter and changes in the curve were delayed. The excretion of phosphate rose steeply, while chloride output fell. Urinary pH fell gradually; blood analysis showed that urea, chlorides, lipoids, ammonia and pH were all within normal limits. The CO_2 combining power of the blood was lower than usual, but still within normal limits. This appears to be due to the absence of greens in the diet. This feature was noted in our previous experiments with ammonium chloride. Serum calcium and phosphorus were higher than usual, but the Ca:P ratio was normal.

In their reaction to calcium chloride the animals formed two distinct groups comprising (*a*) all animals receiving 1·5 to 1·8 g. per kg. plus one animal receiving 2·2 g. per kg.; and (*b*) all animals receiving 2·5 g. per kg. plus one animal receiving 2·2 g. per kg.

Group 1.

Five animals—four receiving 1·5 to 1·8 g. calcium chloride per kg. and one receiving 2·2 g. per kg.

All of the animals in this group were killed on the 5th day of the experimental period. During the first four days of this period the animals showed no signs of toxaemia, ate their food and appeared to be healthy in every way.

During the first 24 hours the urinary volume diminished. Urea excretion ran almost parallel with the output of water, but although the volume of urine was increased at the 48-hour period there was a 24-hours' delay in the rise of urea excretion. Both were increased at 72 hours, but at 96 hours the urea output tended to fall although water excretion remained high. The excretion of phosphates increased slightly during the first 24 hours, but fell moderately although progressively thereafter. Chlorides, naturally, showed a steeply rising curve. Urinary pH fell rapidly and remained round 5. Albuminuria occurred in all cases as soon as the pH fell below 6.

The most marked change in blood chemistry was a fall in CO_2 combining power. Prior to the experimental period the average normal CO_2 combining power was approximately 40 volumes per cent. After calcium chloride the average was 13·5 volumes per cent. At the same time the plasma pH was reduced slightly. Urea was slightly increased; blood cholesterol was almost doubled, and chlorides increased by 25 per cent. Calcium and phosphorus were slightly increased and the blood lipoids remained normal. Table I shows a comparison of average values before and after administration of calcium chloride.

TABLE I.

	Blood urea, mg./100 ml.	CO_2 c.p., vol./100 ml.	pH.	Cl′, mg./100 ml.	Serum Ca. mg./100 ml.	Serum P. mg./100 ml.	Cholesterol, mg./100 ml.	Lipoids, mg./100 ml.	Ammonia, mg./100 ml.
Before . .	42·1	40	7·19	594	12·0	3·76	43·6	0·14	0
After . .	57·7	13·5	6·8	742	13·3	4·25	70	0·13	0

The graph in Fig. 1 shows the detailed changes in Animal 2, which is representative of the group. All of these animals were killed at the beginning of the 5th day of the experimental period by a sharp blow on the back of the neck. At post-mortem the appearances were similar in all cases. The lungs were emphysematous, but showed a few congested areas of collapse. No change could be

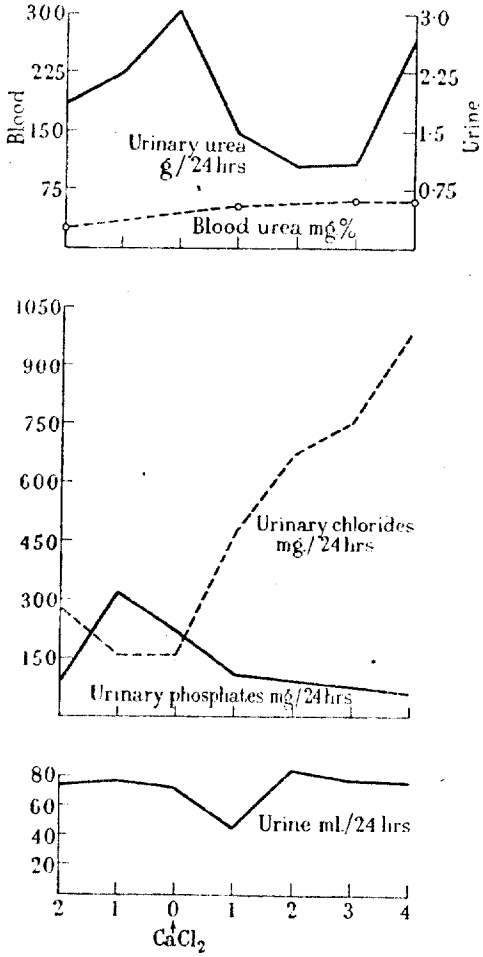

FIG. 1.—Graphs illustrating the effect on Animal 2 of administering 1·5 g. calcium chloride per kg. for 4 days. The animal was killed on the 5th day.

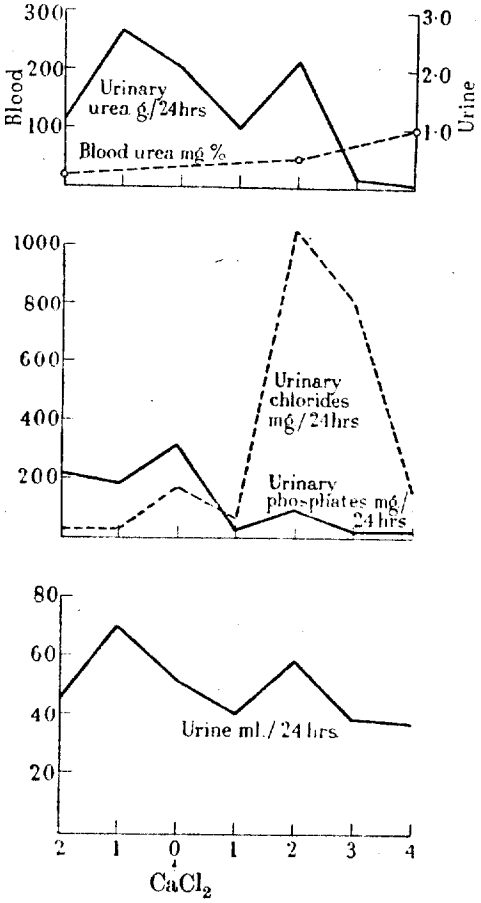

FIG. 2.—Graphs illustrating the effect on Animal 8 of administering 2·5 g. calcium chloride per kg. for 4 days. The animal died on the 5th day.

seen in the heart. The liver was slightly paler than normal, but the spleen showed no abnormality. A distinct zone of pallor was present at the junction of the renal cortex and medulla. Pieces of tissues were taken from all the organs, and fixed and stained as detailed under methods.

Histology.

Lung.—There was marked dilatation of most of the alveoli, but occasional small areas of collapse were present. These areas of collapse were frequently in association with fat emboli, but emboli could also be seen in the emphysematous portions. The number of fat emboli varied in different parts of the lungs, but averaged approximately five per field viewed through a 2/3 in. objective. A marked accumulation of polymorphonuclear leucocytes mainly of eosinphil type was present in the alveolar capillaries, and near the fat emboli these leucocytes appeared to form solid nodules. In one case haemorrhage had occurred into alveoli and bronchi, and free fat globules could be seen in the latter.

Myocardium.—No abnormality could be seen in ordinary paraffin sections of the myocardium. Glycogen was diminished, but most fibres still contained a small amount. Fine fatty globules were present in the myocardial fibres of one animal, but the remainder were normal.

Liver.—No gross abnormality could be seen in paraffin sections, but in some parts the hepatic cells contained collections of greenish-brown granules. Best's carmine stain revealed small deposits of glycogen in the cells surrounding the central veins. Fine droplets of fat were present in the Kupfer cells of the outer and mid zones, and occasionally the parenchymatous cells of the same areas showed a similar change.

Kidney.—The glomeruli seemed to be larger than normal and in many cases slight adhesions appeared to have developed between the tufts and Bowman's capsule. Pronounced catarrhal changes were present in the first convoluted tubules. Distal to this the nephron was dilated and the epithelium of the ascending Henle limb and second convoluted tubule was flattened.

Group 2.

Of 7 animals, 6 received 2·5 g. calcium chloride per kg. and one received 2·2 g. per kg.

Five of these animals died on the 5th day of the experimental period. The remaining two animals, including that receiving 2·2 g. of calcium chloride per kg., were comatose on the same day and were killed. All animals exhibited similar symptoms during the last 24 hours. They became progressively drowsier, gradually losing the power of their limbs and repeatedly falling over. Finally they became comatose and died.

During the first 24 hours of calcium chloride administration urinary volume diminished as in Group 1. Likewise they increased during the second 24-hour period, but by 72 hours they had begun to fall and this fall continued up to the time of death. The excretion of urea ran roughly parallel with that of water, but the rise in urea output at 48 hours did not correspond with the increase in volume. Phosphate showed a progressive fall from its previously high level. Chlorides fell initially, then rose steeply, but during the last 24 hours the output fell once more. The pH, at first reduced, rose, and during the last 2 days was always above 8·0.

In all cases the blood urea rose and the average was around 100 mg. per 100 ml. before death. A marked acidosis was present, and the CO_2 combining power of the blood was reduced to 16 volumes per cent. The pH of the blood dropped, but the fall was no greater than in Group 1. Chlorides increased as before.

Serum phosphorus was doubled and serum calcium also increased. The Ca:P ratio was almost halved. Cholesterol showed a marked increase, almost 200 per cent, and plasma lipoids were augmented by almost 500 per cent. Table II gives a comparison of the average blood values of this group with that of normals.

TABLE II.

	Blood urea, mg./100 ml.	CO_2 c.p., vol./100 ml.	pH.	Cl', mg./100 ml.	Serum Ca., mg./100 ml.	Serum P. mg./100 ml.	Cholesterol, mg./100 ml.	Lipoids, mg./100 ml.	Ammonia, mg./100 ml.
Normals . .	42·1	40	7·19	594·6	12·0	3·76	43·6	0·14	0
After $CaCl_2$.	92	16	7·00	752·6	16·2	8·1	116·0	0·6	5·35

The graph in Fig. 2 shows the changes in Animal 8. At post-mortem the changes were very similar to those seen in Group 1. The depressed, congested areas of collapse in the lung were more numerous but smaller. Marked emphysema was present in the intervening lung tissue. The liver showed a generalized pallor and the lobular markings were blurred. There was well-marked enlargement of the kidneys. The cut surface showed a distinct bulging zone of marked pallor at the junction of cortex with medulla. The cortex also showed pallor, but the medulla was congested. No abnormality could be seen in the other organs.

Histology.

Lung.—Areas of collapse were much more frequent in this group. Fat emboli were present, but were, on the average, only slightly more frequent than in Group 1. There was marked dilatation of the peri-vascular lymphatics, which contained free fat globules. Nodes of eosinophil reaction were numerous (Fig. 3). A few alveolar haemorrhages were present and many of the alveoli contained macrophages, some of which had foamy cytoplasm. In one case a slight fibrinous exudate was present on the pleural surface adjacent to an area showing haemorrhage, oedema and collapse.

Myocardium.—Fine fatty degeneration was present in all cases. Practically no glycogen remained, and the alkaline phosphatase reaction was negative.

Liver.—Glycogen was greatly diminished, but not entirely absent. A few granules could still be seen in the cells surrounding the central vein. Focal degenerative changes were present in the mid-zones of most lobules (Fig. 4).

EXPLANATION OF PLATES.

FIG. 3.—Section of lung from Animal 7, Group 2, showing a solid node of eosinophils in an inter-alveolar septum. H. and E. × 340.

FIG. 4.—Section of liver from Animal 8, Group 2. Note the islands of necrotic cells showing hyperchromatic nuclei. H. and E. × 85.

FIG. 5.—Section of liver from Animal 9, Group 2, showing areas of degeneration with karyolysis of nuclei. H. and E. × 300.

FIG. 6.—Section of liver from Animal 9, Group 2, showing binucleate and trinucleate cells. H. and E. × 340.

FIG. 7.—Section of liver from Animal 11, Group 2, showing greenish-brown granules in cytoplasm of cells. H. and E. × 380.

FIG. 8.—Section of kidney from Animal 9, Group 2, showing degeneration of cells of descending portions of first convoluted tubules. H. and E. × 340.

FIG. 9.—Section of kidney from Animal 8, Group 2, showing dilatation of ascending limbs of Henle. H. and E. × 85.

FIG. 10.—Section of kidney from Animal 10, Group 2, showing pyknosis in the cells of the broad ascending limbs of Henle. H. and E. × 250.

Most commonly these took the form of islands of cells staining strongly with eosin and showing pyknosis of the nuclei. These cells tended to separate from the surrounding columns. Less commonly the nuclei showed karyolysis (Fig. 5), and cytoplasm, staining palely with eosin, was finely vacuolated. Signs of regeneration were present in other parts, the cells being frequently binucleate or even tri nucleate (Fig. 6). Cells in all parts of the lobules contained greenish-brown granules (Fig. 7). Fat was present in the Kupfer cells. The phosphatase reaction was weak.

Kidney.—The degree of renal damage was very variable in these animals. In all of them the glomerular tufts were enlarged, and in the majority light adhesions had formed between the tufts and Bowman's capsule. Albuminoid material was present in the subcapsular spaces. The capillaries were quite patent, and contained a few eosinophil leucocytes in addition to red cells. Marked catarrh was present in the first convoluted tubules and broad descending limbs of Henle. In some cases the lining epithelium was necrotic, the nuclei showing pyknosis and karyolysis. In a few cells the cytoplasm contained brown granules (Fig. 8). The distal portion of the broad ascending limbs of Henle was greatly dilated (Fig. 9). The intervening portion of the nephrons lying in the juxta-medullary region showed necrosis of many cells, mainly in the first part of the broad ascending limb. Pyknotic changes in the nuclei were marked (Fig. 10). This zone was almost completely bloodless, and corresponded to the zone of pallor seen on the cut surface of the kidneys at post-mortem. Colloid casts were common in the collecting tubules. Some of the collecting tubules showed degeneration of the epithelium.

DISCUSSION.

In our paper on ammonium chloride acidosis (Govan and Parkes, 1946) we stated that at least two theories regarding the cause of death in these animals were possible, namely, that (*a*) the animal died of uraemia as a result of nephritis, or (*b*) they died of ammonia poisoning.

Contrary to the findings in acute ammonium chloride poisoning the renal damage in the present experiment was irregular in extent and occurrence, although the symptoms exhibited and the interval of time between administration of calcium chloride and death of the animal were the same. From this alone it would seem that nephritis is not the primary cause of death in these animals, although no doubt it is a contributory factor.

It has been shown that the blood ammonia appears to be greatly increased after administration of 2·5 g. calcium chloride for 4 days. The possibility of spontaneous formation of ammonia from adenosine phosphate (Conway and Cook, 1939) must be taken into consideration. The original concentration may, in this way, be increased many times in a relatively short interval. Benedict and Nash (1926) and Conway (1935) showed, however, that if precautions were taken to prevent exposure to the air, and the estimations were carried out as soon as possible, then the concentration was usually less than 0·05 mg. per 100 ml. In the present series of experiments all possible precautions were taken to guard against this possibility, and it is significant that no appreciable amount of ammonia could be found in the rabbits' bloods prior to administration of calcium chloride. According to McNeil and Levy (1917–18) the blood ammonia in normal animals is always less than 1 mg. per 100 ml. In the present experiment

it rose almost to 6 mg. per 100 ml. The cause of this increase is not quite clear. It is possible that it represents an attempt to neutralize the acidosis, but in the present series the blood ammonia was only increased in the second group of animals, although the degree of acidosis as determined by the CO_2 combining power of the plasma was no greater than in Group 1. A further proof that the blood ammonia levels bear no relationship to acidosis can be seen in the fact that higher levels were found in animals poisoned with ammonium chloride, although the degree of acidosis was similar to that observed in the present series. These findings are in agreement with those of Markert (1935, 1936).

Alternatively the blood ammonia increase may have been due to a change-over from carbohydrate to fat metabolism, as suggested by the histological appearance in the liver and heart. Many workers, McNeil and Levy among them, have shown, however, that the blood ammonia is not raised in diabetic acidosis, where it is definitely established that fat is being metabolized.

The fact that ammonia appeared in the blood only in the case of animals of Group 2 suggests that its appearance was associated with lesions found in the organs of these animals. In this instance the most marked lesions were those of the liver and kidneys. Ammonia is rapidly converted to urea in the normal animal, and according the Svedberg, Maddock and Drury (1938) the sole source of urea in the rabbit is the liver. There would therefore seem to be strong presumptive evidence in the present experiment that the increased blood ammonia is due to liver dysfunction. Also, McNeil and Levy make a series of observations in their paper which tends to confirm the above. These authors only found a raised blood ammonia in acidosis if the liver was damaged. For instance, no increase of blood ammonia could be found in nephritis unless there was a coincidental myocarditis causing venous congestion of the liver. In 13 cases of acidosis, of which 6 were diabetics, only 2 showed an increased blood ammonia. One was a case of general peritonitis with fatty degeneration of the liver, the other a case of persistent vomiting after laparotomy.

All animals receiving 0·75 g. of ammonium chloride per kg. per day died after 4 days of this treatment. Calculating on the basis of chlorine content the corresponding dose of calcium chloride ought to be 0·76 g. per kg. per day. Instead, the dose required was 2·5 g. per kg. It may be that the chlorine of the calcium salt was not available, but the plasma CO_2-combining power was as low after administration of 1·5 g. of calcium chloride per kg. as it was after 2·5 g. per kg. This would suggest that the ammonium salt is more toxic than the calcium, and this must be by virtue of the ammonium ion.

It seems unlikely that the renal tubular necrosis was directly related to the acidosis, since the changes were minimal in Group 1 and irregular in their extent in Group 2, although all animals were profoundly acidotic. The appearance of the kidneys at post-mortem suggests that the ultimate cause of the tubular necrosis was anoxaemia due to a deviation of the blood from the juxta-medullary region. The cause of this deviation is not apparent, but since the avascular zone was present in both groups, it suggests that the altered blood flow was directly related to the acidosis.

Several minor points arise for discussion. It seems from the histological appearances and biochemical findings that fat is mobilized from the depots long before the glycogen stores are exhausted. Fat was present in the Kupfer cells of the liver and in the myocardium, although both organs contained small

amounts of glycogen. Although the fatty change in the myocardium may be the result of degeneration, recent work (Dible, 1934 ; Dible and Gerrard, 1938) suggests that the process is always one of infiltration.

It will have been noted that the serum phosphorus and calcium were both raised prior to administration of calcium chloride. In addition the CO_2-combining power of the plasma was reduced. This latter feature was also found in our ammonium chloride series, and it appears to be related to the diet of oats and bran without greens. The former changes are probably related to this mild acidosis. The change in calcium concentration seems to be secondary to that of serum phosphorus. This is suggested by the fact that urinary phosphorus increased during this period. A further significant feature is that serum phosphorus showed a further increase during the period of calcium chloride administration. These changes would appear to indicate that phosphorus plays an important role in combating the acidosis, and that probably phosphorus is mobilized from body stores.

Finally, attention must again be drawn to the occurrence of pulmonary fat emboli in these experiments. The meaning of this is not apparent, but it is important to realize that the reaction of the lungs to emboli is collapse—a fact which may be of some significance in cases of post-operative collapse. It is also interesting to note the intense eosinophil reaction to these emboli.

SUMMARY.

The oral administration of 2·5 g. calcium chloride per kg. daily to rabbits causes death after four days.

This is accompanied by extensive focal necrosis in the liver, and necrosis of tubules in the juxta-medullary region of the kidney.

The results suggest that death may be due to ammonia poisoning, and that the increase in blood ammonia is related to the hepatic lesions.

Following administration of calcium chloride glycogen stores are rapidly depleted, but before this is complete fat is mobilized from the depots.

This mobilization results in histologically demonstrable deposits of fat in the Kupfer cells of the liver and in the myocardium, and it is accompanied by the formation of fat emboli in the lungs.

The fat emboli are accompanied by a marked eosinophil reaction and cause collapse of the pulmonary alveoli.

There is a retention of phosphorus after calcium chloride administration and the calcium-phosphorus ratio is diminished.

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SERUM SODIUM AND POTASSIUM CONCENTRATIONS IN INFANTILE TETANY

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Tetany, a condition of increased neuro-muscular irritability, is usually considered to be associated with changes in the calcium and phosphorous concentration in the blood. However, changes in the serum potassium concentration may also play a role. Thus, according to Barnett (1956) in experimental animals it was found that, although at a given low level of serum calcium and serum potassium there may be no signs of neuromuscular hyperirritability, yet with the calcium remaining the same, an elevation of the potassium will result in convulsions.

On the other hand, Fourman (1954) reproduced tetany experimentally in potassium deficiency. That tetany might accompany potassium deficiency was first indicated by Darrow *et al.* (1948). Other workers noted tetany in clinical states of potassium deficiency (Eliel *et al.* 1950, Strong 1951 and Roussak 1952).

In the present investigation, the concentration of sodium and potassium in the serum of infants was studied in relation to the condition of tetany.

MATERIAL AND METHODS

Twenty six infants (ranging in age between six months and three years) who suffered from infantile tetany were examined. X-rays showed in 20% of these cases active and in the rest, healing rickets. 40% of these patients had a weight of 70% the normal average for their respective age or even less. Blood samples were obtained from these patients before the start of treatment, which consisted of intravenous calcium gluconate injections together with calcium chloride and sedative by mouth. In cases in which there were respiratory tract infections, antibiotics (penicillin and streptomycin) were used. After the complete disappearance of the manifestations of tetany, usually after 6-14 days, a second blood sample was obtained.

For comparison, thirty two normal children of the same age group as well as twenty-one rachitic infants were also studied. The normal

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TABLE I — *Serum sodium and potassium in normal infants and in cases of infantile tetany and rickets compared with normal adult levels*

Condition	No. of cases	Serum sodium: m Eq/l.			Serum potassium: m Eq/l.		
		mean	range	St. Dev.	mean	range	St. Dev.
Normal adult males (1)	111	144.2	133.5 — 156.0	± 4.25	4.64	3.64 — 5.84	± 0.42
Normal adult females (1)	111	142.2	131.8 — 152.8	± 4.13	4.44	3.46 — 5.62	± 0.42
Normal infants	32	137.7 (2)	132.0 — 150.0	± 4.80	4.05 (2)	3.20 — 5.20	± 0.51
Infantile tetany	26	136.8	128.0 — 150.0	± 5.45	3.70 (3)	2.40 — 5.80	± 0.68
Infantile rickets	21	139.8	128.0 — 152.0	± 4.80	3.92	3.10 — 4.80	± 0.39

1) Habib and Habib (1958)

2) difference from mean of either adult males or adult females significant at 0.1% level.

3) difference from mean of normal infants significant at 5% level.

TABLE II — *Serum sodium and potassium concentration before and after treatment in 26 cases of toxemia.*

Case No.	Serum sodium m Eq./l.		Serum potassium m Eq./l.		Case No.	Serum sodium m Eq./l.		Serum potassium m Eq./l.	
	before treatment	after treatment	before treatment	after treatment		before treatment	after treatment	before treatment	after treatment
1	140	137	5.8	4.4	14	132	132	3.9	3.0
2	137	145	3.6	7.6	15	135	142	3.9	4.1
3	135	145	4.2	3.7	16	135	138	3.8	4.0
4	140	138	3.5	4.5	17	134	134	3.4	3.9
5	142	166	4.4	6.9	18	138	138	3.5	3.4
6	140	140	4.0	3.7	19	132	130	3.2	4.4
7	149	148	3.1	3.6	20	132	138	3.2	4.8
8	135	142	3.4	4.4	21	132	138	3.4	4.0
9	140	148	3.0	3.6	22	135	138	4.1	5.0
10	148	148	2.4	4.0	23	132	135	4.2	4.9
11	140	138	3.6	3.4	24	128	130	3.4	3.0
12	145	142	4.2	3.5	25	130	128	3.7	3.7
13	140	143	4.8	3.5	26	133	140	2.7	3.4

children were free from rickets as verified by the radiological examination of the wrists. In four of the rachitic patients without tetany, the effect on the serum sodium and potassium of treatment with calcium gluconate intravenously and colloidal calcium and vitamin D intramuscularly, was studied after seven days.

In all the infants, blood samples were withdrawn from the external jugular vein and the serum was separated after adequate clotting had occured and at a fixed time interval after sampling. Serum sodium and potassium concentrations were determined by the EEL flame photometer. Student's «t» test (Fisher 1946) was used for the statistical treatment of the data.

RESULTS

No statistically significant difference was found between the normal male and female infants as was established before among adults (Habib and Habib 1958) and accordingly both sexes were grouped together in Table I.

As seen from Table I, the mean levels of sodium and potassium in the serum of normal infants were statistically lower than those in either adult males or females ($P < 0.001$). They are also lower than those obtained by Overman *et al.* (1951) in two-years infants. The mean serum potassium concentration in infants with tetany was statistically lower than that in normal infants ($P < 0.05$). On the other hand, the serum sodium of either infants with tetany or with rickets only and the serum potassium in cases with rickets, were not different from serum sodium or potassium in normal infants. Again, the differences between either the level of sodium or potassium in the serum of infants with tetany before treatment and the levels in rickets only, were not significant.

Table II shows the serum sodium and potassium concentrations before and after treatment in the cases of tetany.

The means of these results were compared with those in four cases of rickets in Table III.

TABLE III.

Condition	No. of Cases	Mean Serum sodium m Eq./l.		Mean Serum potassium m Eq./l.	
		before treatment	after treatment	before treatment	after treatment
Infantile tetany	26	139.8	139.9	3.70*	4.17
Infantile rickets	4	142.2	138.0	4.10	4.05

* difference significant at 5% level.

The only significant difference was an increase in the mean serum potassium concentration in cases of infantile tetany after treatment ($P < .05$) which reached the normal level of potassium in normal infants of the same age group.

DISCUSSION

Fourman (1954) has experimentally obtained tetany during potassium deficiency in normal persons while the absorption of sodium was kept very small. In his experiments, the deficiency of potassium was accompanied by an acidosis of the extracellular fluid and probably with less than the normal amount of (sodium + potassium) in the cells. On the other hand, Black and Milne (1952) produced potassium deficiency in their subjects while the sodium intake was generous and no tetany was produced at any stage of their experiments. Their potassium deficiency was associated with an extracellular alkalosis and probably less (sodium + potassium) than normal in the cells.

In the present investigation, the infants suffering from tetany showed a lower potassium concentration in the serum than in normal infants. It is interesting to note that this level of potassium in the serum was increased after treatment of the tetany although no potassium salts were given during such treatment.

Ibrahim (1959) has also found that the serum calcium, magnesium and phosphorus in active tetany were lower than in normal children and that as a result of treatment, there was an increase in the serum calcium and magnesium levels.

The mechanism of this relation between tetany and serum potassium concentration is open to further study.

SUMMARY

1. The concentrations of sodium and potassium were determined in the serum of 32 normal infants (1/2 - 3 years old), in 26 with tetany and in 21 with rickets.

2) Serum potassium concentration in cases of tetany was statistically lower than that in normal infants.

3) Treatment of tetany with calcium and sedatives resulted in the rise of serum potassium concentration to the normal level although no potassium salts were administered.

4) Serum sodium in tetany cases and both sodium and potassium in rickets were not different from their respective levels in normal children.

We wish to thank Professor S. Abbasy and Professor M. Mahfouz for their encouragement.

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CALCIUM CHLORIDE ACIDOSIS.

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GYÖRGY(1) has shown that calcium chloride, when administered to babies, causes an increased ammonia and acid output in the urine. The experiment here described was made on J. B. S. H. (100 kilos) and lasted for 13 days, during three of which he drank 85 grams (·766 gram-molecule) of calcium chloride in 3·5 litres of water. The food intake was approximately constant through the experiment. The water intake, where above normal, is shown in the table.

Alveolar carbon dioxide was estimated by the Haldane-Priestley method, each daily value being the mean of four pairs of samples. In the urine *p*H was estimated with Clark-Lubs indicators and buffer mixtures, acid excretion by titration to *p*H 9 with ·1 *N* soda and thymol blue, buffer excretion by titrating back to *p*H 3·7 with ·1 *N* hydrochloric acid and thymol blue. A small correction, rarely exceeding 1 p.c., was made for the acid uncombined at the latter reaction. Ammonia was estimated by van Slyke's(2) method, phosphates with uranium acetate, chloride by Volhard's, and calcium by McCrudden's(3) method. A Cole-Onslow(4) comparator was used when necessary. Fæcal carbonate was estimated as carbon dioxide in J. S. Haldane's(5) blood-gas apparatus, fæcal phosphorus with uranium acetate after boiling with nitric and sulphuric acids.

In order to bring ·05 *N* ammonium chloride solution to *p*H 9 it was found necessary to add 16 p.c. of its equivalent of ·1 *N* soda, the salt being partly hydrolysed, while on subsequent addition of formaldehyde only 85 p.c. of its equivalent was needed to bring it back to *p*H 9. Hence if we add the ammonia and acid excretions found in this research, we obtain a value for the total acid eliminated free and in combination with ammonia which is too high by 16 p.c. of the ammonia value. 16 p.c. of the ammonia values have therefore been subtracted from the acid excretion, and the result given as "net acid." If the ammonia is titrated with formaldehyde the total value is correct, except for the small error due to amino-acids, but the ammonia values are too low. The buffering

between *p*H 9 and 3·7 is due in part to the hydrolysed fraction of the ammonia, in part to phosphates, each molecule of which buffers one equivalent of acid. The buffer excretions when these values have been deducted are given as "undetermined buffers." Urines were collected in 12 hour periods from 9.30 to 9.30. Quantities ingested and excreted are given in milligram-molecules, or c.c. *N* solution per 12 hrs.

Calcium chloride in 3 p.c. solution produced no nausea, but there was intense diarrhœa, followed by constipation due to the formation of a large hard fæcal mass. There was great general discomfort, pains in the head, limbs and back, and disturbed nights. These effects never occur with ammonium chloride, and must be attributed to the calcium. The alveolar carbon dioxide fell, while acid, ammonia, and phosphate excretion rose, their relations being similar to those found by Haldane(6) in ammonium chloride intoxication and leaving no doubt that an acidosis is produced. The *c*H soon reached its maximum, followed by acid and phosphate excretion, and slightly later by ammonia and the carbon dioxide minimum. The ammonia excretion was still four times normal when the acid and phosphate had returned to their usual values. This was due to a depletion of the phosphate available for excretion, whilst there were still sources for ammonia. The same cause accounts for the exaggerated diurnal drop in phosphate excretion. The *p*H fell to a fairly steady minimum value of 4·9 comparable to that of 4·7 recorded by Henderson and Palmer(7). It is probably analogous to the maximal concentrations of urea, chloride, and bicarbonate found by Ambard and Papin(8) and Davies, Haldane and Peskett(9). Its early fall may betoken renal fatigue. There was no evidence of a maximal concentration of any other constituent being reached, though on the night of the 20th–21st the ammonia reached ·250 *N* and the acidity ·131 *N*. The buffers varied greatly, the undetermined buffers much less, the extreme values for daily excretion being 75·9 and 57·4, though the results of the 25th suggest that they can be washed out by drinking. Their relative steadiness is intelligible since the most important weak electrolytes in urine unanalysed here are uric acid and creatinine. The phosphates on the average only contributed one-third of the total buffering power, while the ammonia occasionally contributed more than the phosphates.

The calcium excretion fell to nearly normal figures within 12 hours of the last salt being taken. The extra calcium in the urine before this time accounts for only 9·3 p.c. of that ingested. The small increase in the calcium excretion which remained is probably to be attributed to

the acidosis, as in the experiments of Givens and Mendel(10). The chloride figures show that 1·41 gram molecule above normal was excreted within 12 hours of the last salt being taken, out of 1·43 ingested, so the chloride must have been completely absorbed.

The negative balance of urinary phosphorus amounted to 85·8 millimols, or 2·66 grams on Oct. 21st, but taking the experiment as a whole it was only ·37 gram, which may well be due to irregularity of the diet. There can therefore have been little increased loss of phosphorus in the fæces. It was impossible to collect these completely, but a stool of Oct. 19th contained 11·2 p.c. of solids, which gave off 71 c.c. of carbon dioxide at 0° and 760 mm. per gram on the addition of acid. This corresponds to a calcium carbonate content of 31·6 p.c. If all the fæcal phosphorus found was present as calcium phosphate ($Ca_3P_2O_8$), (and according to Rogozinski(11) about half of it is organic) the calcium phosphate only amounted to 9·3 p.c. of the dry weight. So at least 3·5 times as much calcium was being excreted in combination with carbonic as with phosphoric acid. A sample of normal fæces contained only ·16 p.c. of calcium carbonate, even if all the combined carbon dioxide was in this form. No hydrogen sulphide was evolved on adding acid. There can be no doubt that calcium carbonate excretion was the main cause of the acidosis. In the neutral intestinal contents calcium carbonate was precipitated, bringing down the carbonate of the digestive juices and replacing it by chloride which was re-absorbed in accordance with the equation

$$CaCl_2 + 2NaHCO_3 = CaCO_3 + 2NaCl + CO_2 + H_2O.$$

Thus, as has been shown to be the case by Baird, Douglas, Haldane and Priestley(12) in ammonium chloride acidosis, the HCO'_3 of the plasma and tissues is partly replaced by Cl′, the alveolar carbon dioxide falling simultaneously so as to keep the *c*H of the blood approximately constant.

The alveolar carbon dioxide with a normal acid and ammonia excretion was 34–35 mm. as against 39–40 mm. in 1920, and 37–38 in the springs of 1922 and 1923. There was no evidence of kidney disease, and we are unable to account for the changes. The ammonia + acid excretion showed a roughly linear relation with the alveolar CO_2 as in 1920, but in this case the kidneys were somewhat more responsive, excreting as much acid + ammonia at low alveolar CO_2 pressures, and less at high ones.

Mr R. Webb, of the Pathological Laboratory, very kindly estimated

the hæmoglobin of the blood on Oct. 19, 20, and 23, and performed cell-counts on the first two dates. In addition, the hæmoglobin and cells were determined after the close of the experiment. The hæmoglobins were 12·5, 12·5 and 10 p.c. above normal, while the red blood corpuscles were increased by 10 and 8 p.c., the whites by 31 and 30 p.c., the increase in lymphocytes being especially marked. There was thus a concentration of the blood as found by Baird *et al.* (12) with ammonium chloride.

Calcium chloride has been successfully used by Blum (13) and his pupils in the treatment of dropsy, œdema and exudations. They find that large oral doses produce diuresis which may outlast the treatment for days, and great loss of sodium. The plasma proteins become more concentrated, and the weight falls. During calcium chloride ingestion in this experiment there was a large loss of water, partly by diarrhœa, and of sodium or potassium since the chloride excretion increased very much more than that of ammonia. Later there was retention of water, accompanied by thirst from Oct. 21st to 24th inclusive. There was also very great sodium or potassium retention, the ammonia excreted being sometimes enough to neutralize all the phosphoric and hydrochloric acids. Blum attributes the loss of sodium and water to antagonism between sodium and calcium, but this cannot be correct since the effect of ammonium chloride is precisely similar. In both cases HCO'_3 is replaced by Cl' in the blood and tissues. But so great is the loss of salt and water that the total amount of chlorine in the body remains unaltered. This appears from the urine analyses and also from the following observation. During a severe ammonium chloride acidosis 40 grams of sodium bicarbonate were eaten with water in two hours. This would normally cause diuresis and bicarbonate excretion. But neither took place nor did any extra chlorides appear in the urine. All this salt was therefore retained, the change of reaction making this possible.

A possible explanation of these phenomena is as follows. The reaction of the arterial plasma is kept nearly constant by the respiratory centre, but that of the tissues and capillary blood becomes more acid owing to loss of buffering power. For the excess of CO_2 pressure in the tissues over that in the blood, and the excess of CO_2 content of venous over arterial blood presumably remain unaltered, and therefore form larger proportions of the total CO_2. The increase in hæmoglobin and other protein content is not enough to counteract this effect. The increased acidity brings the blood and tissue proteins nearer to their isoelectric points, and they therefore release cations which they are holding in

Donnan equilibrium, and diminish their osmotic pressure, thus losing water. If this view is correct, ammonium chloride or phosphate should prove a valuable alternative to calcium chloride where the latter is indicated as a diuretic.

Summary.

1. Calcium chloride taken orally is mainly excreted in the fæces as calcium carbonate, the Cl′ replacing HCO'_3 in the body and causing acidosis.

2. In calcium chloride and ammonium chloride acidosis the body loses water and salts.

3. Phosphates and ammonia account for less than half the buffering of normal urine between *p*H 3·7 and 9·0, but for more than half during acidosis.

Table.

Date Oct.	$CaCl_2$ taken	Extra water drunk c.c.	Alv. CO_2 mm. Hg.	Vol. c.c.	*p*H	NH_3	Acid	Net acid	Phosphate	Buffers	Undetermined buffers	Ca	Cl
16	—	0	34·4	1005	6·4	24·2	34·2	31·3	19·9	61·2	37·4	2·8	162
16–17	—	—	—	455	5·9 –	22·2	36·9	33·3	19·9	55·0	31·5	1·7	39
17	225	1500	32·2	2815	5·6 –	78·0	56·3	33·8	24·8	71·5	34·2	10·4	443
17–18	—	—	—	1175	4·9	57·3	62·2	53·0	25·7	70·3	35·4	9·6	129
18	275	1000	30·3	1620	4·9	64·2	70·0	53·9	31·8	76·5	34·4	14·6	365
18–19	—	—	—	1450	5·0 –	99·9	85·7	69·7	34·7	88·3	37·6	16·5	333
19	275	1000	27·3	1475	4·9 +	102·4	85·7	69·3	36·1	91·5	39·0	16·2	363
19–20	—	—	—	1705	5·0 +	133·2	93·8	72·5	37·3	95·5	36·9	17·1	381
20	—	0	25·4	880	5·2 –	139·3	78·2	55·9	29·5	88·6	36·8	5·5	189
20–21	—	—	—	480	5·5 +	119·9	62·9	43·7	25·9	78·3	33·2	4·2	75
21	—	500	28·0	600	5·4	128·3	54·9	34·5	17·0	67·3	29·7	4·7	125
21–22	—	—	—	438	5·6 –	105·0	50·0	33·2	17·8	62·5	27·9	3·0	73
22	—	500	32·9	440	5·6	90·9	38·0	23·5	8·2	52·8	30·1	3·7	93
22–23	—	—	—	445	5·7 –	71·4	38·0	26·6	11·9	55·3	32·0	—	64
23	—	600	34·9	1082	5·9	70·1	31·2	20·0	6·3	47·1	29·6	—	105
23–24	—	—	—	561	6·0	42·1	30·6	23·9	14·6	48·9	27·6	—	81
24	—	400	35·0	1258	6·4 +	54·3	27·2	18·5	6·6	43·7	28·4	—	—
24–25	—	—	—	560	6·1	37·4	24·8	18·8	15·0	50·0	29·0	—	—
25	—	*ad lib.*	34·7	1980	6·4	49·9	28·5	20·5	6·8	61·2	46·4	—	—
25–26	—	—	—	502	—	25·0	26·3	22·3	19·2	47·8	24·6	—	—
26	—	*ad lib*	33·9	2020	—	42·8	24·8	18·0	10·3	54·7	37·6	—	—
26–27	—	—	—	473	—	22·8	31·6	28·0	20·3	52·3	28·3	—	—
27	—	*ad lib.*	33·9	1464	—	31·7	21·8	16·7	12·3	54·6	37·3	—	—
27–28	—	—	—	472	—	—	—	—	19·6	—	—	—	—
28	—	*ad lib.*	—	2030	—	—	—	—	13·1	—	—	—	—
28–29	—	—	—	343	—	—	—	—	20·9	—	—	—	—

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[illegible] CHANGES IN THE UPPER INTESTINAL TRACT FOLLOWING THE USE OF CALCIUM CHLORIDE IN NEONATAL TETANY*

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CALCIUM chloride orally in doses as [illegible] high as 6 to 8 grams daily in water or milk is an accepted form of treatment for neonatal tetany, although frequent reports in the literature state that it is irritating and may cause vomiting. Durlacher, Harrison and Darrow[3] are the first to demonstrate by autopsies on 2 infants that calcium chloride may have harmful effects on the stomach, duodenum, and jejunum. One of their patients was given 4 grams of calcium chloride in 25 cc. of water (16 per cent) by gavage for neonatal tetany. Two hours later the child had blood-stained vomitus and blood in the stools. The infant died thirty-six days after birth, and autopsy showed fibrinous adhesions covering the serosa of the stomach with a suppurative peritonitis. The mucosa of the stomach was replaced by opaque yellow calcareous plaques which were firmly attached to the submucosa. Similar changes were present in the duodenum and jejunum. Microscopically there was ulceration and necrosis of the gastric and upper intestinal mucosa with plaques of hyaline scar tissue, some of the scars containing calcium. Some of the arteries in the stomach and intestinal wall showed necrosis and calcification in the media and organizing and calcified thrombi within the vessels.

Their second patient was given 3 grams of calcium chloride in 18 cc. of water (16.6 per cent) by gavage two days after birth and this was followed by 1 gram in each feeding for one day. The infant died on the seventh day. Autopsy showed ulcerated mucosa in the fundus and along the greater curvature of the stomach. The [illegible] of the [illegible] were filled with a white [illegible] [illegible] having the [illegible] [illegible] apparently calcium plaques. Microscopically there was necrosis of the mucosa and of some of the blood vessel walls.

Richards and Eriksen[7] have had a case which will soon be reported in which a newborn infant was given calcium chloride by gavage. Roentgenograms made approximately two and five days after calcium chloride was placed in the stomach showed no calcification in the intestinal tract, but films made nine days after the second examination showed calcification in the stomach, duodenum, and first portion of the jejunum. Calcification was also found in the esophagus at autopsy.

The following case also showed calcium in the stomach wall on roentgenograms, although this infant fortunately survived the severe effects produced by the calcium chloride on the gastric mucosa.

CASE REPORT

One week following a normal birth this infant had three convulsions after vomiting several times. The infant was hospitalized, and 2 grams of calcium chloride in 12 cc. of water (16.6 per cent) was given by stomach tube under the assumption that the convulsions were due to neonatal tetany. The baby had no more convulsions but vomiting and diarrhea became almost continuous after this. There was no blood in the vomitus or stools. Seven days after admission a flat piece of necrotic tissue approximately 4 by 5 cm. in size was discovered by the baby's head. This tissue was sent to the pathology department and microscopically it was thought to be membranous tissue from the stomach although accurate identification was impossible due to marked necrosis.

Thirteen days after admission a roentgenogram of the abdomen showed a continuous calcium outlining part of the stomach [illegible] 1). Re-examination of the abdomen [illegible]

[illegible]

[illegible] in the fundus of [illegible] the calcium deposit in the stomach wall had previously been most [illegible] evidently a [illegible] contraction (Fig. [illegible]). There was no remaining evidence of calcium in the stomach wall on a preliminary film of the abdomen at that time.

It is theoretically possible that the calcium deposit outlining the stomach wall in such cases as the above could represent

Fig. 1. Calcium [illegible] thirteen days [illegible] calcium chloride [illegible]

Fig. 2. [illegible] in the [illegible]

calcium derived from the calcium chloride injected into the stomach by [illegible] with such a theory it is [illegible] the calcium chloride [illegible]

[illegible]

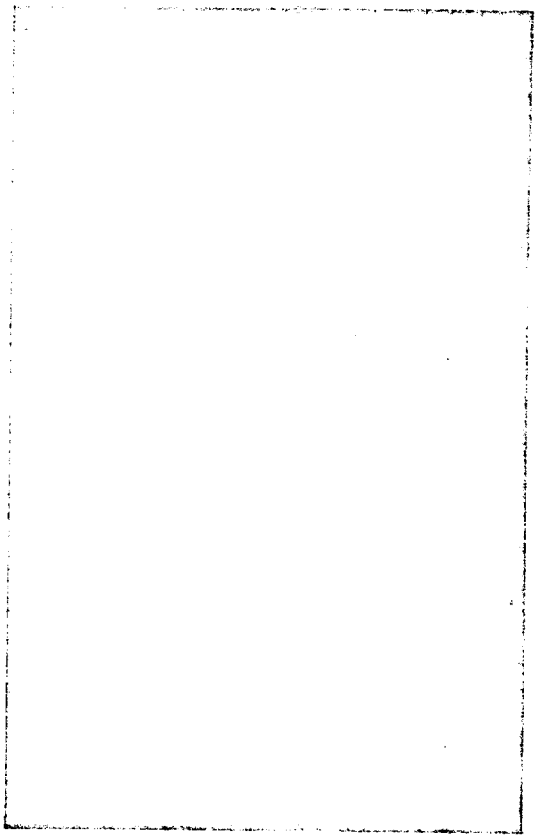

FIG. 3. Lateral film made at the same time as Figure 2 showing the calcification in the stomach wall more clearly.

some time after the tissues have become necrotic and not immediately after calcium chloride is injected into the stomach. This is apparent in the case of Richards and Eriksen[7] where films on the second and fifth days after calcium chloride was placed in the stomach were negative, but films on the fourteenth day showed calcium in the intestinal wall. Even though the exact time at which calcium first appeared in the stomach wall in our case is not known the evidence would point to the same conclusion since there is a definite increase in the amount of calcification in the stomach wall between the first (Fig. 1) and second (Fig. 2 and 3) roentgen examinations. Microscopic sections of the stomach in the case autopsied by Dudacher *et al.*[6] showed calcified thrombi within the lumen of blood vessels as well as calcium in the muscular vessels, which would also indicate a secondary deposition of calcium in the necrotic tissues rather than an original deposit of calcium derived from the calcium chloride injected into the stomach. The deposition of insoluble calcium compounds in necrotic tissue is of very common occurrence and the process is familiar to all physicians, although the way in which it occurs is not clearly understood.

Hamilton[4] has expressed a theory which may explain the necrotizing action of calcium chloride on the intestinal mucosa. He thinks without having performed any experiments for proof, that the calcium chloride in the stomach may undergo some hydrolysis with the formation of a certain amount of hydrochloric acid. This hydrochloric acid may then penetrate into the intestinal wall and produce the necrosis.

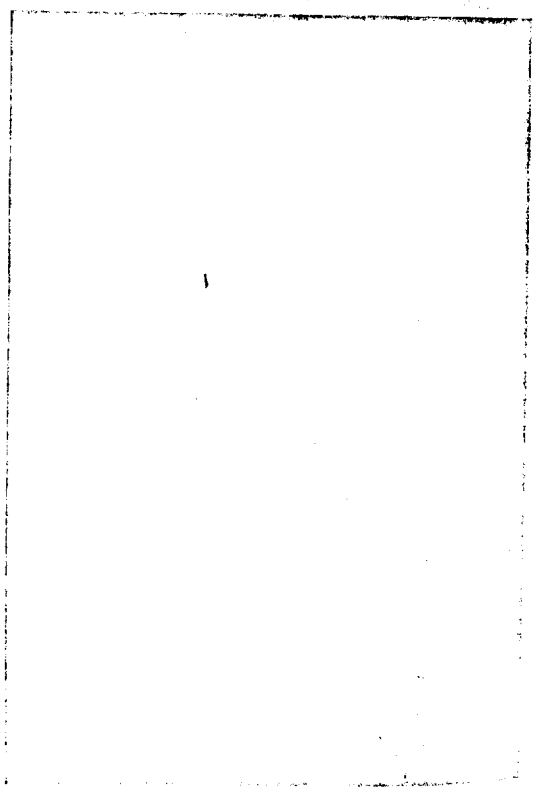

FIG. 4. Film showing constant constriction in the fundus of the stomach approximately eight [illegible] after the first examination.

Experiments by Dunlacker et al on rabbits not only demonstrated the harmful effects of calcium chloride on the stomach but they also showed that these effects were more marked in very young rabbits than in older rabbits. It is quite probable that infants in the neonatal period may likewise be more susceptible to calcium chloride than older children or adults. The author has had personal experience with a man of middle age who took 45 grams of calcium chloride by mouth daily for more than a year without any apparent harmful effects.

SUMMARY

Calcium chloride even in small doses may have very harmful effects on the upper intestinal tract of newborn infants, and necrosis of tissues and death have been reported after its use in neonatal tetany. Secondary calcium deposits in the necrotic tissue in the esophagus, stomach, and jejunal walls followed later by extreme contractures may also occur after its use.

The author is indebted to Dr. Howard Brayton whose case is reported in this article.

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ANN. Rept. Natl. Inst. Nutrition(Tokyo). 1959.

Studies on the Calcium Metabolism of Aged Women (Part II) An Experiment on Skim Milk enriched with two Calcium Salt made under a Special Processing

by

Hiroshi Hayami, Kunihiko Shino and Ayako Kaga

It is believed that the gastric acidity of the aged people is usually lower than that of the adult, and the effect of the solubility of calcium on its utilization is greater in the aged people than the younger adult.

In this experiment, a calcium balance study was performed using as calcium source; skim milk enriched with calcium salts having different solubility, namely calcium carbonate and calcium gluconate.

Experiment. Subjects. Four aged women, 60, 68, 80 and 80 years old, were employed as subjects. Experimental period was 8 days long: from 12 to 19 Dec. 1958, and was divided into 2 stadia of four days each, 1st being a stadium of standard diet, 2nd a calcium adding stadium. Two subjects were given calcium gluconate and the others calcium carbonate, so that the amount of calcium might be equal by calculation.

Results. Absorption rate of calcium of the standard diet in the 1st stadium and the rate of utilization of the added calcium in 2nd stadium were shown on the table 1.

At the calcium intake of 310mg (7.6mg per kg body weight) in the 1st stadium, only one subject showed positive calcium balance.

The absorptions of calcium in the standard diet of the four subjects ranged from 59% to 4.4%.

In the 2nd stadium, 579mg calcium intake (14.2 mg per kg body weight), they showed 100mg retention, and the utilization of calcium salts were 59.2 and 58.8% on calcium carbonate and 59.2 and 17.9% on calcium gluconate respectively.

Conclusion. From the result of this experiment, it was found that the calcium balance was positive when the intake was more than 10mg per kg of body weight, and calcium carbonate was utilized more than 50% so well as water soluble calcium salt.

Table 1. Calcium Balance (mg/day)

Studium	Subjects		S.S.	M.K.	S.A.	T.O.
	Age Body weight		80 40.0kg	80 40.5kg	60 45.0kg	68 38.0kg
I	Intake		308	304	308	318
	Excretion	Feces	208	124	249	304
		Urine	120	183	92	43
		Total	328	303	341	347
	Balance		−20	+ 1	−33	−29
II	Intake	Diet	298	293	298	309
		Ca. Salt	243	316	243	316
		Total	541	609	541	625
	Excretion	Feces	264	299	361	491
		Urine	159	156	76	108
		Total	423	455	437	599
	Balance		−118	+154	+104	+26
Absorption rate of Ca. in I Stad. %			32.5	59.2	19.2	4.4
Utilization of Ca. Salts in II Stad. %			59.2	50.2	58.8	17.9

Ann. Rept. Natl. Inst. Nutrition (Japan)1951-52.

Studies on the Utilization of Calcium
Part VI A Comparative Study of Organic and Inorganic Calcium Salts

by

Hiroshi Hayami, Kikuko Yoshimura Yuki Hirabe,
and Kazuko Mori

From the results of our previous experiments on human beings and animals, we concluded that calcium carbonate may be the most convenient calcium source for Japanese people. But some people think that water-soluble calcium salts are more nutritious than water-insoluble one. The investigation was planned twice to compare the utilization of calcium gluconate (water-soluble) and calcium carbonate (warter-insoluble) as calcium sources.

For preparing diets, instead of calcium lactate of the standard diet, we used 2.25 g Ca-gluconate and 0.5 g Ca-carbonate respectively as their calcium contents were almost equal.

The methods used were same as before.

Results are shown in tables I—V. No great difference was found between them in both experiments.

Table 1. First experiment

Group	No. of rats	Sex	Body weight (g)			Length of Bone (cm)		Length of Body (cm)	Diet	
			Initial	Final	Increase	Femur	Tibia		Total Amount of Food Intake	Utilization of Food (%)
Ca-Gluconate	344	♂	38	99	61	2.36	2.83	16.5	194.5	31.36
	345	♂	36	103	67	2.38	2.84	17.4	234	28.63
	346	♂	34	99	65	2.31	2.82	16.3	218	29.81
	347	♀	36	85	49	2.22	2.72	15.8	196	25.00
	348	♀	32	92	60	2.30	2.79	15.8	217	27.65
	Average				60.2	2.32	2.80			28.49
Ca-Carbonate	349	♂	37	96	59	2.29	2.79	15.8	186	31.72
	350	♂	36	124	88	2.47	2.90	17.5	253.5	34.71
	351	♀	36	101	65	2.30	2.80	16.3	204	31.86
	352	♀	35	106	71	2.30	2.89	16.8	224.5	31.63
	Average				70.8	2.34	2.87			32.48

Table II. First Experiment

Group	No. of Rats	Sex	Calcium Intake			Retention and Utilization of Ca			
			Food intake (g)	Ca content (%)	Amount of Ca (g)	Beginning of Exp.	End of Exp.	Retention (g)	Utilization (%)
Ca-Gluconate	344	♂	194.5	Ca	0.7391	0.2387	0.8047	0.5660	76.58
	345	♂	234	0.380	0.8892	0.2258	0.8532	0.6274	70.56
	346	♂	218	P	0.8284	0.2135	0.8435	0.6300	76.05
	347	♀	196	0.547	0.7448	0.2457	0.7388	0.4931	66.21
	348	♀	217		0.8246	0.2138	0.8070	0.5932	61.94
	Average								72.27
Ca-Carbonate	349	♂	186	Ca	0.6863	0.2319	0.8395	0.6076	88.53
	350	♂	253.5	0.369	0.9354	0.2258	0.9735	0.7477	79.93
	351	♀	204	P	0.7528	0.2457	0.8447	0.5990	79.56
	352	♀	224.5	0.525	0.8284	0.2390	0.8802	0.6412	77.40
	Average								81.36

Table III. First Experiment

Group	No. of Rats	Sex	Femur and Tibia					Carcase (Except Femurs and Tibias)	
			Defatted dry bone (g)	Ash (g)	Ash / Defatted dry bone (%)	Rickets Coefficient	Ca-content of Ash (%)	Ash (g)	Ca-content of Ash (%)
Ca-Gluconate	344	♂	0.2763	0.1425	51.61	1.06	38.35	2.4508	28.37
	345	♂	0.2848	0.1479	51.93	1.08	37.67	2.5865	28.68
	346	♂	0.2815	0.1483	52.85	1.12	38.25	2.4905	29.30
	347	♀	0.2651	0.1358	51.22	1.05	37.80	2.2750	27.96
	348	♀	0.2745	0.1499	54.61	1.20	38.00	2.4429	28.37
				0.1450	52.44	1.10			
Ca-Carbonate	349	♂	0.2715	0.1405	51.78	1.07	38.29	2.3707	30.87
	350	♂	0.3401	0.1733	50.95	1.03	39.51	2.9619	28.24
	351	♀	0.2638	0.1377	52.19	1.09	38.44	2.4578	30.06
	352	♀	0.2620	0.1430	53.34	1.14	39.50	2.5639	30.01
				0.1480	52.07	1.08			

Table IV. Second Experiment (Average)

Group	No. of Rats	Body Weight (g)		Length of Bone (cm)		Length of Body (cm)	Diet		
		Initial	Increase	Femur	Tibia		Total Amount of Food Intake (g)	Utilization of Food (%)	Amount of Ca (g)
Ca-Gluconate	♂ 2 ♀ 1	42.8	62.2	2.40	2.94	16.6	224.5	27.65	0.8531
Ca-Carbonate	♂ 2 ♀ 1	39.3	78.7	2.49	2.97	17.1	261.3	30.03	0.9645

Table V. Second Experiment (Average)

Group	Femur and Tibia				Carcase		Retention and Utilization of Ca			
	Defat. dry bone (g)	Ash (g)	Ash / Def. Dry Bone (%)	Ca-cont. (%)	Ash (g)	Ca-content of Ash (%)	Beginning of Exp. (g)	End of Exp. (g)	Retention (g)	Utilization (%)
Ca-Gluc.	0.3257	0.1624	49.79	38.28	2.6232	30.43	0.2759	0.9222	0.6463	75.65
Ca-Carb.	0.3340	0.1724	51.78	38.1[illegible]	2.8282	29.45	0.2529	0.9643	0.7114	73.62

From these results we concluded that water-insoluble calcium salts are available as well as water-soluble ones as calcium sources.

J. Pharmacol. & Exper. Therap. 60:425-33. 1937.

AN ANALYSIS OF THE CARDIAC IRREGULARITIES PRODUCED BY CALCIUM, AND THEIR PREVENTION BY SODIUM AMYTAL[1]

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I. INTRODUCTION

The intravenous administration of calcium salts is extensively employed in a variety of clinical conditions Occasional reports of serious consequences following this procedure have appeared, and divergence of opinion exists concerning the mechanism of these accidents. Bower and Mengle (1) reported two deaths following intravenous injection of calcium gluconate in patients who had previously received digitalis. In experiments on digitalized dogs they noted an abrupt and dramatic cessation of heart action and the fall in blood pressure to zero following calcium administration. Electrocardiograms taken by Lloyd (2) following self-administration of calcium showed total cardiac arrest after injection of 4 cc. of 10 per cent calcium chloride. Walters and Bowler (3), on the contrary, attributed death from administration of calcium chloride in dogs to ventricular fibrillation.

The studies reported here were undertaken (*a*) to determine the mechanism of calcium syncope, (*b*) to analyse the cardiac arhythmias which appear after calcium injection, and (*c*) to devise a method for their prevention.

II. METHODS

Three series of experiments were undertaken. In the first series 10 normal unanaesthetized rabbits were employed. In the second, 10

[1] This research was aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

unanaesthetized rabbits received 25 to 50 mgm. atropine sulphate intravenously, and in the third, 10 rabbits were anaesthetized with 60 mgm. sodium amytal per kilogram. All of the animals received 2 to 6 cc. of 10 per cent calcium chloride solution intravenously at a rate of 1 cc. per 30 seconds, and serial electrocardiograms were taken from Lead II.

III. RESULTS

A. General calcium effects. In all thirty experiments, whether or not atropine or sodium amytal was given, changes took place in the nature of the ventricular complex, which were so uniform and characteristic that they may be considered specific calcium effects. These were: (*a*) widening of the QRS interval; (*b*) increase in amplitude of S; (*c*) disappearance of the S-T interval; (*d*) displacement of the S-T take off; and (*e*) characteristic changes in the contour of T (figs. 1, 2, 3, 4 and 5).

B. Further changes in the non-anaesthetized heart. Slowing and delay in the A-V conduction regularly appeared after injection of 1 cc. of calcium chloride, and occasionally ventricular extrasystoles were noted. After 2 cc. were injected auricular fibrillation developed in every experiment. Ventricular extrasystoles were encountered with increasing frequency and the P-R delay increased to partial and total A-V block. With larger doses periodic arrest of the heart occurred, alternating with multiple foci ventricular extrasystoles at a slow rate, and finally complete arrest took place. Massage of the heart through the chest wall and diaphragm and artificial respiration restored the heart beat in every instance. Resumption of cardiac activity was characterized by frequent ventricular extrasystoles, auricular fibrillation, subnormal A-V conduction and final reëstablishment of a normal rhythm (fig. 1).

C. Changes in the atropinized heart. When calcium was injected within a few minutes after atropinization, auricular fibrillation appeared in but two of the ten experiments (fig. 4), but then only as a transient phenomenon, after administration of 3 and 4 cc. Very slight slowing occurred, and occasional slight increase in the P-R interval was found. Ventricular extrasystoles were more abundant, and in two experiments ventricular

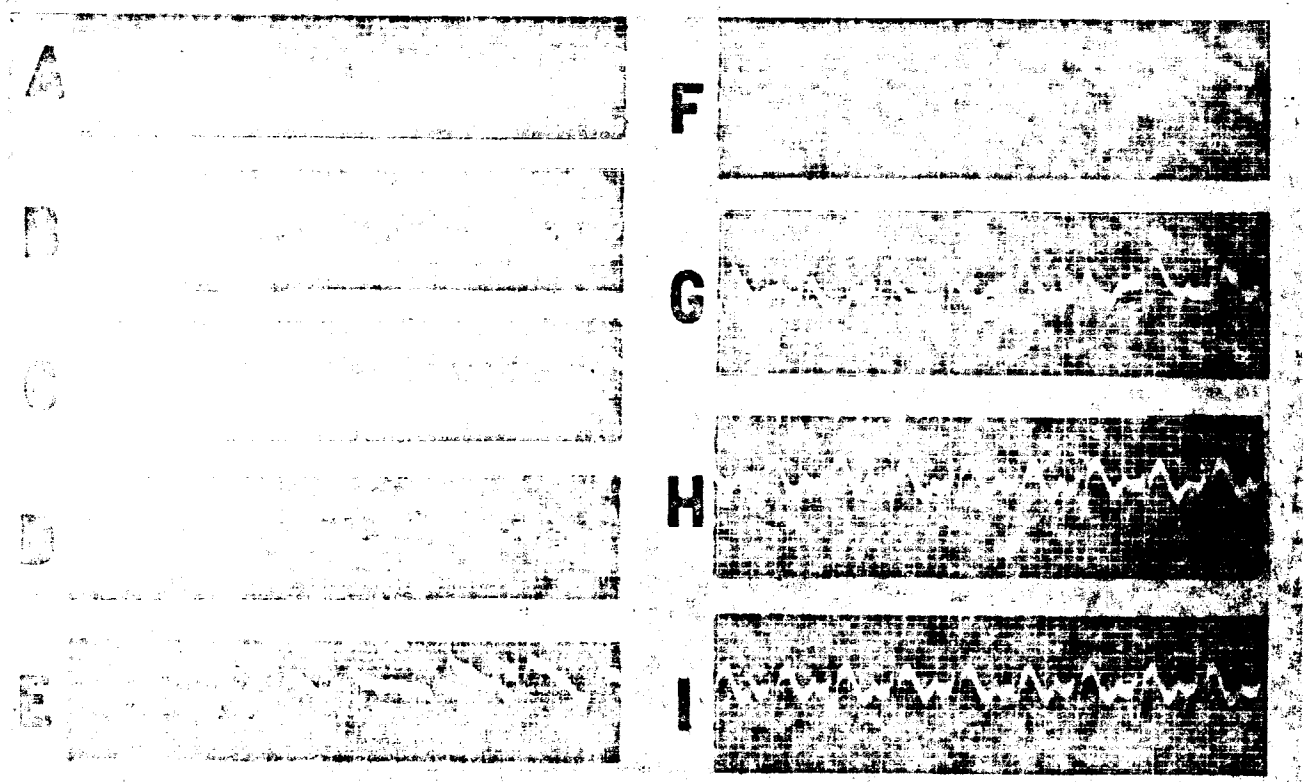

FIG. 1. Normal unanaesthetized rabbit. Lead II. 3 cc. 10 per cent $CaCl_2$ intravenously at 2 cc. per minute. *A*, control. *B*, After injection of 1 cc. *C*, 2 cc. Onset of auricular fibrillation. Changes in QRST. *D*, 3 cc. Occasional return of *P* waves. *E*, immediately after *D*. Auricular fibrillation. *F*, *G*, *H*, and *I*, records at 30 second intervals, showing recovery.

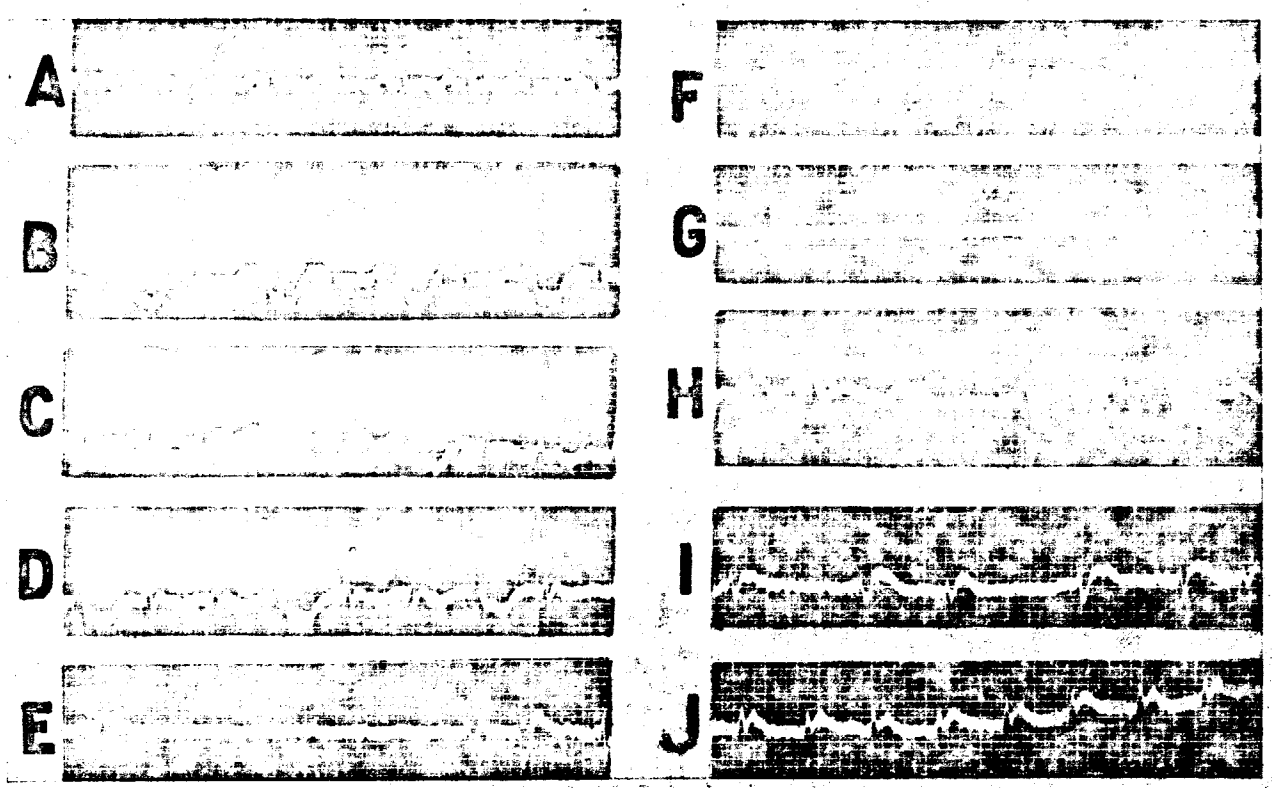

FIG. 2. Normal unanaesthetized rabbit. Lead II, 4 cc. 10 per cent $CaCl_2$ intravenously at 2 cc. per minute

A, control. *B*, 1 cc. $CaCl_2$. Bigeminus rhythm. *C*, immediately after *B*. Auricular fibrillation. Occasional extrasystoles. *D*, 2 cc. *E*, 3 cc. Periods of arrest. *F*, 4 cc. Auricular fibrillation, extrasystoles. *G*, immediately after *F*. Arrest. *H*, 30 seconds after *G*. Multiple foci, extrasystoles. *I*, 20 seconds after *H*. Auricular fibrillation. *J*, 30 seconds after *I*. Return to regular rhythm.

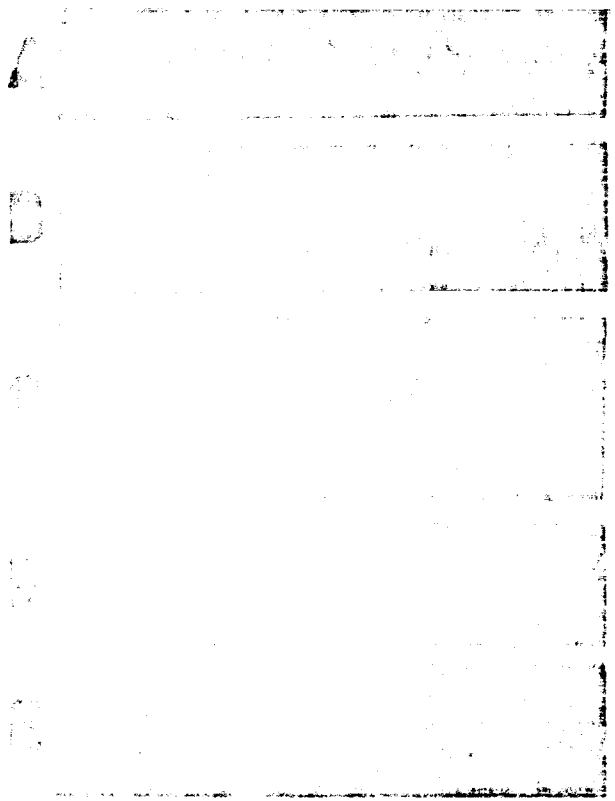

FIG. 3

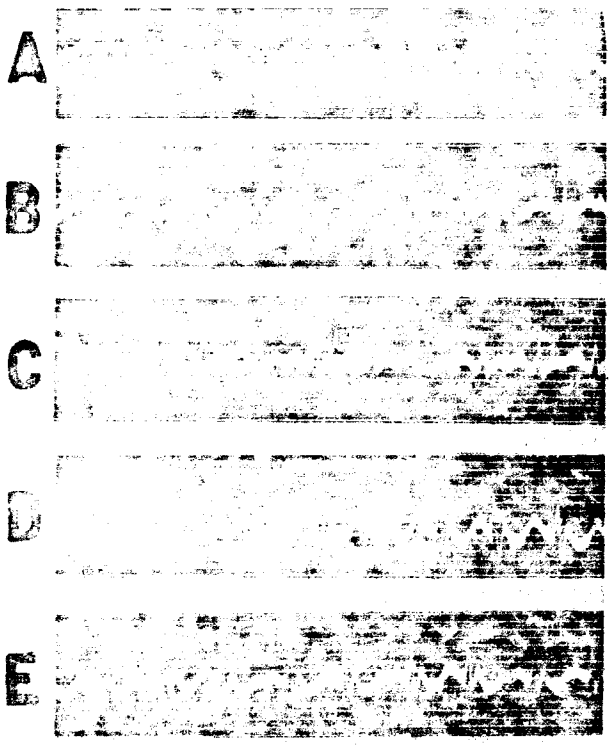

FIG. 4

FIG. 3. Normal rabbit. 0.60 mgm. sodium amytal per kilogram, intraperitoneally. Lead II, 3 cc. 10 per cent $CaCl_2$ intravenously at 1 cc. per minute.

A, Control after anesthesia. *B*, 1 cc. $CaCl_2$. *C*, 2 cc. *D*, 3 cc. *E*, 1 minute after *D*.

FIG. 4. Normal unanesthetized rabbit. Lead II, 25 mgm. atropine sulphate intravenously. 4 cc. $CaCl_2$ intravenously at 20 cc. per minute, started 2 minutes after injection of atropine.

A, control. *B*, 1 minute after injection of atropine. *C*, 1 cc. $CaCl_2$. *D*, 4 cc. $CaCl_2$. *E*, 1 minute after *D*.

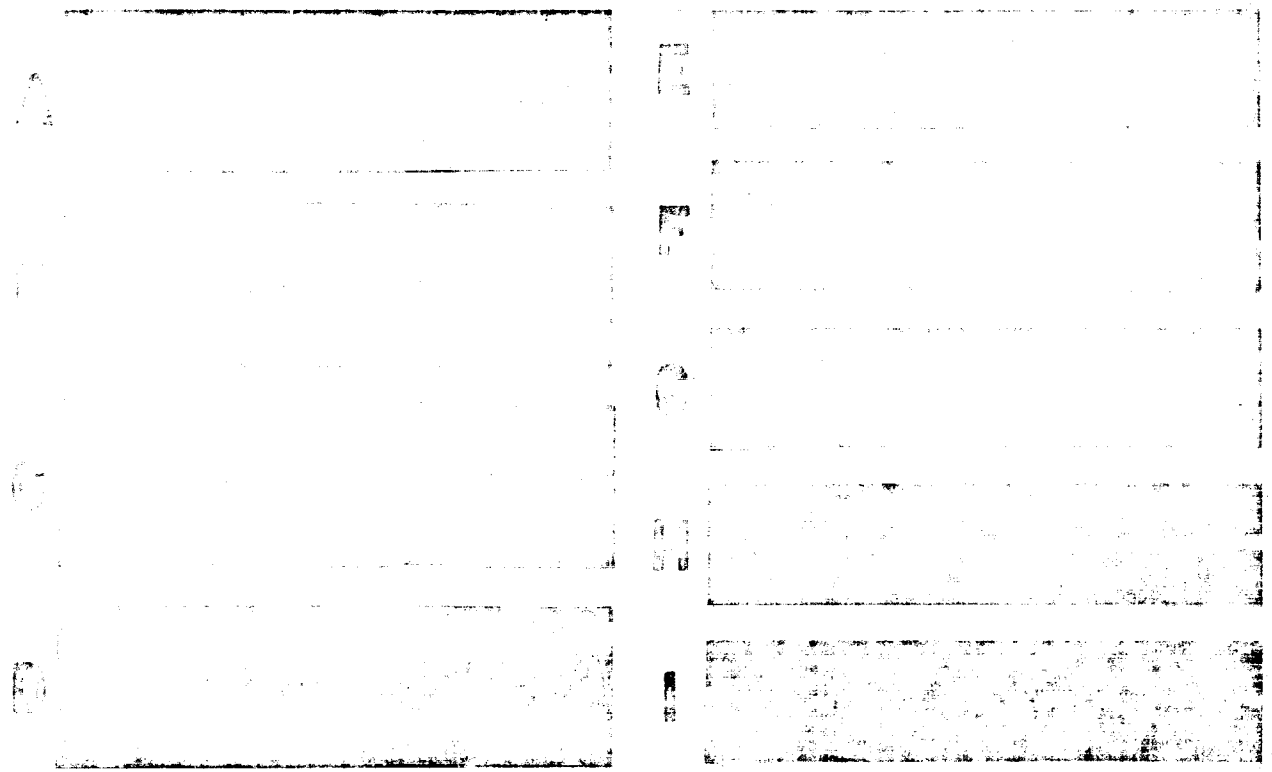

FIG. 5. Normal unanaesthetized rabbit. 25 mgm. atropine sulphate intravenously. 4 cc. 10 per cent $CaCl_2$ intravenously at 2 cc. per minute.

A, control. Rate 270. *B*, 1 minute after atropine. Rate 330. *C*, after 1 cc. $CaCl_2$ injection started 2 minutes after injection of atropine. *D*, 2 cc. $CaCl_2$. Bigeminus rhythm. *E*, 3 cc. Ventricular extrasystoles and ventricular fibrillation. *F*, 4 cc. *G*, periods of arrest and ventricular tachycardia. *H*, arrest, 1 minute after *F*. *I*, recovery.

tachycardia developed. In one of these this culminated in ventricular fibrillation. Periodic cardiac arrest alternating with ectopic ventricular beats (Luciani periods), and final cardiac standstill also occurred in these experiments (fig. 5).

D. Changes in the anaesthetized heart. Following moderately light anaesthesia with sodium amytal, electrocardiograms revealed a rapid heart with a generalized reduction in amplitude of the complexes. In these animals as much as 4 cc. of calcium chloride failed to produce any arrhythmia whatsoever.

E. Other phenomena. In the normal rabbits, calcium invariably provoked an extreme myosis and active intestinal movements. These phenomena were not affected by atropine or amytal.

IV. DISCUSSION

The evidence presented here demonstrates that, in the rabbit, death from poisoning with calcium chloride is due to arrest of the heart. This is in agreement with Lloyd's findings after self-administration of toxic amounts of calcium chloride. Walters and Bowler, however, described ventricular fibrillation as the cause of death in dogs given toxic doses of calcium chloride. One of the experiments in this study terminated in ventricular fibrillation, when large amounts of calcium chloride were administered to an atropinized animal. When the vagus was paralysed in this manner the incidence of ventricular extrasystoles was greatly increased, and ventricular tachycardia appeared in two instances. It is thus apparent that vagal paralysis promotes the appearance of such ventricular rhythms, which are recognized as precursors of ventricular fibrillation.

The mechanism of this phenomenon seems to be the relative sympathetic preponderance resulting from vagal paralysis. Rothberger and Winterberg have called attention to the importance of the sympathetics in the production of extrasystoles by calcium (4), and it is recognized that sympathetic activity plays a significant part in the genesis of extrasystolic irregularities in physiological as well as pathological states (5, 6). The discrepancy between the present results and those of Walters

and Bowler might therefore be due entirely to the existence in their animals of a relative preponderance of the sympathetic nervous system.

All the experiments on normal rabbits revealed a marked slowing of the heart and delay of A-V conduction, with occasional block following even small doses of calcium. The practical absence in the atropinized animals of these phenomena indicates that in the normal animal calcium stimulates the vagal mechanism. The production of myosis and vigorous intestinal movements points to a general action on the parasympathetic system. Vagal effects on the heart have been noted in man by Billigheimer (7) who prevented them by atropine, and by Iwasaki (13) and Berliner (4).

The rôle of the vagus in the production of auricular fibrillation in certain conditions has previously been described (9). In the present experiments also the auricular fibrillation may in part be attributed to vagal activity, since it was regularly observed after injection of 2 cc. of calcium chloride in normal animals, and failed to occur with equal doses in the atropine series. Larger amounts (3 to 4 cc.) failed to produce it except as a transient phenomenon in two out of ten experiments. Loss of P waves, with total irregularity of the heart has been reported following injection of calcium chloride in rabbits by Kraus (10) and Frohlich and Gussenbauer (11) who did not designate it as auricular fibrillation.

The total absence of disturbances of rhythm in the amytalized animals probably may be attributed to the depression of both vagal and sympathetic activity which it induces (14). The protection against final arrest with large doses of calcium provided by amytal anaesthesia, together with the changes induced by amytal in the amplitude of the complexes in the electrocardiogram, seem to be evidence of a direct action on the myocardial fibers, as well as the cardiac nerves.

The characteristic changes in the ventricular complexes produced by calcium have been noticed by others, who attributed them to alterations in intraventricular conduction (10, 11, 12). Inasmuch as they might be due to changes in the action potential

of individual fibers further study is necessary before such a conclusion can be accepted.

SUMMARY AND CONCLUSIONS

1. Intravenous injection of calcium chloride produces a characteristic ventricular electrocardiogram which is not modified by atropine or sodium amytal.

2. One to 2 cc. of a 10 per cent solution of calcium chloride, injected at a rate of 2 cc. per minute produces slowing of the heart, A-V delay, and auricular fibrillation. Larger doses cause extrasystoles from many foci, Luciani periods, and cardiac arrest.

3. Suppression of vagal activity by atropine prevents A-V delay and auricular fibrillation, but promotes the development of ventricular extrasystoles, and leads occasionally to ventricular tachycardia and ventricular fibrillation.

4. Sodium amytal in narcotic doses suppresses all cardiac irregularities due to calcium chloride and prevents arrest of the heart.

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Phosphorus-Free Calcium Supplements in Pregnancy

JEROME J. HOFFMAN, M.D., and GEORGE BLINICK, M.D.

THE METABOLISM OF CALCIUM during pregnancy and the effects of calcium supplements are poorly understood.[1]

Recently Page and Page have advocated the use of phosphorus-free calcium and succinctly reviewed the pertinent literature. The following experiment is an attempt to investigate further the metabolic influence of phosphorus-free calcium supplements in pregnancy.

MATERIAL AND METHODS

A group of 42 service patients attending the Beth Israel Hospital Obstetrical Clinic was selected for a 9-week test period. These patients were in the twenty-first to thirty-first weeks of pregnancies and were chosen whether or not they had experienced leg cramps. Excluded from the group were those who displayed varicosities of sufficient degree to obscure therapeutic results, as were cardiac and diabetic patients who might require unusual dietary regimes. These patients were divided into six equal groups. The control values of serum calcium were determined by the Clark-Collip modification of the Kramer-Tisdall Method; serum inorganic phosphorus by the method of Fiske and Subba Row; and serum total protein by the method of Looney and Walsh.

For successive 3-week periods, each of the groups was given a supplement of either calcium citrate, carbonate, or lactate, 6 capsules a day providing, in each instance, a daily intake of 450 mg. of elemental calcium. Concurrent aluminum hydroxide administration as performed by other investigators was not employed. At the conclusion of each 3-week period, the calcium, phosphorus, and total protein content of the serum were determined, the occurrence or absence of leg cramps noted, and another of the 3 calcium salts was prescribed. These supplements were employed with varied rotation schemes, allowing six permutations. Because of failure to take the supplements in the prescribed manner, faulty clinic attendance, or other technical or personality difficulties, only 36 of the 42 patients successfully concluded the experiment. There was a minimum of 5 patients concluding the experiment in each rotation scheme.

Of these 36 patients, 14 had experienced leg cramps prior to therapy. The others had suffered no previous leg cramps. All patients, however, received the same therapy in the same dosage.

Four successive serum levels of ionized calcium and inorganic phosphorus were determined in the serum of each patient. We took into account not only absolute values, but also fluctuating values, of the ratio of ionized calcium to phosphorus as the treatment progressed, defining a rise as a "good treatment effect" and a fall as a "poor treatment effect." It was then possible to evaluate the behavior of these ions and their ratio during phosphorus-free calcium therapy in later pregnancy. An attempt was then made to establish a correlation between these p[illegible]

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Obstetrics and Gynecology
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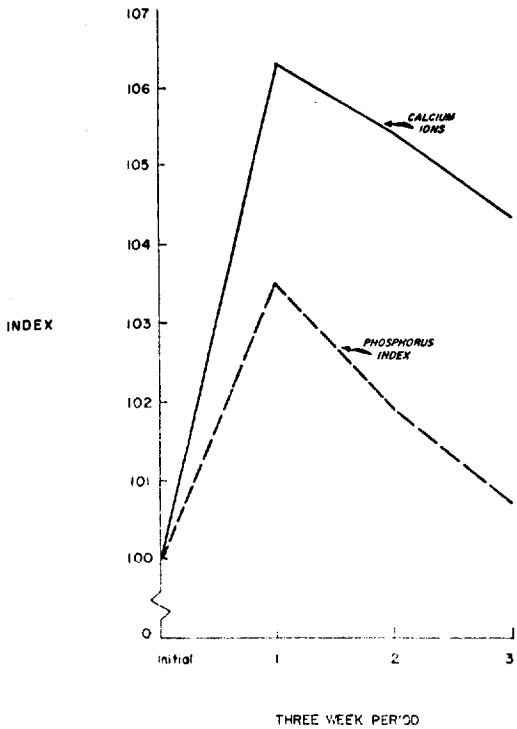

Fig. 1. Phosphorus and ionic calcium by experimental period.

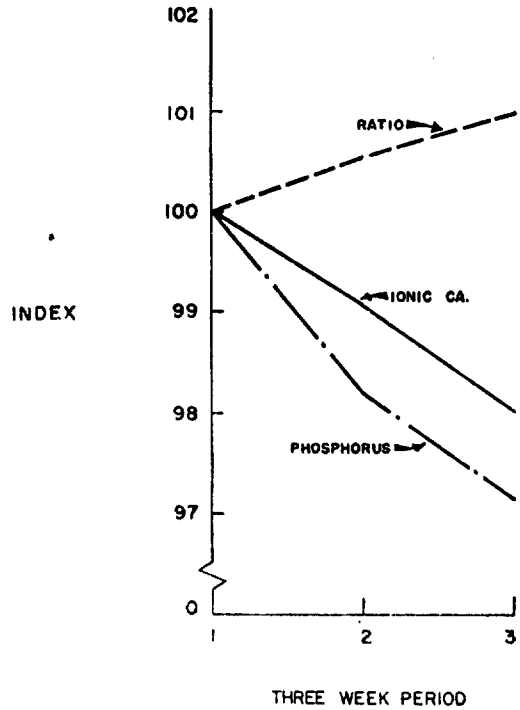

Fig. 2. Ratios of phosphorus to ionic calcium, by experimental period.

terns of behavior and the incidence of leg cramps.

OBSERVATIONS ON CALCIUM AND PHOSPHORUS LEVELS

1. Ionic calcium and phosphorus both showed some increase from their control values during the three test periods of calcium therapy. Since both the numerator and denominator of the ratio increased, the ratios were little changed. This is illustrated by plotting the fluctuation of these ions against an index of percentage rise in Figure 1. It seems likely that previous workers who demonstrated a more dramatic widening of the calcium-phosphorus ratio may have done so by having lowered the denominator (the phosphorus level) by means of concurrent aluminum hydroxide gel administration, and probably not by the addition of calcium.

2. Absolute values of ionic calcium and phosphorus levels, after an initial increase, decrease as the treatment proceeds. This may be due to physiologic hemodilution as well as increased fetal uptake in the latter months of pregnancy. Nonetheless, the values at the end of the 9-week period were still higher than the initial values.

3. Since phosphorus declined more rapidly than ionic calcium, the ratio showed a slight increase from the first to the third experimental period. This is demonstrated in Figure 2. During this time there was also a definite downward trend in the over-all occurrence of leg cramps. At first glance it seemed that there may have been a relationship between the increase of the calcium-phosphorus ratio and the lowered incidence of leg cramps. On closer investigation this proved to be erroneous, since the group with leg cramps and the group without showed no statistically significant differences in the calcium-phosphorus ratios. An observer could not prognosticate which patients may

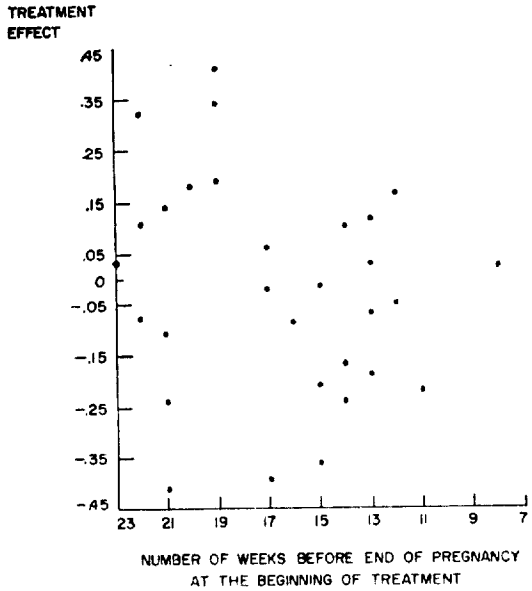

Fig. 3. Relationship of stage of pregnancy and treatment effectiveness.

have experienced leg cramps on the basis of the calcium-phosphorus ratios.

4. In considering the individual patients contributing to the graph in Figure 2, the calcium-phosphorus ratio was increased in some, and decreased in others, as the treatment progressed. There is no evidence that the initial calcium-phosphorus ratios are correlated with the ones observed after the treatments.

5. If calcium supplementation was initiated in early pregnancy, it was noticeably more likely to have a positive treatment effect than if it was initiated late in pregnancy. This is shown in Figure 3 and Table 1. In late pregnancy, negative effects were far more common. We defined "treatment effect" as

TABLE 1. STAGE OF PREGNANCY AND TREATMENT EFFECT

Stage of pregnancy at beginning of treatment	*No. patients*	*Average treatment effect*	*No. patients showing:* *Increases*	*Decreases*
Early	16	+.05	10	6
Late	15	−.08	5	10

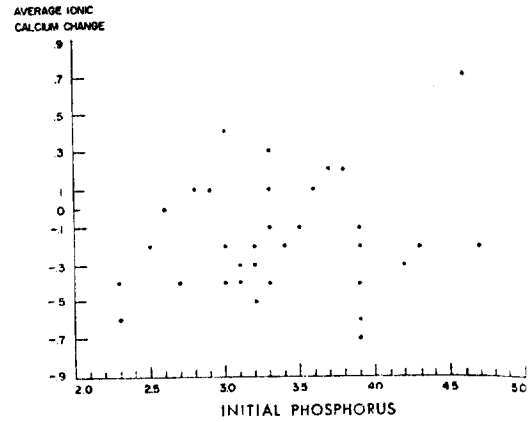

Fig. 4. Relationship of initial phosphorus and ionic calcium change.

the difference between the initial valu[illegible] the calcium-phosphorus ratio and its [illegible] value.

6. Neither the initial values of phosphorus nor changes in phosphorus levels had any effect on ionic calcium change, as shown in Figures 4 and 5. This observation would seem to be most interesting and relevant, since it would cast doubt on the utility of aluminum hydroxide gel to change the ionic serum calcium levels through alteration in uptake of phosphorus. On a theoretical basis, if aluminum hydroxide gel achieves anything, it would seem to be a force [illegible] might, in some instances, lower the i[illegible]

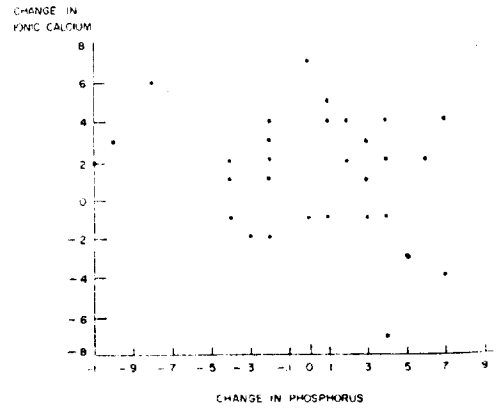

Fig. 5. Relationship of change in phosphorus change in ionic calcium.

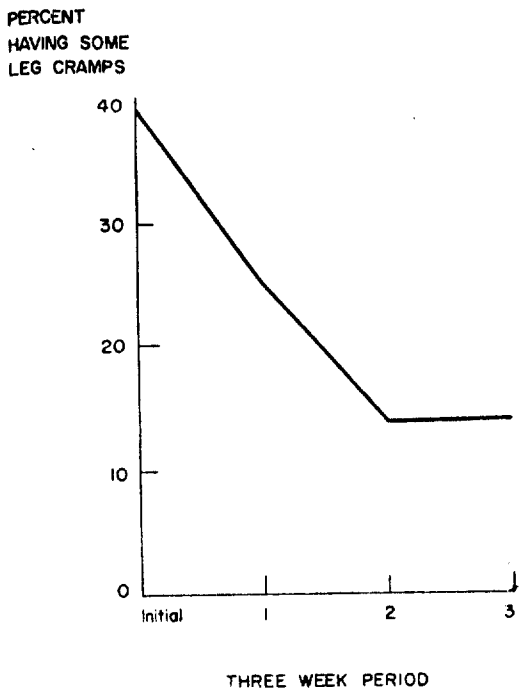

Fig. 6. Leg cramps by experimental period.

phosphorus level and thus cause a widening of the calcium-phosphorus ratio without any elevation of the absolute serum calcium level. This would seem to be opposed to what therapy with aluminum hydroxide seeks to achieve. This requires further investigation.

7. Using the ratio of ionic calcium to phosphorus as the criterion, no significant difference in the ratios at the end of the calcium citrate, lactate, or carbonate treatments could be found. Neither the sequence nor the time period of treatments produced any significant difference in the ratios.

OBSERVATIONS WITH RESPECT TO LEG CRAMPS

1. Of the 36 patients tested, 21 reported 33 incidents of leg cramps. The number of incidents reported on calcium citrate therapy were significantly smaller than those on the other preparations. Although the mean value of the calcium-phosphorus ratio in patients on calcium citrate was the same as the others, the deviations on either side of the mean were more varied.

2. The incidence of leg cramps under treatment was lower than that reported before treatment as shown in Figure 6. The coincidental nature of the rise of the mean calcium-phosphorus ratio has already been pointed out.

3. It was noteworthy that 9 of the 14 women who reported leg cramps prior to treatment also reported leg cramps at a later time. Study of the patterns of the calcium-phosphorus ratios in the remaining five revealed no evidence as to why this change should have taken place. In Table 2 there is some evidence that a higher initial value of calcium is associated with leg cramps. On close scrutiny there was no indication that leg cramps could be related to a change in the calcium-phosphorus ratio, nor could it be related to the initial phosphorus level. Instead, there was definite correlation in the fact that patients with an initial ionic calcium value of 4.2 mg./100 cc. or more and with low ionic calcium gains during the experiment were more prone to leg cramps. On statistical analysis, this was revealed to be the most validly related factor to the appearance of leg cramps either in the consideration of the group as a whole or in studying cases individually.

SUMMARY

1. A test group of 36 women between the twenty-first and thirty-first weeks of pregnancy was placed on 3 three-week courses

TABLE 2. LEG CRAMPS AND IONIC CALCIUM

Average change in calcium during treatment	*Some leg cramps*	*No leg cramps*	*Total no. patients*
+ .2 or more	8	13	21
Less than .2	13	2	15
Total no. of patients	21	15	36
Initial calcium			
4.2 or more	15	6	21
Less than 4.2	6	9	15
Total no. of patients	21	15	36

of calcium lactate, citrate, and carbonate used with varying rotation. Initially, and after each three-week course, determinations of ionic calcium and phosphorus and their ratios were calculated.

2. During the 9-week period, ionic calcium and phosphorus increased from their initial values. The mean calcium-phosphorus ratio showed a slight rise. While coincidental with a diminution of the incidence of leg cramps, there was no correlation in considering individual cases.

3. A rise in the calcium-phosphorus ratio was more easily achieved when therapy was instituted in early pregnancy.

4. The changes in titer of either ionic calcium or ionic phosphorus were shown not to be dependent upon each other's initial values.

5. There were no significant differences in the mean calcium-phosphorus levels produced by the use of either calcium citrate, calcium carbonate, or calcium lactate.

6. The incidence of leg cramps was smallest with the use of calcium citrate, but the mean value of the calcium-phosphorus ratio was the same as with either calcium lactate or calcium carbonate. However, deviations from the mean were more marked and extreme in the calcium citrate group.

7. The incidence of leg cramps during treatment diminished. While no correlation to initial phosphorus level or to the calcium-phosphorus ratio was discernible, there was a definite correlation with the initial ionic calcium level. Patients with a value of 4.2 mg./100 cc. or more and who displayed low ionic calcium gains during pregnancy were more prone to leg cramps.

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2. Page, E. W., and Page, E. P. Leg cramps in pregnancy, etiology and treatment. *Obst. & Gynec. 1*:94, 1953.

EFFECT OF ORAL CALCIUM ADMINISTRATION ON THE CHOLESTEROL AND PHOSPHOLIPID SERUM LEVELS

(Article by J. Kocian and J. Pechar in Casopis lekaru ceskych, 109, 1970, No 8:175-179.)

The work of Schroeder and Brattleboro /15,16/ aroused considerable interest in studying the effect of calcium on lipids as they noticed in their epidemiological studies the negative relationship between the mortality of United States populace afflicted with cardiovascular diseases and the hardness of water in certain areas. They determined a negative correlation coefficient ranging in values r = -0.53 through-0.56. Morris et al. reached similar conclusions a year later in England. They computed isolated correlation coefficients for different substances contributing to the hardness of water and assigned the highest negative correlation coefficient to calcium with r = -0.45 to -0.65 depending on group variations in sex and age. Far lower correlation coefficients were determined for Mg and sulfates. In fact, the mortality of persons afflicted with cardiovascular diseases is 660 cases per 100,000 of populace when Ca^{++} contents in water is lower than 1 mg%, and as the calcium contents in water increases in concentration the mortality rate decreases until it is reduced to 506 cases per 100,000 of populace when the concentration exceeds 10 mg% (see Fig. 1). Similar results were obtained in Scandinavia by Bioerck et al. in 1965 /1/. A somewhat different approach to studying the problem was adopted by Kamiyama et al. /7/ who determined that among Japanese women over 60 suffering from hypertension there is a statistically significant higher percentage of osteoporosis than among normotonic women. They ascribe this phenomenon to a lower intake of calcium and a higher cholesterol level in persons afflicted by the diseases.

Fleischman et al. /3,4/ and Yacowitz et al. /19/ experimentally determined a decrease of lipids in the blood of rats fed a calcium rich diet. The highest rate of decrease during administration of cholesterol and suet occurred when 1.2 % of calcium was added to the diet. There was no corresponding increase in

lipids in the tissue. The authors explain the effect of calcium by increased generation of bile acids in the liver from blood cholesterol and their higher evacuation. They found increased saturation amongthe fatty acids in the stool, but their overall evacuation did not increase. Along the same lines, Iacono et al /5,6/ determined that a daily ration of $CaCO_3$ in 2 g doses prevented a rise in the cholesterol level in rabbits that normally occurs on a starvation diet, and that the administration of calcium negated further adverse effects caused by an injection of fatty emulsion. Schroeder et al. /14/ determined in rats administered potable water containing 5.3% of calcium a decrease in extractable lipids in the aorta while, on the other hand, rats on soft water diet (containing no calcium) became afflicted with hypertension.

The findings made by Kudejka /9/ with rabbits, however, are at variance with the reports quoted above: after administration of a cholesterol and calcium gluconate diet he found an increase in cholesterol esters as well as a cholesterol increase in the serum. He found no correlation between the calcemia and cholesterol levels.

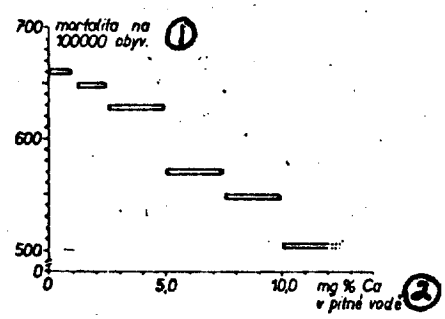

FIGURE 1. Relation between mortality due to cardiovascular diseases and the calcium contents of potable water (based on the findings of Morris et al.)

Key: 1 - mortality per 100,000 of populace;
2 - mg% of calcium in potable water.

The effects of calcium administration on serum lipids and cholesterol in humans were studied by Yacowitz /17/; he found that an addition of 2.66 g calcium (in $CaCO_3$ form) daily to the normal diet produced in four men a decrease in the cholesterol level on the average by 18.5 mg%. He explains the lipid decrease in the serum by the interaction between fatty acids and calcium in the tract, i. e. formation of insoluble soaps that cannot be absorbed and are evacuated to an increasing degree with the stool /18/. Maibach arrived at similar results.

The present authors set out to investigate the possible effects of a short term administration of calcium salts per os on cholesterol and phospholipids and, further, determine the relation between calcemia and the respective levels of the above substances in the serum, as well as to establish the length of time for which any possible changes will persist after discontinuation of calcium administration. A simultaneous objective was to determine the effects of vitamin D -- per se as well as in combination with calcium -- on the above parameters.

The investigation took the form of a double controlled experiment.

The Materials and Methodology

The diagnoses of the 26 outpatients who participated in the experiment are shown in Table 1. The patients were divided into three groups:

TABLE 1. Patients' Diagnoses

Pancreatitis chronica		8
Difuse lesion of intestinal mucous membrane		6
(to include: enteritis chronica	3	
sprue syndrome	2	
scleroderma with gastro-intestinal syndromes	1)	
Resection of the small intestine		3
Regional ileitis (morbus Crohn)		3
Resection of stomach for u. bulbi duod.		1
Osteoporosis (with reduced Ca absorption)		1
Hepatitis chronica		1
Cholecystectomia for lithiasis		1
Colectomy with ileostomy for. ulc. col.		1
Duodenum and pancreas-head resection for carcinoma of third part of the duodenum		1
Total		26

Group 1: Nine patients received 15 g Ca for three weeks (i. e. 16.8 g Calcii gluconici contained in 33 tablets of the calcium preparate supplied by Spofa (TN: CSSR Pharmaceutical Industry Combine); in the final week the dose consisted of 400,000 mouse units of vitamin D per os divided into two doses of 200,000 mouse units each for the 14th and 16th day). For the subsequent three weeks the patients received 3 x 1 g of kaolin in wafers as placebo.

Group 2: Ten patients who received 1.5 g Ca ions for three weeks (the same dose as group 1) and, for the subsequent three weeks 3 x 1 g of kaolin.

Group 3: Seven patients who received for three weeks 3 x 2 Spofavin dragee (i.e. 600 mouse units of vitamin D_2 daily for a total of 12,600 units). For the subsequent three weeks the patients received a dragee produced by Spofa and identical in appearance to the above (a form of sucrose) as placebo.

Blood was drawn from all patients on an empty stomach for calcemia determination, cholesterol and phospholipid counts in the serum were taken before administering medication, after the initial three weeks and again after the second three weeks of placebo administration. Blood was always drawn at 8 a.m. on an empty stomach.

Calcium was determined by means of atomic absorption spectrophotommetry /8/, cholesterol and phospholipids were determined by Novak's modification of the methods of Burchardt and that of Fiske and Subbarow /11,12/.

Results

The average values found for calcemia, cholesterolemia, phospholipids in the serum -- to include significant deviations as well as the ranges between the highest and the lowest values determined -- are listed for all three groups of patients in Table 2. The first column shows the values measured prior to the administration of medicaments, the second column lists the values obtained after the first three weeks of calcium or Spofavit administration, and the last column reflects the final values after the second three weeks of replacing calcium or Spofavit by placebo.

A graphic depiction of the average values of calcemia, cholesterolemia and phospholipids in the serum found during the course of the experiments among the patients of the first group is shown in Figure 2.

Among the patients in group 1 there was a statistically significant rise in calcemia in the first three weeks; average cholestrolemia decreased by 15.8 mg% and phospholipids increased on an average by 9.4 mg% during the same period. The latter two differences are without statistical significance. After the subsequent three weeks of placebo administration, the average cholesterol level in the serum again increased by 16.8 mg%, i.e. by 1.2 mg% more than was the initial value. There was a slight increase in phospholipids. Again, for both differences, there is no statistical significance.

4 Skupina	N		5 Základní			6 Po 3 týdnech terapie			7 Po 3 týdnech placeba		
			Ca	chol.	fosfolip.	Ca	chol.	fosfolip.	Ca	chol.	fosfolip.
1 Pacienti léčení 1,5 g Ca denně + 400.000 m. j. vit. D	9	2 rozmezí	6,6–9,7	119–313	138–273	9,1–12,0	107–272	123–275	8,9–10,7	117–302	126–292
		x̄	8,40	214,3	203,0	10,52	198,5	212,4	9,76	215,3	221,4
		s	1,24	42,81	31,6	0,94	55,42	47,68	0,72	65,04	53,05
2 Pacienti léčení pouze 1,5 g Ca denně	10	3 rozmezí	7,3–9,4	127–380	150–302	8,0–11,2	89–316	116–277	7,6–10,2	121–304	127–301
		x̄	8,80	214,8	193,8	10,04	191,7	206,5	9,42	184,9	211,1
		s	0,66	86,95	54,65	0,84	70,95	55,35	0,79	70,44	66,26
3 Pacienti léčení polyvitaminózním preparátem	7	4 rozmezí	8,8–9,7	131–332	151–358	10,2–11,8	142–300	163–302	9,7–11,2	121–260	121–280
		x̄	9,45	238,8	235,1	10,87	218,2	224,8	10,07	208,6	229,1
		s	0,77	78,79	75,95	0,44	62,01	53,16	0,70	46,52	52,29

TABLE 2. Value ranges, average values and significant deviations for calcemia, cholestrolemia and phospholipids in the serum prior to the commencement of medicament administration, after three weeks of treatment and after subsequent three weeks of kaolin administration as placebo in three groups of patients (in mg%) (Note: For key, see the last page.)

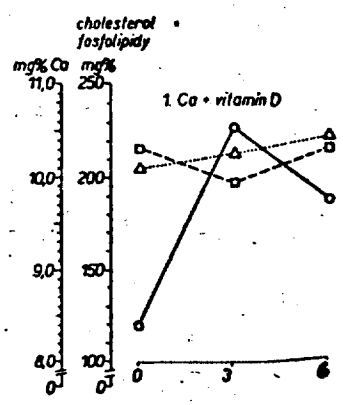

Figure 2. Average values of calcemia (solid line), cholesterolemia (intermittent line) and phospholipids (dotted line) prior to treatment (marked "0"), after 3 weeks of calcium and vitamin D treatment (marked "3") and after 3 more weeks of placebo administration (marked "6") among the second* group of patients. (* According to the text, these data apply to the 1st group.)

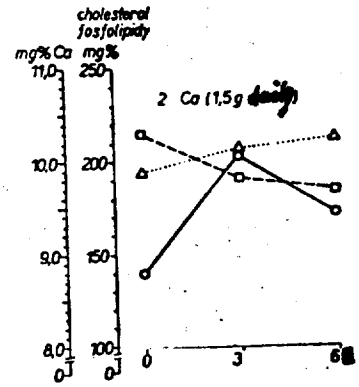

Figure 3. Average values for the second group of patients. Other data as for Figure 2.

A graphical depiction of the average values of calcemia, cholesterolemia and phospholipids in the serum for the second group is given in Figure 3 where the contrary behavior of calcemia and cholesterolemia is once more apparent. The latter average value decreased after the first three weeks of calcium administration by 23.1 mg% and decreased further after three more weeks of placebo administration by another 6.8 mg% so that the overall decrease for cholesterolemia amounted to 29.9 mg%. Only the cholesterolemia value prior to the onset of the treatment and the final value ($0.05 > p > 0.01$) are of statistical significance. Other than the aforementioned variations in the cholesterol level proved of no statistical significance. There was a statistically insignificant increase in the level of phospholipids both after the administration of calcium and of placebo among this group.

A graphical depiction of the average values of calcemia, cholesterolemia and phospholipids in the serum for the third group is given in Figure 4. Average cholesterolemia decreased after the administration of Spofavit by 20.6 mg% (a statistically insignificant decrease), and decreased further after the administration of placebo by another 9.6 mg% (also statistically insignificant). There was an insignificant decrease in phospholipids after the administration of Spofavit and an equally insignificant increase after the administration of placebo.

As it was noticed that hypercholesterolemia patients registered a higher decrease of chlesterol in the serum than did cholesterolemia patients with a normal range of values, all patients were divided -- based on the above criterion -- into a group of 14 "normal cholesterolemia" patients with cholesterol in the serum below 250 mg% and a group of 12 "hypercholesterolemia" patients with cholesterol levels exceeding 250 mg%. The values for the latter two groups are shown in Table 3.

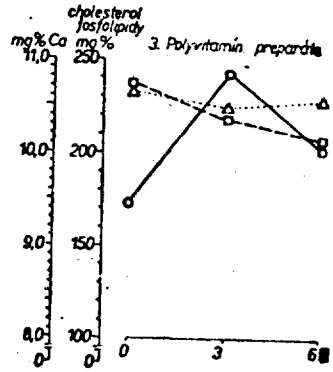

Figure 4. Average values for the third group of patients. Other data same as for Figure 2.

① Skupina	N		② Základní			③ Po 3 týdnech terapie			④ Po 3 týdnech placeba		
			Ca	chol.	fosfolip.	Ca	chol.	fosfolip.	Ca	chol.	fosfolip.
Ⓐ Hypo- a a normo-cholestero-lemici	14	rozmezí	6,6–9,7	119–193	138–220	9,1–12,0	89–189	116–250	7,6–10,6	117–211	121–256
		x̄	8,50	155,7	164,1	10,24	151,9	180,5	9,52	155,7	180,1
		s	1,07	22,12	25,01	1,06	27,86	40,11	0,80	14,30	42,77
Ⓑ Hyper-cholestero-lemici	12	rozmezí	7,3–9,7	260–380	214–304	9,7–11,8	220–316	210–302	9,2–11,2	211–304	215–312
		x̄	9,21	297,3	264,2	10,65	253,9	252,1	10,00	258,8	264,9
		s	0,78	35,06	45,60	1,02	30,73	27,90	0,69	34,15	29,31

TABLE 3. Value ranges, average values and significant deviations for calcemia, cholesterolemia and phospholipids in the serum prior to treatment, after three weeks of therapy and after three more weeks of placebo administration (kaolin) for two groups of patients divided according to the initial cholesterolemia level.

While calcemia in the first group with lower cholesterol registered a statistically highly significant increase ($p > 0.001$) and -- after the administration of placebo -- a statistically significant decrease ($0.01 > p > 0.001$), the cholesterolemia per se remained, for all practical purposes, unchanged; phospholipids showed an insignificant increase in the first three weeks and remained at the lower limit of normal values thereafter.

In the second group with cholesterolemia exceeding 250 mg%, calcemia increased after three weeks of treatment by an average of 1.44 mg% (a highly significant increase, $t = 3.304$, $p > 0.001$), cholesterolemia decreased by an average of 43.43 mg%, a statistically very significant decrease ($t = 7.7696$, $p < 0.001$). After the administration of placebo, cholesterolemia increased by 4.93 mg%, an increase of no statistical significance, however, the difference in cholesterolemia levels (initial and after six weeks) still remained at 38.50 mg%, which is of high statistical significance ($t = 4.3992$, $p > 0.001$). Phospholipids showed an insignificant decrease after three weeks of therapy and after the administration of placebo they returned to their initial value.

Discussion

It follows from the results that calcium administered per os in the form of calcium gluconate decreases the cholesterol level in the serum, particularly in the case of patients afflicted with hypercholesterolemia, while it has practically no effect on the level of phospholipids. This phenomenon could be interpreted in several ways:

1. Calcium salt administered per os will cause calcium in the intestine to bond cholesterol either from food or secreted from the bile. The thus formed compounds which are saponific in nature cannot be reabsorbed and are therefore evacuated in the stool. Thus, cholesterol released from the bile is prevented from being reabsorbed, i.e. its enterohepatal circulation is disturbed. The organism, in order to maintain a stable cholesterol concentration in the bile, absorbs it from the circulation, thus helping to reduce cholesterolemia. This is aided by lower resorption of the exogenous ratio of cholesterol.

In order to find proof that calcium does not internally affect cholesterolemia regulation, a group of five patients with a malabsorption syndrome was selected for treatment by a daily injection of 20 ml of 10% calcium gluconate for a period of three weeks. (The group included three female patients with gluten enteropathy, one with terminal ileitis prior to operation and one patient with staphylococcus enteritis and osteoporosis.) Their average basic calcemia was 7.3 mg% and cholesterolemia 120 mg%. After three weeks of therapy the average calcemia of this group increased to 9.4 mg% which -- in spite of the limited number of cases -- is statistically significant ($p < 0.001$). The cholesterolemia average increased to 138 mg% (of no statistical significance).

An objection could be raised that the overall improvement in the state of the patients after calcium administration there also occurred an improvement in cholesterol resorption, however, since a similar improvement occurred in the patients treated per os, there should have been an increase in cholestrol resorption. It can be seen that after peroral administration of calcium cholesterolemia decreases while, on the other hand, after intravenous administration it registers a slight increase.

2. No attempt was made so far to verify the theory advanced by Fleischman et al /3/ who assume that peroral administration of calcium salts increases the production of bile acids from cholesterol and that choleresis increases. There is also need for animal experiments to establish whether cholesterol is being taken from circulation to be deposited somewhere in the organism together with calcium. The latter verification was undertaken by Fleischman et al /3,4/ on rats administered a suet or coconut oil diet. After addition of calcium to the diet, there occurred a decrease of lipids in the blood without causing an increase in lipids in the tissue.

It is known from experiments with humans that a calcium deficiency in the organism capable of causing osteoporosis is connected with a significantly higher percentage of calcifications in the abdominal aorta detectable by x-ray.

Further efforts ought to be focussed in two directions: first, study the effects of calcium on a group of patients without malabsorption or a group of hypercholesterolemia patients and, second, experiment with various calcium preparations that might eventually prove to warrant lower resorption.

Conclusion

The preceding study of 26 patients, the majority of whom suffered from malabsorption, determined that peroral administration of calcium gluconate in doses corresponding to 1.5 g calcium ions per day, there occurs a decrease in serum cholesterol in direct relation to the initial level of cholesterol. After substituting calcium with placebo, cholesterolemia returns to its original level, but does not reach it even after three weeks time. Calcium gluconate administered intravenously failed to show such effects. It is assumed, therefore, that calcium salt in the intenstine bonds cholesterol from food on the one hand and prevents the resorption of cholesterol secreted from the bile on the other.

Summary

The authors investigated the calcium, cholesterol and phospholipid serum levels after administration of 16.8 g calcium gluconate (i. e. 1.5 g of calcium ions) per day, a combination of calcium and vitamin D and a polyvitamin preparation for three weeks in 26 subjects. The serum calcium level rose in all groups, the cholesterol level declined. The decline was the more marked the higher the initial level. The administration of vitamin D caused a rise of the serum calcium but did not reduce appreciably the cholesterol level. Intravenous administration of calcium gluconate did not reduce the cholesterol level.

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ADDENDUM: Key to Table 2 (p. 5):

1. Patients receiving 1.5 g Ca daily + 400,000 mouse units of vitamin D; 2. Patients receiving 1.5 g Ca daily only; 3. Patients treated with polyvitaminose preparates; 4. Group; 5. Initial; 6. After 3 weeks of therapy; 7. After 3 weeks of placebo administration.

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CEREALS AND RICKETS

X. THE AVAILABILITY OF PHYTIC ACID PHOSPHORUS[1]

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A study of the interrelations of calcium, phosphorus, and vitamin D in the production of rickets has revealed that not all forms of phosphorus are equally available. For a time the total calcium and phosphorus relations were stressed, but of late the emphasis has shifted to comparisons of the availability of phosphorus in different forms. So far all the investigations bearing on the availability of phytic acid phosphorus, with the exception of that of Jones ('39), have failed to recognize the existence of various forms of phosphorus in cereal grain rations with the result that the data have been inconclusive or conflicting.

The object of the present experiments was to compare the availability of the phosphorus of phytic acid with phosphorus from inorganic sources. These were incorporated in a relatively simple ration, low in phosphorus, but complete in other respects. The resultant diets were fed to young rats and compared for their effect on bone ash, size of the costochondral junctions and the width of metaphyses.

EXPERIMENTAL

The basal ration used was the low phosphorus ration (R-14) of Schneider and Steenbock ('39). Its primary ingredients were cerelose 49.0, egg white 18, starch 20, Vitab 4, cottonseed

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

oil 5, low phosphorus salts 4%, respectively. This ration was supplemented with beta carotene fed in Wesson oil by dropper, 10 micrograms per rat per day. All other additions were made at the expense of the cerelose. The basal ration contained 0.04% phosphorus and 0.57% calcium as determined by analysis. The Ca content of the rations was kept constant at 0.57%, supplied as the carbonate (0.33%) and lactate (0.24%). The only variables in the ration were the kind and amount of phosphorus and the presence or absence of vitamin D.

The phytic acid was prepared from crude calcium phytate[3] according to the method of Boutwell ('17). It was added to the ration as a concentrated solution containing 133.8 mg. phosphorus per cubic centimeter. Of this 123 mg. was phytic acid phosphorus. Of the remainder, 6.34 mg. were shown to be inorganic phosphorus. The total and inorganic phosphorus were determined by the method of Fiske and Subbarow ('25) with an Evelyn photoelectric colorimeter. The phytic acid phosphorus was determined by the technique of McCance and Widdowson ('35). The phytic acid was found to be stable in water solution as no hydrolysis had occurred after 3 months standing although the color of its solution had deepened appreciably. The inorganic supplements were incorporated as an aqueous solution of equimolecular amounts of the mono- and dibasic sodium phosphates. The rations were dried before an electric fan at room temperature after addition of the supplements.

Nicolaysen's ('37) data were used in deciding the level of phosphorus to be fed. He pointed out that a 30 to 60 day old rat required 4.5 mg. phosphorus and 7.5 mg. calcium for each gram increase in body weight. Assuming an optimal rate of growth of 4 gm. per day on a food intake of 7 gm., 0.257 gm. "available" phosphorus and 0.428 gm. calcium were required per 100 gm. of ration. This was accepted as the optimal level of intake. Since some of the rats would not gain 4 gm. daily

[3] For this we are indebted to the E. A. Staley Manufacturing Co. of Decatur, Illinois.

even though they consumed 7 gm. of ration, suboptimal amounts of phosphorus were fed in addition.

In all, seven different rations were fed. These included, besides the optimal amount of phosphorus, levels of one-eighth, one-fourth, and one-half the optimal, plus a negative and positive control. The negative control group received as much inorganic phosphorus as was present as a contaminant in the largest addition of phytic acid. The positive control group received inorganic phosphorus equivalent to the total phosphorus. All the above rations were supplemented with 4.5 U.S.P. units of vitamin D (viosterol) per 100 gm. of ration mixed in ether solution. The ether was volatilized before an electric fan and the ration again mixed thoroughly. One additional ration with phytic acid containing one-fourth of the optimal level of phosphorus without vitamin D was included. Analyses of the rations after 2 weeks standing at room temperature revealed no hydrolysis of the phytic acid. The rations were kept in a refrigerator between feedings to avoid the development of rancidity.

Six 50 to 60 gm. rats were placed on each ration. Litter mates were distributed in the series so that no two rats of the same litter were on the same ration. They were kept individually in screen bottomed cages. They received distilled water ad libitum. A careful record of food consumption was kept; however, no attempts were made to equalize consumption. The rats were weighed and inspected weekly. At the conclusion of the experiment, the rats were anaesthetized and blood was drawn from the abdominal aorta for calcium and phosphorus determinations. The calcium was determined by the method of Clark and Collip ('25) and the phosphorus by the method of Fiske and Subbarow ('25). The radii and ulnae were removed for inspection of metaphyses, and the femora for a determination of ash after extraction with alcohol.

It is evident (table 1) that the inclusion of phytic acid in the low phosphorus ration caused a definite improvement in calcification. This became more evident as the amount of phytic acid phosphorus was increased to the optimal level. There

resulted a definite improvement in food consumption, in body weight, in weight of bone and bone ash, and in the percentage of bone ash. That this improvement was not due to the small amount of contaminating inorganic phosphorus is evident from the negative control (ration XII). The deficiency of phosphorus was also reflected in the blood picture. The average value for serum phosphorus for the negative control was

TABLE 1

The effect of increasing amounts of phytic acid phosphorus on calcification

RATION COMPOSITION (ADDITIONS TO BASAL)	NO. OF RATS	NO. OF WEEKS	FOOD CONS.	CHANGE IN WEIGHT	WEIGHT OF BONE	WEIGHT OF ASH	BONE ASH
			gm.	*gm.*	*gm.*	*gm.*	%
VII—0.032 gm.[1] ($\frac{1}{8}$ opt.) phytic acid P plus D[2]	5	14	810	73	0.1773	0.0566	32.4
VIII—0.064 gm. ($\frac{1}{4}$ opt.) phytic acid P plus D	6	14	834	95	0.1770	0.0618	35.5
IX—0.128 gm. ($\frac{1}{2}$ opt.) phytic acid P plus D	6	14	1045	162	0.2594	0.1228	47.3
X—0.257 gm. (opt.) phytic acid P plus D	4	14	1146	186	0.3075	0.1620	56.3
X ———————	2	10	715	135	0.4040	0.2275	52.8
XI—Same as VIII except no vitamin D	6	6	330	39	0.1140	0.0250	22.0
XII—0.0126 gm. inorganic P equal to that in X plus D	5	10	583	63	0.1323	0.0378	28.6
XIII—0.25 gm. (opt.) inorganic P plus D	4	14	1241	208	0.3993	0.2392	61.5
XIII ———————	2	10	877	170	0.4615	0.2831	60.0

[1] Per 100 gm.

[2] 4.5 U.S.P. units vitamin D (viosterol) per 100 gm.

3.5 mg. % as compared with values ranging from 7 to 11 mg. % for the other groups. The serum calcium and phosphorus values for the other rats were of the order expected for mildly rachitic to normal rats.

The rats on the one-fourth optimal phosphorus level without vitamin D (ration XI) did so poorly that they were killed at the end of 6 weeks. Similarly the rats in the negative con-

trol group (ration XII) did poorly; they began to lose weight at the end of 10 weeks when they were killed. Since this latter group served as a control for the rats on the optimal phytic acid (ration X) and inorganic (ration XIII) phosphorus levels, representative rats from these two groups were also killed at the end of 10 weeks for comparative purposes. The remainder of the rats were continued for 14 weeks.

The striking difference between the rats on the one-fourth optimal phytic acid level without vitamin D (ration XI) and those receiving vitamin D (ration VIII) indicated the desirability of another experimental series. In this second series one-half optimal phytic acid and inorganic phosphorus with and without vitamin D were fed. As a negative control, inorganic phosphorus equivalent to that in the phytic acid ration was also fed. Four rats were placed on each ration under the same experimental conditions as in the first series except that the experiment was terminated at the end of 9 weeks. This modification was necessitated by the death of some of the rats during the seventh and eighth weeks. The data on the rats which died were not included in the tabulated results.

It is again evident (table 2) that vitamin D played an important role in the utilization of phytic acid phosphorus. Its effect on inorganic phosphorus was less pronounced. A comparison of the rats on one-half optimal inorganic phosphorus without D (ration XXIV) with those on the same ration plus D (ration XXIII) reveals that the former were only mildly rachitic and hence the increment of improvement which could possibly be achieved by the vitamin D was definitely decreased. A comparison of similar rations containing phytic acid phosphorus instead of inorganic phosphorus (rations IX and XXII) reveals that the rats which did not receive vitamin D were markedly rachitic. In fact the percentage of bone ash of the one-half optimal phytic acid phosphorus group without D (ration XXII) approximated the percentage of bone ash from the negative control rations (ration XXV and XXVI). Apparently under these conditions the phosphorus of phytic acid

was not demonstrably available. Vitamin D was without effect in the negative control group.

As no attempts were made to equalize the phosphorus intake of the rats in the different groups, the results suggested the desirability of carrying out a supplementary series of experiments in which this was done. In this the vitamin D additions were increased tenfold and the experiment was terminated at

TABLE 2

Effect of vitamin D on phytic acid and inorganic phosphorus utilization

RATION COMPOSITION (ADDITIONS TO BASAL)	NO. OF RATS	NO. OF WEEKS	FOOD CONS.	CHANGE IN WEIGHT	WEIGHT OF BONE	WEIGHT OF ASH	BONE ASH
			gm.	*gm.*	*gm.*	*gm.*	*%*
IX—0.128 gm.[1] (½ opt.) phytic acid P plus D[2]	4	9	556	113	0.2013	0.0916	45.4
XXII—Same as IX except no D	2	9	508	66	0.1572	0.0383	24.5
XXIII—0.128 gm. (½ opt.) inorganic P plus D	4	9	683	151	0.3338	0.1681	50.5
XXIV—Same as XXIII except no D	3	9	566	102	0.1440	0.0808	41.9
XXV—0.0063 gm. inorganic P equal to that in IX plus D	4	9	478	60	0.1237	0.0298	24.4
XXVI—Same as XXV except no D	2	9	441	15	0.0984	0.0231	23.5

[1] Per 100 gm.

[2] 4.5 U.S.P. units vitamin D (viosterol) per 100 gm.

the end of 4 weeks. It is evident from the results (table 3) that the presence of vitamin D had no effect on growth. However, vitamin D did have a marked effect on the utilization of phytic acid phosphorus (rations A and B) and a less marked effect on the utilization of inorganic phosphorus (rations C and D). These observations are in agreement with those of the second series. Although the food consumption was almost identical, the rats receiving the inorganic phosphorus, even without vitamin D, were definitely superior to those receiving the same amount of phosphorus as phytic acid with and without vitamin

D. Inspection of the metaphyses and costochondral junctions confirmed the above conclusions which in turn were in direct agreement with the percentage of bone ash. The calcium and phosphorus determinations on the blood sera failed to reveal any anomalies.

TABLE 3

Paired feeding study of the effect of vitamin D on phytic acid and inorganic phosphorus utilization

RATION COMPOSITION (ADDITIONS TO BASAL)	NO. OF RATS	NO. OF WEEKS	FOOD CONS.	CHANGE IN WEIGHT	WEIGHT OF BONE	WEIGHT OF ASH	BONE ASH
			gm.	*gm.*	*gm.*	*gm.*	*%*
A 0.128 gm.[1] (½ opt.) phytic acid P plus D[2]	6	4	215	39	0.1093	0.0405	37.0
C 0.128 gm. (½ opt.) inorganic P plus D	6	4	215	47	0.1482	0.0654	44.1
B Same as A except no D	6	4	211	39	0.0900	0.0194	21.5
D Same as C except no D	6	4	210	47	0.1280	0.0517	40.4

[1] Per 100 gm.

[2] 45 U.S.P. units vitamin D (viosterol) per 100 gm.

SUMMARY

When rats were fed a cereal free ration of normal calcium content the utilization of phosphorus from phytic acid was markedly enhanced by the addition of vitamin D. This effect was less marked with inorganic phosphorus. In no case was the utilization of phosphorus from phytic acid equal to that of inorganic phosphorus.

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CEREALS AND RICKETS

XI. CALCIUM PHYTATE AS A SOURCE OF CALCIUM[1]

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It is generally assumed that the calcium of compounds ordinarily used to supplement grain rations is entirely available. Steenbock et al. ('23) in their experiments with young rats found the calcium of calcium lactate, carbonate, phosphate, silicate, and sulfate to be available to the rat. Buckner and Martin ('29) observed that calcium carbonate, phosphate, and sulfate served equally well as sources of calcium for the growing chick. Bethke, Kennard and Kick ('29) confirmed the work of Buckner and Martin. They extended their studies to the calcium of limestone, steamed bone meal, rock phosphate, phosphatic limestone, and oyster shells, and found no differences in assimilation. However, Mellanby ('37) is of the opinion that phytic acid actually increases rickets in dogs on moderately rachitogenic diets by the withdrawal of calcium from the body tissues. There is the possibility that even the calcium of calcium phytate taken by mouth might be unavailable. This possibility was accordingly put to test. Mellanby's theory regarding the withdrawal of calcium from the tissues by unhydrolyzed phytic acid will be given consideration in a subsequent paper.

EXPERIMENTAL

The calcium phytate used was prepared from crude calcium phytate[2] according to the method outlined by Anderson ('12).

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] Received from E. A. Staley Manufacturing Co. of Decatur, Ill.

Analyses revealed it to contain 16.9% calcium and 17.0% phosphorus. Of the phosphorus, 0.54% was present in the inorganic form. The basal ration in which it was incorporated was essentially the purified low phosphorus ration of Schneider and Steenbock ('39) with the addition of optimal amounts of mono- and di-potassium phosphates, except for the omission of the calcium salts. The composition of the modified salt mixture was as follows: NaCl 183.7, K_2HPO_4 187.83, KH_2PO_4 147.02, KCl 17.3, $MgSO_4$ 90.0, $MnSO_4$ 0.20, $K_2Al_2(SO_4)_4 \cdot 24H_2O$ 0.09, $CuSO_4 \cdot 5H_2O$ 0.39, NaF 0.57, KI 0.05 and Fe citrate 16.15 parts, by weight, respectively.

Two and fifty-seven hundreds grams were incorporated in each 100 gm. of ration. The basal ration contained 0.019% calcium as determined by the method of Meloche, Clifcorn, and Griem ('33).

The level of calcium to be fed was arrived at on the assumption that the calcium was entirely available (Krieger, Bunkfeldt and Steenbock, '40). Calcium as the carbonate or phytate, to the extent of 0.428 gm., was incorporated in each 100 gm. of ration for the optimal level. The experimental conditions and criteria were the same as those of the previous investigation.

A preliminary experiment of 13 weeks' duration in which calcium phytate was fed at the optimal level and at levels of one-half, one-fourth, and one-eighth of the optimal, revealed that in the presence of vitamin D there was a marked improvement in growth, food consumption, weight of bone, weight of bone ash, and consequently, percentage of bone ash with an increase in the calcium intake up to one-half the optimal level. Little or no difference was observed between the rats in the positive control group receiving calcium carbonate and those receiving the same amount of calcium as calcium phytate. However, when vitamin D was omitted from the calcium phytate ration, calcification was markedly reduced. The percentage of bone ash for the group on one-fourth the optimal calcium intake without vitamin D was 45.3% as compared with 54.8% for the same ration supplemented with vitamin D. As calcium car-

bonate was not fed at an identical level without vitamin D, it appeared desirable to run a second experimental series in which this was done. The experimental conditions in this series were essentially the same as in the first.

It is evident (table 1) that in this series the calcium of calcium phytate was utilized as readily as that of calcium carbonate regardless of whether vitamin D was present or not.

TABLE 1

Comparative study of the availability of calcium in calcium phytate and calcium carbonate

ADDITIONS TO THE BASAL RATION [1]		FOOD CONS.	CHANGE IN WT.	WEIGHT OF BONE	WEIGHT OF ASH	BONE ASH
		grams	*grams*	*grams*	*grams*	%
XIV	0.107 gm. (1/4 opt.) Ca as Ca phytate plus D[2]	728	130	0.2588	0.1314	50.8
XVI	0.107 gm. (1/4 opt.) Ca as $CaCO_3$ plus D	692	127	0.2630	0.1290	49.1
XV	Same as ration XIV except no vitamin D	535	76	0.2005	0.0856	42.5
XVII	Same as ration XVI except no vitamin D	548	58	0.1588	0.0691	43.5
XVIII	0.054 gm. (1/8 opt.) Ca as Ca phytate plus D	663	102	0.2102	0.0878	40.9
XX	0.054 gm. (1/8 opt.) Ca as $CaCO_3$ plus D	582	76	0.1776	0.0730	41.5
XIX	Same as ration XVIII except no vitamin D	502	46	0.1576	0.0579	36.8
XXI	Same as ration XX except no vitamin D	493	53	0.1582	0.0582	35.9

[1] Each ration was fed to six rats for 12 weeks. The additions represent those made for 100 gm. of ration.

[2] 4.5 U.S.P. units vitamin D (viosterol) per 100 gm.

Vitamin D produced a definite increase in growth, food consumption, weight of bone and weight of bone ash and consequently percentage of bone ash at the one-eighth and one-fourth optimal levels. This improvement was probably due to the increased absorption of calcium from the gut as has been demonstrated by Nicolaysen ('37). It was again obvious that the amount of calcium present was the limiting factor

since the higher levels (rations XIV, XV, XVI, and XVII) produced the best growth and calcification. This was also borne out by the blood picture. The serum phosphorus values were normal in all cases, but the calcium values were 3 to 5 mg.% below those obtained at the one-half optimal and optimal levels in the preliminary experiment. The metaphyses and the costochondral junctions revealed normal or almost normal calcification except in those animals which received one-eighth the optimal levels without vitamin D.

SUMMARY

When calcium phytate was added to a cereal-free low-calcium ration, its calcium was found to be as readily available to the rat as the calcium of calcium carbonate. Vitamin D improved the utilization of both forms of calcium to the same extent.

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THE SENSITIVITY TO CALCIUM OF ANIMALS GIVEN DIGITALIS

Jean La Barre and Jean Van Heerswynghels (received 3-11-38)

I. Introduction

Digitalis preparations and calcium salts have the property of slowing the cardiac pace and decreasing conduction in the bundle of His. In addition, these two preparations increase excitability and contractility of the myocardium and cause ventricular extrasynstoles, alternating with stoppages occasionally prolonged by the heart in systole.

Concerning the pharmacodynamics of these drugs, Loewi (1) had expressed the opinion, already expressed by Burridge (2), that digitalis sensitizes the myocardium to calcium ions. This hypothesis has been strongly disputed later on and no longer appears to be considered since Mandelstamm (3) has shown that the digitalized body conserves its pharmacologic effects on the isolated perfused heart according to the method of Langendorf by means of a Locke fluid entirely depleted of calcium.

Therefore digitalis seems to act on the heart, not by sensitizing this organ to calcium, but by intervening in an independent manner. It was nevertheless shown that these drugs exert analogous effects on this organ. This fact fairly rapidly attracted the attention of the clinicians. Singer (4) was preoccupied, at first, with the effects of the calcium-digitalis association in the treatment of cardiac attacks of edema. According to this clinician, one should observe in the case of sick people given digitalis and subjected to calcium medication, an increase in ventricular tonicity, a very considerable abatement of the pulse, and an exaggeration of diuretic action. Billigheimer (5) as well as Boger at Diehl (6) have equally recommende this therapeutic association. Nevertheless for these last authors, the simultaneous use of digitalis and calcium would very clearly assist ectopic excitations, which would exclude their utilization in certain cases.

Following the observations of Bower and Mengle (7), which describe two cases of sudden death after the intravenous administration of 10 ml. calcium gluconate or chloride with 10% of the subjects having received digitalis previously i. m., the question was studied again. Bower and Mengle dealt with the question of this medicinal association in a series of experiments using dogs. The animals received, by saphenous vein, therapeutic doses of digitalis followed by the administration of calcium gluconate. After a short increase in the blood pressure, the authors observed cardiac arrest and the death of the animal, which was not produced by the same dose of calcium gluconate not preceded by an injection of digitalis. They concluded that these two compounds exerted an additive effect on the heart such that their simultaneous use was capable of producing the accidents. Golden and Brams (8) confirmed this point of view by observing that after the intra-

venous administration of digalen, dogs, in which calcium gluconate was injected secondarily, died when an amount considerably less than the normal minimum lethal dose of this salt was used. After having determined the dose of calcium chloride necessary for producing momentary heart stoppage in the rabbit, Nahum and Hoff (9) studied the sensitivity of this same substance in animals treated with perliminary strong doses of digifolin. Under these conditions, these last authors did not establish, after digitalization, the reinforcement of the effect of calcium.

In considering the series of experiments, one arrives at the conclusion that a real inconsistency of opinions exists both from the experimental and the clinical point of view on the subjecy of the advisability of prescribing calcium treatment in a digitalized subject.

Therefore we thought that it would be opportune to take up this study in its entirety and to first determine if a hypersensitivity to calcium exists in digitalized animals and after to enentually try to determine its order of magnitude.

II. The Action of Calcium Gluconate On The Heart Of The Digitalized Cat

Right at first, we accounted for unexpected changes in the cardiac muscle while studying in the heart of the digitalized cat rhythmic changes in the ventricle and auricle during 10% calcium gluconate administration.

a) Methods

We used 2 kg. cats, anesthetized by intravenous administration of 10 ml. (per kg. body weight) of a solution of 8 g. chloralose per litre of physiological saline. After placing a tracheal cannula, artificial respiration was established, then the thorax was opened. The pericardium was incised and sutured in the thoracic muscles to obtain a propulsion of the heart forward. After having fixed clamps to the apex of the ventricle and to the auricular wall, rhythmic contractions registered for that organ. We administered intravenously either 10 ml. of a 10% calcium gluconate solution alone or half the lethal dose of isotonic digitalis was infused, then a quarter of an hour later the same quantity of calcium gluconate.

b) Experimental Results

In this first series of experimenta conducted following the technique which has just been described, we proceeded, with 2 kg. cats, with the slow intravenous injection (1 ml. per minute) of 35 ml. of an isotonic digitalis infusion, containing 45 mg. powder per kg. Fifteen minutes later, not one symptom of digitalis intoxication was perceptible when referring to auricular and ventricular tracings, we injected 10 ml. of 10% calcium gluconate, at a speed of 1.3 ml. per second.

FIGURE 1

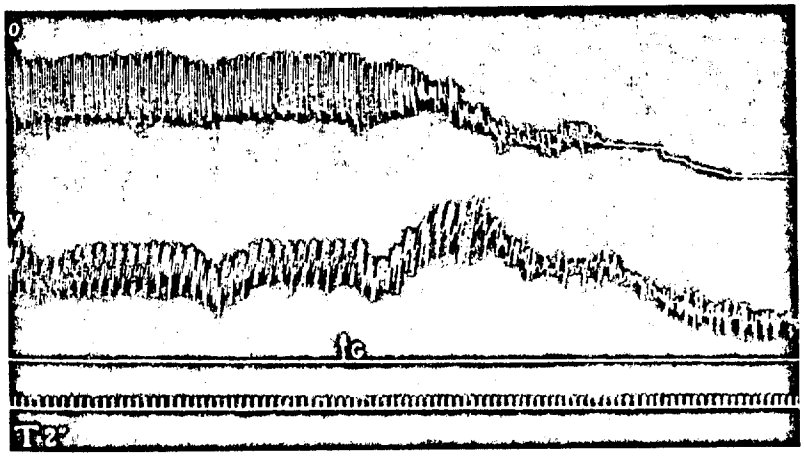


FIG. 1

En O : tracé auriculaire. O : auricular tracing.
En V : tracé ventriculaire. V : ventricular tracing.
En G : injection de gluconate calcique à l'animal digitalisé.
Temps inscrit toutes les 2 secondes. G : injection of calcium gluconate on a digitalized animal.
Time registered every 2 seconds.

FIGURE 2

(translations are the same as for FIGURE 1)

FIG. 2

En O : tracé auriculaire.
En V : tracé ventriculaire.
En G : injection de gluconate calcique à l'animal digitalisé.
Temps inscrit toutes les 2 secondes.

Under these conditions, as figures 1 and 2 indicate, by virtue of the examples, we established that in a very short time after the administration of a solution of gluconate a final stoppage of the auricle occurred at the same time as a ventricular fibrillation followed by death (Fig. 1) or an immediate heart stoppage (Fig. 2)

In the course of the control experiments, we proceeded using chloralased cats with the injection of 10 ml. of 10% calcium gluconate without observing an appreciable change in the auricular or ventricular tracings, as shown by figure 3.

As we can account for the examination of these different tracings, the cats which had been previously digitalized by means of doses which, by themselves, do not produce any change in the rhythem and amplitude of the auricular and ventricular contraction, show a particular sensitivity to the injection of calcium glocunate. A few seconds after the administration of this salt, the heart shows severe disturbances characterized by rapid stoppage of the auricle, soon followed by marked changes in ventricular contraction. In most cases, the animals die shortly after the injection of the calcium gluconate solution.

These statements therefore confirm the opinion by Bower and Mengle and by Golden and Brams.

Based on our concordant results, we believe it necessary to determine more precisely the experimental conditions brought about by the administration of calcium to digitalized animals while trying to detect different stages of poisioning by the electrocardiographic method.

FIGURE 3

Key: O: auricular tracing; V: vnetricular tracing; G: injection 10 ml. of 10% calcium gluconate; time registered every 2 seconds.

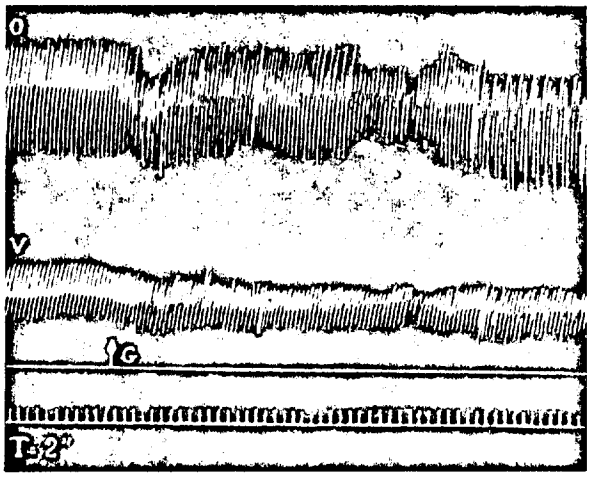


FIG. 3

En O : tracé auriculaire.
En V : tracé ventriculaire.
En G : injection de 10 cc. de gluconate calcique à 10 %.
Temps inscrit toutes les 2 secondes.

III. Electrocardiographic Changes Observed in Digitalized Cats, Treated With Calcium Gluconate

In this second series of experiments, we investigated electrocardiographic technique (1) unexpected changes in the contractile function of the heart under the influence of calcium gluconate and consecutively by the administration of this substance to previously digitalized animals. This method allowed us, moreover, to detect in a precise manner the time of cardiac arrest and to determine under these conditions the doses of calcium gluconate necessary for causing the death of part of the normal animals and of the other part of digitalized cats.

a) Method

After anesthetization with ether, different experimental cats were examined with the electrocardiographic technique, by placing the two electrodes at origin II (before the right leg and behind the left leg). The zinc anodes coated with a salty solution of glycerine were laid on the skin of the previously shaved legs. The tracings were collected at a rate of 5 cm. per second.

b) The Effect of Calcium Gluconate On The Heart.

Lloyd (10), one of the first, to have studied the electrocardiographic changes produced in man by the administration of calcium. After the injection of 4 ml. of a solution of 10% calcium chloride, the electrocardiogram showed a marked slackening of the sinusal rhythm followed by a sinuauricular block then by a return of normal contractions. Berliner (11), in examining 26 normal individuals the electrocardiographic changes produced by the intravenous injection of 10 ml. of a 20% solution of calcium gluconate, established a marked bradycardia, a depression of the P waves, and an inversion of the T waves. In the dog, Lieberman (12) put in evidence that the speed of injection is a very important factor from the point of view of the pharmacologic action of calcium.

Hoff and Nahum (13) observed the general effects of calcium on the electrocardiogram of the rabbit. The first manifestations consist of a widening of the QRS and of consistent changes in a flattening and on inversion of the T wave. The subsequent changes were represented by a slackening of the auriculo-ventricular conduction and in several cases by a fibrillation of the auricle. Larger doses cause ventricular extrasystoles which, in the absence of any sinusal beating, alternate with periodic stoppage till the death of the animal.

We proved from the very first data os Lieberman having treated the importance of the injection speed. Administering the saphenous vein of a cat a solution of 10% calcic gluconate, at a rate of 1 ml. every 15 seconds, the death of the cat was sudden after a dose corresponding to 22 ml. per kg.

On the other hand injecting calcium gluconate, at the rate of 1 ml. every 3 seconds, we have noted smaller lethal doses between 16.8 ml. and 12 ml. per kg. which represents an average of 15 ml. per kg.

The increase in the speed of injection of calcium gluconate was enough therefore to decrease the minimum lethal dose from 22 to 15 ml. per kg.

TABLE 1

Table TABLEAU 1

Chats n'ayant pas reçu de digitale. Cats not having received digitalis		Solution à 10 % de gluconate calcique. 10% calcium gluconate solution	
N° de l'animal No. of animal	Poids en kg. Weight in kg.	cc. nécessaires pour provoquer la mort de l'animal ml. needed to produce the death of the animal	dose mortelle en cc. (par kg.) lethal dose in ml. (per kg.)
1	2.250	38	16.8
2	2.050	34	16.5
3	1.350	19	14
4	2.300	30	13
5	1.300	19	14.7
			moyenne average 15

We had estimated that the most convenient speed of injection to use in establishing the maximum lethal dose of normal and digitalized animals corresponded to 1 ml. every 3 seconds.

Table 1 produced the results observed in 5 cats in which we had administered calcium gluconate under these experimental conditions.

As Table 1 indicates, at the injection speed of 1 ml. every 3 seconds, the smallest lethal dose was an average of 15 ml.

FIGURE 4

Electrocardiographic tracing received in a cat to which we administered a 10% solution of calcium gluconate.

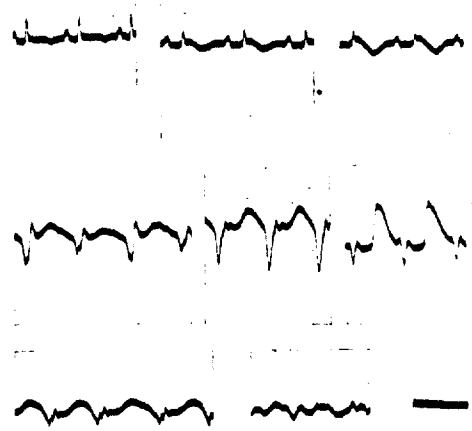


FIG. 4

Tracé électrocardiographique recueilli chez un chat auquel on a administré une solution de gluconate de calcium à 10 %.

When we record the electrocardiographic changes the heart of similar poisonings, we note a series of changes in the tracing which we reproduced at the concentration of figure 4.

This figure shows us a series of electrical changes, at first consisting of a sinusal bradycardia with an elongation of the auriculo-ventricular conduction, a decrease in the amplitude of the R wave as well as a progressive and marked inversion of the T wave. Then ventricular extrasystoles which provide single cardiac contractions. They were followed by a short period of ventricular fibrillation to which death followed.

As seen by this description, our observations are analogous to those of Hoff and Nahum in the rabbit. Apart from the auricular fibrillation that they observed occasionally and that we never found, the electrocardiographic images, which were obtained in the rabbit and that we recorded in the cat, are completely superimposable.

c) The Effect of Calcium Gluconate on Digitalized Animals.

We brought about the digitalization of our animals by injecting them subcutaneously with 1/2 or 2/3 the lethal dose of digitalis in the cat, either 45 or 60 mg. powdered leaf per kg. The minimum lethal dose corresponds, in effect, after Jeanne Levy (14), to 89. 7 powdered digitalis leaf per kg. body weight.

Five cats received 45 mg. and 5 others 60 mg. digitalis powder which was administered in the form of an infusion prepared according to the regulations of the Belgian Pharmacopoeia. In order to approach the utmost human therapy, the digitalis was given in this series of experiments subcutaneously 7 hours before the injection of calcium gluconate. This time lapse appeared to us to be enough so that the digitalization of the animal was completely accomplished. In effect, in most of the cats, the electrocardiographic tracing recorded at that moment of and before every injection of calcium detected the changes which accompany every large digitialization. This one distinguished itself in the majority of cases by the replacement of the sinusal rhythm with an extrasystolic rhyrhm, having at the beginning an influx starting from one or two centers. In this last case, the tracing indicates alternation between two kinds of extrasystoles.

In the animals so treated, the injection of a 10% calcium gluconate solution intravenously, at a speed of 1 ml. every 3 seconds, produces death after introduction into the circulation of 6. 9 ml. to 13. 3 ml. (table II) in cats having received 45 mg. digitalis powder per kg.

TABLE II

Calcium and Digitalis

CALCIUM ET DIGITALE

Table

TABLEAU II

Cats having received 45 mg. digitalis powder per kg. Chats ayant reçu 45 mg. de poudre de digitale par kg.		10% calcium gluconate solution Solution à 10 % de gluconate calcique	
N° de l'animal No. of animal	Poids en kg. weight in kg.	cc. nécessaires pour provoquer la mort de l'animal ml. necessary for producing the death of the animal	dose mortelle en cc. (par kg.) lethal dose in ml. (per kg.)
1	2.355	31	13.1
2	2.400	30	12.5
3	2.155	25	11.9
4	1.450	10	6.9
5	2.300	16	6.9
			moyenne average 10.5

TABLE III

Table

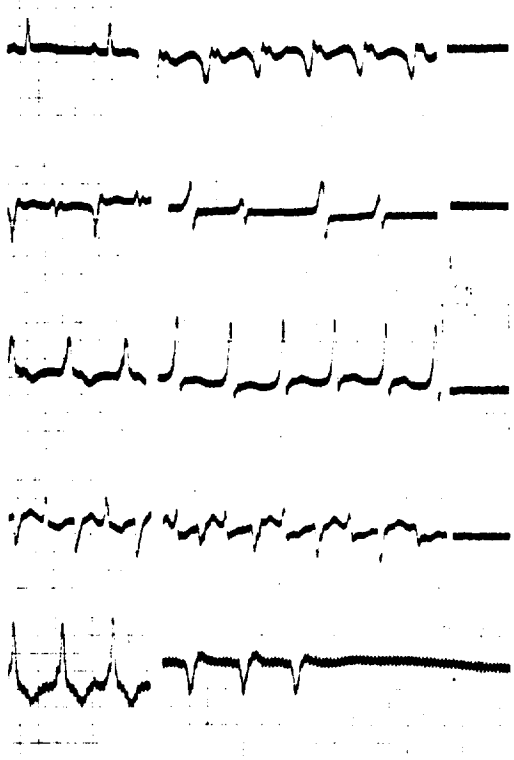
TABLEAU III

Cats having received 60 mg. digitalis powder per kg. Chats ayant reçu 60 mg. de poudre de digitale par kg.		10% calcium gluconate solution Solution à 10 % de gluconate calcique	
N° de l'animal No. of animal	Poids en kg. weight in kg.	cc. nécessaires pour provoquer la mort de l'animal ml. necessary for producing the death of the animal	dose mortelle en cc. (par kg.) lethal dose in ml. (per kg.)
1	1.975	23	11,6
2	2.000	18	9
3	2.100	19	9
4	1.900	16	8.4
5	2.850	23	8
			moyenne average 9,1

In animals having received 60 mg. powdered digitalis leaf per kg., the doses of calcium gluconate necessary for inducing the death of the animal were between 8 and 11.6 ml., or an average of 9.1 mg. (table III0.

From the electrocardiographic point of view, we did not observe electrographic changes of the sinusal complex described in animals treated only with calcium gluconate, but we established the immediate appearance of an extrasystolic rhythm which supersedes the sinusal rhythm in rare cases where this one still existed (Fig. 5, tracing I).

FIGURE 5



Electrocardiographic tracings in cats having received

FIG. 5

Tracés électrocardiographiques de chats ayant reçu 60 mg. de poudre de digitale par kg 60 mg. powdered digitalis per kg.

Dans la premiere colonne sont inscrites les altérations produites par la digitale, dans la deuxième les modifications consécutives à l'administration du gluconate calcique. La troisième colonne enregistre l'arrêt du cœur.

In the first column are inscribed changes produced by digitalis, in the second consecutive changes of calcium gluconate administration. The third column shows heart stoppage.

When this substitution had already been made under the sole influence of digitalis, clacium was limited to changing the character of the ectopic beating. Afterward, as for calcium alone, a ventricular fibrillation appeared soon followed by the death of the animal (Fig. 5, tracings II to V).

Note that the different tracings in figure 5 were recorded in animals having received lethal doses of calcium gluconate indicated in table III. We observed in cat I that it was necessary to give 11.6 ml. calcium gluconate to produce death, which represents a relatively high dose. But, precisely in this case, one sees from the electrocardiographic tracing that it concerns an animal saturated with less digitalis than the other cats, since it maintained its sinusal rhythm.

On the contrary the electrocardiographic tracings (unpublished) of animals having received 45 mg. of powdered digitalis leaf (table II) indicate, that the digitalic poisoning was especially strong in cats IV and V.

IV. General Considerations

The introduction in therapy of calcium gluconate was expected to obtain at the time of its intravenous administration much less pronounced and less frequent accidents than after the injection of calcium chloride or lactate. But, in spite of the great decrease in toxicity of calcium in the form of gluconate, Bower and Mengle nevertheless noted cardiac accidents under the influence of this substance in digitalized patients. These authors thought they were able to exclude as a cause of this phenomenon hepatic changes or a neurovegatative disequilibrium.

The results that we found are in perfect agreement with those of Bower and Mengle and of Golden and Brams. This study confirms the fact that digitalis and calcium exert very additive effects which can cause serious cardiovascular difficulties. Therefore we must prevent the digitalis-calcium association in therapy, and this especially in subjects who already have electrocardiographic symptoms of poisioning produced by digitalis.

The conclusions were contrary to those Nahum and Hoff reached probably from two causes: from the very first test that they used to determine the effect of calcium and which consisted of producing the first momentary heart stoppage, with a return to normal. But, according to our verifications, nothing is more irregular than this momentary stoppage in different animals or even in the same animal in the course of several trials. In another part of the tracings that they published no electro-cardiographic change was observed which indicated that their animals had been digitalized enough previously.

For us the sensitivity to calcium of the digitalized cat is such that in this animal 9.1 ml. of a 10% calcium gluconate solution (per kg.) is sufficient to cause death, whereas under the same experimental conditions 15 ml. are necessary in the normal cat.

Conclusions

1. The hypersensitivity to calcium of digitalized cats has been found by means of the direct recording of heartbeat.

2. The unexpected changes in cardiac contractile function under the influence of calcium gluconate either in cats not given any other treatment, or in cats previously digitalized, have been studied by the electrocardiographic method.

Calcium gluconate causes, in the normal cat, first a sinusal bradycardia with an extension of the auriculo-ventricular conduction, a decrease in the amplitude of the R wave with a progressive inversion of the T wave, next the substitution of the sinusal rhythm by an extra-systolic rhythm soon replaced by a ventricular fibrillation followed by cardiac arrest. Used in the digitalized animal, calcium glutonate causes the immediate appearance of the extrasystolic rhythm after which follows ventricular fibrillation and the death of the animal.

3. The electrocardiographic method allows us to detect precisely the exact moment of cardiac arrest and to determine under these conditions the doses of calcium gluconate necessary to cause death, of a part of the normal cats and another part of digitalized cats. The sensitivity to calcium was increased; the minimum lethal dose of calcium gluconate represents 60% of the quantity needed to cause death in a normal animal.

4. Digitalis and calcium exert additive effects which can cause serious cardiovascular problems, in therapy there is every reason to avoid this medicinal association and especially in patients already showing electro-cardiographic symptoms of digitalis poisioning.

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THE DANGER OF INTRAMUSCULAR INJECTION OF CALCIUM GLUCONATE IN INFANCY

REPORT OF THREE CASES, WITH ONE DEATH

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My purpose in this paper is to stress and reemphasize the danger of using calcium gluconate for intramuscular injection in infants.

In 1936 there were two reports which recorded the effects of calcium deposition in the tissues of 2 infants who had received the calcium intramuscularly. Tumpeer's case[1] was that of an infant who weighed 6 pounds 13 ounces (3.1 Kg.) and received 9 cc. of 10 per cent calcium gluconate intramuscularly on the third day of life. At the age of 5 weeks x-ray examination demonstrated a radiopaque substance in the thigh and buttocks where the injection had been made. A week later there was a slough of the tissue, with the extrusion of calcified material which was identified as calcium phosphate. By the fourth month of life an x-ray of the region was normal. Von Hofe[2] reported the case of an infant who had received calcium gluconate intramuscularly because of twitching on the third, fifth and sixth days of its life. Ten cc. of calcium gluconate had been injected into the gluteal muscles on two occasions, and one dose of 10 cc. had been given intramuscularly. All the areas became indurated at the age of 2 weeks and infection occurred in one of the regions previously injected. X-ray showed calcification in the soft tissues of the gluteal and the scapular regions. Bakwin[3] and Shannon[4] have reported other instances of infection following intramuscular injections of calcium gluconate.

I now report 3 cases of infants who received calcium gluconate intramuscularly with subsequent abscess formation. In the last one gangrene of the tissues and death supervened. As far as I know this is the only recorded death from this complication.

REPORT OF CASES

CASE 1.—R. M. was a 3 months old white boy baby admitted to the Long Island College Hospital on May 30, 1937 because of fever, collapse and convulsions. The family and birth history were negative. The infant had been hypertonic since birth. For a month prior to admission there had been no weight gain. The present illness was characterized by fever of three days' duration. It fluctuated between 100 and 102 F. The cause of the fever was undetermined. Irritability was pronounced, and the infant slept poorly these three days. At 10 o'clock on the morning of admission the patient suffered a vasomotor collapse, including cyanosis and respiratory difficulty. The temperature rose abruptly to 107.5 F. but was reduced by hydrotherapy to 102 F. The stuporous state persisted for four hours, followed by hyperexcitability and irritability. Hospitalization was thereupon advised. Meanwhile, 10 cc. of 10 per cent calcium gluconate was injected into the muscles of the right buttock.

The admission examination revealed that the infant was acutely ill, decidedly cyanotic and in severe respiratory distress. Except for a slight left internal strabismus, the examination was negative. Blood count revealed 5,600,000 red cells, 13,000 white cells and 64 per cent polymorphonuclears. The urine showed 3 plus urine albumin and many red and white cells and cellular casts.

The diagnosis of pyuria was made. The therapy included intravenous glucose and sodium R-lactate injections. There was a favorable response; so that on discharge the patient was afebrile and the urine was normal. However, an abscess had developed at the site of injection, which was about an inch in diameter. This healed after several weeks, but a permanent scar remains.

CASE 2.—C. S. R., a white girl baby born on Nov. 19, 1942, birth weight 7 pounds (3.2 Kg.), had a convulsion on the third day after birth. Ten cc. of 10 per cent calcium gluconate was injected into the right buttock. Forty-eight hours after the injection the buttock became indurated and discharged purulent material for six weeks. After two months healing occurred.

CASE 3.—A. Y., a white girl baby, one of twins, was delivered on July 13, 1943 by low forceps. She weighed 4 pounds 11 ounces (2.1 Kg.). The mother and babies were discharged on

Areas of gangrene in case 3.

the eighth day in good condition. At the age of 14 days the mother observed two convulsions at the 2 a. m. feeding time. These convulsions began as a twitching of the face, followed by clonic movements of the left arm and leg. Her physician sent the baby to the hospital. There the infant received an injection of 10 cc. of calcium gluconate into each buttock for "possible tetany." There were no further seizures. On August 6, when the baby was 24 days old, she was discharged with the advice that a salve was to be applied to the buttocks, because they were infected where the needle had been inserted.

At home the mother noted that her baby "felt feverish" and the buttocks appeared worse. She therefore brought her to the Kings County Hospital (Long Island College Division) the following day, August 7.

The infant was well developed and well nourished and weighed 6 pounds 7 ounces (2.9 Kg.). She appeared acutely ill. Her temperature was 102 F. The examination on admission was normal except for both buttocks. These were equally affected. Each had an area of sloughing gangrene 5 by 5 by 1.5 cm. involving the ischiorectal fat and gluteus maximus, as shown in the illustration. The ischial tuberosities, though not visible, were easily palpated. When the infant bore down, the perirectal tissues bulged into the right ischiorectal space. Two days after the admission a surgical débridement was performed. The slough contained necrotic fat, connective tissue and muscle. Therapy consisted of sulfathiazole, orally and as an ointment for the buttocks, and two blood transfusions. In spite of these

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measures the course of the infant's illness was steadily downhill. On the third day pneumonia set in. This was associated with the development of meningitis. Death followed on the fifth day after admission.

The autopsy was performed by Dr. Caspar Burn. The body, weighing 7 pounds (3.2 Kg.) and measuring 19 inches (48 cm.), appeared to be poorly nourished. There was no evidence of jaundice or petechial hemorrhages of the skin. The scleras and conjunctivas were clear and showed no petechiae, jaundice or hemorrhages. The buttocks presented pronounced ulcerations. The ulceration of the left buttock was oval, measured 2.5 by 5 by 3.5 cm. and was 1 cm. deep. It had a slightly irregular jagged edge and contained a small amount of yellow, necrotic material which covered the adipose tissue and the left gluteal muscles. The ulceration on the right buttock measured 2.5 by 5 by 3 cm. and was 1 cm. deep; this presented a yellow necrotic slough covering the gluteal fat; each ulceration was surrounded by a purple areola 4 to 5 cm. wide.

The pleural surfaces were smooth, glistening and translucent; no evidence of apical scars, tubercles or exudate was noted. The lungs had their usual shape and both lungs appeared to be collapsed except in the right lower lobe, which was firm and prominent. The external surfaces of the left lung showed numerous small, depressed, purple areas, whereas the whole right lung appeared a diffuse purple. On section the lung parenchyma was moist and exuded a frothy, pink material. The right lower lobe was dark beefy red and showed almost complete loss of lobular formation. The cut surface appeared smooth and darker red than usual, with this change being more prominent in the right lung than in the left. The bronchi and blood vessels showed no undue prominence, and the bronchi were lined by a smooth, white, glistening membrane. The hilar and tracheobronchial lymph nodes were not unduly prominent and showed no evidence of tuberculosis.

The scalp over the occiput showed two old hematomas about 6 cm. in diameter, most likely the result of the use of the scalp veins for intravenous therapy ante mortem. The anterior fontanel was diamond shaped, soft, 3 fingerbreadths in diameter, and showed no bulging or recession. The sagittal suture showed no overriding, and the posterior fontanel was closed. The bones of the vault of the skull were soft and showed no irregularities or fractures. The dura showed no evidence of hemorrhage or tearing. The meninges, except over the base of the brain, had lost their usual translucency, and within the subarachnoid spaces in this region there was an accumulation of fluid, greenish, purulent material. The brain was symmetrical and had well formed sulci and gyri, with no evidence of softening. The cerebellum was well developed. The blood vessels were thin walled and showed no aneurysms or arteriosclerosis. On section, the convolutions of the gray matter were pale and indistinct in outline but were regularly arranged. The ventricles appeared somewhat dilated and were filled with granular, puslike material. The medulla and cerebellum showed the usual structures and did not appear unusual.

The skin was lined with stratified squamous epithelium, which stopped short at the edge of the ulceration. The corium of the skin contained sweat and sebaceous glands and hair follicles. The edge of the sinus consisted of collagenous material together with dilated and engorged newly formed capillaries and edema fluid. This process involved the base of the ulcer and to a slight extent the adjacent adipose tissue. There were a few foreign giant cells situated within the newly formed granulation tissue. Occasional polymorphonuclear leukocytes were present within the necrotic zones.

The remaining organs were essentially normal.

Bacteriologic examination of the heart's blood revealed Staphylococcus aureus, of the ulcers of the buttocks Staphylococcus aureus, Bacillus pyocyaneus and Proteus vulgaris and of the meninges Staphylococcus aureus.

The anatomic diagnoses were abscess of buttocks, sepsis, atelectasis of both lungs, bilateral hemorrhagic pneumonia and acute purulent basilar meningitis.

COMMENT

I have referred to the fact that previous articles have stressed the danger of injecting calcium gluconate intramuscularly in infants. The possible explanation lies in the fact that the calcium, which is injected as a gluconate, is precipitated as a phosphate. The reason for this may be linked up with an increased phosphorus content of the blood serum in these cases. This is followed by coagulation within the local blood vessels, accounting for the necrosis and sloughing of the tissues (Tumpeer [1]).

Whatever the reason, the practice would still appear to be quite common. Furthermore, several excellent sources state that calcium gluconate may be used with safety intramuscularly. In Goodman and Gilman's textbook [5] with reference to calcium gluconate the statement is made "It can be administered orally, intravenously or intramuscularly." No mention is made of the danger of using it intramuscularly in infants. The intramuscular injection of calcium gluconate is discussed in New and Nonofficial Remedies,[6] which is issued under the supervision of the Council on Pharmacy and Chemistry of the American Medical Association. On page 454, under actions and uses of calcium gluconate, it states that it "is nonirritant for hypodermic or intramuscular use," "for children, 0.2 to 0.5 Gm. administered every day, on alternate days or every third day."

In view of the previous reports and these additional 3 cases, including a fatality, through sepsis not due to the drug, it would seem advisable to eliminate from the book and drug literature the use of calcium gluconate for intramuscular injection in infancy and specify the use of calcium gluconate in infants orally and intravenously only.

CONCLUSION

Three cases of abscess formation, including one death, following the injection of calcium gluconate intramuscularly in infants have been observed. It would appear advisable to avoid completely the use of calcium gluconate intramuscularly in infants and to restrict its use to the oral and intravenous routes.

340 Henry Street.

J. Pharmacol. + Exptl. Therap.
40: 71-76, 1930

STUDIES ON CALCIUM[1]

II. URINARY OUTPUT OF CALCIUM IN NORMAL INDIVIDUALS AFTER PERORAL ADMINISTRATION OF CALCIUM LACTATE AND CALCIUM GLUCONATE

ARNOLD L. LIEBERMAN, M.D.

From the Hull Physiological Laboratories of the University of Chicago

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It is of practical value to answer for a given drug two questions: How much? When? In so far as peroral administration of calcium salts is concerned, it seems to have been taken for granted that absorption is better on an empty stomach and that the equivalent of 3 to 4 grams of calcium lactate per day is about the right dose for an adult, except in cases of extreme calcium deficiency as in hypoparathyroidism when a much larger amount must be given. It seemed worth while to gather some precise data on this subject.

METHOD

Although less than 10 per cent of peroral calcium is excreted in the urine, it was assumed that the urinary calcium would, nevertheless, serve as a fairly reliable index of the rate of the blood calcium changes. Accordingly, three healthy individuals submitted to the following procedures. As a control, the individual voided hourly from 7 a.m. to 2 p.m. He ate nothing from arising to 2 p.m. He drank a glass of water (250 cc.) hourly from 8 a.m. to 1 p.m. There were no other limitations. The next day calcium lactate was given at 8 a.m. in 250 cc. of water. Everything else was maintained the same. The following day the routine was repeated except that calcium gluconate was given in 250 cc. of water. After an interval of a day, the order of calcium salts was reversed calcium gluco-

[1] Work done under grant from Sandoz Fund.

nate was given first, calcium lactate on the second day. Each subject kept a rather detailed record of pertinent signs and symptoms such as abdominal discomfort, nausea, headache, diarrhea, borborygmi, etc. At first, the subject was given 20 grams of calcium gluconate and 10 grams of calcium lactate but the subjective and objective symptoms (abdominal distress, vomiting and diarrhea) were so violent that the doses in this series were halved, 10 grams of calcium gluconate and 5 grams of calcium lactate being given. The powder was simply dissolved in a glass of water (250 cc.) and drunk. This procedure was then repeated with the important difference that the individual had his usual breakfast about 7:30 a.m. It was uniform each time consisting of two eggs, two cups of coffee, and one piece of toast. For the urinary determinations, Lyman's (1) method was used with only this difference: instead of washing through filter paper, centrifuge tubes were employed. This saved a great deal of time and was as accurate, by actual check, as the unmodified method. The volume of the specimen obtained each hour was recorded and the milligrams of CaO in each specimen determined.

By its very nature, urinary calcium cannot give as true a picture of the level of calcium in the blood as can an actual blood calcium determination. However, for obvious practical reasons, the calcium output in the urine was chosen as the index as to the rapidity and extent of absorption and excretion of the calcium ingested.

RESULTS

Several rather interesting points stand out in the data accumulated. Figure 1 shows graphically the volume of urine. It is the average of 18 determinations each on the calcium gluconate and calcium lactate with 9 controls. As can be seen, ingested calcium salts cause a very definite diuretic effect. Whereas at 2 p.m. the controls showed a volume of only 178 cc., the average volume for the gluconate salt was 360 cc.—a volume twice as great. However this action can be explained on the mere physical basis of the greater volume of water being necessary to put out a larger amount of calcium present. Of course, the individuals here

were presumably healthy so that this would have nothing to do with experiments like those of Barath and Gyurkovich (2) who showed that calcium salts cause a diuresis and a diminution of the albuminuria in nephritic edema. In these cases Blum (3) may be right in saying that the action is due to the dehydration of the blood colloids with resulting hydremia and diuresis.

Figure 2 shows the amount of CaO in milligrams that appeared in the urine during the period of observation. It will be seen that the individual puts out normally somewhere between 2 and 5 mgm. of CaO hourly. After the ingestion of the calcium salt

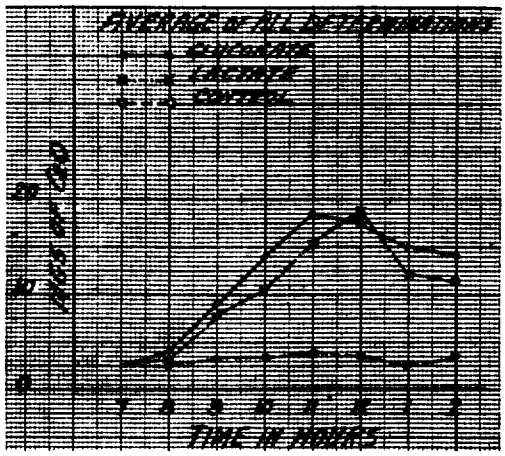

FIG. 1

there is a fairly smooth curve upwards with a maximum attained in some four hours. Then there is a beginning decrease. There is very little to choose between the two salts although a little more of the gluconate salt is recovered. However, there is a vast difference in the subjective symptoms. The gluconate was fairly well tolerated giving rise chiefly to rather annoying borborygmi and some abdominal distress. The lactate salt in equimolecular amounts gave rise to a very disagreeable headache and quite stormy bowel movements accompanied by a good deal of bowel spasm. Altogether, about 5 to 8 per cent of the calcium given was recov-

ered in the urine which is about the amount usually quoted in the literature. A much more interesting and very instructive finding is summarized in figure 3. As can be seen gluconate given on an empty stomach reaches a maximum within two hours and then begins to decrease. When given after a meal it takes longer for it to appear in any amount in the urine but six hours after ingestion the curve had not yet begun to slope downwards. As a net result much more of the calcium gluconate is utilized by the body.

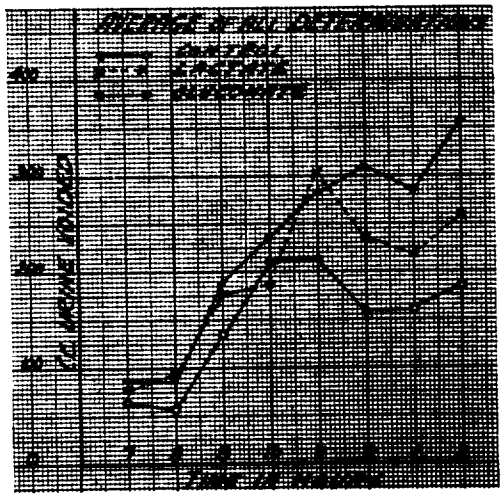

FIG. 2

Also, there was almost complete freedom from subjective symptoms. Except for the slight discomfort right after taking the material, one almost forgot that anything had been ingested. It seems, in this series at least, that there can be no question that better and longer absorption was obtained by taking the salt on a full stomach. The calcium lactate gave a very similar curve except that when given on an empty stomach the plateau of the curve was much flatter. This is probably due to the fact that the hyperperistalsis induced by the lactates caused such a rapid movement of the material through the bowel that proper absorption could not take place. At this point it seems to be worth while

repeating the statement previously made as to maximal optimal doses. The 10 grams of calcium gluconate powder and the equimolecular amount of the lactate is not only the maximal dose from the standpoint of comfort in taking the substances but is also the largest amount that can be taken without having a *decrease* in the amount of calcium ion absorbed. This apparent paradox is readily explicable when we take into consideration the fact that overdosage sets up a violent diarrhea which causes such a rapid passage through the bowels of the salts that proper absorption can not take place.

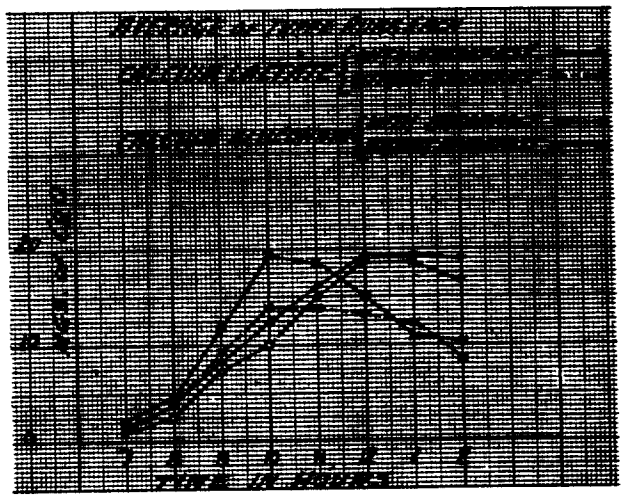

FIG. 3

CONCLUSIONS

1. Using urinary calcium as an index, the extent of calcium absorption following peroral administration of calcium gluconate and calcium lactate has been studied in three healthy individuals

2. There is a slight but very definite diuretic effect in this series.

3. There is a maximal dose beyond which the diarrheal effect begins to outweigh the size of the dose. The salt begins to pass through and out of the intestine too rapidly to be absorbed properly. The gluconate salt gives fewer subjective symptoms as compared with the lactate salt.

4. It is much better to administer the calcium salt after a meal. There is a smoother and greater absorption with a minimum of subjective distress. Also, for a given dose the physiological action of the ingested calcium persists over a more protracted period of time.

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COMPARATIVE STUDIES ON CALCIUM GLUCONATE AND OTHER CALCIUM SALTS*

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About two years ago, Professor Luckhardt asked me to observe the comparative pharmacologic effects following the intravenous injection of calcium lactate and calcium gluconate in unanesthetized dogs. The methods were, briefly, an exposure of the carotid vessels under ether anesthesia, recovery of the dogs from the immediate effects of the operation, and an injection of the salts in question while the animals were awake. Blood pressure tracings, pulse, and respiratory readings were made then over a period of from thirty minutes to one and a half hours. The full details of this series appeared elsewhere,[1] so that it is necessary here only to summarize the results. Charts 1 and 2 show concisely the elevation of the blood pressure with the simultaneous pulse retardation. Also, the relatively evanescent effects of the lactate salt as compared to the gluconate stands out strikingly. Digitalis effects were quite noticeable. Recognizable heart block, arrhythmias, coupling of beats, and other digitalis-poisoning effects

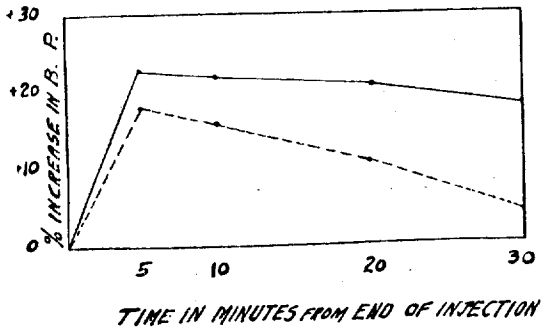

Chart 1.—Average of series of eight determinations: solid line, gluconate; broken line, lactate.

were induced repeatedly. Here, again, the gluconate salt was somewhat slower in producing these results as compared with the lactate compound, but, once produced, they tended to persist a much longer time. They would appear as soon as five minutes after the intravenous administration of the calcium salt and would persist sometimes an hour or more. Billigheimer[2] had previously noted the digitalis effects of calcium which, to me, appear to be well worth further study. Also, emphasis was placed on the danger of intravenous calcium therapy; sudden clotting was noted several times as a result of the well known but frequently forgotten tendency of calcium to increase the coagulability of the blood.

Before proceeding to the study of further cardiac effects of calcium, it was thought advisable to make some detailed observations on the best methods of administering calcium salts. Some of the results already obtained appear to be of immediate practical interest. The changes in the calcium levels of the blood and urine are especially commented on here.

Calcium gluconate can be given subcutaneously in repeated and adequate amounts (parathyroidectomized dogs being kept alive in this fashion[3]) without causing the irritation and necrosis that other calcium salts do when so injected. Szentel.[4] was able to report decreased postpartum hemorrhage by using this compound. That this procedure will promptly elevate the blood calcium was conclusively shown in normal and parathyroidectomized dogs.[5]

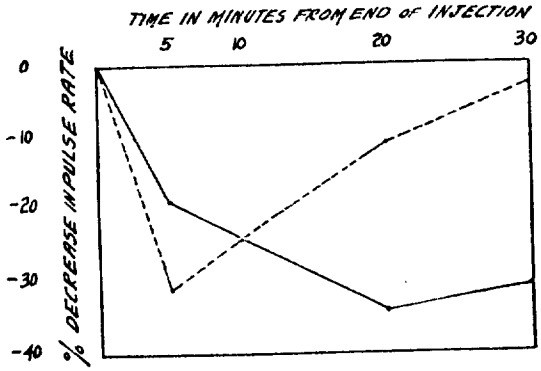

Chart 2.—Average of series of eight determinations: solid line, gluconate; broken line, lactate.

There has been some confusion in the literature as to the proper time of giving calcium compounds when administered orally. Thus, Roe and Kahn[6] showed definitely that large amounts of calcium lactate, when given intimately mixed with food, failed to cause a rise in blood calcium. On these grounds they advocate giving the calcium on an empty stomach. In some studies on urine I[7] came to the conclusion that the amount of intestinal absorption depended to a decisive extent on the length of time the calcium compounds were permitted to stay in the bowel. When given on an empty stomach there appeared to be a marked tendency for the production of diarrhea with its attendant phenomena. As food tended to minimize this hydra-

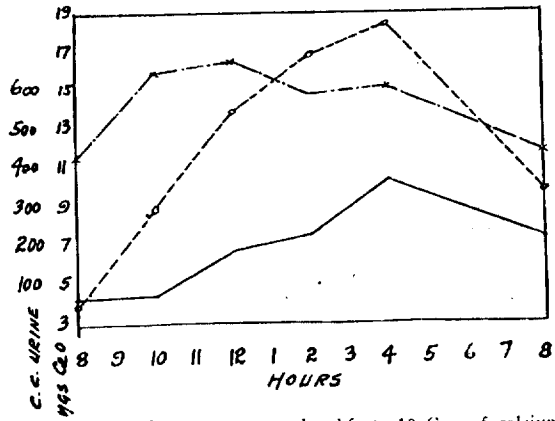

Chart 3.—Average in ten cases; no breakfast; 10 Gm. of calcium gluconate by mouth: solid line, urine volume; broken line with circles, urine calcium; line of dots and dashes with crosses, blood calcium.

gogue salt action of the calcium compounds, it seemed that giving it after a meal would cause greater absorption and less distress.

* Work done on grant from the Sandoz Fund.

* From the Hull Physiological Laboratories of the University of Chicago.

* Read before the Section on Pharmacology and Therapeutics at the Eighty-Second Annual Session of the American Medical Association, Philadelphia, June 10, 1931.

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To settle the point, about thirty individuals were taken and submitted to procedures described in detail elsewhere.[8] The blood and urine calcium changes were traced preceding and following the administration of calcium gluconate as follows: Ten received 10 Gm. orally on an empty stomach; ten received 10 Gm. after a standard breakfast; five had 20 cc. of the 10 per cent solution[9] injected deep in the buttocks while receiving no food; five others had a similar injection following a standard breakfast. Charts 3, 4, 5 and 6 show in summary form the results obtained. Charts 3 and 4 seem to show that calcium gluconate is absorbed a trifle better and gives more persistent elevation of the blood and urinary calcium when given after a meal. Subjectively there was an immense difference: the patients had no complaints to speak of when the compound was given after breakfast, while there was a very definite diarrheal tendency when they received the calcium on an empty stomach. With severe diarrhea there appears to be no blood calcium elevation of any moment, the medication being rushed through and out of the bowel before being given a chance to absorb.[5]

As can be seen, the blood calcium reached its maximum about the fourth hour after oral administration. Intramuscular injection gives the peak within the first hour. This is of practical importance when speed of action is desired. It seems hardly worth while to take a chance on sudden death and give the calcium intravenously when an intramuscular injection will attain the same end in so short a time and without any risk.

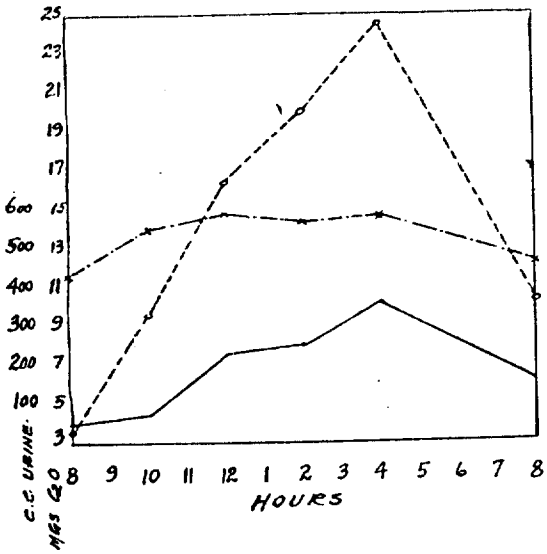


Chart 4.—Average of ten cases; 10 Gm. of calcium gluconate by mouth after standard breakfast: lines as in chart 3.

On further inspection of the urine calcium curves, it will be noted that there is a lag in the rise of the calcium here as compared with the blood level. However, even if there is no quantitative relationship between blood and urine calcium, there appears to be a distinct qualitative correlation. As long as the hourly output of urine in the individual is above, say, 10 mg. of calcium an hour, there is little danger of that individual developing hypocalcemia with its attendant symptoms. Conversely, the continued output of, say, 30 to 40 mg. an hour would be quite clear evidence of a state of hypercalcemia which will lead to its typical signs and symptoms if continued. Because of this factor of lag, however, a low urine calcium followed by a rise some four to six hours after a dose of calcium would be an indication of a fall in blood calcium and of the advisability of repeating the dose. In cases in which parathyroid extract is being given, post thyroidectomy or in infants with tetany, this should be of practical importance. It is certainly much easier to take a specimen of urine than to take a sample of blood.

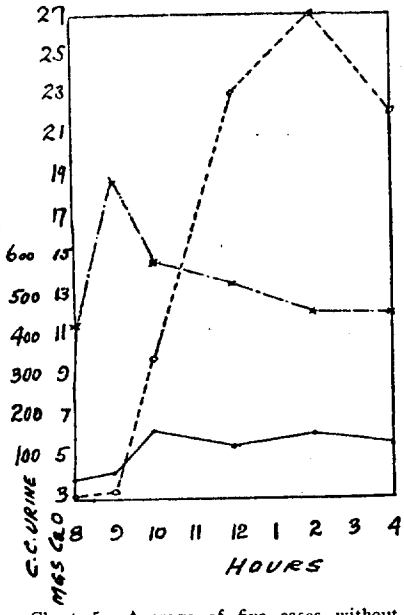


Chart 5.—Average of five cases without breakfast; 20 cc. of calcium gluconate deep in muscles: lines as in chart 3.

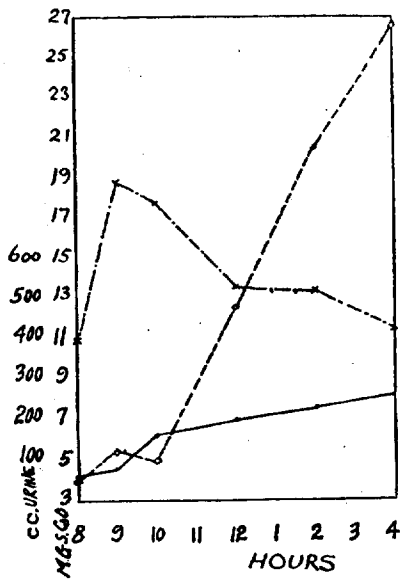


Chart 6.—Average of five cases with standard breakfast; 20 cc. of calcium gluconate deep in muscles: lines as in chart 3.

As regards dosage of calcium gluconate when being given by mouth: After perusing the charts it seems fair to say that 10 Gm. of the powder when split into, say, three doses and given about half an hour after meals should be sufficient to maintain an adequate calcium level without at the same time tending to produce a hypercalcemia.

SUMMARY AND CONCLUSIONS

1. Calcium gluconate was taken as a most convenient representative calcium salt and studied as to dosage and effects on human beings and dogs when given orally, subcutaneously and intravenously.

2. This compound can be given in repeated and adequate amounts subcutaneously without causing either irritation or necrosis.

3. Three or four grams a day after meals appears to be about the right dose for an adult.

4. Blood calcium attains its maximum elevation within an hour after subcutaneous or intramuscular administration of calcium and within four hours after being given orally.

5. Intravenous calcium therapy is dangerous because of the menace of sudden intravascular clotting and death.

6. Urinary calcium appears to be a qualitative index

[8] [illegible] Calcium. J. Pharmacol. & Exper.

is true in all pathologic conditions remains to be seen. A urinary value of about 10 to 20 mg. an hour appears to be most desirable in avoiding either a hypercalcemia or a hypocalcemia.

7. Highly suggestive digitalis-like effects of calcium were observed and the need for further investigation of this phase of the problem is noted.

Acta gerontologica 3:3-12, March-April '53 I

Institute of clinical Medicine and clinical Therapy
of the University of Modena
Director: Prof. M. Coppo

HYPERCALCEMIA DUE TO LOADING WITH CALCIUM CHLORIDE AND VITAMIN D_2 IN PATIENTS WITH GERONTOXON AND AND IN RESPECT TO ACF (*)

Dr. Renzo Lorenzini

In dealing with the pathogenesis of arterial atherosclerosis in the human, Coppo underlined the importance of the metabolic factor in the sense of a disturbance of the lipidic metabolism, and considered it possible that hyperlipidemia, which appears frequently in patients with atherosclerotic lesions, is associated, sooner or later, with a hypervitaminosis D: this in its turn may give some explanation of the tendency to common heterotopic calcifications in advanced age and in the evolution of arterial atheromatosis. (1)

The mineral metabolism is carried out thanks to the intervention not only of hormonal and neurovegetative factors, but also of vitamin precents which, in the specific case of the metabolism of calcium, regulate the absorption, the fixation, and the elimination.

The skeleton, under normal conditions, contains a notable reserve of minerals which is continually being renewed by a progressive, slow elimination and substitution of its mineral components. [Policard et al. (2). Roche (3)].

The intervention of vitamin D is indispensible in the regulation of the exchange of the bone deposits of Ca, not only because of its primary and exquisitely specific action, but also because of a more generic action, common to all the vitamin principles, whatever the effect of their "regulatory" intervention in a general sense (Coppo, 4).

The requirement for vitamin D in man is not always the same, but rather depends on various factors. One of these, for example, is the mineral content of the diet; the arising of symptoms of rickets after infections or diseases of the gastroenteric tube which compromise the normal assumption of the vitamin; the increased demand for vitamin D_2 during pregnancy and in the stage of puberty (Stepp et al., 5).

It should not be forgotten that vitamin D behaves in the organism as a "non-passage substance" (Seiderhelm, 6); that is, it is a vitamin in whose case introduction and elimination are not in balance day after day. In other words, the organism does not need to be supplied with the entire necessary dose of

(*) The question was in part the object of the Communication to the 51st Congress of the Italian Society of Internal Medicine held in Rome (October, 1950).

vitamin D "ex novo" each day, as is the case with vitamin C. Thus it can be understood how a case of hypervitaminosis D can sometimes easily come about, even when relatively small doses of the vitamin are administered.

As concerns the possible relationship between vitamin D and vascular pathology, we recall, among the characteristic effects of hypervitaminosis D, the intervention of this vitamin in the metabolism of fats and the consequent deposition of lipides in the liver, the artery walls and the glands of internal secretion.(Collazo, 7). We recall as well the observations of Baltaceanu et al. (8) on the relationship between vitamin D and vascular pathology; according to these authors, the atheromatosal walls of the aorta, the myocardium and the skeletal muscles of old dogs contain a greater than normal quantity of vitamin D. In this we see the possibility of proposing a hypervitaminotic interpretation of atheroma and cardiovascular sclerosis, and perhaps of senility in general.

Significant in this sense are the observations of Harrison (9) on lesions in the aorta experimentally provoked in rabbits treated with vitamin D and cholesterol. Bertoli et al. (22), studying the effect of the parathormone and vitamin D_2 on the metabolism of Ca in rabbits on a hypercholesterolic diet, verified characteristic alterations of the aortic wall, represented by an evident process of calcification, chiefly of the tunica media of the aorta itself.

Thus, experimental pathology confirms the intervention of vitamin D_2 in the area of arterial diseases, and recognizes in this vitamin a role of no negligible importance in the process of aging of the arterial tree.

The singular frequency of cardiovascular atherosclerotic diseases in our emilian causistics suggested to us the possibility of studying, at various ages, the relationship between the hyperlipidemic rate, which accompanies this pathology, and the behavior of the calcemic curve after leading with a calcium salt in normal subjects and in patients with atheroma of the arteries.

Therefore, a series of experiments was undertaken, for the purpose of determining:

1) the variations of the calcemic curves of leading with $CaCl_2$ resulting from a possible condition of hypervitaminosis D, in relation to age;

2) the behavior, according to the various ages, of the calcemic curves of leading with Ca before and after treatment with vitamin D_2;

3) the repercussions of the hyperlipidemic state on the metabolism of blood Ca.

As the end of our research we considered interesting, even more so than the values of blood Ca in basal conditions and in the course of treatment with vitamin D_2, the study of the calcemic reaction upon leading with a calcium salt. This method, realized by Campana and Gualandi (10) in the case of hyperthyroidism, yielded encouraging results, because it places in evidence a possible deficiency of Ca available for the maintenance of its blood concentration.

The selection of patients with cardiovascular atherosclero

lesions was made, taking into consideration the presence of a corneal "senile arch". This criterion was based on the observations of the school of Coppo (11) and Marfori et al. (12), who confirmed the frequent coexistence of gerontoxon in patients with atherosclerotic vasculopathies. These authors demonstrated that gerontoxon and xanthelasma have not only the value of a localized steatosis, but also often of a lipidosis in the arterial system, and that, in a high percentage of cases, this particular corneal xanthomatosis is accompanied by an intensification of lipidemia (particularly of cholesterolemia).

Method of Research

We studied 41 subjects of the masculine sex, of whom 20 did not have gerontoxon, and 21 had apparent corneal "senile arches". The controls (no gerontoxon) were divided according to age into three groups: the first of four individuals of an average age of 23 years; the second of five individuals of an average age of 51 years; the third of eleven individuals of an average age of 67 years.

The patients with corneal xanthomatosis were divided into two groups: the first of eight individuals, average age of 38 years; the second of 13 individuals, average age of 64. In all these subjectscholesterolemic values superior to the norm were ascertained.

For our case we used volunteers and inmates of a rest home, choosing from among subjects in good states of health.

The Ca injection curves were constructed on calcemic values derived from four samples of blood taken in the morning, the patient having eaten nothing since the preceding evening; after the first sample was withdrawn, an intravenous injection of $CaCl_2$ (10 cc calcium chloride at 10%, equal to 0.182 g of calcium) was immediately made, and the successive samples of blood were withdrawn at respective distances of 3', 30' and 90' from the intravenous injection.

The calcemic curves finished, the treatment with vitamin D_2 was begun, using a phial of 15 mg (600.000 units). Each subject received three intramuscular injections of vitamin D_2 for three consecutive days; on the third day, after the last administration of the vitamin, the curves of Ca leading were repeated with the method described above.

The blood Ca was dosed using the method proposed by Kramer and Tisdall (13): normally the values ranged between 9 and 11% cc of the serum. All the calcemic values reported were obtained by means of two determinations and express in mg the Ca in 100 cc of serum.

Results of the Experiments

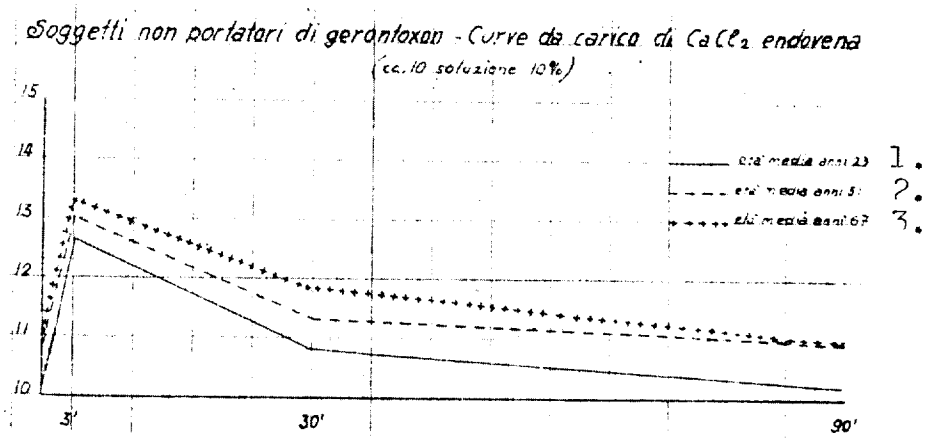
The most significant data obtained from the laboratory protocols, referring to the various groups of subjects examined, are here reported.

1) -- Subjects who were not patients with gerontoxon.

a) The first group of controls, comprising four individuals, lowest age 19, highest 29, average 23, yielded, in basal conditions, calcemic values within the limits of the norm -- from 9.8 to 10.8 mg.%. After intravenous leading of $CaCl_2$ a clear hypercalcemic reaction was observed, very evident after three minutes and still persisting (in three subjects) after a half hour. The control completed at 90' from the injection revealed a calcemic rate about equal to the initial values.

After treatment with vitamin D_2 there was noted in all the subjects a calcemia of fasting superior to that preceding the vitamin effect. As an effect of the injection of $CaCl_2$ a curve was obtained, comparable in movement and intensity of gradients to that obtained before treatment with vitamin D_2, but completely shifted in height. Thus there was a parallel movement of the two curves. (See graph 1).

A.



Graph 1.

Key:
A.= Subjects who are not patients with gerontoxon
Curve of intravenous leading with $CaCl_2$
1.= average age 23
2.= average age 51
3.= average age 67

The variations in increase produced by the vitamin varied between a minimum of 10.9 and a maximum of 13.4 mg per 100 cc of serum.

b) In the second group (five individuals, minimum age 48, maximum age 54, average age 51) the calcemic values in basal conditions varied from 10.8 to 11.2 mg %. the hypercalcemic reaction resulting from leading with $CaCl_2$ was very clear and reached, after 3 minutes, maximum points of between 12.6 and 13.3 mg %, with a tendency to a return to the initial calcemic values of the controls, obtained after 90' from the presentation of the calcium salt.

Also in this group of individuals, after administration of

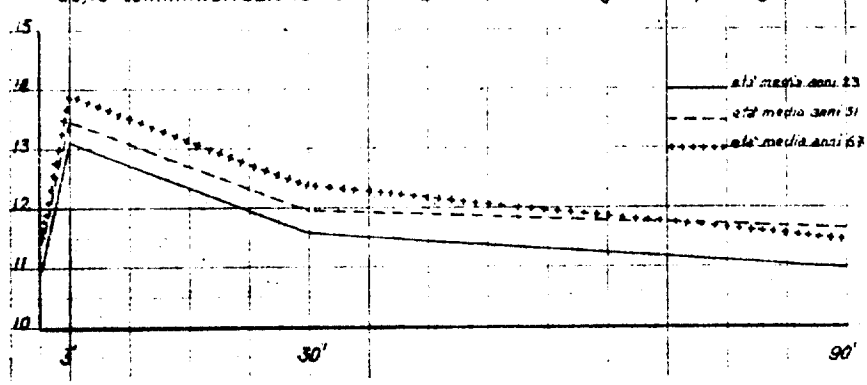
vitamin D_2 , the blood level of Ca in a fasting state was higher than that preceding the vitamin action, and was hypercalcemic in an absolute sense: from 11.4 to 12 mg %.

The variations induced by vitamin D_2 varied, in their maximum points, from 12.9 mg to 13.8 mg %.

c) The third group of controls (eleven individuals, minimum age 60, maximum age 73, average 67) yielded calcemic values in basal conditions of between 10.8 and 11.6 mg %. The venous injection of $CaCl_2$ was followed by a hypercalcemic response which was still evident after 30' from the administration of the calcium salt. Maximum values of between 12.7 and 13.6 mg % were reached, observing the highest differential, based on the respective initial values, in the subjects examined who were not patients with gerontoxon.

In this group of controls, the hypercalcemic response of vitamin D was, on the whole, clearly superior to that verified in the other groups without gerontoxon. From basal values comprising between 11.3 and 13.4 mg %, obviously hypercalcemic, there were reached, after treatment with D_2, maximum values of between 13.5 and 14.6 mg %. Also in this group, after 90' from the presentation of $CaCl_2$ intravenously, the calcemic curve showed a tendency to return to the initial values, with slight variations to a greater or lesser degree. The characteristic movement of the curves before and after administration of vitamin D_2 is reported in graph 1a.

A. *Soggetti non portatori di gerontoxon - Curve da carico di $CaCl_2$ dopo somministrazione di vit. D_2 intramuscolo mg.15 al dì per 3 giorni*



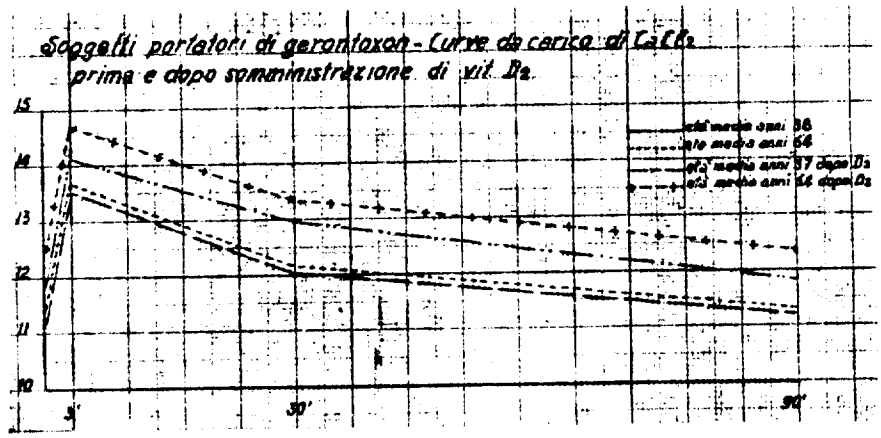
Key:
A.= subjects who are not patients with gerontoxon
curve of intravenous loading with $CaCl_2$ after intramuscular administration of vit. D_2, 15 mg per day for 3 days

The differential between the basal calcemic value and the maximum values of the curve also pointed out, after treatment with vitamin D_2, the most notable differences between the individuals who did not have gerontoxon.

2) -- Patients with gerontoxon

a) the first group, comprised of eight individuals (minimum age 31, maximum age 48, average 38), yielded calcemic values in basal conditions, of between 10.8 and 11.8 mg %. Presentation of $CaCl_2$ into the vein was followed by a clear hypercalcemic reaction, still evident after 30', with maximum points between 12.9 and 14.1 mg %; after 90' the return to the initial values was concordant in all the subjects examined.

After vitamin D_2, the calcium in a fasting state was shown to be increased, in relation to the corresponding control before the vitamin treatment. Presentation of $CaCl_2$ is followed by a curve shifted in height, but comparable in movement to that obtained before vitamin D_2 (see graph 2). The maximum hypercalcemic variations induced by the vitamin varied between 13.2 and 14.6 mg %; the differentials on the initial values touched, in two cases, 3.1 and 3.4 mg respectively.



Key:
A.= patients with gerontoxon
curve of leading with $CaCl_2$, before and after administration of vitamin D_2.
1.= average age 38
2.= average age 64
3.= " " 37, after D_2
4.= " " 64, after D_2

b) In the second group (comprised of thirteen individuals, minimum age 57, maximum age 70, average 64), the calcemic values are still significant, as basal values higher than 11.5 mg % were obtained for the most part, with maxima around 12.5. After presentation of $CaCl_2$, the hypercalcemia became accentuated, touching upon 14.6 mg % after 3' from the presentation, and still intense after 30'. The controls, after 90', also showed in this group a considerable flexion of the calcemic values, with a tendency to a return to the basal values.

The intervention of vitamin D_2 in this group of individuals shifted the basic calcemic level beyond 12 mg % and caused the registration of the maximum calcemic quotas, varying between 13.8 and 15.2 mg %. As for the other groups examined, the hypercalcemia was still notable after 30' from the introduction of $CaCl_2$ into the vein, returning to the basal values of the calcemic

control after 90'.

In this group also, the average curves of calcium leading, determined before and after treatment with vitamin D_2, are characterized by the intensity of the gradients, and present on the whole a parallel trend (see graph 2).

For the purpose of an easier comparison between the various groups of individuals examined, there is reported in the graph the trend of the curve of leading with $CaCl_2$ before and after the administration of vitamin D_2, derived from the average of the values of each group of subjects (graphs 1, 1a, 2).

The results obtained offer incentive for several considerations.

As regards the curve of intravenous leading with $CaCl_2$, we observe that all the subjects examined, regardless of age, responded with a clear hypercalcemic reaction, evident after 3' from the presentation of the calcium salt, and persisting after a half hour: after 90' there was observed a return toward the initial values. This behavior can be considered normal.

The average curves of the three groups of individuals who did not have gerontoxon show a parallel course, but not an overlapping one. In fact, we see that with the increase in age, not only the calcemic level in a fasting state increases, but also, proportionally, all the points of the curve are shifted up.

This observation is also true for the average curves of the two groups of individuals with gerontoxon, curves which are also parallel but not overlapping.

If we compare the relationship between the calcemic level in a fasting state and the ages of the various groups of individuals examined, we find a considerable difference between patients with gerontoxon and those without; the corneal "senile arch" is accompanied by calcemic values considerably higher in individuals of the same age. We see, for example, that the basal calcemia of the first group of patients with gerontoxon (average age 38) can be compared with that of the group of individuals without gerontoxon whose average age was 51; the maximum hypercalcemic response to leading with $CaCl_2$ also yielded nearly average identical values in the two groups of subjects.

After treatment with vitamin D_2 we observed, in all the individuals examined (with or without gerontoxon), that the calcemic fasting value is always higher than the corresponding control before treatment with the vitamin. Moreover, as a result of the leading, a calcemic curve parallel to that preliminarily verified, but always shifted up, was obtained.

As regards the relationship between the calcemic level in a fasting state and age, after treatment with vitamin D_2, the observations already made are valid: in these experimental conditions as well, the highest values, relative to age, are found in the individuals with gerontoxon.

These experiments demonstrate that vitamin D_2, administered in proper doses, can determine a lasting hypercalcemic base, on which the response to leading with $CaCl_2$ in the vein occurs according to the normal form.

The ample literature on the relationship between vitamin D_2 and the blood calcium is not entirely concordant in attibuting

a specific hypercalcemizing effect to this vitamin.

Beside the majority of authors who affirm the possible intervention of vitamin D_2 in the raising of the basal calcemic rate (Aberhalden (14), Maúriquand et al. (15), Brull, Clemens et al. (16), Stepp, Kühnau and Schroeder (5), Mach (17)), there is no lack of those who negate such an action if the vitamin is administered intramuscularly (Gounelle, Bachet and Sassier (18)). Others attribute the hypercalcemizing effect of vitamin D_2 on particular conditions of intolerance to hypervitaminosis (Charpy et al. (19), while Vallery, Milliez and Ryckewaert (20) maintain that there is individual susceptibility to this vitamin, as well as species susceptibility, which explains the accidents of vitaminotherapy D_2, such as the decalcification of the skeleton, hypercalcemia without intoxication, and toxic phenomena without hypercalcemia.

There is also disagreement concerning the physiological mechanism through which vitamin D_2 can produce hypercalcemia. According to the various authors there might occur a stimulation of the parathyroids with the freeing of a hormone having a hypercalcemizing effect; a liberation of Ca from bone substance; an influence of the vitamin on the absorption or the elimination of Ca from the intestine; the possibility has even been proposed that high doses of D_2 decrease the fecal elimination of Ca, detouring it to the kidneys.

Apart from all the possible interpretations of the mechanism of effect of vitamin D_2, the results of our research clearly show that this vitamin, administered by the proper method and in the proper doses, does not interfere in the regulatory mechanism of the elevated experimental calcemic level after leading with $CaCl_2$, but acts rather to elevate the basal calcemic level in a lasting manner. This action was controlled in all the subjects examined, with and without gerontoxon and regardless of age.

Like Lanciotti (21), we also find that the absence of effect of vitamin D_2 on the experimental hypercalcemic curve can lead us to consider a generic influence on the intestinal absorption, the urinary elimination, the relationship of bone Ca to blood Ca, rather than a direct hematic influence, or an indirect one through the neurovegetative nervous system.

The verification in patients with gerontoxon of basal calcemic values habitually greater than those of the controls might be justified by the hyperlipidemic and hypercholesterolemic level, so common in the subjects with corneal xanthomatosis. It is possible that hyperlipidemia can be associated sooner or later with a hypervitaminosis D (Conno, 4), with the consequences on the calcemic level which we have verified.

Hyperlipidemia, hypervitaminosis D, increase of the basal calcemic level, could thus represent the successive stages of a complex phenomenon, and explain the tendency toward heterotopic calcification common in the evolution of human arterial atheromatosis.

Summary

On people affected by gerontoxon and, as a basis of comparison, on others not revealing such a symptom of xanthomatosis,

the behavior of curves of leading with a Ca salt in a vein before and after treatment with vitamin D_2 in so called "deposit" doses, was studied.

The results obtained, taking into consideration of course the age of the subjects examined, evidenced that:

a) in all the subjects examined the intravenous administration of $CaCl_2$ was followed by a hypercalcemic reaction visible three minutes later and still persisted thirty minutes after the administration of the salt; 90 minutes after the introduction, the calcemic rate resumes the initial values;

b) after treatment with vitamin D_2 on an empty stomach, a calcemic value higher than that existing before the vitamin action can be seen; this value, especially in the subjects with corneal xanthomatosis, often reaches a highly hypercalcemic level;

c) owing to the administration of $CaCl_2$, after treatment with vitamin D_2, the calcemic curve can be compared, in trend and intensity of gradients, to the one preliminarily obtained, but it is entirely shifted to higher values; the higher relief caused by D_2 is expressed by an increasing percentage which is constant in both the control subjects and in those with gerontoxon;

d) in people affected by corneal xanthomatosis, basic calcemic values are common, deriving from a calcium or vitamin administration, and are higher that the ones verified in the subjects not affected by gerontoxon, taking into consideration, of course, the age of the subjects compared.

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EXPERIMENTAL PRODUCTION OF GOITER

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Numerous attempts have been made by various investigators to produce goiter experimentally in animals. In a review of the literature it is striking that there are so many diverse experimental solutions of a problem about which so little is known. In many instances the observer was too anxious to note hyperplasia of the thyroid gland, and possibly because of enthusiasm alone he found it. Moreover, the question of control for such experiments repeatedly arises. Were they adequately controlled? It does not seem sufficient, especially in endemic goiter regions, to have the thyroid gland of a dog examined grossly and microscopically for the first time at the end of the period that a trial stimulus has been applied and to pronounce it hyperplastic by comparing it with a gland from an entirely different dog. Because of the great number of the experimental methods that have been reported as successful in the production of hyperplastic changes in the thyroid gland, it is almost impossible to eliminate all except one possible factor in one type of experiment. However, in any one type of experiment the results must be most carefully compared with the normal and, if possible, with a basic normal in the same animal.

The present report is based on experiments performed in an attempt to produce goiter in dogs and in guinea-pigs. In the experiments on dogs an attempt was made to insure a most rigid control by the following procedure:[1] At a preliminary operation the thyroid gland of each dog was measured, and a small piece, 2 by 2 mm., was removed from one of the lobes, usually the right. Subsequently, observations at operation or at necropsy were made, and a similar specimen was removed which was compared with the section obtained at the preliminary operation. In this manner each dog was self-controlled, although there were other controls from which sections were taken but in which no unusual stimulus was applied.

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1. Mahorner, H. R.: Attempts to Produce Hyperplasia in Thyroid by Destruction of Part of Adrenals and by Infection, Proc. Soc. Exper. Biol. & Med. **29**:1076, 1932; Attempts to Produce Hyperplasia of Thyroid Gland by Infection and by Injury to the Adrenal Gland, ibid. **30**:770, 1933.

It is not desirable or feasible to review in detail all the literature pertaining to attempts to produce artificially hyperactivity or hyperplasia of the thyroid gland, but a few articles are cited to show a basis for some of my experiments.

Halsted[2] (1888) concluded from his microscopic studies of the thyroid glands of dogs suffering from low grade peritonitis induced by the intraperitoneal injection of a culture of staphylococci that infection causes hyperplastic changes in the thyroid gland. Womack and Cole[3] reported microscopic changes considered indicative of hyperplasia in the thyroid glands of dogs after implanting infected foreign bodies in the muscles of the necks of the animals. Plummer and Mayo[4] observed clinical hyperthyroidism in certain instances after the onset of an infectious process and expressed the belief that the infection precipitated the hyperthyroidism. McCarrison,[5] working in India, came to the conclusion that goiter is a filth-borne disease. Among numerous other experimental results, he reported[6] enlargement of the thyroid glands of rats kept under insanitary conditions.

According to Hellwig,[7] the theory that a high calcium intake might be responsible for the production of goiter is over one hundred years old. Waller[8] in 1912 suggested that hard water is the responsible factor in the production of goiter. Hellwig,[7] Hibbard[9] and others reported that a high calcium intake caused an increase in the size of the thyroid glands of animals. Bircher[10] in 1914, in experimental work on rats, found enlargement of the thyroid glands of animals which imbibed water of certain municipalities, whereas this observation was not made in controls

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which were given water from other sources. Webster and Chesney[11] reported the occurrence of goiter in rabbits fed on a diet purely of cabbage.

Golyakowsky[12] in 1899 reported that ligation of the adrenal vessels resulted in elevation of the basal metabolic rate. The metabolic rate was said to decrease slowly after an initial rise within ten days followed by a subsequent elevation to plus 50 per cent between the second and the fourth week. A return of the basal metabolic rate to normal or below occurred in the sixth or seventh week. Marine and Baumann[13] in 1921, using rabbits, obtained a rise in the metabolic rate in 57 per cent of instances in which the adrenal glands were injured or removed. They found that dogs died within twenty-four hours after bilateral adrenalectomy, whereas rabbits were able to survive the procedure, probably because of accessory adrenal tissue in the latter. The increased metabolism continued for one, two or three weeks with or without slight remissions. Marine[14] in 1930 expressed the opinion that this experimental condition is similar to exophthalmic goiter.

The following authors, to cite only some of the many who have written reports on the subject, have presented evidence obtained from experiments on animals, either by measuring the metabolism or by histologic and chemical studies of the thyroid gland and its effect on metamorphosis in tadpoles and salamanders, that extracts from the anterior lobe of the pituitary body activate the thyroid gland, diminish the colloid content of the follicle, diminish the iodine content of the gland and elevate the basal metabolic rate: Smith and Smith,[15] Schwartzbach and Uhlenhuth,[16] Silberberg,[17] Uhlenhuth and Schwartzbach,[18] Loeb and

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(Footnotes continued on next page)

The literature on the subject contains numerous reports of changes in the thyroid gland produced by other substances.[36a] Even the foregoing literature contains many conflicting reports. It could easily be shown that the substances which are said to be efficacious in producing changes in the thyroid gland are numerous and that the results reported by different authors are frequently so incongruous that doubt is cast on the reliability of both, and the weight of whichever one is correct is miscarried.

INFECTION AS A STIMULUS FOR THE PRODUCTION OF HYPERPLASIA IN THE THYROID GLAND

Fourteen dogs were used in an attempt to produce hyperplasia of the thyroid gland by means of infection. No attempt was made to produce a deficiency of iodine in the diet in this or in the other experiments. The fourteen dogs were divided into three groups, the first two consisting of four dogs each and the last of six dogs (table 1). At a preliminary operation the thyroid gland of each dog was exposed by a midline incision. The gland was measured, and a piece of tissue approximately 2 by 2 mm. was removed from the anterior border of the right lobe. This was sectioned and stained with hematoxylin and eosin for comparison as a microscopic control with subsequent sections. At the time of the first operation a contaminated foreign body was sewed into the right sternocleidomastoid muscle of the dog. A different contaminating culture was used in each group. In the first group (dogs 20, 21, 22 and 23) the foreign body was a small coil of silver wire contaminated with a pure culture of staphylococci. Each of these wounds remained infected throughout the experiment, and at the end of four, eight, sixteen and twenty-five days, respectively, the dogs were killed. The thyroid glands were measured again, and each was sectioned and stained with hematoxylin and eosin and compared with the section removed at the preliminary operation from the same dog. In no instance was there any definite change in the thyroid gland, either grossly or microscopically.

The experiment on the second group of dogs (53, 54, 55 and 56) was conducted in the same manner as that on the first group, except that the foreign body was a small piece of tape, approximately 0.5 by 0.5 cm., which was contaminated with a mixed culture of excrement obtained during colostomy on a human being. The dogs were killed after eight, seventeen, thirty-two and sixty-four days, respectively. The wound of the first three showed infection at the time the animal was killed. In the last dog the wound was healed. There were no appreciable gross or microscopic changes in any of the thyroid glands when the specimens obtained at necropsy were compared with those obtained at the preliminary operation.

There were six dogs (58, 59, 60, 61, 62 and 63) in the third group. The foreign body was tape contaminated with mixed anaerobic and aerobic cultures

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of excrement obtained at colostomy on a human being. The contaminated tape was sewed into the right sternocleidomastoid muscle at the preliminary operation, at which time the lobes of the thyroid gland were measured and a biopsy specimen was taken from the right lobe. The dogs were killed by electrocution at the end of nine, twenty, twenty-one, thirty and fifty days, respectively, and at the time

TABLE 1.—*Results of Implantation of Infected Foreign Bodies*

Dog No.	Initial Weight, Kg.	Method	Duration of Experiment, Days	Clinical Appearance	Comparison of First and Second Sections of Thyroid Gland
20	8.7	Implantation of silver wire contaminated with staphylococci into muscle of the neck	4	Infected wound	Hyperplasia (?)
21	9.1	Implantation of silver wire contaminated with staphylococci into muscle of the neck	8	Infected wound	No change
22	7.5	Implantation of silver wire contaminated with staphylococci into muscle of the neck	10	Infected wound	No change
23	7.7	Implantation of silver wire contaminated with staphylococci into muscle of the neck	2[illegible]	Infected wound	No change
53	8.18	Implantation of tape contaminated with mixed culture into muscle of the neck	8	Infected wound	No change
54	11.7	Implantation of tape contaminated with mixed culture into muscle of the neck	17	Infected wound	No change
55	...	Implantation of tape contaminated with mixed culture into muscle of the neck	32	Infected wound	No change
56	...	Implantation of tape contaminated with mixed culture into muscle of the neck	41	Normal; wound healed	No change
59	9.8	Implantation of anaerobic and aerobic culture from feces into muscle of the neck	9	Infected wound; culture showed mixed infection, bacilli and cocci	No change
62	9.6	Implantation of anaerobic and aerobic culture from feces into muscle of the neck	20	Infected wound; culture showed mixed infection	No change
63	6.3	Implantation of anaerobic and aerobic culture from feces into muscle of the neck	20	Infected wound; culture showed mixed infection	Retrogressive change
58	9.6	Implantation of anaerobic and aerobic culture from feces into muscle of the neck	21	Infected wound; culture showed mixed infection	No change
60	7.6	Implantation of anaerobic and aerobic culture from feces into muscle of the neck	30	Infected wound; culture showed mixed infection	No change
61	7.0	Implantation of anaerobic and aerobic culture from feces into muscle of the neck	50	Wound healed	No change

each animal was killed the right sternocleidomastoid muscle was explored aseptically and the region of the implanted foreign body located. An abscess was found in all dogs except the last. These abscesses were cultured, and in each instance a mixed culture of organisms was found. At necropsy the size of the thyroid gland was measured. Sections were made of each lobe, and these were stained with hematoxylin and eosin and compared with the section removed at the preliminary operation from the same dog. In no instance was there gross or microscopic evidence of change in the thyroid gland.

The results in these experiments support the conclusion that a focus of infection in the dog does not produce changes in the thyroid gland suggestive of hyperplasia, at least within a period of thirty-two days. Whereas these results do not prove definitely that infection is not a factor in the production of goiter, they do not corroborate the observations (that infection does produce hyperplasia of the thyroid gland) of other investigators whose similar experiments were less well controlled.

I have examined the thyroid glands of a number of other dogs (not included in this series) that died of infections and have never found microscopic evidence that the infection induced hyperplasia. It is true that by careful study one can pick out small individual areas in which the acini are small and have less colloid than other areas of the gland. The colloid may even appear slightly bluish, but this is not unmistakable evidence of hyperplasia, because similar areas can be found in sections from the thyroid glands of other normal control animals. Desquamation of cells into the acini was found in tissue which has undergone postmortem degeneration. Selected microscopic fields are not to be accepted as representative of the appearance throughout an entire section. Unmistakable evidence of diffuse histologic changes is presented in certain experiments reported herein by deliberately avoiding the fallacy of judging the appearance of a whole gland from that of one particular field. By care exerted to show widest discrepancies in microscopic fields and not the average appearance of the section, an author can demonstrate variations that suggest that microscopic changes have taken place when this is not actually true (figs. 1 and 2). Enthusiasm and eager credulousness may be responsible for an incorrect interpretation of small selected areas as evidence of hyperactivity of the thyroid gland. What is really desirable in such experiments is changes so striking that the evidence is incontestable.

DISTURBANCE OR DEFICIENCY IN THE ADRENAL GLANDS AS A STIMULUS FOR THE PRODUCTION OF HYPERPLASIA IN THE THYROID GLAND

Because of the repeatedly expressed opinions that the cause of hyperthyroidism is dysfunction of the adrenal gland and because of the experiments of Marine and Baumann [13] showing that an increase in the basal metabolic rate resulted from removal of part of the adrenal tissue or from freezing the adrenal glands, I attempted to repeat the experiments, using a different method to note the effect of adrenal "deficiency" on the thyroid gland. A section from the thyroid gland of an animal removed after injury to the adrenal gland, the interval after injury varying in different animals, was compared with a section removed at a preliminary operation. Marine and Baumann [13] stated that after decreasing the amount of adrenal tissue by removal in rabbits or after

freezing the adrenal glands the basal metabolic rate was elevated. Marine[44] concluded from this that the elevation of the basal metabolic rate indicates true hyperthyroidism. Granting that the etiologic factor in hyperthyroidism originates outside the thyroid gland, it is safe to conclude that true hyperthyroidism will be attended by some evidence of hyperplasia in the thyroid gland albeit secondary to a stimulus outside the gland. Conversely, it is fair to assume that absence of hyperplasia under similar conditions in which there is elevation of the basal metabolic rate indicates that the elevated basal metabolic rate is not due to true hyperthyroidism.

Dogs were used for this experiment. At a preliminary operation the thyroid gland was exposed through a midline incision and measured. A piece of tissue,

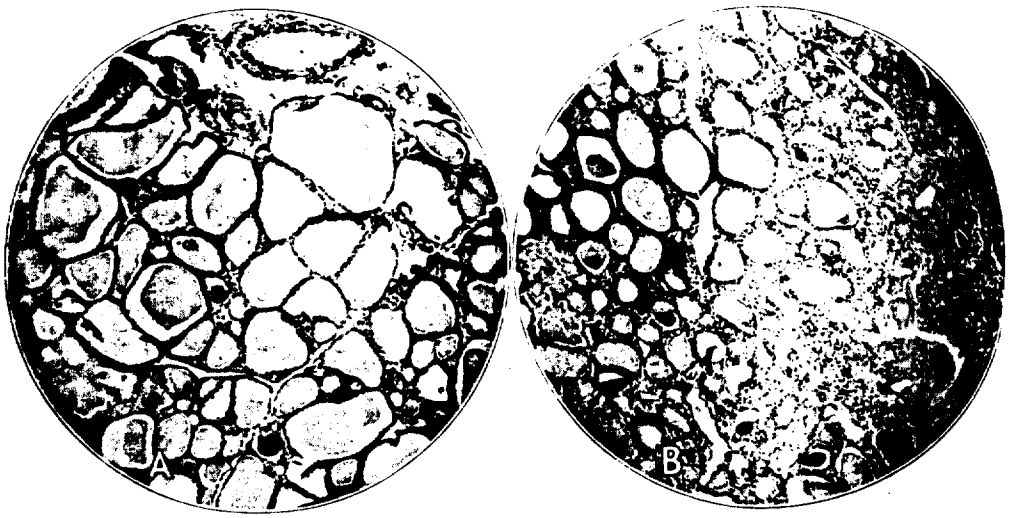

Fig. 1.—*A* and *B* are two different sections taken from the thyroid gland of a dog at the time after the animal had received 1 cc. of the gonadotropic principle of the urine of pregnant women daily for twelve days. There is more colloid and the acini are larger in *A* than in *B*. Tiny vesicles may be seen in the colloid in *B*, and the epithelial cells are more cuboidal, but this section is a selected area. There was no essential difference in the preliminary sections and the twelve day sections. This shows that erroneous results may be demonstrated by selecting foci not representative of the average in a section for photomicrography. The magnification of *A* and *B* is the same.

2 by 2 mm., was removed from the anterior border of the right lobe. The wound was closed, and at a subsequent operation, after the wound had healed, the adrenal glands were exposed either through two lumbar incisions or through a midline abdominal incision. (The former was found to be more satisfactory.) The adrenal glands were crushed with a hemostat, and from one third to one half of one of the glands was left intact. The extent of this procedure is indicated in

table 2. The wound was closed, and the animals either died or were killed in from five to one hundred and twenty-seven days. In the first ten days after the procedure the animals appeared sick, were apathetic and moved slowly, and several of them died. In some of the animals the systolic blood pressure fell from 180 to 80 mm. of mercury. Several of the animals died within the first few days after

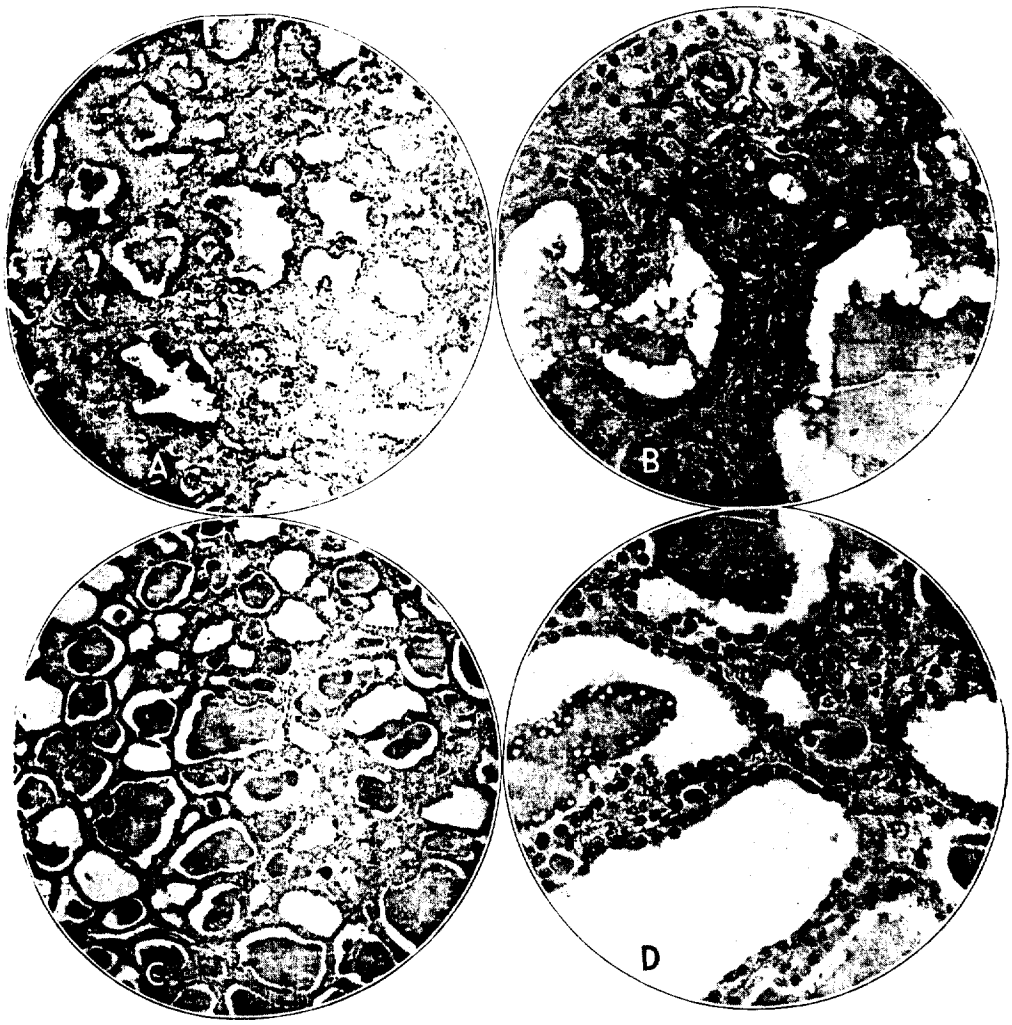

Fig. 2 (dog 61).—*A* and *B* are low and high power photomicrographs of sections of the thyroid gland before implantation of an infected foreign body into the muscles of the neck of a dog, and *C* and *D* are two corresponding magnifications of sections taken fifty days after implantation. Seeing *C* and *D* alone, one might suspect that the thyroid gland in this dog is more active than normal, but with the control there is no doubt that no histologic change has occurred. Note the tiny vesicles (supposed to be absorption vesicles) in the colloid in *C* and *D*.

the operation and are not considered in the report. Three died in from nine to eleven days. There was no suggestion of nervousness or excitability in the dogs. They looked apathetic and sluggish. Those that lived were killed individually in from five to one hundred and twenty-seven days by electrocution. A complete autopsy was done, and the thyroid and adrenal glands were measured and sectioned for microscopic examination. The adrenal glands of animals killed within two weeks showed the result of contusion; those of the animals killed later in some

TABLE 2.—*Results of Crushing the Adrenal Glands*

Dog No.	Initial Weight, Kg.	Method	Interval After Injury to Adrenal Gland, Days	Clinical Appearance	Comparison of First and Second Sections of Thyroid Gland
24	13.7	Entire left and ⅔ of right adrenal gland crushed	5	Looked sick; moved slowly; moribund; killed	No change in thyroid
14	7.1	Entire left adrenal gland crushed	6	Thin; emaciated; looked sick; killed	No change in thyroid; left adrenal ⅔ macerated
25	13.8	Entire left and ⅔ of right adrenal gland crushed	9	Looked normal; killed	No change in thyroid; both adrenals partially destroyed
26	6.6	Entire left and ⅔ of right adrenal gland crushed	10	Sick; died	No change in thyroid; ½ right adrenal remained; no left adrenal
33	...	½ of right and entire left adrenal gland crushed	10	Sick; died	No change in thyroid
29	7.5	Entire right and ⅔ of left adrenal gland crushed	11	Emaciated; died	No change in thyroid; left adrenal ½ normal; right, ⅓ normal size
13	3.9	Entire right adrenal gland crushed	11	Healthy	No change in thyroid; right adrenal missing
8	8.2	Entire left and ⅓ of right adrenal gland crushed	14	Sick; moves slowly	No change in thyroid
8	8.2	.....	103	Healthy	No change in thyroid; adrenals normal size
7	7.5	Entire right and ⅓ of left adrenal gland crushed	14	Sick; moves slowly	No change in thyroid
7	7.5	.....	117	Healthy	No change in thyroid
5	7.1	Entire left and ¼ of right adrenal gland crushed	24	Weak; moves slowly	No change in thyroid; died; no cause found; regeneration of adrenals
6	6.4	Entire left adrenal gland crushed	24	Weak	No change in thyroid
6	6.4	.....	127	Normal	No change in thyroid

instances were smaller than normal, but in all the animals the wounds had healed. In three animals (6, 7 and 8) exploration was done to obtain a biopsy specimen from the thyroid gland between the time of operation on the adrenal gland and the date the animal was killed. Subsequent sections were obtained from the thyroid glands of twelve animals in the following periods after the operation on the adrenal glands: five, six, nine, ten, ten, eleven, eleven, fourteen, fourteen, twenty-four, twenty-four, one hundred and three, one hundred and seventeen and one hundred and twenty-seven days, respectively.

Comparison of the gross measurements and histologic appearance of sections of thyroid gland obtained at the preliminary operation with subsequent sections from the same gland showed no evidence of hyperplasia or retrogression.

FREEZING THE ADRENAL GLANDS AS A STIMULUS TO THE PRODUCTION OF HYPERPLASIA IN THE THYROID GLAND

Seven dogs were used in an attempt to show whether freezing the adrenal glands would produce hyperplasia of the thyroid gland (table 3). At a preliminary operation the thyroid gland of each dog was measured, and a small piece of tissue, 2 by 2 mm., was removed from the right lobe. This was stained as a normal section with which to compare later sections. After the preliminary wound had healed, each adrenal gland was exposed by a lumbar incision and frozen solidly with a spray of ethyl chloride for several minutes. The wound was then closed. One animal died in four days as a result of this procedure. They were all sick, sluggish and apathetic and lost weight. The remaining animals were killed after thirteen, fourteen, nineteen, twenty-five, seventy-one and eighty-five days, respectively. The measurements and microscopic appearance of the thyroid glands were compared with those obtained at the preliminary operation. At necropsy the adrenal glands showed hemorrhage grossly and microscopically. Petechiae were still present in the adrenal glands of the animal killed twenty-five days after freezing. Moreover, in some instances the glands seemed to remain smaller than normal.

TABLE 3.—*Results of Freezing Adrenal Glands*

Dog No.	Initial Weight, Kg.	Method	Interval After Freezing Adrenal, Days	Clinical Appearance	Comparison of First and Second Sections of Thyroid Gland
47	4.9	Both adrenal glands frozen	4	Sick; died	No change in thyroid; adrenals hemorrhagic
32	9.4	Both adrenal glands frozen	13	Sick, sluggish and emaciated; killed	No change in thyroid; left adrenal infected
45	7.4	Both adrenal glands frozen	14	Normal	No change in thyroid; adrenals ⅓ normal size
31	7.0	Both adrenal glands frozen	19	Normal	No change in thyroid; petechiae in right adrenal; hyaline degeneration in left
46	6.8	Both adrenal glands frozen	25	Emaciated; sluggish	No change in thyroid; left adrenal showed petechiae
44	5.5	Both adrenal glands frozen	71	Thin; apathetic	No change in thyroid; left adrenal normal; right adrenal ⅓ normal size
43	5.6	Both adrenal glands frozen	85	Normal	No change in thyroid; adrenals slightly smaller than normal

In no instance was there gross or microscopic evidence of hyperplasia or retrogression of the thyroid gland.

HIGH CALCIUM CHLORIDE INTAKE AS A STIMULUS FOR THE PRODUCTION OF HYPERPLASIA IN THE THYROID GLAND

Six dogs were used in experiments on the effect of a high calcium chloride intake on the thyroid gland (table 4). At a preliminary operation the thyroid gland of each dog was measured, and a small piece of the right lobe, 2 by 2 mm., was removed for microscopic study. Four animals were given 2 per cent calcium chloride in distilled water. They refused to drink this concentrated solution, and

after six days they were given 500 cc. of the solution twice daily, by stomach tube. Because all of the dogs suffered from continued vomiting after four days of this regimen and after one of the dogs (65) died, 0.05 per cent calcium chloride in distilled water was used as drinking water for the remainder of the experiment. Two other dogs (68 and 69) were started on this solution as their only drinking water immediately after the preliminary operation. Four of the six dogs died after six, seven, twelve and ninety-six days, respectively. The remaining two were killed

TABLE 4.—*Results with a High Calcium Intake*

Dog No.	Initial Weight, Gm.	Method	Duration of Experiment, Days	Clinical Appearance	Comparison of First and Second Sections of Thyroid Gland
68	3.8	Calcium chloride 0.05%	6	Died	Hyperplasia (?)
65	7.8	Calcium chloride 2%, 500 cc. daily	7	Sick; died	Smaller acini; less colloid
66	10.2	Calcium chloride 2%, 500 cc. daily	12	Sick; died	No change
69	6.6	Calcium chloride 0.05%	80	Healthy	Definite hyperplasia
64	10.3	Calcium chloride 2%, 500 cc. daily for 8 days and 0.05% for 88 days	96	Sick; died	Hyperplasia (?); postmortem degeneration
67	10.1	Calcium chloride 2% for 8 days and 0.05% for 105 days	113	Healthy	Definite hyperplasia

TABLE 5.—*Blood Calcium Determinations on Dogs Drinking Calcium Water*

Dog No.	Preliminary Estimation of Blood Calcium, Mg. High	Preliminary Estimation of Blood Calcium, Mg. Low	Date of Starting Experiment	Subsequent Calcium Determinations, Mg.
68	10.12	10.06	July 17	July 23, death
65	10.75	10.62	June 14	June 15, 10.52; June 20, 11.01; June 22, death
66	11.19	10.92	June 14	June 15, 11.48; June 20, 11.21; June 26, death
69	11.42	11.42	July 17	July 27, 10.98; August 2, 11.14; August 9, 12.78; August 16, 11.48; August 23, 12.49; August 30, 14.20; September 6, 11.32; September 13, 12.03; September 20, 10.92; September 27, 11.46; October 4, 12.33; October 5, killed
64	10.90	10.51	June 14	June 15, 11.58; June 21, 11.96; June 27, 11.14; July 3, 11.14; July 10, 11.14; July 27, 10.51; August 2, 11.14; August 9, 12.78; August 16, 9.64; August 23, 9.49; August 30, 10.12; September 6, 8.82; September 13, 8.69; September 17, necropsy
67	11.33	10.90	June 14	June 15, 11.2; June 20, 14.61; June 27, 11.28; July 3, 11.28; July 10, 11.14; July 27, 10.51; August 2, 11.14; August 9, 12.78; August 16, 11.64; August 23, 12.25; August 30, 11.70; September 6, 10.17; September 13, 11.23; September 20, 10.92; September 27, 10.26; October 4, 10.64; October 5, killed

after drinking the calcium water for eight and one hundred and thirteen days, respectively. At necropsy the thyroid gland of each animal was measured. Sections stained with hematoxylin and eosin were compared with the original sections removed at the preliminary operation.

Prior to initiating the experiment, determinations were made on the blood calcium in each dog on at least two different days. During the course of the experiment, while the dogs were taking the calcium solution, determinations were made on the blood calcium once a week. The calcium content of the blood was

elevated from 0.5 to 2 mg. by the inhibition of calcium chloride drinking water, but as may be seen from table 5 the elevation was not constant. Determinations were made after the food was removed from the cages over night and the dogs were permitted to drink calcium chloride water.

Gross measurements of each gland showed no appreciable difference between the measurements made at necropsy and those made at the preliminary operation, but microscopically the changes were striking (fig. 3), not only in isolated or selected areas but throughout the gland. There was evidence of hyperactivity as indicated by less colloid in the vesicles, even to complete disappearance; large columnar epithelium, and marked vesicular changes in the colloid, not only at the periphery but throughout the entire acini, so that the colloid looked as if it had degenerated into bubbles. No changes were found in the thyroid glands of dogs dying after seven and twelve days, respectively. There were questionable changes in the thyroid gland in the dog that died after six days, and definite changes in the glands of dogs that died after eighty, ninety-six and one hundred and thirteen days.

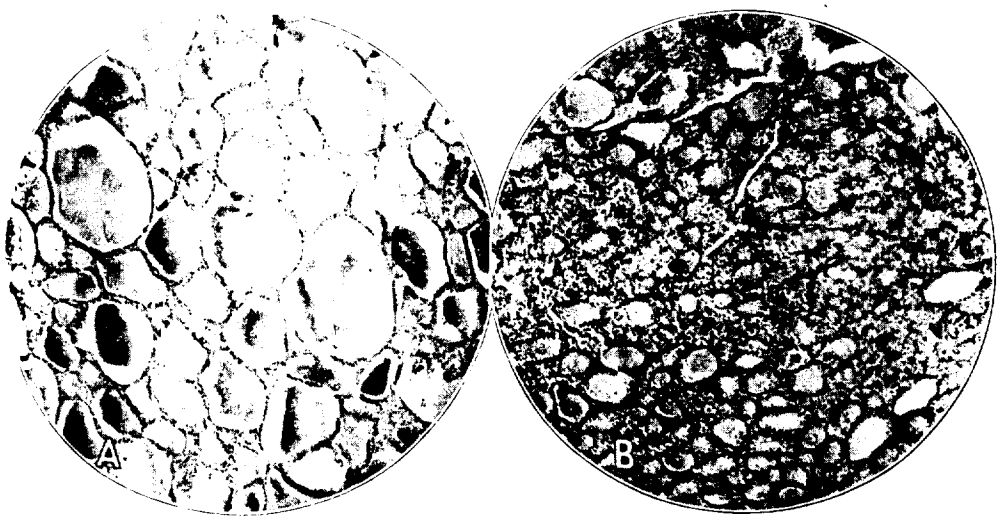

Fig. 3 (dog 67).—*A* is a preliminary section from the thyroid gland of a dog. *B* is a section from the thyroid of the same dog taken one hundred and thirteen days later, after drinking 0.05 per cent calcium chloride water during that interval. The magnification is the same as that of *A*. The entire microscopic appearance has changed. From flat epithelium lining the acini in *A*, the epithelium has changed to a more cuboidal form in *B* but seems in some instances to be several layers thick. The acini are smaller in *B*, and numerous tiny vesicles are found throughout the colloid. These are representative of the entire section and are not selective areas.

These experiments indicate that calcium eliminates the colloid from the thyroid gland and increases the size of the epithelium. Thus, from these experimental results, at least, it does not seem that calcium chloride is the cause of colloid goiter but that it does change the histologic appearance of the thyroid gland.

GONADOTROPIC PRINCIPLE FROM THE URINE OF PREGNANT WOMEN AS A STIMULUS TO PRODUCTION OF HYPERPLASIA OF THE THYROID GLAND

Five dogs were used to determine the effect of the gonadotropic principle from the urine of pregnant women on the thyroid gland. At a preliminary operation the thyroid gland of each dog was exposed and measured, and a piece of tissue, 2 by 2 mm., was removed from the right lobe and sectioned to serve as a normal section for comparison. Thereafter, 1 cc. of the gonadotropic principle[37] was injected intraperitoneally daily for the remainder of the experiment. The remaining dogs were killed in six, eleven, twelve, twenty-five and fifty-one days, respectively. At necropsy the thyroid gland of each animal was removed, measured and sectioned. The material was stained with hematoxylin and eosin and compared with the section removed at the preliminary operation from the same dog.

In none of the dogs was there any gross or microscopic evidence of hyperplasia or changes in the thyroid gland (table 6). These experiments indicate that the gonadotropic principle has no thyrotropic properties.

TABLE 6.—*Results with Gonadotropic Principle from Urine of Pregnant Women*

Dog No.	Initial Weight, Kg.	Method	Duration of Experiment, Days	Clinical Appearance	Comparison of First and Second Sections of Thyroid Gland
70	6.2	1 cc. of gonadotropic principle daily	6	Healthy	No change
72	5.3	1 cc. of gonadotropic principle daily	11	Sick; distemper	No change
73	4.2	1 cc. of gonadotropic principle daily	12	Healthy	No change
74	3.4	1 cc. of gonadotropic principle daily	25	Healthy	No change
71	6.6	1 cc. of gonadotropic principle daily	51	Healthy	No change

EXPERIMENTS ON GUINEA-PIGS TO TEST THE EFFICIENCY OF ANTERIOR PITUITARY EXTRACT AS A STIMULUS FOR THE PRODUCTION OF CHANGES IN THE THYROID GLAND

Eleven guinea-pigs were used to test the efficiency of anterior pituitary extract in the production of changes in the thyroid gland. The disadvantage in using guinea-pigs is that they are too small for preliminary operation and section of the thyroid gland for comparison with later sections. Therefore, the experiments on these animals are not controlled for individual animals. Guinea-pigs are, however, less expensive. Young guinea-pigs about 3 months of age, weighing between 250 and 400 Gm., were chosen. In each instance at the end of the experiment the guinea-pig was killed by injecting 2 cc. of ether into the heart. The thyroid gland was then exposed and measured. In some instances there was a definite isthmus between the two small thin lobes lying close to the trachea. In other instances the isthmus was rudimentary or absent. The parathyroid glands were embedded in the thyroid gland. The thyroid glands were removed and carefully measured, and each gland was sectioned and stained with hematoxylin and eosin.

37. The gonadotropic principle used was antuitrin-S. It was supplied by Parke, Davis and Company.

In each instance the pituitary gland, the pars anterior and posterior, was removed and sectioned for microscopic study in order to determine whether a pituitary extract affecting the histologic structure of the thyroid gland might also affect the pituitary gland.

Of the eleven guinea-pigs, three were used as controls. They had been kept in the cages for ten, fourteen and sixty-two days, respectively, while their companions were receiving injections of anterior pituitary extract daily. At the time they were killed they weighed 310, 400 and 650 Gm., respectively. These weights gave some basis for comparison of the size of the thyroid glands of those animals which weighed approximately the same but which had been subjected to intraperitoneal injections of anterior pituitary extract.

Eight of the guinea-pigs were given daily intraperitoneal injections of anterior pituitary extract (1 cc.), an extract of the pars anterior of the pituitary gland. No iodine was used in the experiment, and the guinea-pigs were killed after two, four, six, eight, ten, nineteen, thirty-two and sixty-four days, respectively, by injecting 2 cc. of ether directly into the heart. The thyroid and pituitary glands were recovered and compared microscopically with themselves and with the controls. The extract apparently did not produce any abnormal increase in weight. The sixty-six day control, weighing 650 Gm. (initially 400 Gm.), was compared with guinea-pig 6, weighing 600 Gm. (initially 400 Gm.), after sixty-four daily injections of anterior pituitary extract. Similar comparisons to show this point may be seen in the protocol.

Results of the experiment show that the extract apparently produces some gross change in the thyroid gland. Although the method of measurement was crude, the thyroid gland of the guinea-pig (weight, 600 Gm.) that had received the extract for sixty-four days was slightly greater than that of the control guinea-pig (weight, 650 Gm.) which had been observed for sixty-six days. Similar comparison may be made with other animals in the series with a control of analogous weight. There was no apparent change in the conformity of the gland, and the difference in size detectable by this method is not accepted as unequivocal proof that there is a gross change under these circumstances.

In every instance the pituitary gland showed no recognizable changes microscopically. However, in the thyroid glands from the animals which had received a large number of injections there was unquestionable evidence of hyperplasia (fig. 4). This evidence was seen not only in selected areas but throughout the sections. The microscopic changes indicative of hyperplasia were:

1. The vesicles were smaller and contained less colloid. This reduction in colloid was approximately one third of that in the acini of the controls.

2. In the colloid of the normal guinea-pig, after the thyroid gland was sectioned and stained with hematoxylin and eosin, minute, apparently clear, rounded vesicles were found. These were near the periphery of the colloid. They may be interpreted as evidence of colloid absorption, but this has not been established unequivocally as an accurate interpretation. However, in the guinea-pigs which had received daily doses of anterior pituitary extract over a prolonged period and in which the colloid in the vesicles was diminished, these vesicles were much more numerous and larger and in some instances (guinea-pig 16) were so plentiful that practically all the remaining colloid seemed to be nothing but bubbles.

38. The anterior pituitary extract used was antuitrin. It was supplied by Parke, Davis and Company.

5. I do not believe that the sign of basophilic staining of the colloid is reliable as a guide to absorption of colloid or hyperactivity of the thyroid gland. It is true that a bluish tinge to the colloid does occur. It was found in the animals that had received anterior pituitary extract over a long period of time, but I also

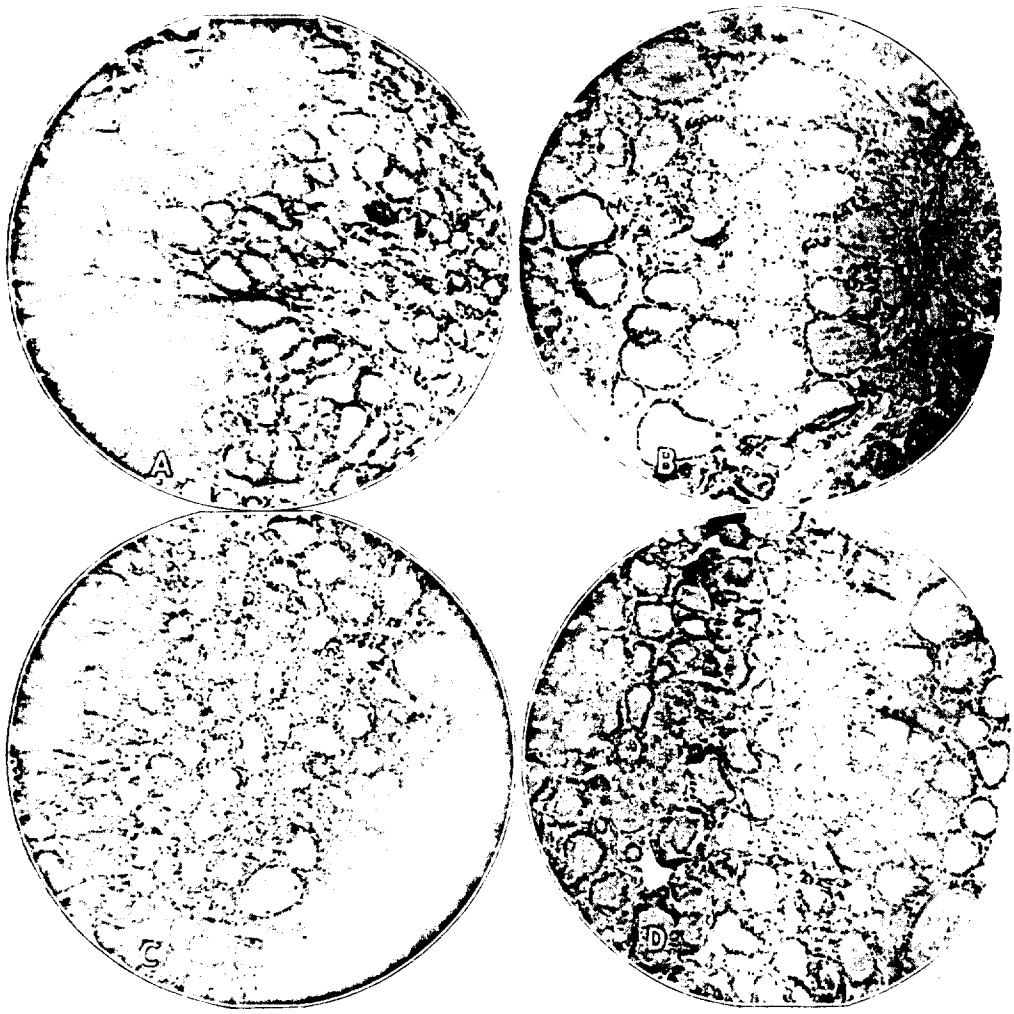

Fig. 4.—Photomicrographs (all identical magnification) of sections of the thyroid gland of four different guinea-pigs. *A* and *B* are sections from normal controls. *C* is a section from the thyroid gland of a guinea-pig which had received daily intraperitoneal injections of anterior pituitary extract for nineteen days. *D* is a section from a guinea-pig which had received injections of anterior pituitary extract daily for ten days. *C* and *D* show smaller acini, more cuboidal cells, less colloid and more vesicles in the colloid than the sections from the controls. The changes in the ten day section are more marked than those in the nineteen day section, but the length of administration does not parallel the degree of change.

found it in some areas in the normal controls. Some normal glands exhibited basophilic colloid more than others, and whereas it seemed to be more constant in animals which had received the extract, its presence is so unreliable in its associations that using it as a sign of activity carries only the weight of presumption.

4. The cells lining the acini of the thyroid gland were definitely more columnar in the animals which had received the extract. The flattest cell in the animals receiving the extract was more columnar than the tallest cells in the acini of the controls. In some acini of the thyroid glands which had been stimulated by

TABLE 7.—*Results with Anterior Pituitary Extract*

Guinea Pig No.	Stimulus	Duration of Experiment, Days	Weights, Gm. Initial	Weights, Gm. Final	Clinical Appearance of Dog	Thyroid Gland: Measurements	Thyroid Gland: Microscopic Appearance	Microscopic Appearance of Pituitary Body
10	Control	10	500	510	Healthy	R.: 1.0x0.4 cm. L.: 0.9x0.3 cm.	Normal	Normal
1	Control	14	400	400	Healthy	R.: 0.8x0.5 cm. L.: 1.0x0.5 cm.	Normal	Normal
2	Control	36	400	650	Healthy	R.: 1.3x0.6 cm. L.: 1.5x0.5 cm.	Normal	Normal
12	Anterior pituitary extract, 1 cc. daily intraperitoneally	2	220	300	Healthy	R.: 0.7x0.3 cm. L.: 0.6x0.3 cm.	Slight (?) hyperplasia	Normal
13	Anterior pituitary extract, 1 cc. daily intraperitoneally	4	340	380	Healthy	R.: 1.6x0.5 cm. L.: 1.0x0.3 cm.	Questionable hyperplasia	Normal
14	Anterior pituitary extract, 1 cc. daily intraperitoneally	6	260	300	Healthy	R.: 1.0x0.3 cm. L.: 1.0x0.4 cm.	Questionable hyperplasia	Normal
15	Anterior pituitary extract, 1 cc. daily intraperitoneally	8	260	320	Healthy	R.: 1.0x0.3 cm. L.: 1.0x0.4 cm.	Slight hyperactivity	Normal
16	Anterior pituitary extract, 1 cc. daily intraperitoneally	10	260	320	Healthy	R.: 1.2x0.4 cm. L.: 1.5x0.5 cm.	Definite hyperactivity	Normal
4	Anterior pituitary extract, 1 cc. daily intraperitoneally	19	420	535	Healthy	R.: 0.9x0.3 cm. L.: 1.0x0.5 cm.	Hyperactivity	Normal
5	Anterior pituitary extract, 1 cc. daily intraperitoneally	32	330	300	Healthy	R.: 1.0x0.5 cm. L.: 0.9x0.5 cm.	Hyperactivity	Normal
6	Anterior pituitary extract, 1 cc. daily intraperitoneally	64	400	600	Healthy	R.: 1.5x0.5 cm. L.: 1.6x0.5 cm.	Normal	Normal

the anterior pituitary extract there were high columnar epithelial cells similar to the epithelial cells lining the follicles in the thyroid of a patient with exophthalmic goiter. In no instance were there any infoldings or papillary projections.

The most marked evidences of hyperactivity in these sections occurred in the animals receiving the injection for from ten to thirty-two days. It had disappeared in the sixty-four day specimen. Several authors [39] have reported evidence indicating that continued injection of extract of the anterior lobe of the pituitary gland produces changes in the thyroid gland which retrogress toward the normal after different periods of time.

39. Loeb and Friedman,[25] Siebert and Thurston,[29] Collip and Anderson,[37]

COMMENT

The conclusions to be drawn from these experiments are merely supportive or nonsupportive of certain still controvertible ideas that extraneous substances stimulate or inactivate the thyroid gland in such a manner as to produce goiter. The experiments support strongly the view that the excess administration of calcium chloride results in changes in the histologic appearance of the thyroid gland. These changes indicate hyperactivity. These experiments do not corroborate the findings of other investigators that infection in the organism and injury to the adrenal gland affects the histologic picture of the thyroid gland.

At the present time these are all merely accumulations of facts which may encourage one to believe that an explanation of the etiology of goiter is nearer. It is still debatable whether the true cause is positive and extraneous, such as calcium or an unknown goitrogenic substance, or positive and autogenous, such as a hormone of the pituitary gland, or negative and autogenous, such as the absence of a hormone, or negative and exogenous, such as a deficiency of some necessary exogenous regulator, for example, iodine. Many conflicting data exist in the literature on the etiology of goiter, but in the main the accumulating weight of evidence is that a hormone of the anterior lobe of the pituitary gland can stimulate the thyroid gland to hyperactivity. It is a much further step to prove that this hormone is the factor outside the thyroid gland which whips it up to hyperactivity in acute hyperthyroidism. It is my experience, as well as that of others, that the thyroid gland removed at operation for hyperthyroidism in patients with acromegaly is a meaty type of thyroid, very parenchymatous and with relatively little colloid in the follicles. In comparing the clinical with the experimental evidence, the interesting question arises whether hyperthyroidism in cases of acromegaly, which is akin to, but clinically not exactly the same as, exophthalmic goiter, results from long-continued excess of this particular thyrotropic hormone of the anterior lobe of the pituitary gland, or whether exophthalmic goiter actually is acute acromegaly or at least a fraction of it. Experimental evidence has far from answered this question.

At the present time there are other possible factors with as much accumulated experimental support, if not in quantity at least in quality. Calcium, an entirely extraneous factor, undoubtedly produces changes in the thyroid gland. Will excess calcium be responsible for one type of goiter and the anterior pituitary extract for another?

Iodine undoubtedly is a factor, but it appears to be an objective and not a subjective one. It is lessened in the goitrous gland, but it seems that something else either fixes it in the blood stream to prevent it from being utilized by the gland or drives it out of the gland by the

hyperactivity of the parenchyma. It is true that even when the intake of iodine is undoubtedly adequate under ordinary conditions, the thyroid gland may undergo changes histologically and functionally. Iodine in excess of the normal intake may mitigate the action of the additional factor, but the additional factor is the important one, and that factor as yet has not been found with certainty.

SUMMARY

Experiments on dogs and guinea-pigs gave results which might be safely interpreted to support the following conclusions: Infection in the organism has no specific effect on the histologic picture of the stage of activity of the thyroid gland or on the size of the gland. Injury to or freezing of the adrenal gland in a series of animals resulted in no gross or microscopic changes in the thyroid gland, thus intercepting the inference that elevated basal metabolic rates after similar procedures in the lower animals are the result of hyperthyroidism.

Anterior pituitary-like gonadotropic substances obtained from the urine of pregnant women had no effect on the histologic structure of the thyroid gland when injected daily over varying periods of time. But an extract of the anterior lobe of the pituitary gland produced a histologic picture in the thyroid gland which might be interpreted as increased activity of the gland, viz., disappearance of colloid or diminution of the amount of colloid in the acini; an increase in the size of the epithelial cells, changing their form from low cuboidal to high columnar epithelium, and an increase in the number of vacuoles in the colloid, possibly an indication of its absorbability.

Calcium chloride when added to the drinking water produced definite histologic changes in the thyroid gland of animals which had been on the regimen eighty days or longer. The histologic changes were similar to those described as the result of the administration of anterior pituitary extract; viz., less colloid, larger epithelial cells and more vacuoles in the remaining colloid.

[GANN, Vol. 44: March, 1953]

THE CYTOLOGICAL EFFECTS OF CHEMICALS ON ASCITES SARCOMAS
II. SELECTIVE DAMAGE TO TUMOR CELLS BY $CaCl_2$, $AlCl_3$ AND H_2O_2

With Plate V

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In the preceding paper of this series, the effects of podophyllin, the mitotic poison, on cell division in the ascites tumors of rats and its influence on the growth of this type of tumor were reported (Makino and Tanaka 1953). It was concluded that suppression of tumor growth and considerable prolongation of the tumor-bearing rat's life occurred as a result of complete damage to most of the tumor cells by the judicious injection of podophyllin. However, some of the tumor cells remained unaffected and became the primary source of renewed malignant groth. Why some of the tumor cells should remain alive and undamaged by the drug is entirely unknown. A possible view to this phenomenon is that in a transformation into the resistant form, the surface of these cells may be altered in some manner, which renders them impermeable to the noxious substance in the surrounding medium. The present investigation was undertaken to confirm the occurrence of this phenomenon and, if possible, to examine the effects of some chemicals which act as a dehydrating agents on cells.

The authors acknowledge with pleasure the valuable advice of Dr. T. Sakamura and his cooperation in supplying chemical agents used here. This work was done in the Zoological Institute of Hokkaido University, Sapporo, Japan, and the manuscript was prepared by the senior author during his stay at the Tissue Culture Laboratory of George Washington University Cancer Clinic, Washington, D. C., U. S. A., through the generosity of Dr. Ivor Cornman, to whom the senior author is greatly obliged.

The chemicals used in these experiments were the dehydrating agents, $CaCl_2$, $AlCl_3$ and H_2O_2. The experiments were carried out with the Yoshida sarcoma and MTK-sarcomas, all being ascites tumors of white rats kept in our laboratory.

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intraperitoneal injections of these chemical solutions were made in the animals on the 6th day after transplanatation of the tumor. At this time the most active proliferation of the tumor cells is to be obtained in the peritoneal cavity of the host. For observations, smear preparations were made from a droplet of the tumor ascites obtained by abdominal puncture at appropriate intervals following the application of the chemical (for technique, see Makino and Tanaka 1953). In each series of experiments, injections were made in five or six tumor-bearing animals, with the same number of untreated tumor-bearing animals as control. The following observations and discussion were described on the basis of the summarized results from the experimental animals in comparison with those from control animals.

OBSERVATIONS

1. Effect of $CaCl_2$

After several tests employing different concentrations, it was found that a 0.25 M solution of $CaCl_2$ prepared with Ringer's solution exerted a selected effect on tumor cells.[2] Two cc of this solution were injected with a glass pipette into the peritoneal cavity of a 6th-day-tumor animal bearing the Yoshida sarcoma. The observations were based on smear preparations made at various intervals following the injection of $CaCl_2$.

Fifteen to twenty minutes after injection, the tumor ascites displayed a large number of damaged tumor cells (Figs. 4–5). The drug damaged the mitotic somewhat more than resting cells. This solution first exerted its influence on the cytoplasm. The cells were damaged by a breaking down of the cell bodies, followed by a pycnotic disintegration of the naked nuclei, or of the chromosomes after irregular thickening (Fig. 6). The effect of the chemical seems heightened on cells of relatively large size and those showing mitotic abnormalities.

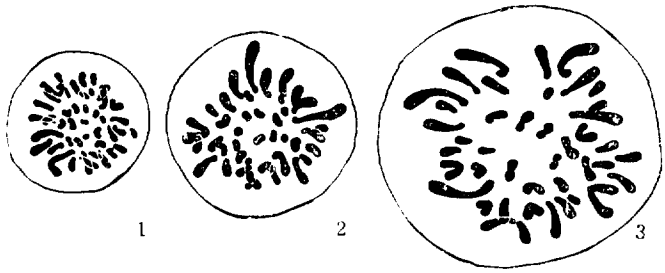
The number of affected cells increased with time following injection. The extent of cellular disintegration was manifested by the amount of cellular debris in the ascites. In the ascites 30 minutes after injection, more than one third the number of tumer cells under observation were disintegrating cells. Forty to fifty minutes after injection, about half the number of the observed cells were found damaged. In the tumor ascites sampled 60 to 70 minutes after injection, most tumor cells were in damaged condition. Rough counts in this stage indicated that over 70 per cent of the tumor cells were undergoing degeneration. By this time there appeared in the tumor ascites a number of eosinophilic leucocytes; they were entirely unaffected by the chemical. It was also found that, interspersed among damaged

[2] Preliminary accounts on the effects of this chemical have been given in the paper of Makino, 1952 b.

tumor cells, there were a certain number of tumor cells of small size, which persisted undamaged by the chemical. These were characterized by a small amount of cytoplasm and a single, well-defined uncleus. In the meantime, these small-sized tumor cells began to divide (Fig. 7). The dividing cells increased with time. Three to four hours after injection, the multiplication of these residual cells became a conspicuous phenomenon showing many dividing figures in the tumor ascites. As a result of multiplication of these cells, the tumor renewed growth.

As thus far noted, injection of a sublethal concentration of $CaCl_2$ solution into the peritoneal cavity of tumor-bearing rats resulted in damage to the greater number of tumor cells. Yet, part of the tumor cells remained unaffected by the chemical. The existence of these resistant tumor cells[1] results in a renewed development of the tumor in the treated animals.

Attention should be called on to the nature of the small-sized tumor cells which remain undamaged by the chemical. They divide in an almost regular mitotic manner. The behavior of their chromosomes during division is apparently also regular. Detailed observations were made on the chromosomes of these tumor cells by morphological analysis of the individual chromosomes which revealed that the chromosome complex of these cells is essentially similar to that previously found in the tumor stem (or strain) cells by the present authors (Makino 1952 a, b; Makino and Kano 1953; Makino and Tanaka 1953). The resistant tumor cells



Figs. 1-3. Metaphase complexes of resistant tumor cells, showing subdiploid chromosomes (= 40). From $CaCl_2$ experiments. Fig. 1, dividing cell, 60 minutes after injection of 0.25 M $CaCl_2$. Fig. 2, the same, 3 hours after injection of 0.25 M $CaCl_2$. Fig. 3, dividing cell observed 2 days after injection of the tumor ascites from the animal killed by the injection of 0.5 M $CaCl_2$.

of the Yoshida sarcoma showed at metaphase a characteristic complex of approximately 40 chromosomes, consisting of 22 to 24 rod-shaped chromosomes and 16 to 18 J- and V-shaped ones (Figs. 1-3). On the basis of this finding, there is no doubt that the small-sized, resistant tumor cells, which have persisted without damage by the chemical, are none other than the tumor stem (or strain)

1) Borst (1936) described the existence of the resistant cells in tumor.

cells. The numerical data derived from the study of the material three hours after injection clearly illustrate the above fact by indicating that almost all the dividing cells derive from the tumor stem cells, as evidenced by the presence of approximately 40 chromosomes having their own peculiar characteristics (Table 1, No. 1).

Higher dosages of $CaCl_2$ solution, such as 0.5 M and 0.75 M solutions, produced greater damage to tumor cells, but were lethal to the host animals. For instance, the injection of 0.5 M solution of this chemical killed the animals after about one and half hours. The observation of the tumor ascites from these dead specimens showed a large number of damaged tumor cells and together with them a number of tumor cells at the resting stage characterized by a small amount of cytoplasm and single, compact nuclei, namely, the resistant tumor cells. On examination of the tumor ascites about two hours after death of the host, the degeneration of damaged cells was more pronounced than in the former, but a certain number of the resistant tumor cells still remained free from damage. About 0.3 cc of the tumor ascites taken from these dead specimens about three hours after death, were injected into the peritoneal cavities of new rats. About 48 hours after this injection, many dividing figures were observed in the peritoneal cavities of new hosts. At this time, almost all dividing cells were derived from the tumor stem cells, as indicated by their approximately 40 chromosomes of the characteristic shapes and sizes (Fig. 3). Numerical data illustrating these facts are summarized in Table 1, No. 2.

2. Effects of $AlCl_3$

Experiments were performed with both the Yoshida sarcoma and MTK-sarcoma I as material. These tumors proved to respond in essentially similar manner to the $AlCl_3$ solution, hence the following descriptions apply to both cases. After

Table 1. Results of observations on dividing tumor cells with approximately 40 chromosomes and those showing mitotic abnormalities, after treatment with $CaCl_2$, $AlCl_3$ and H_2O_2 in the ascites sarcomas of rats.

	Chemicals applied	Hrs. after injection	Cells with chrom. numbers of 39–42	Cells with chrom. numbers of 36–38 or 43–46	Cells showing abnormalities
No. 1	0.25 M $CaCl_2$	3 hrs.	89.5%	6.5%	4%
No. 2	0.5 M $CaCl_2$	48 〃	88.5%	10.5%	1%
No. 3	0.125M $AlCl_3$	6 〃	91.0%	4.4%	5.6%
No. 4	2% H_2O_2	10 〃	91.5%	5.0%	4.5%
	〃	12 〃	88.0%	7.0%	5.0%
	〃	15 〃	82.7%	8.1%	10.2%

preliminary tests, it was found that the application of one cc of 0.125 M solution

of $AlCl_3$ prepared with Ringer's solution exerted destructive influence on tumor cells. As in the former case, injection rf the chemical was made in the tumor animals on the 6th day after transplantation of the tumor, and observations were made with smear preparations prepared at intervals after injection of the chemical.

The examination of the slides showed that the $AlCl_3$ solution produced cytological effects essentially similar to those observed in the case of the $CaCl_2$ injection. Following application of this solution, first the cell body broke away and then the disintegration of the naked nucleus followed (Fig. 9).

In tumor ascites taken about one hour after injection, most of the tumor cells were found damaged. After two hours the cell damage was much more pronounced; however, the ascites contained a certain number of tumor cells of small-sized resistant form remained unaffected by this chemical. The unaffected tumor cells began to divide about six hours after injection. An examination of the chromosomes in these dividing cells indicated that the majority of them possessed approximately 40 chromosomes and the characteristic complex peculiar to the stem cells. Numerical data clearly illustrate this feature (Table 1, No. 3). Hence, the renewed growth of the tumor was caused by the proliferation of the resistant tumor stem cells in this case, too.

3. Effects of H_2O_2

The Yoshida sarcoma and MTK-sarcoma displayed a similar response to H_2O_2. The cytological effects of this chemical are apparently the same as those produced by the former two chemicals. The intraperitoneal injection of one cc of 2 per cent H_2O_2 in the tumor-bearing animals induced breaking down of the cell body followed by the pycnotic degeneration of the naked nucleus in tumor cells (Figs. 10–12). As compared with the former two chemicals, this chemical seems to show a more severe action in damaging cells. About one hour after injection, over 70 per cent of tumor cells observed were undergoing degeneration. Two hours after application the damaged cells increased to about 90 per cent of the observed cells. The eosinophilic leucocytes shrank considerably. The neutrophilic leucocytes were actively increasing in number. At this time, there were present in the tumor ascites a certain number of tumor cells of small size, which remained without damage. These unaffected tumor cells began to divide about eight to nine hours after application of the chemical. They divided more actively with time, and the tumor ascites sampled from twelve to fifteen hours after injection contained many dividing cells. Close investigation of the chromosomes in these dividing cells made clear that most of them were the tumor stem cells, because they possessed approximately 40 chromosomes with the characteristic constitution. Numerical data showing this feature are given in Table 1, No. 4. With the passage of time the number of tumor cells increased through multiplication, causing

the renewed growth of the tumor in the treated animal.

It should be added here that the application of H_2O_2 resulted in a prolongation of the life of some treated animals. They lived for about 20 days; namely some 10 days longer than untreated animals.

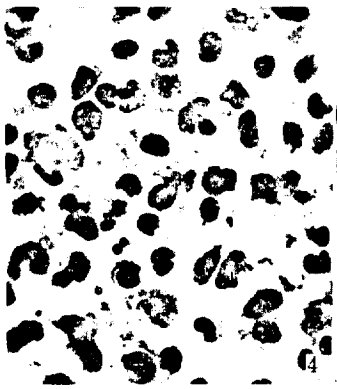
DISCUSSION

As described above, the cytological effects of $CaCl_2$, $AlCl_3$ and H_2O_2 on the tumor cells of ascites sarcomas of rats are similar for all three substances in the concentration used. There is first a break-down of the cytoplasm followed by disintegration of the resulting naked nucleus. Generally, those showing mitotic abnormalities are damaged first by these chemicals. Those in process of division seem also to be sensitive to the action of the chemicals. Of the three substances tested, H_2O_2 produced the most damage. Furthermore, this chemical was not completely selective for the tumor cells, since some leucocytes were also affected.

After the use of each of the chemicals, it was found that a certain number of tumor stem cells remained unaffected and alive. Though there occurred a temporary reduction of growth of the tumor as a result of the damage to most of the tumor cells, there was no complete recovery from the tumor. Upon cessation of the action of the action of the chemicals, the resistant stem cells began division and caused the renewed growth of the tumor in the treated animals. Complete recovery can be expected only after complete damge to the resistant tumor stem cells. Thus, the general results obtained here are similar to those attained in the previous study with podophyllin by Makino and Tanaka (1953).

The resistant tumor stem cells are generally characterized by a small amount of cytoplasm and a single, well-defined compact nucleus with distinct nucleoli. They divide with regular mitotic behavior. They possess a characteristic chromosome complex which is specific to the kind of tumor (see Makino 1952 a, b; Makino and Kano 1953). The individuality of the chromosomes in the tumor stem cells remains unchanged during successive transplant generations. The continuity and the growth of the tumor during successive transplant generations are primarily due to the presence and protiferation of these stem cells.

The question why a few of the tumor stem cells should remain undamaged by the chemicals applied, whereas the majority of tumor cells are damaged, is one of the important subjects of investigation in cancer therapy. It is highly probable that, when exposed to injurious conditions, some tumor stem cells are able to protect themselves by transforming into the resistant form. Possibly, this transformation may be accomplished by the cells becoming impermeable to the noxious substance in the surrounding medium. In other words, the resistant nature of the stem cells may result from a physiological change or changes in the cell membrane.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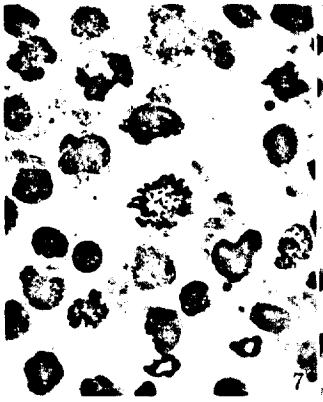
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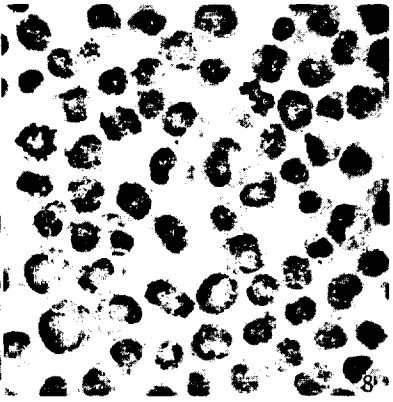
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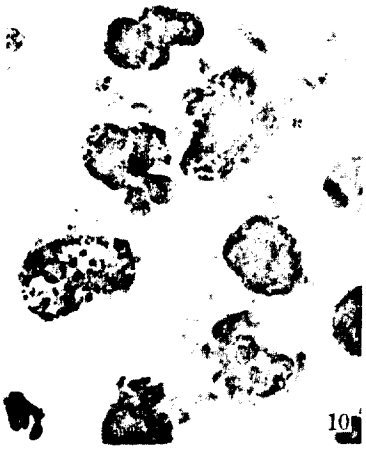
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The former study with the application of a mitotic poison, podophyllin (Makino and Tanaka 1953), and the present investigation with dehydrating agents, such as $CaCl_2$, $AlCl_3$ and H_2O_2, have shown that the resistant tumor stem cells of ascites tumors of rats can not be completely damaged with doses of the drugs not lethal to the hosts. Further, the resistant tumor cells can live for a time after the death of the host. It is these tumor cells that cause the renewed growth of the tumor in the treated animals. The physiological nature of these stem cells remain entirely unknown. The necessity arises thus, to investigate the general physico-chemical properties of these tumor cells, in particular such properties as permeability, excitability phenomena, metabolism and adsorption effects.

SUMMARY

Two cc of 0.25 M $CaCl_2$ solution, 1 cc of 0.125 M $AlCl_3$ solution, or 1 cc of 2 per cent H_2O_2 were injected into the peritoneal cavity of the tumor-bearing rats of ascites sarcomas, and the effects of these chemicals were investigated with smear preparations made at various intervals after injection of the chemicals. It was found that these chemical first exerted their influence on the cytoplasm, invariably inducing the breaking down of the cell body of the tumor cell. Then the pycnotic disintegration of the resulting naked nuclei followed. There occurred a temporary retardation of growth of the tumor to greater or less degree in every experimental animals as a result of damage to the majority of the tumor cells by the application of these chemicals, but a certain number of the resistant tumor cells always remained unaffected by the chemicals applied, so long as the dosage employed was sublethal to the host. Upon cessation of the action of the chemicals, these resistant tumor cells began multiplication and caused the renewed growth of the tumor in the treated animals. There is a great need to study the physico-chemical properties of these resistant tumor stem cells in connection with cancer chemotherapy.

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EXPLANATION OF PLATE V

All are photomicropgraphs. Figs. 4-7, from $CaCl_2$ experiments. Fig. 4, destruction of tumor cells. 30 minutes after injection of 0.25 M $CaCl_2$. 400×. Fig. 5, the same, 60 minutes after iejection. Tumor cells of small size have remained unaffected (indicated by arrows). 400×. Fig. 6, abnormal condensation of chromosomes in a tumor cell, observed 60 minutes after injection of 0.25 M $CaCl_2$. 1000×. Fig. 7, dividing cell showing subdiploid chromosomes, observed 10 hours injection of 0.25 M $CaCl_2$. 600×. Fig. 8, tumor cells just before injection of $CaCl_2$. From an animal on the 6th day after transplantation of the tumor. (Control). 400×. Fig. 9, destruction of tumor cells. 1 hour after injection of $AlCl_3$ (0.125 M). 800×. Figs. 10-12, from H_2O_2 experiments. 1000×. Figs. 10, breaking down of the cytoplasm remaining the nuclei naked. About 2 hours after injection of 2% H_2O_2. Fig. 11, irregular thickening of chromosomes occurring in a naked tumor cell. 2 hours after injection. Fig. 12, disintegration of chromosomes in a tumor cell. 2 hours after injection. Figs. 4-8, from Yoshida sarcoma. Figs. 9-12, from MTK-sarcoma I.

要　旨

薬品の白鼠腹水肉腫に及ぼす細胞学的影響（第二報）

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2 cc の 0.25 M $CaCl_2$, 1 cc の 0.125 M $AlCl_3$ 及び 2% H_2O_2 を，移植 6 日目の吉田肉腫ならびに MTK 肉腫ネズミの腹腔に注射して，腫瘍細胞への影響を細胞学的に観察した。この3種の化学薬品は例外なく，まず腫瘍細胞の細胞質を破壊し，ついで裸出した核が退化を起す，大部分の腫瘍細胞が破壊されるので，肉腫の成長はここで一時的に退行するが，いずれの場合においても，一群の母系細胞は薬品によつて破壊されることなく生きのびるので，やがてそれらの分裂増殖によつて，再び腹水肉腫が形成される。如何なる機構によつて母系細胞のある者は薬品の破壊をまぬかれるか，これは諸の化学薬剤においてまず第一に研究しなければならぬ問題である。

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EFFECT OF CALCIUM SALTS IN THE DIET ON THE DEPOSITION OF STRONTIUM-90 IN THE BONES OF RATS

(Vliyanie razlichnykh solei kal'tsiya v ratsione na otlozhenie strontsiya-90 v kostyakh krys)

R.M. Malkina and S.M. Puchkova (Moscow)

Since the question of protecting man against strontium-90 first arose, the attention of workers has been drawn to the so-called protective effect of calcium. Calcium, being the nearest chemical analog of strontium, is able to reduce the absorption of strontium within certain limits, if the two

elements simultaneously enter the gastrointestinal tract. The absorption of calcium depends on the anion with which it is ingested (Kaplanskii; Klinke).

The basic foodstuffs — milk, vegetables, bread, meat — contain various calcium salts, which are absorbed with different degrees of facility. Milk contains calcium chloride, acid calcium phosphate, calcium citrate, and calcium combined with protein. These forms of calcium readily dissociate at the pH values found in the intestine, and are readily absorbed.

In vegetables and certain cereals, the calcium is mainly combined with oxalic and phytic acids. These calcium compounds pass through the gastrointestinal tract practically without dissociation and they are therefore poorly absorbed.

The present study concerns the absorption and deposition of strontium-90 when ingested together with various calcium salts which are present in the principal foodstuffs.

The work was carried out on Wistar strain rats, weighing 160-180 g on the average. All the groups of 20 animals each were kept on a special diet, which was physiologically complete except that it contained only 3 mg of calcium. However, each group also received 23-25 mg daily of a different calcium salt; the total amount of calcium satisfying the physiological requirements for rats of this age (Ichikawa, Enomoto).

The experimental diet was based on the Na form of ionite milk, which ensured the full value of the diet (optimum quantities of phosphorus, fat, lactose, vitamins D and B) and the good assimilation of calcium.

The number of rats used, the calcium salts, the composition of the diet and the amounts of strontium-90 ingested are shown in Table 1.

The Na-milk was prepared by passing ordinary milk through a column with containing a KU-2 ion-exchange resin in the Na-form. This treatment only removed the calcium, the milk retaining its full value in all other respects.

TABLE 1

Content of Ca and Sr^{90} in the diets of rats

Group of animals	Source of calcium	Amount of calcium, mg/day	Amount of strontium-90 ingested per rat per day, μc	Diet
1st	$CaCl_2$ $CaHPO_4$	12 11	0.4	Na-milk, 20 ml, sugar 5 mg
2nd	CaC_2O_4	25	0.4	Starch 4 g, hulled millet 5 g
3rd	Ca citrate $CaCl_2$ $CaHPO_4$	15 5 5	0.4	Gelatine 0.02 g, cod liver oil 0.2 g, yeast 0.2 g
4th	Natural milk	23	0.006	

Four weeks prior to the experiment, all the animals received the same diets as during the experiment, but with ordinary milk, so that their calcium metabolism was stabilized by the beginning of the experiment. The experimental period lasted for 14 days. Animals in the first group received calcium salts with an anion composition corresponding approximately to that of meat (calcium chloride and phosphate, 1:1). A second group of animals received calcium oxalate, which forms an essential part of the calcium compounds of vegetables. A third group received calcium salts, the anion composition of which was approximately that of milk (calcium citrate, acid calcium phosphate, calcium chloride). For 14 days the rats in the 3 groups also daily received 1 ml strontium nitrate with an activity of 0.4 μc, administered per os by means of a special stomach tube. Milk obtained from a cow which had daily received strontium-90 nitrate was also administered to the 4th group, through the stomach tube, in doses of 10 ml daily, which contained 0.006 μc of strontium-90.

The third and fourth group of animals provided comparison of the absorption of calcium from salts added to the diet and from natural milk.

Three rats from each group were kept in metabolic cages throughout the experimental period. Their excreta were collected daily, for radiometric assay and for the determination of calcium (by the trilonometric and oxalate methods). The amount of strontium-90 in the skeleton was estimated from its content in the thigh and shin bones. Samples of bones and excreta were ashed at 600-700°. The activity of samples was determined by means of a B-2 device with a BFL-25 counter, with a counting error of 5%.

TABLE 2

Ca content of the diet and excreta, and Sr^{90} content of the skeleton of experimental rats

Group of animals	Source of calcium	Amount of calcium in the diet, mg	Calcium content in excreta		Sr^{90} content in the skeleton, % of the administered dose
			urine, mg/24 hr	feces, mg/24 hr	
1st	$CaCl_2$	23	0.66 ± 0.08	5.4 ± 0.62	12 ± 5.0
2nd	$CaHPO_4$ CaC_2O_4	25	0.42 ± 0.12	14.5 ± 0.40	42 ± 1.0
3rd	Ca citrate $CaCl_2$	25	0.78 ± 0.06	9.0 ± 0.42	30 ± 2.0
4th	$CaHPO_4$ Natural milk	23	0.72 ± 0.06	10 ± 0.5	35 ± 3.0

Analysis of 24-hr specimens of the excreta of all the rats showed that the absorption of calcium was poorest in the case of the group which received calcium oxalate. This group excreted 14.5 mg Ca per day in the feces, i.e., 50% of the ingested calcium, whereas the group which received calcium salts with an anion composition similar to that of meat excreted 5.4 mg Ca per day, which was only 20% of the daily intake.

The deposition of strontium-90 in the skeleton displayed a reverse relationship to that of calcium absorption. The amount of deposited strontium-90 was 42% of the total ingested strontium in rats which received calcium oxalate, as compared to only 12% in the first group of rats. Statistical analysis showed that there was only a slight difference (30 and 35%) in the strontium-90 content of the skeletons of rats from the third and fourth groups.

As already described, our experimental conditions were equally favorable for the absorption of calcium in all groups of animals. Yet, the absorption differed in different groups, despite the absolutely identical conditions and the equal quantities of calcium ingested. It must therefore be assumed that this difference depends on the anions together with which the calcium entered the gastrointestinal tract.

According to several authors, the degree of absorption is partially indicated by the excretion of the element in the urine of the experimental animals (Milhaud et al.).

A comparison of the calcium excretion of the second and the third groups of animals shows that the rats which had been given calcium salts corresponding to these of milk excreted nearly twice as much calcium as those which received calcium oxalate (0.78 mg/day as against 0.42 mg/day). The second group of rats excreted 50% of the ingested calcium in the feces, and these animals actually absorbed an insufficient amount of calcium (Kaplanskii; Petrun'kina). This was due to the insolubility of calcium oxalate at the pH of the intestine and its poor absorption (Abrosimova; Gorb, Maksakov).

Calcium was satisfactorily assimilated from milk (fourth group) and from the mixture of salts approximating the anion composition of calcium in milk (third group). Thus, according to our experiment, the assimilation was the same for calcium of biological origin and for that ingested in the form of salts. This would appear to justify the addition of calcium to diets containing insufficient amounts of this element, or containing calcium in poorly assimilated forms.

Studies of the absorption of strontium-90 and its deposition in the skeleton in relation to different calcium salts showed that the assimilation of strontium-90 depends on that of the calcium which is ingested simultaneously. Of the strontium-90 ingested by the second group of rats, 42% was deposited in the skeleton, while the accumulation of strontium-90 by the first group amounted to 12% of the ingested dose. The highest content of strontium-90 in the urine was found in the second group ($6 \cdot 10^{-8}$ curie per rat per day) but only about half of this quantity was found in the first group ($3 \cdot 6 \cdot 10^{-8}$ curie per rat per day). There is thus a correlation between the strontium-90 content in the urine and its deposition in the skeleton. A similar finding was displayed by the third and fourth groups of rats, which had received calcium salts and ordinary milk, respectively. In these groups, the excretion of calcium in the urine was approximately the same, 0.78 and 0.72 mg/day, and the difference in the deposition of strontium-90 in the skeleton was accordingly small (30 and 35%, respectively). These findings are also supported by our previous work, which established the similar assimilation of strontium-90 from naturally contaminated milk and from milk to which the isotope had been added in the form of a solution of strontium-90 nitrate.

Our results hold out the hope that it will be possible to reduce the deposition of strontium-90 in the skeleton by the addition of an optimum anion composition of calcium salts to the diet.

Conclusions

1. Calcium from different compounds was found to be differentially absorbed.
2. The deposition of strontium-90 in the skeleton of rats is affected by the anion composition of calcium salts.
3. The greatest effect is obtained by the addition of $CaCl_2$ and $CaHPO_4$ in a ratio of 1:1.

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SUMMARY

Effect of Calcium Salts in the Diet on the Deposition of Strontium-90 in the Bones of Rats

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Experiments performed on rats demonstrated that calcium was not absorbed uniformly. The extent of calcium absorption depended on the anions with it entered the gastrointestinal tract. The calcium was best absorbed from chloride and phosphate salts in the ratio of 1:1. The calcium oxalate was absorbed to an insignificant extent and the main mass of calcium passed through. Thus the anion composition of calcium salts affected the strontium-90 deposit in rats' skeleton.

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INTESTINAL ABSORPTION OF CALCIUM

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The measurement of the intestinal absorption of calcium in man has been made difficult by the fact that the calcium of fecal materials does not come simply from the calcium ingested with foods but also from the calcium which is not absorbed and which comes from the digestive juices and secretions (Fig. 1). The latter is commonly called endogenous fecal calcium (EFC).

The studies made by means of metabolic balances , before it was possible to use radioisotopes, could only measure the difference between:

$$Ca_i - Ca_f$$

where Ca_i is the calcium ingested and Ca_f is the total fecal calcium. This difference is referred to by some authors as net calcium absorption.

The possibility of using radioisotopes of calcium (Ca*), first of all Ca^{45} and then Ca^{47} , permitted the use of various methods for measuring the absorption of this element. In this paper we will analyze the various methods employed in determining the intestinal absorption of calcium and some of the physiological factors which affect this phenomenon.

Methods for Determining Intestinal Calcium Absorption in Man

1. Balance combined with the determination of the EFC by radioisotope methods (1).

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* Member of "Carrera del Investigador" (Research Career) C.N.I.C. and T.

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The true absorption (Av.) is measured using the following formula:

$$Av. = \frac{Ca_i - (Ca_i - CFE)}{Ca_i} \times 100$$

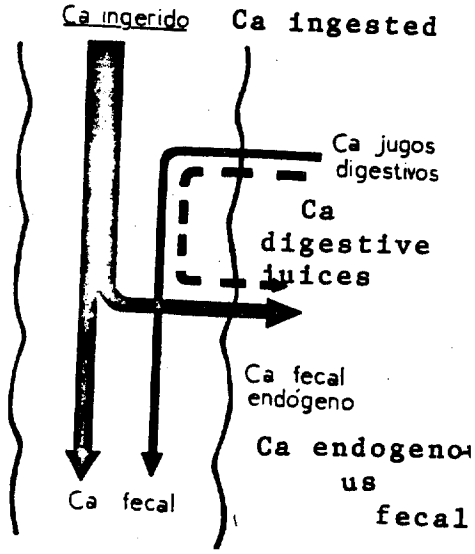

Figure 1

The subjects being studied must be in conditions of metabolic balance. Ca_i and Ca_f are measured by the usual methods during a period of approximately one week. Simultaneously Ca^* is injected endovenously and the activity in fecal materials is measured. The EFC is calculated by one of the methods described (2).

This method has the advantage of measuring the absorption of the calcium present in the foods of normal diet. Therefore it is a test of digestion and absorption. Also it can be combined with a kinetic study of the calcium metabolism, permitting a more complete evaluation of the patient.

The disadvantages of this technique are that the subjects to be studied must be subjected to a prolonged metabolic balance which, in addition to its high cost, is not within the reach of many laboratories. Furthermore the complexity of the procedure introduces an error of regular magnitude in the final result (3).

Measurement of the Fecal Excretion of an Oral Dose of Ca^*

If the quantity of Ca^* appearing in the fecal materials is measured after the administration of an oral dose of Ca^* then the absorption, without correction, is equal to:

$$Asc = \frac{Ca_i - Ca_f}{Ca_i} \times 100$$

We call this absorption without correction because this method does not correct the error coming from the EFC. This error has been estimated at 3.2% ± 1.8%(limits ? 7%) in 19 studies (4). In 14 subjects studied by us we obtained results whihh were almost identical : 3.3 % ± 1.8% (range 1.1-7.5%) (5).

Another source of error comes from the length of the period of time during which the samples are collected. A shorter period would lose part of the Ca^* which is not absorbed. On the other hand, a very long period would add a significant quantity of Ca^* coming from the EFC. The most appropriate method consists in addition of a substance which does not absorb, such as chromium, marked or stable, and in collection of all of the samples which contain the non-absorbable substance. If this aid is not available we take into account the samples in which the activity of the Ca^* is greater than 1 % of the dose administered, or we directly determine a period of time, generally 6 days. Figure 2 shows the average fecal excretion of an oral dose of Ca^{47} in 20 normal subjects (5).

3. Measurement of the Fecal Excretion of an Oral Dose of Ca^* corrected by the simultaneous measurement of the endogenous contribution (4)

If, at the same time as the oral administration of a dose of Ca^*, we inject endovenously another different calcium isotope (usually Ca^{47} and Ca^{45}) the measurement of the fecal materials in the latter makes it possible to make the corrections to determine the true absorption (Av.) . Here it is assumed that the fraction of the oral dose which was absorbed is subject to the same distribution and excretion process as the endovenous dose. Therefore:

$$Av = \frac{1 - ko}{1 - kv} \times 100$$

where ko is the fraction of the oral dose present in the fecal materials and kv is the fraction of the endovenous dose present in the same samples of fecal materials. The derivation of this formula can be found in the original paper (4).

This method has the advantage of measuring the true absorption without requiring special conditions of balance and with a relatively low total error. The disadvantages are the following: 1. The determination of the Ca^{45} in the fecal materials requires the calcination and processing of these materials before they can be measured, 2. The Ca^{47} contains small quantities of Ca^{45} but they contaminate the corresponding samples. Furthermore, the Ca^{47} and the Sc^{47} (its related isotope) have β activity. This requires measuring the samples after a prolonged period of time (more than 40 days) in order to prevent, by decay, the activity of the Ca^{47} and Sc^{47}. Furthermore it is necessary to calculate the β activity of the Ca^{47} standard and to correct in each sample the number of counts coming from the Ca^{45} present as a contaminant. These disadvantages persist and increase the complexity of the calculations to obtain the final result.

4. Double Isotope Relation in Blood and Urine

The methodology is essentially the same as indicated in the preceding technique. If two different isotopes of the same element are administered orally and intravenously in such a way that both reach the blood at the same time then the quotient between the fraction of dose,present in a sample of blood or urine, of the isotope administered orally over the fraction of dose,present in the same sample, of the isotope administered intravenously, gives a measurement of the true absorption:

$$\text{Av (true absorption)} = \frac{ko}{kv} \times 100$$

where ko is the fraction of the oral dose in a sample of plasma or urine and kv is the fraction of the intravenous (endovenous) dose in the same sample of plasma or urine.

In actual practice, while the intravenous dose reaches the blood instantaneously, the fraction of the oral dose which was absorbed does this over a lapse of time of approximately two to four hours. It is therefore recommended that the intravenous dose be injected two hours after the oral dose, coinciding approximately with the maximum level reached in the blood by the isotope administered orally. In order to reduce the error caused by the different time of arrival of the two isotopes to the rapidly interchangeable pool of calcium, an adequate lapse of time must be provided to permit calcium equilibrium. De Grazia (6) recommends taking urine samples no sooner than 24 hours before the test is begun. Samachson (7) generally analyzes the values of 24 hours, even though he admits that valid results can be obtained in the plasma even after 4 hours.

This method has the advantage that it permits the determination of the true absorption, completely omitting the fecal materials. This totally eliminates the errors coming from the loss of fecal materials. Theoretically it is possible to take only a single sample of blood or urine on the day following the start of the test, thus requiring minimum effort on the part of the patient and on the part of the laboratory to carry out the studies.

The same disadvantages indicated in beginning the preceding method are applicable in this case with respect to the contamination of Ca^{47} with Ca^{45}. The error with which the Ca^{47} and the Ca^{45} are measured and the correction by contamination produce an error in the final result, which is necessarily greater than in the preceding method. Nevertheless, the greatest source of error, and consequently the greatest disadvantage of the method, comes from the interval existing between the administration of the intravenous dose and the complete absorption of the isotope given per os. De Grazia (6) observed that the results obtained are erroneous if the intravenous is injected two hours before the oral dose instead of two hours after. The same thing occurs if the test is not carried out while fasting because the absorption of the isotope is slower than when ingested with the stomach empty. Therefore in actual practice it is necessary to take blood samples during the first four hours after the ingestion of the oral dose and to trace a plasma absorption curve to corroborate or reject the results of the double isotope method.

5. Body Counters

The total body retention after an oral dose of Ca^{47} drops rapidly during a period of from two to four days. The slope of the retention curve tends to straighten out from that time on. The rapid initial loss in activity is due primarily to the fecal elimination of the Ca^{47} which has not been absorbed and in a secondary manner to the urinary and fecal elimination of the absorbed Ca^{47}. The total body retention, once the phase of rapid loss of the unabsorbed isotope is ended, can be taken as an absorption index.

For Deller (8) the absorption equals the activity of day 0 (100%) minus the activity of day 7.

The comments on the errors coming from the use of a body counter and the cost disadvantages are outside of the scope of this paper. It suffices to state that very few laboratories in the world are equipped to do this type of study.

6. External Radioisotope Counting of the Activity in the Forearm

To employ this method recently described by Curtis et al. (10) it is necessary to have a counter for the β activitywhich permits measurement of the activity present in the forearm. The quotient between the fraction of activity encountered in the forearm after an oral dose of Ca^{47} over that found after an intravenous dose provides a measurement of the true absorption.

$$Av = \frac{\text{fraction of activity oral dose}}{\text{fraction of activity intravenous dose}} =$$

$$= \frac{(A_2 - A_1\ \beta)\ /\ \text{oral dose}}{A_1 /\ \text{intravenous dose}}$$

where A_1 is the activity 23 hours after the intravenous dose of Ca^{47}, A_2 is the activity 48 hours after the intravenous dose of Ca^{47} and 25 hours after the oral dose of Ca^{47}, β is the biological decay in the interval of time between the determination of A_1 and A_2.

Theoretically this method has the same sources of error as the double isotope methods in plasma or urine. Nevertheless, in the article mentioned (10) a very acceptable correlation was found between the results which were obtained using this technique and those which were determined simultaneously with the method of cumulative fecal excretion with correction of the endogenous contribution. If these initial observations are corroborated then the method is of great simplicity and will permit determination of the true intestinal absorption without any manipulation of plasma, urine or fecal materials and with minimum effort on the part of the laboratory.

Non-quantitative Methods for Measuring Intestinal Calcium Absorption

1. Maximum activity in the blood

The plasma activity after an oral dose of Ca^* increases during a period of time which varies from one to three hours and then gradually decreases once the maximum level has been attained.

In the studies made by Bhandarkar (11), Jaworski (12), Kinney (13), and by our group (5) the maximum values in the plasma were observed by about the second hour. The quantity of carrier used varied between 200 and 300 mg of calcium ion. Avioli (14) used 20 mg of calcium ion as the carrier in 5 cubic centimeters of water and he observed that the maximum level is reached in 60 minutes.

The values obtained have been correlated with the quantitative results of intestinal absorption of calcium determined by some of the methods which have already been commented upon. Whereas Jaworski, Kinney and we reached the conclusion that the two parameters do not correlate or that they correlate very poorly, Bhankdarkar and Avioli found a satisfactory correlation. Avioli, in particular, observed a very significant correlation ($r = + 0.89$).

The plasma level after an oral dose of Ca^* depends on the rate of stomach emptying and intestinal transit, the absorption capacity of the intestine, the size of the rapidly interchangeable calcium pool and the rate of re-exchange of this pool (15). Thus it should not be surprising that the correlation between the plasma level and the absorption is generally weak in normal subjects and is non-existent in some pathological cases which change the intestinal physiology or the interchangeable calcium pool.

Factors Affecting the Determination of the Intestinal Absorption of Calcium

A number of experiments carried out both on humans and on laboratory animals made it possible to obtain information on the factors affecting the intestinal absorption of calcium. A summary of this information is presented below:

Quantity of calcium in the normal diet: The work done by Malm (16) showed in a conclusive manner that normal subjects are able to adapt to a low-calcium diet by increasing the intestinal absorption of this element. The period of time necessary to

achieve this adaptation can last several months. Kenney (13) observed a negative correlation between calcium ingestion and intestinal absorption. Nevertheless, the variations observed were not very significant when the quantity of calcium in the diet fluctuated between 500 and 100 mg, which would include the greater part of the population in our area. Spencer (17) observed that the average intestinal absorption of calcium increased from 30% to 60% in 10 normal subjects when the determinations were made during consumption of a diet rich in calcium and poor in calcium, respectively, the latter containing a quantity of calcium 10 times smaller than the former.

Age: Dowdle (18) and Kimberg (19) encountered a significant decrease in the calcium transport capacity of the intestine when the age of the rats increased from one and a half to twelve months.

Avioli (20) observed a significant correlation between the maximum plasmatic peak and the age. This , however, is not a quantitative absorption parameter and in its turn it is affected by various factors which are not related to it. Although supposedly the increase in age causes a decrease in calcium absorption, as of the present time no study has been made of normal subjects of different ages to arrive at a true conclusion in this regard.

Carrier: The type and quantity of ion calcium used as a carrier have a definite affect on the percentage of element absorbed.

As has been shown in studies on normal subjects, the calcium chloride salts which dissociate easily are absorbed in a greater percentage than those of lactate and the latter are absorbed more than the gluconate ones. This aspect is discussed in greater detail in the section devoted to the results obtained on normal subjects. Bhandarkar (11) observed that when using calcium chloride higher levels of plasmatic Ca^* are obtained than when calcium carbonate was used as the carrier.

Intestinal Absorption of Calcium in Normal Subjects. Results

Figure 3 summarizes the results provided in 13 projects coming from 9 different research centers and published in the last three years. Examination of this table shows that,with the exception of the work done by Avioli, in all of the others they used different types of carrier or different quantities of ion calcium in the carrier and different methods of study, thus making it difficult to analyze the results. It is obvious that to make comparisons of the values obtained in different research centers it is necessary to standardize the conditions and the methodology of this study, as is proposed further on.

Figure 3 groups the studies according to the type of carrier, leaving aside the differences in quantity of ionic calcium and methods used. For example, the three groups of subjects in which milk was used as the carrier are analzed as a whole, although they were studied by three different methods (body counter, double isotope and fecal excretion without correction). Also the studies made with calcium chloride as the bearer were analyzed together although the quantity of ionic calcium varied from 10 to 200 mg

and two different methods were used (fecal excfetion without correction and corrected).

Analyzing the results in this manner and taken the groups as a whole, it would appear that the intestinal absorption of calcium varied basically with the type of carrier, depending to a lesser extent on the quantity of ionic calcium of it and the methodology used.

The most numerous group is the one studied with calcium chloride. Fifty one normal controls were studied with an average of 45.8% (limits 30 to 60%). It is significant here that similar results were obtained using 10 mg (De Grazia) and 200 mg (our work) of ionic calcium as the carrier. Using calcium gluconate, De Grazia found a very significant difference in the percentage absorbed when he used 10 and 200 mg of ionic calcium as the carrier. At this time we do not have any explanation to justify the similarity of the results with different quantities of calcium chloride as the carrier in contrast to the dissimilarity observed in varying the quantities of calcium gluconate.

The three groups of subjects studied with milk as the carrier show considerable similarities in the absorption averages. There was a total of 33 controls with an average absorption of 26.3% (limits 10-46%). As a rule these figures indicate that when the Ca^* is administered with milk, the intestinal absorption drops practically to half of that obtained when mixed with an easily dissociable salt such as calcium chloride.

The absorption of the calcium of the foods averaged 39.4% in 13 subjects studied in two different centers (limits 25-57%). These figures would indicate that the calcium of the foods as a whole is absorbed more than that of milk but less than that of an inorganic salt (Cl_2 Ca). Nevertheless, the different methodology used to study the absorption of the calcium in food makes it necessary to take this observation with caution.

Lastly, with equivalent quantities of ionic calcium, the 15 subjects (2 groups) who received calcium lactate absorbed an average of 38.7% and the 16 subjects who received calcium gluconate absorbed 26.6% of it.

Conclusions

Despite the fact that numerous factors affect the intestinal absorption of calcium (age, diet, vitamin D, physical activity, etc.) we believe that it can be considered that when calcium chloride is used as the carrier 30% to 60% of the dose administered is normally absorbed. When milk is used as the carrier normally 15% to 35% of the dose is absorbed. Normal subjects who habitually ingest diets very poor or very rich in calcium, less than 400 mg or more than 1500 mg per day, can present values outside of the limits mentioned.

The dissimilarity of the methods used makes it difficult to compare the results obtained in different laboratories. For this purpose we propose the following standard procedure for studying the intestinal absorption of calcium.

a. Patient should be without food (fasting) 12 hours before and 4 hours after the test begins;

b. Carrier: Cl_2Ca when it is desired to study only the absorption. Milk when it is desired to study the digestion and absorption (this may be necessary in diseases of the digestive system),

c. Quanaity of Ca^{++} in the carrier: 200 mg (physiologically similar to that ingested per meal),

d. Method: Administer a calcium radioisotope orally and another intravenously . Determine the cumulative fecal excretion of the first (oral administration) and correct the endogenous contribution in accordance with the fecal secretion of the second (i.e. intravenous).

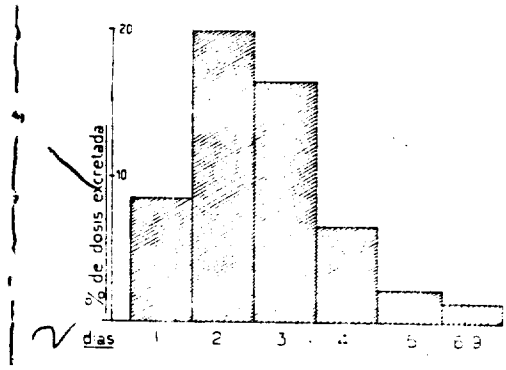

Figure 2. Average fecal excretion of an oral dose of Ca^{47} in 20 normal subjects.

1= percentage of dose excreted

B = days

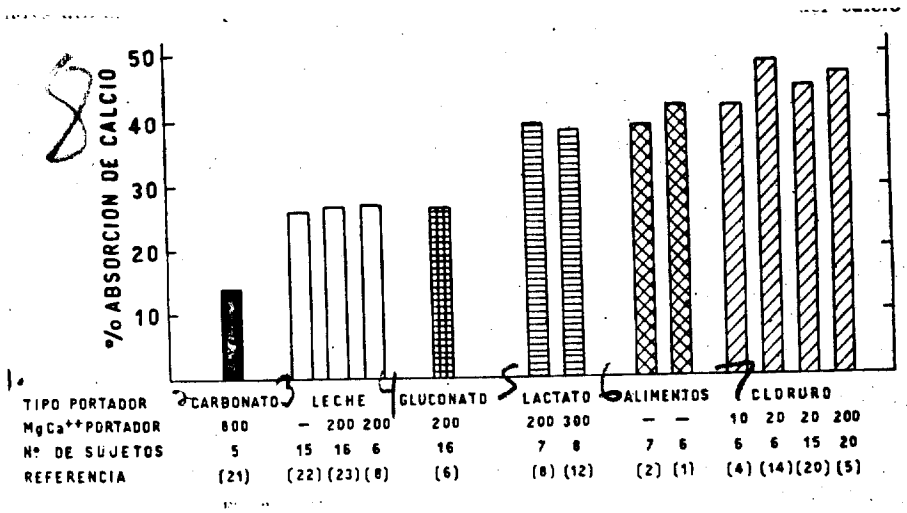

Figure 3. Intestinal absorption of Ca^{*} in normal subjects.

1 = Carrier type $MgCa^{++}$ carrier
$MgCa^{++}$ carrier
No. of subjects
Reference
2= Carbonate
3= Milk
4= Gluconate
5= Lactate
6= Foods
7= Chloride
8= % of calcium absorption

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THE EFFECT OF A LARGE INTAKE OF CALCIUM CITRATE IN NORMAL SUBJECTS AND PATIENTS WITH CHRONIC RENAL FAILURE

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CHRONIC renal failure is nearly always associated with a diminished absorption of calcium from the gut and a low urinary calcium excretion. Often there is also a low plasma calcium. Isolated cases suffer from osteodystrophy. It has been suggested that the disturbance of calcium metabolism is due to an impaired ability to absorb calcium due to vitamin D resistance (Liu and Chu, 1943). This paper describes the effect of a high intake of calcium citrate in patients with chronic renal failure and normal subjects. In both, calcium absorption increased to about the same extent. The results do not support the hypothesis that patients with renal failure have an impaired ability to absorb calcium.

EXPERIMENTAL PROCEDURE

The investigation was carried out in the metabolic ward according to the principles of Reifenstein, Albright and Wells (1945). Six patients with chronic renal failure and two subjects who were convalescent from duodenal ulcer were studied; at the time of the study the latter were symptomatically and radiologically normal. Details of the individuals studied are given in Table 1. The two normal subjects and patients No. 3, 5 and 6 were up, out of bed and active during the day. Patient No. 4 was up throughout the day but sat in an armchair for most of the time. Patients No. 1 and 2 were in bed night and day. There was no symptomatic or radiological evidence of bone disease in any of the subjects studied; in addition the serum alkaline phosphatase levels were all in the normal range. The diets on which subjects were to be maintained were arranged so that each continued to receive approximately the same daily intake of calcium and phosphorus that he had been having before admission to hospital. After an initial period of 7 to 10 days on this diet the balance study was begun. There were two or three control periods after which the calcium intake was increased by 10 g. of calcium citrate per day in all individuals, except in patient No. 1 and subject A who received an increase of 10·5 g. The high calcium intake was maintained for three periods in two patients (No. 1 and 5) and for four in the remainder. After this, the added calcium citrate was stopped and there were two final two periods at the original level of calcium intake. An outline of one study is shown in Fig. 1. All the calcium citrate to be used in a study was weighed out from one batch of the salt before the balance started, and its calcium content estimated. The intake of each patient therefore remained constant throughout the period of high calcium intake. The average amount of calcium as calcium citrate administered to patients No. 2, 3, 4, 5, 6 and subject B was 2112 mg./day and in patient No. 1 and subject A it was

TABLE

Details of the patients with renal failure an

Patient No.	Age yr.	Diagnosis	Weight kg.	Blood pressure mm.Hg.	Hb g./100 ml.	Blood urea mg./100 ml.
1	54	Polycystic disease	69	180/100	7·1	200
2	16	Chronic glomerular nephritis	57	140/100	12·0	145
3	48	Chronic glomerular nephritis	66	210/90	6·8	315
4	51	Polycystic disease	58	160/100	9·1	114
5	70	Chronic glomerular nephritis	50	160/90	9·1	73
6	68	Chronic glomerular nephritis	68·5	200/110	14·0	39
Subject No.						
A	42	Normal	62·5	110/70	15·2	21
B	36	Normal	80	110/70	16·1	27

* This patient has been treated with $NaHCO_3$.

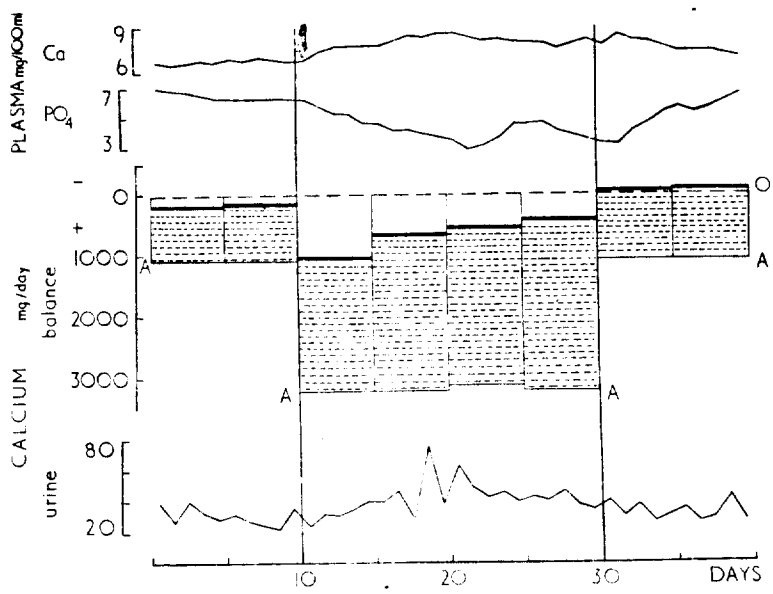

Fig. 1. *This figure illustrates the plan of the experiment.* It has been constructed from some of the results in patient No. 3. The area extending down from the broken horizontal line to the line (A) represents calcium intake. The hatched area extending upwards from (A) represents faecal calcium excretion; the thick black area immediately above this represents urinary calcium excretion. The two vertical lines indicate the beginning and end of the high calcium intake. The balance data is given in five day periods.

1

the normal men who were the subject of the study

Creatinine clearance ml./min.	Plasma total CO_2 content mMol./100 ml.	Plasma calcium mg./100 ml.	Plasma phosphate mg./100 ml.	Urinary calcium mg./24 hr.	Serum alkaline phosphatase K.A.U./100 ml.	Serum proteins g./100 ml.	
						Total	Albumen
5·5	9·0	5·1	14·0	58·8	6·2	5·1	3·2
5·8	29·0*	9·6	3·9	13·6	5·0	5·3	3·7
6·0	13·5	7·0	6·4	27·3	12·5	6·1	4·2
10·0	21·0	9·8	5·1	43·5	3·6	6·6	4·2
30·0	21·0	8·3	4·4	58·1	5·2	5·8	2·5
43·0	25·0	8·9	2·2	53·0	9·6	6·6	4·5
96·0	28·0	9·8	3·1	291	5·5	6·2	3·8
134	31·5	9·4	3·0	245	8·0	6·7	4·0

2210 mg./day. Table 2 gives the details of the amounts administered to each subject. To facilitate administration, calcium citrate for the day was suspended in distilled water in a flask and given in four equal doses. The container was rinsed at the end of the day and the rinsing added to the last dose.

Fæcal collections were made over five or six day periods. Carmine markers (5 g.) were used in all subjects; as an additional marker patient No. 3 was given chromium sesquioxide labelled with ^{57}Cr. Consecutive 24 hour urine collections were made, beginning and ending at 7.30 a.m. At this time, when the patient was fasting, venous blood was taken into heparinised tubes and the plasma separated within five to ten minutes. In order that sphygmomanometer artefacts might be avoided, the blood was taken without stasis. Blood was obtained daily from all subjects except one (Patient No. 2) a young girl in whom venepuncture proved difficult as the result of many months previous investigations. For this reason blood samples were obtained from her on only 7 occasions before the high calcium intake, three times during, and only once after the period of calcium citrate administration. The following estimations were performed on each sample of blood: plasma calcium, phosphate and creatinine. In patients No. 1 and 5 plasma bicarbonate estimations were performed approximately twice a week. The urinary excretion of calcium, phosphate and creatinine were measured daily.

METHODS

Fæces were stored at −15°C. until the period was completed. They were then pooled, thawed and homogenised. For calcium analysis duplicate 20–30 g. aliquots of homogenate were weighed into vitrosil beakers, dried under an infra-red lamp and ashed in a muffle furnace at 600°C. for 24 hr. The ash was eluted with 20–30 ml. of 20% HCl and made to volume. This solution, suitably diluted, was analysed for calcium in a flame photometer. For phosphate analysis 0·3–0·5 g. aliquots of homogenate were weighed into Kjeldahl type flasks calibrated at 25 ml., and wet-ashed with 3 ml. of 70% perchloric acid for 3 hr. Suitable dilutions of this solution were used to estimate phosphate by a modification of Hall's method (1963). The procedure for analysing diet was the same as for fæces.

Calcium estimation

An Eppendorf flame photometer was used throughout. This instrument measures calcium emission at 620 mμ in an acetylene-air flame and employs interference filters and a photomultiplier. The calcium reading is enhanced by sodium, potassium and phosphate ions though the concentration of phosphate and potassium ions in plasma is too low to do this. The effect of plasma

TABLE 2 (a)
Balance data in patients with chronic renal failure

Patient No.	Period	Calcium mg./day					Phosphate mg./day				
		Intake	Fæces	Absorption	Urine	Balance	Intake	Fæces	Absorption	Urine	Balance
1	5 days	454	642	−188	57	−245	817	430	+387	292	+95
	5 days	454	690	−236	62	−298	817	416	+401	329	+72
	5 days	2650	2029	+621	55	+566	817	508	+309	294	+15
	6 days	2650	2139	+511	71	+440	817	425	+392	219	+173
	5 days	2650	2565	+85	55	+30	817	508	+309	170	+139
2	5 days	323	437	−114	12·8	−127	599	374	+225	235	−10
	5 days	245	308	−63	9·7	−73	542	238	+304	195	+109
	5 days	300	480	−180	12·3	−192	650	435	+215	217	−1
	5 days	2400	2238	+162	21·1	+141	650	563	+87	98	−11
	5 days	2400	2182	+218	15·3	+203	650	452	+198	58	+140
	5 days	2400	2042	+358	13·7	+344	650	482	+168	63	+105
	5 days	2400	2271	+129	14·1	+115	650	496	+154	34	+120
3	5 days	1074	867	+207	33	+174	1255	707	+548	268	+280
	5 days	1066	813	+253	28	+225	1270	730	+540	246	+294
	5 days	3166	2119	+1047	32	+1015	1255	1049	+206	168	+38
	5 days	3166	2506	+660	46	+614	1270	889	+381	138	+243
	5 days	3106	2554	+552	48	+504	1255	870	+385	146	+239
	5 days	3166	2735	+431	41	+390	1270	910	+360	132	+228
4	5 days	551	760	−209	51	−260	723	419	+304	711	−407
	5 days	554	620	−66	53	−119	722	323	+399	435	−36
	5 days	2651	1608	+1043	58	+985	723	335	+388	334	+54
	5 days	2654	3239	−585	71	−656	722	488	+234	350	−116
	5 days	2651	2293	+358	64	+294	723	368	+355	177	+178
	5 days	2654	2270	+384	58	+326	722	376	+346	264	+82
5	5 days	1110	1200	−90	72	−162	1470	800	+670	419	+251
	5 days	1110	1272	−162	35	−197	1470	880	+590	561	+29
	5 days	3260	2267	+993	32	+961	1470	730	+740	271	+469
	6 days	3260	3082	+178	31	+147	1470	745	+725	254	+471
	5 days	3260	3335	−75	27	−102	1470	1150	+320	228	+92
6	5 days	854	747	+107	63	+44	1124	351	+773	789	−16
	5 days	855	789	+66	52	+14	1124	401	+723	826	−103
	5 days	2902	2284	+618	150	+468	1124	443	+681	751	−70
	6 days	2918	2904	+14	175	−161	1124	555	+569	504	+65
	5 days	2921	2351	+570	144	+426	1124	430	+694	583	+111
	5 days	2921	2271	+650	144	+506	1124	422	+702	557	+145

TABLE 2 (b)
Balance data in normal subjects

Normal Subject	Period	Calcium mg./day					Phosphate mg./day				
		Intake	Fæces	Absorption	Urine	Balance	Intake	Fæces	Absorption	Urine	Balance
A	5 days	1213	995	218	295	−77	1610	726	+914	882	+32
	5 days	1213	1070	+143	295	−152	1640	770	+878	743	+135
	6 days	3438	2680	+758	444	+314	1632	860	+772	596	+176
	6 days	3438	3125	+313	428	−115	1640	950	+690	704	−14
	5 days	3438	2770	668	411	+257	1648	785	+863	653	+210
	5 days	3438	2870	+568	415	+153	1632	785	+847	684	+163
B	5 days	1302	1130	+172	260	−88	1775	880	+895	962	−67
	5 days	1302	1115	+187	281	−94	1775	875	+900	957	−57
	5 days	3471	2788	+683	382	+301	1775	1097	+678	786	−108
	5 days	3471	3401	+70	337	−267	1775	1070	+705	725	−20
	5 days	3471	3080	+391	348	+43	1775	1018	+757	725	+32
	6 days	3471	2780	691	335	+356	1775	925	+850	728	+122

sodium is sufficiently constant for it to be eliminated by zeroing the instrument with a sodium solution similar in concentration to that of the 1 in 20 dilution of plasma required by the sensitivity of the instrument, and including sodium at the same concentration in the calcium standards. The final dilution of the urine and of the solution obtained from the ashed diet and fæces were made in a sodium chloride-magnesium sulphate diluent to remove the phosphate interference and raise the sodium concentration to approximately that in the standard. Corrections were made for sodium and potassium concentrations which were greater or less than that in the standard. In this way recovery of calcium added to 27 urines in which the sodium concentration varied from 108–280 mEq./l. was 101·3% ± 2·25 S.D. The mean plasma value for 50 normal subjects was 9·55 mg./100 ml. ± 0·75 S.D.

Phosphate estimation

Urine and plasma phosphate were estimated in an autoanalyser employing the method of Fiske and Subbarow (1925). The perchloric acid digest of diet and fæces was neutralised to litmus with 20% NaOH and diluted to contain 0·1–0·2 mg. P/100 ml. Ammonium molybdate was added to 4 ml. aliquots and the phosphomolybdate complex extracted into a butanol-petroleum ether solvent and reduced with stannous chloride. This manœuvre separates phosphomolybdate from silicomolybdate which is present owing to dissolving of silicates from the glass digestion flask during wet-ashing with perchloric acid. The mean plasma value for 40 normal subjects was 3·7 mg./100 ml. ± 0·8 S.D.

Creatinine was measured in an auto-analyser.

Proteins were measured by a Biuret method (Weichselbaum, 1946).

Urea was estimated by the method of Fawcett and Scott (1960).

RESULTS

During the administration of calcium citrate there was a substantial rise in calcium absorption in both patients with chronic renal failure and the normal subjects. In addition, changes in calcium and phosphorus balance, in plasma calcium and urinary calcium excretion also suggested that there was a rise in calcium absorption.

TABLE 3

The average results in each individual obtained in the calcium and phosphate balances before and during the period of high calcium intake in six patients with chronic renal failure (No. 1–6) and two normal subjects (A and B). The urinary excretion of calcium after the high calcium intake is also shown

Patient No.	Period	Calcium mg./day					Phosphate mg./day				
		Intake	Fæces	Absorption	Urine	Balance	Intake	Fæces	Absorption	Urine	Balance
1	Before	454	666	−212	59	−271	817	423	+394	310	+84
	During	2650	2333	+317	64	+253	817	463	+354	197	+157
	After				59						
2	Before	289	408	−119	12	−131	597	349	+248	216	+32
	During	2400	2165	+235	14	+221	650	477	+173	52	+121
	After				11						
3	Before	1070	840	+230	31	+199	1263	719	+544	257	+287
	During	3146	2598	+548	45	+503	1265	890	+375	139	+236
	After				29						
4	Before	552	690	−138	52	−190	723	371	+352	573	−221
	During	2653	2601	+52	64	−12	722	411	+311	264	+47
	After				47						
5	Before	1110	1236	−126	54	−180	1470	840	+630	490	+140
	During	3260	3197	+63	29	+34	1470	929	+541	242	+299
	After				25						
6	Before	855	768	+87	58	+29	1124	376	+748	808	−60
	During	2920	2533	+387	156	+231	1124	474	+650	545	+104
	After				51						
Subject A	Before	1213	1033	+180	295	−115	1644	748	+896	813	+83
	During	3438	2934	+504	419	+85	1640	847	+793	682	+111
	After				224						
B	Before	1302	1123	+179	270	−91	1775	878	+897	960	−63
	During	3471	3068	+403	340	+63	1775	999	+776	726	+50
	After				246						

Table 2 gives some of the detailed results from the balances in each individual. Table 3 shows the average results obtained before and during the period of high calcium intake in each subject. In calculating these results the first five day period of high calcium intake was used as an equilibration or transitional period (Reifenstein *et al.*, 1945) and omitted. In this table and Figs. 2 to 6 the results before the high calcium intake are the average of the initial control periods; the results during the high calcium intake are the average of the ten or fifteen days following the equilibration period. In Fig. 4 the results after the high calcium intake are the average of the ten days which immediately followed the return of the calcium intake to normal.

Calcium absorption (Table 3 and Fig. 2)

Before the calcium intake was increased in four of the patients with chronic renal failure the calcium content of the fæces was greater than that of the diet, in the other two patients with renal failure and the two normal subjects the calcium absorption was positive. During the high calcium intake, calcium absorption increased in all patients with chronic renal failure and both normal subjects. The average increase was 311 mg./day (range 189 to 529 mg./day) in patients with chronic renal failure compared with 274 mg./day (224 and 324 mg./day) in the two normal subjects.

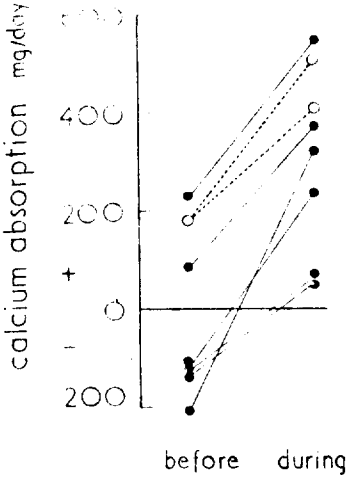

Fig. 2.

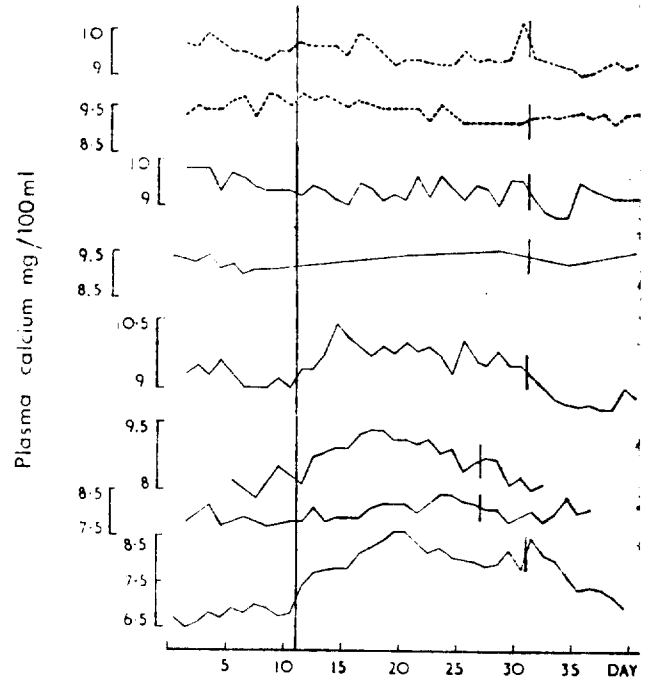

Fig. 3.

Fig. 2. *Average calcium absorption before and during the period of high calcium intake.* Normal subjects - - - - - - - - -. Patients with chronic renal failure ●————●.

Fig. 3. *Plasma calcium concentration before, during and after the period of high calcium intake.* The number or letter at the right hand side of each curve identifies the subject. They are arranged in descending order of control plasma calcium levels and the scale for each subject is shown at the left. The continuous vertical line represents the start and the smaller vertical lines the end of the period of high calcium intake. Normal subjects - - - - - - - -. Patients with chronic renal failure ——— ———.

Plasma calcium concentration (Fig. 3)

Before the calcium intake was increased the plasma calcium concentration in three of the patients with chronic renal failure was below normal; in the other patients and the two normal subjects the concentration was within normal limits. During the high calcium intake there was a rise in the plasma calcium concentration of 0·45 to 1·7 mg./100 ml. in the three patients with initially low plasma calcium concentrations, and also in one patient with an initially normal plasma calcium. There was little change in plasma calcium concentration in the other two patients in whom the initial plasma calcium levels were within normal limits. In the two normal subjects there was a slight fall in plasma calcium concentration during the high calcium intake.

After the high calcium intake, the plasma calcium fell to control levels or below in the four patients with chronic renal failure in whom the concentration had increased during the high calcium intake. One of the other two patients with chronic renal failure had a sustained rise in plasma calcium of about 0·6 mg./100 ml. for three days following the end of the high calcium intake. The two normal subjects showed little change at the [illegible].

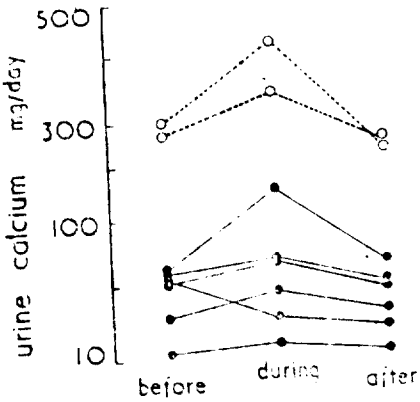

Fig. 4.

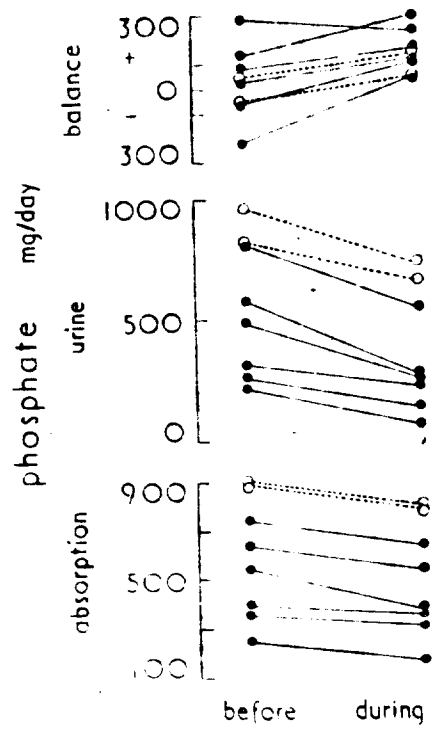

Fig. 5.

Fig. 4. *Average urinary calcium excretion before, during and after the period of high calcium intake.* The scale is logarithmic. Normal subjects ○- - - - - - - - -○. Patients with chronic renal failure ●— — — — —●.

Fig. 5. *Average phosphate balance, urinary phosphate excretion and phosphate absorption before and during the period of high calcium intake.* Normal subjects ○- - - - - - - - -○. Patients with chronic renal failure ●— — — — —●.

Urinary calcium excretion (Table 3 and Fig. 4).

Before the calcium intake was increased the average urinary calcium excretion in the patients with chronic renal failure was low (12 to 59 mg. 24 hr.); in the normal subjects it was 270 and 295 mg./day. During the period of high calcium intake, the urinary calcium excretion rose by 2 to 14 mg./day in four, and by 98 mg. in one other of six patients with chronic renal failure and by 70 and 124 mg./day in the two normal subjects. After the high calcium intake, there was a fall in calcium excretion in all individuals.

Phosphate balance (Table 3 and Fig. 5).

Before the high calcium intake, two patients with chronic renal failure and one normal subject had a negative phosphate balance. The other four patients with chronic renal failure and the remaining normal subject had a positive phosphate balance. During the high calcium intake there was a rise in phosphate balance in five of the six patients with chronic renal failure and in both normal subjects. [illegible] All eight subjects showed a diminution of phosphate absorption [illegible] The rise in phosphate balance [illegible] absorption being less than [illegible]

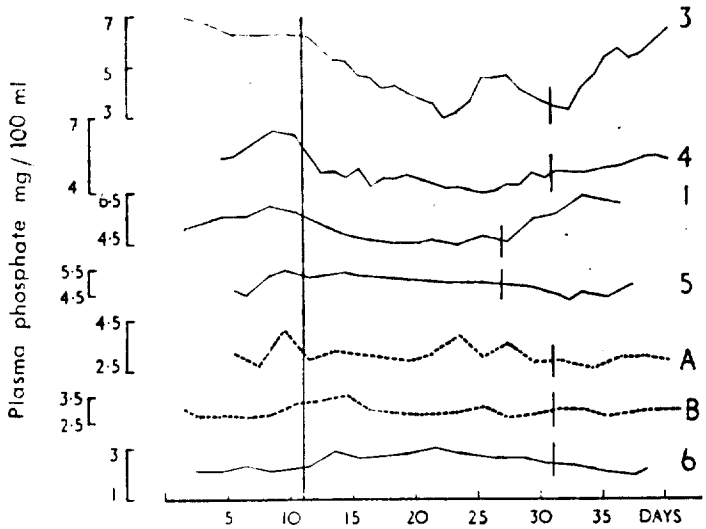

Fig. 6. *Plasma phosphate concentration before, during and after the period of high calcium intake.* All patients except No. 2 are shown. Each is identified by the number or letter at the right hand side of the curve. They are arranged in descending order of control plasma phosphate concentration. The scale for each subject is shown at the left. The continuous vertical line represents the start and the smaller vertical lines the end of the period of high calcium intake. Normal subjects - - - - - - - -. Patients with chronic renal failure ————.

Plasma phosphate concentration (Table 3 and Fig. 6)

Before the calcium intake was increased three of the patients with chronic renal failure had abnormally high plasma phosphate concentrations. In two other patients with chronic renal failure and the two normal subjects the plasma phosphate concentrations were within normal limits.

During the high intake of calcium the plasma phosphate concentrations fell in the three patients with chronic renal failure in whom it was high during the control period. In the others it showed little change.

After the high calcium intake the plasma phosphate concentrations rose to control levels in the three patients with chronic renal failure in whom the concentration had decreased during the high calcium intake. In the other four subjects there was little change.

In the remaining patient with chronic renal failure (patient No. 2) the plasma phosphate concentration was measured on seven occasions before the intake of calcium was increased; it ranged between 5·6 and 6·4 mg./100 ml. No further plasma phosphate estimations were obtained until ten days after the high calcium intake had ceased when it was 4·6 mg./100 ml.

Creatinine clearance and plasma bicarbonate

There were no consistent changes in creatinine clearance during the intake of calcium citrate. The plasma bicarbonate, however, in patient No. 1 rose by 2 mEq., and in patient No. 5 by 5 mEq.

Discussion

The data presented in this paper shows that when the intake of calcium was increased with calcium citrate, calcium absorption rose to about the same extent

in both the patients with renal failure and in the normal subjects. This deduction is derived from the calcium balance data and is supported by the reversible rise that took place in the urinary calcium excretion and plasma calcium levels, and also by the increase in phosphate balance.

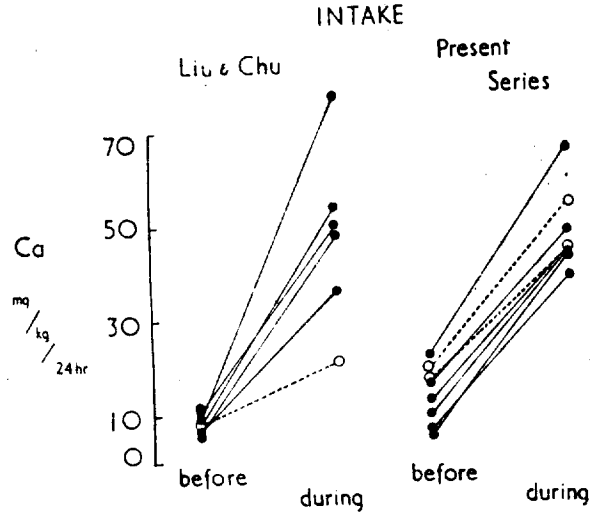

Fig. 7. *The daily calcium intake per kilogram body weight before and during the administration of large amounts of calcium lactate in Liu and Chu's* (1943) *subjects, and calcium citrate in the present series.* Normal subjects ○- - - - - - - - -○. (In Liu and Chu's series this dotted line represents the average for 12 subjects.) Patients with chronic renal failure ●————●.

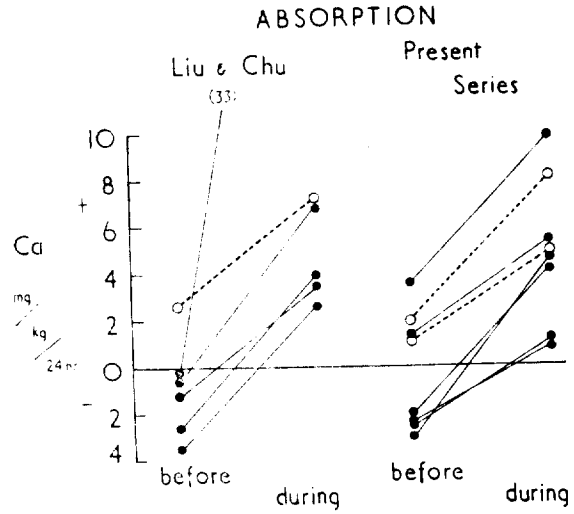

Fig. 8. *The daily calcium absorption per kilogram body weight before and during the administration of large amounts of calcium lactate in Liu and Chu's* (1943) *subjects, and calcium citrate in the present series.* Normal subjects ○- - - - - - - - -○. (In Liu and Chu's series this dotted line represents the average for 12 subjects.) Patients with chronic renal failure ●————●.

Estimated calcium absorption from the gut increased in all individuals during the period of high calcium administration. A similar increase was obtained by Liu and Chu in 1943. They studied the effect of increasing the dietary calcium with calcium lactate in five patients with chronic renal failure and renal osteodystrophy, and in twelve normal subjects. In Figs. 7 and 8 it can be seen that the amount of calcium administered by Liu and Chu as calcium lactate was comparable to that administered in the present series as calcium citrate, and that the increase in calcium absorption which then occurred was also comparable. Stanbury and Lumb (1962) also obtained a rise in calcium absorption upon increasing the calcium intake in one patient with azotæmic hyperparathyroidism after parathyroidectomy. Pre-operatively the calcium content of the fæces was 25–31 mg. per day greater than the oral intake of 875 mg. per day, whereas post-operatively when the calcium intake was increased to 1,875 mg. the absorption rose to 120 mg. per day. One of Dent, Harper and Philpot's (1961) patients, however, who was on a similar high intake of calcium did not have a positive balance of calcium. It is difficult to compare this patient with those mentioned above as no data is given of the calcium intake and absorption before the calcium intake was increased to 1,900 mg. per day.

Urinary calcium excretion increased during the high calcium intake in all but one subject and then fell on returning to a normal diet. The extent of the changes varied with the degree of impairment of renal function, the smaller changes occurred in patients with the lowest creatinine clearances. It is improbable that these increases in calcium excretion were due to an increase in urinary citrate excretion for the oral administration of calcium citrate is unlikely to have increased the citrate concentration of the plasma. In two normal subjects the plasma citrate concentration one hour after the oral administration of 4·75 g. calcium citrate showed no significant change (personal observation). In addition it has been shown that the administration of sodium citrate in normal man does not affect urinary calcium excretion (Farquharson, Salter, Tibbets and Aub, 1931).

Three of the patients with chronic renal failure whose initial control concentration of plasma calcium was low, had a rise in plasma calcium during the high calcium intake which returned towards control values when the high calcium intake ceased. Another of the patients with renal failure, who had an initial control concentration of plasma calcium which was normal, had a similar rise in plasma calcium during the high calcium intake, and again the plasma calcium fell steeply upon returning to the control diet, but in this patient to values which were well below those seen in the first control period. One other patient with renal failure who also had an initial control concentration of plasma calcium which was normal had little change in plasma calcium concentration during the period of high calcium intake but afterwards the plasma concentration slumped and remained low for three days in a manner similar to the previous patient. If the slump which occurred in both these cases is due to reducing the dietary calcium from high to normal then it probably represents a change in calcium absorption. If this is accepted these changes in plasma calcium concentration suggest that five of the patients with chronic renal failure had an increase in calcium absorption during the high calcium intake. The concentration of plasma calcium in the two normal subjects fell slightly throughout the high calcium intake and did not show any tendency to return to control levels afterwards. These changes are similar to that which Sanderson (1959) reported in normal rats fed on a high calcium diet.

The phosphate balances [illegible] in all individuals except one during the high calcium intake. At the same time there was a diminution both in phosphate absorption from the gut and excretion of phosphate in [illegible] individuals. The increase in phosphate balance was because the diminution in urinary phosphate excretion was greater than the decrease in [illegible] absorption. It is probable that the

fall in phosphate absorption during the high calcium intake was due to the increased calcium present in the gut binding some of the phosphate. The rises in phosphate balance were accompanied either by a fall in plasma phosphate, particularly if the initial plasma phosphate was high, or by little change. This suggests that phosphate moved from the extravascular compartment either into cells or into bone. As this occurred during the administration of large quantities of calcium, and at a time when there was evidence of an increase in calcium absorption, presumably the phosphate accompanied the calcium into bone. Simultaneous nitrogen balances, however, were not made and it is not possible to be certain. The cause of the fall in plasma phosphate during the high intake of calcium in some of the patients with renal failure is not clear. Dent (1962) has shown that in certain circumstances there may be a reciprocal relationship between plasma phosphate and calcium concentration, and that this may be precipitated by diminishing phosphate absorption. If, however, the plasma calcium and phosphate concentrations are compared in patients No. 6 and No. 3 it can be seen that in one there is a rise in plasma calcium concentration with no change in plasma phosphate while in the other there is no change in plasma calcium concentration and a fall in plasma phosphate. There is only one individual (No. 3) (Fig. 1) who shows any degree of reciprocity in plasma calcium and phosphate concentrations.

It should be noted that the normal subjects had a much greater content of phosphate in their fæces than the patients with chronic renal failure. This is additional evidence against an early hypothesis (Mitchell, 1930) that the diminution of calcium absorption in renal failure is due to an excess fæcal excretion of phosphate.

The results presented here do not support the hypothesis that in chronic renal failure there is an impaired ability to absorb calcium (Stanbury, 1962; Dent, 1962). It is more probable that the low absorption of calcium in renal failure is secondary to the low urinary calcium excretion and that the decrease in calcium excretion is due to the low glomerular filtration rate or some concomitant intrarenal change. In other words, if only negligible quantities of calcium are lost in the urine because of renal disease, only negligible quantities of calcium need be absorbed from the gut.

It is also difficult to reconcile these findings with the theory that in chronic renal failure the lack of rise in calcium absorption after the administration of physiological amounts of vitamin D is due to vitamin D resistance (Liu and Chu, 1943). It is possible, however, that such a response is normal. It was Chu, Liu, Hsu, Chao and Chen (1941) themselves, who demonstrated that the administration of physiological amounts of vitamin D to normal man on a normal diet does not increase calcium absorption from the gut. It is difficult to understand, therefore, why a similar lack of response in chronic renal failure should have been considered to be abnormal. Chu *et al.'s* (1941) observations have since been confirmed by Jackson and Dancaster (1962). And Carlsson and Lindquist (1955) have demonstrated in rats that increasing vitamin D intake one hundred times does not alter calcium absorption. It would appear that once physiological amounts of vitamin D are being ingested, insensitivity to a further physiological amount is not a pathological disturbance but a normal physiological state.

Summary

1. Two normal subjects and six patients with chronic renal failure were given 2,000 mg./day of calcium orally as calcium citrate for 15 to 20 days.

2. During the high calcium intake there was a rise in calcium absorption from the gut in the normal subjects and in the patients with chronic renal failure to about the same extent.

3. At the same time there were changes in phosphorus balance, plasma calcium, and urinary calcium excretion which were consistent with an increase in calcium absorption having taken place.

4. These results do not support the hypothesis that the low absorption of calcium in chronic renal failure is due to an impaired ability of the gut to absorb calcium.

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CASE REPORTS

IDIOPATHIC HYPOPARATHYROIDISM: REPORT OF A CASE *

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EMIR H. ADLE, M.D., *Teheran, Iran*

INTRODUCTION

IDIOPATHIC hypoparathyroidism is a relatively rare disease. In 1952 Steinberg and Waldron [1] collected 51 cases from the literature and added one of their own. They further reported that, out of 213,025 patients admitted to Queen's Hospital over a period of 15 years (1935–1950), only one was diagnosed as idiopathic hypoparathyroidism. Wise and Hart [2] (1952) found only three such cases in a 10-year survey at the King's County Hospital, where the yearly admission rate is 62,000.

Recent reviews of the clinical features of idiopathic hypoparathyroidism are found in the papers of Grant [3] (1953), Sugar [4] (1953), de Mowbray [5] (1953) and Robinson et al.[6] (1954). A case recently studied and treated at Jefferson Medical College Hospital is reported in this paper because the patient presented some unusual features, characterized by exceptionally long duration and advanced severity of symptoms prior to diagnosis, followed by a remarkable recovery.

CASE REPORT

A 30 year old white woman was admitted to the Neurological Service, Jefferson Medical College Hospital, Philadelphia, on March 25, 1957, with the chief complaint of painful muscle spasms. The history was obtained from the patient's mother and husband on admission, and later verified by the patient after her condition improved.

At 14 years of age she had begun to lose some of the hair of her scalp at fairly regular yearly intervals. During the four years prior to admission her hair had been very thin, without regrowth. She had had one generalized convulsion, unrelated to other illness, at the age of five months, and at the age of 22 years she had had a "spell" during which she fell to the floor, stretched out, became rigid and vomited. The episode lasted 10 minutes and was associated with excessive sweating. During a two-year period beginning at the age of 26 (four years prior to admission), she had had several generalized convulsions with urinary incontinence, but other details concerning these seizures were not known.

During the last four years she had had frequently recurring, transient, painful cramps and spasms of the muscles of all extremities, neck and back. There was no loss of consciousness during these episodes, but the patient was unable to speak, respond to commands or make voluntary movements. She was reported to have

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screamed or groaned as if suffering great pain, while her arms and hands were tightly drawn up and her legs were either forcefully extended or flexed involuntarily. There were slow twisting movements and arching of her body during these episodes, which varied in intensity and duration; the frequency varied from several attacks on one day, often repeated on several successive days, to one attack weekly.

During the last three years she had been unable to carry out her duties as a mother and had been mostly confined to bed. She had had impairment of memory and inability to concentrate or think clearly for about three years. She had been unable to walk for a year because of unsteadiness and spastic torsion movements of her trunk and limbs. She had difficulty in pronouncing words, and at times her speech was unintelligible. She lost all interest in social activities and events taking place in her home. She was unable to care for her personal habits of cleanliness. Her appetite had been extremely poor for over a year and was associated with considerable loss of weight.

She was delivered at term with the use of forceps, without complications. Her growth and development were normal. She had had no significant diseases other than the usual childhood illnesses. During adolescence and early adulthood she lost all her teeth as a result of gum infections and tooth decay. She had had some loss of hearing on the left side since early childhood. She had had two uneventful pregnancies, seven and five years prior to admission. Menarche had occurred at the age of 14, and the menses had always been regular. During the last four years her muscle pain had increased during each menstrual period.

The patient's father suffered from diabetes, but there were no other significant diseases in her family. Her social history revealed that she had progressed with difficulty but without failure through high school, and had subsequently worked as a machine operator in a dress factory. Her intellectual interests consisted of card playing and reading simply written religious material or comic books. She did not smoke or drink alcoholic beverages.

Physical examination on admission revealed pronounced generalized wasting of subcutaneous tissue and muscles, a weight of 86 pounds, considerable thinning of the hair of the scalp, and dark, periocular pigmentation. The blood pressure was 110/68 mm. of Hg; pulse rate, 80/minute. Neurologic examination revealed the following abnormal findings: There were impairment of comprehension, inability to attend to the interview, delayed and incomplete responses to commands, disorientation as to time and place, and intellectual deterioration. Her speech was dysarthric and unintelligible during tetanic episodes. There were incomplete lens opacities, denser on the left, where the fundus could not be visualized. The right fundus was normal. Vision appeared to be intact, but visual acuity and fields could not be reliably tested. Hearing was grossly intact, but the results of tuning fork tests were unreliable. Her station and gait were characterized by unsteadiness and hypertonicity of muscles, generally with twisting movements of the limbs and trunk. Motor power was good in all extremities, but there were increased tone and dyskinetic incoördination of all movements. The deep tendon reflexes were generally diminished. Pain, temperature and touch sensations were intact, but vibration and position senses could not be evaluated. Chvostek's and Trousseau's signs were positive.

The hemoglobin was 12.3 gm.%, the hematocrit value 37%, and the white blood cell count 5,500 per cubic millimeter, of which 55% were neutrophils, 36% lymphocytes and 8% eosinophils. The urine contained a faint trace of protein and many epithelial cells. Sulkowitch's test was negative. There was 35% excretion of phenolsulfonphthalein in 30 minutes. The Venereal Disease Research Laboratories' and Kolmer's tests were negative. The cerebrospinal fluid was under a pressure of 120 mm. water and contained less than 1 cell per cubic millimeter, 15 mg.% protein, 68 mg.% glucose [illegible] chlorides. The cerebrospinal fluid con-

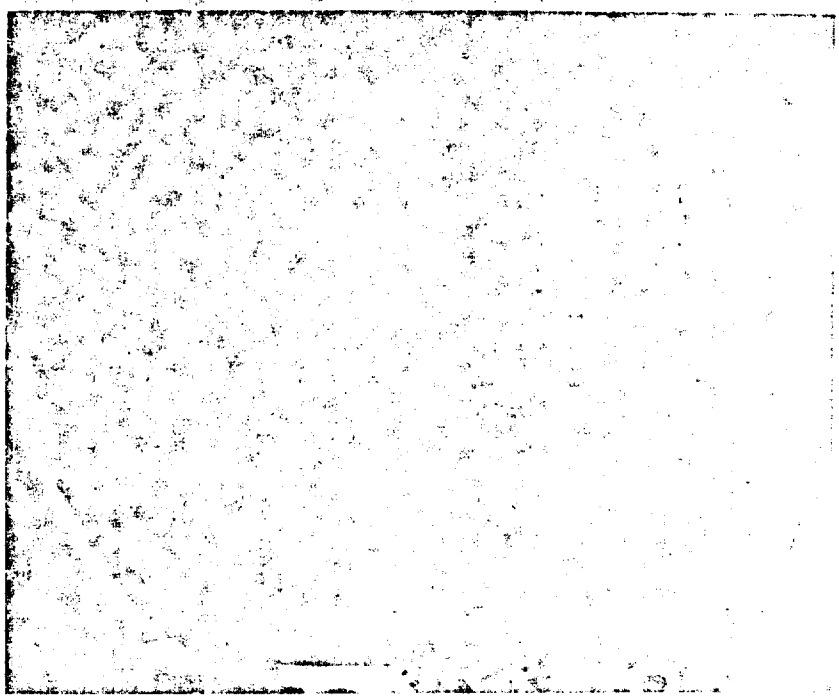

FIG. 1. Laminagram showing intracerebral calcification bilaterally in the basal ganglia.

plement fixation test was negative, and the colloidal gold curve was 0[10]. Roentgenograms of the chest yielded normal findings. Roentgenograms of the skull revealed fine intracranial calcifications symmetrically placed on either side of the midline in the region of the basal ganglia (figure 1). Roentgenograms of the entire spine, pelvis and long bones revealed numerous growth arrest lines and increased density of the vertebral centra. Initially the serum calcium was 4.4 mg.%, the inorganic phosphorus 7.4 mg.%, and the alkaline phosphatase 0.1 Bodansky unit. The fasting blood sugar was 103 mg.%; serum potassium, 5.0 mEq./L.; serum sodium, 137 mEq./L.; serum chlorides, 94.6 mEq./L.; total proteins, 7.0 gm.%; albumin, 5.0 gm.%; globulin, 2.0 gm.%; prothrombin time, 90%; total cholesterol, 170 mg.%; cholesterol esters, 115 mg.%; blood ureau nitrogen, 16.4 mg.%. The fat content of the stools was 4.3%. The protein-bound iodine was 7.9 μg/100 ml., and the 24-hour uptake of I^{131} was 29%, with a conversion ratio of 28%. An initial basal metabolic rate of plus 67 was unreliable; when repeated after therapy, it was plus 10. The

TABLE 1

Blood Calcium and Phosphorus

Date	Ca mg. %	P mg. %	Alk. Phos. Bodansky Units	Treatment
3/28/57	4.4	6.3	—	
4/3/57	5.6	7.4	0.1	*4/5/57:*
4/8/57	7.2	7.6	1.4	Vit. D, 150,000 U/D
4/10/57	6.6	7.8	1.3	Ca gluconate, 12 gm./D
4/12/57	7.4	6.5	1.9	Low P diet
4/15/57	8.2	—	3.2	
4/17/57	7.4	6.1	1.3	
4/19/57	7.4	6.7	2.9	
4/22/57	7.8	5.6	1.8	
4/24/57	8.5	5.6	2.7	
4/29/57	8.5	6.5	1.7	
5/1/57	8.9	4.7	0.7	
5/3/57	9.4	6.1	0.3	*5/3/57:*
5/6/57	8.7	3.8	2.2	Amphojel R,
5/8/57	9.0	6.1	—	15 c.c. t.i.d.
5/10/57	9.2	5.6	2.6	*5/9/57:*
5/13/57	8.5	5.6	2.5	Vit. D, 200,000 U/D
5/17/57	9.6	5.3	0.9	

urinary 17-ketosteroid excretion was 7.1 mg.; the 17 OH corticosteroid excretion, 7.3 mg. per 24 hours. The electrocardiogram on admission revealed prolongation of the QT interval to 0.42 second, mainly due to lengthening of the ST segment. The electro-encephalogram on admission revealed normal voltage; absence of alpha rhythm, a basic rhythm of six per second, and slow waves of four per second in all leads seen best on hyperventilation.

The patient's course in the hospital was remarkable in several respects. Prior to the initiation of specific therapy she experienced many episodes daily of painful spasms of the muscles of the extremities, neck and trunk, with carpopedal spasm, extension of the legs and arching of the back. There were periods of unresponsiveness during which she appeared to be awake, and other periods lasting for several hours during which she was in a comatose state. These inactive periods alternated with restlessness and attacks of tetany.

Therapy was begun with oral administration of 12 gm. calcium gluconate and of 150,000 units of vitamin D_2 (calciferol) daily. The patient was given a diet free of dairy products. Serum calcium, inorganic phosphorus and alkaline phosphatase

were determined three times weekly, and Sulkowitch's test was done frequently. The level of serum calcium rose gradually, and became nearly normal (8.5 mg.%) after three weeks of therapy, but the serum phosphorus level remained elevated (5.6 mg.%). Aluminum hydroxide gel, 15 ml. three times daily, was therefore added to the regimen. The daily dose of vitamin D was increased to 200,000 units five weeks after therapy was begun because the serum phosphorus level still remained somewhat elevated (table 1).

The patient's clinical response to therapy was rapid and one of steady improvement. Within 72 hours there was a noticeable decrease in muscular tonicity, so that movements became less jerky and better coördinated. The frequency and severity of the recurrent attacks of tetany began to decrease. She became more alert and her speech more intelligible, and all aspects of mental function showed improvement. On the sixth day of therapy the neurologic examination revealed an alert patient and intact orientation, but there were impairment of retention and recall, and inability

FIG. 2. The patient on admission (left) and six weeks after therapy was begun (right).

to concentrate and to perform simple arithmetic problems. She had poor insight concerning her illness and current events. She manifested a moderate degree of intellectual retardation. By the fifth day she was having only slight tetanic spasms, but muscle tone and coördination of movements were normal. Chvostek's and Trousseau's signs had disappeared; she was able to walk [illegible] but with a stooped gait due to a kyphoscoliosis. Her speech [illegible] lisp that had been a lifelong characteristic [illegible] tetany had completely disappeared [illegible] the exception of moderate intellectual deficit [illegible] prior to this illness. She began [illegible] became an energetic, [illegible] weight had increased to [illegible] (figure 2).

Subsequent [illegible] three weeks after therapy was begun [illegible] generalized paroxysmal

dysrhythmia in the form of bursts of 6/second waves, slowing to 4/second on hyperventilation. This abnormal activity was somewhat improved with the appearance of some alpha rhythm five weeks after the clinical manifestations of tetany, and her mental state had cleared. At the same time the electrocardiogram improved, so that about 12 days after the onset of therapy the hypocalcemic changes were less marked, and three weeks later the electrocardiogram was entirely normal (five weeks after the onset of therapy).

Discussion

This patient presents many of the classic features of hypoparathyroidism. The mental picture and tetany were striking. The alopecia, history of convulsions, and cataract, and the roentgenographic, electrocardiographic and electroencephalographic changes are not unusual. Features which are common but which this patient did not manifest are papilledema, increased intracranial pressure, and trophic changes of skin and nails. The absence of digestive and hematologic disturbances and the presence of low blood calcium, low alkaline phosphatase, high phosphorus and normal amounts of fat in the stools permitted the exclusion of tetany associated with steatorrhea. There was no evidence of kidney disease or of pseudohypoparathyroidism, and no history of thyroid surgery or neck irradiation. Finally, the thyroid, adrenocortical and gonadal functions were normal. Thus the monoglandular nature of the insufficiency involving solely the parathyroids was established. Hypoparathyroidism associated with insufficiency of other endocrine glands has been reported by Perlmutter et al.[7] (1956), Whitaker et al.[8] (1956) and Leonard[9] (1946). There was no evidence of moniliasis. In the absence of any detectable cause, the case was classified as idiopathic hypoparathyroidism.

The most noteworthy feature of this case is the rapid and complete clinical recovery following an unusually prolonged and severe course of mental deterioration. The patient had had a moderate degree of intellectual deficiency prior to the onset of the disease, and this original level of mental function became evident after adequate therapy. Mental deterioration had apparently begun three years prior to admission and was progressive, leading to severe impairment of sensorium and mental function in all spheres. She manifested noticeable improvement within 72 hours after therapy was instituted, two weeks before the serum calcium reached even the low limit of normal and when the phosphorus level was still very high. This response is unusual, since most patients with such mental changes require several weeks or longer to return to their previous mental state. Indeed, a case has been reported by Mortell[10] (1946) in which there was no change in the pronounced mental symptoms while other features improved with treatment. Idiopathic hypoparathyroidism may be associated with such symptoms as depression, excitement, toxic delirium, personality changes of various types, intellectual deficiency, mental deterioration, mental dullness and impairment of sensorium as described by Grant[3] (1953), Sugar[4] (1953), de Mowbray[5] (1953), Robinson et al.[6] (1954) and Simpson[11] (1952). These mental changes have not been reported as the initial symptoms, and are usually manifest only after prolonged hypocalcemia in idiopathic hypoparathyroidism.

Postoperative hypoparathyroidism is commonly associated with an early onset of mental symptoms constituting a toxic psychosis as reported by Sugar[4] (1953),

de Mowbray[5] (1953) and Greene[16] (1941). Greene[16] pointed out that delirium, which may be followed by dementia, may be the first and only clinical manifestation of postoperative hypoparathyroidism, and that it improves rapidly with a return of the electrolytes to normal levels. The cause of the mental changes is not known.

Tetany is the most common presenting symptom of idiopathic hypoparathyroidism, as reported in 70% of the 57 cases in the literature reviewed by de Mowbray[5] (1953). Alopecia appeared to be the earliest symptom in our patient, since it began 12 years prior to the onset of painful muscle cramps. The increased density of bones seen in our patient is occasionally found in hypoparathyroidism. It may well be that the unusually long duration of the disease prior to therapy caused the increased mineral content of bones to reach a level permitting detection in the roentgenogram.

Seizures are the second most common presenting symptom, according to de Mowbray[5] (1953), occurring in 42% of the 57 cases reviewed. Reports as to the frequency of the occurrence of generalized seizures in tetany differ widely. Simpson[11] (1952) states that seizures are common and may occur prior to the onset of tetany. Sugar[4] (1953), on the other hand, states that most patients with hypocalcemia do not have seizures. The mechanism of production of seizures is unknown. Sugar[4] (1953) discusses the theory that seizures are due to water retention and/or a disturbance in surface function of brain-cell membranes due to the low calcium, which permits the building up of abnormal electrical potentials.

The various aspects of papilledema and seizures were reviewed by Grant[3] (1953), who found the occurrence of convulsions to be the feature most commonly associated with papilledema and tetany, although they may occur without papilledema. Some authors, such as Gotta and Odoriz[12] (1948), believe that the low calcium and elevated phosphorus precipitate seizures in individuals with low thresholds and idiopathic seizure states, but Grant[3] (1953), Gotta[13] (1951) and others do not accept this explanation. Grant[3] (1953) pointed out that the convulsions are controlled by primary therapy of the hypoparathyroidism and not by anticonvulsants.

In this patient's history the occurrence of two convulsions prior to the onset of tetany suggests the possibility of a preëxisting seizure state. However, convulsions were a prominent feature during the first two years of the tetanic illness. No convulsions were recognized during the latter two years, in which the mental symptoms were pronounced. The occurrence of seizures early in the illness was probably responsible for failure to recognize the disease.

The electro-encephalographic changes in hypoparathyroidism have been described by numerous authors including Grant[3] (1953), Sugar[4] (1953), de Mowbray[5] (1953), Simpson[11] (1952), Gotta and Odoriz[12] (1948), Gotta[13] (1951), Grieve[14] (1955) and Odoriz et al.[15] (1944). Abnormal electro-encephalographic records are said to be found in over 80% of cases of chronic tetany by Klotz and Stroun[16] (1955), who found that such a record has no practical significance in the differential diagnosis of the cause of tetany. Grant[3] (1953) and others have found that the electro-encephalogram is usually abnormal in chronic hypoparathyroidism, with or without the occurrence of seizures. There are no characteristic changes, but usually there are little or no alpha

rhythm, an occasional increase in the amount of fast activity, simple spikes and, commonly, slowing to from three to seven waves per second. Hyperventilation accentuates these and brings out latent changes. There is no exact correlation between the electro-encephalographic changes and the serum calcium, but the duration of the hypocalcemia appears to be more important than the calcium level. The electro-encephalogram returns to normal several weeks after the serum calcium and phosphorus have been maintained at normal levels (Grant [3] (1953); Sugar [4] (1953) and Schalk an and Ratner [17] (1955)). Often the degree of improvement in the electro-encephalogram is incomplete after treatment (Sugar [4] (1953)). Grant [3] (1953) stated that this delayed response suggests an intracellular fluid and metabolic disturbance that require readjustment.

The electro-encephalographic changes of our patient conform to those described by others. It is of interest that after three weeks of adequate treatment the pattern changed from a generally slow record to one of generalized paroxysmal cerebral dysrhythmia which persisted throughout the remainder of the hospitalization. Whether the persistently abnormal electro-encephalographic record is indicative of a preëxisting epileptic state or secondary to permanent changes within the brain is uncertain.

Although papilledema due to increased intracranial pressure has been reported in cases of long standing idiopathic hypoparathyroidism, it is said by Grant [3] (1953) to be relatively rare. Our patient illustrates the fact that mental symptoms may be pronounced without elevated intracranial pressure. Cataracts resulting in decreased visual acuity are a common feature of this disease and may be the presenting symptom (de Mowbray [5] (1953)). A cataract was found in the left eye, but the patient herself failed to report decreased vision. Headaches may be severe, as reported by Simpson [11] (1952), but were absent in our patient.

Extrapyramidal dyskinesias, such as hypertonicity and choreiform movements, have been reported in hypoparathyroidism by Simpson [11] (1952) and Gsell [19] (1950). These features were present in our patient and were accentuated by attempted voluntary movements, especially walking. These signs cleared rapidly with treatment.

Intracranial calcifications are not unusual in postoperative hypoparathyroidism, but are relatively rare in the idiopathic form, according to Sugar [4] (1953) and Burghardt [20] (1953). Originally, following the early descriptions by Pick [21] (1902) and Fahr [22] (1930), it was thought that the tetany was the result of the cerebral calcifications, but the cerebral lesions are now generally considered to be caused by hypoparathyroidism (Sugar [4] (1953) and Wagner et al.[23] (1954)); however, the mechanism is not known. In roentgenograms the calcifications are usually seen as bilaterally symmetric collections of fine-to-heavy densities in the area of the basal ganglia, as was the case in our patient. The pathologic findings at autopsy on patients with idiopathic as well as postoperative hypoparathyroidism have been reported by Sugar [4] (1953), Burghardt [20] (1953), Wagner et al. [23] (1954), Eaton and Haines [24] (1939), Eaton et al.[25] (1939), and Jordan and Kelsall [26] (1951). The changes are chiefly those of [illegible] and calcification of capillaries and smaller arteries and veins, [illegible] in the caudate and globus pallidus, but to a slight[illegible] and occasionally in other areas of [illegible] chiefly in the [illegible] the parenchyma.

Degeneration and loss of ganglion cells have been reported in areas of the basal ganglia where the vascular degeneration is severe (Sugar[4] (1953)). Why the basal ganglia are primarily involved is not known, but Sugar[4] (1953) postulated that the characteristics of the circulation in these areas may be pathogenetically related, since these are also the areas involved in disease states producing cerebral hypoxia. The occurrence of calcification in the basal ganglia is not restricted to hypoparathyroidism, and is not necessarily accompanied by neurologic symptoms. There seems to be no correlation between the clinical picture and the degree of calcification. The calcification has not been reported to regress following therapy.

The electrocardiographic changes characteristic of hypocalcemia reverted to normal only when the blood calcium level became normal. This special behavior of electrocardiographic changes due to hypocalcemia has previously been recorded by White et al.[27] (1929) and Barker et al.[28] (1937), and more recently by Schulman et al.[17] (1955). The dissociation between the response of tetany and of the electrocardiogram to calcium administration was reported by Sussman et al.[29] (1957).

Summary

A case of idiopathic hypoparathyroidism occurring in a young woman is presented. The four-year duration, pronounced severity and relative completeness of symptomatology and rapid recovery are of interest. The clinical features of this condition are discussed.

Summario in Interlingua

Es presentate un caso de hypoparathyroidismo idiopathic, occurrente in un femina de racia blanc de 30 annos de etate. Aspectos de interesse es que le morbo habeva durate quatro annos quando illo esseva diagnosticate, que le symptomatologia esseva relativemente complete e sever, e que le restablimento del patiente esseva rapide.

Al initio—i.e. quatro annos ante le admission del patiente al Hospital del Collegio Medical Jefferson a Philadelphia—le morbo esseva characterisate per perdita de capillo. Attaccos de tetania generalisate de severitate e frequentia progressive e convulsiones generalisate predominava durante le sequente duo annos. Disturbation de omne aspectos del functionamento mental deveniva apparente e progredeva in le curso de tres annos usque a grados sever de deterioration. Durante le quarte anno le patiente esseva incapace de ambular a causa de spastic movimentos torsional de su trunco e de su extremitates. Illa non poteva attender a su exigentias personal, e su parola esseva inintelligibile. Su historia medical revelava un annue perdita partial de capillo depost le etate de 14 annos, le perdita del complete dentatura al etate adolescente, e duo pregnantias sin incidente que habeva occurrite septe e cinque annos ante le hospitalisation.

Le examine del patiente revelava le sequente significative constatationes anormal: Un peso de 86 libras, alopecia quasi complete, imperfectiones de comprehension e attention, responsas tardive, disorientation, deterioration intellectual, parola dysarthric, opacitate del lente sinistre, hypertonicitate e movimentos dyscinetic del trunco e del extremitates, e positive signos de Chvostek e Trousseau. Le patiente habeva frequente generalisate spasmos tetanic de grado sever, e su stato de conscientate alterava inter semistupor e coma. Aspectos del morbo que es commun sed que non esseva manifeste in le presente caso es papilledema, augmentate pression intracranial, alterationes trophic del pelle e del ungulos, e moniliasis.

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Le constatationes laboratorial que esseva congruente con le diagnose de hypoparathyroidismo includeva un basse nivello sanguinee de calcium, un basse nivello de phosphatase alcalin e un alto nivello de phosphoro, le alterationes electrocardiographica typica de hypocalcemia, calcification intracranial in le area del ganglios basal, e un generalmente lente activitate electro-encephalographic. Varie altere examines excludeva specialmente le possibilitate de steatorrhea, morbo renal, e altere dysfunctiones endocrin.

Le therapia consisteva del administration oral de gluconato de calcium, vitamina D_2 (calciferol), e un dieta libere de productos lactee. Subsequentemente, gel de hydroxyde de aluminium esseva adhite al regime proque tres septimanas post le initiation del medication le phosphoro del sero monstrava ancora nivellos elevate. Le plus notabile aspecto de iste caso es le rapide restablimento clinic post un inusualmente longe e sever maladia. Melioration symptomatic esseva recognoscibile in omne respectos intra 48 horas. Illo esseva complete intra un septimana, con le exception del recrescimento de capillo e le augmento del peso corporee. Istos progredeva rapidemente. Le melioration clinic precedeva le restitution de nivellos normal de calcium e phosphoro in le sero. Le normalisation de iste magnitudes requireva tres e sex septimanas, respectivemente. Le electro-encephalogramma e le electrocardiogramma attingeva lor maximos de melioration tres septimanas post que le calcium del sero habeva redevenite normal. Le moderate residue defectuositate mental que persisteva es probabilemente un phenomeno que esseva presente ante le declaration del symptomas de hypoparathyroidismo.

Un revista del litteratura pertinente al aspectos clinic de hypoparathyroidismo idiopathic es presentate. Le datos es discutite in connexion con le aspectos correspondente del presente caso.

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THE UTILIZATION OF CALCIUM FROM LACTATE, GLUCONATE, SULFATE AND CARBONATE SALTS BY YOUNG COLLEGE WOMEN [1, 2]

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ONE FIGURE

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Calcium salts are often used as a source of calcium for comparison in experiments designed to study utilization of calcium from different foods. It is also a common practice for the physician to recommend the use of tablets of calcium salts to supplement that from food sources at times when large amounts are needed, such as during pregnancy and lactation. It has been found in studies of the dietary habits of older persons that many of them consume diets inadequate in calcium, and if they have not been accustomed to drinking milk, it is difficult to get them to increase their calcium intake by this means. Calcium salts have been used in such cases. In order to improve diets of the general population, particularly under emergencies such as war conditions, calcium salts have been used in the enrichment of breadstuffs and meat products. Under still other conditions, due to unequal distribution or inadequacies of food supplies, calcium salts have been recommended as a means of increasing calcium intakes. Hence it seemed worthwhile to compare the utilization of calcium from some of the salts that are used as supplements.

[1] This paper is condensed from the dissertation submitted by Mary Brown Patton in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Foods and Nutrition in the Graduate School of The Ohio State University.

[2] Journal Article 35-52, Ohio Agricultural Experiment Station.

Studies in which salts have been the sole source of calcium in the diet have been limited almost entirely to those with experimental animals since it is very difficult to obtain permission of human beings to subsist on a purified diet for a sufficient length of time to obtain reliable results. However, in one study three human subjects were maintained for 7 weeks on a purified diet in which the calcium was furnished by a mixture of phosphate and carbonate salts at a level of 879 mg of calcium daily. A natural diet supplied 789 mg daily for a three-week period (Hathaway, '49). The calcium from food sources was better utilized than that from the salts in all three cases.

Studies have been made with human beings in which the calcium in a diet of natural foods has been supplemented by that from various salts. The purpose of most of these studies has been to compare the utilization of calcium from food sources rather than that from the different salts. Two studies made among young children may be cited in which the generalization was reached that calcium from salts is retained as well as that from milk (Kempster et al., '40; Stearns and Jeans, '34). Several studies made among adults may be cited in which it was shown that calcium in salts was utilized to approximately the same extent as that in milk (Drake et al., '49; McQuarrie et al., '47; Schroeder et al., '46; Steggerda and Mitchell, '39, '46). In another study (McCance and Widdowson, '42) the conclusion was reached that the form of the salt used in bread as enrichment was not important, but that the type of flour was a factor due to the amount of phytic acid present.

PROCEDURE

The young women selected as subjects had been college students for at least one year, had no strong dislikes or allergies for foods in the diet, and all were considered healthy by the examining physician.[3]

[3] Dr. Keith Frankhauser, medical officer in charge of a Nutrition Unit of the U. S. Public Health Service stationed in Ohio in 1948–1950.

The diet differed from day to day but remained the same from week to week. Each individual food was weighed for each subject although certain ones may have been combined and served. Aliquots of a weekly food composite were dried in an oven at 60°C. and samples were burned in an oxy-calorimeter. The diet contained an average of 2179 cal. daily according to this method of determination (Fuqua, '48). Those subjects who required additional calories were given weighed amounts of foods of low calcium content. The fact that all of these young women maintained their weight within the expected fluctuations of slight weekly gains and losses indicates that the diet was probably adequate in calories.

The protein content of the diet was determined by the method of the Association of Official Agricultural Chemists ('45); the calcium by the Stearns ('29) modification of the McCrudden method; and the phosphorus by the Fiske and Subbarow ('25) molybdate method. The diet supplied an average of 57 gm protein, 347 mg calcium, and 758 mg phosphorus daily.

The ascorbic acid, thiamine, and riboflavin contents of the diet were calculated from food tables. The diet was estimated to be adequate in all respects except riboflavin and calcium. To bring the riboflavin intake to the recommended level each subject received one milligram of riboflavin daily in tablet form. The basal diet, containing 347 mg of calcium per day, was supplemented by 400 mg of calcium daily from the salts which were added in approximately equal portions at the beginning of each meal. The total of approximately 700 mg was the amount considered to be just below the critical level, that level above which an additional dietary increment would not be expected to result in additional storage but have a retention value of zero. As recommended by the Food and Nutrition Board of the National Research Council ('48), the diet was further supplemented with 500 I.U. of vitamin D in the form of "Viosterol" given daily at breakfast time.

Excreta were collected according to the customary procedures. Carmine was used as a fecal marker. Calcium was

precipitated from aliquots of an acidified composite of urine. Aliquots of feces and food composites were wet ashed and calcium determined by a modification of the McCrudden method as described by Stearns ('29).

TABLE 1

Plan of study of the utilization of 4 calcium salts

PERIOD	LENGTH OF PERIOD	SUBJECTS				
	day					
I	2–7	I No supplement	II No supplement	III No supplement	IV No supplement	V No supplement
I	2–7	VI No supplement	VII No supplement	VIII No supplement	IX No supplement	
II	2–7	I Lactate	II Sulfate	III Gluconate	IV Carbonate	V Gluconate
II	2–7	VI Sulfate	VII Lactate	VIII Carbonate	IX Gluconate	
III	2–7	I Carbonate	II Lactate	III Sulfate	IV Gluconate	V Carbonate
III	2–7	VI Gluconate	VII Sulfate	VIII Lactate	IX Carbonate	
IV	2–7	I Gluconate	II Carbonate	III Lactate	IV Sulfate	V Lactate
IV	2–7	VI Carbonate	VII Gluconate	VIII Sulfate	IX Lactate	

Serum-calcium levels were followed for each subject on blood samples collected at the beginning of the study and at the close of each experimental period. Determinations were made in the laboratory of the Ohio Department of Health.[4]

The Statistical Laboratory of The Ohio State University planned an experimental design (table 1) in which 8 subjects (Subjects I to VIII, inclusive) were to be studied. A 9th subject was included and she received the supplements indicated in table 1.

[4] These determinations were made by E. C. Tabor, chief chemist with a Nutrition Unit of the U. S. Public Health Service stationed in Ohio in 1948–1950.

RESULTS AND DISCUSSION

The calcium balances of 9 subjects during periods II, III, and IV are reported in table 2. A statistical analysis of the data for Subjects I to VIII, inclusive, was made by the method of least squares and no significant effects of salt, individual, or time (order in which the various salts were taken) could be found. If it is assumed that significant differences do exist, the question arises as to why these data fail to reveal such differences. These suggestions are offered: too few subjects, sensitivity of statistical method, and individual differences offset treatment differences.

TABLE 2

Calcium balances of 9 young women during three experimental periods when a low calcium diet was supplemented by 400 mg of calcium from salts

PERIOD	SUBJECT								
	I	II	III	IV	V	VI	VII	VIII	IX
	mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
II	24	70	76	47	17	32	— 25	53	73
III	— 90	— 13	65	18	— 48	10	6	88	— 1
IV	— 4	— 48	— 61	— 27	— 83	— 36	157	18	— 60

Among the differences in expected and observed values of the 24 balances of these 8 subjects, the two largest were associated with Subject VII. Observation of the values in table 2 reveals that this subject did show unusual variation, particularly as far as the time element was concerned. Inspection of the fundamental data shows that she was in negative balance during the first experimental period while all the other subjects were in positive balance. During the third experimental period this subject showed a comparatively large positive balance and all other subjects, except one, were in negative balance.

If Subject VII is replaced by Subject IX, who was observed at the same time, the symmetry in treatments will be lost. However, if it is assumed that the treatment effects are zero, an analysis of these new data may be made to see if the

statistical method used would then reveal any significance in time or in individual subject differences. When this replacement was made the mean retention on the sulfate treatment was increased from 27.33 to 31.60 mg; the mean for the lactate treatment was reduced from −11.67 to −17.50 mg; the mean for carbonate treatment was increased from −20.30 to −17.57 mg; and the mean for the gluconate treatment was decreased from 45.67 to 31.67 mg.

Based on the values when Subject IX is substituted for Subject VII, it was shown by analysis that the individual differences in the subjects cannot be considered as significant but that time produced a difference in calcium balances among these subjects that is significant at the 5% level.

Further analysis of the data, using all 9 subjects, was made to determine the percentage of utilization of each salt according to the method used by Steggerda and Mitchell ('46). In 4 cases the balance on a supplement was less than that on the basal diet alone and these cases were termed zero utilization. The mean value for carbonate utilization was 10.79%; for sulfate, 20.92; for lactate, 11.45; and for gluconate, 28.79. The less soluble salts, carbonate and sulfate, were as well utilized as the more soluble ones, gluconate and lactate. Schroeder et al. ('46) reported an average of 23.65% utilization of the calcium of calcium sulfate. Average utilizations of 20 and 25% have been reported for gluconate in two studies by Steggerda and Mitchell ('39, '46). Stearns and Jeans ('34), in a study with young children, reported variable results for lactate, gluconate and carbonate but fairly consistent results with phosphates. They reasoned that the difference was due to the variation brought about in the calcium-phosphorus ratio. The senior author of the present study was unable to relate balance to calcium-phosphorus ratio in a study among a group of 18 young college women (Patton et al., '52). The values reported above for the 4 salts used in the present study are within the range reported by these other workers and within the range of that reported for milk, the food recognized by nutritionists as the best source of calcium.

In studying the percentage utilization of the salts by the 9 subjects reported herein, it was noted that 7 of the 9 subjects utilized to the largest percentage the salt taken first, regardless of which salt it was (fig. 1). The correlation coefficient of utilization and rank was 0.67 which is significant for this number of cases. The time at which a salt was taken was shown to be a factor when the data for the 8 subjects (Subject IX substituting for Subject VII) were analyzed by the method of least squares.

Blood samples were taken from each subject at the beginning of the study and every two weeks thereafter and analyzed for

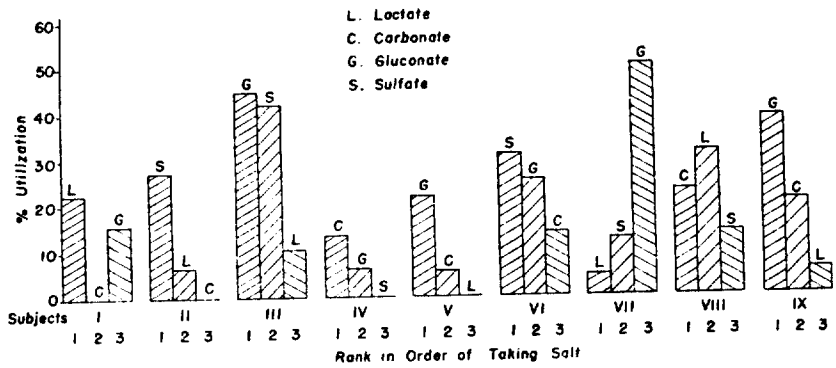

Fig. 1 Relation of the per cent utilization to the rank in order of taking a salt.

serum-calcium. The average value at the beginning was 9.8 mg % and at the end of the no-supplement period, 10.5. The levels for the periods when each of the salts was given are practically the same, 11.3, 11.5, 11.5, and 11.8, respectively, for gluconate, carbonate, lactate, and sulfate. All of the values were within the normal range of 9 to 11 mg %. The increase in blood level that was noted when 400 mg of calcium were added to their diets as salts is not in agreement with the statement by Sherman ('47) that "in normally nourished individuals, the intake of food calcium or even the administration of calcium salts alone has hardly a measurable direct influence upon the blood calcium level."

The average utilization of calcium from the three salts taken by each subject was compared with the basal heat production per square meter per 24 hours. Three subjects, whose basal metabolic rate was slightly above the Aub-DuBois predicted standard but within normal range, showed an average utilization of 22.7%. A 4th subject, whose rate was slightly below the standard but within the normal range, showed an average utilization of 21.5%. The basal metabolic rates of the remaining 5 subjects were below their predicted standard by from 7 to 27%. The average utilization of the salts by these 5 subjects was only 14.0%. These results suggest a possible relationship between calcium utilization and basal metabolic rate. Johnston and Maroney ('36) have shown that poor diets in general result in low energy metabolism. Although a record was made of the foods eaten by each subject in the study reported herein on the day previous to the beginning of the study, it has not been considered as an evaluation of their customary eating habits. This observation emphasizes the importance of noting previous dietary habits in studies such as the one reported.

The difficulty of separating feces into portions that represent those from the several periods offers a possible source of errors in studies such as this one. Two subjects, who were very regular in their habits of elimination, had formed stools in which the carmine usually appeared in from 24 to 36 hours, and the demarcation was clear cut, showed a fairly consistent utilization of calcium from the salts that supplemented their diets. One subject was constipated and the carmine usually remained in the digestive tract for from 4 to 5 days. However, this subject showed the greatest average percentage retention of calcium of any of the subjects studied. The remaining 6 subjects varied considerably as to the nature of their stools and the time of passing the carmine.

Leichsenring et al. ('51) reported that a group of 18 college women were able to adjust to the level of calcium in a basal diet similar to the one used in this study within one week. For this reason that period was used for each of the experi-

mental periods in this study. The data show that, regardless of the nature of the salt taken as a supplement, if it happened to be the first salt following the preliminary adjustment period when the intake was 300 mg, its retention was greater than that for the same or any other salt at a later period. This casts some doubt as to whether or not these 9 subjects made the adjustment from the 300 to the 700 mg level during the one week allowed for adjustment.

Another implication from these data might be that human beings do not efficiently utilize calcium from salts for periods of more than a couple of weeks. A study has been carried out in this laboratory to obtain additional information on this subject and a report of the findings is in preparation.

SUMMARY

A study was made of the utilization of calcium by 9 college women from 4 salts (gluconate, lactate, carbonate, and sulfate) given in amounts equivalent to 400 mg of calcium daily. The basal diet contained an average of 347 mg of calcium daily and calculation of other nutrients showed that the diet was adequate.

Statistical analysis of the balance data was made by the method of least squares. No significant differences in the utilization of calcium from these salts were found, the calcium from the less soluble salts being utilized as well as that from the more soluble ones.

The order in which the salts were taken was shown to be a significant factor in the utilization. In the majority of the cases, the salt taken first was utilized to the largest percentage regardless of which salt it was.

The average percentage utilization from all salts for all subjects was approximately 18%, a value within the range of those reported in the literature for utilization of calcium from food sources.

Young women having normal basal metabolic rates showed somewhat greater percentage utilization of calcium from salts than those whose rate was below the predicted normal.

The serum-calcium levels were normal, 9 to 11 mg per 100 ml, during the 8 weeks of the study and were practically the same for the periods when the 4 salts supplemented the basal diet.

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FURTHER EXPERIMENTS ON THE UTILIZATION OF CALCIUM FROM SALTS BY COLLEGE WOMEN [1]

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ONE FIGURE

(Received for publication September 15, 1954)

In an earlier study made by Patton and Sutton ('52) in this laboratory on the utilization of calcium from 4 salts there were indications that the order in which the salts were taken was a factor in the utilization of calcium from these sources. In that study a subject was given one of the salts for two 7-day periods, then a second salt for the same period of time and finally a third one. It was observed that, in general, the calcium from the first salt taken was utilized to a greater percentage than subsequent ones. This posed the question as to whether or not the utilization of calcium salts by human beings was limited by the time factor. The present study was planned to give a subject the same salt for a period of 6 weeks with the objective of determining whether or not she would utilize the calcium from the same salt equally well from week to week.

Milk was given to another group for the same length of time in quantities sufficient to supply a comparable amount of calcium for comparison of utilization with that from the salts.

The data from the earlier study (Patton and Sutton, '52) also gave indication of a relationship between calcium utilization and basal metabolic rate. The present study was designed to give further consideration to this relationship.

[1] Journal Article No. 42-45, Ohio Agricultural Experiment Station.

PROCEDURE

The design of the experiment was developed by the Statistics Labortaory of The Ohio State University. Nine college women, considered by a physician to be in good health, were selected for study. The basal metabolic rates were observed and the subjects were classified into three groups on the basis of their variation from normal standards for their age, sex and body surface. In each group the basal metabolic rates of the three subjects varied from approximately 12% below the Aub-Dubois standard to approximately the predicted normal rate of the same standard. One group received milk as a supplementary source of calcium in the basal diet; the second group, calcium gluconate; and the third group, calcium carbonate.

The basal diet of the earlier study by Patton and Sutton ('52) was modified by substituting French bread for white bread in several meals during the week in order to reduce the calcium content of the daily diet to a level of approximately 300 mg. It met the recommended allowances of the National Research Council in all other nutrients except riboflavin, which was given at the 1 mg level in tablet form. The diet was further supplemented by 400 I.U. vitamin D. Seven menus were planned and repeated 8 times during the study. The first week was termed an adjustment period and no balance was determined. The customary balance techniques were followed during the remaining 7 weeks. The first experimental period was used to establish the base line for calculation of the utilization of calcium in the supplements taken during the succeeding 6 weeks. Milk supplied 305 mg of calcium and the gluconate and carbonate salts each furnished 280 mg. The basal diet plus supplements provided these subjects with a daily calcium intake of approximately 600 mg. Apples, a food low in calcium, were given ad libitum to provide additional calories for those whose appetites were not satisfied with the basal diet. All food portions were weighed to 0.1 gm and were completely consumed by all subjects.

Aliquots of 24-hour urine specimens were preserved with concentrated hydrochloric acid. Portions of the weekly composite were analyzed for calcium by the McCrudden method as modified by Stearns ('29) without preliminary ashing. Using the method of this same investigator, hydrochloric acid digests of food and feces composites were wet ashed, the calcium precipitated as the oxalate, and titrated against 0.01 N potassium permanganate.

The experiment took place in January and February so there was no appreciable loss in perspiration.

RESULTS

For a period of one week the 9 subjects were fed the basal diet which contained the daily average of 307 mg of calcium in order that they might become acquainted with the routine and make adjustments from their freely-chosen diets to the experimental diet. Collection of excreta and food began on the 8th day and continued through the 49th day. The balances for the 9 women during the 7 weeks of observation are reported in table 1. All subjects lost calcium on the basal diet, the amounts varying from 9 to 113 mg with an average of 48 mg.

By adding approximately 300 mg of calcium to the diet in the form of carbonate or gluconate salts or milk it was expected that the subjects would attain equilibrium. It will be noted from table 1 that 7 of the 9 subjects were in positive balance during the first week of supplementation. All subjects that were receiving milk and calcium carbonate in addition to the basal diet (with an average intake of 645 and 623 mg of calcium, respectively) were in positive balance. Only one of the three subjects receiving calcium gluconate was storing calcium. It will be noted that one of the subjects in the gluconate group who was losing calcium, P. R., continued to lose calcium for the duration of the study; the other, R. S., lost during 4 of the 6 periods of supplementation. The motility of the gastro-intestinal tract may have been a factor

in the case of P. R. since she passed an average of two stools per day.

The balance data for the second, third, 4th, 5th and 6th periods indicate a pattern of loss and storage which occurs with some rhythm on alternate weeks for 4 of the subjects.

TABLE 1

Calcium balances, in milligrams, of 9 college women on a basal diet and during 6 periods of supplementation with milk, calcium gluconate, and calcium carbonate

SUBJECT	PERIOD OF BASAL DIET [1]	PERIOD OF SUPPLEMENTATION [1]					
		1	2	3	4	5	6
			Milk				
J. M.	— 42	57	— 10	67	— 13	19	72
M. E. W.	— 59	41	— 66	34	— 106	— 76	2
L. Y.	— 113	30	— 80	— 37	— 90	— 21	60
			Calcium gluconate				
O. D.	— 9	47	39	— 2	30	13	72
P. R.	— 26	— 83	— 33	— 26	— 71	— 4	— 13
R. S.	— 48	— 7	— 52	49	— 64	— 44	87
			Calcium carbonate				
E. H.	— 25	62	— 29	23	— 31	25	70
R. J.	— 78	44	— 87	— 4	— 29	— 22	11
D. S.	— 34	26	— 44	20	— 71	94	0

[1] All periods were of 7 days duration.

As pointed out above, P. R. was in negative balance during the 6 periods and O. D., in the same group, was storing or in equilibrium (— 2 mg) during the 6 periods.

These data were analyzed by the Statistics Laboratory of The Ohio State University to determine the significance of the differences in balances due to form of calcium used to supplement the basal diet. The method of least squares was used to determine the significance of these differences. It was shown that the differences in balances on the three treat-

at the beginning. The retentions of 7 of the 9 subjects were largest during the 5th and 6th periods; the remaining two subjects retained the largest amounts in the first period. Since the kind of supplement was not a factor in utilization this comparison may indicate a trend of inverse relationship as shown by the balance data using the initial basal metabolic rate. Subsequent studies of calcium utilization will be designed to obtain additional information on this relationship.

SUMMARY

The calcium balances of 9 college women consuming approximately 600 mg of calcium were studied in relation to period to period variation, source of calcium used to supplement a low-calcium diet, and the effect of basal metabolic rate on utilization.

Approximately one-half of the calcium consumed came from foods commonly served in American diets; the other half was supplied by milk to three subjects, calcium carbonate salts to three subjects and calcium gluconate salts to the remaining three. Statistical analysis of the data showed that under present experimental conditions, milk, calcium gluconate, and calcium carbonate were utilized equally well by these subjects as a source of calcium.

Differences in balance from week to week were significant but the relative importance of the time factor was reduced during the final two weeks, the 5th and 6th periods, from that in the preceding periods.

Basal metabolic rate was inversely related to calcium utilization. In general, the basal metabolic rates of the subjects were lower at the close of the study than at the beginning and this may be related to the greater utilization during the 5th and 6th periods.

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Effect of Calcium Administration and Deprivation on Serum and Urine Calcium in Stone-forming and Control Subjects

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Idiopathic hypercalciuria is a syndrome characterized by a raised urinary calcium associated with normocalcaemia and a tendency to hypophosphataemia (Albright *et al.*, 1953; Harrison, 1959; Edwards and Hodgkinson, 1965). Hypercalciuria is common in patients with renal stone disease (Flocks, 1939; Hodgkinson and Pyrah, 1958), but is also present in a proportion of the normal population (Knapp, 1947; Hodgkinson and Pyrah, 1958; Watson and Dale, 1966; Nordin *et al.*, 1967), the exact prevalence depending on how it is defined.

The cause of idiopathic hypercalciuria has not been established, but increased calcium absorption is known to be a feature of the condition (Henneman *et al.*, 1958; Hodgkinson, 1961). Some workers believe this hyperabsorption of calcium to be primary, while others regard it as a response to increased calcium excretion (Jackson and Dancaster, 1959). In previous papers we have shown that tubular reabsorption of calcium is generally normal in idiopathic hypercalciuria (Nordin *et al.*, 1967; Peacock and Nordin, 1968). The present paper gives further data on the relation between calcium ingested, plasma calcium, and urinary calcium in normal and hypercalciuric subjects and describes a simple procedure for the diagnosis of the condition.

Clinical Material and Methods

The patients consisted of nine male idiopathic renal stone-formers who showed persistent hypercalciuria on a free diet according to the definition of Hodgkinson and Pyrah (1958)—that is, over 300 mg. daily. Their ages ranged from 24 to 54 years. In no case was there evidence of renal failure, systemic acidosis, or endocrine disease. The controls consisted of nine healthy males, all members of staff, aged 17 to 40 years, none of whom was hypercalciuric.

The hypercalciuric patients were first studied in the non-fasting state when simultaneous samples of blood and urine were obtained for measurement of calcium and creatinine. Subsequently the patients were admitted to the ward fasting and observed for one day of total starvation. Tap-water was given at a rate of half a pint (285 ml.) an hour to ensure adequate urine flow and bladder emptying. Urine samples were collected every two hours and blood was taken at the midpoint of each collection. The test usually started at 8 a.m. and was completed by 6 p.m. On another day the same patients were again admitted to the ward after an overnight fast. A two-hour specimen of urine was collected with a blood sample taken at the midpoint and the patients were then given an oral load of calcium (100 mg./kg.) as a calcium citrate suspension over a period of 15 minutes. Urine samples were collected for eight hours, hourly for the first four hours and two-hourly thereafter. Blood samples were taken at the midpoint of each urine collection. A light calcium-free breakfast was allowed half an hour after the calcium load and a light low-calcium lunch three hours later. The patients were not confined to bed. The calcium citrate was well tolerated by all of them and did not cause vomiting or diarrhoea.

In the nine controls blood and urine samples were obtained after an overnight fast for comparison with the stone cases, and the calcium citrate load was given and samples were obtained as described for the nine cases of idiopathic hypercalciuria.

Calcium was estimated with *o*-cresolphthalein complexone in serum and urine by AutoAnalyzer techniques as modified by Knowles (1968). Creatinine was estimated with picric acid by the standard AutoAnalyzer method (Technicon Methods Manual, N-11b).

Urine calcium is expressed as calcium excreted per 100 ml. of glomerular filtrate (Ca_E), which is calculated as follows (Nordin *et al.*, 1967; Peacock and Nordin, 1968):

$$Ca_E \text{ (mg./100 ml. of G.F.)} = \frac{\text{urine calcium} \times \text{plasma creatinine}}{\text{urine creatinine}}$$

when all concentrations are expressed in mg./100 ml. The range of plasma creatinine was the same in stone-formers and controls (0.7–1.1 mg./100 ml.).

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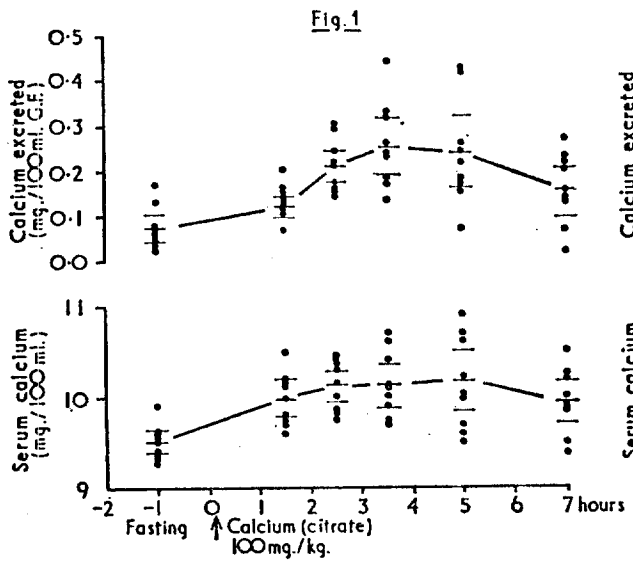
Results

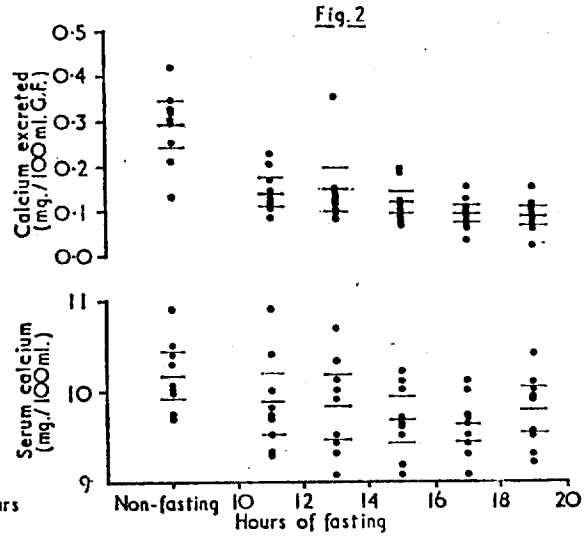
In the control cases the mean fasting serum calcium was 9.51 ± 0.063 mg./100 ml. and the mean fasting urine calcium was 0.077 ± 0.016 mg./100 ml. of glomerular filtrate (Table I and Fig. 1).

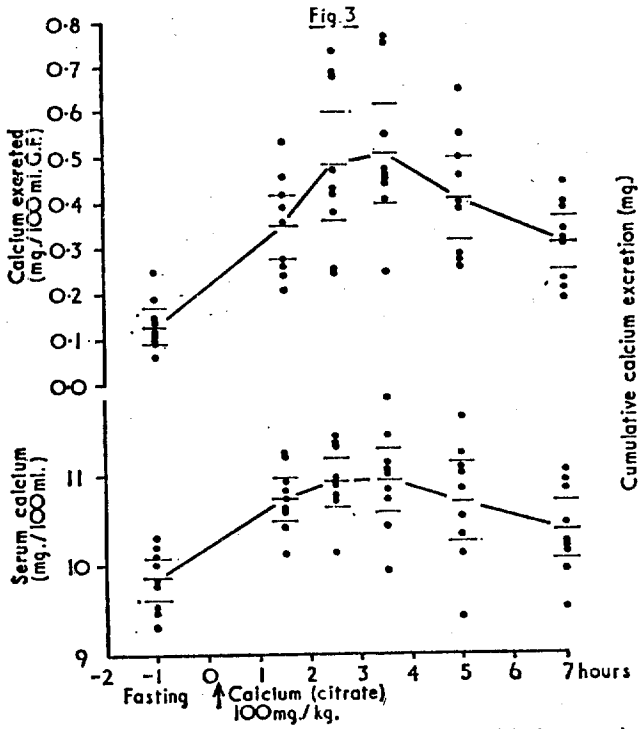
In the hypercalciuric stone-formers the mean outpatient non-fasting serum calcium was 10.17 ± 0.13 mg./100 ml. and the urine calcium 0.30 ± 0.027 mg./100 ml. of glomerular filtrate (Table II and Fig. 2). Both these values are significantly higher than the fasting normal values ($P<0.001$). Fasting overnight reduced these non-fasting values to 9.84 ± 0.18 and 0.15 ± 0.015 respectively. The fall in serum calcium is not significant, but the fall in urine calcium is ($P<0.001$) (Fig. 2 and Table II). The fasting values are still significantly higher than the fasting normal values, but only just ($P<0.05$).

TABLE I.—*Serum and Urine Calcium in Control Subjects and Hypercalciuric Stone-formers Fasting and Three and a Half Hours After an Oral Dose of Calcium Citrate (100 mg. Calcium/kg.)*

	Serum Ca (mg./100 ml. ± S.E.)				Urine Ca (mg/100 ml. of G.F. ± S.E.)			
	Fasting	3½ Hours after Load	"t"	p	Fasting	3–4 Hours after Load	"t"	p
Controls (9) ..	9·51 ± 0·063	10·13 ± 0·12	4·55	< 0·001	0·077 ± 0·016	0·26 ± 0·032	5·21	< 0·001
Stone-formers (9)	9·83 ± 0·12	10·90 ± 0·18	4·92	< 0·001	0·13 ± 0·020	0·51 ± 0·055	6·49	< 0·001
t	2·45	3·49			2·18	3·92		
p	< 0·05	< 0·01			< 0·05	< 0·01		







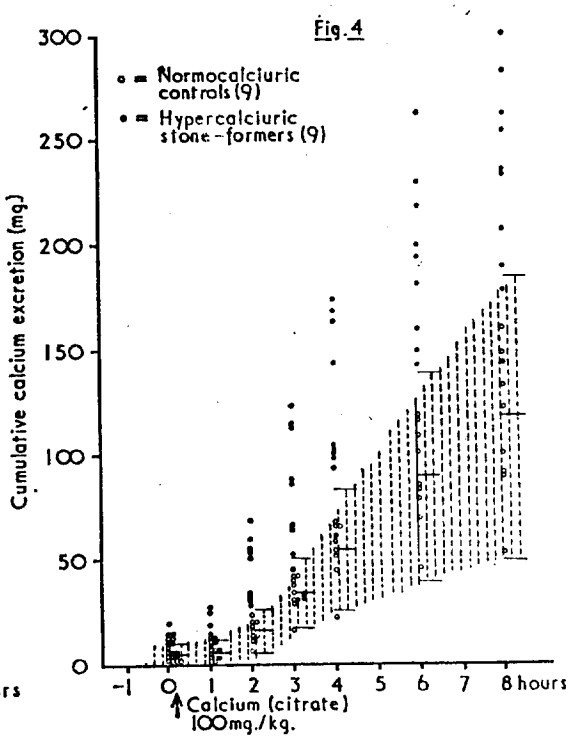


FIG. 1.—Serum and urine calcium in nine normocalciuric control subjects after overnight fasting and after administration of an oral load of calcium citrate (mean values ± 2 S.E.). FIG. 2.—Serum and urine calcium in nine cases of hypercalciuric stone disease, non-fasting (left) and during prolonged calcium deprivation (mean values ± 2 S.E.). FIG. 3.—Serum and urine calcium in nine patients with hypercalciuric stone disease fasting and after an oral dose of calcium citrate (mean values ± 2 S.E). FIG. 4.—Cumulative calcium excretion in nine normocalciuric control subjects and nine patients with hypercalciuric stone disease after an oral dose of calcium citrate (mean values ± 2 S.D).

fasting for a further six hours, however, reduced the mean serum and urine calcium values to levels which were not significantly higher than the normal fasting values.

The effect of the oral calcium load on serum and urine calcium in the control subjects is shown in Fig. 1. The mean serum calcium rose from 9.51 to 10.13 mg./100 ml. by three and a half hours, after which it remained steady for a further two to three hours. The mean urine calcium rose from 0.077 mg./100 ml. of glomerular filtrate to 0.26 at three to four hours, and was falling with the serum calcium at seven hours.

TABLE II.—*Serum and Urine Calcium in Hypercalciuric Stone-formers Before and During Prolonged Fasting*

	Serum Ca (mg./100 ml. ± S.E.)			Urine Ca (mg./100 ml. of G.F. ± S.E.)		
		"t"	p		"t"	p
Non-fasting	10·17 ± 0·13			0·30 ± 0·027		
Fasting						
10 hours	9·84 ± 0·18	1·47	<0·2	0·15 ± 0·015	4·78	<0·001
16 hours	9·60 ± 0·11	3·35	<0·01	0·096 ± 0·012	6·74	<0·001

In the hypercalciuric cases the calcium load raised the serum and urine calcium more rapidly and to a higher level than in the controls (Fig. 3). The mean peak serum calcium was 10.90 mg./100 ml. and the mean peak urine calcium 0.51 mg./100 ml. of glomerular filtrate. Both these peak values are significantly higher than the corresponding values in the controls ($P<0.01$) (Table I).

In both groups the rise in serum and urine calcium produced by the oral load was highly significant ($P<0.001$) (Table I).

The cumulative absolute calcium excretion after the oral calcium load in both groups is shown in Fig. 4. The basal urine calcium excretion tended to be higher in the hypercalciurics than in the control cases, but the overlap was considerable. After two hours there was an absolute separation of the two groups and the difference between them became progressively greater with time.

After eight hours all the hypercalciurics had excreted over 174 mg. of calcium and all the controls less than this.

Discussion

We have shown that in idiopathic male stone-formers with hypercalciuria calcium excretion is increased in the non-fasting state but generally falls to normal after an overnight fast. In those cases in which it remains raised, further fasting for six to eight hours almost invariably brings the urine calcium into the normal range. The non-fasting serum calcium tends to be at the upper limit of normal but falls to normal with calcium deprivation, generally to less than 10 mg./100 ml. In only one of our cases did the urine calcium remain high after 24 hours of calcium deprivation. Thus differences in the severity of calcium deprivation may explain in part the conflicting results obtained by previous workers. Some have reported that urinary calcium falls to normal on a low calcium diet (Henneman *et al.*, 1958 ; Harrison, 1959 ; Gill and Bartter, 1961 ; Peacock *et al.*, 1967), while others record that urinary calcium remains high (Jackson and Dancaster, 1959 ; Edwards and Hodgkinson, 1965 ; Phillips and Cooke, 1967).

It will be noted that urinary calcium after the standard calcium load was higher in the stone-formers than in the controls whether it was expressed in absolute terms or in units per 100 ml. of glomerular filtrate. This excludes the remote possibility that idiopathic hypercalciuria might be an artifact occurring in subjects with an exceptionally high glomerular filtration rate. In general, as we have discussed elsewhere (Nordin *et al.*, 1967), we advocate for most purposes that calcium excretion be expressed in mg./100 ml. of glomerular filtrate, since this eliminates differences in calcium output due to glomerular function per se.

Our results suggest that this form of hypercalciuria is generally due to hyperabsorption, as Caniggia *et al.* (1965) have claimed. This is borne out by the response to oral calcium. In normal subjects there is a measurable and significant rise in serum calcium, which reaches its peak at three to four hours, and a corresponding rise in urine calcium, presumably due to the increase in filtered load. Subjects with idiopathic hypercalciuria show a similar response, but their blood and urine values rise to a significantly higher level. This procedure may be used to distinguish subjects with absorptive hypercalciuria from normocalciuric individuals, and in view of the great variation in urine calcium of hypercalciuric persons on a free diet this may be a better way of identifying hypercalciuria than an outpatient 24-hour collection.

Summary

In nine hypercalciuric patients with renal stone disease non-fasting serum and urine calcium were significantly raised. After overnight fasting, both serum and urine calcium values had fallen almost into the normal range and calcium deprivation for a further eight hours yielded completely normal values in all but one case.

Oral administration of a large dose of calcium citrate (100 mg. calcium/kg.) produced a significant rise in serum and urine calcium in both groups, but the rise was significantly greater in the hypercalciuric subjects than in the controls.

The cumulative eight-hour calcium excretion after the oral load was more than 174 mg. in all the hypercalciuric cases and less than this amount in the controls.

These observations tend to show that idiopathic hypercalciuria is absorptive in origin and emphasize the importance of measuring serum and urine calcium in the fasting state. It is suggested that oral calcium loading may constitute a better diagnostic test of this condition than a 24-hour urine collection on a free diet.

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A CALCIFYING CARDIOPATHY PRODUCED BY STRESS AND CALCIUM ACETATE

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In a previous paper (Selye and Prioreschi, 1960), we described an electrolyte-steroid-induced cardiopathy characterized by calcification (ESCC) in the myocardium, coronary arteries, and kidney. This syndrome was produced by administering 9 α-fluorocortisol and calcium acetate to rats exposed to stress.

The purpose of this paper is to show that under suitable conditions, a similar cardiopathy can be produced, even without exogenous corticoids, by the combined action of stress and calcium acetate.

MATERIALS AND METHODS

Sixty female Sprague-Dawley rats from the Holtzman farm, with a mean initial body weight of 96 g. (range: 90–110 g.), were divided into 6 equal groups and treated as indicated in the Table. As a stressor we used forced restraint, which consisted in fastening the animal to a board with adhesive tape, in the prone position, for 24 hr. (Groups 2 and 3) or 5 hr. (Groups 5 and 6). Calcium acetate was given at the beginning of the experiment in 3 ml. of water, by stomach tube, at the dose level of 2 or 4 mM; Groups 1 and 3 received an additional gavage 8 hr. later, all other conditions being equal.

The experiment lasted 24 hr. for Groups 1–3, and 8 hr. for Groups 4–6. At the end of these periods all surviving animals were killed with chloroform. The hearts, kidneys and stomachs were first inspected by means of a binocular loupe and then fixed in alcohol-formol (Renaud, 1959) for the subsequent demonstration of calcium with the von Kóssa technique. The mean grades of the myocardial lesions, nephrocalcinosis, and gastric lesions were based on examination of the entire freshly dissected organs with a binocular loupe, while the grades of "coronary calcification" were based on microscopic readings. The severity of the lesions was assessed in terms of an arbitrary scale of 0–3 (0 = no lesions, 1 = barely detectable lesions, 2 = moderate lesions, 3 = most severe lesions); the mean grades are listed with standard errors in the Table.

TABLE.—*Production of Cardio-Renal Lesions by Calcium Acetate and Stress*

Group	Treatment	Myocardial lesions	Coronary calcification	Nephrocalcinosis	Gastric lesions	Mortality per cent
1	Calcium acetate 2mM × 2	0	0	0	0	
2	Restraint 24 hr.	0	0	0	1·1 ± 0·20	0
3	Calcium acetate 2mM × 2 + restraint 24 hr.	2·4 ± 0·30	2·0 ± 0·45	1·7 ± 0·30	2·8 ± 0·17	60
4	Calcium acetate 4mM × 1	2·0 ± 0·43	1·0 ± 0·45	1·5 ± 0·25	3·0 ± 0	10
5	Restraint 5 hr.	0	0	0	0·4 ± 0·17	0
6	Calcium acetate 4mM × 1 + restraint 5 hr.	2·0 ± 0·45	1·7 ± 0·27	1·2 ± 0·38	3·0 ± 0	90

* Fellow of the Life Insurance Medical Research Fund.

RESULTS

The lesions were typical of the ESCC ; macroscopically, the heart often had a " tigroid " appearance, because of the distribution of calcification in parallel lines. The coronary arteries showed sclerosis of the Mönckeberg-type (Fig.). In the kidneys, necrosis and calcification affected the proximal convoluted tubules of the cortex preferentially. In the animals given calcium acetate, and especially in Groups 3, 4 and 6, the ventriculus (but not the proventriculus) showed a deeply penetrating, necrotizing gastritis, while in the rats subjected to restraint alone

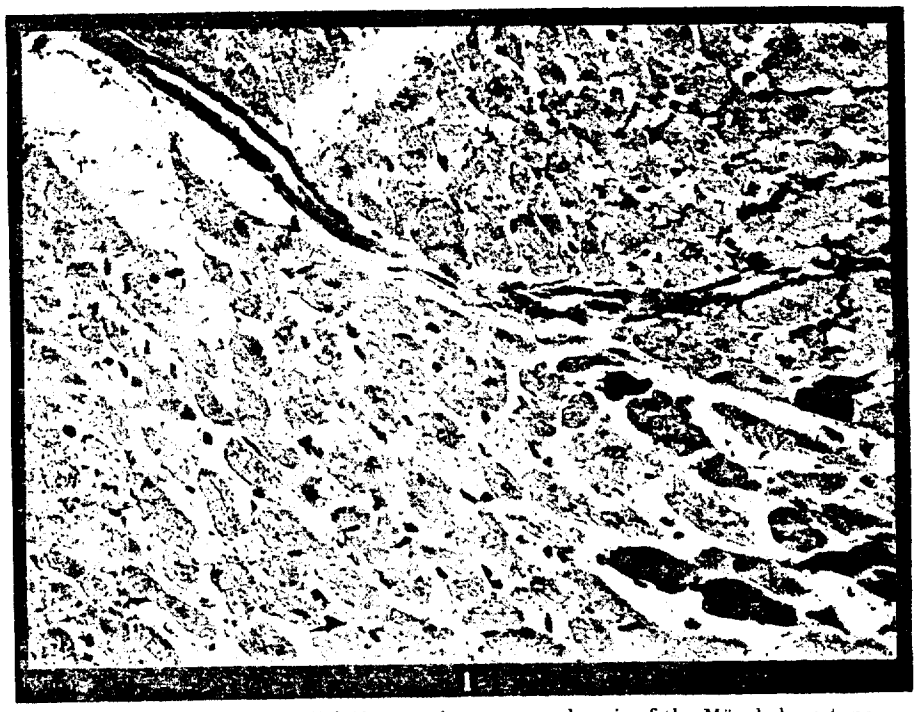

FIG.– Calcification of myocardial fibres and coronary sclerosis of the Mönckeberg-type (von Kóssa × 420).

(Groups 2 and 5), the gastric lesions consisted only of the usual, limited, haemorrhagic erosions characteristic of the alarm reaction.

As shown in the Table, cardio-renal lesions were obtained only in animals treated with either 4 mM of calcium acetate (with or without restraint) or 2 mM of this salt plus restraint. When given alone, both the stressor and the lower dose level of the salt were completely ineffective.

DISCUSSION

The role of stress in the production of this experimental cardiopathy seems to be fundamental. Restraint was necessary for the production of pathological changes in the calcium acetate-treated rats of Group 3 (2 mM twice), while the animals

given this dose of the electrolyte alone (Group 1) showed no noteworthy cardiac, renal or gastric lesions. The rats of Group 4 were given the same amount (4 mM) of calcium acetate as those of Group 1, but in 1 dose instead of 2 ; this method of administration resulted in a very intense necrotizing gastritis often accompanied by thrombosis of the splenic vessels and consequent infarction of the spleen. In these rats, stress was so intense that severe cardiac lesions ensued without any additional treatment. The intensity of these lesions could not be further aggravated by the additional stress of restraint (Group 6), which, in this case, increased the mortality rate from 10 per cent to 90 per cent (cf. Groups 4 and 6).

Although investigations on a necrotizing type of experimental cardiopathy (electrolyte-steroid-induced cardiopathy with necrosis—ESCN) (Selye, 1958) have proven that the cardiotoxic effect of stress is not mediated through the adrenals (Selye, Prioreschi and Jean, 1960), only further studies in adrenalectomized animals will show if this is also the case for the calcifying lesions described here.

Like the other experimental syndromes described elsewhere—the electrolyte-steroid-induced cardiopathy with hyalinization (ESCH) (Selye, 1958), the ESCN, and the ESCC—the cardiopathy described here seems to be pluricausal. Its development depends, not upon a single pathogen, but upon the creation of complex pathogenic situations in which stress and " adaptive hormones " play particularly important roles.

SUMMARY

Calcifying cardiovascular lesions can be produced by the administration of calcium acetate with or without restraint. The cardiopathy is characterized by calcified streaky necroses (" tigroid ") of the myocardium, and coronary sclerosis of the Mönckeberg-type ; it is usually accompanied by nephrocalcinosis. Stress appears to play a decisive role in the production of such lesions.

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(From the Pharmacological Laboratory of the Chemical Factory,(formerly SANDOZ) at Basel).

EXPERIMENTAL INVESTIGATIONS CONCERNING THE RESORPTION AND EFFECT OF CALCIUM GLUCONATE.

By
E. ROTHLIN

With 5 illustrations in text.

(Recived on July 25,1929).

In an earlier work[1], we demonstrated for calcium gluconate the equivalence, in equimolar quantities, with $CaCl_2$ in vitro and in vero, using various test subjects such as isolated frog hearts, isolated intestine and uterus in rabbits under magnesium narcosis according to AUER and MELTZER[2], etc. From the viewpoint of toxology, calcium gluconate proved to be less toxic in mice and rabbits after intravenous application than calcium chloride or preparations containing the latter. An interesting fact was discovered in the course of these investigations, namely that calcium gluconate can be subcutaneously and intramuscularly injected into animals in comparatively large amounts without the appearance of the known necrotizing properties of the calcium chloride or of other soluble Ca salts, even when these are supplied in very small amounts. The intracutaneous injection of a 10 to 20% solution into the rabbit ear took a practically non-reactive course. KOTTMANN[3] was first to confirm our findings obtained from animal tests in man: he was able to recommend that the intramuscular injection of calcium gluconate be given to the amount of 10 ccm, in a 10% solution. Since that time, various other authors confirmed in the course of therapeutic experiments in practice that Ca gluconate is well-tolerated when intramuscularly applied.

We have since continued the development of research work with Ca gluconate, from the viewpoint of animal tests; a report on these investigations follows below.

I. Behavior of the blood calcium level after oral, subcutaneous and intravenous application of Ca gluconate.

Oral application. Evidence of Ca resorption through the stomach-intestinal tract, verified from the increase of the blood calcium level (a much-discussed question) can be regarded as confirmed today in man and animal. The more recent works of HJORT[5], IRVING[6], ROE and KAHN[7], IPONSUGI[8], HICKMANS[9], BILIDA and WALDIMIROWA[10], JANSEN[11], LASCH and NEUMAYER[12], SCHLOSSMANN[13], KRANE[14] etc. supply numerical proof for the above. Most of the investigations refer to $CaCl_2$; ROE and KAHN demonstrated the satisfactory resorption of Ca-lactate in rabbits in in humans.

SCHLOSSMANN compared the resorption of $CaCl_2$ with the resorption of Ca-citrate and likewise found that the resorption of both salts was satisfactory.

TABLE I. Behavior of the blood calcium level, after the oral, subcutaneous & intravenous application of various Ca gluconate quantities, in rabbits.

Blutkalkspiegel in mg-% nach *oraler* Verabreichung				Blutkalkspiegel in mg-% nach *subcutaner* Verabreichung				Blutkalkspiegel in mg-% nach *intravenöser* Verabreichung		
Zeit	70 mg p. kg	Zeit	126 mg p. kg	Zeit	20 mg p. kg	45 mg p. kg	80 mg p. kg	Zeit	21 mg p. kg	29 mg p. kg
—	11,2*	—	11,2	—	12,5	14,0	12,0	—	12,0	12,4
90′	15,9	80′	16,9	20′	15,0	17,4	18,5	5′	22,4	29,0
160′	13,4	195′	17,4	40′	14,5	18,0	19,0	30′	16,9	17,2
270′	12,4	270′	15,0	80′	13,4	18,1	17,9	60′	14,3	14,6
360′	12,0	375′	12,6	120′	12,9	15,8	16,5	90′	13,0	13,3
430′	11,0	460′	12,1	240′	—	—	16,0	120′	12,2	12,4
—	—	—	—	360′	—	—	14,0	—	—	—

1) Blood calcium level in mg-%, after oral application.
2) Blood calcium level in mg-% after subcutaneous application.
3) Blood calcium level in mg-% after intraveous application.
4) Time.

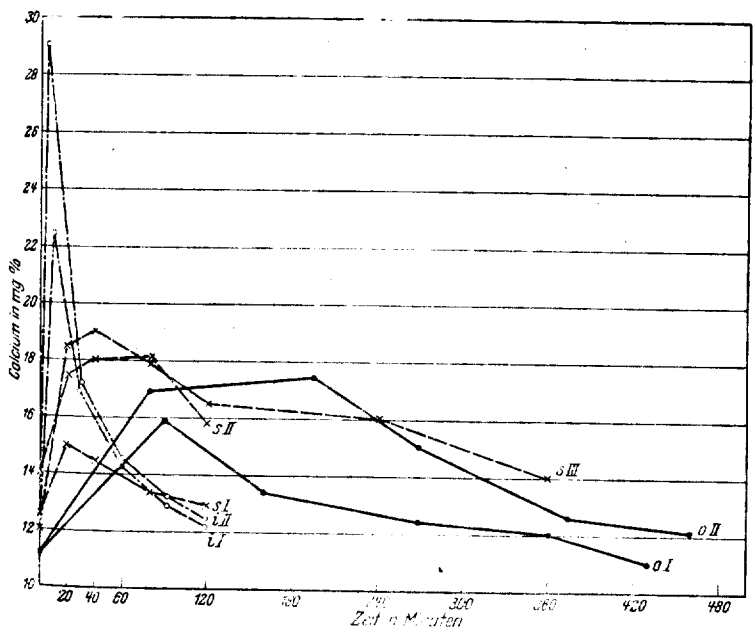


Illustration 1. The illustration shows the values of the blood calcium increases of Table I, after oral application .--. (oI=70 mg, oII=126 mg Ca per kg); after subcutaneous application x--x (sI=20 mg, sII=45 mg, sIII=126 mg Ca per kg); after intravenous application O-.-.O (iI=21 mg, iII=29 mg Ca per kg).

* The determination of Ca was carried out with serum according to KRAMER and TIDSALL: J. biol.chem., 47,476 (1921).

The intestinal resorption of Ca-gluconate results from the tests on Table I and Ill.1, whereby 2 and 4 g Ca-gluconate in a 20% solution or 0,070 and 0,126 g Ca per kg respectively, was administered to fasting rabbits with the stomach probe. Depending on the dose, the maximum of the blood calcium level is found after 1 and 2 hours respectively; after the application of 0,07 g Ca per kg said level returns to normal after 7 hours; but it is then still slightly above normal following the application of 0,127 g Ca per kg. The maximum increase for 0,07 g Ca per kg amounts to 42%; the maximum increase for 0,127 g Ca per kg is 53%. The course of both Ca-gluconate curves shows that the once reached maximum is not decisive for the magnitude of the resorption; instead, the question how long the increased calcium level is maintained is decisive for the magnitude of the resorption.

The Ca gluconate values for rabbits coincide well with those of HJORT[9] for dogs, with a 17 to 48% increase of the blood calcium level after 0.193 g Ca per kg, in the form of Ca-lactate; IRVING[6] found in the dog 40 to 214%, after direct introduction into the duodenum of 0,04 to 0,24 g Ca per kg, in the form of Ca lactate. LASCH and NEUMAYER[12] obtained in the cat a maximal blood calcium level increase of 50 to 69% after 0,44, and an increase of 100 to 172% after 0,88 g Ca per kg; in the dog the increase amounted to 28 to 53% after 0,44 g Ca per kg in the form of $CaCl_2$,

TABLE 2.

Tabelle 2.

1 Verabreichungsart und Menge Ca in mg per kg	2 Ausgangswert des Blutkalkspiegels in mg-%	3 Maximale Erhöhung über den Ausgangswert in %	4 Zeit nach der Injektion in Minuten	5 Mittlere absolute Erhöhung des Blutkalkspiegels: 6 für die verabreichte Gesamtmenge Ca per kg in %	7 für 1 mg verabreichtes Ca per kg in %	8 Prozentige Resorptionswerte	9 Bemerkungen
70 oral	11,2	42	90	18,60	0,265	100	Werte für die Versuchsdauer von 6 Stunden nach der Injektion 10
127 oral	11,2	53	195	38,90	0,306	115	
80 subcutan	12,0	58	40	36,00	0,450	172	
70 oral	11,2	42	90	25,30	0,361	108	Werte für die ersten 3 Stunden nach der Injektion 11
127 oral	11,2	53	195	41,10	0,326	100	
80 subcutan	12,0	58	40	39,10	0,489	150	
70 oral	11,2	42	90	25,60	0,365	131	Werte für die ersten 2 Stunden nach der Injektion 12
127 oral	11,2	53	195	34,90	0,277	100	
20 subcutan	12,5	20	20	10,66	0,533	191	
45 subcutan	14,0	29	40	27,20	0,600	217	
80 subcutan	12,0	58	40	40,00	0,500	180	
21 intravenös	12,0	87	5	26,40	1,269	458	
29 intravenös	12,4	134	5	32,30	1,114	402	
45 subcutan	14,0	29	40	26,66	0,592	117	Werte für die erste Stunde nach der Injektion 13
80 subcutan	12,0	58	40	40,47	0,506	100	
21 intravenös	12,0	87	5	44,00	2,100	415	
29 intravenös	12,4	134	5	56,45	1,946	384	

1) Application method and quantity of Ca in mg per kg.
2) Initial value of the blood calcium level in mg-%.
3) Maximal increase over the initial value, in %.
4) Time after the injection, in minutes.
5) Average absolute increase of the blood calcium level:
   6) for the total quantity of the applied Ca per kg in %;
   7) for 1 mg of applied Ca per kg ,in %.
8) Percentual resorption values.
9) Remarks
10) Values for a test duration of 6 hours after the injection.
11) Values for the first 3 hours after the injection.
12) Values for the first 2 hours after the injection.
12) Values for the first hour after the injection.

Explanations for Table 2: With reference to the maximal increases above the initial value (column 3): this is understood to be the maximal difference between the initial value and the verified peak values of the blood calcium level in % for the duration of the respective test. With reference to the average absolute increase of the blood calcium level (column 4): these absolute values were obtained by planimetric measurement of the calcium level increase area over the intial value, for the duration of the corresponding test, in quadrimillimeters, starting from the original curve and using millimeter paper. The pertinent area of the normal blood calcium level is obtained by multiplying the normal value and the duration. The average absolute increase of the blood calcium level for the duration of the respective test is recomputed from these two values in %. For example: for a test after oral application of 70 mg Ca per kg, the result of the measurement was 3750 qmm for the average increase during 6 hours; the pertinent area of the normal value is 112 qmm (= normal value) x 180 qmm (= duration of the test) = 20,160 2mm. 3750 is 16,60% of 20,160. Accordingly, 18,60% represents the average absolute increase of the blood calcium level during 6 hours after the oral application of 70 mg Ca per kg. The division of said value by the number of the supplied Ca milligrams(in this case, by 70) yields the average absolute value for 1 mg : 18,60 : 70 = 0,265%.

With reference to the percentual resorption values (column 5): Here the minimal value for the absolute increase of the blood calcium level per 1 mg of the supplied Ca is specified as equal to 100. Other values correspond to the intensified rise of the blood calcium level per 1 mg Ca and are found according to the following equation: 0,265 = 0,306: x, result: 11,6.

It is obvious that the increase of the blood calcium level is a function of the supplied quantity. When the area of the increase (of the blood calcium level) is planimetrically measured in quadrimillimeters for both tests, the result for 6 hours , with 0,07 and with 0,127 g Ca per kg,will be: 3750 and 7850 qmm respectively. According to the equation 0,07 : 3750 = 0,127 x, the expected result during the second test would be 6800 qmm. The difference between the verified and the computed value amounts to 1050 qmm, or 14% respectively. Therefore the conclusion can be reached that the ratio of the applied quantity as compared to the resorbed quantity is comparatively favorable.

Reliable evidence of a considerable blood calcium level increase caused by intestinal Ca resorption is an adequate basis for explaining the clinically confirmed therapeutic effect of calcium salts administered per os. This finding, in animal and man, likewise proves that the calcium resorption proceeds faster than the excretion through kidneys and intestine; this makes sufficient time available for the calcium to deploy its physiological and therapeutic effect respectively.

ROE and KAHN[7] proved that the increase of the blood calcium level is of long duration in man as well; this applies likewise to perorally supplied therapeutic doses of Ca-lactate. A comparison between the above-mentioned data concerning the increase of the blood calcium level following the oral application of $CaCl_2$ and Ca lactate and our tests shows that the resorption ratio for Ca-gluconate is just as favorable as (the ratio) for the listed Ca salts.

2. Subcutaneous application. The subcutaneous and intramuscular injection of soluble calcium salts was tested to a lesser extent on account of the necrotizing effect and because therapeutic use in practice is not possible. HEUBNER and RONA[15] determined a quite considerable blood calcium level increase (up to 40% above the initial value) in cats following the subcutaneous application of 0,17 to 0,25 g $CaCl_2 + 6\ H_2O$, i.e. 0,03 to 0,045 g Ca per kg. Resorption occurs after the subcutaneous application of Ca-salts; this results from the functional evidence of the calcium effect, as known from the tests concerning the termination of magnesium narcosis, performed by AUER and MELTZER[2], by CHIARI and JANUSCHKE[17] as well as by GOLD[18] for the purpose of obtaining evidence on the secretion-inhibiting effect of experimetally generated exudates.

However, the behavior of the blood calcium level after subcutaneous or intramuscular application respectively, of Ca-gluconate is of special interest because the latter application method can be used for man as well without causing side-effects. Table 1 and Illustration 1 show the effect of three different doses of Ca-gluconate on the blood calcium level in case of subcutaneous application.

During these tests 0,020 g, 0,045 g and 0,080 g of Ca per kg was administered to rabbits in the form of Ca-gluconate, given subcutaneously. It is evident from the course of the three curves that the maximum of the blood calcium level is reached within 20 to 60 minutes; the maximum for the 3 above-mentioned doses is 20, 30 and 58%. It is likewise obvious in case of subcutaneous application that the attained maximum is not decisive for the magnitude of the resorption; instead, the more or less long period during which the considerably increaed calcium level remains constant is the decisive factor. The planimetric measurement of the blood calcium increase area shows a very good ratio between supplied and resorbed Ca-quantity. Accordingly (Table 2, column 4) for a 2-hour test, it was found that the average absolute increase of the blood calcium level, can be expressed as 10,60% for 20 mg, 27,20% for 45 mg and 40,00% for 80 mg Ca per kg. The proportionate series for the supplied doses is 1 : 2,2 : 4,0, and the proportionate series for the blood calcium increase is: 1 : 2,55 : 3,98.

Presupposing that the increase of the blood calcium level represents the expression of the resorption magnitude, the percentual resorption values were calculated from the obtained figures; thereby the percentual increase of the blood calcium level for 1 mg of the supplied Ca per kg served as a basis. These values are compiled for various test periods in column 5 of Table 2. The lowest value for the respective test duration is supposed to be 100% in all cases. During 6 hours 70% more is subcutaneously resorbed than in case of oral application; during 3 hours 50%, and for 2 hours roundly 100%. When comparing the course of the curves for oral and subcutaneous application, it results that while the blood calcium level increase is not completely over after 6 hours, both curves run parallely towards the initial value.

It results that the conditions regarding the duartion of the blood calcium level increase are analoguous with oral and subcutaneous application. However, in case of the latter, the maximum is reached within 20 to 60 minutes, while it is reached within 1 to 2 hours after oral application. ROE and KAHN[7] found that when Ca-lactate is orally supplied to man, the maximum is reached even later. The identical duration, the higher maximum of the blood calcium level increase readily explain the higher resorption value of 70% for subcutaneous application. Said difference in the behavior of the blood calcium level after oral and subcutaneous application corresponds to the difference of the phsyiological and therapeutic effects. The effect occurs faster after subcutaneous injection, it is more intensive and of the same duration as after oral application.

3. Intravenous application. Resorption shortcomings are eliminated with this application method. As known, the blood calcium level increases rapidly after intravenous application of $CaCl_2$; it drops to standard value within a comparatively short period of time. In Ill. 2 two examples show the behavior of the blood calcium level after administration of 21 and 29 mg Ca per kg respectively, in the form of Ca-gluconate. 5 minutes after the injection a maximal increase of 87 and 134% respectively is found. With this increased blood calcium level, the animals do not show any specific symptoms; it is certain, however, that such a high blood calcium level would cause in the long run malfunctions of the heart and central nervous system; this results especially from experiences made by COLLIP[19] with parathyroid incretion (Parathyreoideainkret).

The course of the curves 1[1] and 2, in Illustration 1, shows that the maximum is followed by a comparatively rapid decrease, and that the initial value is reached again after approximately 2 hours. The two curves show differences during the first 30 minutes; from then on their course is completely parallel.

As HEUBNER and RONA[15] have already demonstrated in the course of their thorough tests, the view of REY[24] must be considered erroneous: he believes that the blood calcium level can be increased for the duration of a few days by means of a single calcium injection.

Table 2 contains the analogously calculated values as compared to the subcutaneous and oral application. The value resulting from the percentual resorption values of the test series for the duration of 2 hours is four times higher than the value with oral application, and twice as high as (the value) with subcutaneous application. It must be taken into consideration in this context that the duration of the increase is only 2 hours; therefore we must compare the corresponding values after oral and subcutaneous application for the total duration (of 6 hours for our tests). We thus obtain for 1 mg of supplied Ca an absolute blood calcium level increase of 0,285% (average for both tests) for oral, of 0,450 with subcutaneous, and of 1,191% with intravenous application, which corresponds to the following resorption values: 100, 160, 400. It is understood that the figures refer only to the part of the resorbed Ca-volume which is detectable in the blood after oral and subcutaneous application; no numerical conclusions concerning the total resorption quantity can be reached from the above, because this presupposes knowledge of the quantities transferred from the blood into the tissues and body fluids, as well as of those which were excreted through the kidneys and through the intestine. The fact, however, that after intravenous application the blood calcium level returns to normal within approximately two hours, and that respectively, all calcium above normal value disappears from the blood, leads to certain conclusions concerning resorption in case of oral and subcutaneous application. This is possible because it can be assumed that the trend to eliminate calcium above normal value from the blood and to transfer the same into the tissues is just as strong after the two last-mentioned application types as it is after intravenous injection. In view of the long duration and comparatively strong increase of the blood calcium level, specifically after subcutaneous application, it is therefore presumable that quite favorable resorption conditions exist for Ca-gluconate with these application types as well.

When the normal total of the blood calcium content is calculated in tests with intravenous application, it will amount to (4,35 kg) 34,80 mg Ca for the first test, to (3,32 kg) 27,28 mg for the second test. A total of 93 mg Ca was supplied during both tests. By adding this amount to the normal content, a theoretical maximum content of 127.80 is computed for the first test, and of 120,28 for the second test. 5 minutes after the injection 64,96 mg and 63,80 mg Ca was found in the total blood. It can be determined from the difference between the theoretical and the detected milligram Ca in the total blood (volume) how much Ca had already disappeared from circulation at the time when the maximum was specified. This amounts to 127,80 - 64,96 = 62,84 mg Ca for the first test and to 120,28 - 56,48 mg Ca respectively, for the second test.

Therefore during the first test, 62,84 mg, i.e. 67,57% of the intravenously supplied 93 mg Ca, and during the second test 56,48 mg, i.e. 60,73 % (of the supplied Ca volume)--which are values corresponding well to each other--vanished from the blood, or more correctly: from the blood serum through diffusion, already within 5 minutes; a small part of the above infiltrated the erythrocytes which under normal conditions are practically Ca-free. Furthermore, excretion through kidneys and intestine must be taken into account. It can be stated concerning the diuretic effect of Ca-gluconate : in the acute test it is quantitatively comparable to the diuretic effect of NaCl and can therefore presumably be primarily attributed to osmotic changes. It is unlikely that a substantial part of the intravenously applied calcium left the organism in this way within the first 5 minutes.

HETENYI[31], taking into consideration the calcium which was excreted through the kidneys and intestine, found that after 3 hours approximately half of the intravenously introduced calcium remains in the body.

This speedy transfer from the blood into the tissues and tissue fluid proves that the Ca-effect must be very rapid after intravenous application, which likewise results from the experimental and clinical symptoms of the Ca-effect. Thus animals awaken from magnesium narcosis during the injection. Electrocardiographic examinations show evidence of an immediate effect of Ca on the heart function. Sub-lethal and lethal doses very rapidly produce intoxication symptoms.

II. Comparative investigations of $CaCl_2$ and Ca-gluconate

Table 3 and Illustration 2. record differences in the behavior of $CaCl_2$ and Ca-gluconate in case of subcutaneous application of equal quantities of Ca per kg. In both cases the maximum of the blood calcium level is reached after approximately 1 hour; it amounts to 28,33% for $CaCl_2$ and to 46,96% for Ca-gluconate. But the curves differ, especially in their further course: after $CaCl_2$ the curve drops comparatively fast to a minor increase above the normal value, remains there for about 4 hours and reaches the norm after about 8 hours. After Ca-gluconate, on the other hand, the blood calcium level remains stable at the maximum for more than 1 hour and subsequently decreases gradually but continuously, reaching the initial value after the 7th hour.

Table 3. Blood calcium level after the subcutaneous application of calcium chloride and calcium gluconate.

1 Kaninchen von 3,120 kg erhält 0,060 g per kg in Form von Calciumchlorid					2 Kaninchen von 3,100 kg erhält 0,060 g per kg in Form von Calciumgluconat				
	3 η Blut	4 η Serum	5 Ca mg-%	6 Zunahme in %		3 η Blut	4 η Serum	5 Ca mg-%	6 Zunahme in %
7 1. Normal	3,6	1,65	12,00	—	1. Normal	3.2	1,55	13,20	
8 Injektion von 11.5 ccm $CaCl_2+2H_2O$ in 6%					Injektion von 20 ccm Ca-Gluconat in 10%				
9 2. Nach 1h	3,5	1,65	15,40	28,33	2. Nach 1h	3.2	1,55	19,40	46.96
3. „ 2h15′	—	1,65	12,90	7,50	3. „ 2h10′	3.2	1,55	19,80	50,00
4. „ 4h10′	3,4	1,60	13,70	14,16	4. „ 4h15′	3.1	1,50	16,50	25.00
5. „ 6h10′	—	1,58	12,90	7,50	5. „ 6h	2.8	1,48	13,80	4.54
6. „ 8h	3,2	1,52	12,30	2,50	6. „ 7h30′	2.65	1,45	13,10	1,51
					7. 8h nach der letzten Bestimmung	2.75	1,50	12,80	—3,03

1) Rabbit weighing 3,120 kg receives 0,060 g per kg in the form of calcium chloride.
2) Rabbit weighing 3,100 kg receives 0,060 g per kg of calcium gluconate.
3) Blood; 4) serum; 5) Ca mg-%; 6) increase in %.
7) Normal . . .
8) Injection of 11,5 ccm $CaCl_2 + 2H_2O$ in 6%
9) After . . . hour
10) 8 hours after the last specification. . .

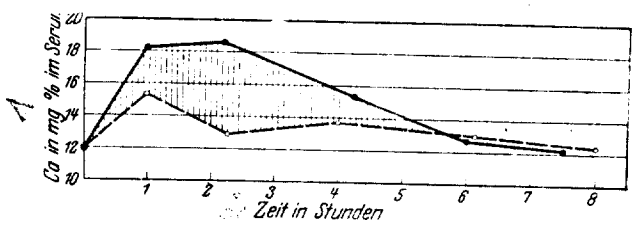
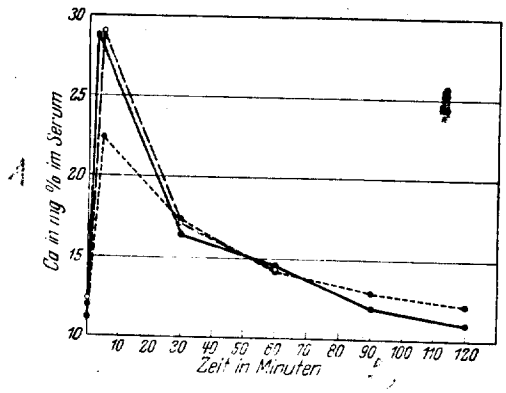


Illustration 2. Illustration 2 shows the values of the blood calcium increase of Table 3 after the subcutaneous application of Ca gluconate (.--.) and $CaCl_2$ (Θ--Θ)/ The extent of the lesser resorption after $CaCl_2$ results from the shaded area.

1) Ca in mg-% in the serum. 2) Time in hours.

The difference is illustrated by the shaded field in Ill.2. The planimetric calculation yields 820 qmm for $CaCl_2$ and 2430 qmm for Ca gluconate. Accordingly, the average absolute resorption for Ca gluconate is three times in excess of the average absolute resorption for the chloride. This substantionally lesser resorption of $CaCl_2$ is evidently no accidentally observed fact, and it originates from differences between the local effects of the two Ca-salts. The gluconate is smoothly reabsorbed without irritating effect, while the chlorid, applied in said doses, causes violent local symptoms; this results in a delay or inhibition respectively, of the resorption. Obviously, part of the injected $CaCl_2$ volume is then left behind in situ and causes inflammation and necrosis symptoms.

Such differences are not foreseeable when these two calcium salts are intravenously administered . Illustration 3 shows an example for equal quantities of Ca per kg and per animal. The increase and the decrease are absolutely parallel. The Ca-gluconate disappears as rapidly from the blood as does the $CaCl_2$. This behaviour is the best confirmation of findings described earlier, i.e. that equimolar Ca quantities of the two salts, applied intravenously, have the same effect on magnesium narcosis.



<u>Illustration 3.</u> Ill.3 shows the analogous behavior of the blood calcium level after intravenous application of equal Ca-quantities of $CaCl_2$ (.--.) and Ca gluconate (O--O); the curve (.--.) corresponds to ½ of the Ca gluconate volume.

1) Ca in mg % in the serum.
2) Time in minutes.

In the course of a test series, the relative viscosity of the non-coagulated blood and of the serum was determined during the various stages of the blood sampling; this was done in addition to the determination of the Ca content of the serum. The determination was carried out with the HESS laboratory viscosimeter. Table 3 shows such values for two tests with Ca gluconate and $CaCl_2$. Analogous conditions exist in both cases.

No difference, as compared to the initial value, exists as yet between the first three blood samplings. The subsequent specifications show a gradual decrease of both viscosity values. The viscosity decrease for blood and serum is more extensive after Ca gluconate than after $CaCl_2$; it amounts to 0,4 for η of the blood, and to 0,13 for η of the serum after $CaCl_2$. After Ca gluconate, it amounts to 0,55 of the for the blood, and to 0,10 of the η for the serum. Since the number of the erythrocytes is more significant for the viscosity of the blood than the serum, said difference can be explained by the fact that the standard values of the erythrocytes vary; this, however, was not determined simultaneously. The decrease of the serum values --as the decrease of the total blood values- can be attributed to the thinning of the blood as a consequence of the blood sampling, which amounted to approximately 8 ccm for each test. No refractometric specification was performed either; it could have supplied information on the modifications of the hydration condition by means of the Ca-ions of the serum's protein material.

III. Intracutaneous and subcutaneous tolerability of the Ca gluconate.

1. Intracutaneous application. The ear of the rabbit is especially adequate for testing whether a medicament can be intracutaneously injected. It is therefore relatively easy to cause an intracutaneous swelling (Quaddel). Three different Ca-salts were compared with this method and the observed symptoms were described in the listed work[1]. Thereby only the Ca gluconate was found to be very well tolerated, while $CaCl_2$, $CaCl_2$ plus urea, mannitic sulfate of Ca, even in minor concentrations, proved to be irritating, caused inflammation and had a necrotizing effect.

$CaCl_2$ is undoubtedly the most irritating of all investigated Ca-salts. Even a small volume of 0,10 ccm in 1% solution caused swelling, hyperemia, slight inflammation and the formation of superficial scars. This coincides with the experiences of SELIG[20], who found that 1% solutions, subcutaneously applied to guinea pigs, were not innocuous. According to said author, 2% solutions cause a violent inflammatory reaction. It is principally remarkable that the inflammation symptoms caused by all irritating Ca-salts take a lengthy course and tend to heal slowly. This finding coincides with clinical experiences, since inflammation sysmptoms occuring together with necrosis after unsuccessful intraveous application of $CaCl_2$ last for weeks, even for months.

Two examples, for which we supply characteristic illustrations, show the behavior of $CaCl_2$ and Ca gluconate. Albino rabbits qualify best for obtaining good visual evidence of vessels and inflammation symptoms. (Text continued on page 14 of translation)

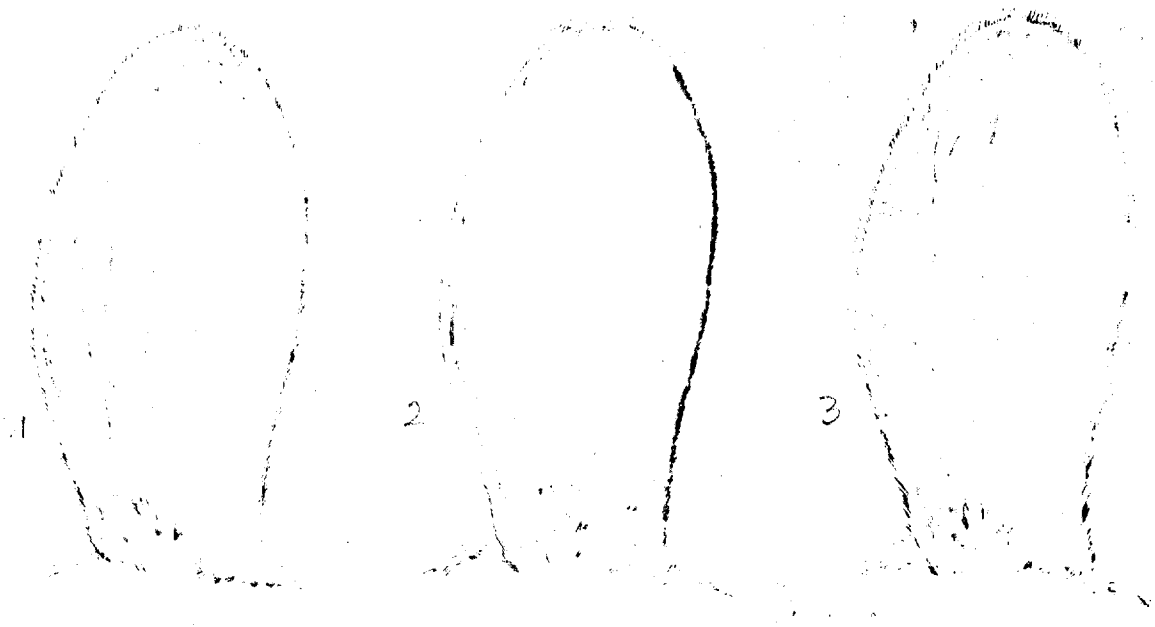


Illustration 1. Before the injection.
Illustration 2. 24 hours after the injection. Slight hyperemia without swelling.
Illustration 3. 48 hours after the injection. Once more, normal aspect.
Illustration 4. Ca gluconate (Calcium SANDOZ); tolerability, tested subcutaneously in the rabbit ear, proves to be very good.

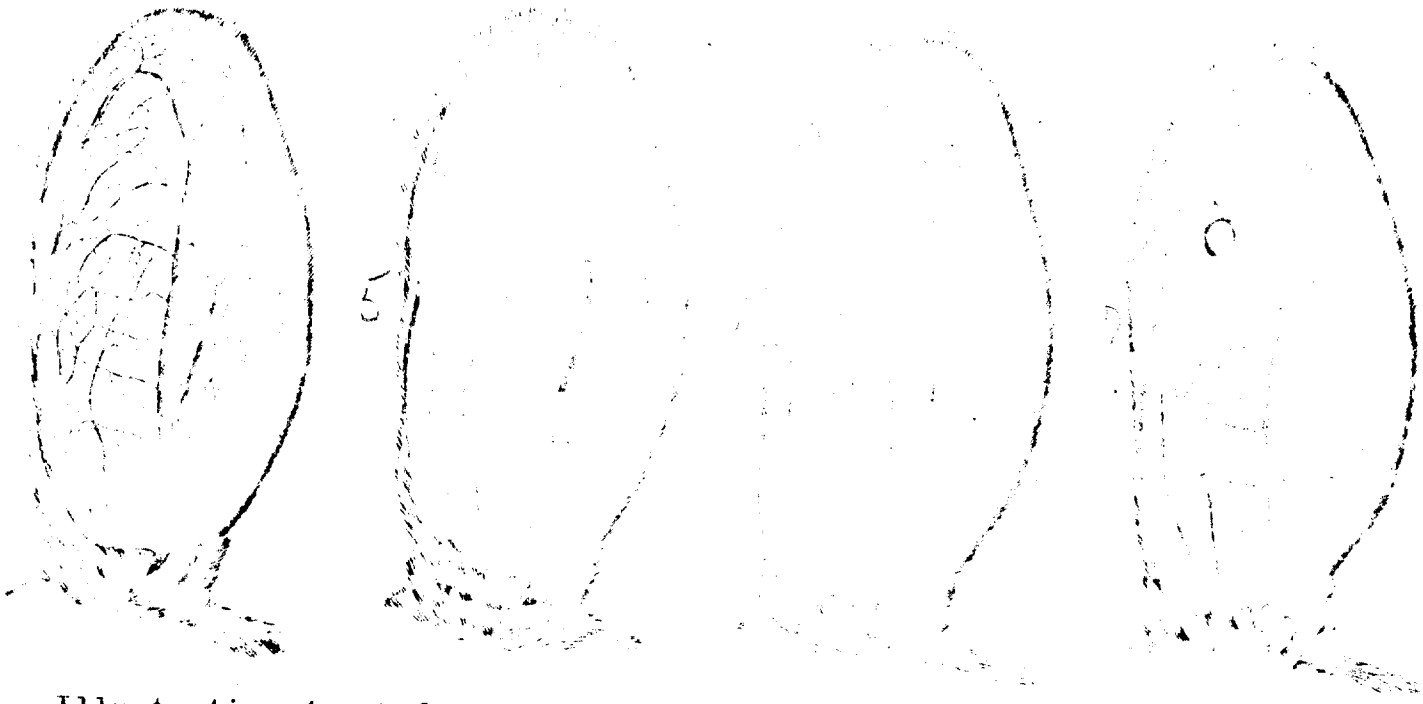


Illustration 4. Before the injection.
Illustration 5. 48 hours after the injection.
Illustration 6. After 35 days.
Illustration 7. After 68 days.
Illustration 5. Ca chloride causes lengthy inflammation and necrotic destruction in the rabbit ear.

Explanation of the illustrations.

Left ear.

Injection on November 27,1928: 0,20 ccm of a 20% solution of calcium gluconate, i.e. 0,0037 g Ca.
Illustration 1: before the injection, on November 27,1928.
Illustration 2: 24 hours after the injection, Nov. 28,1928. Slight hyperemia without swelling at the site of the injection.
Illustration 3: 48 hours after the injection on November 29,1928. The hyperemia disappreared completely, the ear has, and retains, a quite normal aspect.

Right ear.

0,10 ccm of a 5% calcium chloride solution, i.e. 0,0018 g Ca.
Illustration 4: before the injection, on November 27,1928.
Illustration 5: 48 hours after the injection. The site of the injection is dark red, partially with brownish-black discoloration; evident swelling and very strong reddening of the surrounding area. A well-defined hyperemia is evident on the entire surface of the ear. After 6 days, the injection site shows an increasingly blackish discoloration; its outline is sharp; the reddened marginal zone is somewhat reduced. The lighter areas are necrotized tissues.
Illustration 6: on January 9, 1929, 35 days after the injection: the inflammation progresses very slowly; it shows a protracted course. The necrotized parts of the skin are clearly visible, in the shape of scar tissue. In the meanwhile, the process extended to the interior of the ear as well, where analogous symptoms are in evidence.
Illustration 7.: on February 2, 1929, 68 days after the injection. The necrotized central sector of the injection site could be easily removed; it formed a cohesive crust. The lips of the lesion are still inflamed, with minor necrotized sectors.

(Text continued from p. 12 of translation):

The corresponding illustrations originate from the same test animal and were obtained as follows: the vessel network of both ears was photographed prior to the start of the test, with permeating light. A vessel dilatation is evident, caused by the heat effect of the strong light source. The vessel system was reproduced full-scale from the photographic plate and subsequently an artist painted a few characteristic stages of the inflammation from nature.

2. Subcutaneous application. SEELIG[20] proved that $CaCl_2$, subcutaneously administered in 1% solution, cannot be regarded as harmless for guinea pigs, and that a 2% solution causes inflammation and necrotizing symptoms. We used 14 animals to test whether 10% Ca gluconate can be subcutaneously injected in increasing volumes. The strong doses were administered in intervals of 2 to 3 days. Table 4 shows a compilation of said tests and records the observed symptoms.

Table 4. Subcutaneous tolerability of Ca gluconate in guinea pigs.

1 Nr.	2 Anzahl der Injektionen		3 Injektionsmenge der 10%ig. Lösung in ccm	4 g Ca per kg pro Injektion	5 Total injizierte Menge Ca in g per kg	Verhalten des Körpergewichts 6		9 Bemerkungen
						7 Anfangsgewicht in g	8 Endgewicht in g	
1	92	täglich	0.50	0.0104	0.94	445	540	Keine lokalen oder allgemeinen Erscheinungen 12
2	92		0,50	0,0104	1,05	455	450	
3	92	täglich	1,00	0,0186	1,71	500	600	Keine lokalen oder allgemeinen Erscheinungen 12
4	92		1,00	0,0182	1,66	510	550	
5	47	alle	2,00	0,0365	1,72	510	525	Keine lokalen oder allgemeinen Erscheinungen. 12
6	33	2 Tage	2,00	0,0310	1,23	600	464	Nach der 26. Injektion Gewicht 625 g, sodann allmähliche Gewichtsabnahme 13
7	27	alle	3,00	0,0508	1,37	550	470	Keine lokalen Erscheinungen 13/A
8	27	2 Tage	3,00	0,0498	1,34	560	470	Bei beiden Tieren Gewichtsabnahme während den ersten 18 Injektionen, nachher Gewicht stabil; keine lokalen oder allgemeinen Erscheinungen 14
9	33	alle 3 Tage	3.00	0,0465	1,53	600	560	Exitus nach der 33. Injektion. Leichte Gewichtsabnahme, keine lokalen Erscheinungen 15
10	20	alle 3 Tage	3,00	0,0430	0,85	650	445	Exitus nach der 20. Injektion. Allmähliche Gewichtsabnahme seit Beginn des Versuchs. Keine lokalen Erscheinungen 16
11	4	alle 3 Tage	4,00	0,0586	0,234	635	555	Wird am folgenden Tag nach der 4. Injektion tot aufgefunden. Keine lokalen oder allgemeinen Erscheinungen 17
12	14	alle 3 Tage	4,00	0,0484	0,678	770	701	Stirbt 2 Tage nach der 14. Injektion. Keine lokalen Erscheinungen 18
13	4	alle 3 Tage	5,00	0,0940	0,376	495	473	Beide Tiere sterben 3 Tage nach der 4. Injektion. Bei beiden Tieren leichte lokale Reizerscheinungen, welche aber sicher nicht den Tod herbeigeführt haben 19
14	4		5,00	0,10	0,400	465	355	

1) No. 2) Number of injections; 3) volume of injected 10% solution in ccm; 4) g Ca per kg per injection; 5) total injected volume of Ca in g per kg; 6) behavior of body weight; 7) initial (body) weight in g; 8) final (body) weight in g; 9) remarks.
10) daily; 11) every 2nd day. 11/A every 3rd day.
12) No local or general symptoms.
13) Weight after 26th injection: 625 g, followed by gradual weight loss.
13/A No local symptoms.

(Text of Table 4 continued):

14) Both animals lose weight during the first 18 injections; subsequently the weight remains stable; no local or general symptoms.
15) Exitus after the 33rd injection. Slight weight loss, no local symptoms.
16) Exitus after the 20th injection. Gradual weight loss since the start of the test. No local symptoms.
17) Is found dead on the day following the 4th injection. No local or general symptoms.
18) Dies on the 2nd day after the 14th injection. No local symptoms.
19) Both animals die 3 days after the 4th injection. In both animals slight symptoms of local irritation, which however certainly did not cause death.

These tests demonstrate once more that Ca gluconate is well-tolerated when subcutaneously applied. A slight local irritation was observed in 2 cases only, whereby it remains undecided whether this was due to the Ca-gluconate or to the technique of the injection. From a quantitave viewpoint, the Ca ions prove to be quite effective pharmacologically, with subcutaneous administration. The volume of 0,10 g Ca per kg of animal can be given 4 times; 0,04 to 0,05 g per kg is tolerated when applied during up to 33 days, every 2nd to 3rd day. Said quantities are close to the tolerance limit; 0,03 g per kg was well-tolerated 47 times by an animal, while the parallelly treated animal showed a weight loss under identical conditions after the 26th injection. On the other hand, 0,010 to 0,018 g per kg was well-tolerated when applied daily on 92 occasions, without resulting in any local or general symptoms. On the basis of these values a conclusion can be reached concerning the optimal quantity of Ca per injection for introducing the maximum of Ca ions into the guinea pig subcutaneously.

The observed side-effects are rather inconspicuous with lethal doses as well. The animals show less appetite and their behavior is calmer than the behavior of normal animals. No symptoms similar to those variously described as evident, especially in cats and dogs, after the prolonged application of $CaCl_2$; symptoms as observed in the course of a few tests with Ca gluconate do not occur either. These symptoms principally are: nausea, vomiting, pronounced and increasing paralysis, specifically of the rear extremities, and finally, complete paresis followed by exitus. The reactions of rabbits are similar to those of the guinea pigs; as known, these herbivores vomit with difficulty, or not at all.

Said good tolerability of the Ca gluconate when applied subcutaneously in effective quantities is unexpected, principally because it has been demonstrated with physiological testing as well as with clinical application that equimolar quantities are as effective as $CaCl_2$. This involves an interesting biological problem: a similar physiological effect on the one hand, and on the other hand, an enormous difference between the side-effects of $CaCl_2$ and Ca gluconate.

Without further analysis of this difficult question, it should be pointed out that the good tolerability of the Ca gluconate prompted the manufacturing of a series of known and new gluconates*), specifically from those metals which, in the form of salts known so far, likewise showed a pronounced irritating effect, similar (to the effect of) $CaCl_2$. It was not possible to demonstrate an inflammation-inhibiting effect even approximately as pronounced as for example, the effect of the gluconic acid calcium.

IV. Investigations concerning the effect of Ca gluconate on experimental pleuritis.

The favorable effect of calcium application in cases with various forms of exudation (urticaria, edemas, serum exanthema) was first determined clinically by WRIGHT[21]. WRIGHT errneously attributed the effect to the intensification of blood coagulation, since according to CHIARI and JANUSCHKE[16] the calcium effect also occurs when blood coagulation is prevented with the aid of the (anticoagulant) hirudin. The last-mentioned authors provided the experimental basis for proving the exudation-inhibiting effect of calcium; they showed that the subcutaneous and intravenous administration of $CaCl_2$ inhibits, or prevents respectively, the development of of exudation- and inflammation processes. This calcium effect is said to be connected with a decreased vessel permeability. LEO[17] confirms the favorable effect of $CaCl_2$ on mustard - oil conjuntivitis in rabbits, first observed by CHIARI and JANUSCHKE[16]. Recently GOLD[18] reported on the prophylactic effect of $CaCl_2$ on pleuritis which w experimentally generated with $CuSO_4$. Tests concerning the effect of Ca gluconate related to this problem were not perforamed so far. We report our experiences in this respect below. We used a 1% $CaSO_4$ solution as irritant.

Methodology: Ether narcosis. Skin incision to the right over the intercostal area, directly below the point of the scapula; blunt removal of the muscle system to the pleura. Perforation with cannula without point. The perforation of the rather tough pleura parietalis is easily detectable. No resistance should now be felt during the injection; otherwise, the cannula is not intrapleurally inserted, but reaches into the lung instead. Minor resistance during injection is a reliable criterion for an interpleurally given injection. On the other hand, the increase and decrease of the resistance synchronizes with ex- and inspiration respectively, a safe criterion of the intrapulmonary injection.

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*) These preparations were made available for me by Prof. Dr. A. STOLL.

With this method, 9 control tests and 13 tests with Ca treatment were performed. They are compiled in the two Tables No. 5 and 6. The control of the exudate was always carried out 20 to 24 hours after the operation. The prophylactic Ca-administration was adapted to the resorption conditions specified above; in other words: the subcutaneous injection was given in each case 30 minutes prior to the intrapleural application of $CuSO_4$ and the intravenous injection was given in each case 5 to 10 minutes prior to the same.

Table 5. Control tests with experimental pleuritis using $CuSO_4$ in rabbits.

1 Nr.	2 Gewicht	3 $CuSO_4$ 1% in ccm	4 Exsudat in ccm			8 Calcium in mg pro 100	
			5 rechts	6 links	7 total	9 in Serum	10 in Exsudat
1	3,870	2,0	27,0	3,5	30,5	11,40	10,10
2	2,500	1,0	27,5	6,5	34,0	—	—
3	2,940	1,0	14,0	2,0	16,0	11,20	10,50
4	3,290	1,0	20,5	4,5	25,0	9,90	10,00
5	2,750	1,0	11,0	11,0	22,0	11,20	9,80
6	2,040	0,5	13,0	1,5	14,5	—	
7	2,210	0,5	15,0	2,0	17,0	—	—
8	2,180	0,5	10,0	2,0	12,0	—	—
9	2,210	0,5	11,0	1,5	12,5	—	—

Mittel von 4 Versuchen mit 0,5 ccm $CuSO_4$ 1% = *14 ccm.* 11
Mittel von 4 Versuchen mit 1,0 ccm $CuSO_4$ 1% = *24 ccm.* 12

1) No.; 2) weight; 3) $CuSO_4$ 1% in ccm; 4) exudate in ccm; 5) right; 6) left; 7) total; 8) calcium in mg per 100; 9) in the serum; 10) in the exudate.
11) Average of 4 tests with 0,5 ccm $SuCO_4$ 1% = 14 ccm.
12) Average of 4 tests with 1,0 ccm $SuCO_4$ 1% = 24 ccm.

The calcium gluconate undoubtedly has an exudate-inhibiting effect, as demonstrated already for $CaCl_2$ by various authors. With a single exception, the inhibition of the pleuritic exudate caused by the prophylactic application of calcium gluconate is evident in all tests. Our tests results with calcium gluconate using rabbits, compare favorably with the tests of GOLD[18] with a cat, in which case $CaCl_2$ was subcutaneously applied.

Our control tests primarily confirm the reliability of the method indicated by GOLD for generating the experimental pleuritic exudate with $CuSO_4$. However, said method does not work quantitatively; but this whould not be expected from such a biological method. In any case, the above-mentioned method with $CaSO_4$ is probably much more reliable than the method with NaJ and diphteria toxin, as used by CHIARI and JANUSCHKE. LEVY[22] was unable to obtain a pleura exudate with the latter method regularly; STRANGMEYER[22] likewise questions the correctness of the statements made by CHIARI and JANUSCHKE. Recently GOLD failed to obtain a result with NaJ. However, we also experienced failures as observed by GOLD in 2 of 10 control tests with $CuSO_4$, and we obtained a small quantity of exudate in the course of tests 3 and 4 (Table 2).

Table 6. Effect of calcium gluconate on pleuritis, experimentally induced with $CuSO_4$ in rabbits.

1 Nr.	2 Gewicht	3 $CuSO_4$ 1% in ccm	4 Verabreichte Menge Ca-Gluconat: 5 subcutan Zeit	8 g Ca per kg	6 intravenös 9 Zeit	10 g Ca per kg	11 total Ca in g per kg	12 Exsudat in ccm: 13 rechts	14 links	15 total	16 Calcium in mg-% in: 17 Serum	18 Exsudat
10	2.910	2,0	60′	0,063	10′	0.063	0.126	20.0	10,0	**30,0**	10,50	10,80
11	2.760	2,0	40′	0,070	—	—	0.070	12,0	4,0	**16,0**	14.80	12,20
12	2.890	1,5	60′	0,064	10′	0,032	0,096	6,5	2,0	**8,5**	9,00	8,00
13	3.170	1,5	60′	0,058	10′	0,029	0,087	4,5	1,0	**5,5**	11,30	11,25
14	2.830	1,0	60′	0,066	10′	0,033	0,099	1,2	0	**1,2**	—	—
15	4.140	1,0	60′	0,044	10′	0,044	0,088	0	0	**0**	—	—
16	2.650	1,0	60′	0,070	—	—	0.070	7,0	2,0	**9,0**	12,00	10,60
17	4.120	1,0	55′	0,045	10′	0.022	0.067	11,5	1,0	**12,5**	—	—
18	2.080	0,5	—	—	5′	0,044	0.044	4.5	0,5	**5.0**	—	—
19	2,680	0,5	55′	0,077	—	—	0,077	5,0	0	**5,0**	—	—
20	2,320	0,5	—	—	10′	0,060	0,060	2,0	0	**2,0**	—	—
21	2,350	0,5	30′	0,114	—	—	0.114	1,0	0	**1,0**	—	—
22	2,770	0,5	45′	0,033	5′	0.033	0,066	0	0	**0**	—	—

Mittel von 5 Versuchen mit 0.5 ccm $CuSO_4$ = *2.6 ccm.* 19
Mittel von 4 Versuchen mit 1.0 ccm $CuSO_4$ = *5.7 ccm.* 20

1) No.; 2) weight; 3) $CuSO_4$ 1% in ccm; 4) applied quantity of Ca gluconate; 5) subcutaneous; 6) intravenous; 7) time; 8) g Ca per kg; 9) time; 10) g Ca per kg; 11) total Ca in g per kg; 12) exudate in ccm; 13) right; 14) left; 15) total; 16) calcium in mg% 17) in the serum; 18) in the exudate.
19) Average of 5 tests with 0,5 ccm $CuSO_4$ = 2,6 ccm.
20) Average of 4 tests with 1,0 ccm $CuSO_4$ = 5,7 ccm.

In our opinion, these failures are not caused by the ineffectiveness of the $CuSO_4$ or by individual resistance, because it could in each case be unequivocally proven during the autopsy following these tests that the injection was not intrapleural; instead, it was mostly given intrapulmonarily, which could be easily detected from the localized lung necrosis. These tests are excluded from our tabulation.

GOLD showed that the degree of the exudative pleura irritation depends on the $CuSO_4$ dose. Our average values for 0,5 ccm and 1,0 ccm $CuSO_4$ of 1% show this quite obviously; they amount to 14 and 24 ccm exudate respectively. However, the animals even tolerate large quantities--1,5 to 2,0 ccm of 1% $CuSO_4$ solution--without showing specific side-effects. We nevertheless confirm GOLD's finding according to which this does not increase the exudate volume substantially within 1 to 2 days.

A single prophylactic administration, given subcutaneously or intravenously, of Ca gluconate in doses of 0,044 to 0,126 g Ca per kg of animal, with 0,5, 1,0 and 1,5 ccm $CUSO_4$, regularly caused partially complete suppression of the pleuritic exudation.

In the two tests with 2,0 ccm the Ca effect is evidently present with the lesser dose (Test 11); in test 10, on the other hand, the dose seems to have failed completely. GOLD was likewise unable to obtain an exudation inhibition in a cat with 2,5 ccm of 1% $CuSO_4$. The $CuSO_4$ is a violent cell toxin; it is therefore evident that even optimal Ca doses cannot prevent the strong pleuritic irritating effect of Cu.

Furthermore, the conclusion can be reached from our tests that hardly any substantial difference exists between intravenous and subcutaneous application regarding exudate inhibition (see Tests 16 and 17, 18 through 21). It is of course necessary thereby to take into account the differences of behaviour concerning time of the blood calcium level in case of subcutaneous and intravenous application. Furthermore, an optimally effective Ca-volume obviously exists, i.e. still higher calcium doses do not improve the effect (see Tests 10 and 11). GOLD used up to 0,5 g $CaCl_2$ in cats; this means that when $CaCl_2$ plus 2 H_2O was used, 0,123 g Ca per kg was subcutaneously applied; however, the last-mentioned doses of $CaCl_2$ are certainly too high because GOLD reports that the cats died within a few days. The following symptoms were observed: nausea, vomiting, loss of appetite, loss of weight, general debility and paralysis, bloody diarrhea. In view of our specification, no additional statement is needed on the fact that $CaCl_2$ in such doses, administered subcutaneously, results in extensive necrosis at the site of the injection.

No unequivocal proof is obtainable from the tests at issue to confirm the superiority of one or another application form, as well as of combined subcutaneous and intravenous application. A thorough investigation of this question is in course. However, our tests show that no favorable effect can be expected from excessively high Ca-doses. This point is especially important for the clinical evaluation of the tests because Ca doses similar to those given to animals here cannot be taken into consideration for man. But it should be pointed out in this context that the intramuscular and intravenous prophylactic administration of 10 to 20 ccm 10% Ca gluconate solution before applying the artificial pneumothorax inhibits pleuritic exudation, as reported by a reliable source.

Various factors must be considered when explaining the secretion-inhibiting effect of calcium. Substantial influence of the blood pressure could certainly be excluded. The intravenously applied doses cause bradycardia and blood pressure changes; but they last for a few minutes only. If the doses in question are given subcutaneously, no blood pressure drop is registered in the course of 2 hours. But in the test for the inhibition of pleuritic exudation the subcutaneously application yields the same result as does the intravenous application. The sealing effect*) of Ca, as first assumed possible by H.H. MEYER,[25] is foremost when attempting an explanation.

*) <u>abdichtende Wirkung</u>

The aforementioned sealing effect, acting on the capillaries, the cell boundary areas and on the cell substance respectively, has not been precisely verified in conjunction with the phenomena at issue here; it complies with the finding according to which the Ca-ions cause the shrinkcage of the colloids. We believe, however, in agreement with ZONDEK[26] that this physical-chemical effect is not sufficient for a complete elucidation of the inflammation-inhibiting action of the calcium; we believe instead that the specific processes from which the secretion or inflammation respectively, originates are also affected by the Ca. The nervous, parasympathetic processes are presumably likewise involved in the mechanism of the secretion. It is known in this context that an intensified functioning of the vagus occurs in cases of rhinitis vasomotorica and asthma bronchiale; these vagotonic conditions, however, are favorably affected or inhibited respectively, by the calcium.

V. Therapeutic conclusion.

It is undoubtedly possible, starting from the results of tests concerning the behavior of the blood calcium level, to raise said level after oral application. Depending on the course of the resorption, a mild but long-lasting effect can be expected. It is unlikely that several applications of Ca gluconate, distributed in the course of a day, would fail to raise the blood calcium level in all cases, as a result of habituation. Accordingly, a way must also be found for the permanent raising of the blood calcium level to some extent with oral application. The inhibiting effect of food on the resorption, demonstrated by ROE and KAHN[7], has thereby to be taken into consideration. The specific behavior of the resorption when Ca is orally administered indicates that this application method should be used whenever an effect of some duration seems desirable. This applies specifically to cases of <u>metabolic Ca disorders, involving demineralization</u>, in particular when hypocalcemia is present. The effect of Vitamin D and the incretion of the parathyroidea are closely linked to the metabolic problem. The physiological importance of these substances, however, has not yet been fully clarified. The results obtained so far neverthless speak unequivocally in favor of a regulating, controlling function which affects the Ca metabolism. Vitamin D has an important role in the Ca-fixation within tissues. The action mechanism remains obscure; the functional result is experimentally and clinically evident. This becomes strikingly obvious from the tests of KRAITMAIR and HINTZELMANN[27] as well as from the tests of WENZEL[28]. In the course of said tests, excessively high dosages caused Ca deposits in the vessel-walls and in various organs, specifically in the normal tissues. Vitamin D is a highly effective substance which has to be therapeutically used in adequate dosages.

On the other hand, the incretion of the parathyreoidea is able to mobilize calcium from the tissues into the blood. The parat-hormone prepared by COLLIP[14] can raise the blood calcium level by 100 to 400%; we do not know exactly from where the calcium originates. It was ascertained only that the calcium is of endogenous origin because this effect occurs without exogenous supply of Ca. The stabilizing function of Vitamin D as well as the mobilizing effect of the parathreoidea incretion depend on a sufficient calcium supply available in the organism. This is the case principally for conditions of demineralization (rachitis, tetany), when the Ca-metabolism faces increased demands, as it occurs during pregnancy, lactation, growth. Increased exogenous Ca-supply is indicated also when when parathyreoidea incretion is adminsitered, because the generated hypercalcemia intensifies the excretion of calcium through the kidneys and intestine.

Speedier results are obtained with parenteral application of calcium, especially with intravenous injection. The widespread view according to which the effect of a parenteral Ca injection is quite transient is presumably not true for intravenous injection and even less for intramuscular application as it became feasible with Ca gluconate. The intravenous effect on the blood calcium level during animal tests lasts for 2 hours, while the intramuscular effect lasts for 6 to 8 hours, i.e. approximately as long as it does after oral administration. The question arises regarding the intravenous injection whether the effect actually lasts only as long as a blood calcium increase can be verified. This is the case only when, on the one hand, the corresponding surplus calcium excretion took place within said period of time, and on the other hand, when the entire Ca effect is unconditionally linked to an increase of the blood calcium level. None of these two conditions presumably exist. The Ca excretion proceeds at a slower rate than the drop of the blood calcium level following intravemous administration; a series of physiological and clinical findings speak in favor of a longer-lasting Ca effect. The conclusion can be reached from the above-mentioned results (Table 6) concerning the inhibition of the pleuritic exudation through intravenously injected Ca gluconate that the effect is not transient, and that it is instead a comparatively very persistent type. If the effect of the intravenous application were transient, then approximately equal volumes of exudate would have to be found with and without calcium application, since the duration of the test is 20 to 24 hours. But this is not the case. Instead, the exudation inhibition is clearly evident after a single intravenous application as well.

The behavior of the blood calcium level leads to the conclusion that the intravenous injection of calcium, in the first place, produces a very rapid effect; therefore its therapeutic use is mainly indicated for pathologic conditions of a more acute nature;

in other words: whenever calcium is involved, not as normal metabolic product, but as a specific pharmacopoeia, as in case of hemorrhages, vagotonic conditions (asthma, rhinitis, diarrhea during intestinal tubeculosis), shock effects (salvarsan), allergic symptoms (serum disease, urticaria, hay fever etc.), in case of heart ailments for intensifying the effect of glycosides, during exudative processes for the inhibition of the secretion.

Furthermore, a quantitavily better utilization is achieved with intramuscular administration, as compared to oral application. These favorable resorption conditions justify the use of intramuscular application of Ca gluconate for therapeutic purposes similar to those requiring oral application, i.e. in case of demineralization or hypercalcemia respectively.

The considerable pharmacological effectiveness of the Ca ions must be taken into consideration in conjunction with the parenteral, and specifically in conjunction with the intravenous application of Ca. This results from numerous experimental and clinical experiences which supply information on the acute and chronic types of intoxication symptoms. The first are mainly related to the circulatory system, while the latter are related to the functional disorders of the central nervous system, principally as paralysis. HEUBNER[15] and RONA[16] correctly emphasized this fact. CAZZOLA[29] as well as HAFFNER[30] demonstrated that the speed of the injection is primarily responsible for the above. The slight toxicity of the Ca gluconate, as compared to $CaCl_2$, was initially mentioned. It explains why no intoxication symptoms were observed after intravenous application, despite the frequent use of Ca gluconate.

The intramuscular application which can be performed at this time with Ca gluconate only without undesirable local side-effects, presumably deserves special interest from a therapeutic viewpoint as well. On the one hand, the danger of causing acute intoxication symptoms is practically excluded for therapeutic doses; on the other hand, the behavior of the blood calcium level indicates quite favorable resorption conditions. The effect occurs with comparative speed and lasts almost as long as during oral resorption. This new application method is therapeutically adequate, not only as an application form used alone, but it also serves for supporting the intravenous injection with the long duration of the blood calcium increase; it is helpful in case of oral application by means of a stronger and speedier Ca-effect.

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CALCIUM AND THE COLLOIDS OF THE ORGANISM

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As a sequel to a work which one of us has already published (Scaglioni), we wished, with the present research, to establish whether there is any adsorbtion of Ca on the part of the colloids which constitute the blood and the various organs and, in the case that this may be verified, to determine the capacity of adsorption.

The wide use of Ca in therapy, increased in past years with the adoption of calcium cure through intravenous injection, might suggest that by now the destination of this element in the organism is known. On the contrary, the extensive bibliography concerning this question, almost exclusively disputing the question of calcium balance, only offers very approximate and often uncertain data, because it is always difficult to calculate exactly the contribution of Ca of a non-unilateral diet, and the Ca eliminated by the various routes of excretion. Besides this, even in the case of a precise active balance, it cannot be established how much Ca added to an appropriate diet is kept by the organism, due to the fact that elimination may occur at the expense of this portion of the Ca, and the Ca may be in organic combination with the nutritive material, that really fixed by the organism.

The studies of the modifications of the normal calcemia in relation to the diet and the administration of calcium salts are interesting. Many authors, among them Clark (1), have found that a diet rich in Ca does not modify the calcemic level; only after ingestion of considerable quantities of Ca does hypercalcemia occur. According to some authors, of the elements which constitute $CaCl_2$, only Cl^- is adsorbed by the intestine. This does not correspond to the results obtained in isolated tops of the empty stomach, in which the Ca^{++} of $CaCl_2$ is in part adsorbed (Condorelli, 2).

The introduction of Ca by the endomuscular or hypodermic route produces, on the other hand, after a brief lapse of time, hypercalcemia, which then decreases little by little, until it arrives at the normal or slightly lower values. Rothlin (3), Bufano (4) and others have observed in these conditions, that during the descent of the calcemic curve there may appear a new ascent of the calcemic level. According to Di Natale and Cancer (5), this phenomenon is due to a movement toward the emunctory organs of the Ca injected into the organism and temporarily retained by the alleged organs of storage, or by the Ca of bone origin, mobilized under the stimulation of that organ.

The intravenous injection of Ca leads to a more rapid and notable increase of the calcemia, and the calcemic curve, followed for several hours, presents the trend described by others (Rothlin, Bufano, Di Natale and Canger, Jansen (6), Podrella (7), Archi (8), etc.).

In general it is observed that after the peroral or parenteral administration of Ca, there is an increase in the elimination of this element by way of the principal emunctory organs (kidney, liver, intestine). But it is not possible to establish with exactitude how much Ca is globally eliminated, not only because we cannot rely upon the elimination of a single organ, since this function is carried out in a very variable manner, under the influence of various factors, both endogenous and exogenous, but also because the fecal content of Ca is in part bound to the diet. Besides this, there always remains the question of calculating how much Ca is eliminated and then again adsorbed by the intestine, even if in transition.

But returning to the starting point, can we, with our actual knowledge, establish where the Ca of heterological origin, abandoning the blood flow little by little, is directed to? The opinions on this question are diverse; and thus while some maintain that the Ca introduced into the organism is immediately eliminated or at the very most temporarily stored and then eliminated, others maintain than at least a part of the Ca is really adsorbed by the organism.

The dosage of Ca into the isolated organs does not decide whether the organ in question has adsorbed the Ca introduced artificially into the organism or not, since it is impossible to perform this investigation on the same organ before and after the experiment. Certainly the experiments of Heteniy (9) are not very precise; he dosed Ca into the paw of a rabbit, removed before the intravenous injection of Ca, and from the Ca contained in the other limb after such an injection and the increase of Ca observed in it, calculated that 90% of the Ca introduced was deposited in the bones.

We must take into consideration the investigations on the variation of the calcemic rate in the efferent and afferent blood of the same organ, although these investigations are not very extensive and often discordant as well.

A part of the Ca contained in the organism is deposited in the bones, another part is certainly bound to the colloids, and finally one part is found in the interstitial and circulating fluids. Of the total blood Ca, a certain quantity is fixed by the colloids, the other, being partly free, is able to ooze and, through the emunctory organs, to pass to the exterior. It seems that only 5-6 mg% of the blood Ca is ultrafiltrable, though to Pons and Takahashi (10) these values seem to small.

According to modern views, the Ca in the serum is presented in three forms in balance with each other: the ionic form (Klinke, 11) = 1/6 of the total Ca; the non-ionic form, soluble, molecularly dispersed and dialyzable; and the non-dialyzable form, which is adsorbed by the proteins.

Thus it appears of great scientific and practical importance to study and establish which and how much of the Ca contained in several of the salts most used in therapy is adsorbed by the

colloids of the blood and the various organs, because it is this Ca whose degree of utilization by the organism we can evaluate.

EXPERIMENTAL PART

Method. -- In our experiments we kept to the method of ultrafiltration suggested by Bechhold-König (12), which is described with the modifications made in it for our particular investigations, in the account of one of us (Scaglioni, 15), as regards the adsorption of quinine by the colloids of the blood and the various organs. It must be kept in mind that, for the construction of the ultrafilte we always used the collodion at 10% in glacial acetic acid, with 2.5% K_2CO_3, stabilized in heat.

The calcium compounds taken into consideration were $CaCl_2 \cdot 6H_2O$ and $[CH_2OH.(CH.OH)_4.COO]_2Ca \cdot H_2O$, which are commonly used in therapy, and besides having a diverse chemical nature, also have diverse anionic functions (Bufano, 4).

The solutions used were very diluted and were calculated so that they had a quantitative of about mg 17% of Ca. Both the calcium chloride and the calcium gluconate were loosened in the liquid of Ringer (NaCl 0.75%, $CaCl_2$ 0.024%, KCl 0.042%, - $NaHCO_3$ 0.01%, at a pH = 7.57), by way of subtraction of its $CaCl_2$ content (*). Thus in the first case we used a Liquid of Ringer with a $CaCl_2$ content higher than normal, and in the other case a liquid of Ringer with calcium gluconate in place of calcium chloride. In both, therefore, the saline solution was not more biologically balanced, and its pH was shown to be inferior to that noted in the organic material (blood, blood serum of a sheep) used in the research. Since we were not able to used the buffer solution of Sorensen at a known pH with the Ca, we added to the two original solutions several drops of NaOH (1 drop NaOH 10% in 10 cc H_2O) until we obtained a pH of 7.70-7.73.

The solutions of calcium chloride and calcium gluconate, although they are not isoionic, are, however, isotonic, and the ionic-saline balance is such as to lead to a favorable experimental condition.

The determination of the Ca in the original solutions and in the various mixtures of the original solutions with organic liquids was done according to the method of Kramer and Tisdall (14).

EXPERIMENTS ON PURE BLOOD

To three parts of an original solution of calcium chloride and calcium gluconate we added respectively:

a) One part of the liquid of Ringer;

b) One part of whole defibrinated blood of mutton;

c) One part of blood serum of mutton;

d) One part red corpuscles of mutton, obtained by means of repeated washing with a physiological solution, and then added with a physiological solution to the volume of whole blood from which they derive.

(*) In our experiments with Ca, when we speak of the liquid of Ringer, we are always referring to the liquid Of Ringer deprived of its Ca content.

Ultrafiltration was performed on 40 cc of each single mixture, while in the other 2 cc we analyzed the percentual Ca content.

For the moment we must occupy ourselves with the first series of experiments, conducted with 30 cc of the mother solution of calcium chloride and calcium gluconate, plus 10 cc liquid of Ringer. The ultrafiltrate of these solutions of Ca was gathered in 6 test-tubes, graduated to 1/10 cc, the first of which contained 1 cc of ultrafiltrate, the others 2.5 cc. Thus there were in total 13.5 cc of ultrafiltrate divided into 6 portions; only from the last 5 test-tubes did we withdraw 2 cc of liquid for a quantitative analysis of the Ca.

The values of these first experiments are reported in table I. From the results noted we deduce:

1) The Ca increases in concentration in the ultrafiltrate in proportion as the ultrafiltration proceeds; however, after 11 cc of ultrafiltrate, that is, in the last portion (see column C VI), we find a content of Ca lower than the initial values, noted in the liquid to be ultrafiltrated (column B). This is due to the fact that the ultrafilter is soaked with distilled water, which dilutes the ultrafiltrate in an ever-decreasing degree with the progress of the ultrafiltration; at a certain point, all the water would have to be removed, and then the ultrafilter would assume the same concentration of ultrafiltrating liquid. However, we stopped with only 13.5 cc of ultrafiltrate, that is a quantity certainly inferior to that necessary to remove all the water contained in the thickness of the ultrafilter walls, and we did this in order not to prolong the time of the experiment and consequently to reduce the evaporation of the liquids being tested.

TABLE I

1. Soluzione in esame	Soluz. madre Ca = mgr%	Soluz. madre + Ringer (1:1) Ca = mgr%	ULTRAFILTRATO porzioni di cc 2,5 Ca = mgr. II	III	IV	V	VI	Soluz. di rimanenza nell'ultrafiltro dopo la ultrafiltrazione Ca mgr%	Ca assorbito dall'apparecchio ecc. (II. C VI) Ca mgr%
	A)	B)	C)					D)	E)
2. Ca-cloruro	16.85	12.70	7.60	8.90	10.80	11.00	11.95	12.10	0.75
3. Ca-gluconato	17.35	13.05	7.80	9.40	11.90	12.80	12.80	12.80	0.25

Key:
1.= solutions being tested 2.= calcium chloride 3.= Ca-gluconate
A.= mother solution B.= mother solution + Ringer
C.= ultrafiltrate, portions of 2.5 cc
D.= Solution remaining in ultrafilter after ultrafiltration
E.= Ca absorbed by apparatus, etc.

2) The Ca contained in the ultrafilter (see column B) is diminished when ultrafiltration is performed on it (column D). Given the preceding observation on the ultrafilter, it could be assumed that the Ca contained in the ultrafiltrating flask, after

the experiment, maintains or increases the original concentration. Thus the diminution can in a word be referred to the absorption of Ca on the part of the ultrafilter (flask of porous porcelain, collodion membrane).

3) The difference between the values of Ca contained in the mixture to be ultrafiltrated (column B) and those noted in the last portion of the ultrafiltrate (Column C IV), indicates in the aggregate the quantity of Ca adsorbed by the apparatus and the dilution it undergoes by the water still left in the ultrafilter after the passage of the first 11 cc of ultrafiltrate. This difference is reported in column E and was considered the differential value for the successive experiments on the organic liquids where, as we shall see, this could not be calculated.

Before beginning the experiments with Ca and the blood elements, we conducted to preliminary tests; in the first we dosed the Ca contained in the organic liquids being tested, in the second we researched how much of this Ca contained in the organs can pass through the ultrafilter. The results of the first experiment were the following:

Defibrinated blood of mutton	mg 8 = 8.5% Ca
Blood serum of mutton	" 11 = 12% "
Red corpuscles of mutton	" 1 = 1.5% "

For the second test we united 10 cc blood, 10 cc serum and 10 cc red corpuscles respectively to 30 cc liquid of Ringer, and then these mixtures were ultrafiltrated in the method described. The Ca was evaluated in the liquid to be ultrafiltrated and in the last portion of ultrafiltrate. Results obtained were the following:

TABLE II

1. Sostanza in esame	Miscela da ultrafiltrare Ca = mgr F)	Ultrafiltrato Ca = mgr G)
2. Sangue in toto	2.5	Tracce indosabili
3. Siero	3.0	» »
4. Globuli rossi	0.5	» »

Key:
1.= substances being tested 2.= whole blood 3.= serum 4.= red cells
F.= mixture to be ultrafiltrated C.= ultrafiltrate
a) undosable traces

From these results it appears evident that the Ca contained in the elements of the blood only ultrafiltrates in slight quantity. It can also be thought that the modest quantity of Ca to be ultrafiltrated can only pass through the ultrafilter in a very small percentage, because for the most part it is absorbed by the ultrafilter itself. This occurs in small proportions, since with very diluted water solutions it was seen in part that the Ca in

the ultrafiltrate is always found in concentrations not very inferior to the initial ones.

These data of ours are however in contrast with those of Cushny (16), Richter-Quittner (17), Meysemburg-Cann (18), Meysemburg, Pannenheimer, Zucker and Murray (19), Peassen (20), Cruickshank (21), who claim that the Ca of the plasma passes into the ultrafiltrate though not entirely. But the authors cited did not use these diluted solutions of blood and prolonged the ultrafiltration enormously besides, so that it is permissible to suppose that in our ultrafiltrate there is ionized Ca, but in an amount so slight that it is not dosable with our means of indagation.

After these investigations we passed on to the ultrafiltration with the blood elements (whole blood, blood serum, red cells), added to the mother solution of Ca.

Here as well we did not occupy ourselves with the analysis of the first 11 cc of ultrafiltrate, because we considered as the ultrafiltration of balance that which occurs between the 11 and 13.5 cc of ultrafiltrate; thus only in the last 2.5 cc of ultrafiltrate did we calculate the percentage of Ca contained in them. The mixture of 30 cc of mother solution of calcium chloride and calcium gluconate with 10 cc of the organic substances named above, before being ultrafiltrated, was left untouched for 15 minutes at an atmospheric temperature of 17° C.

The results of these experiments are recorded in table III.

TABLE III

1. Sostanze in esame	Soluz. madre Ca mgr% H)	Soluz. madre + Ringer Ca mgr% I)	Soluz. madre + elementi sospesi (1, 1) Ca mgr% L)	Ultrafiltrato Ca mgr% M)	Ca assorbito colloidi + Ca assorbito apparecchio mgr% N)
Ca-cloruro					
2. Sangue in toto + soluz. madre (1:3)	16.85	12.70	14.95	11.75	0,95
3. Siero di sangue + soluz. madre (1:3)	16.85	12.70	15.60	11.60	1.10
4. Globuli rossi + soluz. madre (1:3)	16.85	12.70	12.95	11.90	0.80
Ca-gluconato					
2. Sangue in toto + soluz. madre (1:3)	17.35	13.05	15.30	12.30	0,75
3. Siero di sangue + soluz. madre (1:3)	17.35	13.05	16.00	12.15	0,90
4. Globuli rossi + soluz. madre (1:3)	17.35	13.05	13.30	12.85	0,20

Key:
1.= substances being tested
2.= whole blood + mother solution 3.= blood serum + mother solution
4.= red cells + " "
H= mother solution I= mother solution + Ringer
L= mother solution + blood elements M= ultrafiltrate
N= Ca absorbed by colloids + Ca absorbed by apparatus

From these data it is seen that the Ca is always in a lesser

quantity in the ultrafiltrate than in the respective mixture to be ultrafiltrated. This would demonstrate that part of the Ca is taken up by the colloids of the blood elements (*). The evaluation of this colloidal adsorption now must be made on the basis of the concentration of Ca really contained in the mixture to be ultrafiltrated (column L).

Thus it has been ascertained in the preceding preliminary experiments (see table 2) that the Ca contained in the blood elements, given our special experimental conditions, only passes through the ultrafilter in very small, altogether negligible amounts, so that this Ca, at the end of the calculations which we propose, must be neglected. Now it is necessary to deduct from the ultrafiltrant mixtures (column L) the fraction of Ca contained in the elements of the blood, that is to say to calculate the concentration of Ca artificially added to these mixtures, so that we can with greatest precision establish the amount, on the basis of that which is obtained by diluting the mother solutions of Ca with a dose of Ringer liquid, corresponding to the quantity of whole blood, blood serum, and red cells used (columns B and I).

TABLE IV

Sostanze in esame	5. Ca assorbito dai colloidi senza correzione mgr% O)	con correzione mgr% P)
Ca-cloruro		
Sangue in toto + soluz. madre (1:3)	0.20	0.73
Siero di sangue + soluz. madre (1:3)	0.35	0.61
Globuli rossi + soluz. madre (1:3)	0,05	0.37
Ca-gluconato		
Sangue in toto + soluz. madre (1:3)	0,50	1,05
Siero di sangue + soluz. madre (1:3)	0,65	0.92
Globuli rossi + soluz. madre (1:3)	—	0.33

Key:
1.- 4 see table III. 5.= Ca adsorbed by colloids

O.= without correction P.= with correction

In the third table (column P) are reported the results obtained from the difference between the calcium content of the sample solutions (= mother solutions + Ringer liquid, column I), and that of the ultrafiltrate (column H). However, these figures do not yield the effective quantity of Ca adsorbed by the colloids being tested because, as has been seen, part of the Ca is also absorbed by the ultrafilter, and besides, the Ca, during ultra-

(*) In these experiments, and also in those with the organs we must make one reservation; that is, that the Ca of the two salts being tested, placed into contact with the organic substances (blood, blood serum, organ pulp) can not meet with any chemical combinations which might render it, even in part, insoluble or unfiltrable. If this occurs -- which at present is solely hypo-

thetical -- and if consequence it is possible to evaluate the amount of this Cs incapable of ultrafiltration, it would then be necessary to deduct it from our final results; logically, this aliquot of Cs would no longer be considered as retained by the ultrafilter, as such as adsorbed by the organic substances under examination.

filtration, is somewhat diluted by the water still remaining in the apparatus. Thus it is necessary to subtract from the figures obtained (column N) these 2 differential values, which we have already calculated in the first experiments (table I, column E).

These final results, noted in table IV, column O, represent the part of the Ca adsorbed by the blood colloids, the blood serum colloids, and those of the red corpuscles.

For a calculation even closer to the truth, it is necessary to keep in mind that the mother solutions of Ca, added to the liquid of Ringer, present an easily calculable and dosable dilution (table I, column B; table III, column I), while the kind of dilution obtained with the addition of the blood elements does not occur, where the effective dilution must be inferior to this latter, because it is involved with protein substances which are definitely not participants in the processes of dilution , and with water bound to the colloidal . It is impossible to calculate the effective dilution obtained in these latter cases; however, as one of us has done (Scaglioni), it is possible, by means of the dry residue, to calculate at least the maximum dilution which is obtained, whenever the organic substances being examined, composed essentially of one solid part (dry residue) and one liquid part capable of behaving like free water, are considered. By means of the dry residue we calculate the greatest dilution which the mother solution can undergo in contact with blood, blood serum, and the red cells; that is, this dilution corresponds to that considered thus far in the sample solutions (mother solution + Ringer = 3:1), increased by the percentage of the respective dry residue.

The dry residue, derived from the constant weight of the organic substances being tested, after heating in the oven at 105°C, and in the dessiccator, was 16.85% for the blood, 8.30% for the serum, and 30.60% for the red cells.

Since the substances named were diluted in the proportion of 1:3 with the mother solutions of Ca, and since the red corpuscles used came from 10 cc of blood (and thus represent only about 1/3 of this quantity), the following factors of correction were found:

	Calcium chloride	gluconate
whole blood	4.20% = 0.53 mg	0.55
blood serum	2.075% = 0.26 mg	0.27
red cells	2.55% = 0.32 mg	0.33

In column P are reported the values inherent to the Ca adsorbed by the colloids of the blood, the blood serum and the red cells, increased by these factors of correction.

From our research the following conclusions can be drawn:

a) The Ca added to the blood and the blood serum in the form of calcium chloride and calcium gluconate is adsorbed by the colloids of these substances in very small amounts which, if they are not always constant, may be considered null, because they re-enter the limits of error forecast by the method of experimentation. However, the whole blood adsorbs more Ca than the serum.

b) The red cell either do not adsorb Ca or adsorb it in minute quantities, practically negligible ones. The adsorption in question should also be referred to the extra-globular protein substances which can also be found among the red cells, after repeated washing with a physiological solution of defibrinated blood.

c) The Ca-gluconate appears to be more adsorbed by the colloids of the blood and the serum, than the Ca contained in the chloride.

EXPERIMENTS ON THE ORGANS

We then extended our experiments to the various organs: the muscles, the liver, the spleen, the lungs and the encephalus. These organs were withdrawn from the animal (sheep) immediately after it was butchered and, after being weighed, were pounded in a morter with an equal amount of glass powder, until a uniform powder was obtained. Then a 6 times greater amount of the mother solution of calcium chloride or calcium gluconate was added, tempered at 18°C and containing about 15 mg% of Ca.

Here as well, the mother solutions of Ca were made with the liquid of Ringer, deprived of its Ca, to which were added drops of a very diluted solution of NaOH, for the purpose of obtaining a pH of 7.70-7.73.

Said mixtures with the organs were left for 10 minutes at an atmospheric temperature of 17°C, and then centrifugated at a thousand turns per minute for 3 minutes; on 40 cc of the decanted liquid ultrafiltration by the customary method was conducted. The first 10 cc of ultrafiltrate were not taken into consideration, and the quantitative analysis of the Ca was made only of the last 3.5 cc.

Before performing the ultrafiltrations with the organs, we performed a series of orientation experiments.

1) Dosage of the Ca in mixtures composed of 6 parts Ringer liquid plus one part of organ pulp of the various organs;

2)Research on the Ca in the ultrafiltrate, obtained from 40 cc of the preceding mixtures.

3) Determination of the Ca in the mother solutions of Ca-chloride and Ca-gluconate; in the mixtures composed of 6 parts of these solutions plus 0.4 of Ringer liquid; and finally in the ultrafiltrates, obtained from 40 cc of the indicated solutions.

In the first tests the analysis of the Ca was negative. With this we do not wish to exclude that Ca was not contained in the organs studied, but, since it is for the most part fixed to the colloids, and free it undergoes a strong dilution by the Ringer liquid, it is no longer dosable with our methods.

In the second experiment the Ca was not found in the ultrafiltrate, and this appears to us to be a logical consequence of the outcome we formerly obtained.

The results obtained in the third experiment are recorded in table V, because they will be of use to us in the final calculations.

In these experiments we see that the ultrafiltrate (column B) is poorer in Ca than the solution from which it comes (column A) and that moreover, in the solution remaining from the ultrafiltration (column C) the Ca is decreased. We already know that these facts (see table I) depend, on the one hand, on the adsorption of Ca due to the materials which compose the ultrafilter, and on the

other hand, on the dilution caused by the water still remaining in the apparatus after the passage of the first 10 cc of ultrafiltrate. The values reported in column D, resulting from the difference between the concentration of Ca to be ultrafiltrafiltrate and the respective concentration of Ca in the ultrafiltrate, include in the aggregate both the factors reported.

TABLE V

A. Soluzioni	Ca mgr% nella soluzioni A)	Ultrafiltrato Ca mgr% B)	Soluz. residua dopo ultrafiltrazione Ca mgr% C)	Ca assorbita dall'apparecchio (A - C) Ca mgr% D)
N. 1 Soluz. madre Ca-cloruro	15.00	14.35	14.25	0,65
N. 2 Soluz. madre Ca-gluconato	15.20	14.90	14.80	0,30
N. 3 Soluz. Ca-cloruro + Ringer (6:0.4)	14.15	13.55	13.40	0,60
N. 4 Soluz. Ca-gluconato + Ringer (6:0.4)	14.30	14.10	14.10	0,20

Key:
A.= solutions 1.= mother solution of Ca-chloride
2.= mother solution of Ca-gluconate
3.= Ca-chloride solut. + Ringer
4.= Ca-gluconate + Ringer

Once the above investigations were completed, we went on to the ultrafiltration of 40 cc mother solution of calcium chloride and calcium gluconate added to the powder of the organs. The results of these new experiments are recorded, for purposes of brevity in table VI.

TABLE VI

		1. Soluz. madre + organi (6 : 1)	2. Concentraz. Ca nella soluz. madre mgr% E)	3. Concentrazione Ca soluz. madre + organi a) Miscela ultrafiltrate mgr% F)	b) Ultrafiltrato mgr% G)
A.	*Ca-cloruro*	Muscoli	15. -	15.50	14.65
B.		Encefalo	15.-	15.60	13.
C.		Polmoni	15.	15.10	12.
D.		Fegato	15.-	15.00	14.-
E.		Milza	15.	15.00	13.
F.		Reni	15. -	15.	13. -
A.	*Ca-gluconato*	Muscoli	15.20	15.50	14.90
B.		Encefalo	15.20	15.20	13.10
C.		Polmoni	15.20	15.30	12.30
D.		Fegato	15.20	15.50	13.25
E.		Milza	15.20	15.50	13.
F.		Reni	15.20	15.20	13.10

Key:
1.= mother solution + organs
2.= concentration of Ca in the mother solution
3.= concentration of Ca in mother solution + organs
a) ultrafiltrant mixture b) ultrafiltrate
A.= muscles
B.= encephalus
C.= lungs
D.= liver
E.= spleen
F.= kidney

Here as well we find that the Ca appears in a lesser quantit in the ultrafiltrate than in the ultrafiltrant liquid. This demonstrates that the colloids of the muscles, the encephalus, the lungs, the liver, the spleen and the kidneys have adsorbed Ca. Now, in order to calculate this adsorption it is necessary to subtract the Ca of the ultrafiltrate from the relative ultrafiltrant liquid. We do not do this, because we prefer, for greater exactness, to lead our operations on the mother solutions and on the basis of it to calculate , with as much more approximation is conceded to us, the dilution which it certainly undergoes in the organs being tested.

The differential values between the mother solution and the ultrafiltrate in question, deprived of the Ca taken up by the ultrafiltrating apparatus, would indicate the Ca adsorbed by the colloids of the pulps of the various organs, if these pulps behaved in the face of the mother solution as solid substances, that is, with a completely nonexistent power of dilution, But in the face of these data it is logical to think the opposite, that is, that all or part of the liquid content of the organs studied may enter into the processes of dilution. These eventualities are also sufficiently calculable, by means of the dry residue which, determined in the aforementioned method for blood, yielded:

	1. Residuo secco	2. Acqua totale
Muscoli	23.0%	77.0%
Encefalo	18.0%	82.0%
Polmoni	18.4%	82.0%
Fegato	24.0%	76.0%
Milza	20.0%	80.0%
Reni	25.5%	74.5%

1.= dry residue 2.= total water
A-F see above

It immediately appears that between the possibility that all the water contained in the organs may enter into the process of dilution (possibility of maximum limit) and that previously considered (at zero limit), is where the truth is found. In fact one of us (Scaglioni), in previous works, found that the effective value of the free water must comprise between 10% and 70% of the quantity of the organ used; thus fixing the average value as 40%, we find that the organs added to the mother solution

of Ca in a proportion of 1:6, will increase the liquid of the said solutions of Ca. Thus we have a concentration of calcium chloride and calcium gluconate in the mother solution, diluted by the addition of the organs in parts of 6 or 6.4, and this corresponds respectively to 14.06 mg % and 14.25 mg %, instead of the 15 and 15.20 mg % noted in table VI, column E. These two values have a differential of plus or minus 0.65%, according to which we consider the maximum or minimum possible dilution of the mother solutions; that is, corresponding to its descent to the limit of 6.7 (= 70% H_2O in the organs) or increase to 6.1 (= 10% H_2O in the organs).

After these premises of finding the Ca adsorbed by the colloids of the organs examined, nothing can be done except to subtract from the actual concentration of Ca in the original solution, mixed with the organs (table VII, column H), the Ca adsorbed by the ultrafiltrating apparatus, calculated in No. 3 and 4 of table V, column D; and finally the Ca which ultrafiltrates is deducted (table VI, column C). These final results are recorded in table VII.

TABLE VII

1. Sostanze in esame		2. Concentrazione effettiva del Ca: nelle miscele avanti l'ultrafiltrazione Ca = mgr	come H), ma diminuito del Ca assorbito dall'apparecchio. Ca = mgr %	Ca assorbito dai colloidi degli organi mgr %
		H)	I)	L)
	Ca-cloruro			
A.	Muscoli	14.05 ± 0.65	13.45 ± 0.65	— ± 0.65
B.	Encefalo	14.05 »	13.45 »	0.45 »
C.	Polmoni	14.05 »	13.45 »	1.45 »
D.	Fegato	14.05 »	13.45 »	— »
E.	Milza	14.05 »	13.45 »	0.45 »
F.	Reni	14.05 »	13.45 »	0.45 »
	Ca-gluconato			
A.	Muscoli	14.25 ± 0.65	14.05 ± 0.65	— ± 0.65
B.	Encefalo	14.25 »	14.05 »	0.95 »
C.	Polmoni	14.25 »	14.05 »	1.75 »
D.	Fegato	14.25 »	14,05 »	0,80 »
E.	Milza	14.25 »	14.05 »	1.05 »
F.	Reni	14.25 »	14.05 »	0.95 »

Key:
1.- substances being examined 2.- actual concentration of Ca.
H.- in the mixtures before ultrafiltration
I.- like H, but decrease of the Ca absorbed by the apparatus
L.- Ca adsorbed by the colloids of the organs
A-F see table VI.

These results, though subject to quite a large margin of

cures, allow us to make the following conclusions:

a) The colloids of all the organs in the experiment, with the exception of the muscles, adsorb Ca.

b) The lung is the organ which adsorbs the Ca with greatest intensity: it is followed by, in descending order, the spleen, the kidneys, the encephalus and the liver.

c) The organs which adsorb the Ca always showed a greater affinity for calcium gluconate than for calcium chloride.

GENERAL CONCLUSIONS AND SUMMARY

1. -- The Ca, in the form of calcium chloride and calcium gluconate, put into contact with the blood and pulps of organs, is in part adsorbed by their colloids. The adsorption, however, occurs less intensely in the case of blood, than in the case of the organs.
2. -- Only the muscles do not adsorb Ca.
3. -- The strongest adsorption of the Ca was in the lung, and then in descending order, in the spleen, the kidneys, and the encephalus. The liver possesses the least power of adsorption, and only for calcium gluconate, while this power is totally lacking for calcium chloride.
4. -- The whole blood adsorbs Ca in greater proportion than the related serum; on the other hand, the red cells do not exercise this function, because the small amount of Ca taken up by them is attributable to the extra-globular protein substances still remaining in the blood from which the cells are derived.
5. -- The colloids under examination always showed a greater affinity for calcium gluconate than for calcium chloride; it is, in fact, with the former that we observed the greatest adsorption of Ca.
6 -- From our experiments, we are nonetheless unable to establish a judgment on the manner (physical, chemical, physico-chemical) in which the Ca is fixed by these colloids.

Summary -- The colloids of the various organs adsorb the calcium of the calcium chloride and the calcium gluconate. Only the colloids of the muscles do not have this adsorbing power. Of the organs examined, the lungs adsorb more intensely , followed then by the spleen, the kidneys, and the brain. The liver does not adsorb the calcium chloride, and the calcium gluconate only slightly.

The blood in toto adsorbs the calcium chloride and gluconate more than the serum; the red cells, instead, seem incapable of adsorbing calcium. The blood elements adsorb less calcium than the above-named organs.

The examined colloids have constantly demonstrated a greater affinity for the calcium chloride than for the calcium gluconate.

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STUDIES ON CALCIUM METABOLISM WITH Ca^{45}

I. The effect of citric and tartaric acid on the calcium metabolism

by

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(received on February 22, 1956)

The question as to which form, structure and accompanying substances calcium (Ca) should have in food in order to be optimally absorbable has in no way been finally answered. This is shown by the continuously growing publications having to do with this complex of problems (composite literature in Nicolaysen, see also Schreier and Wolf).

Along with vitamin D , such acids are of particular interest whose alkali salts are capable of complex formation with Ca ions. Since the description of the antirachitic effect of citric acids (Ci) by Shol, Hamilton and Dewar, Rominger, its action in the calcium metabolism has been studied many times (Schreier, Steggerda and Mitchell, Theopold and associates, Minder and Gordonoff, Harrison and Harrison, Klinke et al., etc.).

The results and the interpreatation are not uniform. In continuation of previous experimental studies on children (Schreier and Wolf) we believed that we would be able to provide a contribution to the explanation of this complex of problems through the use of marked Ca (Ca^{45}) as indicator for the course of the process of calcium metabolism.

Since tests on healthy sucklings and children were forbiddenm we used rats for our tests and we used both normal and rachitic animals. The normal rats received a standard diet with sufficient vitamin D and calcium content. The rachitic animals were fed with the rachitogenic food according to McCullum. The rachitis was determined by x-ray[1].

The test was conducted as follows:

The radioactive Ca^{45} was given to the animals in the form of Ca^{45} Cl_2 . They received uniformly in all tests 51.1 mg with Ca^{45} marked $CaCl_2$, corresponding to 20 mg Ca. To these constant-held amounts of Ca^{45} we gave in the various individual tests different amounts of sodium citrate, sodium phosphate and sodium tartrate and we dissolved the salt mixture in distilled water or full milk (see ahead).

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1. We are grateful to Mrs. Dr. Muller for the rachitic rats .

milk (see further below).

The solutions were administered to the animals by stomach probe whereby the probe and sprays were flsshed each time with distilled water.

The probe feeding yielded in the corresponding routine an equally great storage of Ca^{45} in the bones and teeth as the allowance for of Ca^{15} to the food (Antoni and Cremer).

Six hours before the start of the test the animals were given no food. After the probe they came into metabolism cages which permitted a separate determination of stool and urine. Eighteen hours after the probe they were sacrificed without their having received any food in the meantime. Water was given as desired (ad libitum).

We determined the radioactive Ca^{45} content in the following tests:

a. Blood serum (after dealbuminization with trichloracetic acid),
b. Femur, tibia snd fibula of both rear extremities,
c. Rodent teeth,
d. abdominal-intestinal tract and feces,
e. Urine.

Preparation of the probes. The bones, teeth and abdominal-intestinal tract with feces were dried previously in porcelain pans in dry oven at 120-150° C for a few hours and then incinerated in muffle oven at a temperature of 800° C. In the urine the fluid was evaporated and the solid components were incinerated in incinerating baskets with concentrated salpeter acid and perchlric acid (1:1).

The incineration remains were brought to a volume between 2 cm^3 and 10 cm^3 according to the degree of expected activity with rarefied salpeter acid (D 1.1). Then the radioactivity of the fluid probes was determined.

Measurement methdd: For the activity measurement we used a counter of the Friesecke and Hoepfner company (FH 49) and a bell counting tube with thin coating. For the measurement of the fluid samples we used glass basins with a diameter of 15 mm and a depth of 10 mm into which we pipetted 20 cm^3 of the radioactive fluid with calibrated pipettes. The distance between the fluid surface and the counter tube window was 6 mm.

The measurement of fluid samples, when the radiation of the isotope is sufficiently penetrating, presents the advantage of great simplicity in preparing the and considerable savings in time in comparison to the preparing of solid samples (Cremer and Herr).

The activity of each preparation was determined as the mean value from 4 to 5 measurements over 10 minutes.

Then we studied the effect of various amounts of citric acid and sodium phosphate (Na_2HPO_4) on the resorption and retention of radioactive Ca^{45} in normal and rachitic rats.

The first test series included 12 normal rats which were divided up into 4 groups of 3 rats each.

Ca^{45}, Ci and Na_2HPO_4 was fed the rats of the individual groups in the following composition:

Group I: 51.1 mg with Ca^{45} marked $CaCl_2$

Group II: 51.1 mg $CaCl_2$ + 4 mg trisodium citrate

Group III: 51.1 mg $CaCl_2$+10 mg trisodium citrate

Group IV: 51.1 mg $CaCl_2$+4 mg trisodium citrate + 20 mg Na_2HPO_4

The salt mixture was dissolved in 1 cubic centimeter full milk.

The test results are given in table 1.

The total absorption of radioactive Ca^{45} was calculated as the difference between the amount of Ca fed and the amount of Ca secreted in the digestive tract and feces. The amount which is actually absorbed is naturally somewhat greater since a part of the absorbed Ca is still in the intestinal wall. This amount is so small, however, that it can be disregarded (Lindquist).

In another test 12 normal rats were studied (4 groups of 3 rats each). These rats were fed the following salts:

Group I : 51.1 mg with Ca^{45} marked $CaCl_2$

Group II : 51.1 mg with Ca^{45} marked $CaCl_2$+ 13 mg trisodium citrate

Group III: 51.1 mg with Ca^{45} marked $CaCl_2$+20 mg Na_2HPO_4

Group IV : 51.1 mg with Ca^{45} marked $CaCl_2$+13 mg trisodium citrate + 20 mg Na_2HPO_4

The salt mixture was dissolved in distilled water.

The test results are given in table 2.

In the next test series we studied 12 rachitic rats to which we fed the salts in the same dosage as in the preceding test.

The results are shown in table 3.

Tabelle 1

Vers.-Gruppe	Salze	Körpergewicht (g)	Ca^{45} in % der zugeführten Dosis: Resorption	Serum (1 ml)	Knochen	Incisoren	Urin
I	$Ca^{45}Cl_2$	91,0	50,0	0,4	9,5	1,2	2,0
		84,5	50,7	0,3	9,9	1,1	1,8
		86,5	51,6	0,4	9,6	1,2	1,9
II	$Ca^{45}Cl_2$	78,0	56,8	0,3	10,8	1,1	1,4
	+Na-Citr.	91,0	55,2	0,4	11,8	1,3	1,7
	(4 mg)	84,0	53,7	0,3	11,1	1,1	1,8
III	$Ca^{45}Cl_2$	102,0	54,2	0,6	10,2	0,9	1,5
	+Na-Citr.	120,0	57,0	0,6	10,5	0,9	1,8
	(10 mg)	110,5	55,9	0,4	11,3	0,9	1,9
IV	$Ca^{45}Cl_2$	88,5	52,3	0,4	9,5	1,3	2,0
	+Na-Citr.	68,0	51,4	0,4	9,3	1,2	2,0
	$+Na_2HPO_4$	104,5	53,7	0,5	9,4	1,4	2,0

Tabelle 2

Vers.-Gruppe	Salze	Körpergewicht (g)	Ca^{45} in % der zugeführten Dosis: Resorption	Serum (1 ml)	Knochen	Incisoren	Urin
I	$Ca^{45}Cl_2$	65,0	50,3	0,4	9,2	1,2	1,7
		74,0	50,0	0,5	9,0	1,1	1,8
		74,0	49,7	0,4	9,3	0,9	1,5
II	$Ca^{45}Cl_2$	52,4	54,6	0,3	9,9	1,5	1,1
	+Na-Citr.	54,2	52,2	0,4	9,7	1,2	1,3
		57,6	55,4	0,4	10,9	1,3	1,1
III	$Ca^{45}Cl_2$	88,5	40,9	0,5	7,3	0,9	0,9
	$+Na_2HPO_4$	80,8	41,7	0,4	6,9	1,0	1,1
		75,7	43,9	0,4	7,7	1,2	1,0
IV	$Ca^{45}Cl_2$	70,3	52,8	0,3	8,9	1,2	1,1
	+Na-Citr.	75,5	53,9	0,3	8,4	0,9	1,2
	$+Na_2HPO_4$	55,6	51,8	0,3	8,3	1,1	0,9

1. test group; 2. salts; 3. body weight (grams);
4. Resorption; 5. Ca^{45} in % of the dose administered;
6. serum (1 ml), 7. bones; 8. incisors; 9. urine

Tabelle 3

Vers.-Gruppe	Salze	Körpergewicht (g)	Ca^{45} in % der zugeführten Dosis: Resorption	Serum (1 ml)	Knochen	Incisoren	Urin
I	$Ca^{45}Cl_2$	54,8	37,4	0,4	5,1	0,8	2,7
		65,4	41,0	0,4	6,2	0,8	2,5
		50,7	36,1	0,4	5,0	0,8	2,6
II	$Ca^{45}Cl_2$	60,3	45,9	0,5	7,7	1,0	2,6
	+Na-Citr.	57,3	47,5	0,5	7,5	1,0	3,3
		51,2	44,7	0,5	9,2	0,9	2,9
III	$Ca^{45}Cl_2$	41,0	40,8	0,3	3,5	0,7	3,4
	$+Na_2HPO_4$	52,0	37,0	0,5	4,0	0,7	3,9
		56,8	44,9	0,5	4,4	0,7	3,2
IV	$Ca^{45}Cl_2$	63,3	49,3	0,5	7,3	0,9	4,1
	+Na-Citr.	64,2	48,2	0,5	6,9	1,0	5,2
	$+Na_2HPO_4$	56,8	47,9	0,5	7,4	0,9	5,3

(same captions as the other tables).

Furthermore, the administration of 20 mg sodium tartrate increases the incorporation of $Ca^{45}Cl_2$ in the teeth and bones, as shown by the following test: (mean values of 10 animals).

Tabelle 4

Vers.-Gruppe	Salze	Körpergewicht (g)	Ca^{45} in % der zugeführten Dosis: Resorption	Serum (1 ml)	Knochen	Incisoren	Urin
10 Tiere	$Ca^{45}Cl_2$	108	50,6	0,4	9,6	0,9	2,3
10 Tiere	$Ca^{45}Cl_2$ +Na-Tartr.	105	53,8	0,6	11,0	1,1	2,0

1. test groups, 2. salts, 3. body weight (g), 4. Ca^{45} in % of dose fed, 5. resorption, 6. serum (1 ml), 7. bones, 8. incisors, 9. urine, 10. animals, 11. +sodium tartrate.

Coleman found that the rachitic skeletal changes only healed if the phosphorus content of the food was not proportioned too low. These observations were supplemented by studies made by Theopold and Hennig who found that the Ci-citrate complex only turns out to be effective when optimal amounts of phosphorus are present.

Based on these observations we compared the resorption and retention of Ca^{45} from a mixture of 75 mg with Ca^{45} marked Ca^{44}-citrate +11 mg with Ca^{45}-marked $Ca^{45}{}_3(PO_4)_2$ with that from 51.1 mg$Ca^{45}Cl_2$, 95 mg Ca^{45}-citrate and finally from 95 mg Ca^{45}-citrate+ 320 mg citric acid+ 68 mg KH_2PO_4 (= equivalent Ca amounts). We obtained the following results:

Tabelle 5

Vers.-Gruppe	Salze	Körpergewicht (g)	Ca^{45} in % der zugeführten Dosis: Resorption	Serum (1 ml)	Knochen	Incisoren	Urin
I	$Ca^{45}Cl_2$	98,0	49,9	0,7	6,0	1,5	1,4
		101,5	50,4	0,7	6,3	1,4	1,5
		205,0	58,8	0,6	7,9	1,5	1,6
		141,0	52,0	0,5	6,3	1,2	1,4
		119,0	51,6	0,6	6,3	1,2	1,7
		173,5	51,6	0,7	5,6	1,1	1,6
II	Ca^{45}-Citr.	139,5	50,2	0,9	8,2	1,3	2,4
		137,5	52,0	0,8	8,5	1,3	2,4
		220,0	58,7	0,9	9,2	1,5	3,2
		177,0	56,4	0,8	8,4	1,6	3,1
		156,0	53,0	0,9	8,8	1,6	2,8
III	Ca^{45}-Citr. +$Ca_3{}^{45}(PO_4)_2$	146,0	61,2	1,1	8,9	1,4	1,1
		186,0	63,4	1,1	9,6	1,3	1,2
		87,0	59,7	1,1	8,7	1,2	1,2
		128,5	61,0	0,9	8,8	1,3	1,1
		127,0	63,9	1,0	8,3	1,4	1,2
		141,0	62,8	1,0	9,4	1,5	1,3
IV	Ca^{45}-Citr. +Ci+KH_2PO_4	161,5	54,3	1,0	6,4	1,5	1,5
		161,0	49,2	1,1	9,4	1,5	1,5
		161,0	56,6	1,1	10,8	1,5	1,5
		181,5	55,8	1,2	7,7	1,5	1,4
		137,0	53,0	1,2	6,0	1,4	1,5
		142,5	50,5	1,1	6,4	1,5	1,4

The addition of 4 mg and 10 mg sodium citrate to the $Ca_3^{45}(PO_4)_2$ causes a definite increase in the resorption and retention of this salt.

The average values of the 4 animals were:

	bones	teeth	
$Ca_3^{45}(PO_4)_2$	1,6%	0,3%	of the dose administered
$Ca_3^{45}(PO_4)_2$ + 4 mg Na-Citr.	2.1%	0,4%	
$Ca_3^{45}(PO_4)_2$ + 10 mg Na-Citr.	2,2%	0,4%	

The small number of laboratory animals can be explained by the almost insurmountable difficulties in sampling (probing) this salt quantitatively.

Discussion of the Test Results

In confirmation of our calcium balance tests in children, these tests on rats showed that the addition of sodium citrate (and also sodium tartrate) results in an increase in calcium resorption and also retention in normal and rachitic rats. It is worthy of note that in the case of the rachitic animals the effect of the calcium metabolism by citrate is expressed more strongly than in the case of the normal animals. It is also to be pointed out that the calcium supply of our rats was in accordance with the conventional international standard. The calcium doses of the balance tests had to be selected so high because of the fact that the specific activity of the marked $CaCl_2$ which we had available was not very high.

The fact of the resorption improvement of Ca by Ci should be considered as proved through a statistical evaluation of the results. A large number of publications have already reported positive findings. Older studies came, for example, from Hathaway and Meyer; Henry and Kon; Lanford; Day et al. With marked Ca, Gordonoff and Minder studied the effect of 2 drops of a 25% sodium citrate solution per day (approximately 25 mg sodium citrate) on the metabolism of rachitic rats. The rachitis was cured, but the amount of stored Ca^{45} did not show any significant difference in comparison to the animals without citrate administration. This would mean that the decomposition processes in the bone were suppressed by Ci. Mallon and Jones, as well as Steggerda and Mitchell, however, did not find any effect of citric acid or orange juice on the Ca balance. Recently Antoni and Cremer reported on Ca^{45} tests in rats. They used, among other things, tertiary Ca-citrate , secondary Ca-phosphate and mixtures of Ca-citrate and phosphate. They found without vitamin D that the utilization of lactate and chloride was better than that of Ca-citrate. Vitamin D brought about a very significant improvement in the Ca-citrate utilization. The citrate-phosphate mixtures resulted in a worsening of the Ca utilization with respect to the citric acid salt. As an explanation for the differences in the results of our tests and those of Antoni and Cremer we can perhaps give the difference in the food of the laboratory animals and in particular the vitamin D content in the food.

In order to also verify the effect of citric acid (Ci) on the resorption and retention of $CaCl_2$ mathematically, we subjected our figures to a statistical examination with the aid of the T test. The difference in both the resorption and retention in the bones and teeth is highly significant within all groups and also in the comparison of all animals without citrate with those which received this salt. The values were between 6.2 and 7.6 In the calculation we used the known formula:

$$M_1 - M_2 \cdot \sqrt{\frac{n^2}{2n}}$$

$$\sqrt{\frac{c_1 + c_2}{2n - 2}}$$

$c = (M - a)^2$ nach STUDENT[1].

1. according to Student

The increase in resorption by Ci is apparently dependent upon Ca hunger of the body (rachitis!) since naturally under these circumstances a more favorable drop occurs for the resorption.

The capacity of the Ci to bring about Ca complex formation is generally accepted as the cause for the increase in resorption. The individual authors, however, do not agree on the form and structure of the complex salt, or better still the complex salts (literature references for example in HEINZ). We hold that the concept is correct that the complex salt formation takes place in the sense of a dynamic equilibrium which is dependent upon the various anions and cations which are also present in the fluid. These accompanying substances , as well as the pH of the solution, affect the structure and the dissociation stages of the complex salt (see also KLINKE).

To study the question of whether in vivo Ca-complex salt forms actually affect the resorption and retention of Ca, the effect of various polymer phosphates was studied in an extensive test series (Mattern and Schreier). It turned out that the administration of 10 mg pyrophosphate , tripolyphosphate and Graham salt together with 10 or 20 mg Ca in form with $CaCl_2$ marked with Ca^{45} did not have any effect on the resorption and retention of Ca. Also the daily intake of 10 and 20 mg Graham salt for 10 days did not have any effect on the calcium retention in the bones and teeth. On the other hand the same amount of orthophosphate leads to an average reduction in resorption of about 35% and to a reduction in the retention of about 36%. The polyphosphates consequently demonstrated a significantly lower obstructing effect on the Ca resorption than the orthophosphate. Borenstein and Schwartz, as well as Schwartz and Jones, even found a promoting effect on the Ca resorption by polyphosphates. One will have to assume that these differences are to be attributed to the fact that the polyphosphates with Ca form water-soluble complex salts.

In a study which is worthy of attention , Yendt and Howard found that cartilage sections of rachitic rats which had been administered Ci shortly before,in a corresponding medium store calcium in considerably lower Ca and phosphate amounts than in the case of control animals. This seems to provide proof that the Ci penetrates into the bone substance not only in the resorption processes but also in the retention process of the calcium salts. This is also shown by the values which are indicated here, since, as already pointed out, the retention of the Ca^{45} is also significantly increased in the presence of citrate in the intestine. Furthermore, Heinz, Muller and Rominger already were of the opinion a long time ago that the Ci interferes in the mechanism of the calcium retention in the bones, since the increase in the resorption already is not enough to explain the anti-rachitic action of the Ci, since otherwise every good water-soluble and absorbable calcium salt would cure rachitis. In this regard the findings of Dickens and recently of Teufel and Krusen are interesting, i.e. that in florid rachitis the Ci content of the bones is considerably smaller than in the case of healthy control animals. Bellin and Steenbock found that vitamin D greatly increases the excretion of Ci in the urine and that this action occurs both in the case of alkaline reaction of the urine and in the case of acid reaction in the urine. Harrison and Harrison found that a reduction in the serum-Ci level occurs in rachitis Antoni and Cremer confirmed in rats the results of Schreier and Wolf that vitamin D increases still further the calcium resorption from citric acid calcium. In the case of subcutaneous administration , furthermore, the Ci should not develop any antirachitic action (Nicolaysen and Nordbo, as well as Hurni).

Table 5 gives a special indication. It is impressively shown here that a certain amount of phosphate is of great significance for the optimum incorporation of the calcium in the bones. A particularly favorable proportion seems to be in the ratio of 7 parts calcium citrate to one part calcium phosphate[1]. Calcium citrate surpasses both the resorption and the retention of this salt mixture , but also a salt from calcium citrate and potassium phosphate with a considerably higher phosphate content which the animals of the 4th group received. With the opinion of Klinke that the sudden change of the Ca in the organism proceeds without any increase in the serum values we cannot agree. Our isotope studies, which make it possible to study the "flow equilibrium", show that an increased resorption is accompanied almost always by an increase in the Ca^{45} level of the blood. The negative findings of Klinke are probably to be attributed to the method of calculating calcium which unfortunately is still not sufficiently accurate.

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1. A salt made in this way is being marketed by the J. A. Benckiser Co.

Summary

A report is given on tests on calcium resorption and retention in normal and rachitic rats. It was found with the aid of Ca^{45}-marked calcium salts that the addition of sodium citrate in doses of up to 4 mg per animal results in a statically verified increase in the retention and resorption of Ca^{45}. Sodium tartrate also significantly increased the calcium absorption and calcium retention in comparison to calcium chloride. A certain amount of phosphate turned out to be essential for the calcium retention and absorption in the bones. A mixed salt, which has calcium citrate and calcium phosphate in a proportion of about 7 to 1 shows a particularly good resorption and retention of Ca^{45}.

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CLINICAL OBSERVATIONS ON THE VALUE OF CALCIUM CHLORID AS A DIURETIC AND ON ITS INFLUENCE UPON THE CIRCULATORY MECHANISM.

(Seventeen Cases of Cardiac Failure, One of Tuberculous Effusions in Serous Cavities, One of Hepatic Cirrhosis with Ascites and Two Normal Controls.)

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Literature. In 1918 Schutz reported beneficial results from large doses of calcium chlorid administered by mouth in cases of war

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nephritis with edema. In a number of his cases this therapy alone did not cause diuresis, but on the addition of digitalis a marked diuresis resulted with the reduction of edema. He inferred from these observations that calcium chlorid did not affect the cardiac factor in the etiology of edema in these cases.

On the other hand, Singer in 1921 found that the intravenous injection of calcium chlorid (0.5 gm.) or the oral administration of calcium lactate (3 gm. per day) caused a temporary diuresis in cases of cardiac failure as well as in so-called cardiorenal cases with edema. He observed that these calcium salts also caused temporary strengthening of the heart action, slowing of the heart rate and lowering of blood pressure. He also states that the best results are obtained by giving these calcium salts and digitalis simultaneously. Cheinisse also reports that calcium chlorid given intravenously in doses of 1 cc. of a 10 per cent solution is a good adjuvant to digitalis in cases of cardiac failure with edema.

More recently, Walters and Bowler investigated the effect of intravenous injection of calcium upon the heart experimentally in normal and jaundiced dogs. They found that therapeutic doses (82.5 mg. per kilogram of body weight injected in a 10 per cent solution at the rate of 1 cc. per minute) quickened the pulse somewhat but they did not find any change in blood pressure even during gradual increase of the dose to the lethal amount by the gradual injection of the 10 per cent solution at the rate of 1 cc. per minute. Lethal doses caused tachycardia, ectopic origin of impulses, disturbances in conduction and finally ventricular fibrillation followed by death of the animal. They refer to the occurrence of tachycardia and the sensation of flushing of heat all over the body in patients during the intravenous administration of calcium chlorid. However, with Hirschsohn and Maendl, they attribute these phenomena to the "dynamic effect" of intravenous injection *per se*, for similar phenomena have been observed following the injection of solutions of quinin, dextrose, hexamethylenetetramin and urea. It is probable that the observations made by Singer correspond to this temporary reaction.

Blum and his collaborators have reported results which indicate that calcium chlorid is an effective diuretic and causes elimination of fluid from serous cavities in cases of inflammatory effusions in the pleural sacs or in cases of hepatic cirrhosis with ascites. Krummenacher and others have reported similar results. Blum and his collaborators, however, sounded a warning against the use of calcium chlorid administered by mouth in cases of edema of cardiac failure, since in their experience the condition of such patients became aggravated as a result of this therapy.

Lowenberg recently reported investigations which led him to conclude that in small doses of 0.1 to 1 gm. calcium chlorid has a cardiotonic but no diuretic action, whereas this latter effect takes

place only after the administration of doses larger than 1.5 gm. From these premises he infers that the diuresis caused by calcium chlorid is not due to its cardiotonic influence.

In 1920 Denis and Minot drew attention to the fact that the upper level of calcium content of blood serum tends to be fixed and could be raised for but a very short period of time (if at all) by the ingestion of large quantities of calcium chlorid. Their observations have been repeatedly corroborated by other investigators. It would seem, therefore, that a continued diuretic effect during and after calcium chlorid administration by mouth is not the result of increased calcium content of the blood.

In 1911 Meyer and Cohn showed that the oral administration of calcium salts to normal infants causes decrease in weight due to loss of water from the body. These investigators found that the calcium ion is excreted mainly in the stool, that the chlorin ion combines with other bases and that the resulting chlorids are excreted in the urine. More recently, Jansen has shown that even after intravenous injection of various calcium salts, including calcium chlorid, the calcium ion is largely excreted in the stool.

In 1921 Vlum and his colleagues found that with the administration of calcium salts, when no diuresis occurred, sodium was retained, while during diuresis an increase in sodium excretion was found. This they attributed to the antagonistic action between the calcium and sodium ions. They therefore advocated the use of salt-free diet together with calcium chlorid in the treatment of nephritis with edema.

Atchley, Loeb and Benedict studied a case of diabetic coma with edema treated by the oral administration of large doses of calcium chlorid per day, and observed a marked diuresis which began at the end of the first twenty-four hours of this therapy. They found the chlorin ion was excreted largely in the form of ammonium chlorid, while sodium excretion was also increased. They compared the data in this case with the data in a case of diabetes with spontaneous diuresis and found no essential difference. They also studied the effect of 20 gm. of calcium chlorid given by mouth to a normal individual; in this case marked diuresis occurred and they found an increase in the amount of sodium, chlorin, potassium and ammonia excretions.

Haldane, Hill and Luck also found increased ammonium chlorid excretion following the ingestion of large doses of calcium chlorid by a normal person. They believe that the diuresis may be due to lowering of osmotic pressure in the tissues, which is caused by disturbance of the Donnan equilibrium as a result of the slight acidosis developed during the ingestion of large doses of calcium chlorid. Gamble, Ross and Tisdall offer a similar explanation for the diuresis which results from the administration of hydrochloric acid, ammonium chlorid and calcium chlorid. Most recently, however,

Salvesen, Hastings and McIntosh have reviewed the subject of the effect of calcium salts on the inorganic composition of the blood, and from their work as well as from that of others they conclude that the question whether the diuretic effect of calcium salts is dependent on the anion and is in some way connected with the production of acidosis is as yet unsettled.

Rockwood and Barrier report 7 cases of edema of nephritic and diabetic origin treated by the oral administration of calcium lactate or calcium chlorid or both in doses of 12 to 18 gm. per day. Marked diuresis occurred as a result of this therapy in 6 cases; in the seventh diuresis occurred but was not attributed to the calcium therapy. They also found that large doses of calcium chlorid do not increase the amount of serum calcium. In several cases the chlorid was more effective than the lactate salt and they conclude that the former is the better diuretic. Keith *et al.* also report a significant diuresis in 1 case of subacute glomerular nephritis and in 1 of nephrosis.

This brief review of the literature indicates that calcium salts may cause diuresis in normal individuals as well as in individuals with a variety of diseases, including nephritis, cardiac failure, diabetes with edema, inflammatory effusions in the pleural sacs, hepatic cirrhosis with ascites and others. The influence of the calcium salts given orally in therapeutic doses seems to be related to the metabolism of water and inorganic salts in the tissues rather than directly to its effect upon the circulatory mechanism.

Our interest in studying the value of calcium chlorid as a diuretic was aroused by the striking results obtained in a case of heart disease with congestive failure and edema (Case III) when, after digitalis, diuretin and calomel began to fail or to be sluggish in their diuretic effect, calcium chlorid was most effective.

The chief object of our investigation has been to test the value of calcium chlorid as a diuretic and to determine its effect upon the circulatory mechanism as measured by clinical methods, particularly with reference to a possible digitalis-like action.

Present Investigation. Method. Crystalline calcium chlorid was administered in the form of a solution almost invariably, in 2.5 gm. doses diluted in 50 to 100 cc. of water. In experiments upon a normal individual doses of 5 gm. and 8 gm. each were taken. In the majority of cases the daily amount consisted of 15 gm. given in 6 doses of 2.5 gm. each at two-hour intervals. With the smaller doses the only handicap was the unpleasant taste of the drug; this may be avoided by giving the dry salt in capsules, each containing 0.5 gm. The larger doses of 5 and 8 gm. each caused a burning sensation in the epigastrium which was readily relieved by taking some food, less readily by drinking water. Diarrhea did not occur in any case; rather was there a tendency to constipation.

The "fluid intake" includes only liquids, like soups, milk, water, and not the water in solid or semisolid foods. In all but 2 cases

(II and XVII) the diet was a mixed ordinary hospital diet and salt was not restricted. In the few cases in which salt intake was restricted this fact is mentioned.

The pulse rate represents the resting pulse rate taken in the morning before breakfast at about seven o'clock.

The blood pressure was recorded by the auscultatory method and in cases of auricular fibrillation the point at which beats of an average rate of 80 to 90 per minute were first heard was recorded as the systolic pressure and that point at which beats at a similar rate ceased to be heard or became very faint was recorded as the diastolic blood pressure.

Vital capacity was determined with an ordinary water spirometer and is expressed in the tables in the terms of cubic centimeters of air, thus furnishing evidence of changes in the individual case.

By the term "digitalization" is meant the administration of 0.1 gm. digitalis leaf per 10 pounds of body weight within forty-eight hours and the usual maintenance dose prescribed in our clinic is 0.1 gm. of digitalis per day. Almost invariably digitalis leaf in pills of 0.1 gm. each is the preparation used; in a few instances digifolin was administered by mouth or intramuscularly.

Diuresis in Normal Persons During Calcium Chlorid Administration. CASE I.—H. N. S., male, aged twenty-six years. In order to determine the effect of calcium chlorid when administered to normal individuals with no cardiac or renal disease, two volunteers took the drug. The first series of observations on Case I were made under the following conditions: low calcium and salt-poor diet, which contained 0.093 to 0.140 gm. calcium and 1 to 2 gm. sodium chlorid per day, with a calorific value of 10 per cent or more over the basal requirements; continuation of subject's usual activities throughout the fifteen days of the experiment; blood pressure and vital capacity determined at midday before luncheon. The relatively low daily fluid intake of the subject during this experiment is his normal fluid intake. The calcium chlorid was taken in doses of 5 gm. diluted in 100 cc. of water three times a day with each meal for two days, on the third day 15 gm. in 200 cc. of water in one dose with the midday meal and during the next three days 8 gm. in 125 cc. of water three times a day, with each meal. Thus a total of 117 gm. was taken in six days.

The subjective symptoms were chiefly related to the unpleasant taste of the solution of calcium chlorid and the sensation of burning referred to the epigastrium which followed the larger doses. This discomfort lasted for one-half to one hour. With the smaller doses of 5 gm., each taken with a meal after about half of the food had been consumed, there was no epigastric burning. Occasional griping occurred during the days when the larger doses were being taken and there was a tendency to constipation; at no time did diarrhea occur.

The data in Table I show evidence of some diuresis during the course of calcium chlorid ingestion. The daily changes in the electrocardiogram are not conclusive evidence of the effect of calcium chlorid administered by mouth since similar changes occurred before and after this therapy.

TABLE I.—EFFECT OF CALCIUM CHLORID INGESTION IN A NORMAL PERSON. (CASE I.)

Date. 1923.	Calcium chlorid, gm.	Fluid intake, cc.	Urine, cc.	Body weight, kilos.	Blood pressure.		Vital capacity, cc air.	Heart rhythm.	Electrocardiogram.		T wave in Lead II, mv.
					Sys.	Dia.			Rate.	P-R interval second.	
Nov. 20	0	780	720	70.0	109	64	3600	Normal	80	0.16	+0.65
21	0	790	720	70.0	115	68	3600	Normal	70	0.16	+0.65
22*	0	790	700	70.0	110	65	3620	Normal	75	0.16	+0.70
23	15	790	820	70.0	120	70	3640	Normal	90	0.15	+0.50
24	15	780	1240	70.0	112	64	3700	Normal	70	0.17	+0.80
25	15	720	960	69.5	115	65	3690	Normal	80	0.16	+0.75
26	24	900	720	69.5	115	68	3800	Normal	75	0.17	+0.70
27	24	1420	2400	69.0	115	70	3790	Normal	70	0.17	+0.65
28†	24	960	1560	68.5	108	62	3800	Normal	75	0.17	+0.75
29	0	1200	1500	68.5	110	65	3810	Normal	90	0.16	+0.45
30	0	960	1250	69.0	112	65	3800	Normal	75	0.16	+0.45
Dec. 1	0	1000	950	69.0	115	71	3800	Normal			
2	0	920	800	70.0	115	61	3800	Normal			
3	0	960	720	70.5	110	60	3810	Normal			

* 8.5 mg. calcium per 100 cc. blood.
† 8 mg. calcium per 100 cc. blood.

TABLE II.—EFFECT OF CALCIUM CHLORID INGESTION IN A NORMAL PERSON.* (CASE I.)

Date. 1924.	Fluid intake, cc.	Urine, cc.	Calcium chlorid, gm.	Date. 1924.	Fluid intake, cc.	Urine, cc.	Calcium chlorid, gm.
Mar. 18	2160	900	0	April 1	2100	1400	0
19	2100	950	0	2	1200	1400	0
20	2100	1150	0	3	1700	1100	0
21	1800	750	0	4	1600	900	0
22	1500	660	0	5	1800	850	0
23	2100	1200	0	6	1700	800	10
24	2000	1000	0	7	2100	650	10
25	2000	1400	0	8	2200	1350	10
26	1900	1300	0	9	2000	1300	10
27	1400	700	0	10	1900	1500	10
28	1400	600	0	11	2000	1400	10
29	1800	780	0	12	1900	1350	10
30	1800	900	0	13	2000	1400	10
31	1800	1500	0	14	1800	1200	10

There was apparently no significant change in vital capacity or blood pressure and none in the rate or rhythm of the heart; no extrasystoles were noted.

In Table II are represented the results in the same subject on a liberal mixed diet, a forced intake of 2 liters of water per day, and only 10 gm. of calcium chlorid per day. Only a tendency to diuresis is evident.

Acidosis was quite pronounced in this subject during the administration of the calcium chlorid, as proved by laboratory tests.

CASE II.—A. E. K., male, aged twenty-seven years, the second volunteer, also had a liberal diet and took about 2 liters of fluid per day, which is the amount he usually consumes. It is interesting to note that he eliminated about four-fifths of his fluid intake per day, under normal conditions. Calcium chlorid was administered in solution, 5 gm. in 100 cc. of water per dose, and 2 such doses were taken per day, one at the midday meal and the other at the evening meal. On the second day of this therapy a marked diuresis occurred. This however continued only for another day, then ceased and did not recur during the remaining seven days of calcium chlorid therapy (see Table III).

TABLE III.—EFFECT OF CALCIUM CHLORID INGESTION IN A NORMAL PERSON. (CASE II.)

Date. 1924.	Calcium chlorid, gm.	Fluid intake, cc.	Urine, cc.	Date. 1924.	Calcium chlorid, gm.	Fluid intake, cc.	Urine, cc.
Mar. 18	0	2100	2100	April 3	0		
19	0	2100	2150	4	0	2000	1875
20	0	2100	1900	5	0	2000	1800
21	0	2100	1900	6	0	2100	1900
22	0	2100	1900	7	10	2100	1875
23	0	2100	1925	8	10	2100	3200
24	0	2100	1625	9	10	2100	3000
25	0	1900	1700	10	10	2100	1800
26	0	1200	1400	11	10	2100	2000
27	0	2100	2100	12	10	2100	1820
28	0			13	10	2000	1490
29	0	2100	1800	14	10	2100	1900
30	0	2100	1800	15	10	2100	1900
31	0			16	10	2100	1750
April 1	0	1800	1450	17	10	2100	1800
2	0	2100	1900				

Discussion. In these two normal individuals the diuresis was slight or moderate in degree and its duration varied. Thus in Table I the diuresis is seen to begin on the second day of calcium chlorid therapy and to continue for two days after discontinuation of the drug. In Table II, however, it is seen that only slight if any diuresis occurred in the same subject with the administration of 10 gm. of calcium chlorid per day. The other normal subject, A. E. K., had a striking diuresis on the second day of calcium chlorid therapy but this ceased quite suddenly and in spite of continued ingestion of the drug there was no further diuresis.

Effect of Calcium Chlorid in Cases of Cardiac Failure. CASE III.—N. C. M., male, aged fifty-three years, a merchant, always very active, began to notice dyspnea on exertion in 1921. In February, 1922, his doctor found him to have a systolic blood pressure of 170. Cardiac failure with edema of the legs, ascites and congestion of the lungs first occurred in May, 1922, when he was found to have hypertensive heart disease with a blood pressure of 260 systolic and 160 diastolic. He subsequently had frequent recurrences of congestive failure. There was no evidence of chronic nephritis. He was digitalized in May, 1922, and continued to take an average of 0.2 gm. of digifolin per day. In addition to rest in bed and digitalis, diuretics were always necessary in the treatment of the attacks of congestive failure with general anasarca. He responded very readily to calomel, 0.7 to 1 gm. per day for three days; sometimes diuretin or theocin was given following a course of calomel. These diuretics produced very marked diuresis, as much as 6½ liters of urine a day being passed until all the edema, ascites and hydrothorax disappeared.

In April, 1923, he became less responsive to these diuretics and continued to gain weight, with increasing edema even while taking calomel. A diuresis followed the second of two courses of calomel. Edema did not completely disappear and diuresis having ceased after the second of these two courses of calomel it was decided to try calcium chlorid: this was the first case in which we used this drug as a diuretic.

Well-marked diuresis occurred promptly after the beginning of and during the administration of calcium chlorid by mouth and stopped when this was discontinued only to begin again with resumption of the calcium chlorid therapy. Moreover, a constant negative fluid intake and output balance was maintained by the regular administration of calcium chlorid by mouth, 10 to 15 gm. every other day alternating with 0.2 gm. of digitalis. This régime was continued for two weeks after he left the hospital, then he took digitalis 0.2 gm. every day and calcium chlorid 12 gm. only once a week. He remained free from edema for about six weeks and then in spite of daily doses of digitalis (0.2 gm.) and calcium chlorid (12 gm.) he again developed general anasarca and died on July 23, 1923.

This patient derived much benefit from diuretics. When calomel, theocin and diuretin began to fail in their effectiveness, calcium chlorid produced very prompt diuresis. These results led us to try the use of calcium chlorid as a diuretic in other cases of cardiac failure with edema as well as in 1 case of polyserositis and in 1 case of ascites due to cirrhosis of the liver, conditions in which a number of investigators have reported calcium chlorid to be an effective diuretic.

CASE IV.—J. B. (Hospital No. 260088), male, aged fifty-one years, a coal miner, noticed the gradual onset of shortness of breath on exertion in 1921. After "a cold" in February, 1922, he had swelling of the legs; general weakness and shortness of breath became more marked. After a rest of two weeks he felt better, but not well enough to return to coal mining. He changed his occupation to shoe repairing. In September, 1923, dyspnea and general weakness were very marked; edema of the extremities and "filling up" of the abdomen recurred. After three weeks of rest and treatment with "medicines" he felt well enough to be up and about. In November, 1923, he again became "sick" with swelling of the legs and abdomen; during two weeks of treatment at home with rest in bed and "medicines" he grew steadily worse so that he was brought to the hospital in a desperate condition. He appeared to be in a moribund state, with very intense cyanosis, marked general anasarca, ascites and hydrothorax. There was no evident enlargement of the heart, the blood pressure was 120 systolic and 90 diastolic, the heart sounds were of fair quality, and there was no evidence of valvular or pericardial pathology; there were frequent extrasystoles with pulsus bigeminus for long periods of time (hours). He had marked emphysema and this condition together with possible anthracosis were considered as important factors in the causation of the deep cyanosis.

Soon after his arrival in the ward abdominal paracentesis was performed and a liter of pale straw-colored fluid was removed; 0.8 gm. of digifolin were administered intramuscularly during the first twenty-four hours and because he had nausea only 0.2 gm. of digitalis leaf per day was given during the next three days. This had no noticeable beneficial effect: on several occasions he lapsed into unconsciousness with very weak pulse and intensified cyanosis; the prompt administration of oxygen by the simple funnel method seemed to revive him.

On the eighth day after his admission to the hospital calcium chlorid therapy was started. From the data in Table II it appears that marked diuresis occurred during this therapy and the hydrothorax disappeared; the patient's general condition improved with dramatic rapidity. At the end of three weeks' stay in the hospital he was able to be up and about the ward. There was no perceptible diminution in cyanosis, the blood pressure remained at a relatively normal level, two attacks of paroxysmal fibrillation occurred and ventricular extrasystoles persisted, but were less frequent. There was no change in the size of the heart as measured by percussion, and the heart sounds did not become altered. He became much less orthopneic and the vital capacity increased by more than 300 per cent; whereas the pulse was relatively rapid before marked diuresis began, it became slow and less fluctuating in rate during and after the diuresis. It is doubtful whether this was the result of the action

of calcium chlorid on the heart directly. During the administration of this drug for five days prior to the onset of marked diuresis the pulse remained as it was before calcium chlorid therapy was begun. The diminished frequency of ventricular extrasystoles also began only after the onset of diuresis and it is presumed as most likely that in this case the reduction in number of extrasystoles may have been due to either the excretion of all digitalis which the patient had taken before and after his entrance into the hospital, or, if digitalis was not the cause of the extrasystoles, to improvement in circulation by reduction of edema *per se*.

The evidence in this case may be summarized as follows: probable arteriosclerotic heart disease with cardiac failure and marked general anasarca; digitalis given in large doses (probably causing pulsus bigeminus and nausea) failed to cause diuresis or any clinical improvement in the patient's serious condition; five days after the beginning of calcium chlorid therapy a marked diuresis began; this persisted until all the edema, ascites and hydrothorax had disappeared and some diuresis continued for six days after the drug was discontinued; coincidentally with the diuresis the patient's clinical condition changed so that he was able to be up and about. It is presumed from the evidence in this case that if calcium chlorid were responsible for the improvement in this patient's condition its effect consisted in producing a diuresis not primarily by an effect upon the heart itself, but by influencing water metabolism. The improvement in circulation is, therefore, considered as secondary to the reduction of edema.

Case V.—C. W. C., (Hospital No. 260263), aged seventy-five years, male, janitor, was first observed in 1920 when he was found to have arteriosclerotic and hypertensive heart disease, auricular fibrillation and congestive failure with edema of both lower extremities and congestion of both lungs. He did not complain of dyspnea or orthopnea. Following digitalization and while continuing to take 0.1 gm. digitalis leaf per day he remained free from edema until January, 1923, when he was admitted to the hospital ward with general anasarca. This had been developing during the previous month when he ceased taking digitalis because the supply of pills became exhausted and he procrastinated in buying more of them. Fibrillation continued but the pulse rate was quite low, varying between 50 and 60 per minute, apparently due to an organic lesion causing *A-V* block. There was no evidence of chronic nephritis.

On the third day after admission a thoracoparacentesis was performed and 1200 cc. of transudate were removed. Two days later calcium chlorid therapy was begun. A prompt diuresis occurred soon after the beginning of and during calcium chlorid administration. There was no appreciable change in heart rhythm or rate, or in blood pressure, and vital capacity increased only

slightly; no change was detected in the electrocardiographic curves which could be ascribed to the effects of calcium chlorid on the heart directly, and it is therefore inferred that the diuresis was caused by the influence of calcium chlorid on water metabolism in the tissues.

CASE VI.—W. G., (Hospital No. 260230), male, aged sixty-five years, clerk. This patient stated that he was in very good health until four days before he came to the hospital (in December, 1923), when he began having a dry cough and discovered that his feet and ankles were swollen. He was found to have arteriosclerotic and hypertensive heart disease, auricular fibrillation and congestive failure with a moderate amount of edema of both legs and feet and some congestion at the bases of both lungs. He also had marked emphysema. There was no evidence of nephritis.

During the first nine days he was merely kept in bed on a liberal diet with restriction of the total fluid intake to 2 liters per day. During this period of time all edema and congestion disappeared but no very marked diuresis occurred. Then, during the administration of calcium chlorid, the fluid intake and output balance changed from positive to negative and continued so until the cessation of this therapy when a positive balance was reëstablished and persisted later during digitalization.

In this case calcium chlorid therapy caused diuresis in the absence of any evident edema, without effecting any change in the heart rate or rhythm, or any change in the shape of the electrocardiographic curve or in vital capacity. Furthermore the absence of diuresis during digitalization suggests that calcium chlorid has not a digitalis-like action but that its effect is on water metabolism in the tissues rather than on the circulatory mechanism directly.

CASE VII.—E. M., (Hospital No. 256940), female, aged twenty-four years, housewife, at the age of sixteen had rheumatic fever and two years later, shortly after she was married, became aware of shortness of breath on exertion. Two normal pregnancies and one miscarriage were unassociated with cardiac failure. In the fifth month of the most recent pregnancy she began to have marked dyspnea on going up one flight of stairs, edema of both legs and feet and cough with occasional bloody sputum. She was admitted to the hospital in June, 1923, two weeks after a normal delivery and was found to have rheumatic heart disease with mitral stenosis (marked) and aortic regurgitation (slight), congestive failure with ascites and edema. She also had phlebitis of the left brachial and internal jugular veins. There was no evidence of nephritis.

Digitalization and 4 gm. diuretin, the latter given in one day, had no significant diuretic effect. The administration of calcium chlorid one week later brought about a negative balance of fluid intake and output which continued for one day after the cessation

of this therapy; edema and ascites disappeared. In spite of continuation of digitalis to maintain a state of digitalization a positive balance of fluid intake and output became reëstablished. The pulse rate became somewhat slower only after twelve days of rest in bed and nine days after digitalization; there was no significant change in blood pressure.

From the evidence in this case it may be inferred that the effect of digitalis on the circulatory mechanism was not enough to cause diuresis, but that with the aid of the influence of calcium chlorid on water metabolism in the tissues the output of urine was greatly increased and edema and ascites disappeared.

CASE VIII.—R. B., (Hospital No. 253977), male, aged sixteen years. Since the age of seven he had been having recurrent attacks of rheumatic fever and some choreiform movements in the intervals between these attacks. He first came under our observation in January, 1923, with rheumatic fever and was found to have rheumatic heart disease with mitral stenosis and aortic regurgitation. In spite of tonsillectomy which was performed in February, 1923, he had another attack of rheumatic fever the following month while at home. In April, 1923, was again taken into the hospital with rheumatic pericarditis and congestive failure with edema of feet and legs, ascites and congestion at the bases of both lungs; at this time he also developed bronchopneumonia. With rest in bed, salicylates, digitalization and recovery from the respiratory infection, the edema, ascites and congestion of the lungs gradually disappeared and he was again sent home to convalesce, with instructions to continue taking 0.1 gm. digitalis per day in order to maintain digitalization. Contrary to advice, however, he discontinued the digitalis and returned to the out-patient clinic five weeks later with marked ascites, edema of the legs and congestion at the bases of both lungs. He was sent home with instructions for digitalization and constant rest in bed. He took the digitalis but a week later edema had increased and he was more orthopneic than ever before. Consequently he again entered the hospital and the results of digitalis and calcium chlorid therapy were similar to those in Case VII inasmuch as in the presence of normal rhythm digitalis caused little or no diuresis, while calcium chlorid was more effective.

In Table IV are represented the results of the intravenous injection of 75 cc. of a 2 per cent solution of calcium chlorid in this case (VIII); an increased production of urine must have started soon after the injection, for 350 cc. were eliminated within an hour and another 400 cc. in the following two hours. Electrocardiograms taken just before and immediately after the injection of calcium chlorid showed no differences. These results as regards diuresis are similar to those obtained by Dr. Koehler while working in the Massachusetts General Hospital. He has made numerous obser-

vations of this nature and has found that the calcium content of the blood remains slightly elevated only for one-half to one and a half hours after the injection of calcium chlorid and that there is a coincident moderate but definite decrease in the pH of the blood. These observations are in accord with those of other authors who have investigated these problems.

TABLE IV.—INTRAVENOUS INJECTION OF 75 CC. OF 2 PER CENT SOLUTION CALCIUM CHLORID AT THE RATE OF 5 CC. PER MINUTE.

Time, P.M.		Urine, cc.	Electrocardiogram, etc.
3.30	....	..	Normal rhythm; rate, 110; *T* wave in Lead II = −0.1 mv.; *P–R* = 0.26 seconds.
4.00	....	75	
4.35 to 4.50	Injection of calcium chlorid		
4.55	....	..	Electrocardiogram; exactly the same as at 3.30.
5.30	....	..	Desire to pass urine.
5.45	....	350	
6.30	....	310	
7.30	.....	90	

Discussion. In all of these 6 cases of cardiac failure with edema some combination of the usual measures of causing diuresis was tried before the administration of calcium chlorid was begun. Rest in bed alone or with digitalization, and sometimes the addition of calomel or diuretin, or both, were either moderately or little if at all effective in reducing the edema. With calcium chlorid therapy, diuresis began on the first to the fifth day and in 3 cases continued for one or more days after the drug was discontinued. No changes occurred in pulse rate, blood pressure or vital capacity which could be attributed to the effect of the calcium chlorid therapy on the circulatory mechanism directly; nor can we unreservedly attribute to it the slight changes in the electrocardiogram. It would appear therefore that calcium chlorid when taken by mouth is not similar to digitalis in its action but causes diuresis by influencing water metabolism in the tissues.

CASE IX.—L. M., (Hospital No. 261196), male, aged fifty-four years, tailor, was a moderately obese man who had had asthma for twenty years. He was first observed in the hospital in 1920, when it was found that in addition to asthma and emphysema he had arteriosclerotic heart disease, angina pectoris (rare attacks) and some congestive failure. His symptoms of dyspnea on exertion, orthopnea, general weakness and slight swelling of the ankles gradually increased in severity. The attacks of angina pectoris became more frequent, as many as four per week. In February, 1924, he was admitted to the hospital with congestive failure, edema of legs and feet and

congestion at the bases of both lungs. There was no evidence of nephritis. The cardiac rhythm was normal.

With rest in bed and an ordinary mixed diet he slowly improved and the edema decreased while the output of urine increased slightly. When calcium chlorid therapy was begun 6 days after admission to the ward some edema over the sacrum was still present, and with the marked diuresis which followed, this and the crackling rales at both lung bases also disappeared. Some diuresis continued for eight days during which the patient received no medication; with the resumption of calcium chlorid administration no diuresis occurred.

The data in this case indicate a diuretic influence of calcium chlorid, although diuresis occurred after the first course and not after the second. During neither period was there any significant change in heart rate or rhythm, in the electrocardiographic curve or in vital capacity which could be attributed to the direct action of calcium chlorid on the circulatory mechanism. If the diuresis during and after the first course of calcium chlorid therapy was due in part to the action of this drug it is fair to infer that the effect was on water metabolism in the tissues and not on heart action.

Case X.—A. J. P., (Hospital No. 260490), male, aged fifty-two years, butcher, became aware of dyspnea on exertion subsequent to a cold with sore throat, cough and some fever in January, 1924. He continued at work lifting quarters of beef, weighing as much as 150 pounds, until he came into the hospital the following March, when he was found to have arteriosclerotic heart disease, auricular fibrillation and slight congestive failure evidenced by moist crackling rales at the bases of both lungs. There was no ascites or edema of the extremities and no evidence of nephritis.

A moderate diuresis occurred for 2 days with a régime of complete rest in bed only and continued without any significant increase during 10 days of calcium chlorid administration (15 grains daily). The diuresis ceased on the second day after discontinuing calcium chlorid and subsequent digitalization with effective slowing of the heart rate had no diuretic effect.

The heart rate became somewhat slower during rest and calcium chlorid therapy, but a relatively large apex-radial pulse deficit continued until after digitalization. Electrocardiographic tracings showed certain slight but definite changes; the *T* wave in Lead II was isoelectric before and somewhat negative during and after calcium chlorid therapy and the rate of auricular fibrillation became somewhat slower.

There was no significant change in blood pressure and after the patient had become proficient in the practice of forced expiration no appreciable change in the vital capacity occurred.

Restoration to normal rhythm with quinidin sulphate therapy

was not accompanied by any objective signs of improvement in cardiac function, but the patient felt greatly improved. He was no longer conscious of his heart beating and felt "as if everything were normal again."

The evidence in this case indicates that calcium chlorid had little or no influence on the heart muscle, the slowing of the ventricular rate and the slight negativity of the *T* wave in Lead II probably resulting from other causes. The constant rest in bed alone seems to have been responsible for the moderate diuresis although it is possible that calcium chlorid had some additional diuretic influence.

Case XI.—M. C. F., (Hospital No. 259937), female, aged sixty-three years, cashier, during the summer of 1923 began to notice palpitation on exertion and also had to begin using two pillows instead of one to avoid slight difficulty in breathing at night. Otherwise she felt well and was able to carry on her work as a cashier. Six weeks before coming to the hospital she had a slight cold with several chills and about this time began to notice swelling of feet and legs which extended up to thighs and abdomen in a few weeks. When she entered the hospital in December, 1923, she was found to have arteriosclerotic and hypertensive heart disease and congestive failure with general anasarca. There was no evidence of nephritis. As a result of rest in bed, an ordinary mixed diet, and slight limitation of total fluid intake she had a moderate diuresis, lost much weight, and the edema and ascites entirely disappeared in eleven days. Subsequently, administration of calcium chlorid for three days did not cause any significant diuresis.

This case again illustrates the effect of rest alone in improving cardiac function with consequent reduction of edema. No striking objective signs of improvement in cardiac function were observed, the pulse rate remained between 75 and 85 per minute, but there was some increase in vital capacity and an increase in pulse pressure as a result of increase in the systolic and decrease in the diastolic pressures.

There was slight diuresis on the third, the last, day of calcium chlorid therapy, but none occurred during the previous two days or after the drug was discontinued.

Case XII.—E. H., (Hospital No. 261936), male, aged thirty-nine years, farmer, at the age of twenty-nine years had an attack of rheumatic fever which caused him to remain in bed for seven months; subsequently he had several milder attacks. At the age of thirty-five he began to notice palpitation and dyspnea on exertion and had to cease working. During the next four years these symptoms became progressively more marked. In January, 1924, two months before he came to the hospital, he took to bed because of marked dyspnea and orthopnea, swelling of legs and abdomen, and cough with bloody sputum.

He was found to have rheumatic heart disease, mitral stenosis and regurgitation, cardiac enlargement and signs suggesting adhesive pericarditis. There were right hydrothorax, congestion in base of left lung, ascites, and edema over the sacrum and of both lower extremities below the hips. The patient appeared very sick; he was cyanotic and markedly orthopneic.

Marked diuresis and subjective improvement developed gradually during a week of complete rest in bed, limitation of fluids, Karrel diet and digitalization. As the anasarca diminished the extent of diuresis waned until a positive fluid intake and output balance became established.

All therapeutic measures were then discontinued for three days and thereafter calcium chlorid was administered for five days; a slight diuresis occurred only on the second and third days of this therapy. When administration of calcium chlorid was started the patient was feeling quite comfortable and there was no apparent hydrothorax, ascites or edema of the extremities: crackling rales at both lung bases persisted however.

There were no apparent changes in the circulatory mechanism and the subjective symptoms were not noticeably changed by the calcium chloride therapy.

Case XIII.—C. P., (Hospital No. 259245), male, aged fifty-seven years, laborer, since his illness with typhoid fever in 1917 had had shortness of breath on exertion and edema of the feet and legs which increased during the day and diminished over night. In September, 1923, he came to the out-patient cardiac clinic and was found to have arteriosclerotic heart disease with congestive failure (edema over both shins): the electrocardiogram showed sinoauricular bradycardia, the rate varying from 45 to 55 per minute. He was digitalized and continued to take a maintenance dose of 0.1 gm. and 0.2 gm. of digitalis leaf on alternate days. His symptoms and edema were somewhat diminished in severity but he returned in two weeks with persistent edema of both legs. Diuretin, 3 gm. per day for a week, was added to the digitalis therapy and this resulted in the entire disappearance of edema and the loss of 3.5 kilos of weight in one week. However he returned again the next week with a re-accumulation of edema of both legs and was taken into the hospital.

In this case calcium chlorid given together with digitalis at that time for 5 days was associated with but slight diuresis. There were no significant changes in rhythm or in vital capacity. In the electrocardiogram the *P-R* interval became lengthened, while the negative *T* wave in Lead II tended to become positive.

Case XIV.—H. P., (Hospital No. 260405), male, aged twenty-one years, was first observed in May, 1920, when he was found to have

rheumatic heart disease, mitral stenosis and regurgitation, aortic regurgitation, and fibrinous pericarditis. During the next three years he continued a well-ordered convalescent existence, being greatly limited in his activity by marked shortness of breath on exertion. On several occasions after undue exertion he became very weak, with feet and legs swollen, and had to remain in bed. For the first four or five days of rest in bed he would continue to feel weak and orthopneic, passed very small amounts of urine and the swelling would increase; then on the fifth or sixth day he would suddenly begin to pass a great deal of urine, sometimes with frequency (every fifteen minutes). He took little digitalis (never more than 30 drops of tincture per day) during these periods.

In January, 1924, he came to the hospital again in a state of marked cardiac failure with general anasarca and orthopnea. In addition to the previous cardiac findings he also had auricular fibrillation. There was no evidence of nephritis.

During eight days of calcium chlorid therapy, and restricted low fluid intake, the amount of urine per day was about equal to the total fluid intake excepting on the eighth day, when more marked diuresis occurred. The slight diuresis during the first seven days can hardly be ascribed to the calcium chlorid therapy alone and it is more likely that the rest in bed and restricted low fluid intake were chiefly responsible. The patient did not improve symptomatically and edema seemed to increase. The heart rate became somewhat slower (probably from complete rest in bed), but it remained relatively rapid and the large apex-radial pulse deficit persisted. The change in the *T* wave of Lead II from slightly diphasic to upright may be ascribed to elimination of the digitalis which he had taken before coming to the hospital. Because of the poor condition of the patient it was necessary to apply more orthodox measures for his relief and after digitalization and the administration of large doses of diuretin, a marked diuresis occurred, the apex-radial pulse deficit disappeared, edema gradually became reduced, he lost 10 kilograms in weight and there was a marked subjective improvement in symptoms.

The evidence in this case indicates that calcium chlorid has not a digitalis-like action in the treatment of auricular fibrillation with cardiac failure and edema.

CASE XV.—S. S., (Hospital No. 263703), female, aged sixty-two years, housewife, an obese, short, elderly woman, complained of dyspnea and palpitation on exertion, and nocturia (3 or 4 times), which she began to notice in February, 1924. Four months later she came to the hospital and was found to have hypertensive and arteriosclerotic heart disease with slight congestive failure (edema of feet and ascites) and general arteriosclerosis. Heart rhythm was normal. Renal functional tests (Schlayer concentration test,

chlorid excretion, and nonprotein nitrogen in the blood) did not suggest nephritis but were compatible with passive congestion and arteriosclerosis of the kidneys.

Calcium chlorid together with the maintenance dose of digitalis was given for five days; no diuresis occurred until the fifth day and then it ceased with the discontinuation of calcium chlorid therapy. While this slight temporary diuresis may have been caused by the calcium chlorid therapy we can draw no definite conclusions from the data in this case.

CASE XVI.—H. H., (Hospital No. 263735), male, aged fifty-nine years, janitor, an elderly obese man, had been suffering from dyspnea on exertion for about five years. His symptoms became greatly aggravated following pneumonia a year and a half before coming under our observation in June, 1924. For the last year he had been confined to bed because of marked swelling of the legs and abdomen, and orthopnea. Fluid had been oozing through the skin of his legs and this was followed by a very painful dermatitis. He also gave a history of typical attacks of angina pectoris. He was found to have hypertensive and arteriosclerotic heart disease, and marked congestive failure with general anasarca. Tests of kidney function were consistent with marked passive congestion of the kidneys, there being no data pointing to acute or chronic nephritis.

Neither diuretin, digitalis nor calcium chlorid had any significant diuretic effect on this patient. Neither digitalis nor calcium chlorid had any effect on blood pressure or on pulse rate. Edema increased and he grew steadily weaker and died of cardiac failure on the twentieth day of his stay in the hospital. The significant finding at autopsy was a large heart; the kidneys presented a typical appearance of chronic passive congestion without any evidence of acute or chronic nephritis.

CASE XVII.—P. J. B., (Hospital No. 259681), male, aged forty years, seaman, had had "attacks of kidney trouble" during the past eight years, corresponding to acute and subacute nephritis; he was treated with [illegible], low-protein diet and rest in bed for periods varying from [illegible] days to weeks. Dyspnea on exertion and nocturnal paroxysms of orthopnea had occurred only in the last two years. He had been [illegible] for six weeks before coming to the hospital, presenting evidence of hypertensive heart disease with congestive failure, [illegible] nephritis (nitrogen retention type) and a moderate [illegible]. He was markedly orthopneic, both lower extremities and the sacral region were edematous, and moist crackling rales were heard at the bases of both lungs. True pulsus alternans was detected by the blood-pressure method; halving of the pulse rate occurred between 260 and 240 mm. of mercury, systolic pressure.

Treatment with salt-free, low-protein diet, diuretics, including calcium chlorid, and digitalis had no beneficial effect. In the fifth week of his stay in the hospital he developed erysipelas, uremia became more marked and he died two weeks after the onset of this infection. Permission for an autopsy was not granted.

In this case of edema resulting from cardiac failure and chronic nephritis, neither calcium chlorid together with a salt-free, low-protein diet nor digitalis caused diuresis; nor had these measures any discernible effect on the circulatory mechanism directly. The changes which occurred in the electrocardiographic curve during calcium chlorid administration are similiar to those which were observed immediately before and after this therapy and therefore cannot be ascribed to it but were due more probably to the retention of toxic substances which caused the uremia. (See paper by Wood and White.)

Discussion. The above 9 cases include 5 in which diuresis occurred during calcium chlorid therapy. In 2 of these (1 with normal rhythm and the other with auricular fibrillation) it occurred in association with the beginning of the régime of rest in bed, and therefore the diuresis cannot be entirely attributed to the calcium chlorid although this drug may have had some influence. In the other 3 a slight transient diuresis occurred after all apparent edema had been reduced with other measures, namely rest alone in 1 case and with the addition of digitalis in the other 2. Of the remaining 4 cases, 1 with rheumatic heart disease, auricular fibrillation and anasarca failed to respond to calcium chlorid but digitalization and the administration of diuretin was followed by marked diuresis and by great improvement in the patient's condition. Two cases of cardiac failure without nephritis and 1 with nephritis and uremia did not respond to any medication, digitalis, diuretin, calcium chlorid, alone or in combination.

In none of these 9 cases was there any clinical evidence to indicate that calcium chlorid administered by mouth had any effect on the circulatory mechanism. The slowing of the heart rate in the 2 cases of auricular fibrillation may be accounted for by the rest in bed alone. The persistence of a large apex-radial pulse deficit in both of these cases and the fact that no diuresis or other beneficial effect occurred in the one with anasarca while digitalization and the administration of diuretin were followed by marked diuresis and general improvement indicate that calcium chlorid had not a digitalis-like action in cases with auricular fibrillation.

Effect of Calcium Chlorid in a Case of Tubercular Polyserositis. CASE XVIII.—H. A. S., (Hospital No. 260418), female, aged fifty-five years, housewife, had polyserositis, pulmonary tuberculosis, cardiac enlargement and auricular flutter. Her illness began grad-

TABLE V.—EFFECT OF CALCIUM CHLORID AND OF DIGITALIS IN A CASE OF TUBERCULOUS POLYSEROSITIS AND AURICULAR FLUTTER WITH VARYING DEGREES OF BLOCK. (CASE XVIII).

Date, 1924.	Therapy.	Fluid intake, cc	Urine, cc	Body weight, kilos.	Pulse rate.	Blood pressure.		Vital capacity, cc. air.	Paracentesis. Electrocardiograms.
						Sys	Dia.		
Jan. 4	.....	...	...	...	..	..	..	....	Abdominal paracentesis, 5 liters. Auricular flutter; auricular rate, 260; 2:1 to 4:1 block; ventricular rate, 80 to 125; *T* wave in Lead II diphasic.
5	Calcium chlorid, 15.0 gm.	1400	600	37.3	108	..	..	600	
6	Calcium chlorid, 12.5 gm.	1900	900	...	100	.	..	....	Right thoracoparacentesis, 900 cc.
7	Calcium chlorid, 12.5 gm.	2400	700	...	100	120	85		
8	Calcium chlorid, 12.5 gm.	2100	600	41.3	90	..	..	600	
9	Calcium chlorid, 12.5 gm.	1800	300	41.5	80	..	..	....	Electrocardiogram same as on January 4, except ventricular rate, 100 to 125.
10	Calcium chlorid, 12.5 gm.	2000	500	41.5	120	..	..	650	
11	Calcium chlorid, 12.5 gm.	1800	400	...	110	110	80		
12	Calcium chlorid, 12.5 gm.	1600	500	...	120	..	..	750	
13	Calcium chlorid, 12.5 gm.	2100	600	...	110				
14	Calcium chlorid, 12.5 gm.	2100	600	...	110				
15	Calcium chlorid, 12.5 gm.	1800	750	...	120				
16	Calcium chlorid, 12.5 gm.	1300	650	...	100				
17	Digitalis, 0.6 gm.	2600	900	44.0	108	115	85	700	
18	Digitalis, 0.1 gm.	2500	1000	...	122	..	..	....	Electrocardiogram same as on January 4.
19	Digitalis, 0.1 gm.	2250	800	...	118	..	..	750	Electrocardiogram same as on January 18.
20	Digitalis, 0.1 gm.	1700	700	45.3	100	..			
21	Digitalis, 0.1 gm.	1200	600	...	90	120	80		
22	No medication	1500	700	...	100	..	..	....	Auricular fibrillation (coarse); ventricular rate, 90; inverted *T* wave in Lead II = − 0.2 mv.
23	No medication	1500	600	...	90	..	..	700	Abdominal paracentesis, 5 liters.
24	No medication	1500	650	...	110	115	80		

ually six years before entrance to the hospital on January 4, 1924; fluid accumulation in abdomen and chest had been progressive, requiring paracentesis several times. There was slight fever only. Hemoptysis had occurred two years previously.

On physical examination at the time of entrance to the hospital ward she was emaciated and showed signs of old tubercular processes at both apices, marked ascites, bilateral pleural effusion, enlargement of liver and of heart, and auricular flutter with auricular rate of 270 and ventricular rate varying from 80 to 130. Abdominal and thoracic paracentesis yielded much fluid (5000 cc. from former and 900 cc. from latter) with specific gravity of 1011 to 1015 and lymphocytic smear.

Calcium chlorid therapy was begun January 6, and continued for eleven days at a dose of 15 gm. the first day, 10 gm. the next four days and 7½ gm. thereafter. It was absolutely without effect either on the ascites, pleural effusion, weight of patient, auricular flutter or electrocardiogram. (See Table V.) Fluid accumulated steadily in the serous cavities in spite of its use. On January 18 the calcium chlorid was discontinued and digitalis was begun and continued for three days. A reduction of the ventricular rate resulted without appreciable improvement in the patient's condition. Ascites reaccumulated.

This case of polyserositis showed no beneficial effect from calcium chlorid.

Effect of Calcium Chlorid in a Case of Hepatic Cirrhosis with Ascites. CASE XIX.—F. H. S., (Hospital No. 261984), male, aged forty years, automobile mechanic, had alcoholic cirrhosis of the liver with marked ascites and jaundice. He died on the tenth day of his stay in the hospital following a severe hemorrhage from an esophageal varicose vein.

There was no diuretic effect from calcium chlorid. It is interesting that although there was no evidence suggestive of nephritis of either the chlorid or nitrogen-retention type, the urine output remained about the same during large or small fluid intake. Fluid reaccumulated after both abdominal paracenteses.

In this case of hepatic cirrhosis with ascites calcium chlorid affected neither the amount of ascites already present, the reaccumulation of ascitic fluid nor the elimination of urine.

Discussion. These 2 cases belong to the types in which Blum and his associates, and subsequently other authors, have observed beneficial results from calcium chlorid therapy. The administration of calcium chlorid had no apparent effect on the case of probable tuberculous effusion into serous cavities, nor on the patient with hepatic cirrhosis and marked ascites. In these cases also there was no evidence of any influence of calcium chlorid on the circulatory

mechanism; the auricular flutter in Case XVIII was not affected in any way.

TABLE VI.—CHANGES IN HEART RATE, RHYTHM AND ELECTROCARDIOGRAM DURING CALCIUM CHLORID THERAPY.

	Change.	Cases in which change occurred.
Rhythm	Normal to abnormal	None.
	Abnormal to normal	None.
Rate	Increase	None.
	Decrease	IV, X, XIV.
Extrasystoles	Newly produced or increased	None.
	Decrease	IV.
P wave	Positive	IV.
	Negative	None.
P-R interval	Lengthened	XIII, IV.
	Shortened	XIII, IV.
Q-R-S complex, Lead II	Slurred or notched	IV.
	Widened	None.
T wave, Lead II	Positive	I, IV, VI, XIII, XVII.
	Negative	I, V, VI, XIV, XVII.

Effect of Calcium Chlorid Administration on the Electrocardiogram. In 11 cases electrocardiograms were taken before, during and after calcium chlorid therapy and some change or other was noted in 8. In no case did any change in rhythm occur and the variations that were observed were not uniform. In 3 cases there was a decrease in rate which, however, is to be attributed to the fact that this change occurred during the first part of the patient's stay in the hospital under a régime of constant rest in bed. In Case III a prolongation, then shortening, of the *P-R* interval and also an increase in positivity of the *T* wave in Lead II occurred during calcium chlorid therapy. In Cases I, VI, and XVII the *T* waves of Lead II varied in their changes from day to day, sometimes toward negativity and at other times toward positivity. Case XVII had uremia, and, as Wood and White have shown, such changes in the *T* wave occur in cases of nitrogen retention probably as a result of toxic effects on the myocardium. The electrocardiogram of Case XIV showed auricular fibrillation without any change in this rhythm but slight negativity of the *T* wave in Lead II. Case IV showed numerous changes, including prolongation of the *P-R* interval, increased positivity and notching of the *P* wave, increased negativity of the *T* wave and change from slurring to notching of the *R* wave in Lead II; in this case the frequency of extrasystoles and the heart rate were diminished only after marked diuresis had caused much improvement in the general condition of the patient. In view of the fact that in the other 18 cases the heart rate was not affected by calcium chlorid therapy, it is inferred that in this case the slowing of the heart rate was secondary to the effect of diuresis on the circulatory mechanism rather than that the

slowed heart rate was responsible for the diuresis. As regards the other changes it is probable that they were as likely due to sclerotic changes in heart muscle as to the direct effect of calcium chlorid upon the myocardium.

In view of the lack of uniformity of the changes and the simultaneous existence of other variable factors it is not possible to draw any absolute conclusions as to the effect of oral administration of calcium chlorid upon the electrocardiogram. It appears probable that there are no direct effects.

In Case IX electrocardiograms taken just before and immediately after the intravenous injection of 1.5 gm. of calcium chlorid showed no change as a result of this procedure.

Summary. The administration of large doses of calcium chlorid by mouth in two normal individuals was accompanied by a moderate temporary diuresis and no apparent significant changes in the circulatory mechanism as studied by clinical methods. Acidosis accompanied the diuresis.

Of the cases of heart disease with congestive failure and edema a group of 6 which had not responded well to treatment with rest in bed only, or together with digitalis and various diuretics, showed a well-marked diuresis during the administration of calcium chlorid. The diuresis began on the first to the fifth day of calcium chlorid therapy and in 3 cases did not cease until this drug had been discontinued for one or more days. In another group of 9 similar cases (excepting 1, XVII, who also had chronic nephritis and uremia) no significant diuresis occurred which could be attributed to calcium chlorid and in 3 of these digitalis alone or together with calcium chlorid or other diuretics also failed to cause diuresis. One case of this group (XIV), a patient with auricular fibrillation, after eight days of calcium chlorid therapy without beneficial effect responded readily and well to digitalization and the administration of diuretin.

In 2 miscellaneous cases, namely, 1 with probable tuberculous polyserositis and another of hepatic cirrhosis with marked ascites there was no diminution of the amount of fluid in the serous cavities nor was the continued accumulation of the fluid arrested by calcium chlorid therapy.

Data obtained upon heart rate, blood pressure, and vital capacity showed no evidence of direct effect of calcium chlorid administration upon the circulatory mechanism. Whereas some changes or other were noted in the electrocardiograms of 8 patients, there was no effect on rhythm or rate and the variations were not consistent either in different cases or in individual cases. Other variable factors, like recovery following previous digitalis therapy, uremia, improvement in circulation with marked diuresis from constant rest in bed, etc., were present in these cases and therefore it is not possible to draw any definite conclusions as to the effect of calcium chlorid

therapy on the electrocardiogram. It is obvious however, that calcium chlorid has no digitalis-like action upon the heart. From this it may be inferred that the diuretic effect of calcium chlorid is due to its influence upon water metabolism in the tissues rather than upon the circulatory mechanism.

In no case did we observe any deleterious effects on the patient's condition as a result of calcium chlorid therapy.

Conclusions. 1. In cases of cardiac failure with edema in which constant rest in bed, digitalization, and the administration of various diuretics have not resulted in a satisfactory diuresis, calcium chlorid may be employed as a diuretic.

2. No deleterious effects were observed from the oral administration of calcium chlorid, 10 to 15 gm. per day, in any of the cases studied.

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DIETARY SENSITIZATION FOR PARATHYROID HORMONE[1]

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ABSTRACT

Sensitization to parathyroid hormone by the oral administration of calcium acetate, sodium phosphate, and corn oil was studied comparatively in the rat.

Calcium acetate greatly faciliates the induction by parathyroid extract of calcification in the cardiovascular system, skeletal musculature, and renal cortex, as well as the production of osteitis fibrosa, particularly if, at the same time, the intake of fat is augmented. Sodium phosphate likewise increases the activity of parathyroid hormone, though in equimolecular amounts this salt is less effective than calcium acetate; moreover, sodium phosphate—unlike calcium acetate—changes the quality of the resulting morbid lesions in that the cardiac calcification is accompanied by suppuration, and the nephrocalcinosis shifts to the corticomedullary junction. The effects of combined treatment with parathyroid hormone and sodium phosphate are likewise aggravated by orally administered fat-supplements.

Attention is called to the fact that the same sensitizing agents (calcium acetate, sodium phosphate, dietary fat-supplements) that are effective in increasing the actions of parathyroid hormone also proved to be potent sensitizers for the induction of cardiovascular lesions by certain vitamin-D derivatives and corticoids.

THE oral administration of sodium phosphate not only increases the actions of parathyroid hormone, but qualitatively changes them. Normally, excessive amounts of this hormone produce cortical nephrocalinosis and ectopic calcium deposition in the cardiovascular system of the rat. These changes are not merely aggravated by concurrent sodium phosphate treatment, but are so altered that calcium deposition in the kidney tends to predominate at the corticomedullary junction, while the cardiac lesions assume a definitely suppurating character (1). In all these respects, sodium phosphate modifies the effects of dihydrotachysterol intoxication in the same manner (2, 3). Oral administration of corn oil

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[2] Fellow of The Rockefeller Foundation.

TABLE 1. DIETARY SENSITIZATION FOR PARATHYROID HORMONE
(First experiment)

Group	Treatment*	Cardiac lesions (Scale: 0–3)	Nephrocalcinosis		Mortality (%)
			Degree (Scale: 0–3)	Type	
1	None	0	0.6 ±0.20	Cortical	0
2	Na_2HPO_4	0	0.8 ±0.20	Corticomedullary	0
3	Ca-acetate	1.0 ±0.30	1.8 ±0.20	Cortical	0
4	Corn oil	0	1.0 ±0.23	Cortical	0
5	Corn oil + Na_2HPO_4	0.3 ±0.10	2.0 ±0.15	Corticomedullary	30
6	Corn oil + Ca-acetate	2.4 ±0.33	2.4 ±0.33	Cortical	90

* In addition to the treatments listed in this column, the rats of all groups received 50 U.S.P. units of *parathyroid horomone* twice daily, as indicated in the text.

greatly augments the toxic actions of dihydrotachysterol overdosage, but does not change their character (4).

In this communication, we wish to report upon experiments showing that orally administered calcium acetate, unlike sodium phosphate, increases the effectiveness of parathyroid hormone without changing the quality of the response and that corn-oil supplements further augment this increase.

MATERIALS

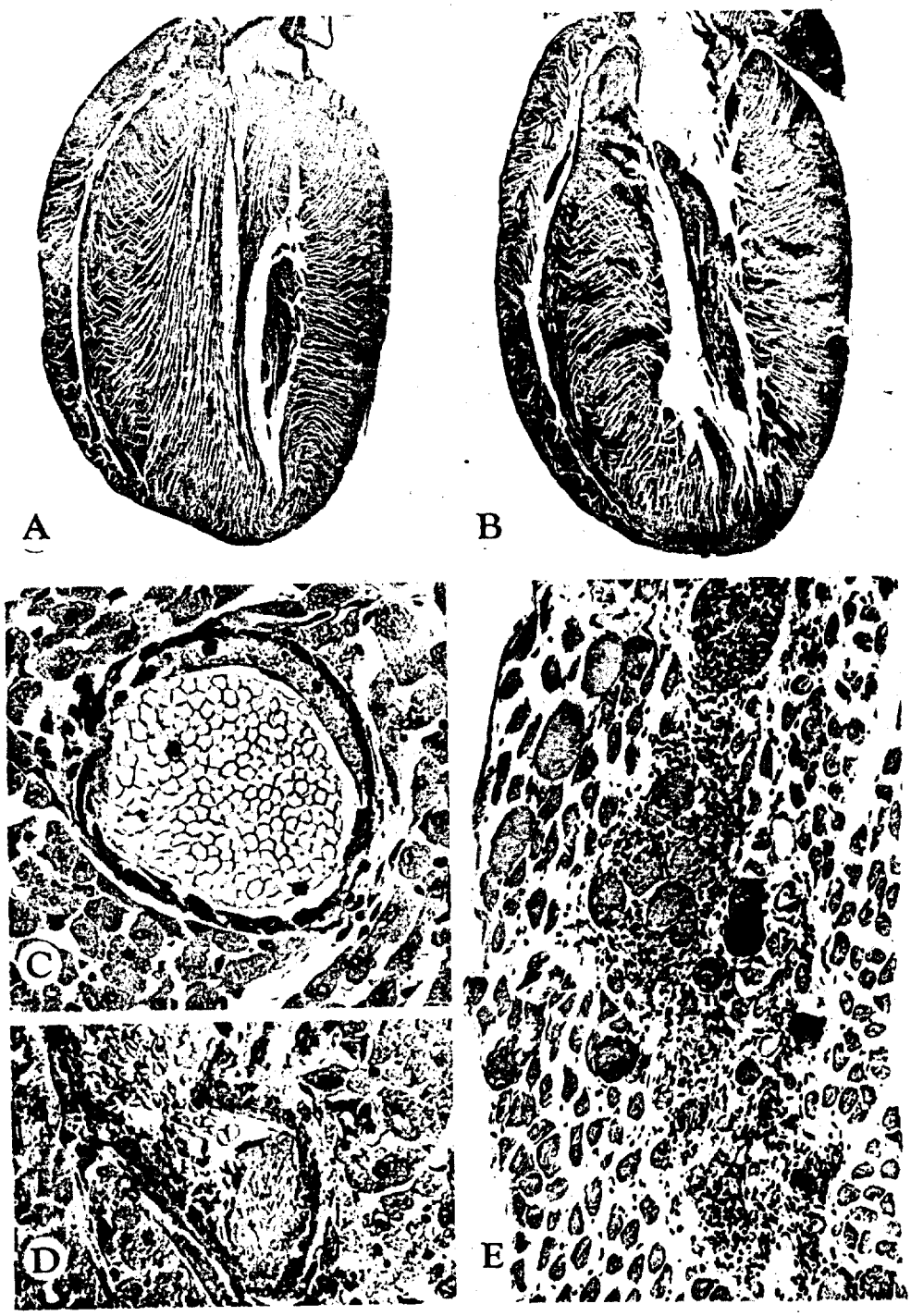
Eighty female Holtzman rats, with a mean initial body weight of 100 gm. (range: 95–106 gm.), were subdivided into eight equal groups and treated as indicated in Tables 1 and 2. In the first experiment (Table 1), parathyroid extract (Lilly) was injected subcutaneously at the dose of 50 U.S.P. units in 0.5 ml. of water, twice daily; Na_2HPO_4 and calcium acetate were given in the amount of 1 mM in 2 ml. of water by stomach tube, twice daily, while corn oil was administered by the same route at the dose of 1 ml., likewise twice daily. The experiment was terminated after three days.

In the second experiment (Table 2), treatment with acetate and corn oil was the same, but in order to permit more prolonged survival the individual dose of parathyroid extract was reduced to 30 U.S.P. units in 0.3 ml. of water. The experiment was terminated on the eighth day.

In both experiments, the rats were maintained exclusively on Purina fox chow (Purina Co. of Canada) and tap water. All survivors were killed with chloroform at the end of the observation period and the hearts, quadriceps femoris muscles, and kidneys

TABLE 2. DIETARY SENSITIZATION FOR PARATHYROID HORMONE
(Second experiment)

Group	Treatment	Cardiac lesions (Scale: 0–3)	Nephrocalcinosis		Osteitis fibrosa (Scale: 0–3)	Mortality (%)
			Degree (Scale: 0–3)	Type		
1	Paraffin oil + Ca-acetate + Parathyroid hormone	0.5 ±0.15	0.4 ±0.17	Cortical	1.2 ±0.20	20
2	Corn oil + Ca-acetate + Parathyroid hormone	2.5 ±0.20	2.7 ±0.20	Cortical	2.8 ±0.23	80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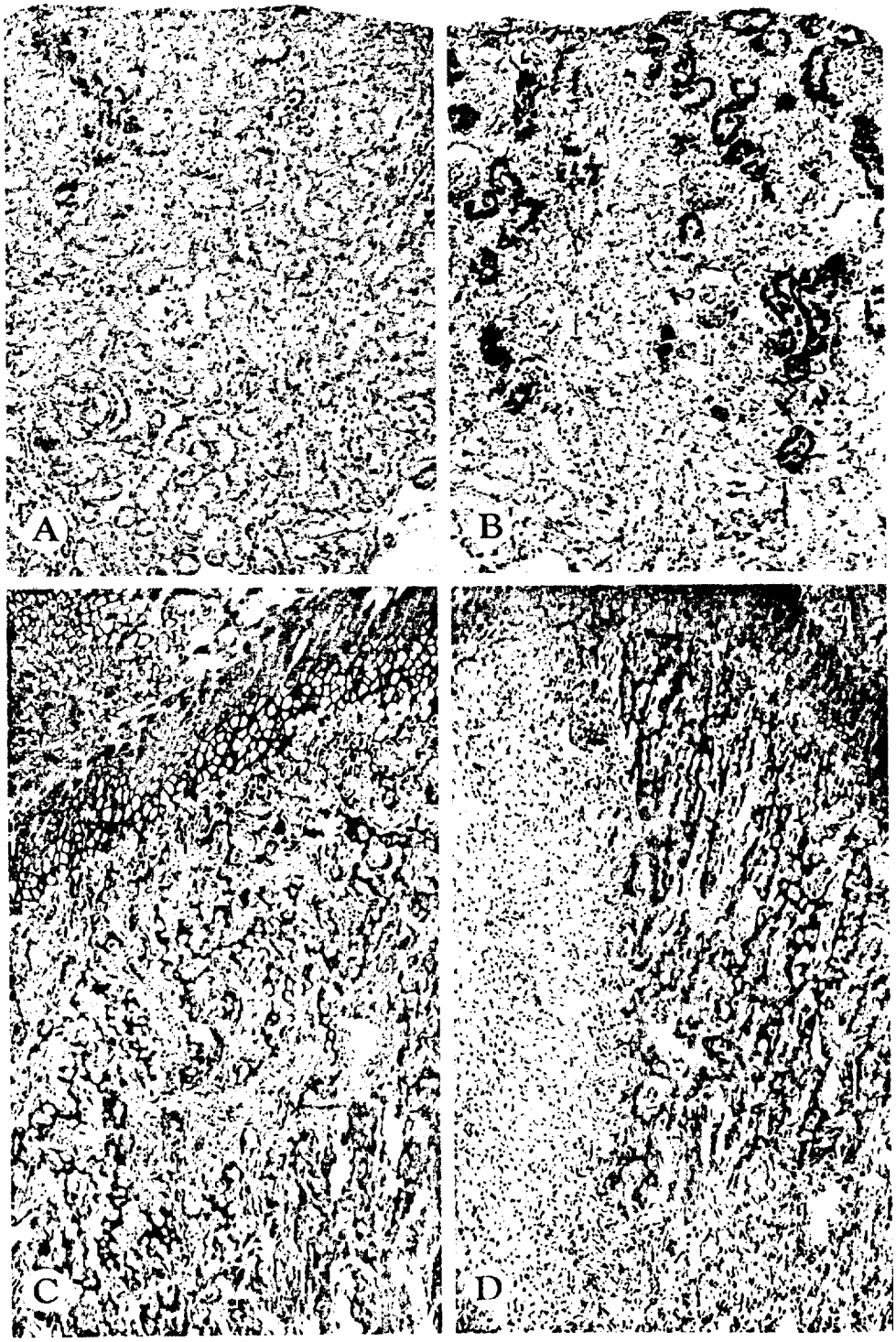
were fixed in alcohol-formol for the subsequent histochemical demonstration of calcium with the von Kóssa method. In addition, one femur was fixed and decalcified, in Susa solution saturated with picric acid, and subsequently stained with the PAS procedure and hematoxylin-phloxine. The organ lesions were graded in terms of an arbitrary scale in which 1 designates barely detectable, 2 marked, and 3 most severe, lesions. The means of these readings (with standard errors) and the percentual mortality rates are listed in the tables.

RESULTS

First experiment (*Table 1*).—The first experimental series showed that, when given in equimolecular amounts, calcium acetate is much more effective than Na_2HPO_4 in aggravating the syndrome of parathyroid-hormone intoxication. Indeed, in this short-term experiment, parathyroid extract produced marked *cardiac lesions*, only when given in combination with calcium acetate. The effect of this combined treatment (Group 3) was slightly, but significantly ($P < 0.05$), aggravated by corn oil (Group 6). Whether or not oil-supplements were given, the lesions consisted of virtually pure calcification, sometimes accompanied by the subintimal deposition of fibrinoid material but never by suppuration (Fig. 1A, B, C, and D). Conversely, the lesions induced by parathyroid extract in combination with Na_2HPO_4+corn oil (Group 5), though very mild, were associated with suppuration.

The *nephrocalcinosis* produced by parathyroid hormone was augmented both by Na_2HPO_4 and by calcium acetate; but while the former salt induced calcium deposition almost exclusively at the corticomedullary junction, parathyroid hormone alone or in combination with calcium acetate produced a predominantly cortical nephrocalcinosis. The apparent aggravation of the nephrocalcinosis by corn oil was statistically significant only in the rats receiving parathyroid hormone+Na_2HPO_4 (Group 2 compared with Group 5, $P < 0.01$), although the mean values were slightly higher in all the fat-treated groups than in the corresponding controls. Since almost all the rats treated with parathyroid hormone in combination with corn oil+calcium acetate died during the first two days of the experi-

FIG. 1. *A.*—Essentially normal heart of rat (second experiment, Group 1) treated with paraffin oil+calcium acetate+parathyroid hormone (von Kóssa ×8). *B.*—Numerous foci of calcification throughout the myocardium and its vessels in a rat (second experiment, Group 2) treated with corn oil+calcium acetate+parathyroid hormone (von Kóssa ×8). *C. and D.*—Higher magnification of cross sections through two small arteries of the heart shown in Fig. B. There are calcium deposits (black) in the arterial wall and subintimal accumulations of fibrinoid material (gray) in the form of crescents or cushions (von Kóssa ×590). *E.*—Typical skeletal lesion in the quadriceps of a rat from Group 2 of the second experiment. Swelling, homogenization, and calcification of certain muscle fibers, with inflammation in the surrounding area (von Kóssa ×180).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ment, the survival period might not have been sufficiently long to permit more intense calcium deposition (Fig. 2A and B).

Osteitis fibrosa was more pronounced in all groups treated with corn oil than in the corresponding controls, but the lesions were not sufficiently pronounced to permit clear-cut quantitative evaluation.

Pronounced *calcification, necrosis, and inflammation in the skeletal musculature* (Fig. 1 E) was evident only in the rats treated with parathyroid extract+calcium acetate+corn oil (Group 6).

Interestingly, in many animals receiving Na_2HPO_4 or calcium acetate, in addition to corn oil+parathyroid hormone, a pronounced *lipemia* developed, which imparted a milky appearance to the blood.

Second experiment (*Table 2*).—In order to obtain more definite proof of the sensitizing effect of corn oil a second experiment was performed, in which the control rats were given paraffin oil, and the experimental animals received corn oil, in addition to calcium acetate+parathyroid hormone. Otherwise, the conditions of experimentation were the same as in the first experiment, except that—as explained in the Materials section—the individual dose of parathyroid hormone was decreased from 50 to 30 U.S.P. units to permit longer survival.

Here, corn oil significantly aggravated the cardiac, muscular, renal, and skeletal lesions, as well as the mortality, produced by parathyroid hormone+calcium acetate. In both groups, the *cardiovascular lesions* consisted of pure calcium deposition without suppuration, and the *nephrocalcinosis* was limited to the cortex. In this more chronic experiment, the *muscular lesions* were particularly severe and it became obvious that these as well as the *osteitis fibrosa* induced by parathyroid hormone are likewise greatly intensified by the fat-supplements (Fig. 2 C and D). A striking degree of *lipemia* was evident in virtually all the animals treated with corn oil +parathyroid extract. This unexpected effect of the hormone upon lipemia will be the subject of a more detailed chemical study; let us point out here, however, that in numerous experiments—performed for different purposes but under similar conditions—corn oil did not produce any comparable degree of lipemia in otherwise untreated control rats.

FIG. 2. *A.*—Mild cortical nephrocalcinosis in a rat (Group 1 of second experiment) treated with paraffin oil+calcium acetate+parathyroid hormone (von Kóssa ×120). *B.*—Intense cortical nephrocalcinosis in a rat (Group 2 of second experiment), following treatment with corn oil+calcium acetate+parathyroid hormone (von Kóssa ×120). *C.*—Mild osteitis fibrosa in the subepiphyseal layer of the femur of the rat whose kidney is shown in *A* (PAS×85). *D.*—Particularly pronounced osteitis fibrosa in the corresponding region of the femur of the rat whose kidney is shown in *B* (PAS×85).

DISCUSSION

From these observations, it appears that the effect of parathyroid hormone can be significantly increased, not only by the oral administration of calcium salts or of phosphates, but also by lipid-supplements. It is noteworthy that the same food constituents that proved to be effective in sensitizing for the actions of parathyroid hormone were previously found to be particularly potent sensitizing agents for the production of cardiovascular lesions by vitamin-D derivatives (e.g., dihydrotachysterol) and by corticoids (2, 3).

It would be premature to speculate upon the mechanism through which these electrolytes and lipid-supplements exert their sensitizing action. In essence, the present experiments merely confirm that the observations made previously regarding dietary sensitization for dihydrotachysterol also apply to parathyroid hormone. It is of interest, however, that not only the intensity but even the quality of the response to this hormone can be altered by natural food constituents. Very little is known about the participation of the parathyroids in the pathogenesis of diseases other than simple primary hyperparathyroidism with osteitis fibrosa and ectopic calcification. Yet, it is possible to produce selective cutaneous calcinosis and scleroderma-like connective tissue proliferation in a predetermined skin region of parathyroid-hormone treated animals, merely by exposing it to mechanical or chemical trauma (5). In this respect, the actions of the hormone can also be duplicated by dihydrotachysterol (6).

All these observations suggest the possibility that parathyroid hormone and vitamin-D derivatives may participate in the pathogenesis of diverse, seemingly unrelated, morbid lesions in various organ systems (e.g., the heart, vessels, skeletal muscles, kidney, bone, skin), whose selective sensitivity to the potential pathogenic actions of these agents is largely dependent upon topical and systemic conditioning factors.

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ANAPHYLACTIC SHOCK AS A RESULT OF SENSITIZATION TO CALCIUM CHLORIDE

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Calcium chloride is a highly popular agent for the treatment of very different illnesses. This is explained both by its effectiveness over a range of serious conditions (parathyroprival tetany, spasmophilia, hemorrhage, allergic conditions, etc.) and by (its) high permeability. Of the adverse effects of calcium chloride, an irritating endemic reaction to the preparation, especially marked during its entry into a tissue substurface, and the sensation of fever when injecting solutions of the preparation into a vein, are known (V. V. Zakusov; M. D. Moshkovskiy).

Until recently, calcium chloride hardly figured as an agent causing sensitization. Only I. V. Chernusskiy has reported on an allergic reaction, with an increase in temperature to 38.5 degrees over the course of 5 days, widespread polymorphic erythema of the skin and clear mucous membranes, and hemopathy (l. 7900 [leucocytes], e. 11% [erythrocytes], yu. 2% [immature erythrocytes], p. 20% [platelets], s. 49% [serum], lim. 16% [lymph], mon. 2% [monocytes]), setting in directly after the repeated injection of 10 ml. of a 10% calcium chloride solution into a vein. The preparation was used in connection with occupational (benzine) eczema of the hand of a 2-year duration in a sick airplane mechanic. The first injection of calcium chloride was carried out for 17 days, whereupon a portion of the solution got under the skin. At the place of the injection, a patch of skin necrosis formed

on a square 15 by 18 centimeters. The overall condition of the patient did not suffer. In childhood, the patient had scrofula; in recent years (he) couldn't stand betts and milk. The author assumed that the subcutaneously injected calcium chloride acted continously and sensitized the organism; also that it caused a violent allergic reaction in the patient to the repeated introduction of the preparation.

Loiseleur and Sauvage experimentally caused the formation of antibodies to calcium chloride. We observed severe anaphylactic shock following the introduction of calcium chloride.

Patient A, 30 years of age, suffered from chronic purulent bilateral mesotympanitis for a period of 8 years; for 2 years an asthenic condition was noted, with cardioneurosis, treated with various preparations. For a few years the patient had stomatitis. Over the course of the last year of her treatment, eosinophilia (to 12%), nettle rash, and external allergic eczematous otitis of the left (ear) (were) (?observed?) one at a time. In relation to a relapse of the latter (disease) (suffered) by her, the application of calcium chloride (in the form of a 10% solution in [a dosage] of 10 ml. intravenously) was begun. The injections were carried out daily, with the exception of Sundays, (were) technically accurate and without complications. The patient stood up to eight injections well. Immediately after the next injection, the ninth, which was carried out on the 11th day of treatment, she unexpectedly developed an acute cardiac condition (and) approached death. Revival measures proved ineffective.

During a pathologoanatomical study of the symptoms of some illnesses which possibly served as the direct cause of the patient's death, none (were) discovered. During a histological study, plethora and non-uniform blood aggregate of the internal organs and of the brain, minute perivascular hemorrhage, edema of the brain, and symptoms of acute emphysema of the lungs (were) exposed.

During a forensic-chemical study of the brain and other

internal organs, toxically active substances (were) not discovered. The erroneous introduction of another preparation (was) eliminated.

Thus, a woman 30 years of age died from anaphylactic shock, caused by the intravenous injection of calcium chloride, to which the patient had apparently developed an increased sensitivity in the course of treatment. As a benefit of this (case), it is claimed (that) the allergic anamnesis of the patient, the periods of development (Milian syndrome, 9-12th day), the specific clinical picture (the unexpected comatose condition and death), immediately after the intravenous injection of the preparation, (are) typical morphological findings. The ineffectiveness of revival measures is possibly explained by the presence of a drug-antigen (calcium chloride) directly in the blood, which eliminated the possibility of neutralizing its effect on the organism.

During the analysis of medical documentation, one pays attention to the absence of sufficiently serious indications on the use of calcium chloride by means of intravenous injection. Consequently, we think it beneficial to once ag in pay attention to doctors concerning the need to avoid the sweeping assignment of medicines, particularly intravenous (types). As apparent from the case presented, the random intake of medicines might facilitate the development of specific or paraspecific sensitization, with subsequent allergic reactions, among them, a fatal (reaction), during the use of medicines, the purpose of which is indicated.

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GOITROGENIC ACTION OF CALCIUM AND VITAMIN D

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In goiter surveys and experimental studies, calcium has been suggested as a goitrogenic agent. Surveys by Orr ('31) and Stott ('32) have shown that goiter often occurs in limestone regions. It is now commonly accepted that the thyroid influences calcium metabolism. However, there is no general agreement on the goitrogenic role of calcium in the reported experimental studies. Thompson ('36) found that the thyroid size varied directly with the calcium concentration of the diet, but Levine, Remington and von Kolnitz ('32) with the same low iodine diet observed no effect from calcium. Although Hibbard ('33) and Mahorner ('37) obtained a goitrogenic effect from calcium chloride, Hibbard concluded from studies with sodium chloride that chloride and not calcium was the cause of enlarged thyroids.

Experimental goiter in nearly all reports has been of the hyperplastic type. Hellwig ('35) introduced a new problem by asserting that calcium with a low iodine intake produced a hyperplastic thyroid and that a slight increase in iodine intake caused a colloid goiter. Both types of goiter were prevented by further increasing the iodine intake.

If calcium is goitrogenic it should be possible to demonstrate the fact experimentally with a diet that is known to produce a slight thyroid enlargement. It is assumed that

under these conditions the thyroid would be sensitive to substances with only slight goitrogenic action. This is the report of such a study in which the goitrogenic effect of three calcium salts (chloride, phosphate and lactate) was determined. Also reported is a study of Hellwig's diet of corn meal and rolled oats, undertaken to determine whether results similar to his could be obtained under our experimental conditions.

When the results of the above investigation were found to be negative, a study was made of the influence of vitamin D on the alleged goitrogenic action of calcium. According to Abelin ('28) calcium decreases the metabolic effect of thyroxine and therefore should increase the requirement. If calcium should act in this manner, vitamin D, through its action on absorption, would be as important as the calcium intake.

EXPERIMENTAL

The experimental conditions were the same as those previously described (Sharpless, Pearsons and Prato, '39). Both male and female rats, 4 to 5 weeks old, were used. The sexes were kept separate and two or three animals were placed in each cage. Littermates were distributed through the diets as much as possible. Of the three calcium salts studied, the chloride was prepared from calcium oxide and hydrochloric acid. The lactate and phosphate were fed without purification but a correction was made for the iodine they contained so that the total iodine added was 4.0 μg. per 100 gm. of diet. The basal diet had the following percentage composition: Soybean flour (unprocessed) 75.0, dried brewers' yeast 3.0, calcium carbonate 0.5, sodium chloride 1.0, butterfat 5.0, sucrose 15.5, and 4.0 μg. of iodide as potassium iodide. Two per cent calcium chloride or an equivalent amount of calcium in the other salts was added to the basal diet in place of an equal weight of soybean flour. In the experiment with calcium in the water, 1% calcium chloride was added to the drinking water. Hellwig's diet had the following percentage composition: Corn meal 50.0 and rolled oats 50.0. Two per cent calcium chloride was given to these animals in their drinking water.

RESULTS

The thyroid weight, iodine content and concentration for rats fed the different calcium salts are recorded in table 1. Littermate controls were fed the basal diet in all of the studies and the results were sufficiently uniform to combine them in one group. The thyroid weight per 100 gm. of body weight of these animals was 17 mg. which is approximately twice the normal thyroid weight and one-half the weight obtained when the diet is not supplemented with iodine. None of the

TABLE 1

Effect of calcium salts on weight and iodine content of the thyroid.

DIET	NO. OF RATS	THYROID WEIGHT		THYROID IODINE	
		Ave. total	Per 100 gm. body wt.	Total per gland	Dry basis
		mg.	*mg.*	*µg.*	%
Basal	212	22	17 ± 0.21	1.5	.026
1% $CaCl_2$ in drinking water	24	21	17 ± 0.7	1.7	.025
2% $CaCl_2$ in basal diet	71	23	17 ± 0.47	1.6	.029
6% calcium lactate	15	18	18 ± 0.8	1.2	.026
2% calcium carbonate	23	25	17 ± 0.97	1.2	.020
2% $Ca_3(PO_4)_2$	11	20	19 ± 0.8	1.8	.030
Rolled oats 50.0% Corn meal 50.0%	12	12	14 ± 0.5	3.3	.11
2% $CaCl_2$ in drinking water	16	16	12 ± 0.5	2.1	.06

calcium salts produced a real enlargement of the thyroid. The slight enlargement obtained with calcium lactate and calcium phosphate proved to be insignificant by mathematical analysis. The iodine concentration on the dry weight basis was almost identical for all four diets.

The diet composed of 50% corn meal and 50% rolled oats (Hellwig's diet, table 1) was unsatisfactory for growth. Although Hellwig does not state that rats fed this diet need any vitamin D supplement, the rats showed tetany and died within 4 or 5 weeks unless vitamin D was given. The usual dosage of 15 drops of viosterol per kilogram of food proved ineffective, but 2 drops per rat per day maintained life and

stimulated some growth in those getting no calcium chloride. The diet containing calcium chloride was supplemented with 3% of dried yeast after 6 weeks in order that the rats might survive. Yeast contains only a small amount of iodine and good results have been obtained with it in soybean goitrogenic diets. Only those animals which lived for the full 12 weeks are recorded in the table. Rats given calcium chloride had an average thyroid weight of 12 mg. per 100 gm. of body weight compared with 14 mg. for their littermate controls. The difference is not statistically significant and because the parathyroids of rats fed the low calcium diet were very large, the difference is not so great as the figures imply. Because of their intimate relation with the thyroid, the parathyroids must be weighed with it.

Examination of some glands from each group showed no real differences in the microscopic structure. Some of the glands from animals fed calcium chloride appeared more hyperplastic than those of littermate controls, but the differences were slight. The 2% calcium chloride fed in the diet is a large amount when compared with any normal diet. Because of this large amount of calcium, the slight hyperplasia obtained, while it supports the results of Mahorner with dogs, is believed to have no real significance from the standpoint of goiter incidence.

These results demonstrate that a high calcium intake does not affect the size of the thyroid. However, if calcium should influence the oxidation processes that are controlled by the thyroid the effect would be obtained from its action within the body and the factors that influence calcium metabolism should receive consideration. One of the principal factors affecting absorption and utilization of calcium is vitamin D.

The results of experiments with small and large amounts of vitamin D are shown in table 2. In the basal diet without iodine supplement, viosterol in either small or large amounts had no real effect on the size or iodine content of the thyroid. Calcium carbonate either alone or along with 2% viosterol caused no difference in iodine concentration of the glands and

TABLE 2

Effect of viosterol and calcium on weight and iodine content of the thyroid.

BASAL DIET SUPPLEMENT	NO. OF ANIMALS	BODY WEIGHT		THYROID WEIGHT		IODINE IN GLAND	
		Initial	Final	Ave. total	Per 100 gm. body wt.	Ave. total	Dry basis
		gm.	*gm.*	*mg.*	*mg.*	*μg.*	*%*
Basal —40 μg. I_2 per kg.	36	65	160	53	33 ± 1.1	0.7	.007
15 drops viosterol per kg.	29	68	131	44	34 ± 1.4	0.9	.009
2% viosterol	39	60	147	50	34 ± 1.1	0.6	.006
2% $CaCO_3$	17	73	145	55	37 ± 2.1	0.7	.007
2% $CaCO_3$ + 2% viosterol	17	70	134	49	37 ± 1.2	0.6	.006
Basal	82	65	139	23	17 ± .40	2.2	.038
I_2 + 2% viosterol	28	61	133	24	18 ± .72	2.3	.043
I_2 + 2% $CaCl_2$	70	65	128	23	18 ± .47	2.0	.038
I_2 + 2% $CaCl_2$ + 15 drops viosterol	55	67	141	28	20 ± .52	2.0	.033
I_2 + 2% $CaCl_2$ + 2% viosterol	27	63	134	29	22 ± 1.0	1.62	.029
I_2 + 2% $CaCO_3$	23	72	145	25	17 ± .97	2.2	.039
I_2 + 2% $CaCO_3$ + 2% viosterol	24	69	128	21	16 ± .63	2.0	.040

only a slight enlargement which was not mathematically significant. In the basal diet, however, both concentrations of viosterol produced an increase in thyroid weight when calcium chloride was fed, but no effect with calcium carbonate. The average thyroid weights per 100 gm. of body weight of rats fed the diets containing calcium chloride with either 15 drops or 2% viosterol were 20 mg. and 22 mg., respectively; mathematical analysis showed that, because of the greater individual variation in the latter group, both had the same significance, viz., a "P" value of .00 (Fisher, '34).

While 15 drops per kilogram of diet had no effect, 2% viosterol raised the average serum calcium from 12.6 to 13.3 mg. per 100 cc.

DISCUSSION

The two most evident facts in the results are (1) that elevated serum calcium has no tendency to increase the thyroid

weight, and (2) that calcium chloride, the salt that has been used in many experimental studies, has a significant goitrogenic effect when vitamin D is present.

Hibbard's ('33) results in which calcium chloride but not other calcium salts was found to produce goiter, are therefore confirmed. However, Hibbard's second conclusion that chloride is goitrogenic is not supported by these results since without vitamin D, calcium chloride did not cause an increase in thyroid weight.

When thyroid enlargement occurs and an increase in the iodine intake brings about a normal gland, the accepted explanation is that a relative iodine deficiency exists. The principal, if not the only, site of iodine storage is in the thyroid gland. Therefore, the iodine content of the thyroid gives a direct measure of the amount of iodine stored in the body and is a more reliable indication of the iodine balance than its determination from the food and excreta. The thyroids of rats at the start of the experiment contained approximately 2.0 μg. of iodine or 0.13% of dry weight. From the results it can be seen that with the exception of diets lacking iodine supplement the net iodine balance for the experiment is practically zero. Furthermore, it can be calculated that a 150-gm. rat with a normal thyroid weight of 0.0105 gm. and an iodine concentration of 0.1% of the dry weight would contain 2.1 μg. of iodine. Since glands with an iodine concentration of 0.1% dry weight have a normal microscopic appearance, it must be concluded that within the limits of 1.6 to 2.3 μg. (table 2) the total is within the normal range. Since additional iodine in the diet will produce an approximately normal gland, these results are interpreted as evidence of a relative iodine deficiency. Balance studies which have been proposed for this experiment could indicate only the summation which is really a determination of absolute iodine deficiency.

A relative iodine deficiency could occur (1) when there is an excessive excretion of iodine before it can be utilized, (2) when the requirement for thyroxine is increased, or (3) when the mechanism for thyroxine formation is blocked. All of these conditions assume that the iodine is absorbed in

available form. While the iodine content of the glands mentioned above is within the calculated normal range for these animals, the iodine concentration is low. Chlorides tend to decrease the concentration and the greatest reduction occurred when calcium chloride was fed with 2% viosterol. Growth and food intake were similar in all diets. Iodine storage was slightly less in rats fed calcium chloride and 2% viosterol, which suggests that it could have been excreted before being utilized. Other data not given here demonstrate that chloride can cause a reduction in the content and concentration of iodine in the thyroid.

The possibility that viosterol, by way of its alleged effect on metabolism (Deutsch, Reed and Struck, '36), might influence the results is ruled out by control experiments in which it did not influence the size, iodine content or iodine concentration of the thyroid.

Hellwig's ('35) hypothesis that goiter is caused by a positive factor along with an iodine deficiency is partially supported by these results, namely, that an apparent decrease in available iodine occurred when calcium chloride produced thyroid enlargement. Because calcium chloride was the only salt that was goitrogenic, neither of its components should be classed as having this property. Furthermore, the compound should be considered as only a potential goitrogenic agent.

The fact that calcium carbonate plus 2% viosterol caused a slight but not significant decrease and calcium chloride caused an increase in thyroid weight raises the question whether acidosis might play a role in thyroid enlargement. Experiments on this point have not provided consistent results. Direct determinations were not made but some evidence is provided in these results. Calcium chloride without vitamin D had no significant goitrogenic effect. Vitamin D causes a shift in calcium excretion from the feces to the urine, thereby increasing the acidity of the feces. It is assumed that this would tend to relieve a condition of acidosis if it was present, yet calcium chloride was goitrogenic only when vitamin D was given. Loose stools were common in rats receiving calcium chloride but extensive diarrhea was not observed.

Other experiments not reported here show that inclusion in the basal diet of hydrochloric acid, equivalent to 70% of the acid producing effect of 2% calcium chloride, had no significant effect on the thyroid weight.

SUMMARY

The purpose of these experiments was to determine whether calcium could be classed as a goitrogenic substance. When other factors are kept constant, all of the evidence indicates that it does not influence the size of the thyroid gland. However, along with vitamin D calcium chloride can act as a goitrogenic agent. While the mechanism of this action cannot be absolutely determined from these experiments, the results suggest that chloride causes some loss of iodine which is followed by an increase in thyroid weight when an excess of calcium is absorbed.

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A COMPARISON OF THE EFFECTS OF LARGE DOSES OF CALCIUM GLUCONATE-IDONATE, CALCIUM GLUCONATE, AND CALCIUM CHLORIDE*

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THE wide use of intravenous calcium therapy makes the pharmacologic action of a readily soluble calcium salt a matter of some interest. Calcium gluconate-idonate† is a very soluble salt. Hence a study of its action, comparing it with calcium chloride and with calcium gluconate, has been made.

PROCEDURE AND RESULTS

Chronic Studies in Rats.—The drugs in solution or suspension were administered by stomach tube to rats six days weekly throughout the experiment. The animals were weighed at least once a week. Certain tissues from animals dying during the experiment, as well as those surviving for its duration, were examined histologically. Calcium gluconate-idonate, calcium chloride, and calcium gluconate were all given in amounts equivalent to 0.4 Gm. calcium per kilogram of body weight daily, the first two in solution and the third in a heavy suspension. The gluconate-idonate and the chloride were also tested at levels equivalent to 1 and 2 Gm. calcium per kilogram per day. Ten animals comprised each group except that receiving 1 Gm. calcium per kilogram as gluconate-idonate; it contained 20 rats.

All the animals receiving calcium at the 2 Gm. per kilogram level died after a single dose; hence they are not included in Fig. 1, where the average weight of each group is plotted against the survival time in days. A cross bar indicates the death of an animal. Administration of 1 Gm. of calcium per kilogram of body weight as calcium chloride produced death after one or two doses, but as calcium gluconate-idonate 4 animals survived the entire period. In a dose of 0.4 Gm. calcium per kilogram, of the rats receiving calcium chloride 5 of the original 10 survived the entire period, while all those receiving calcium gluconate-idonate or calcium gluconate of the same calcium content survived. Two of the gluconate animals died (at 56 and 63 days, respectively), but the entire gluconate-idonate group was still alive.

Pathology.—The heart, kidney, and liver tissue from animals given calcium gluconate-idonate equivalent to 1 Gm. of calcium per kilogram per

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The calcium gluconate-idonate used in this study was supplied by Charles Pfizer & Co., Inc., who have contributed generously to the support of this research.

†Calcium gluconate-idonate is soluble in water to the extent of 411 Gm. per liter of solution at 25° C. It is possible to prepare a temporarily supersaturated solution of about 45 per cent at room temperature. The calcium content of the salt is approximately 9.3 per cent. Idonic acid is the sugar acid derived from the hexose idose and is isomeric with gluconic acid.

day for seventy days were examined histologically. These tissues were essentially normal in appearance, showing no abnormalities which could be ascribed to the effect of the drug. Likewise, neither calcium gluconate for seventy days nor calcium chloride for sixty-five days each, given in amounts containing 0.4 Gm. of calcium per kilogram per day, produced any apparent change in these tissues.

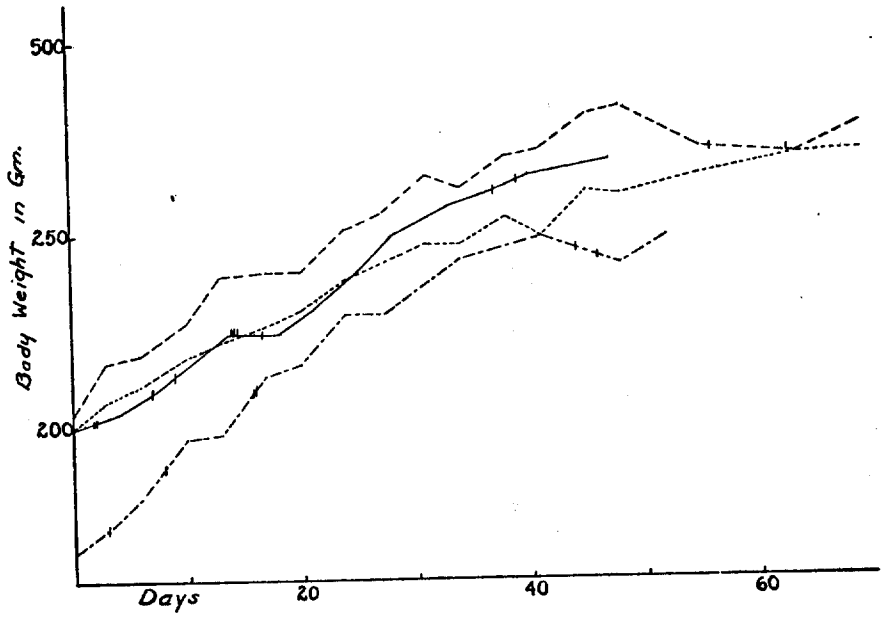

Fig. 1.—Average growth curves of rats receiving indicated doses of calcium salts daily. Cross bars indicate deaths of animals.
——— Calcium chloride = 0.4 Gm. Ca per kg.
- - - - - Calcium gluconate = 0.4 Gm. Ca per kg.
---------- Calcium gluconate-idonate = 0.4 Gm. Ca per kg.
———— Calcium gluconate-idonate = 1.0 Gm. Ca per kg.

Acute Studies.—Eighteen adult dogs were used. The animals received morphine sulfate subcutaneously, 10 mg. per kg. body weight, thirty minutes before injection of the calcium salt was begun. The calcium solution* (0.205 M) was injected through a cannula into the femoral vein at varying rates. A blood sample was withdrawn from the femoral vein just prior to cannulation; a second was obtained at death by direct cardiac puncture. The concentration of calcium in the serum was determined by the method of Kramer and Tisdall.[3] Electrocardiograms† from Lead II were taken at frequent intervals during the injection.

The protocols from all experiments are summarized in Table I. The results of Hoff, Smith, and Winkler,[1, 2] who used the same procedure with calcium chloride, are included for comparison.

*To prepare the calcium gluconate solution it was necessary to heat the mixture on the steam bath to bring about solution. Dextrose 5.0 per cent, citric acid 0.37 per cent, and lactic acid 1.0 per cent were then added to stabilize the supersaturated solution which was filtered and diluted to volume after recooling to room temperature.

Calcium gluconate-idonate in 20 per cent solution (0.507 M) was also injected intravenously into each of 2 dogs in quantities of 3 c.c. per kg. at rates of 1.76 and 2.14 c.c. per kg. per minute without subsequent ill effect.

†Dr. H. E. Hoff and Dr. P. K. Smith kindly assisted in obtaining the electrocardiograms.

TABLE I

DOG	CALCIUM CHLORIDE					CALCIUM GLUCONATE-IDONATE					CALCIUM GLUCONATE				
	KG.	C.C.	C.C./KG.	C.C./KG. MIN.	MG. % CA	KG.	C.C.	C.C./KG.	C.C./KG. MIN.	MG. % CA	KG.	C.C.	C.C./KG.	C.C./KG. MIN.	MG. % CA
1	12.2	99	8.1	0.5	51.4	12.5	193	15.4	0.81	111	12.5	856	68.7	1.01	192[A]
2	9.1	280	30.8	0.7	45.6	9.0	92	10.2	1.12	100	10.8	63	5.8	3.89	182
3	10.5	66	6.3	0.8	53.0	5.8	166	28.6	1.22	231	10.7	110	10.3	1.72	100
4	6.8	149	21.9	0.8	106.4	6.8	296	43.5	1.34	290	10.2	89	8.7	1.29	88
5	12.6	89	7.1	1.0	56.8	6.2	37	6.0	5.97	170	9.0	368	40.9	1.14	175
6	7.5	113	15.1	1.1	70.8	6.9	84	12.2	0.94	76	18.1	368	20.3	0.96	89
7	7.5	142	18.9	1.1	60.0	7.0	214	30.6	1.22	127	12.5	108	8.6	0.86	98
8	8.8	209	23.8	2.6	52.4	10.7	610	57.0	1.66	242[A]	10.1	203	20.1	1.25	152
9	11.6	86	7.4	7.4	95.4	11.6	524	45.2	1.10	168[A]					
10	7.0	155	22.1	1.1	126.0	8.0	120	15.0	5.45	110					
11	17.5	230	13.1	1.1	138.4										
12	6.4	142	22.2	1.6	114.0										
13	9.6	43	4.5	2.6	86.0										
	Mean		15.5		81.2			26.4		162.5			17.8		134.5
	S.D.		8.4		31.8			17.4		71.0			12.32		45.0
	PEm		1.57		5.9			3.7		16.0			3.14		10.7

[A]—died in cardiac arrest.

Changes in the electrocardiogram followed the sequence reported by the above-mentioned authors for calcium chloride. An initial phase of inhibition, both of rate and of auriculoventricular conduction, was followed by a period of excitation, during which ectopic rhythms appeared, and which often culminated in ventricular fibrillation. In experiments in which ventricular fibrillation did not appear during the rapid phase, a second period of inhibition occurred, and the animal died of cardiac arrest. In experiments in which calcium gluconate-idonate or gluconate were employed, certain variations were detected in their effect on the heart. First, corresponding with the greater amounts of calcium required to kill, the duration of the various phases was increased. Second, the degree of inhibition during the first stage was increased, so that arrest of as much as eight to ten seconds was observed. Thus, the tendency of the ventricles to fibrillate persisted even after the second slowing phase began, so that ventricular fibrillation frequently terminated a burst of tachycardia after an arrest of fifteen seconds or more. Because of this extension of the period during which ventricular fibrillation might be expected, 8 of 10 animals given calcium gluconate-idonate, and 7 of 8 given calcium gluconate died of fibrillation, compared with 6 of 13 given calcium chloride.

Appreciably larger quantities of calcium as gluconate-idonate or as gluconate are required to produce death than are needed when calcium is given as the chloride. This is reflected in the terminal serum calcium values, the means of those for both organic salts being significantly higher than that with the chloride. The gluconate-idonate and the gluconate do not appear to differ appreciably in their effects. It is possible that the difference between the chloride and the sugar acid salts can be explained in part by the different amounts of calcium ions they yield.

SUMMARY

Calcium chloride, whether given orally to rats or intravenously to dogs, is definitely more toxic than calcium gluconate-idonate or calcium gluconate.

Calcium gluconate-idonate and calcium gluconate differ very little in their toxicity for dogs and rats. Over a long period of time (seventy days) calcium gluconate proved lethal to 2 of 10 rats, and calcium gluconate-idonate to none of 10.

Intravenous injections of 0.205 M solutions continued to the point of cardiac arrest (usually with ventricular fibrillation) revealed no differences between the effects of gluconate and calcium gluconate-idonate.

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Range-Finding Toxicity Data: List VII

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Acute toxicity and irritation data on about 200 compounds are presented, accumulated in a continuing program for screening potential commercial products.

DURING THE SEVEN years since the appearance of our last communication on the range-finding test,[1] many additional data have become available for presentation. Table I lists these data under names conforming to the nomenclature used in *Chemical Abstracts*. Table II lists only the oral LD_{50} values for a number of inorganic compounds. All materials included are either in commercial production or have been evaluated for commercial potential within the past few years.

As has been stated in earlier communications,[1-6] the range-finding test is relied upon only to allow predictions of the comparative hazards of handling new chemicals. Acute toxicity studies, no matter how precisely executed, yield no more than an indication of the degree of care necessary to protect exposed workmen or lead to an opinion that certain technically feasible applications of a chemical may or may not eventually be proved safe.

Our last paper[1] included data under the name of triisooctyl phosphine. The compound should have been entered as triisooctyl phosphite.

Experimental methods have remained unchanged. They are described in our 1962 publication.[1]

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TABLE I
Range-Finding Toxicity Data

Material Studied	Single Oral LD_{50} for Rats ml/Kg	Single Skin Penetration LD_{50} for Rabbits ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
Hydrocarbons								
2-, 4-Cyclohexenyl -bicyclo-(2.2.1)-hept-5-ene	4.29 (3.07 – 5.98)	3.54 (2.19 – 5.72)	8 hr.	–	–	–	5	2
Cyclopentene	2.14 (1.45 – 3.17)	1.59 (0.97 – 2.59)	5 m. §	16,000	4	4/6	5	5
5-Ethylidene-2-norbornene	2.83	9.17 (1.35 – 6.33)	–	4,000	4	5/6	5	2
p-Menth-3-ene	20.75 (15.36 –28.03)	5.66 (2.59 -12.3)	30 m.	2,000	4	2/6	5	1
2-Norbornene	11.3 (7.0 –18.3)*	> 5	5 m.	–	–	–	1	7
1,7-Octadiene	19.7 (15.0 –25.8)	14.1 (4.66 42.9)	15 m.	8,000	4	2/6	4	1
3a,4,7,7a-Tetrahydro-4H-indene	3.73 (2.68 – 5.21)	14.1 (8.75 – 22.9)	4 hr.	–			3	2
Tetralin	7.35 (6.73 – 8.43)*	14.1 (9.63 – 20.77)	–	4,000	4	1/6	4	9
4-Vinylcyclohexene	3.03 (2.49 – 3.81)	20	15 m.	8,000	4	4/6	4	2
5-Vinyl-2-norbornene	5.19 (4.22 – 6.41)	15.9 (11.7 – 21.5)		4,000	4	4/6	5	2
Hydroxyl Compounds								
[illegible]	3.25 (2.48 – 4.26)	2.52 (0.93 – 8.58)	8 hr.		–	–	6	9
[illegible]	2.83 *	3.48 (1.98 – 5.18)*	8 hr.		–		–	9
[illegible]	0.47 (0.34 – 0.66)	0.18	1 hr.	500 ?	4	0/6	2	5
1,3-Dioxolane-2-methanol	1.62 (1.24 – 2.13)	0.46 (0.29 – 0.54)					2	6
[illegible]	>64	>20	8 hr.	–	–		3	1
[illegible]	2.46 (1.82 – 3.33)	2.38 (1.76 – 3.34)	8 hr.	–	–	–	3	5
Hydroxyethylcyclopentanol	3.25 (2.41 – 4.99)	3.15 (2.33 – 4.26)	8 hr.		–	–	5	9
2-Methylcyclohexanol	7.07 (4.82 – 10.4)	3.97 (2.93 – 5.37)	8 hr.		–	–	2	8
Methylcyclohexylcyclopentanol	1.41	0.62 (0.42 – 0.92)	8 hr.	–			3	8
[illegible]	1.62 (1.06 – 2.50)	2.14 (1.52 – 2.98)	4 hr.	–			6	10
2-Nitrobutanol-1-ethanol	1.62 (1.24 – 2.13)*	0.80	8 hr.	–	–	–	3	9
[illegible]	32.5 (29.1 – 36.3)	7.13 (4.41 – 11.52)	8 hr.		–	–	2	1
Ethers								
[illegible]-alkyl ether	3.73 (2.43 – 5.74)	5.95 (3.57 – 9.09)	8 hr.		–	–	3	1
[illegible] ether	11.3	1.59 (1.03 – 2.59)	4 hr.				5	2
[illegible] propene ether	19.0 (13.4 – 27.0)	>10	5 m.	8,000	4	0/6	2	2
[illegible] ether	8.16 (7.03 – 9.48)	>20		64,000	4	0/6	1	1
Methoxyethyl [illegible] ether	11.3	10.0 (4.56 – 21.9)	8 hr.		–		2	3
[illegible] Methylbenzyl ether	9.80 (6.38 – 15.06)*		8 hr.	–	–		4	1
2-Methoxypropene	1.87 (1.26 – 2.76)	>20	–	64,000	4	4/6	1	1
1,1,1-Trichloroethoxycyclohexane	4.09 (3.52 – 4.76)*	–	8 hr.	–		–	2	2
Hydroxy Ethers								
1-Butoxyethoxy-2-propanol	4.00 (2.55 – 6.27)	2.83 (1.75 – 4.57)	8 hr.	–	–	–	3	5
3-Butoxy-1-propanol	5.95 (3.65 – 9.68)	1.59 (1.17 – 2.15)	8 hr.	–	–		2	7
Diethoxytetraethylene glycol	4.29 (2.90 – 6.34)	6.35 (3.89 – 10.4)	8 hr.	–	–		2	2
3-Ethoxy-1-propanol	13.0 (8.46 – 20.0)	2.83	8 hr.	–			2	3
2-Ethoxybutoxypropanol, mixed isomers	3.56 (2.21 – 5.74)	6.0	8 hr.				2	4
2-Hexyloxy-2-ethoxyethyl ether	3.73 (2.68 – 5.21)	6.30 (4.66 – 8.52)	8 hr.	–			2	3
Isobutoxypropanol, mixed isomers	7.5	3.56 (1.95 – 6.52)	8 hr.	–			3	7

TABLE I
Range-Finding Toxicity Data (Continued)

Material Studied	Single Oral LD50 for Rats ml/kg	Single Skin Penetration LD50 for Rabbits ml/kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
2-Isopropoxyethanol	[illegible]	[illegible]	2 hr.	4,000	4	4/6	3	5
3-Methoxy-2-propanol	[illegible]	[illegible]	8 hr.		–		1	5
2-Propoxyethanol	[illegible]	[illegible]		–	–	–	2	8
Propoxypropanol, mixed isomers	[illegible]	[illegible]	8 hr.		–		2	7
Alcohols, Ketones								
Cyclohexanone	[illegible]	[illegible]	30 m.	2,000†	4	1/6	1	5
3,5,5-Trimethylcyclohexanone	[illegible]	[illegible]	8 hr.	–	–	–	2	1
Hexanal, mixed isomers	[illegible]	[illegible]	30 m.				2	5
2-Methylcyclohexanone	[illegible]	[illegible]		2,300	4	3/6	3	5
Methyl 5-[illegible]-2-pentanone	[illegible]	[illegible]	8 hr.		–	–	5	5
[illegible]	[illegible]	[illegible]	8 hr.		–		4	3
Vinylcyclohexene	[illegible]	[illegible]	15 m.	1,000	4	3/6	2	5
Acids, Anhydrides, Esters								
2-Hydroxyethyl acrylate	[illegible]	[illegible]	8 hr.	–	–	–	5	5
2-Methyl-[illegible]-acrylic acid	[illegible]	[illegible]		–	–	–	1	4
Methylbis(2-ethylhexyl) phthalate	[illegible]	[illegible]	8 hr.	–	–	–	1	9
Trioxyethylene acrylic acid hydrazide	[illegible]	[illegible]		–	–	–	4	7
Azelaic acid, mixed esters	[illegible]	[illegible]	8 hr.	–	–	–	6	9
Esters, Acetates								
Acetic acid, 2-[illegible]-2-propanediol diester	[illegible]	[illegible]	8 hr.	–	–	–	3	2
Acetic acid, 2-methoxy-2-propanol 1-[illegible] diester	[illegible]	[illegible]	1 hr.	62.5	4	5/6	5	9
Acetic acid, phenyl ester	[illegible]	[illegible]	8 hr.	–	–	–	2	2
Acetic acid, propyl ester	[illegible]	[illegible]	30 m.	8,000	4	4/6	1	2
Acetic acid, triethylene glycol diester	22.6	8.0 (1.91 -33.5)	8 hr.	–	–	–	1	1
Acrylic acid, 2-butoxyethoxy ester	6.50 (4.72 - 8.95)	0.64 (0.39 - 1.04)	8 hr.	–	–	–	5	2
Acrylic acid, decyl ester, mixed isomers	12.3 (9.98 - 15.3)	3.54 (1.17 -10.7)	8 hr.	–	–	–	5	1
Acrylic acid, hexyl ester	26.0 (18.9 - 35.8)	5.66 (3.50 - 9.14)	4 hr.	–	–	–	5	1
Acrylic acid, 2-hydroxypropyl ester	1.23 (0.89 - 1.70)	0.16 (0.10 - 0.26)	8 hr.	–	–	–	5	7
Acrylic acid, 2-methoxyethoxy ester	0.81 (0.59 - 1.21)	0.25 (0.17 - 0.37)	15 m.	500	4	3/6	4	5
Acrylic acid, 2-norbornyl ester	5.66	0.88 (0.54 - 1.43)	8 hr.	–	–	–	5	2
Azelaic acid, dihexyl ester	16.0 (13.2 - 25.0)	>20	–	–	–	–	2	1
Benzoic acid, 2-ethylhexanediol diester	29.2 (15.8 - 53.9)	20	8 hr.	–	–	–	2	1
Fumaric acid, ethyl 2,3-epoxypropyl ester	1.62 (1.06 - 2.50)	0.35 (0.22 - 0.57)	–	–	–	–	5	8
Fumaric acid, dimethyl ester	2.24 (1.15 - 4.37)*	1.25 (0.84 - 1.85)*	8 hr.	–	–	–	5	9
Malonic acid, diethyl ester	14.9 (10.7 - 20.8)	>16	8 hr.	–	–	–	2	5
Methacrylic acid, allyl ester	0.43 (0.31 - 0.60)*	0.50 (0.37 - 0.68)	8 m.	250†	4	0/6	5	2
Methacrylic acid, butyl ester	22.6	11.3 (5.19 -24.7)	8 hr.	–	–	–	5	1
Phthalic acid, butyl decyl ester	20.8 (16.8 - 25.7)	15.9 (11.7 -21.5)	8 hr.	–	–	–	2	1
[illegible]	49.4 (39.9 - 61.0)	>20	8 hr.	–	–	–	2	1

(Continued on Next Page)

Table I
Range-Finding Toxicity Data (Continued)

Material Studied	Single Oral LD_{50} for Rats ml/Kg	Single Skin Penetration LD_{50} for Rabbits ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
Phthalic acid, decyl octyl ester	45.2 (28.0 - 73.2)	>20	8 hr.				2	1
Propionaldehyde dicrotyl acetal	2.14 (1.54 - 2.99)	3.54 (2.19 - 5.72)	30 m.	2,000	4	5/6	4	2
Succinic acid, di-2-[(2-ethylbutoxy)ethoxy] ester	3.3	* 7.11 (6.58 - 7.69)					3	2
Epoxy Compounds								
Bicyclononadiene diepoxide	2.14 (1.54 - 2.99)	1.77	8 hr.				3	5
Bis(3,4-epoxybutyl) ether	1.07 (0.77 - 1.50)	0.25 (0.17 - 0.37)	8 hr.				5	7
Bis(3,4-epoxycyclohexylmethyl) adipate	4.39 (3.07 - 5.98)	>20	8 hr.				2	1
N,N-Bis(2,3-epoxypropyl)aniline	1.62 (1.18 - 2.24)	3.56 (1.68 - 7.57)	8 hr.				3	1
Butyl 2,3-epoxypropyl fumarate	0.71 (0.48 - 1.04)	1.26 (0.93 - 1.70)	8 hr.				5	8
p-Butylphenyl 2,3-epoxypropyl ether	3.73 (2.68 - 5.21)	5.66	8 hr.				5	2
1,2-Epoxycyclohexane	1.09 (0.66 - 1.80)	0.63 (0.30 - 1.33)	90 m.	2,000	4	1/6	3	8
2,3-Epoxypropyl methacrylate	0.77 (0.57 - 1.04)	0.45 (0.21 - 0.95)	2 hr.				5	4
Propylene oxide	1.14 (1.05 - 1.23)	1.50 (1.07 - 21.0)	5 m. §	4,000	4	1/6	1	5
Heterocyclic Oxygen								
2-Aminomethyl-2,3-dihydro-4H-pyran	1.00 (0.64 - 1.57)*	0.18	4 hr.				6	9
2,3-Dimethoxytetrahydropyran	2.46 (1.79 - 3.39)	1.41 (0.65 - 3.08)	4 hr.				4	2
3,9-Diethyl-2,4,8,10-tetraoxaspiro[5.5]undecane	3.25 (2.48 - 4.26)*	7.92 (5.87 - 10.73)	8 hr.				2	2
2-Ethoxy-4-methyl-tetrahydropyran	5.16 (4.17 - 6.38)	>5	4 hr.				2	3
Aliphatic and Alicyclic Amino Compounds								
3-Aminomethyl-3,5,5-trimethylcyclohexanol	1.36 (1.04 - 1.75)	-	8 hr.				7	9
2-Aminomethyltetrahydropyran	0.71 (0.44 - 1.13)	0.71 (0.44 - 1.14)	4 hr.				6	9
2-Aminopropoxy-2-ethoxy ethanol	6.56 (4.95 - 8.53)	5.09 (4.27 - 8.42)	8 hr.				3	-
Amyl amine, mixed isomers	0.47 (0.33 - 0.65)*	1.12 (0.61 - 2.05)	30 m. §	2,000	4	4/6	6	9
Bis(2-dimethylaminoethoxy)ethane	2.83	1.2					-	8
Bis(2-dimethylaminoethyl) ether	1.23 (0.94 - 1.62)	0.28	8 hr.				6	9
Cyclohexylamine	0.71 (0.51 - 0.97)	0.32 (0.24 - 0.43)	2 hr.	1,000	4	0/6	7	10
1,8-Diamino-p-menthane	0.77 (0.62 - 0.95)	0.63 (0.47 - 0.85)	4 hr.				7	9
2,4-Diamino-1-methylcyclohexane	1.41	0.50 (0.31 - 0.82)	8 hr.				6	9
Di-3-aminopropyl ether of diethylene glycol	4.29 (3.07 - 5.98)	2.50 (1.14 - 5.48)	4 hr.				6	8
Di-3-aminopropoxyethane	2.83 (1.76 - 4.55)	1.25 (0.84 - 1.85)	8 hr.				6	8
N,N-Dimethyl-N-cyclohexylmethylamine	1.23 (0.94 - 1.62)*	0.21 (0.15 - 0.30)	8 hr.				7	9
N-Methyl-N-cyclohexylmethylamine	1.41	* 0.63 (0.47 - 0.85)	4 hr.				6	9
N,N,N',N',N''-Pentamethyldiethylenetriamine	1.63 (1.24 - 2.13)	0.28	8 hr.				7	9
N,N,N',N'-Tetramethyl-1,3-butanediamine	0.93 (0.67 - 1.30)	0.40 (0.29 - 0.54)	8 hr.				6	9
N,N,N',N'-Tetramethyl-1,3-propanediamine	0.41 (0.31 - 0.53)	0.30 (0.21 - 0.42)	15 m.	1,000	4	2/6	5	9
Tetramethyl-2-tetrazene	1.78 (1.12 - 2.83)		4 hr.				2	4
Tripropylamine	0.0961 (0.071 - 0.13)*	0.57 (0.26 - 1.23)	8 hr.	250	4	3/6	4	4
Aromatic Nitro and Amino Compounds								
8-Aminopyrene	1.07 (0.77 - 1.50)*	>1	8 hr.					8
N-Methyl-1-naphthylamine	1.41 (1.03 - 1.95)		8 hr.				2	1

TABLE I

Range-Finding Toxicity Data (Continued)

Material Studied	Single Oral LD_{50} for Rats ml/Kg	Single Skin Penetration LD_{50} for Rabbits ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
Amides								
N-2-Acetoxyethyl-N-methylacetamide	20.8 (16.8 - 25.7)	>10	8 hr.	–	–	–	2	4
Caprolactam	2.14 (1.54 - 2.99)*	1.41	4 hr.	–	–	–	–	7
N-Ethyl-N-methylacetamide	2.46 (1.88 - 3.23)	1.25 (0.84 - 1.85)	4 hr.	2,000	4	3/6	2	5
Propyl-N,N-diethylacetamide	12.93 (8.42 - 19.87)	>20	8 hr.	–	–	–	4	5
Heterocyclic Compounds								
3,5-Dimethyl-1-(2-hydroxymethylpiperidino)-pyrazole	0.57	0.20 (0.15 - 0.27)*	4 hr.	–	–	–	6	10
Isopropylmorpholine	0.71	0.10 (0.07 - 0.15)	15 m.	500	4	5/6	6	7
Isopropylpiperazine	2.83	0.64 (0.47 - 0.86)	8 hr.	–	–	–	6	9
3-Methylpyridine	0.71 (0.40 - 1.24)	0.14	1 hr.	250†	4	0/6	6	8
N-Methylcaprolactam	1.62 (1.18 - 2.24)	1.41	8 hr.	–	–	–	3	5
2-Methylpiperazine dihydrate	7.13 (3.32 - 15.30)	10.8 (6.17 - 16.5)	8 hr.	–	–	–	1	4
1-Methyl-3-indolinone	1.39 (0.71 - 2.37)	0.80 (0.54 - 1.18)	4 hr.	4,000	8	4/6	5	8
1-(2-Hydroxyethyl)-mercapto-pyrazole	0.35 *	0.063 (0.047 - 0.085)	–	200	1	6/6	7	9
1-(2-Ethoxyethyl)-mercapto-3-methylpyrazole	1.01 (0.81 - 1.28)	0.079 (0.059 - 0.107)	2 m. §	–	–	–	7	8
Oxygen Compounds								
Bis-(2-methoxyethyl) carbonate	1.83 (1.21 - 2.79)	>12	8 hr.	–	–	–	3	9
Bis-(2-methoxyethyl)-4-cyclohexene-1,2-dicarboxylate	10.4 (5.55 - 19.4)	> 8	8 hr.	–	–	–	5	5
Bis-(2-methoxyethyl)-5-methacrylamido-2,3-tricarboxylate	22.6 (13.4 - 38.2)	16.1 (4.5 - 57.2)	8 hr.	–	–	–	5	5
3-Butenenitrile	0.115 (0.097 - 0.138)*	1.41	5 m.	250†	4	0/6	2	3
N,N-Dibutyl-3-aminopropionitrile	3.25 (2.48 - 4.26)	5.04 (3.73 - 6.82)	8 hr.	–	–	–	2	2
1,6-Hexamethylene diisocyanate	0.71	0.57 (0.35 - 0.91)	8 hr.	–	–	–	8	9
Lactonitrile	0.087 (0.060 - 0.125)*	0.020 (0.015 - 0.027)	–	62.5†	4	0/6	2	–‡
3-Methoxypropionitrile	4.39 (3.07 - 5.98)*	>10	8 hr.	–	–	–	2	5
Methyl isocyanate	0.071 (0.027 - 0.18)*	0.22 (0.12 - 0.41)	–	31.25†	4	0/6	6	10
Tridecanitrile, mixed isomers	3.73 (2.68 - 5.21)	3.18 (1.43 - 7.06)	8 hr.	–	–	–	4	5
Halogen Compounds								
1-Bromooctane	4.49 (2.09 - 9.64)	8.00 (4.93 - 13.0)	–	–	–	–	5	1
2-Chlorobutane	20.0 (12.8 - 31.3)	20	5 m. §	8,000	4	3/6	1	2
Chloromethyl ether	0.21 (0.12 - 0.37)	0.28 (0.13 - 0.62)	–	8†	4	1/6	6	10
Dibromobicycloheptane, mixed isomers	0.21 (0.15 - 0.30)*	0.25 (0.19 - 0.34)	1 hr.	–	–	–	6	–
Dichlorobenzyl alcohol, mixed isomers	0.81 (0.50 - 1.31)	0.40 (0.24 - 0.65)	8 hr.	–	–	–	5	9
1,2-Dichloropropane (propylene dichloride)	1.9 (1.7 - 2.1)	8.75 (8.31 - 9.20)	10 m.	2,000	8	3/6	1	2
trans-2,3-Dichloro-1,4-dioxane	1.41	0.44 (0.27 - 0.71)	4 hr.	–	–	–	5	7
1,2-Dichloroethane (ethylene dichloride)	0.77 (0.67 - 0.89)*	3.89 (3.40 - 4.46)	–	–	–	–	2	3
Di(2-chloroethyl)acetal	0.31 (0.24 - 0.40)*	0.20 (0.14 - 0.30)	1 hr.	–	–	–	3	2
Fumaroyl chloride	0.81 (0.51 - 1.30)	1.41	–	500	4	3/6	6	10
1,1,1,3,3,3-Hexachloro-2,2-difluoropropane	0.54 (0.30 - 1.01)	4.53 (2.80 - 7.32)	–	–	–	–	3	2
[illegible]	2.46 (1.79 - 3.39)	0.79 (0.49 - 1.30)	[illegible]				[illegible]	[illegible]

(Continued on Next Page)

TABLE I
Range-Finding Toxicity Data (Continued)

Material Studied	Single Oral LD_{50} for Rats ml/Kg	Single Skin Penetration LD_{50} for Rabbits ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
Phenyl chloroformate	1.41 (0.83 - 2.40)	3.97 (2.93 - 5.37)	15 m.	44	4	3/6	6	10
Propylene chlorohydrin	0.22 (0.19 - 0.27)	0.48 (0.34 - 0.67)	15 m.	500	4	1/6	1	8
Tetrachloroethane	0.20 (0.16 - 0.27)	3.99 (3.10 - 5.13)		1,000	4	3/6	6	
1,1,2,3-Tetrachloropropene	0.35	* 0.40 (0.27 - 0.59)	30 m.	250	4	5/6	5	9
1,1,2-Trichloroethane	0.58 (0.47 - 0.71)	3.73 (3.30 - 4.21)		500	8	4/6	2	2
Trichloroethylene	4.92 (3.75 - 6.46)	>20		8,000	4	4/6	5	4
Phosphorous Compounds								
Bis 2-chloroethyl phosphite	0.26 (0.19 - 0.35)*	0.141 (0.065 - 0.308)	8 hr.				2	3
Diethyl phosphite	5.19 (2.81 - 9.58)	2.02 (1.24 - 3.29)	4 hr.				1	5
Trimethyl phosphate	2.83 (1.75 - 4.57)	2.87	8 hr.				4	2
Tris dipropylene glycol phosphite	19.7 (15.0 - 25.8)	>10	8 hr.				2	1
Tris dipropylene glycol phosphonate	29.9 (21.4 - 41.6)	15.9 (11.7 - 21.5)	8 hr.				3	1
Sulphur Compounds								
Bis 2-acetoxyethyl sulfone	14.1	11.3	8 hr.				4	2
Bis 1,2-dichloroethyl sulfone	0.25 (0.19 - 0.32)*	1.00 (0.49 - 2.51)	8 hr.				7	9
2,3-Epithiopropyl methoxy ether	2.14 (1.54 - 2.99)	>5	1 hr.				3	2
n-Hexyl vinyl sulfone	0.55	0.84 (0.50 - 1.40)					7	5
Isopropyl sulfate	1.06 (0.61 - 1.87)	1.41 (0.88 - 2.29)	1 hr.				6	5
2-Methoxyethyl ethanol ethanol	13.00 (7.79 - 21.93)	5.04 (3.09 - 8.23)	8 hr.				2	5
Methylvinyloxyethyl sulfide	3.25 (2.16 - 4.26)	>8		3,200	6	4/6	2	2
Tetrahydrothiophene-1,1-dioxide = sulfolane	1.54 (1.25 - 1.91)	3.18 (2.35 - 4.29)	8 hr.				2	4
1,2-Dichloroethanol diacetate	9.25 (7.51 - 12.9)	>16	8 hr.				3	1
Trichloromethyl allyl perthioxanthate	2.13 (1.39 - 3.42)*	0.6					6	1
Trichloromethyl methyl perthioxanthate	1.54 (0.89 - 2.68)*	1.3					6	2
Silicon Compounds								
Amyl trimethoxy silane	4.92 (3.75 - 6.46)	10.00 (4.56 - 21.9)	4 hr.				4	1
2,3,4-Epoxycyclohexyl ethyl trimethoxy silane	12.3 (9.98 - 15.3)	6.30 (4.66 - 8.52)	8 hr.				3	1
3-(2,3-Epoxypropoxy) propyl trimethoxy silane	22.6	3.97 (2.93 - 5.37)	8 hr.				3	2
2-Ethylbutyl silicate	19.7 (15.0 - 25.8)	>10	8 hr.				3	1
2-Mercaptoethyl trimethoxy silane	2.46 (1.88 - 3.23)	—	2 hr.				3	2
3-Mercaptopropyl trimethoxy silane	2.83 (1.61 - 4.98)	5.66					3	2
Methyl trimethoxy silane	12.5 (9.98 - 15.3)	>10	1 hr.				3	2
Trimethoxy silane	9.33 (6.69 - 13.0)	6.30 (4.66 - 8.52)	15 m.	62.5	4	5/6	3	5
Vinyl trimethoxy silane	11.3	3.54 (2.03 - 6.16)	2 hr.	4,000	4	1/6	2	1

* as gm/kg in a suitable vehicle

§ inhalation time shown killed all six rats

† twice concentration shown killed all six rats

‡ caused death when introduced into the rabbit eye

TABLE II

Acute Oral Toxicity of Some Inorganic Compounds for Rats

Material*		Formula	Single Oral LD_{50} for Rats gm/Kg	Concentration Intubated gm/ml**	
Aluminum isopropylate	T	$Al[OCH(CH_3)CH_3]_3$	11.3 (7.0 -18.3)	0.200	A
Aluminum nitrate		$Al(NO_3)_3 \cdot 9H_2O$	4.28 (3.86 - 4.76)	0.100	
Ammonium metavanadate	T	NH_4VO_3	0.16 (0.12 - 0.21)	0.0025	B
Ammonium paratungstate	T	$(NH_4)_6W_7O_{24} \cdot 6H_2O$	11.3	0.400	A
Ammonium persulfate		$(NH_4)_2S_2O_8$	0.82 (0.53 - 1.26)	0.200	
Borax		$Na_2B_4O_7 \cdot 10H_2O$	5.66	0.010	
Boric acid		H_3BO_3	5.14 (4.75 - 5.58)	0.200	
Calcium acetate		$Ca(OOCCH_3)_2 \cdot H_2O$	4.28 (3.86 - 4.76)	0.100	
Calcium acrylate	T	$Ca(OOCCH=CH_2)_2 \cdot H_2O$	4.92 (3.75 - 6.46)	0.200	A
Calcium cyanide		$Ca(CN)_2$	0.039(0.030- 0.051)	0.001	
Calcium hydroxide		$Ca(OH)_2$	7.34 (4.83 -11.14)	0.100	
Calcium propionate	T	$Ca(OOCCH_2CH_3)_2 \cdot H_2O$	5.16 (4.17 - 6.38)	0.100	
Chromium acetate	I	$Cr(OOCCH_3)_3 \cdot H_2O$	11.26	0.200	
Chromium chloride (ions	I	$CrCl_3$	1.87 (1.21 - 2.87)	0.050	
Chromium nitrate (ic		$Cr(NO_3)_3 \cdot 9H_2O$	3.25 (2.11 - 4.99)	0.200	
Cobalt oxide	I	CoO	1.70 (1.07 - 2.82)	0.100	A
Copper acetate		$Cu(OOCCH_3)_2 \cdot H_2O$	0.71 (0.60 - 0.83)	0.010	
Copper nitrate		$Cu(NO_3)_2 \cdot 3H_2O$	0.94 (0.61 - 1.43)	0.200	
Copper sulfate		$CuSO_4 \cdot 5H_2O$	0.96 (0.71 - 1.30)	0.050	
Cuprous oxide	T	Cu_2O	0.47 (0.34 - 0.66)	0.100	A
Diammonium decaborane	I	$(NH_4)_2B_{10}H_{10}$	3.56 (1.85 - 6.86)	0.050	
Dicumene chromium	I	$(C_6H_5)_2Cr$	0.81 (0.62 - 1.07)	0.100	M
Ferric nitrate		$Fe(NO_3)_3 \cdot 9H_2O$	3.25 (2.56 - 4.26)	0.200	
Ferrous ammonium sulfate		$(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$	3.25 (2.48 - 4.26)	0.300	
Lithium carbonate		Li_2CO_3	0.74	0.200	
Magnesium chloride		$MgCl_2 \cdot 6H_2O$	8.1 (7.3 - 9.1)	0.100	
Manganous acetate		$Mn(OOCCH_3)_2 \cdot 4H_2O$	3.73 (2.68 - 5.21)	0.200	
Nickel nitrate		$Ni(NO_3)_2 \cdot 6H_2O$	1.62 (1.06 - 2.50)	0.200	
Potassium acetate		$KOOCCH_3$	3.25 (2.48 - 4.26)	0.100	
Potassium carbonate		K_2CO_3	1.87 (1.34 - 2.60)	0.200	
Potassium hydroxide		KOH	1.3 (0.80 - 1.89)	0.100	
Potassium permanganate		$KMnO_4$	1.09 (0.70 - 1.71)	0.020	
Silver oxide	T	Ag_2O	2.82	0.200	A
Sodium arsenate		Na_3AsO_4	0.041 (0.031- 0.053)	0.010	
Sodium cyanide		$NaCN$	0.015 (0.011- 0.021)	0.0004	
Sodium fluoride		NaF	0.18 (0.12 - 0.26)	0.005	
Sodium nitrite		$NaNO_2$	0.18	0.010	
Sodium pyrophosphate (dibasic)		$Na_2HPO_4 \cdot 12H_2O$	12.93 (9.85 -16.97)	0.200	
Sodium silicate (metasilicate)		$Na_2SiO_3 \cdot 5H_2O$	4.6 (6.81 - 8.03)	0.200	
Sodium tripolyphosphate	I	$Na_5P_3O_{10}$	6.50 (4.03 -10.48)	0.200	
Stearic acid		$C_{18}H_{36}O_2$	2.14 (1.54 - 2.99)	0.250	
Vanadium (III) chloride	I	VCl_3	0.54 (.38 - 0.75)	0.010	A
Vanadium (III) oxide	I	V_2O_3	0.14 (.09 - 0.22)	0.050	M
Vanadium (V) oxide	I	V_2O_5	[illegible]	0.100	M
Vanadyl sulfate	I	$VOSO_4$	[illegible] (.2 - 0.57)	0.100	
Zinc acetate		$Zn(OOCCH_3)_2 \cdot 2H_2O$	[illegible] (1.60 - 3.78)	0.200	
Zinc sulphide	I	[illegible]	[illegible] (3.75 - 9.46)	0.200	L

I = Pro[illegible] ... [illegible] grade

T = [illegible]

M = [illegible]

L = [illegible]

A = [illegible]

B = [illegible]

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Comparative Absorption of Calcium from Calcium Gluconate and Calcium Lactate in Man [1]

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ABSTRACT Calcium balance and radiocalcium (^{47}Ca) studies were performed under controlled metabolic conditions in 8 patients during the intake of calcium gluconate and of calcium lactate in order to determine the absorption of calcium from these 2 salts. The calcium balances were somewhat more positive in all but one of the patients during the intake of calcium lactate than that of calcium gluconate, the average improvement in calcium balance being statistically significant at a level of $P < 0.05$. This improvement was due mainly to a slight but consistent decrease in fecal calcium excretions. The average net absorption was increased by 96 mg in the lactate study, significant at a level of $P < 0.001$. ^{47}Ca studies also indicated greater absorption of calcium from calcium lactate than from calcium gluconate, as evidenced by the increase in the average ^{47}Ca plasma level and the decrease in average fecal ^{47}Ca excretions during the intake of calcium lactate. The increased absorption of calcium on calcium lactate intake was also observed at different intake levels of protein and calcium in a patient receiving long-term corticosteroid therapy.

The availability of calcium from different sources has been the subject of investigation for many years (1–3). The utilization of different calcium salts has been reported to be similar (2, 3) as well as dissimilar (4, 5) in experimental animals. In studies performed in man the absorption of calcium from different calcium salts has been reported to be similar (6,7).

Supplemental calcium is indicated in certain conditions of calcium deficiency and this type of treatment has been suggested for patients with osteoporosis (8–10). The present studies were performed to investigate the intestinal absorption of calcium from 2 calcium salts, calcium gluconate and calcium lactate, under controlled dietary conditions in man, using tracer techniques and metabolic balances.

EXPERIMENTAL

The comparative absorption of calcium, administered as the gluconate and as the lactate was studied under controlled dietary conditions in 8 male patients on the Metabolic Research Ward. The age and diagnoses of the patients and the type and duration of the studies are listed in table 1. All patients were fully ambulatory and in good physical condition despite the diagnoses indicating minor chronic abnormalities; the renal and intestinal function and the clinical laboratory tests of all patients were normal. All patients received the same constant, analyzed low calcium diet, which contained an average of 200 mg calcium and 820 mg phosphorus/day. The composition of the basal diet is listed in table 2. The patients received this diet from 30 to 60 days before the studies were performed. The energy intake was not the same for all patients and adjustments in calories and in nitrogen intake were made according to the ideal weight, the previous dietary habits, the activity and the age of the patients. Although the same food lots were used, there was some variability in nitrogen content of the 6-day food aliquots, especially due to the differences in nitrogen content of the meats. Supplements of calcium in equivalent amounts were added to this diet as the gluconate or as the lactate, all other constituents of the diet remaining unchanged. During supplementation with calcium gluconate, the calcium intake averaged 1,657 mg/day; during supplementation with calcium lactate, 1,672 mg/day. The average intake of phosphorus in the 2 study phases was 865 mg and 870 mg/day, respectively.

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TABLE 1

List of patients studied

Patient	Age	Diagnosis	Study	
			Type	Days
1	61	Pulmonary calcifications	calcium gluconate	66
			calcium lactate	30
2	51	Osteoporosis, mild	calcium gluconate	36
			calcium lactate	38
3	51	Osteoarthritis	calcium gluconate	40
			calcium lactate	14
4	40	Peripheral neuropathy	calcium gluconate	50
			calcium lactate	24
5	43	Peripheral neuropathy	calcium gluconate	24
			calcium lactate	32
6	47	Osteoporosis	calcium gluconate	48
			calcium lactate	38
7	42	Osteoporosis	calcium gluconate	48
			calcium lactate	18
8	48	Cortisone osteoporosis	calcium gluconate [1]	30
			calcium lactate [1]	30
			calcium gluconate [1]	30
			calcium gluconate [2]	42
			calcium lactate [2]	32
			calcium gluconate [3]	54
			calcium lactate [3]	24

[1] During high protein-high calcium intake.
[2] During normal protein-high calcium intake.
[3] During high protein-medium calcium intake.

TABLE 2

Composition of metabolic low calcium diet (2223 kcal) (basal diet)

	Protein	Carbohydrate	Fat
	g/day	*g/day*	*g/day*
Fruit juice, 200 ml		24	
Rice,[1] 30 g		24	
Bread, 150 g	22	78	
Spaghetti,[1] 20 g		15	
Jelly, 120 g		78	
Potatoes,[2] 100 g	5	19	
Canned vegetables, 175 g		15	
Sweetened canned fruit, 225 g		44	
Sugar, 28 g		28	
Meat (beef and turkey), 200 g	44		28
Butterfat, 50 g			41
Cream, 20 g			2
Total	71	325	71

[1] Weight of raw food.
[2] Weight of cooked food.

The average duration of the calcium gluconate studies was 45 days and of the calcium lactate studies, 28 days. The patients did not receive any medications, except for patient 8, who received 20 mg prednisone/day throughout all study phases. In this latter patient, the comparative effect of calcium gluconate and calcium lactate was studied during 2 intake levels of both calcium and protein.

The calcium lactate studies followed the calcium gluconate studies immediately in 4 patients (patients 4, 5, 6, and 8) and were performed from 2 weeks to 2 months

after the calcium gluconate study in 3 patients (patients 1–3). In patient 7, the time interval between the calcium gluconate and calcium lactate study was 7 months. The studies were performed from October to April in 5 patients (patients 1, 3–6), from January to April in patient 2 and continuously from February to July in patient 8. In patient 7, the calcium gluconate studies were performed in November and December and the calcium lactate studies in June and July.

Complete collections of urine and stool were obtained throughout the studies. Demineralized water containing 4 to 5 mg calcium/liter, was used for drinking and food preparation. The body weight, the fluid intake, the urine output and the urinary excretions of creatinine, calcium and phosphorus were determined daily. Metabolic balances of calcium, phosphorus and nitrogen were determined on aliquots of 6-day pools of urine and stool and on aliquots of the diet in each 6-day metabolic period.

Radioisotope studies were carried out during the intake of both calcium gluconate and calcium lactate. A single tracer dose of ^{47}Ca as the chloride (30 to 50 μCi) was administered orally with breakfast in each study. The plasma levels of ^{47}Ca were determined at 1, 4, 8 and 24 hours after the oral administration of ^{47}Ca, daily in the first week of the study and at less frequent intervals thereafter. The urinary ^{47}Ca excretions were determined daily on aliquots of each 24-hour urine collection and the fecal ^{47}Ca excretions on each stool specimen. The studies averaged 24 days during the intake of calcium gluconate and 20 days during the intake of calcium lactate.

The radioassays of ^{47}Ca in plasma, urine and stool were performed in a well-type NaI crystal γ-scintillation counter equipped with a single channel pulse height analyzer. Standards equivalent to the administered dose were always counted along with the samples of plasma, urine and stool. The ^{47}Ca level in plasma was expressed as percentage of the administered dose per liter, the urinary ^{47}Ca excretion as percentage of dose per total urine volume per day and the fecal ^{47}Ca as percentage of dose per stool specimen.

The net absorption of ^{47}Ca, as percentage of the administered dose, was determined by subtracting the fecal ^{47}Ca excretion (in per cent) from 100. The true absorption was calculated by correcting for endogenous fecal ^{47}Ca excretion, determined from excretion values following the intravenous injection of ^{47}Ca to the same patient. For patients who did not receive ^{47}Ca intravenously, the endogenous fecal ^{47}Ca excretion was estimated to be 15% of the absorbed dose. This estimate is usually very close to the true value, and the error introduced is small (11).

Urinary calcium was determined by a modification of the method of Shohl and Pedley (12), serum calcium by a modification of the Kramer-Tisdall method (13), nitrogen by the Kjeldahl method (14) and phosphorus by the method of Fiske and Subbarow (15). Stool calcium and phosphorus were determined on dry-ashed aliquots of 6-day stool pools which were dissolved in hydrochloric acid.

RESULTS

The results obtained in the calcium gluconate and calcium lactate studies are shown in table 3. The slight variations in nitrogen and calcium intake from patient to patient and also in the same patient in the 2 study phases are due to differences in food intake, to inherent differences in the composition of the different food lots and to rejection of small amounts of food. The lower protein and calcium intake of patient 5 was due to caloric adjustments which had to be made in accordance with the previous dietary habits of this patient. In patients 1 to 5 the average urinary calcium excretion was similar during the intake of calcium gluconate and of lactate; in patients 6 and 7, the calciuria was approximately 50 mg and 100 mg higher per day during the intake of calcium lactate, respectively. The fecal calcium excretion decreased in 6 of the 7 patients (patients 1, 3–7), during the intake of calcium lactate but was little changed in patient 2. The calcium intake of patients 2 and 4 was slightly greater during the intake of calcium lactate than during the intake of calcium gluconate. The calcium balances became more positive in patients 1 to 7 during the intake of calcium lactate, the

TABLE 3

Calcium balances of patients receiving calcium gluconate and calcium lactate supplements

Patient	Study days	Study	Calcium					Phosphorus		Nitrogen	
			Intake	Urine	Stool	Balance	Change in balance	Intake	Balance	Intake	Balance
			mg/day	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*
1	66	Calcium gluconate	1894	163	1727	+ 4		874	+ 82	14,893	+ 1750
	30	Calcium lactate	1864	130	1558	+ 126	+ 122	938	+ 93	14,876	+ 1622
2	36	Calcium gluconate	2022	45	1757	+ 220		924	+ 225	15,552	+ 4025
	38	Calcium lactate	2107	43	1792	+ 272	+ 52	892	+ 161	15,035	+ 4300
3	40	Calcium gluconate	1813	64	1568	+ 181		882	+ 183	14,838	+ 3052
	14	Calcium lactate	1900	67	1455	+ 278	+ 97	816	+ 156	15,826	+ 2923
4	50	Calcium gluconate	1065	76	892	+ 97		835	+ 198	14,852	+ 3796
	24	Calcium lactate	1113	69	863	+ 181	+ 84	859	+ 169	14,468	+ 3388
5[1]	24	Calcium gluconate	853	111	808	+ 66		802	+ 64	11,346	+ 1494
	32	Calcium lactate	796	108	598	+ 90	+ 156	794	+ 132	12,761	+ 1967
6	48	Calcium gluconate	2391	321	1650	+ 230		929	+ 83	15,177	+ 2922
	30	Calcium lactate	2260	376	1575	+ 309	+ 79	949	+ 153	15,396	+ 2649
7	48	Calcium gluconate	1732	140	1500	+ 112		780	+ 93	13,421	+ 1762
	18	Calcium lactate	1766	246	1351	+ 169	+ 57	837	+ 131	13,326	+ 1275

[1] The lower nitrogen and calcium intake of patient 5 was due to adjustments in food intake (see text).

improvement in balance ranging from 52 to 156 mg/day. The average improvement in calcium balance for all patients including patient 8 (table 4), during the intake of calcium lactate as compared with calcium gluconate, was 71.6 mg/day, which is significant at a level of $P < 0.05$. The net absorption of calcium which is the difference between intake and fecal excretion was greater in the calcium lactate phase by 96 mg/day with a significance of $P < 0.001$. The changes in the phosphorus balances were consistent with the changes in calcium and nitrogen balances in most patients. The nitrogen balances of all patients were positive and showed fluctuations during the two study phases.

Although the average urinary calcium excretion did not differ markedly in the calcium gluconate and calcium lactate studies, in some of the patients the urinary calcium excretion increased distinctly during the initial phase of calcium lactate intake. Figure 1 shows that the urinary calcium excretion of patient 6 was about 300 mg/day in a 36-day study, during the intake of calcium gluconate. When calcium lactate was substituted in equivalent amounts for calcium gluconate, the uri-

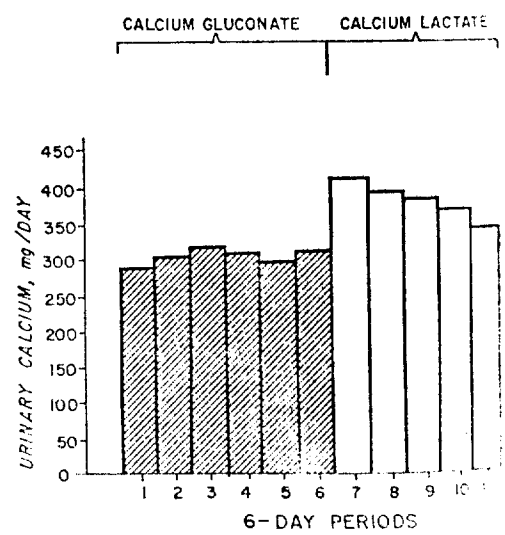

Fig. 1 Urinary calcium excretion during intake of calcium gluconate and calcium lactate. Each bar represents the average urinary calcium excretion per day for each 6-day metabolic period. Equivalent amounts of calcium were given as calcium gluconate and as calcium lactate to patient 6.

TABLE 4

Calcium, phosphorus and nitrogen balances of patient 8 [1] (long-term corticosteroid therapy)

Calcium intake	Protein intake	Study days	Study	Calcium					Phosphorus balance	Nitrogen	
				Intake	Urine	Stool	Balance	Change in balance		Intake	Balance
				mg/day	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*
High	high	30	calcium gluconate	1901	551	1533	−183		+ 6	18,044	+1829
		30	calcium lactate	1923	582	1428	+ 87	+ 96	−49	19,001	+1391
		30	calcium gluconate	1990	541	1593	−144		−45	18,919	+2051
High	intermediate	42	calcium gluconate	1979	547	1484	− 52		−67	11,141	+ 86
		32	calcium lactate	1978	560	1364	+ 54	+106	−29	10,594	− 71
Intermediate	high	54	calcium gluconate	922	411	682	−171		+53	19,631	+2529
		24	calcium lactate	960	384	640	− 64	+107	−29	19,303	+1595

[1] The patient received 20 mg of prednisone/day in all study phases.

nary calcium excretion increased promptly by an average of about 100 mg/day in the first 6 days of calcium lactate administration and decreased gradually in the subsequent 6-day periods. However, the urinary calcium excretion was still higher after 30 days of calcium lactate intake than during the intake of calcium gluconate. A prompt increase in calciuria was also observed during the intake of calcium lactate in patient 7 (fig. 2). The urinary calcium excretion of this patient ranged from 173 mg/day to 132 mg/day in the six 6-day periods of calcium gluconate intake. When an equivalent amount of calcium was administered as the lactate 7 months later, the urinary calcium excretion was considerably higher. The average urinary calcium excretion per 6-day period ranged from 260 mg/day to 236 mg/day. When calcium lactate was then replaced by equivalent amounts of calcium as the gluconate, the daily urinary calcium excretion decreased promptly (fig. 2).

The mineral and protein balances of patient 8 are listed in table 4. This patient had received corticosteroid therapy for 8 years and continued to receive 20 mg prednisone/day during the present study. Two intake levels of protein and of calcium were used. During the high protein-high calcium intake, the urinary calcium excretion was high, approximately 500 mg/day, with an intake of either calcium gluconate or calcium lactate. The average calciuria was, however, slightly higher (by an average of 31 mg/day) in the calcium lactate study and decreased promptly by an average of 41 mg/day when calcium lactate was again replaced by calcium gluconate. The average fecal calcium excretion was lower by approximately 100 mg/day during the intake of calcium lactate than during the intake of calcium gluconate and the calcium balance improved by approximately 100 mg/day in the calcium lactate study. On re-administration of calcium gluconate, the fecal calcium increased to about the same level as in the first calcium gluconate study, and the calcium balance became somewhat more negative. During the intake of the intermediate amount of protein, the calcium intake remaining unchanged, similar observations were made: the fecal calcium de-

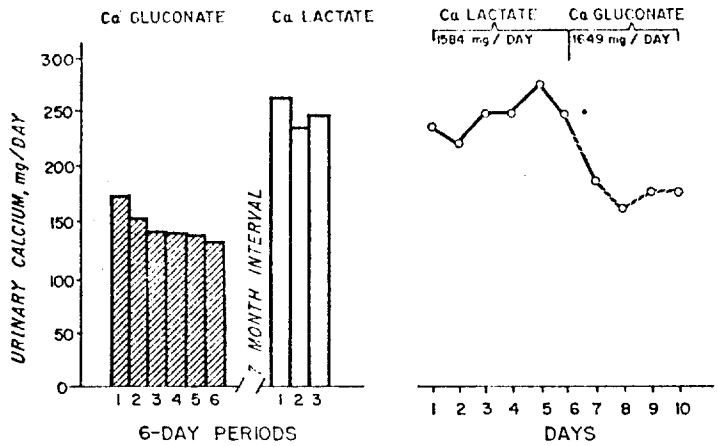

Fig. 2 Urinary calcium excretion during intake of calcium gluconate and calcium lactate. Each bar represents the average urinary calcium excretion per day for each 6-day metabolic period. The daily urinary calcium excretions during the intake of calcium lactate and calcium gluconate are those of the second study phase. Patient 7.

creased by 120 mg/day during the administration of calcium lactate and the urinary calcium excretion increased very slightly, resulting in an improvement of the calcium balance by 106 mg/day. When the calcium intake was decreased from an average of 1979 mg to 922 mg/day, the protein intake remaining high, the urinary calcium excretion was lower than during high calcium intake and averaged 411 mg/day in the calcium gluconate phase. When calcium gluconate was replaced by calcium lactate during this high protein-intermediate calcium intake, both the urinary and the fecal calcium excretions decreased slightly and the average calcium balance improved by 107 mg/day. The phosphorus balances of this patient varied with the changes in calcium and nitrogen balances, similar to the changes observed in the remaining patients. The nitrogen balances were positive during high protein intake; they became markedly less positive and were even slightly negative when the nitrogen intake was decreased to approximately 11,000 mg/day.

Table 5 lists the cumulative fecal ^{47}Ca excretions in the calcium gluconate and calcium lactate studies. The fecal ^{47}Ca excretions were consistently lower during the intake of calcium lactate than during the intake of calcium gluconate, although the differences were not great in most patients. The average difference of 7.9 was significant at a level of $P < 0.05$.

Figure 3 shows that the plasma levels of ^{47}Ca of patient 1 were higher after the oral administration of this tracer during the intake of calcium lactate than during the intake of calcium gluconate. Similar results were obtained in five other patients (patients 1, 2, 4, 6 and 7) while they remained in the same range in both studies in 2 patients (patients 5 and 8), and were slightly lower in the calcium lactate study in patient 3 (table 6). The

TABLE 5

Fecal ^{47}Ca excretion in patients receiving calcium gluconate and calcium lactate supplements

Patient	Cumulative ^{47}Ca excretion [1,2]		Difference [3]
	Calcium gluconate	Calcium lactate	
	% of dose	% of dose	
1	78.9	68.4	10.5
2	78.6	73.7	4.9
3	78.5	71.9	6.6
4	72.3	70.6	1.7
5	63.0	60.5	2.5
6	65.5	61.4	4.1
7	85.9	61.4	24.5
8	64.3 [4]	56.1 [4]	8.2

[1] A single tracer dose of ^{47}Ca was given on the first day of the study.
[2] The excretions represent the cumulative fecal excretions up to the time when less than 1% of dose was excreted daily in stool.
[3] Difference between fecal ^{47}Ca excretion during intake of calcium gluconate and calcium lactate.
[4] With high protein diet. This patient received 10 mg prednisone per day in both study phases.

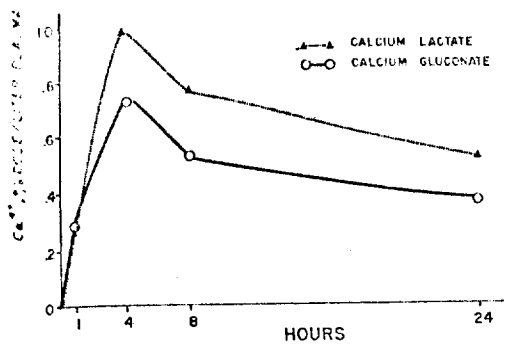

Fig. 3 Plasma levels during intake of calcium gluconate and calcium lactate (oral ^{47}Ca). A single tracer dose of $^{47}CaCl_2$ was given orally during the intake of calcium gluconate and of calcium lactate.

^{47}Ca plasma levels at 4, 8 and 24 hours, during the intake of calcium lactate, were compared with those of the calcium gluconate study and a ratio was obtained which indicated the relative absorption during the intake of calcium lactate and calcium gluconate. The average value of this ratio for the 8 patients was 1.19, significantly higher than 1.00, at a level of $P < 0.05$, indicating that the absorption of ^{47}Ca was higher during the ingestion of calcium lactate than of calcium gluconate.

DISCUSSION

Several reports in the literature have indicated that calcium lactate is a poor source of calcium (2). Most studies, however, were performed in young animals or in young adults in whom the difference in utilization of calcium from various calcium salts may not be appreciable.

In experiments continued over 3 generations in rats, the addition of calcium lactate and of disodium phosphate to a low calcium-low phosphorus diet was reported to be as effective as milk in promoting growth in the first generation of rats but was less effective in maintaining this growth rate in succeeding generations (4). Calcium acetate, chloride, levulinate and gluconate have been used in calcium absorption studies with isolated loops of the upper part of the small intestine of rats (16). The absorption of calcium from the isolated loops was found to be higher from

TABLE 6

^{47}Ca Plasma levels during the intake of calcium gluconate and calcium lactate (oral administration of ^{47}Ca)

Patient	Study	^{47}Ca plasma levels				^{47}Ca plasma ratio: [2]
		Hours [1]				Ca lactate / Ca gluconate
		1	4	8	24	
		% of dose/liter of plasma				
1	Ca gluconate	0.28	0.73	0.53	0.36	1.40
	Ca lactate	0.27	0.98	0.76	0.52	
2	Ca gluconate	0.54	0.54	0.48	0.26	1.38
	Ca lactate	0.51	0.71	0.63	0.43	
3	Ca gluconate	0.62	1.87	1.53	1.02	0.88
	Ca lactate	0.45	1.60	1.30	1.01	
4	Ca gluconate	0.38	0.78	0.64	0.39	1.20
	Ca lactate	0.42	0.84	0.74	0.60	
5	Ca gluconate	0.58	1.01	0.78	0.55	0.99
	Ca lactate	0.39	0.93	0.83	0.56	
6	Ca gluconate	0.65	0.96	0.78	0.50	1.24
	Ca lactate	0.52	1.22	0.96	0.59	
7	Ca gluconate	0.21	1.02	0.78	0.51	1.41
	Ca lactate	0.38	1.41	1.08	0.76	
8	Ca gluconate	0.82	1.50	1.08	0.74	1.02
	Ca lactate	0.59	1.48	1.13	0.76	

[1] Hours after the oral administration of ^{47}Ca.
[2] Values are averages. Ratios were determined by relating the sum of ^{47}Ca plasma levels at 4, 8 and 24 hours in the 2 study phases.

calcium gluconate than from other salts. However, no differences in absorption of calcium from the various salts were observed in intact rats (16). In another study, calcium from calcium lactate was found to be absorbed to a lesser extent than from calcium chloride when introduced into Thiry-Vella fistulas in dogs (5). More recently, studies of the absorption of calcium in dogs with Thiry-Vella fistulas have been reported (17). In a limited series of experiments the absorption of calcium from the chloride, lactate and gluconate in distilled water or in a hypotonic solution has been found to be similar.[2]

In studies performed in man, Patton and Sutton (6) found no significant difference in the utilization of calcium from the lactate, gluconate, sulfate or carbonate in young college women. However, the order in which the calcium salts were administered was found to be a significant factor and the salt taken first was utilized to a greater extent, regardless of which salt was used. In a subsequent study in college women Patton (7) found that calcium in milk, calcium carbonate, and calcium gluconate were utilized to the same extent. In a study in children comparisons were made on the effect of calcium lactate with that of milk, and the criteria used were those of height and weight increments (18). Since milk supplies many nutrients, in addition to calcium, that favorably influence growth, the effect of a calcium salt alone cannot be used for comparison.

The results obtained in the present investigation indicate that calcium is absorbed better from calcium lactate than from calcium gluconate in man as judged by three separate sets of analytical data, i.e., the changes in stool calcium analyses and calcium balances, in fecal ^{47}Ca excretions and in ^{47}Ca plasma levels. The differences in the results obtained with the 2 calcium salts for the group of patients are statistically significant for each of these criteria. It cannot be predicted whether the fecal excretions of calcium and of ^{47}Ca would have increased if calcium lactate had been administered for a prolonged period of time. The increase in calcium balance in patient 2 in the calcium lactate phase cannot be interpreted with certainty because the calcium intake was 85 mg higher in this study than in the calcium gluconate study.

All patients were studied with calcium gluconate before they were studied with calcium lactate. Since the sequence of administration of the calcium salts was reported to influence the absorption of calcium (6), the order of administration of the calcium salts in the present study should have favored the absorption of calcium from the gluconate. The greater absorption of calcium lactate than of calcium gluconate in the present study would, therefore, be even more significant.

The difference in the results reported here and those obtained by other investigators (5, 6) may be due in part to the fact that the amount of calcium added to the diet in the present study was high in comparison with the dietary calcium intake. The amount of calcium contained in the calcium supplements constituted from 87% to 90% of the total calcium intake in this study. In investigations reported in the literature the total calcium intake was often relatively low and the amount of calcium given in the form of calcium salts represented 50% or less of the total calcium intake. The estimation of the effects of the calcium supplements may be difficult under these circumstances. For instance, in the studies of Patton and Sutton (6), the amounts of calcium added as gluconate, lactate and in forms of other salts was low, 400 mg/day, and the diet contained a similar amount of calcium, [illegible] mg calcium/day. In a study of the comparative utilization of calcium in milk and of calcium contained in carrots (19), the dietary calcium averaged 270 mg/day, the additional calcium supplied by 7[illegible] carrots/day was 202 mg. In the present study, the lower absorption of calcium from carrots than from milk may have been due to the low concentration of calcium in a relatively large mass of absorbable vegetable material.

In most of the patients in the present study the urinary calcium excretion was similar during the intake of calcium gluconate and of calcium lactate except patients 6 and 7. The increased cal

[2] Cramer, C. F., personal communication

was probably the result of greater intestinal absorption of calcium from calcium lactate than from calcium gluconate. The increase in urinary calcium excretion in patient 7, during the intake of calcium lactate, must be interpreted with caution, since the calcium lactate study was performed 7 months after the calcium gluconate study and the possibility of a spontaneous increase in urinary calcium excretion must be considered. However, the prompt decrease in urinary calcium excretion upon replacing calcium lactate by calcium gluconate in this patient (fig. 2), indicated that the increase in calciuria most probably resulted from the higher absorption of calcium lactate; the lower excretion of fecal calcium during the intake of calcium lactate was also indicative of greater absorption of calcium.

It is of interest that the results in patient 8 were similar to those of the other patients despite prolonged corticosteroid therapy. Also, the effect of calcium lactate in decreasing fecal calcium excretion was independent of the intake level of protein and of calcium. The slightly higher urinary calcium excretions during supplementation with calcium lactate on intermediate as well as on high protein-high calcium intake, also indicated better absorption of calcium lactate than of calcium gluconate. The decrease in urinary calcium excretion during the intake of the intermediate level of calcium as the lactate in patient 8 may have been a consequence of lowering the intake of calcium.

It has been claimed that calcium gluconate causes diarrhea in man (20). Both calcium gluconate and calcium lactate in the amounts given were well tolerated by patients reported here and neither of the 2 calcium salts caused untoward side reaction.

Gluconate salts have been used as complexing agents, but the binding constant, K, for calcium gluconate, is low, 1.21, and is almost the same as the value of 1.07 given for calcium lactate (21). As complexing agents, neither lactate nor gluconate ions would be expected to bind calcium more strongly than the proteins and carbohydrates of food. The reason for the better absorption of calcium from calcium lactate than from calcium gluconate in man remains conjectural.

ACKNOWLEDGMENT

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Calcium ^{45} in Metabolic Studies

Staub, H. Schweizerische Med. Wochenschrift No. 18, p. 499-506 (1954).

In animal bodies, 99% of the calcium is contained in the skeleton. Depending on the metabolic position, this bone mineral plays not merely the role of a building stone of the supporting material, but represents the depot and supplier in the Ca metabolism of the entire organism. If Ca resorption is inadequate or if substantial amounts of Ca are lost in the urine because of acidosis, the Ca requirements of body fluids and soft parts are supplied by the mineral depositions in the bones; the skeleton is the first to exhibit demineralization. The fact that the C:P ratio in nutrition is decisive with respect to bone metabolism, the Ca balances the Ca needs, is important and has been known for a long time. For mammals, the optimum ratio is approximately 1 to 1.7.

If, as customary, Ca balances are to be established in metabolic experiments, it must be considered that the relatively slight amount of approx. 1.0 g per day in the food of humans compares with approx. 1 kg of available Ca deposits in the bones. The magnitude of elimination in the feces and urine may be affected by storage or release by this reservoir so that the balance between and elimination does not represent an assured relationship of resorption. Although in experimentation with small animals the mineral content of the skeleton can be determined directly by processing the entire animal and thus a better insight into the mineral balance can ve obtained, today, if Ca metabolism investigations are to be conducted in the living organism to determine the progress in time and location of the resorption and elimination of Ca, salts of Ca^{45} are used to advantage. The application of the isotope yielded knowledge also concerning the distribution of Ca introduced in the body and the utilization of Ca preparations under different physiologicak, pathological and therapeutical conditions.

Of the two synthetic isotopes of Ca, Ca^{49} and Ca^{45}, Ca^{49} cannot be used in investigations of the relatively slow Ca metabolism, due to its short half-life of 2.4 hours. Ca^{45}, on the other hand, has a half-life of 152 days. The particles of the soft Ca^{45} radiation can be reliably measured with the new sensitive counters. Ca^{45} is now available without a carrier.

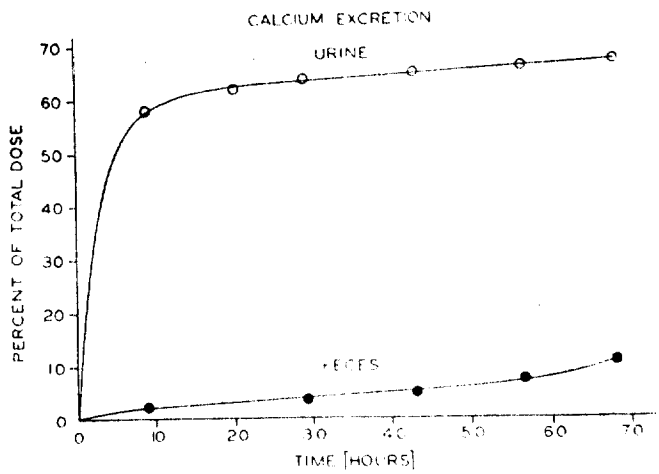
I

In the following, a condensed review of important biological results obtained with Ca^{45} is presented. Such summaries have been given by J. Sacks (Isotopic tracers in biochemistry and Physiology, McGraw-Hill, 1953, pp. 284ff.) or Cremer & Herr (Artificial Radioactive Isotopes in Physiology, Diagnostics and Therapy, Springer Press, 1953, pp. 370ff.)

a) Resorption, Distribution and Elimination in Healthy Organisms

The first experiment with Ca^{45} was performed by Campbell & Greenberg /1/ in 1940. They administered Ca^{45} lactate (35 mg $Ca^{\cdot\cdot}$) to an adult rat with a pharyngeal probe. During 69 hours the activities were determined in the urine and feces (see Fig. 1). The animal was then killed and the amounts of radioactive calcium found was as given in Table 1. The applied activities were recovered quantitatively. 10.8% were found in the feces, 89.2% were reabsorbed; probably somewhat more because a small portion of the reabsorbed amount was secreted during the observation period by the intestines.

FIGURE 1. Ca^{45} Elimination of Rats Following the Administration of Ca^{45} Lactate (Cambell & Greenberg /1/)



In comparision with other human and animal experiments, this resorption is remarkably high. Within the first 9 hours, 57.9 were eliminated in the urine and 65.9% in 69 hours. Principal secretion through the kidneys thus occurs in the first hours after administration with more than 50%. Retention amounted to 23.6%, most of it naturally in the bones, i.e. 19.9%. Highest concentrations were found in bones and teeth (2.23 and 2.75% of the radioactive Ca supplied). Lower activities were found in the intestines, heart, lungs and skin.

TABLE 1. Distribution of Ca^{45} in Rats Following the Administration of Ca^{45} Lactate with a Pharingeal Probe (Campbell & Greenberg /1/

Gewebe (1)	Gewicht in g (2)		Inhalt im Gesamtgewebe (5)	Totaldosis in % (6)	
	frisch (3)	trokken (4)		Inhalt per g Frischgewicht (7)	Inhalt per g Trockengew. (8)
Knochen (9)		8,94	19,9 ± 1,76		2,23 ± 0,20
Zähne (10)		0,410	1,13 ± 0,25		2,75 ± 0,61
Blutserum (11)	5,095	0,358		0,007 ± 0,001	0,094 ± 0,009
Muskeln (12)		45,08	0,76 ± 0,066		0,017 ± 0,001
Haut, Haare (13)	51,19	23,88	1,43 ± 0,15	0,028 ± 0,003	0,060 ± 0,006
Magen (14)	1,86	0,544	0,008 ± 0,004	0,004 ± 0,002	0,015 ± 0,008
Dünndarm (15)	5,87	1,65	0,099 ± 0,008	0,017 ± 0,0014	0,060 ± 0,005
Dickdarm (16)	1,66	0,62	0,037 ± 0,006	0,022 ± 0,004	0,060 ± 0,010
Leber (17)	9,62	3,06	0,049 ± 0,005	0,005 ± 0,0001	0,017 ± 0,002
Niere (18)	2,07	0,53	0,007 ± 0,001	0,003 ± 0,0004	0,013 ± 0,002
Milz (19)	0,79	0,19	0,008 ± 0,003	0,010 ± 0,0037	0,042 ± 0,015
Herz (20)	0,84	0,19	0,017 ± 0,004	0,020 ± 0,0047	0,089 ± 0,021
Lungen (21)	0,45	0,27	0,018 ± 0,006	0,040 ± 0,013	0,067 ± 0,022
Hoden	5,48	2,71	0,031 ± 0,006	0,006 ± 0,0012	0,012 ± 0,002

Key: 1. Tissue; 2. Weight in g; 3. Fresh; 4. Dry; 5. Content in tissue, total; 6. Total dose, %; 7. Content per g of fresh weight; 8. Content per g of dry weight; 9. Bones; 10. Teeth; 11. Blood serum; 12. Muscles; 13. Skin, hair; 14. Stomach; 15. Small intestines; 16. Colon; 17. Liver; 18. Kidney; 19. Spleen; 20. Lungs; 21. Testicles.

With radioactive strontium, again high concentrations were detected in the bones of malignoma patients, while soft parts showed only very slight activities (Lawrence, /2/). It is known of strontium that it interchanges at interfaces in the bones in the manner of calcium, but that its behavior with respect to stay time in the body and to elimination is entirely different (Tweedy & al. /3/ Jones & Copp /4/). This concentration in the bones does not, however, represent new formations of bone salts, but may be explained by interchange between serum Ca and bone Ca; this relatively rapid "turnover" will be discussed later.

Bellin & Laszlo /5/ recently investigated the elimination and distribution in malignoma patients after i.v. injection of 0.75-100 millionC/kg of body weight in the form of $Ca^{45}Cl_2$. The application of such high activities to cancer patients has been authorized by the U.S. AEC. Following the i.v. administration of e.g. 57.6 million C of $Ca^{45}Cl_2$, within 24 hours 7.4% of the dose was eliminated in the urine and in 2 days 0.8% in the feces. In the blood, 5 minutes after i.v. administration only 26% of the dose could be detected and only 4.3% after 24

hours. Over a period of 14 days, 32.7% were evacuated in the urine and 6.8% in the feces. During the 14 days preceding exitus, approx. 35 millionC were retained in the body; the entire activity was confined to the bones and the myeloma tumor of the skeleton. Elimination through the urine and feces was much slower in these human experiments than in the experiments with rats by Campbell & Greenberg (see also subsequent experiments by Geissberger on humans). Parenteral application leads to increased secretion in uring and to more storage.

Particularly interesting is the observation of these authors that upon the i.v. continuous administration of the sodium salt of ethylenediaminotetra-acetic acid (EDTA) whereby 10 times more Ca^{40} and Ca^{45} is secreted through the kidneys. Since the ca^{45}/Ca ratio remains unchanged in EDTA calciuria, it may be assumed that the Ca originates in a homogeneous depot. Further, the rapid and substantial secretion of Ca as an EDTA complex salt leads to the safe conclusion that Ca^{45} has not been incorporated as a bone salt but that it has been deposited in the bones by exchange reaction and was available relatively freely for the complex formation.*

Special investigations with Ca^{45} concerning Ca resorption and secretion in the intestines were especially informative. Ordinary chemical equilibrium studies did not provide accurate information concerning the localization, magnitude and progress in time of Ca in the stomach-intestine tract. Three publications on Ca^{45} in rats yielded accurage information with respect to conditions in animals.

Greenberg & Troescher /6/ found in rats with biliary fistula a total of 4-5% of the Ca^{45} introduced in the bile within 60 hours; i.e. roughly $\frac{1}{2}$ to $\frac{1}{4}$ of total elimination in urine and feces. The greatest amount was secreted during the first two days. Purely chemical experiments ondogs with biliary fistula by Gillert /7/ yielded a Ca secretion in urine: bile of 3:2.

Harrison & Harrison /8/ administered to rats with a pharingeal probe 1 cm^3 of a $Ca^{45}Cl_2$ solution with a total of 10 mg Ca^{++} and less than 1.0 millionC.

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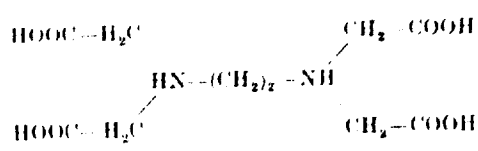
*EDTA is a very strong complex former for alkaline earth ions (Schwarzenbach & Ackermann /5a/).



This amount of Ca corresponds roughly to the normal daily requirements. After 2, 4, 24 and 72 hours, groups of animals were killed and different intestine sections examined for their Ca^{45} content. Ca^{45} was also determined in the prepared skeleton and in urine. It is shown that after 24 and 72 hours the sum of the Ca^{45} in the urine and the skeleton amounted to 94% of the absorbed Ca^{45}. The amount was calculated from the difference of activities supplied and the activity found in the intestine tract and the feces. Resorption is most rapid during the first 4 hours and takes place in the proximal 2/3 of the small intestine and amounts to approx. 30% of the Ca administered. After 4 hours the greater part of the Ca introduced is found in the distal third of the small intestine and in the colon. Within 24 hours, another 30% are absorbed from the distal parts of the intestine. With young, growing rats of 6-8 weeks, 24-hour resorption amounted to 65%, while older animals of 16-20 weeks absorbed only 40%. It may be concluded that skeleton building in young growing rats favors resorption in the intestines, presumably because diffusion gradients exist. In any case, the heretofore unknown fact that Ca is being absorbed in distal sections of intestines, but not by the rectum, must be noted (Geissberger /22/).

The secretion of Ca^{45} in the gastrointestinal tract of young and adult rats was investigated by Wallace & al. /9/. In the intestinal wall and content of different sections Ca^{45} was determined separately. It is shown that as early as 15 minutes after i.m. injection of approx. 17 millionC of Ca^{45} appreciable amounts of the isotope are present in the stomach and intestine walls, less in the intestine content. Feces analyses show that 2 weeks later young rats secreted 15% and adults 41.% in the feces. Within the first 15 minutes and during the first hours activities are distributed uniformly over the turns of the entire intestine, with preferential participation of the small intestine. Secretion is preceded by concentration in the intestine wall. Six or more hours later the highest concentration is found in the colon, as expected, considering the motion of the intestine content. Young rats exhibit lower Ca^{45} in intestine walls and intestine content 6 and more hours later than adults; this may be explained in part by better retention and in part by re-resorption. Reabsorption from the colon must be assumed, because young rats lose more of their intestine contents in 6 to 24 hours than adults (Fig. 5a and b: note the difference in scales of the ordinates in Fig. 5a and b). Apparently this represents active secretion by the mucous membrane of the intestine, because in the case of a low-Ca diet, i.e. with no increase in serum Ca, twice the Ca intake may be eliminated in the feces. Ca secretion in the bile naturally also represents an active secretion process. Table 2 (Wallace & al. /9/) presents the amounts of Ca^{45} secreted after different periods of time in the urine and feces, following i.m. application, as percentages of the applied doses. The amounts and proportions differ from those determined by Bellin & Laszlo, because in the latter case much more has been eliminated in the feces, although it was also applied parenterally.

FIGURE 2. Secretion of Ca^{45} in the Feces and Urine and Serum Values Following the i. v. Application of 57. 6 millionC to Myeloma Patients (Bellin & Laszlo /5/).

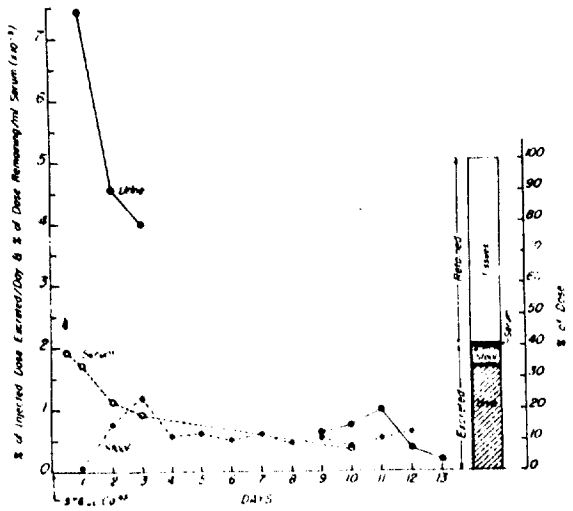


FIGURE 3. Serum Values and Urine Secretion in the First 24 Hours After the i. v. Acministration of 57. 6 MillionC on Adult Humans (Bellin & Laszlo /5/)

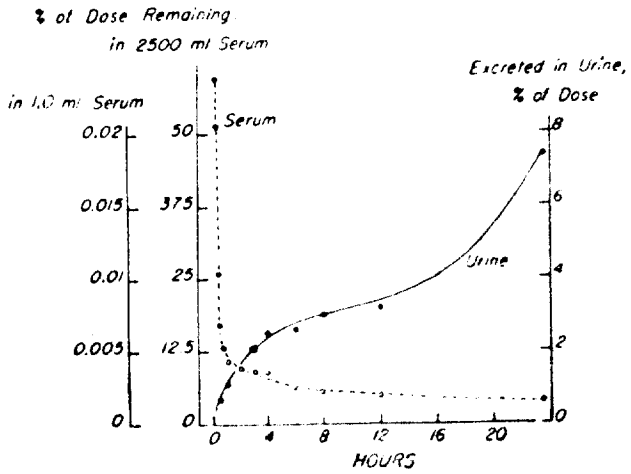


FIGURE 4. Accereration of Urinary Secretion of Ca^{45} During the i. v. Continuous Infusion of 4 g Sodium Ethylenediaminetetra acetate For 3 Days. In addition to the increased Ca^{45} secretion, the constancy of the Ca^{45}:Ca^{40} Ratio is Remarkable (Bellin & Laszlo /5/)

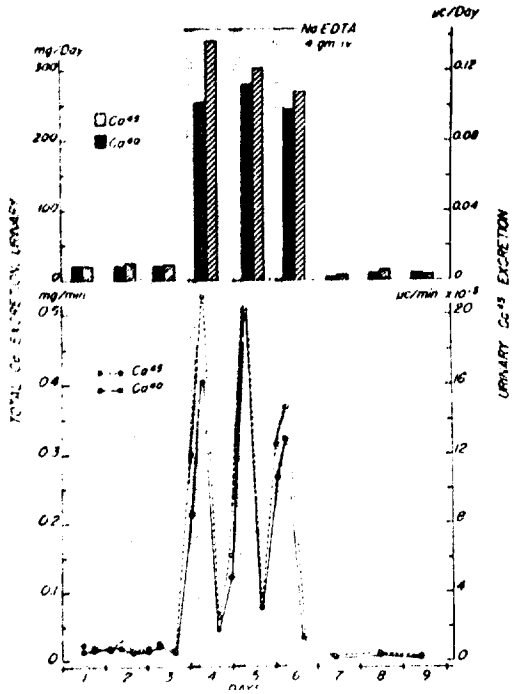


FIGURE 5a. Distribution of Ca^{45} in the Wall of the Stomach-Intestine Tract at Different Times After i. m. Injection of 17 C Ca^{45} Cl_2 in Young and Adult Rats (Wallace & Al. /9/).

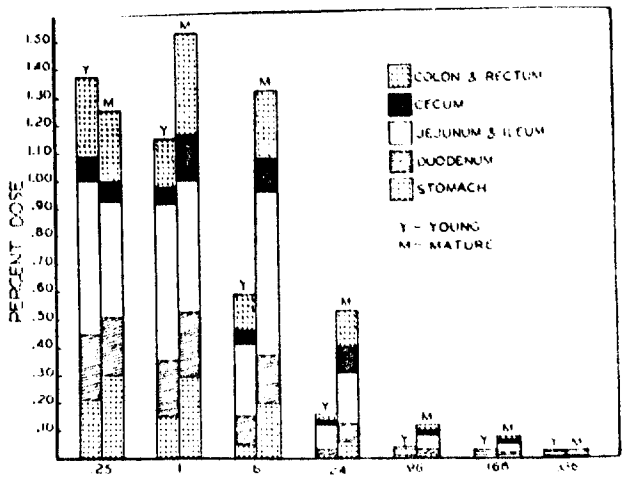


FIGURE 5b. Distribution of Ca^{45} over the Content of Intestines After i.m. Application of $Ca^{45}Cl_2$ to Young and Adult Rats (Wallace & al. /9/)

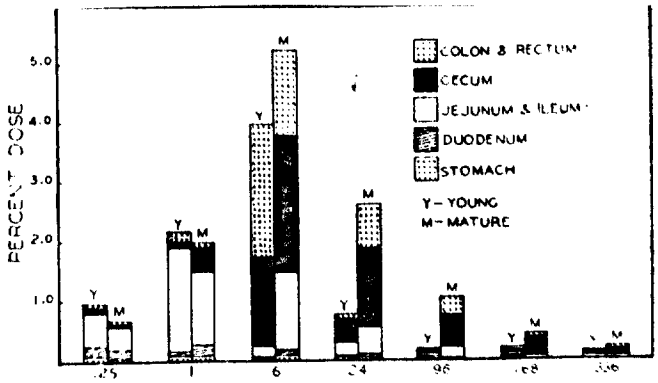


TABLE 2. Ca^{45} Secretion in the Urine and Feces After the i.m. Administration of 17 millionC of $Ca^{45}Cl_2$ to Young and Adult Rats in % of Dose Intorduced (Wallace & al. /9/).

Key: 1. Hours after administration; 2. Young rats; 3. Urine; 4. Feces; 5. Adult rats.

Stunden nach Verabreichung (1)	Junge Ratten (2)		Erwachsene Ratten (5)	
	Urin (3)	Stuhl (4)	Urin	Stuhl
½	0,00	0,00	0,00	0,00
1	0,00	0,01	0,00	0,84
6	1,08	0,49	0,38	0,20
24	0,56	6,60	2,21	10,26
96		9,31	1,35	28,54
168	3,07	12,49	3,59	35,27
336	1,66	15,22	2,10	41,55

CA^{45} passes through theplacenta into the fetus and the milk; the Ca content of milk evidently originates mainly in the food, to a lesser extent in the bone deposits (Pecher /9a/); (Erf & Pecher /9b/).

b) Bone System

While the application of the isotope yields accurate information on intestinal resorption and secretion with respect to time and location and also permits the highly accurate and convenient observation of secretion in feces and urine, isotope technology provides less conclusive results for bone metabolism. As mentioned above, this is related to the face that a continuous exchange relationship exists between the calcium of the bones and of serum. This "turnover" parallels metabolic processes leading to the formation of hydroxyapatite and the formation and decomposition of bone substance. The "turnover" can be simulated in vitro, e.g. with bone powder in a $P^{32}O_4$ - containing solution.

It is generally known that in a system where solid crystals are present in a solution containing an isotope of one of the elements forming the solid phase, the isotope will be absorbed on the crystal, without increasing the mass of the solid phase. In this manner, even from e.g. a dilute $P^{32}O_4$ solution a high % of P^{32} can be absorbed without affecting the phosphate content of the liquid phase analytically. In the process, unmarked P is released by the solid phase so that the P equilibrium is maintained. The velocity of this "turnover" for P^{32} is rapid in the first phase and includes within the first two hours approx. 1/5 of bone salts. A slower second phase, extending over many hours, follows. These exchange reactions have been demonstrated by Hevesy & al. (Nature 136, 754, 1935; Biochem. J. 34, 543, 1940) and by Manly & al. (J. Biol. Chemistry 134, 393, 1940) with radioactive P.

The rate of absorption is a function of crystal size and surface. The larger the crystals, the slower the first phase of the exchange reaction. For this reason, the P "turnover" is more rapid for bones than for dentin and is slowest for enamel (Neuman & Riley /10/; Falkenheim & al. /11/.). For Ca^{45} the same exchange reaction is valid both in vivo and in vitro.

Hydroxypatite crystals -- $Ca_{10}OH(PO_4)_6$ -- are relatively small and have very great surface areas per unit weight. Specific surface areas, measured by N. V. Wood (see l.c. 11) on powder material, are 99.3 for bones, 51.1 for apatite, 2.4 for dentin and 1.8 m^2/g for enamel. One gram of bone thus possesses an internal and external surface area of approximately 0.025 acre. It is readily understandable that on surface area substantial and rapid exchange reactions are possible.

According to Harrison & Harrison /12/ the period of time during which 50% of the skeleton Ca is exchanged for serum Ca amounts to 45 hours in 6-8 week old rats. In older animals the process is slower; vascularization may be playing a certain role. According to the authors cited above /12/, on occasion all of the skeleton Ca may be exchanged against the Ca of the body fluids. Harrison & Harrison /12/ also were able to prove in particularly beautiful experiments the existence of the two phases od Ca absorption on the skeleton. In the first rapid phase, within the first two hours after the pharingeal application of Ca^{45}, the element is absorbed primarily in the epiphysis of the bones, later in the diaphysis part. The slow phase lasts more than 72 hours; Table 3 shows that equilibrium between the specific activities (= activities per mg Ca) of serum and bones is attained only after two weeks following the administration of the isotope.

TABLE 3. Progress of the Ca^{45} Exchange Reaction Between the Serum Ca^{45} and Different Parts of the Bone Skeleton After Pharingeal Application of Less Than 1.0 millionC of $Ca^{45}Cl_2$ to Healthy and Rachitic Rats and Rachitic Rats Treated with Vitamin D (Harrison & Harrison /12/. The values represents pulse counts per second/mg Ca. The data for the 72-hour period are averages of values determined for 3 animals of each group. The other values refer to single animal determinations.

Key: 1. Time; 2. Group; 3/ Serum; 4. Epiphysis bones; 5. Diaphysisis bones; 6. Total skeleton; 7. Rachitic; 8. Control; 9. Treated; 10. Hours; 11. Weeks.

Zeit (1)	Gruppe (2)	Serum (3)	Epiphysen-knochen (4)	Diaphysen-knochen (5)	Gesamt-skelett (6)	Epiphyse / Diaphyse
2 Std.	Rachitisch	68	2,4	0,9	1,2	2,7
	Kontrolle	175	5,4	2,1	3,6	2,6
	Behandelt	212	10,8	2,7	5,4	4,0
4 Std.	Rachitisch	83	4,2	1,6	3,0	2,6
	Kontrolle	37	3,1	1,0	2,4	3,1
	Behandelt	72	8,2	2,5	5,3	3,3
72 Std.	Rachitisch	9,3	7,8	3,6	5,2	2,2
	Kontrolle	7,8	7,6	3,8	5,3	2,0
	Behandelt	10,1	9,4	4,3	7,1	2,2
2 Wo.	Rachitisch	2,1	2,5	2,5	2,5	1,0
	Kontrolle	3,1	3,1	2,5	3,2	1,2
	Behandelt	2,6	2,6	2,4	2,8	1,1

The fact that initially more Ca^{45} is deposited in the epiphysis is probably related to the more rapid new bone formation in this location and to the loose structure and thus greater exchange surface. In the more compact diaphysis the "turnover" is slower, due to structural or vascular conditions. Armstrong & Barnum /12a/ found the highest turnover rate in bone marrow, a lower one in the epiphysis and the lowest in the diaphysis.

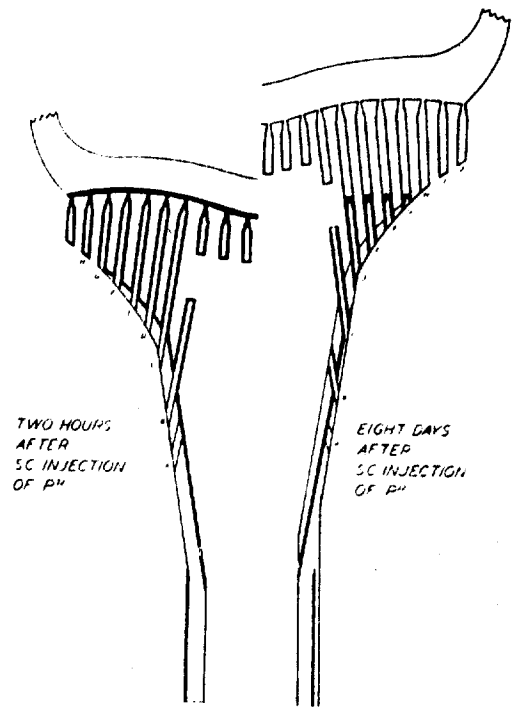
The variation of activities in bones after the supply of radioactive Ca is thus a function on the one hand of physical absorption in the bone, which depends on the anatomy and crystal structure of the latter, and on the other of bone metabolism with the formation and decomposition of bone particles. The simultaneous existence of the two mechanisms renders investigations of the balance of skeleton metabolism with isotopes uncertain.

The incorporation of isotopes in different parts of bones has been demonstrated very clearly with P^{32} phosphate by Leblond & al. /13/. Bone sections of the tibia and humerus of newly born and adult rats were examined at different times after the injection of P^{32} by radioautography. In part they were histologically stained to determine the relationship of P^{32} with osterblasts and osteoclasts. In these experiments, the absorbed unstable exchange P^{32} can be distinguished by a diffuse, rapidly disappearing reaction from the precipitated, well localized stable P^{32} converted to bone salt, with constant reaction. This latter part of the autoradiograph is a sign of new bone formation.

According to these investigations, the longitudinal growth of the diaphysis takes place in three different locations, i.e. periosteally in an intermediate diaphysis part, endosteally in the vicinity of the epiphyseal plate and endochondrally directly under the epiphyseal plate. This simultaneous addition in three different locations insures the extension of the bone without altering its general structure, such as shown clearly in the diagram by the authors (Fig. 6). Initially, however, these locations represent preferred places of the first phase of the exchange reaction. The radioautographs proved to be highly valuable for the study of the function in time and space.

FIGURE 6. Diagram of Bone Growth with Retention of Shape and Internal Structure. The left side exhibits with thick lines the surfaces of the tibia head of a rat, on which radioactive P is concentrated 2 hours after the s.c. injection of P^{32}. The three preferred locations are indicated in the text. The right side shows conditions 8 days later, when the growth of locations marked on the left side had progressed another step. (Leblond & al. /13/.)

SEE NEXT PAGE FOR FIGURE 6 ---------



Osteoblasts are present in all locations were P^{32} is deposited. Osteoclasts are found mainly at some distance from the epiphyseal lines toward the diaphysis; i.e. where body cells are decomposed and Howship's lacunae are formed. Previously, histologists had assumed that osteoblasts would secrete bone salts, however, radioautograms did not show insoluble phosphates in the plasms of the cells.

Shortly after the injection of P^{32}, on the other hand, an appreciable amount of the stable, insoluble phosphate appears in the vicinity of the osteoblasts. It may be assumed, therefore, that the osteoblasts facilitate the precipitation of the salt. It is likely that the osteoblasts secrete the phosphatase which liberates the phosphate used in bone formation, because at the location of P^{32} deposition, phosphatase is also detected.

The fact that phosphatase produces precipitation of tagged phosphate as early as 5 minutes after the injection of P^{32}, is explained in the following manner: tagged PO_4 increases the concentration of phosphate in the immediate vicinity

of the phosphatase* and when the ferment cleaves the organic P bond, the concentration rises to the point of precipitation. Obviously, tagged PO_4 is included in the precipitation and tagged bone salts are formed. Osteoblasts and osteoclasts are required for new bone formation and longitudinal growth with shape retention.

Following the oral administration of $Ca^{45}Cl$ to rats, the specific activity in the serum of rachitic animals in the first few hours is only 1/3 or $\frac{1}{4}$ of that of controls or animals treated with vitamin D. This is shown clearly in Table 3 above.

It is remarkable that the turnover rate of controls, with the exception of the first hours, does not substantially exceed that of rachitic animals; in pathological processes the curative effect of Vitamin D is more pronounced. It is uncertain whether the increased concentration of Ca^{45} in the bones under the effect of Vitamin D represents enhanced bone salt deposition. Underwood & al. /15/ observed similar Vitamin D effects on the skeleton of rachitic and healthy young rats. Harrison & Harrison further showed that the initial rapid resoprtion following oral application in the priximal section of the small intestine is not affected by Vitamin D. On the other hand, an increase in Ca resorption under the effect of Vitamin D from the distal intestine can be observed between 4 and 24 hours after the administration of Ca^{45}. Untreated rachitic animals do not exhibit during this experimental period Ca resorption, even when ample amounts of Ca are available in the content of the intestine. Since the Ca residue of food passing through the first phase without being absorbed is present during subsequent periods in the distal section mainly in the form of slightly soluble salts, it may be assumed that the mechanism of the enhancement of resorption by Vitamin D is related to the increase in solubility of Ca salts in the intestine. Harrison & Harrison, however, do not emphasize this possibility and postulate, quite uncertainly in the present state of knowledge, that Vitamin D enhances intestinal Ca resorption in the intestine liquor under the unfavorable condition of very low concentrations of soluble Ca salts. The authors support this by the statement that with relatively high concentrations of Ca dissolved in the intestine liquor in the proximal section resoprtion is high and is unaffected by Vitamin D.

Vitamin D deficiency is also assumed to inhibit the incorporation of Sr^{89} in fractured bones of rats; it is locally enhanced by the administration of Vitamin D (Copp & Greesberg /16).

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* The administration of radioactive P does not account of and by itself for the increas in P concentration.

Isotope investigations thus did supply essential information concerning the understanding of the pathogenesis of rachitis and the mechanism of the action of Vitamin D. In the case of rachitis, the Ca turnover between bones and the serum is slowed -- a half time value of 65 hours against 45 hours in the normal case -- and the intestinal Ca resorption from the distal intestine sections is reduced. Vitamin D accelerates the turnover and improves resorption.

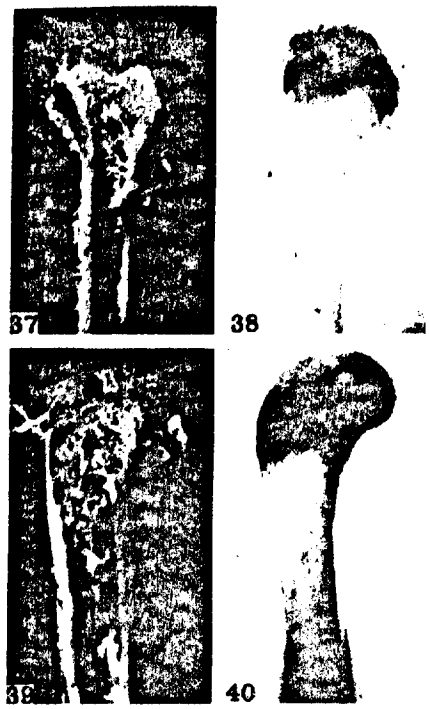
Bone fractures: In rats, increased activity has been detected both with Ca^{45}, and p^{32} during the healing of fractures in the ashes of damaged bones. The increase in activity rises from 30% during the first week to 75% three weeks after the fracture. Adjoining bone sections participate in the reaction. The accelerated exchange reaction apparently introduces the consolidation of the bone; the improved blood supply also plays a role (Bohr & al. /16a/).

d) Hormone Effects

Tweedy & al. /17/ found in rats -- the epitheal corpuscles of which had been removed alons, or together with their thyroid glands -- in addition to a decrease in serum Ca, a reduced Ca^{45} secretion in the feces during the first 14 days. Secretion in the urine was unchanged in comparison with the controls, as was the distribution in the bones and soft parts (24 hours after the operation and 18 hours after the subcutaneous or intraperitoneal injection of 0.3 millionC of $Ca^{45}Cl_2$. When 500 E parathyroid extract was administered to the animals after the operation, the Ca^{45} injected one hour earlier concentrated in the kidneys. In normal animals, following the administration of toxic doses of the parathyroid extract, in addition to the known increase of Ca in the serum, an increase in Ca^{45} in the urine and a decrease in the feces also occur. It is known from earlier investigations that excessive doses of parathyroid hormone lead to enormous increases of Ca in the kidneys and to the calcification of heart and kidneys. Investigations with radioactive Ca thus did not yeild further explanation of this hormone mechanism. The fact that the metabolism of Ca and P are largely parallel again does not represent new information.

FIGURE 7. Humerus Head of Grown Rat. 37 and 39 represent bone sections, 38 and 40 are corresponding radioautographs. Upper figures represent the state after 5 minutes, lower figures 2 days after subcutaneous injection of P^{32} (Leblond & al /13/.)

SEE NEXT PAGE FOR FIGURE 7 ---------



If hypophysectomized rats are given growth hormones for three weeks, two hours after the i. v. administration of $Ca^{45}Cl_2$ the activity in the tibia bones is approx. twice that of controls. The total Ca content in the ashes of the tibia bone does not change, however, so that during these first two hours the exchange reaction is accelerated esclusively. The Ca^{45} content of the serum and the urine and feces secretion of Ca^{45} are not affected. Autoradiography confirms the concentration in the vicinity of the epiphysis (Ulrich & al. /18/.)

According to Becks & al. /19/ and Baker & Ingle /19a/, ACTH delays the chondrogenesis and osteogenesis of the tibia of rats. Ulrich & al. /19b/ investigated the radioactive Ca metabolism in intact and hypophysectomized young rats, which exhibited a cessation of growth due to the ACTH treatment. The ACTH was administered during 39 days; the animals were killed one hour after the i. p. Ca^{45} injection. In normal animals, the Ca^{45} incorporation of the femur was not affected by the ACTH intake; in hypophysectomized animals it was reduced to about one third compared with the controls. According to published tables, however, the weight of the bones and of the bone ashes is also reduced to about ont-third, so that apparently the animals were in a highly reduced condition and were incapable of maintaining the normal turnover due to the atrophy of the femur. Controls with radioautography of the head of the tibia yielded different results so

that the effect of ACTH on the salt metabolism of the bones remains unexplained.

II.

Resorption Experiments in the Medical Clinic in Basel with Ca^{45} Glucomate.

It has been contended for a long time whether rectal Ca resorption exists. Heubner & Rona /20/ stated in 1919, following their experiments with cats, that rectal Ca resorption is slow and incomplete. Bircher & Rothlin /21/ found no increase in blood Ca after rectal administration to humans and concluded that no Ca is absorbed. Other authors -- the relevant literature has been cited and critically discussed by Geissberger /22/ -- observed on the basis of mostly insignificant increases in serum Ca, possibly also because of methodically inadequate Ca determinations or by indirect arguments (increased rest tonus of the uterus) an excellent and rapid rectal resorption of Ca.

In a very time-consuming chemical analyses, Geissberber & al. /23/ showed that no preferential rapid resorption of Ca takes place. Their analyses of rectal rinsing liquids lead to the assumption that even in the most favorable cases only a few % of Ca are absorbed by the rectum. Apparently, Ca^{45} is capable of resolving this argument and Geissberger /22/, in his many experiments on humans in the Basel Medical Clininc, showed decisively that no rectal Ca resorption takes place.

Notes on Potential Toxicity

Radioactive Ca was obtained in the form of $Ca^{45}CO_3$ from the Harwell Isotope Division (England). The Dr. Wild Co., Basel, synthetized the radioactive Ca^{45} gluconate and made it available for the experimenta.

Ca^{45} belongs, together with Co^{60}, Sr^{89}, Zr^{95}, etc. to the group of isotopes which, due to their long half-life and selective concentration in bones, may be considered dangerous clinically, if activity doses are not kept especially low.

According to the catalog, $CaCO_3$ from Harwell contains 115 million C/g. Ca. With respect to the gluconate applied $(C_{12}H_{22})_{14}Ca + H_2O$ = 448 mol. weight, a content of 10.0 millionC/g gluconate is calculated. By direct measurements, however, only 0.17 million C/g were determined. The loss of activity is evidently attributable to the preparation of the gluconate from carbonate. In accordance with the rule of thumb customary in the USA (Ebert /24/ p. 118.) no more than 30 million C/kg of body weight should be used on humans. Assuming that practically all of the Ca^{45} is stored in the bone system, and assuming 7 kg bones in a man weighing 70 kg, 1 g Ca^{45} gluconate introduces 0.024 million C/kg bones, i.e. less than 1/1000 of the activity permitted by the empirical American

rule. This estimate is based on the worst possible case wint respect to potential damage: all of the Ca^{45} is absorbed, nothing is secreted and all activities are effective in the bones.

Ca^{45} gluconate was administered in amounts of 1.0 or 0.5 g in an aqueous solution or in the form of suppositories either i.v., p.o., or rectally. Activities in the urine and feces were calculated with respect to the activity of 1 g gluconate in 20 cm^3 water = 100%. The 20 cm^3 were administered i.v. or orally. Either 10 cm^3 of the 5% solution or suppositories with 1.05 g Ca^{45} gluconate were introduced rectally. If only 10 cm^3 of a solution are instilled rectally, it is certain that no resportion occurs from the sigmoid. The urine of 24 hours was processed in toto or in two fractions, Ca was precipitated as the oxalate, dissolve in HCl and diluted to 100 cm^3 with water. Each individual bowel movement was dried, calcined to ashes, the ashes dissolved in HCl and diluted to 100 cm^3 with water. Activities were measured in the usual manner in 0.5 cm^3 liquid in the cavity of a sample holder. Values given represent averages of 10 measurements each.

The values in Table 4 and the graphical presentation in Fig. 8 shows clearly that following rectal administration, 94-99% of the Ca are found in the feces within 1-2 hours, while nothing is secreted in the urine. The stay time of the Ca in the rectum is immaterial, because no resorption takes place, even if the first bowel movement occurs as late as 48 hours after the introduction of Ca^{45}. Folowing oral application, 64-83% were found in the feces and 0.6-6.5% in the urine, after i.v. application of Ca^{45} to humans thus again prove that the mucous membrane of the intestine secretes sizable amounts of Ca. Actual resorption can be calculated within 4 days after oral administration by deducting the amount found in the feces from the total amount introduced and adding the amount an average daily value for the Ca^{45} resorbed and again secreted in the intestine. The average daily secretion in the intestine after Ca elimination following i.v. administration can be assumed to be approx. 2% of the amount injected. This value is probably somewhat too low because some of the Ca secreted in resorved in lower sections of the intestine. On the other hand, with oral application the amount in the blood is not 100% and the concentration gradient toward the intestine is less. Let us assume that the two opposing uncertainly factors compensate each other, so that with oral administration a daily resorption of 2%, due to the resecretion of already resorbed Ca must be accepted.

TABLE 4. Feces and Urine Secretion Following a Single i.v., Oral and Rectal Application of Ca^{45} (Geissberger /22/)

Key: 1. Time; 2. i.v., 3. Feces.

SEE NEXT PAGE FOR TABLE 4. ---------

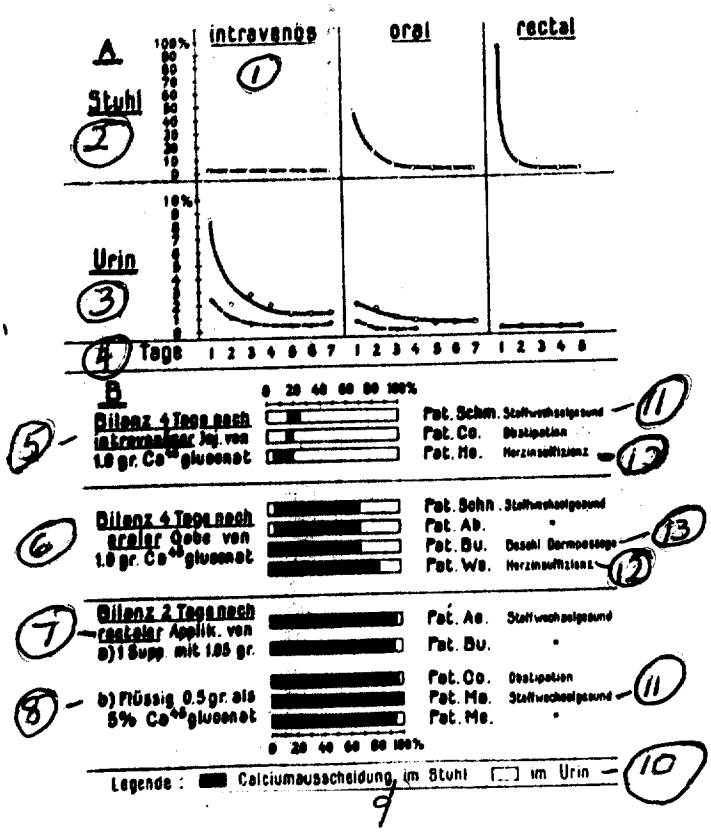
Zeit (1)	Intravenös (2)		Oral		Rectal	
	Urin	Stuhl (3)	Urin	Stuhl	Urin	Stuhl
1. Tag	8,8	2,5	1,6	51,8	0	93,0
2. Tag	1,8	2,3	1,3	7,3	0	0,6
1.-2.	10,6	4,8	2,9	59,2	0	93,6
3.	2,4	1,8	1,3	3,8	0	0,2
4.	2,6	2,4	0,9	2,0	0	0
1.-4.	15,6	9,0	5,1	64,9	0	93,8
5.	1,5	2,0	0,4	1,0	0	0
6.	1,5	1,9	0,5	1,5	0	0
7.	1,5	1,8	0,5	1,0	0	0
1.-7.	20,1	14,7	6,5	68,4	0	93,8
1.	8,1	1,6	2,1	49,6	0	92,0
2.	2,4	1,5	1,9	10,3	0	4,0
1.-2.	10,5	3,1	4,0	59,9	0	96,0
3.	3,2	1,8	0,5	1,8		
4.	1,5		0,4	2,0		
1.-4.	15,2	4,9	4,9	63,7		
1.	2,4	3,1	0,9	47,0	0	83,0
2.	1,1	4,7	0	20,0	0	14,0
1.-2.	3,5	7,8	0,9	67,0	0	97,0
3	0,6	4,5	-	1,6	0	0
4.	0,8	2,4	-	-	0	0
1.-4.	4,9	14,7	0,9	68,6	0	97,0
1.	8,5	2,2	0,6	35,8	0	91,0
2.	2,0	1,8	0	32,2	0	1,4
1.-2.	10,5	4,0	0,6	68,0	0	92,4
3.			0	11,2	-	1,5
4.			0	3,6	-	0
1.-4.			0,6	82,8	0	93,9
1.					0	99,0
2.					0	0
1.-2.					0	99,0

After i. v. and oral administration, a positive Ca balance is attained; Ca retension after i. v. administration amounts to roughly 80%, after p. o. administration to 20-25%. Following rectal application, both in solution or as a suppository, since there is no resorption, nothing is retained. This proves all reports of recal Ca resorption to be untrue.

According to Fig. 8A, in humans, after i. v. application, 8-9% Ca^{45} is secreted in the urine on the first day; after this, calciuria rapidly drops to a lower value of about 2% of the Ca^{45} introduced per 24 hours.

FIGURE 8. Urine and Feces Secretion of Ca^{45} and Ca^{45} Balance Following a Single Application of Ca^{45}, i. v. or Rectal to Humans (Geissberger /22/.)

SEE NEXT PAGE FOR FIGURE 8. ---------



Key: 1. i.v.; 2. Feces; 3. Urine; 4. Days; 5. Balance 4 days after i.v. injection of 1.0 g Ca^{45} gluconate; 6. Balance 4 days after oral application of 1.0 g Ca^{45} gluconate; 7. Balance 2 days after rectal application of a)-1 suppository with 1.05 g; 8. b)-liquid, 0.5 g as 5% Ca^{45} gluconate; 9. Ca elimination in feces; 10. In urine; 11. Patient Schm. - healthy metabolism; 12. Cardiac insufficiency; 13. Accelerated intestine passage.

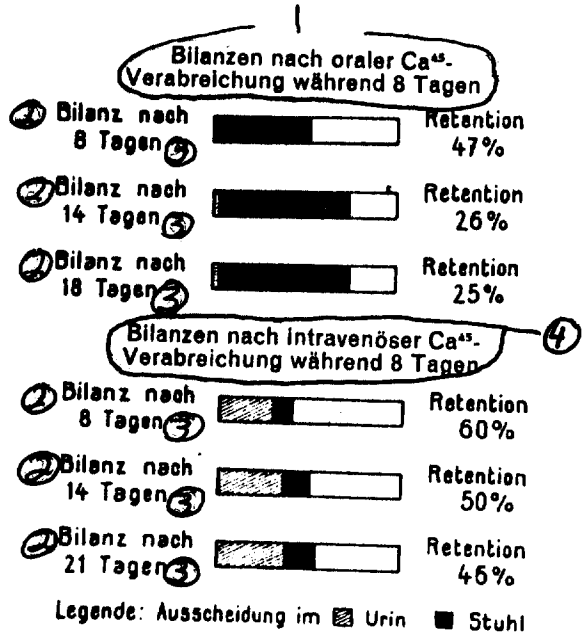
In the case of cardiac insufficiency, the secretion of Ca^{45} in the urine is reduced, both after i.v. and p.o. application (dotted line in Fig. 8A, lower row). The balance after oral administration is lower with an 18% retention in the case of the cardiac patient, than for a healthy person with 30 and 31%. Cardiac patients resorb and retain less Ca after oral application; the Ca turnover is slower.

In another series of experiments, Geissberger /22/ administered to two persons with healthy metabolisms for eight successive days a faily dose of 1.0 Ca^{45} gluconate in 20 cm^3 water; orally to one, i.v. to the other. It is shown that in both types of application the new value of Ca in the serum remains practically unchanged. In both experiments, Ca^{45} was determined in the urine and the feces for 20 to 40 days by the balance method. Graphical representation shows that after oral application even in the chronic experiment, the major part of the Ca^{45} is secreted without resorption in the feces; with i.v. administration, elimination is mainly through the urine. Retension after i.v. application is twice the value after oral administration. Ten days after oral application, a positive balance of 25% remains; this value is +46% after 13 days for i.v. application.

Geissberger's careful investigations contirbuted on the one hand to an understanding of Ca resorption and secretion in humans and, on the other, yielded the therapeutically important fact that a Ca therapy is most effective when administered i.v. and that rectal Ca applications represent a symbolic act.

FIGURE 9. Balance Experiments after Chronic, Oral and i.v. Application of Ca^{45} (Geissberger /22/).

Key: 1. Balance after 8 days oral administration of Ca^{45}; 2. Balance after; 3. Days; 4. Balance after 8 days i.v. administration of Ca^{45}.



III.

The use of Ca^{45} in physiological and pathological and potentially in diagnostic examinations has certainly not been exhausted in its possibilities. Many of the investigations mentioned exhibit methodological possibilities which permit primarily the observation of metabolic processes with respect to time through the use of radioactive Ca. Perhapy the Ca^{45} experiments performed on humans in our clinic will result in additional experiments with this long-lived isotope on humans. The use of activity doses of less than 1/1000 of the toxic dose on humans can certainly be justified. Subsequent controls 1-2 years after the application of Ca^{45} of some of the experimental persons found them well and with normal blood conditions.

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THE CALCIUM REQUIREMENT OF ADULT MAN AND THE UTILIZATION OF THE CALCIUM IN MILK AND IN CALCIUM GLUCONATE [1]

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The calcium requirement of the adult organism relates to the replacement of endogenous losses of calcium from the body, mainly through the kidneys and the alimentary tract. While the cause of these losses is quite obscure, their existence has been definitely established in many published experiments on human subjects. We may consider the endogenous loss of calcium per day as measuring the requirement for maintenance. It is undoubtedly dependent upon body size, but whether it varies directly with body weight, body surface or with some other power function of body weight is not at present known. However, until this question is settled the endogenous losses of calcium may be tentatively expressed to the kilogram of body weight.

The efficiency of dietary calcium in replacing endogenous losses may be expected to vary with the kinds of food materials contained in the diet. Hence the dietary requirement of calcium for maintenance will vary with different diets and will probably always be much greater in magnitude than the endogenous loss. In a compilation and analysis by Mitchell and Curzon ('39) of available information on the subject including 139 average observations on 107 human subjects,

[1] This investigation was aided materially by the donation of funds from the Graduate School of the University of Illinois, for which grateful acknowledgment is made.

only eighteen of whom were males, it was found that the average endogenous loss of calcium was 3.1 mg. per day per kilogram of body weight, while the average intake of dietary calcium required for calcium equilibrium was 9.75 mg. per day per kilogram of body weight. Thus, slightly more than 3 gm. of dietary calcium is required on the average to replace an endogenous loss of 1 gm. But considerable disparity exists among the individual data here summarized, partly the result of metabolic differences among the various subjects studied, partly the result of the short experimental periods usually employed in human metabolism studies, and partly the result of the many different types of diets employed, containing different sources of calcium.

It seems important to know the extent to which the calcium in human foods is utilized by the adult, particularly those foods that supply important amounts of calcium to the American diet. Most of the experimental evidence concerning the utilization of the calcium of foods by human subjects reviewed by Mitchell and Curzon ('38) is of comparative, not absolute, significance. In none of the experiments on adults there reviewed is it possible to compute from the reported data the per cent to which the dietary calcium was used in the body in the replacement of endogenous losses of calcium.

The experiment to be reported below was designed to measure the calcium excretion of an adult human on a low calcium diet, and to assess the value of the calcium in milk solids and in an inorganic salt, calcium gluconate, in replacing these losses and in maintaining a condition of calcium equilibrium. Since the human organism, perhaps to a greater extent than the lower animals, is subject to wide fluctuations in its utilization of dietary minerals, the experiment was planned to extend over much longer than the usual periods of time. A total of 168 days of experimental observation is included in this experiment.

PLAN OF EXPERIMENT

The subject of the experiment was one of the authors (F.R.S.), about 35 years of age, weighing normally about 85 kg., and 176.5 cm. high.

The plan of the experiment was to subsist throughout upon a low-calcium diet, upon which the daily loss of calcium in urine and feces would be determined. Then graded additions of calcium in the form of skim milk powder or of calcium gluconate were made to the diet and the calcium balance determined on each level in the attempt to find out how much calcium in the two forms would be required for calcium equilibrium. Such data would also permit the determination of the utilization of the calcium in each case by relating increments in calcium balance to increments in calcium intake.

The collection periods were of 4 days duration, though each experimental period of definite calcium intake lasted from 12 to 32 days. The feces of successive collection periods were separated with the aid of carmine markers. Each day samples of the foods consumed, in the proportions in which they were eaten, and equal in amount to one-fourth of the day's consumption, were collected, and every 4 days these samples were composited for analysis for calcium. Modifications of the McCrudden method were used in the analysis of food, feces and urine.

The low-calcium basal ration that was used throughout the experiment was as follows:

Breakfast	
Orange juice	100 cc.
Oatmeal	224 gm.
Lunch	
Bread	50 gm.
Bacon	48 gm.
Oatmeal cookies	60 gm.
Apples	180 gm.
Dinner	
Potatoes, baked	160 gm.
Beef	80 gm.
Beets	100 gm.
Prunes	120 gm.

The ratio of calcium to phosphorus in this day's menu was found by actual analysis to be 0.3 to 1. The day's intake of protein and energy in the basal diet amounted to about 64 gm. and 2627 calories, respectively, including the sugar and butter supplements given below.

Tea, made with distilled water, was taken with the noon and evening meals and all water drunk was distilled. During the day 94 gm. of sugar, partly in the form of candy, and 56 gm. of butter were also consumed. Vitamins A and D were provided daily in a vitamin capsule (Parke, Davis and Company) equivalent to 3 teaspoonfuls of cod liver oil. The skim milk powder supplements, ranging from 10 to 50 gm. daily, provided additional energy, as well as calcium, a circumstance that was considered favorable in view of the tendency for the body weight to decrease during the experimental periods. Following the intermission during the latter part of March and the first few days of April, and continuing throughout the calcium gluconate periods, the basal diet was supplemented with a further addition of 40 gm. of sugar daily in order to raise the energy intake to the level of the preceding periods when milk powder supplements were fed. The preparation of calcium gluconate used contained 9.084% of calcium.

The experiment consisted of forty-three 4-day periods, the sequence of which was interrupted only twice, from December 20th to January 6th and from March 23rd to April 7th. Throughout the period of experimental feeding the subject was in good health, and in fact felt better than usual.

EXPERIMENTAL RESULTS

The calcium balance data are summarized in table 1. The basal diet provided an average of 195 mg. of calcium daily, on which the average daily balances for the three basal periods were, in order, —99, —121 and —109 mg. daily, averaging —110 mg. The calcium excreted daily on this low-calcium diet averaged 304 mg., of which 68% was excreted by way of the intestinal tract. The close agreement of the metabolism

TABLE 1

The calcium metabolism data of the experiment

Period	Inclusive dates	Body weight	Calcium supplement consumed daily	Daily calcium metabolism: Intake	Feces	Urine	Balance
		kg.		*gm.*	*gm.*	*gm.*	*gm.*
1[1]	Nov. 6–9	85.5	None	0.160	0.358	0.105	—0.303
2	Nov. 10–13		None	0.180	0.197	0.086	—0.103
3	Nov. 14–17		None	0.164	0.193	0.102	—0.131
4	Nov. 18–21	82.7	None	0.189	0.182	0.092	—0.085
5	Nov. 22–25		None	0.164	0.205	0.086	—0.127
6	Nov. 26–29		None	0.166	0.199	0.063	—0.096
7	Nov. 30–Dec. 3	80.8	None	0.228	0.204	0.070	—0.046
8	Dec. 4–7	81.0	None	0.198	0.226	0.072	—0.100
Averages		82.5		0.184	0.201	0.082	—0.099
9	Dec. 8–11	80.5	10 gm. milk solids	0.280	0.262	0.089	—0.071
10	Dec. 12–15		10 gm. milk solids	0.292	0.282	0.086	—0.076
11	Dec. 16–19	80.5	10 gm. milk solids	0.290	0.281	0.111	—0.102
Averages		80.5		0.287	0.275	0.095	—0.083
12[1]	Jan. 6–9		None	0.195	0.286	0.073	—0.164
13	Jan. 10–13		None	0.210	0.209	0.093	—0.092
14	Jan. 14–17		None	0.182	0.203	0.130	—0.151
Averages		...		0.196	0.206	0.111	—0.121
15	Jan. 18–21		40 gm. milk solids	0.692	0.575	0.140	—0.023
16	Jan. 22–25		40 gm. milk solids	0.709	0.584	0.127	—0.002
17	Jan. 26–29		40 gm. milk solids	0.716	0.650	0.118	—0.052
18	Jan. 30–Feb. 2	80.7	40 gm. milk solids	0.705	0.589	0.129	—0.013
19	Feb. 3–6	80.1	40 gm. milk solids	0.725	0.552	0.117	+0.056
Averages		80.4		0.709	0.590	0.126	—0.007
20	Feb. 7–10	80.3	50 gm. milk solids	0.832	0.755	0.189	—0.112
21	Feb. 11–14		50 gm. milk solids	0.856	0.652	0.163	+0.041
22	Feb. 15–18		50 gm. milk solids	0.856	0.751	0.143	—0.038
23	Feb. 19–22		50 gm. milk solids	0.850	0.697	0.136	+0.017
24	Feb. 23–26		50 gm. milk solids	0.868	0.677	0.144	+0.047
25	Feb. 27–Mar. 2		50 gm. milk solids	0.880	0.792	0.130	—0.042
Averages		80.3		0.857	0.721	0.151	—0.015
26	Mar. 3–6		40 gm. milk solids	0.718	0.582	0.126	+0.010
27	Mar. 7–10		40 gm. milk solids	0.726	0.609	0.121	—0.004
28	Mar. 11–14	79.8	40 gm. milk solids	0.745	0.605	0.126	+0.014
29	Mar. 15–18		40 gm. milk solids	0.721	0.634	0.096	—0.009
30	Mar. 19–22		40 gm. milk solids	0.758	0.647	0.147	—0.036
Averages				0.734	0.615	0.123	—0.004
31[1]	April 7–10	81.2	None	0.197	0.369	0.090	—0.262
32	April 11–14		None	0.209	0.214	0.098	—0.103
33	April 15–18	79.6	None	0.185	0.206	0.093	—0.114
34	April 19–22		None	0.196	0.215	0.106	—0.125
35	April 23–26	79.6	None	0.225	0.219	0.102	—0.096
Averages		80.1		0.204	0.213	0.100	—0.109
36	April 27–30		7.038 gm. Ca gluconate	0.816	0.640	0.160	+0.016
37	May 1–4		7.038 gm. Ca gluconate	0.884	0.600	0.186	+0.098
38	May 5–8	78.7	7.038 gm. Ca gluconate	0.796	0.577	0.172	+0.047
Averages		78.7		0.832	0.606	0.173	+0.053
39	May 9–12		5.304 gm. Ca gluconate	Collections lost			
40	May 13–16	78.9	5.304 gm. Ca gluconate	0.682	0.565	0.149	—0.032
41	May 17–20		5.304 gm. Ca gluconate	0.689	0.484	0.155	+0.050
42	May 21–24	78.2	5.304 gm. Ca gluconate	0.679	0.610	0.165	—0.096
43	May 25–28		5.304 gm. Ca gluconate	0.683	0.540	0.166	—0.023
Averages		78.5		0.683	0.550	0.159	—0.026

[1] The data for these periods are not included in the averages.

data in the three periods (November 6th to December 7th, January 6th to 17th, and April 7th to 26th) of subsistence on the basal diet alone is significant.

Forty grams of skim milk powder daily, providing about 542 mg. of calcium (1.355%) was sufficient in addition to the 195 mg. of calcium in the basal diet, to maintain the subject in approximate equilibrum, the average balance over a 20-day period being —7 mg. daily and over a subsequent 20-day period, —4 mg. daily. In the intervening 24-day experimental period, a supplement of 50 gm. of milk powder was no more effective than 40 gm. in maintaining calcium equilibrium, indicating the sufficiency of the latter supplement for calcium equilibrium.

For the 12 days from April 27th to May 8th, a daily supplement of calcium gluconate providing 639 mg. of calcium promoted some calcium storage averaging 53 mg. per day. During the following 20 days, this supplement was decreased to provide only 482 mg. of calcium daily. The contemporary balances of calcium were generally negative and averaged —26 mg. for the last 16 days for which balances were determined. The results indicate that 639 mg. of calcium added to the basal diet as the gluconate salt were more than adequate for the maintenance of calcium equilibrium, while a supplement of 482 mg. was inadequate. If interpolation is permissible on the basis of the observed balances of calcium, it appears that 534 mg. of added calcium as the gluconate would have established calcium equilibrium. This estimate is practically identical with the quantity of milk calcium found to be adequate—or only very slightly inadequate—for equilibrium, i.e., 542 mg.

DISCUSSION

The equation that Mitchell and Curzon ('39) derived to describe the average relationship in adult human subjects between the output of calcium and its intake is:

$$y = 0.6826\,x + 3.0940$$

where y is the calcium output in milligrams per kilogram of body weight per day and x is the intake of calcium expressed in the same fashion. In the experiments reported above, the average body weight of the subject in the three experimental periods in which no calcium supplement was added to the basal diet was 81.1 kg. His average daily intake of calcium per kilogram of body weight was therefore $195 \div 81.1 = 2.40$ mg. His average output on the same basis was $304 \div 81.1 = 3.75$ mg. Placing the former value for x in the above equation, gives a value for $y = 4.73$ mg., about 26% higher than the observed output, 3.75 mg. per kilogram. This divergence from the average relationship is perhaps not surprising in view of the disparity existing among the data from which the equation was derived. The coefficient of x in this equation indicates an average utilization of 32% ($[1 - 0.6826] \times 100 = 31.74$) of the dietary calcium.

About 540 mg. of calcium either in skim milk powder or in calcium gluconate seemed sufficient to attain calcium equilibrium in this subject in addition to the 195 mg. of calcium in the basal foods. Equilibrium was thus attained on a total of 735 mg. of calcium, equivalent to about 9.2 mg. per kilogram of body weight, as compared with the average of 9.75 mg. derived by Mitchell and Curzon ('39) from available data in the literature.

The daily addition of 540 mg. of calcium in the form either of milk solids or of calcium gluconate to the basal diet changed the daily calcium balance from an average of —110 mg. to approximately 0. In other words 540 mg. of calcium in these forms were required to replace an endogenous loss of 110 mg., indicating a utilization of the dietary calcium of about 20%.

This is a much lower utilization of milk calcium, or of dietary calcium of any origin, than is frequently assumed. It is difficult to find in the literature, however, any precise determinations of calcium utilization, since the information sought and obtained has been comparative in significance; for example, the relative utilization of the calcium of milk and of carrots. Such evidence as the following seems worthy of

citation. Mallon, Jordan and Johnson ('30) found that an intake of 9.6 mg. of calcium per kilogram of body weight daily, 94% of which was derived from milk, induced a small storage of calcium in one woman subject but a sizable loss of calcium in another. Mallon, Johnson and Darby ('32) reported that 9.3 mg. of calcium per kilogram of body weight per day of which 87 to 88% was supplied by milk was slightly inadequate for calcium equilibrium for two women subjects. In a study reported the following year ('33), the same investigators showed that a diet providing 9.8 and 10.5 mg. of calcium per kilogram of body weight daily, 88% of which was derived from milk, promoted a slight storage of the element in two female subjects. Using a group of ten college women as experimental subjects, Kramer, Potter and Gillum ('31) were unable to secure positive calcium balances on daily intakes of calcium ranging from 5.7 to 9.0 mg. per kilogram of body weight, 78 to 80% of the food calcium being derived from either milk or ice cream. These experimental results are consistent with the results reported in this paper, indicating a requirement of 9.2 mg. of calcium per day per kilogram of body weight for equilibrium, milk or calcium gluconate furnishing about 73% of the calcium consumed.

On the other hand, investigations by Adolph and Chen ('32), Rose and MacLeod ('23), Rose ('20) and Kramer, Latzke and Shaw ('28) indicate that diets providing daily from 8.2 to as low as 6.4 mg. of calcium per kilogram of body weight, supplied largely from milk, may promote calcium storage in adult human subjects for short periods of time, at least.

If these short-time calcium balance studies on adult subjects may be taken at their face value, they suggest that the utilization of calcium in any particular food, such as milk, is subject to considerable variation depending upon unknown factors, dietary or non-dietary or possibly both. It is not clear that the potential acidity or alkalinity of the diet, the proportion of fibrous materials in it, the proportion or character of the fat or mineral ratios are of preponderate importance at least when adequate amounts of vitamin D are available. The

possibility of raising the utilization of dietary calcium in the adult by varying the above (or other) factors is an important problem in human nutrition.

Turning to the calcium metabolism of children, it appears that, while the infant may utilize milk calcium to the extent of 60% or so (Mitchell and Curzon, '39), older children do not do so well. Sherman and Hawley ('22) placed three children, varying in age from 4 to 12 years, upon graded supplements of milk added to a low calcium diet. Comparing successive periods in these experiments, in which the milk intake was increased or decreased at the rate of 250 cc. daily, it may be shown that the increments or decrements in calcium retention average only 24% of the simultaneous increments or decrements in intake. Unpublished experiments of Outhouse, Kinsman and others at the University of Illinois on eleven preschool children afford excellent evidence of the efficiency in the utilization of milk calcium. Measuring the utilization of milk calcium by relating increments in calcium retention to corresponding increments in intake of milk calcium within ranges of the latter not exceeding the requirements for maximum retention, the percentages of utilization varied from 14 to 25 and averaged 20, exactly the same as the value secured on an adult reported in this paper.

SUMMARY

The calcium balance of an adult human subject receiving a basal low-calcium diet with varying supplements of calcium in milk solids and in calcium gluconate was followed throughout forty-three 4-day periods.

For equilibrium 9.2 mg. of calcium per kilogram of body weight per day were required, about three-fourths of which were supplied either in milk solids or in calcium gluconate. The calcium in both of these forms was equally well utilized, but only to the extent of 20%.

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THE GLUCONATES

*By George B. Stone**

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THE physiological essentiality and nutritional indispensability of certain metals have interested a large number of investigators over the past century. The necessity for such elements as calcium, magnesium, sodium, potassium and iron for the maintenance of life has been accepted in fact, although the actual biological mechanisms through which these elements act have still not been specifically elucidated. These metals are required in relatively large quantities for vital processes as compared to other items of the diet or when employed therapeutically. Only recently has more complete information been reported as to the metabolic role and therapeutic value of magnesium and potassium.

In addition to these five metals, certain others appear to be required in very small amounts. These so-called "trace" elements are presumed to be essential, but their indispensability in some cases is still a matter of conjecture. The trace metals which appear to be necessary for life are copper, zinc, manganese, and cobalt. The actual metabolic role of all these metals is, for the most part, obscure, and the deficiency symptoms which are produced by their omission from the diet in many cases are neither striking nor spectacular. For this reason, over the past two decades we have seen the emphasis in clinical and nutritional research placed on the role and action of such substances as vitamins and hormones, as for example vitamin B_{12} and cortisone, deficiencies or imbalance of which produce definite symptoms and where their therapeutic use produces dramatic recoveries from the observed conditions. In fact the recent investigations relative to potassium and magnesium occurred principally because the therapeutic use of these metals brought about almost amazing results.

In the past, where diseases or deficiency symptoms have been thought to be the result of mineral deficiency, inorganic salts of the metal concerned have usually been employed. Normally, the anion in such compounds exerts its own physiological action, thereby producing undesirable side reactions and lowering the patients' tolerance to the compound, as well as occasionally increasing the toxicity. In other instances, a lack of solubility prevented effective administration of the compound.

Thus the therapeutic or nutritional use of metallic salts of a compound, the anion of which, in itself, exerts little or no pharmacological action would be of distinct value. Such compounds are to be found in the metallic salts of gluconic

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acid. The gluconate anion is without any apparent pharmacological action, and the toxicity of the gluconate radical is of a distinctively low order. Hence, the metallic gluconate may be administered as an effective source of the metal without any complications arising from the presence of the gluconate radical. All of the metallic gluconates are quite soluble in water.

Some word might be brought forth concerning the parent radical of these metal salts, namely, gluconic acid. Gluconic acid is a pentahydroxy caproic acid derived by the oxidation of the aldehyde group in the common sugar, d-glucose. It was first isolated about 1870 and was prepared by chemical oxidation reactions and later by electrolytic methods. In 1922, the French chemist, Molliard, discovered that gluconic acid was the metabolic product of certain molds, and the commercial production of gluconic acid today is principally through the fermentative oxidation of glucose.

Gluconic acid is a very mild acid and does not display any marked physiological actions. In fact, it has been demonstrated that gluconic acid may be tolerated in the daily diet for years without injury or disturbance to the digestive system. Upon parenteral injection the gluconate radical is also of relatively low toxicity.

Calcium Gluconate

The low order of toxicity of gluconate salts and the high tolerance with which the body absorbs and metabolizes these salts is perhaps best illustrated by the most commonly used gluconate salt, Calcium Gluconate U.S.P. Since this compound was first placed on the market some twenty years ago, it has received almost worldwide acceptance in the medical field as a therapeutic source of calcium.

Calcium, in addition to having the primary function for the formation of bones and teeth, is involved in a large number of physiological processes. Among these may be mentioned the blood coagulation process, the regulation of heart beat, the transformation of chemical energy into muscular contraction, the maintenance of normal membrane permeability, and regulation of the osmotic, water and acid-alkaline balance of the circulatory system.

The principal use of calcium gluconate has been in the treatment of conditions predisposing to rickets or tetany where a dietary insufficiency or imbalance of calcium is observed. The high absorption rate of calcium gluconate as well as the relatively large dosages which can be administered orally over prolonged periods of time without digestive disturbances or injected parenterally without irritation is one of the main advantages of calcium gluconate.

In recent years, a large number of ailments have been reported as being benefited by the use of calcium gluconate in conjunction with other medicinals. This is not unexpected in view of the large number and variety of biological functions in which calcium demonstrates some physiological action. In a recent monograph some forty reports indicated the value of calcium gluconate in the treatment of a wide variety of ills and it is anticipated that many more will be reported in future medical literature. For this reason, the pharmaceutical application of calcium gluconate is being extended to a rather large variety of proprietary products. In addition to its use in tablet and capsules or in sterile solutions for parenteral injection where the active ingredient is calcium gluconate alone, we now find that calcium gluconate is being employed in such products as antacid preparations, analgesics, vitamin and mineral products and many other similar preparations. From a purely pharmaceutical viewpoint, calcium gluconate is stable in such mixtures and in most cases is compatible. At the present time, it is possible to employ calcium gluconate not only as a source of calcium but as a dry base for oral products such as tablets and capsules. Because of its relatively high solubility as far as calcium salts are concerned, calcium gluconate may also be employed as a source of calcium in liquid preparations, for example multivitamin emulsions or multi-mineral products.

Ferrous Gluconate

The necessity for iron in the human system has been *(Continued on page 266)*

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RESEARCH COMMISSION

HEMOSTATIC EFFECTS OF ADMINISTRATION OF CALCIUM, VIOSTEROL, STYPTYSATE, AND CEANOTHYN BY MOUTH*

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IN many dental procedures, bleeding has to be especially combated because it obscures the field, makes the operative procedure more difficult and is, of course, deleterious to the patient. There is, therefore, a constant need for therapeutic agents which will accelerate the coagulation of blood, so that bleeding will be diminished to a point where it is no longer troublesome. We are not referring here to the pathologic condition of hemophilia, since this condition is rarely, if ever, encountered in ordinary practice. But certain, otherwise normal, persons have a bleeding time of from ten to fifteen minutes without any other serious deviation from normality in the blood-clotting mechanism. These cases are the source of the major difficulties in hemorrhage in dental practice.

The normal coagulation mechanism involves a combination of reactions between tissue or blood ferments, the protein fibrinogen produced by the liver and calcium. These various elements in the blood are seldom seriously deficient, even in patients who bleed too freely. These patients are, therefore, essentially normal persons whose blood clots by the normal mechanism, but too slowly. Attempts to hasten the coagulation process have been numerous, and the reports on them have generally been characterized by great optimism on the part of the proponents of a particular method or drug. Too many articles have been written on the results in half a dozen coagulation time measurements, wherein the writer has contented himself with comparing the times observed in single control observations with subsequent times observed after a drug was administered. It is thoroughly established that the coagulation time, when measured by ordinary clinical methods, is somewhat variable, and therefore it is to be expected that, about half the time, any two successive readings may show a

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[illegible] of a drug on the rate of coagulation, without first firmly establishing the normal time to be expected and the range of variability, not on a very insecure and unreliable foundation. The literature is almost entirely lacking in studies in which the normal coagulation times of individuals have been determined repeatedly until the average values and their statistical variability have been established. Without this, any apparent subsequent change in the observed time cannot be reliably interpreted.

Therefore, the present study has been directed at repeated measurement of the coagulation time of a group of individuals until the average values of the times observed have been established with a high degree of certainty. Then a drug has been administered over a period of days or weeks, and the coagulation time under the influence of this medication has been similarly established by repeated measurements. These tests have been controlled further by subsequently observing the subject for a similar period without medication, in order to have a second control period for comparison with the period of medication. By carrying on the observations in each period for a sufficient number of days, and by making a sufficient number of observations to permit reliable averages to be calculated, the results obtained by this method afford information not obtainable by the more usual method of conducting coagulation studies. Our method also shows the effect of prolonged administration of the drugs in question, which has been almost entirely neglected in the past. The other common method, that of comparing a single control coagulation time before medication with that after medication, can be so affected by the time of day, the diet, variations in activity and other conditions not completely understood that the results can seldom be accepted as convincing.

[illegible] to the effect of administration of calcium, vitamin K, styptysate and cemoctyn by mouth, since the practicing dentist is only to a limited extent interested in hemostatic measures which require injection of a drug. In a subsequent paper, we shall discuss results with other hemostatics, primarily derived from [illegible] animal tissues, such as tissue extracts and related materials.

METHODS

The coagulation times were measured by the capillary tube method, since this is the most common clinical method for determining the coagulation time, or for predicting a possible hemorrhagic tendency. Seven subjects were used in all, in a total of fifteen complete experiments, to determine the effects of four different drugs. A total of 1,699 coagulation time measurements were made in [illegible]. The procedure was as follows: The lobe of the ear was pricked with a mechanical lancet, adjusted by a guard to give constant depth of penetration. The capillary tube was filled immediately from the drop of blood, and held in a fold of the palm of the hand to maintain a fairly constant temperature. Every ten to fifteen seconds, a short piece of the capillary tube was broken off, and the coagulation time taken as that when a filament of fibrin strung out between the two pieces of tubing. The time was measured in seconds from the first instant a drop of blood appeared on the ear to the observed time of clotting. Each subject made observations on his own clotting time, taking blood from the ears alternately, usually twice a day, for about two weeks. The coagulation times were always taken at the same time of day and with a constant relationship to meals and other activities and were so arranged as to be taken at the time of the supposed maximum action of the drug. This procedure was then continued for a further two-week period, during which the drug in question was taken. The

[illegible] the effect of subcutaneous injection of a drug on the rate of coagulation, without first firmly establishing the normal time of coagulation and its range of variability, rest on a very [illegible] and controllable foundation. The literature is almost entirely lacking in studies in which the normal coagulation times of individuals have been determined repeatedly until the average values and their statistical variability have been established. Without this, any apparent subsequent change in the observed time cannot be reliably interpreted.

Therefore, the present study has been directed at repeated measurement of the coagulation time of a group of individuals until the average values of the times observed have been established with a high degree of certainty. Then a drug has been administered over a period of days or weeks, and the coagulation time under the influence of this medication has been similarly established by repeated measurements. These tests have been controlled further by subsequently observing the subject for a similar period without medication, in order to have a second control period for comparison with the period of medication. By carrying on the observations in each period for a sufficient number of days, and by making a sufficient number of observations to permit reliable averages to be calculated, the results obtained by this method afford information not obtainable by the more usual method of conducting coagulation studies. Our method also shows the effect of prolonged administration of the drugs in question, which has been almost entirely neglected in the past. The other common method, that of comparing a single control coagulation time before medication with that after medication, can be so affected by the time of day, the diet, variations in activity and other conditions not completely understood that the results cannot be accepted as convincing.

[illegible] to the effect of oral administration of antivitamin, styptic and anticoagulant drugs, since the practising dentist is only to a limited extent interested in hemostatic measures which require injection of a drug. In a subsequent paper, we shall discuss with other hemostatic drugs, primarily derived from, or associated with, animal tissues, such as tissue extracts and related materials.

METHODS

The coagulation times were measured by the capillary tube method, since this is the most common clinical method for determining the coagulation time, or for predicting a possible hemorrhagic tendency. Seven subjects were used in all, in a total of fifteen complete experiments, to determine the effects of four different drugs. A total of 1699 coagulation time measurements were made in the series. The procedure was as follows: The lobe of the ear was pricked with a medical lancet, adjusted by a guard to give a constant depth of penetration. The capillary tube was filled immediately from the drop of blood, and held in a fold of the palm of the hand to maintain a fairly constant temperature. Every ten to fifteen seconds, a short piece of the capillary tube was broken off, and the coagulation time taken as that when a filament of fibrin strung out between the two pieces of tubing. The time was measured in seconds from the first instant a drop of blood appeared on the ear to the observed time of clotting. Each subject made observations on his own clotting time, taking blood from the ears alternately, usually twice a day, for about two weeks. The coagulation times were always taken at the same time of day and with a constant relationship to meals and other activities and were so arranged as to be taken at the time of the supposed maximum action of the drug. This procedure was then continued for a further two-week period, during which the drug in question was taken. The

period of medication was followed by a second control period of similar duration to that of the first.

The average coagulation times for each period were calculated and the standard deviation of the mean or average (SD_m) derived by the usual formulas. Comparison of the control with the medication periods was made by calculating the standard deviation of the difference between the means ($SD_{diff.\ means}$). An estimate of the reliability of the observed difference was then obtained by calculating the critical ratio (CR), which is the ratio of the difference between the two means to the $SD_{diff.\ means}$, and the probability of significance of this secured from standard probability tables. As it requires a CR of 2.0 or more to give a probability of 20 to 1 or better that the result may not be due to chance, it is believed that any CR less than 2.0 is not proof of a probably true difference between the two series. It must be emphasized at this point that when the CR is greater than 2.0, the differences are not necessarily biologically or clinically significant. The fact is simply established that the odds are very great against chance alone being responsible for a difference of the magnitude observed. Then, if it is accepted that the difference between the average or mean results was not a chance variation, it is in order to inquire whether this difference is of sufficient magnitude to be of any importance in the practical application of the drug in question.

For example, it might be perfectly feasible, by performing a sufficiently large number of experiments, to show that some given drug would change an average coagulation time of 200 seconds to 195 seconds, and it could then be predicted with a high degree of certainty that, if the medication were repeated over and over again, this would almost always be the average result. However, such a point having been proved, the interpretation would still be quite obvious that, for all practical purposes, the medication in question was of no particular value, because, if it could only produce a shortening of coagulation time of five seconds out of a total time of 200 seconds, it would have so little influence under normal conditions as to be practically useless. Therefore, the statistical treatment of the data in this paper does not, in itself, prove that the hemostatic agents in question are or are not valuable drugs to use. It merely allows a determination with certainty as to whether the data are reliable enough to use the averages as a basis for conclusions. The results may now be described according to the agents tested.

CALCIUM

Calcium is known to play an indispensable rôle in the coagulation of blood. It is by no means established, however, that a prolonged coagulation time is frequently associated with an inadequate blood calcium content. In the years 1893 to 1905, Wright and his co-workers advocated the use of calcium by mouth for a wide variety of clinical conditions, including the control of hemorrhage and the acceleration of the coagulation process. There is considerable evidence that injection of calcium can shorten the coagulation time, but there is less certainty that the same is true of oral administration.

In spite of negative observations made under reliable conditions by Addis,[1] Dale and Laidlaw[2], and Loewenstein and Politzer[3] reports appear from time to time of the effective action of calcium when taken by mouth, and textbooks and writers of clinical articles advocate the use of calcium by mouth preoperatively to diminish bleeding. Examples of observation of positive results from oral administration of calcium are those of Colemann[4] and Hinman and Sladen.[5] More recent advices to use calcium chloride or calcium lactate in this way are those of Thibault[6] and Fitzgerald.[7] There is some evidence, although not very convincing, that calcium gluconate is better

absorbed than either of the other two salts, and therefore it might have a better prospect of being effective orally. Further evidence that improved absorption of calcium might be desirable is seen in the results of Matusevich,[8] who found that the addition of ammonium chloride, which is known to increase absorption of calcium, shortened the coagulation time more than addition of calcium chloride alone.

In order to fix firmly the normal value and its natural range of variability, in none of these positive studies was the coagulation time measured over a long period before medication was started. By the use of a more arduous technic, our study affords adequate control observations, which have hitherto been lacking. It also shows any protracted or slowly developing effects, which might be missed in observations for only a few days.

In our tests with calcium, the four subjects took control observations for from thirty-two to forty-five days, divided into control periods before and after medication. Calcium gluconate was administered for from fourteen to twenty days, during which time the coagulation time observations were continued. The dosage of calcium gluconate was 13.5 gm. a day, taken in the form of chocolate-coated tablets with each meal.*

Figure 1 shows that the greatest decrease in the average coagulation time was thirty-four seconds, in one subject. In another subject, the average time was increased by fifteen seconds. In the two others, the changes were so slight as to be of no significance. If the two control periods are considered together for comparison with the period of medication, as shown in accompanying table, in only one subject was there enough change to have a CR over 2; that is, beyond the range of chance fluctuation. The average change in coagulation time produced by the administration of calcium [illegible] subjects was only a decrease of [illegible] seconds out of an average of [illegible] is therefore quite certain that [illegible] tion of calcium in the [illegible] dose, for [illegible] cannot be relied [illegible] lation time by [illegible] The small decrease [illegible] ject may be much [illegible] ascribed to [illegible] tion, or to a [illegible] to diet, etc., that [illegible]

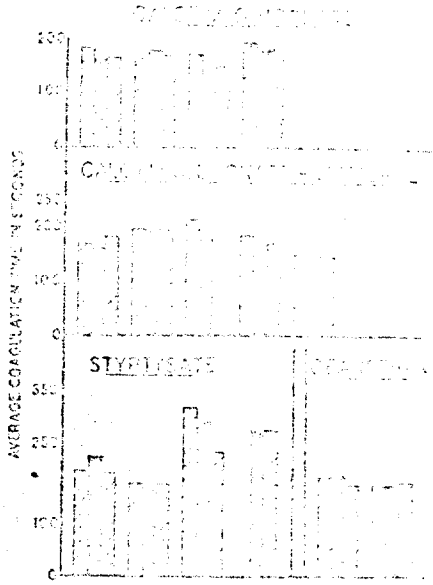

Fig. 1.—Variation in coagulation time.

the drug, inasmuch as no similar effects were seen in the other three subjects.

CALCIUM GLUCONATE AND VIOSTEROL

In view of the fact that Matusevich observed a shortening of coagulation time with calcium when absorption was increased by the use of ammonium chloride, an attempt was made to obtain the same result by a more normal means of increasing calcium absorption; that is, by the administration of vitamin D. [illegible]

*We are indebted to E. R. Squibb & Co. for the calcium gluconate and viosterol used in this study.

AVERAGE COAGULATION TIMES IN CONTROL PERIODS AS COMPARED WITH PERIOD OF MEDICATION*

Subject	Control Period				Medication Period					
	Days No.	Observations No.	Coagulation Time Sec.	SD mean Sec.	Days No.	Observations No.	Coagulation Time Sec.	SD mean Sec	SD diff. means Sec.	Critical Ratio
					Calcium gluconate, 13.5 gm. a day					
Tn	32	64	170	3.8	19	38	182	6.0	7.1	1.70
Tn	35	70	176	3.8	17	33	164	14.7	15.2	0.79
Er	45	45	190	2.1	14	14	183	4.0	4.5	1.60
Ci	34	34	161	5.2	20	20	140	4.7	7.0	3.00
Average coagulation time			174				167			
					Calcium gluconate, 13.5 gm. and viosterol 1 cc. a day					
Tn	29	58	197	3.7	19	38	194	5.1	6.3	0.47
Tn	29	58	178	3.3	15	29	162	2.9	4.4	3.60
Ed	14	28	215	3.9	12	24	180	8.2	9.1	3.80
Er	45	45	183	2.9	14	14	134	8.0	8.5	5.80
Ci	30	30	151	4.4	14	14	150	6.0	7.4	0.14
Average coagulation time			185				164			
					Styptysate, 9 tablets per day					
Tr	30	60	171	4.4	15	30	160	5.8	7.3	1.50
Br	20	39	274	9.6	11	22	290	13.0	16.2	0.99
Tn	31	59	195	2.2	14	28	223	8.4	8.7	3.20
Bs	9	12	275	8.4	24	27	273	7.8	11.5	0.17
Average coagulation time			229				237			
					Ceanothyn, 30 cc. a day					
Tn	31	61	184	6.4	14	28	190	11.9	13.5	0.44
Tr	30	60	177	3.6	10	17	173	5.3	6.4	0.63
Average coagulation time			181				182			

* $SD_{diff.\ means}$ is the standard deviation of the difference between the mean or average of the control period and the period of medication. Critical ratio is the difference between these two means divided by the $SD_{diff.\ means}$.

established that vitamin D largely controls the normal calcium absorption from the gastrointestinal tract, and that administration of increased amounts of this vitamin brings about an increase in calcium absorption. Apparently, however, the appli-

[illegible] of this fact to shortening of coagulation time clinically has received little attention. Bilyey[illegible] in 1931, gave massive, and probably toxic, doses of irradiated ergosterol to rats and observed a decrease in the coagulation time. Similar observations were made by Phillips, Robertson, Carson and Irwin in 1931.[10] The only similar clinical report we have seen is that of Bingham,[11] who, in 1930, reported that administration of cod-liver oil or viosterol in therapeutic doses reduced the coagulation time by from one-half to two-thirds. However, in his experiments, there was apparently only a single control observation on each subject before medication, and this lack of adequate control leaves the validity of the observations in question.

In our study, calcium gluconate in a dose of 13.5 gm. was given as before, and with this there was taken daily a dose of 1 cc. of viosterol. This has a vitamin D potency of 10,000 U.S.P. vitamin D units per gram, so that our subjects were getting about three times the usual maximum therapeutic dose. The control periods were maintained before and after the medication, as before. The accompanying graph shows that, in the first, third and fourth subjects, there were decreases in the average coagulation time, but only in the third and fourth subjects were they of any appreciable magnitude. The greatest effect observed was a drop from 187 seconds in the precontrol period to 134 seconds under medication, followed by recovery to 172 seconds during the second control period. When the control periods are taken together for each subject, the results, as shown in the accompanying table, were in at least three of the subjects, large enough in comparison to the variability of the data to be statistically reliable. That is, they had a CR over 2, and so there was little chance that the decreases in average coagulation times observed were due to chance fluctuations. However, in these three subjects, the average coagulation time in subject Tn was decreased only sixteen seconds, in Ch, thirty-five seconds, and in Ec, [illegible] seconds.

Hence these results, even though they might be reliable as far as the [illegible] probably, still are not of [illegible] to be of any [illegible] except perhaps in subject Ec. [illegible] of the values [illegible] of only twenty-one seconds, or [illegible] per cent, and a change of this [illegible] is not great enough to be of any [illegible] value. Therefore, it is concluded from these data that, although there is evidence that massive doses of [illegible] viosterol together may decrease the coagulation time in short periods in some subjects, the changes that can be produced by these means are too small to be of any [illegible] use. Such a result is in accord with [illegible] concepts of the stability of the [illegible] calcium, since it is generally [illegible] that changes in the amount of [illegible] calcium in the blood are produced [illegible] with great difficulty.

STYPTYSATE

Styptysate is made by an unknown method from Capsella bursa-pastoris, or "shepherd's purse." It came into prominence in Germany during the War, for the control of uterine bleeding, as a substitute for ergot. From this use, styptysate came to be used by some for control of hemorrhage from other regions, despite a lack of adequate consideration of the differences in the mechanisms involved in bleeding from the uterus and in urinary vascular injuries. Styptysate is now the subject of occasional favorable reports,[12] and it is frequently referred to in dental texts for use before operations. This compound has been the subject of adverse reports by both the Council on Pharmacy and Chemistry of the American Medical Association[13] and the Council on Dental Therapeutics of the American Dental Association.[14] The conclusions in these reports were that no evidence was available that styptysate had any [illegible]

on the coagulation time in ordinary bleeding.

In our study, styptysate was taken by four subjects, in a daily dose of nine tablets a day, or three with each meal, a dose which is as large as, or larger than, that clinically recommended. The accompanying graph shows that, in the second subject, there was a very slight decrease in coagulation time; and in the third subject, a decrease when compared with the precontrol period, but an increase over the postcontrol period. In the fourth subject, there was no change at all; whereas, in the first subject, there was a rather definite delay of coagulation. When the control periods are averaged, as shown in the table, it is seen that the only change of statistical reliability was the increase in average coagulation time seen in subject Tu, and this was an increase of twenty-eight seconds. The average result for the entire series was an increase in the average time of eight seconds. There can be no question, therefore, that, in this study, where an adequate number of control data are available for comparison, styptysate completely failed to decrease the coagulation time. Therefore, it has no therapeutic justification for use as a hemostatic.

CEANOTHYN

Ceanothyn is said by the manufacturer to be an extract of the alkaloids of Ceanothus Americanus L., or "Jersey tea." It is said to have been recommended originally, in 1836, in the *Boston Medical and Surgical Journal*,[15] and also to have been referred to by Hammond as a hemostatic in the *Georgia Eclectic Medical Journal*, in 1879.[16] Reports bearing on the chemistry and pharmacology of these alkaloids have been recently published.[17, 18, 19] The claims made were that administration of ceanothyn to experimental animals or patients by mouth, or by injection, shortens the coagulation time by as much as one-half the original. As none of these studies were adequately controlled, the validity of the conclusions may be questioned. Moreover, Gibbs, in a critical study, adequately controlled, showed that even as much as 80 cc. of ceanothyn taken by mouth did not reduce the coagulation time.[20] However, one article has been published in which the coagulation time was measured in 225 patients, with a decrease in the time required for clotting in every single instance.[21] Apparently, the author made single control observations, followed by the administration of 15 or 30 cc. of ceanothyn by mouth, and then, at the end of about one hour, took a second coagulation time measurement. The average decrease was about 22 per cent. A decrease after medication in every single instance in a series of 225 patients raises a serious question as to whether there was some systematic error in the method, or in the treatment which the patients were receiving simultaneously. Other writers also occasionally advocate the use of ceanothyn before operations and apparently have some confidence in its efficacy.[22] Ceanothyn has been reported on by the Council on Pharmacy and Chemistry of the American Medical Association, with rejection of claims for effects on the coagulation process.[23]

In our studies, adequate controls were established as previously described, so that the defect in some of the previous positive reports would not be present. Only two subjects were used because the result came out so convincingly negative that it did not seem worth while to extend the series further. As shown in the graph and table, the subjects had control observations for a total of one month each and periods of medication of ten and fourteen days, respectively, during which time they took 30 cc. of ceanothyn daily. In one patient, the average coagulation time was increased by six seconds; in another, it was decreased by four seconds. It is apparent that no significant change in the coagulation time was produced by the administration of maximum doses of this product.

COMMENT

It is generally conceded by the critical workers in the field of hemostatics that the use of these materials by mouth has no real effect on the speed of the coagulation process. Our results, which supply, for the first time, measurements made enough times that a reliable average figure could be obtained, confirm this sound clinical opinion. Therefore, the results of single measurements made by the more reliable workers in the past, and the repeated measurements made by us on the protracted actions of the drugs, are in essential agreement; namely, that administration of calcium, viosterol, styptysate and ceanothyn by mouth does not reduce the coagulation time of normal patients by an amount great enough to be of any clinical value. There would, therefore, seem to be little reason for the clinician to continue the use of these materials.

Out of a desire to do everything possible for the patient in difficult cases of bleeding undoubtedly arises the uncritical but hopeful use of these drugs. However, it is believed that, although the motives may be above question, the result of using these drugs cannot but be disadvantageous to the patient. The surgeon who relies on such ineffective measures to control bleeding is apt to disregard or to slight other more effective measures; so that the result of relying on these valueless drugs is to run the danger of depriving the patient of more adequate means to prevent the hemorrhage or to control it. Therefore, we believe that the use of these alleged hemostatic products is irrational, unscientific and thoroughly unjustifiable in clinical practice.

SUMMARY AND CONCLUSIONS

1. The coagulation time of healthy human subjects was measured once or twice daily for about six weeks under constant conditions. The first and last two weeks of this period were taken as control periods, and the average times observed were compared with the [illegible] of the middle two weeks period during which medication was administered. [illegible] observed were averaged, [illegible] statistically computed to [illegible] degree of variability and the [illegible] that the differences [illegible] effects of the drugs [illegible] fluctuation.

2. Administration by mouth of calcium gluconate in daily doses of [illegible] produced an average decrease in coagulation time in four subjects of only [illegible] seconds, which was within the range of natural variability and was not [illegible] be of therapeutic value.

3. The same doses of calcium gluconate with the addition of [illegible] U.S.P. daily, in five subjects decreased the average coagulation time for the whole series by twenty-one seconds, or [illegible] cent. However, there was [illegible] change in two of the five subjects [illegible] the remainder, the changes were so small as to be clinically of little, if any, value.

4. In four subjects, nine tablets daily of styptysate failed to reduce the coagulation time, the average change for all four being an increase of eight seconds.

5. Administration of ceanothyn in a daily dose of 30 cc. to two subjects left the coagulation time completely unchanged.

6. The results of this study, in which unusual care was taken to establish satisfactory control values and their change variability, show convincingly that even two weeks continuous administration of these alleged hemostatic agents fails to modify significantly the coagulation time of the blood when used by mouth in maximum therapeutic dosage. Therefore, there is no justification for their continued clinical use with the hope of shortening coagulation time or reducing bleeding.

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The Availability of Phytic Acid Phosphorus for Chicks.

3. EFFECT OF CALCIUM AND VITAMIN D_3 LEVELS ON THE UTILIZATION OF CALCIUM PHYTATE[1]

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PREVIOUS reports (Harms *et al.*, 1962; Waldroup *et al.*, 1964) have indicated that phytic acid phosphorus and dicalcium phosphate were of equal availability to the chick. However, a widening of the calcium:phosphorus ratio decreased the relative availability of the phosphorus in phytic acid to a greater degree than phos-

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phorus from dicalcium phosphate. The effect of Ca:P ratio on phytin phosphorus availability was previously reported by Vandepopuliere *et al.* (1961). They reported that plant source phosphorus was readily available to support growth in chicks when fed at a Ca:P ratio of 1:1 but did not permit increased bone ash as did dicalcium phosphate when the ratio narrowed from 4:1 to 1:1. The availability of phytic acid phosphorus to the rat has been reported to be markedly affected by the calcium and vitamin D content of the diet (Krieger and Steenbock, 1940).

In view of these reports, experiments were conducted to further define the influence of calcium and vitamin D_3 levels on the utilization of phosphorus from calcium phytate, which has been shown to be of lower availability than phytic acid or sodium phytate phosphorus (Waldroup *et al.*, 1964).

MATERIALS AND METHODS

A practical type diet using degerminated corn meal as the major energy source and dehulled soybean meal as the major protein source served as the basal diet (Table 1). This diet was analyzed to contain 0.30% phosphorus (all from plant sources), and was calculated to contain 20 percent protein, and 1,000 kilo-calories of productive energy per pound of feed.

Day-old broiler type chicks obtained from a commercial hatchery were sexed, debeaked, wing banded, and intraocularly vaccinated for Newcastle disease and infectious bronchitis. They were randomly assigned to pens in electrically heated battery brooders with raised wire floors. Three pens, each containing 5 male and 5 female chicks, were assigned to each of the experimental treatments. The experimental feeds and tap water were supplied *ad libitum*.

TABLE 1.—*Composition of basal diet*

Ingredient	%
Degerminated corn meal	56.7
Dehulled soybean meal (50% protein)	34.0
Dehydrated alfalfa meal (20% protein)	3.0
Iodized salt	0.4
Micro ingredients[1]	0.9
Variable ingredients[2]	5.0

[1] Supplied per kilogram of diet: 6,930 I.U. vitamin A, 770 mg. choline, 40 mg. niacin, 4.4 mg. riboflavin, 2.0 mg. calcium pantothenate, 22 mcg. vitamin B_{12}, 20 mg. iron, 2.0 mg. copper, 198 mcg. cobalt, 11 mg. iodine, 100 mcg. zinc, 220 mg. $MnSO_4$, and 12.5 mg. ethoxyquin.

[2] Consisted of calcium carbonate, phosphorus source, and oat hulls in amounts calculated to attain desired calcium and phosphorus levels.

The data were subjected to analysis of variance as outlined by Snedecor (1956) with significant treatment means determined by the multiple range test of Duncan (1955).

Experiment 1. Calcium phytate was compared to dicalcium phosphate at levels of 0.05, 0.10, 0.20, and 0.30 percent added phosphorus at calcium to total phosphorus ratios of 0.8:1, 1.4:1, and 2:1. A level of 360 I.C.U. of vitamin D_3 per pound was used in each of these diets. In addition, vitamin D_3 (1440 I.C.U. per pound of feed) was added to diets supplemented with 0.20 percent phosphorus from calcium phytate at each of the three calcium: phosphorus ratios.

The calcium phytate supplement,[4] described as phytic acid calcium-magnesium salt with an analysis of 12 percent calcium, 1.5 percent magnesium, and 22 percent phosphorus, was in the form of a white odorless powder insoluble in water.

At twenty-one days of age the chicks were individually weighed and two male and two female chicks from each pen were sacrificed. The right tibia was re-

[4] Corn Products Co., New York, N. Y.

moved, cleaned of adhering tissue, and bone ash determined by the A.O.A.C. (1960) method.

Experiment 2. A 2^4 factorial arrangement of two phosphorus sources (calcium phytate *vs.* dicalcium phosphate), two supplemental phosphorus levels (0.10 *vs.* 0.20 percent), two calcium levels (0.50 *vs.* 1.00 percent), and two vitamin D_3 levels (360 *vs.* 6,000 I.C.U. per pound) was used in this test. The chicks and the basal diet were prepared as in Experiment 1. The experimental diets were fed for 21 days at which time individual body weights were obtained.

RESULTS

Experiment 1. Twenty-one day body weight and tibia ash were significantly affected by the source and level of phosphorus, the Ca:P ratio and the vitamin D_3 level of the diet (Tables 2 and 3). As expected, there were significant interactions among the factors of phosphorus source, level of added phosphorus, and the calcium:phosphorus ratio of the experimental diets. At the lowest Ca:P ratio, (0.8:1), the addition of 0.05 or 0.10 percent phosphorus from calcium phytate increased body weight and tibia ash, with the increase from the 0.10 percent level statistically significant. A further increase in phosphorus from this source had no additional effect. In fact the addition of 0.30 percent phosphorus from calcium phytate resulted in body weight and tibia ash values similar to those observed on the basal diet with no added phosphorus. In comparison, levels of dicalcium phosphate up to 0.30 percent added phosphorus significantly increased body weight and tibia ash.

A comparison of the two sources at the 0.8:1 Ca:P ratio indicates that, with the exception of the diet containing 0.30 percent added phosphorus from calcium phytate, there were no significant differences in body weight or tibia ash at comparable phosphorus levels. In several instances, body weight was numerically greater for the calcium phytate groups (Table 2), while dicalcium phosphate tended to produce a greater degree of bone calcification (Table 3).

An increase in the Ca:P ratio to 1.4:1 significantly depressed body weight and tibia ash of the chicks fed diets supple-

TABLE 2.—*Twenty one-day body weight of chicks fed different phosphorus sources, levels of vitamin D_3, and calcium:phosphorus ratios (Experiment 1)*

Phosphorus source	% Added phos.	Vit. D_3 I.C.U./lb.	Calcium:Phosphorus ratio 0.8:1	1.4:1	2:1	Average
			Body weight (grams)[1]			
Basal[2]	0	360	238^{fg}	262^{hij}	202^{e}	234
Calcium Phytate	0.05	360	251^{gh}	229^{def}	184^{b}	221
	0.10	360	281^{klm}	238^{fg}	186^{b}	235
	0.20	360	279^{jkl}	213^{cd}	163^{a}	218
	0.20	1,440	284^{lmn}	268^{hijkl}	175^{ab}	242
	0.30	360	235^{efg}	220^{de}	160^{a}	205
Dicalcium Phosphate	0.05	360	264^{hijk}	267^{hijkl}	228^{def}	255
	0.10	360	272^{ijkl}	274^{ijkl}	259^{hi}	278
	0.20	360	274^{ijkl}	300no	285^{lmn}	275
	0.30	360	315°	299no	297^{nm}	304

[1] Means bearing same superscripts do not differ significantly (Duncan, 1955). Means are average weights of 15 male and 15 female chicks.
[2] Basal diet contained 0.30% phosphorus, all from plant sources.

TABLE 3.—*Twenty one-day tibia ash of chicks fed different phosphorus sources, vitamin D_3 levels and calcium:phosphorus ratios (Experiment 1)*

Phosphorus source	% P Added	Vit. D_3 I.C.U./lb.	Calcium:Phosphorus ratio 0.8:1	1.4:1	2:1	Average
			Tibia ash (%)[1]			
Basal[2]	0	360	30.91[cd]	29.40[bc]	25.88[a]	28.73
Calcium Phytate	0.05	360	33.32[de]	27.45[ab]	26.48[a]	29.08
	0.10	360	33.79[e]	26.48[a]	25.71[a]	28.78
	0.20	360	34.37[ef]	25.34[a]	27.63[ab]	29.11
	0.20	1,440	33.42[de]	33.52[e]	27.57[ab]	31.50
	0.30	360	29.46[bc]	25.12[a]	25.16[a]	26.58
Dicalcium Phosphate	0.05	360	34.23[ef]	29.90[be]	25.26[a]	29.80
	0.10	360	35.03[ef]	35.04[ef]	31.17[cd]	33.75
	0.20	360	36.54[fg]	39.80[hi]	37.71[gh]	38.02
	0.30	360	37.87[gh]	41.28[i]	41.03[i]	40.06

[1] Means bearing same superscripts do not differ significantly (Duncan, 1955). Means are average tibia ash of 6 male and 6 female chicks.
[2] Basal diet contained 0.30% phosphorus, all from plant sources.

mented with phosphorus from calcium phytate. A further increase in the Ca:P ratio to 2:1 severely lowered body weights but had little additional effect on bone ash of chicks consuming diets supplemented with calcium phytate. Chicks fed comparable diets supplemented with phosphorus from dicalcium phosphate were not as severely affected by increases in the Ca:P ratio. This was observed as a source×ratio interaction that was highly significant for both body weight and tibia ash.

At the 2:1 Ca:P ratio, higher levels of added phosphorus from calcium phytate (*i.e.*, 0.20 and 0.30 percent) produced a more severe depression in body weight than did lower levels. However, this effect was not as severe when tibia ash was the criterion. Chicks fed the diets containing dicalcium phosphate tolerated the 2:1 Ca:P ratio to a greater extent than did chicks fed the calcium phytate supplemented diets. As the level of phosphorus from dicalcium phosphate increased, less depression in body weight or tibia ash was observed.

Increasing the level of vitamin D_3 (1440 vs. 36 I.C.U. lb.) in the diet supplemented with 0.20 percent phosphorus from calcium phytate significantly improved body weight and tibia ash at a Ca:P ratio of 1.4:1 (Tables 2 and 3). At Ca:P ratios of 0.8:1 and 2:1, body weight was numerically but not significantly increased by the addition of the higher level of vitamin D_3. However, little effect was observed on tibia ash at these Ca:P ratios.

Experiment 2. Calcium phytate was inferior to dicalcium phosphate as a phosphorus source at all levels of phosphorus, calcium and vitamin D_3 supplementation as measured by body weight (Table 4). The availability of both sources was significantly affected by alterations in the calcium and vitamin D_3 levels in the diet.

At either level of calcium supplementation, increasing the vitamin D_3 level of the diet from 360 to 6,000 I.C.U per pound resulted in an increase in 21-day body weight. This increase was statistically significant at all levels of supplementation with calcium phytate and at the 0.10 percent phosphorus level of supplementation with dicalcium phosphate. The increase in body weight resulting

from the increased vitamin D_3 supplementation at higher levels of supplementation with dicalcium phosphate was numerically superior at the 1.0 percent level of calcium and significantly superior at the 0.5 percent level of calcium.

Chicks fed diets supplemented with phosphorus from calcium phytate were heavier when the diet contained 0.5 percent calcium that at 1.0 percent calcium. Diets containing 0.10 percent phosphorus from dicalcium phosphate also supported greater growth at the lower calcium level, but when the supplemental phosphorus level was increased to 0.20 percent from dicalcium phosphate, growth on the higher calcium level was favored when both levels of supplemental vitamin D_3 were combined. However, the maximum growth at this level of phosphorus supplementation resulted when the calcium level of the diet was 0.50 percent and the vitamin D_3 level was 6,000 I.C.U. per pound.

DISCUSSION

The results of these experiments demonstrate a close relationship between calcium and phosphorus levels and ratios and the vitamin D_3 level of the diet upon the utilization of the phosphorus from calcium phytate. Low calcium levels or low Ca:P ratios tended to improve the availability of the calcium phytate phosphorus. In several instances the improvement in phosphorus availability was such that calcium phytate phosphorus was equal to dicalcium phosphate phosphorus in promoting growth or bone calcification. These low calcium levels, however, were not the most desirable levels for optimum performance on diets supplemented with dicalcium phosphate. Higher levels of calcium or wider Ca:P ratios were required for optimum performance on dicalcium phosphate supplemented diets; these higher calcium levels lowered the availability of the calcium phytate phosphorus.

Increasing the level of vitamin D_3 in the diet (1,440 or 6,000 *vs.* 360 I.C.U./lb. of feed) improved the utilization of phosphorus from both calcium phytate and dicalcium phosphate at certain Ca:P levels and ratios. This would be expected since Waldroup *et al.* (1963) reported the vitamin D_3 requirement of chicks to be increased at unfavorable calcium:phosphorus ratios.

SUMMARY

Two experiments were conducted to study the effect of calcium and phosphorus levels and ratios and vitamin D_3 levels on

TABLE 4.—*Twenty one-day body weight of chicks fed different phosphorus sources with varying levels of calcium and vitamin D_3 (Experiment 2)*

Phosphorus source	% Phos. added	Vit. D_3 I.C.U./lb.	1% Calcium 360 D	1% Calcium 6,000 D	0.5% Calcium 360 D	0.5% Calcium 6,000 D
			Body weight (grams)[1]			
Basal[2]	0		114^{a}	208^{d}		
Calcium Phytate	0.10		129^{b}	227^{efg}	206^{d}	251^{hi}
	0.20		134^{b}	217^{de}	217^{de}	268^{j}
Dicalcium Phosphate	0.10		218^{e}	241^{gh}	276^{j}	294^{k}
	0.20		265^{ij}	277^{j}	237^{fgh}	298^{kl}

[1] Means bearing the same superscript do not differ significantly (Duncan, 1955). Means are average body weights of 15 male and 15 female chicks.
[2] Basal diet contained 0.30% phosphorus, all from plant sources.

the utilization of the phosphorus of calcium phytate as compared to dicalcium phosphate. Low calcium levels and narrow Ca:P ratios tended to improve the relative availability of calcium phytate phosphorus. These low levels of calcium were not the most desirable levels for optimum performance on diets supplemented with dicalcium phosphate.

Increasing the vitamin D_3 level of the diet above 360 I.C.U./lb. of feed tended to improve the utilization of phosphorus from both calcium phytate and dicalcium phosphate.

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The Problem of Drug Addiction*

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WHEN the Harrison Narcotic Act was passed by the Federal Government in 1915, it had as its basic premise the restriction of the use and sale of morphine and its derivatives. It was further designed that these drugs would not be legally available for drug addicts. The law required that the doctor who prescribed the drug to his patient should give the patient's name and address as well as his own and his registration number, and be prepared to give any other essential features justifying the use of the drug. This Act is administered by the Division of Narcotics of the Bureau of Internal Revenue, which regulates the distribution of opium and its derivatives in the United States and its possessions.

In spite of this law there is now flourishing in the United States an illegal narcotic trade greater than ever. Its ramifications and extension even to teen-agers and high school and college students are of servious concern to the Federal Government so that new laws have been passed to punish more severely those who sell or deal in this traffic.

The governments of the world have more or less agreed on certain laws as to the sale, import and cultivation of this drug because of the evil that has been associated with it.

These drugs while of greatest use in the relief of pain, gastro-intestinal upsets and colds, when used in excess soon produce addiction. Here certain personality changes take place. The drug soon undermines the emotions and morals of its user—perhaps more than any other drug. At first it expands the ego and gives him an exaggerated personal value of happiness and contentment—a feeling of well-being never previously attained. But by the same capacity that it takes the individual to such heights of ecstasy, it can also produce feelings of depression and wretchedness never previously experienced. It is during this period that the patient loses all sense of responsibility, becomes antisocial and shows criminal tendencies as pity and remorse are removed and the need for the drug becomes the paramount factor in daily life.

The drugs considered here are opium and its derivatives morphine, heroin, eukadal and codeine, although the last-named rarely have ever caused addiction. Of greatest concern and use, however, is heroin which is being considered in this paper.

The patient, after using this drug for a while, soon comes to the conclusion that he is going in a direction not conducive to his best interest. But he also finds he cannot stop as readily as he desires. He has become addicted. When he tries to stop the experience is a most painful one, and he becomes deathly sick. Symptoms of yawning, lacrimation, rhinorrhea, perspiration, goose flesh, tremors, twitching of muscles, dilated pupils, nervousness, restlessness, aching legs and bones, hyperpnea, elevation of blood pressure, loss of weight, and fall in B.M.R. are noted when the drug is withdrawn and are characteristically known as "withdrawal symptoms."

The Federal Government, in order to cope with addicts, has set up an institution in Lexington, Kentucky to treat such patients. It is my opinion that this institution cannot take care of this problem alone. Besides, there are some features that make treatment there somewhat objectionable as the distance from home, the inability to follow up these cases after they are discharged, and the need to remove the psychology of punishment from the treatment.

Drugs and preparations used in the treatment of the withdrawal reaction in drug addiction are as numerous as those used in the treatment of arthritis. There were over fifty-five found in the literature, some of which are here given:

1. Electric shock
2. Galvanic current
3. Apomorphine
4. Insulin
5. Prolonged sleep
6. Thiamin chloride and nicotinic acid complex
7. Prostigmin
8. Cannabis indica

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9. Tolserol
10. Curare—curare-like preparations
11. "Cold Turkey"
12. Belladonna preparations
13. Glucose treatment
14. Vitamin C
15. Penicillin
16. Dilantin sodium
17. Barbiturates
18. Lobotomy
19. Pyrobenzamine
20. Auto-sero therapy
21. Lecithin-glucose combined
22. Benzedrine and its derivatives
23. Xanthine derivatives (diuretics)
24. Sodium chloride
25. Sulfonamides
26. Purgatives
27. Hormones
28. (Synthetic) Demerol
29. Histamines
30. Slow withdrawal—substitute therapy (codeine or morphine)

It is equally important that nearly all investigators claimed some degree of success with their particular methods, therefore, any new method or drug added to the list must seek to lessen confusion and contribute considerably to the therapy.

Some years ago when forced by an addict to relinquish my medical bag and its contents at the point of a gun, I was at the same time forced to administer to this patient nicotinic acid and calcium gluconate intravenously. As these were the only preparations I then possessed, I explained that these drugs were not harmful and would have a minimal or limited value in his condition. When forced to give him this medication as his friend held the gun, to his amazement and mine, he was definitely relieved of his withdrawal symptoms following the intravenous administration of calcium gluconate. He confiscated the two other vials of calcium gluconate and then returned to the bag.

This factor was never lost sight of for in the intervening years, we too found calcium gluconate to be most efficacious in the handling of the withdrawal symptoms of drug addicts. In the course of handling such patients, therefore, a routine was established which consisted of: 1) relief from the withdrawal symptoms; and 2) attempts to get the individual readjusted to society.

Patients seen during the withdrawal period were given 10 c.c. of a 10-20 per cent solution of calcium gluconate (Sandoz) five times daily for four days, and four times daily for three days, and thereafter only periodically to control anxiety. In instances where such patients were extremely agitated and restless about 2 grs. q.i.d. of barbiturates were given intravenously daily in the calcium gluconate for two or three days, and this usually sufficed.

Pain of muscles and bones, and sleep were usually two problems that persisted. It was observed that patients given intravenously 2500 c.c. of glucose in N/solution daily slept better and showed little or no pains whatever. Vitamins were usually given to induce appetite.

Equally important to the patient was the "why" of the use of drugs. Psychotherapy was carried out to help the patient get a proper insight into this condition. Unfortunately this was the greatest source of failure.

While many patients often took treatment only long enough so as to be able to reduce the intake commensurate with their income, by far the overwhelming number were those who failed to follow through psychotherapeutically. Once the withdrawal symptoms were gone these patients for the most part felt quite capable of coping with any and all situations and soon found or gave what to them were excellent reasons why they could no longer continue treatment. The result has been quite a number of repeaters; but even after repeating once they were well the results were often the same again—the refusal to follow through from the viewpoint of psychotherapy. It is conceivable that some legislation might be of some aid in this respect.

TWO CASES ARE HERE BRIEFLY CITED

R. J., a 26-year-old boxer while celebrating after a successful fight, was advised if he wanted to get real "high" it could be arranged. He declared he had no idea what was given to him, but that while under the influence of alcohol he submitted to this treatment. The next night he went to another place of amusement. There again the same offer was made and once more he accepted, thinking these individuals were trying to make him feel happy. The following evening he reported to one of those who had given him this drug how miserable he felt, and the next day he was advised that he had been given too much "stuff" and thus submitted to a third dose which made him feel equally miserable. He was advised by someone with whom he discussed the matter that he had been given "drugs" and he decided to discontinue its use. However, about ten days later when he went back to practice he felt effortless, and

his manager felt his "timing" was off. About two days before his next fight, he again took heroin and he felt much better. While he was good in the early rounds, he was mercilessly beaten in the end. Eventually he became a regular "user" of drugs and this ended his career as a boxer. The patient when hospitalized was given 10 c.c. of a 20 per cent solution of calcium gluconate (Sandoz) eight doses daily for four days, followed by four doses daily for three days, and one dose daily for three days. Withdrawal symptoms disappeared on the fifth day. During this time the patient also received Brewer's yeast tablets (12 daily), and 2000 c.c. of 5 per cent glucose in N/saline intravenously. Sleep as a factor was not much disturbed as the patient had difficulty sleeping only the first two nights, and received intravenously 2 grs. of Nembutal combined with 2 grs. of sodium amytal. Psychotherapy was carried out daily for ten days, but on the eleventh day the patient decided to leave the hospital pointing out how wonderful he felt, and that he had a job he had to accept right away. We had no choice in this matter.

J.S., a 17-year-old high school student who was given quite a large allowance by indulging parents, was soon urged to smoke reefers, and later introduced to "snuff" by peddlers since he was afraid of being stuck by a needle. Eventually the "snuff" was withdrawn and he had to accept being stuck by a needle. When his grades fell down, his parents soon found out he had been addicted and owed quite a large sum of money which his parents paid. The patient was hospitalized and given treatment similar to that of the previously mentioned patient. However, he had a desire to sleep all the time, probably being ashamed of being in a hospital. He was given four capsules of sodium amytal (1 gr. each) at bedtime for six days, and thereafter only when he requested it. Psychotherapy was disrupted because he wanted to return to school and not lose the semester. He broke appointments frequently at first, but upon strong pressure from his parents he continued this phase of his treatment until he had good insight. He completed his high school work and then went on to college.

COMMENT

The use of calcium gluconate in the use of drug addiction could be considered as a complete withdrawal in the sense that no morphine or codeine in reduced dosage is used. On the other hand, it might also be considered substitute therapy since another drug is used in the place of morphine or its derivatives and administered similarly in gradually reduced dosage. Of import is the fact that the patient experienced no pain with the use of calcium gluconate.

So far as we are now aware no contraindications have been noted, except for bad or poor veins. The greatest difficulty is to get the patients to accept this preparation. They often wait quietly for their symptoms to return in order to be given a "shot." When given calcium gluconate a second or third time and pains continue to be absent, they then gradually began to accept this therapy. The success in combatting pain or the control of pain gave these patients the greatest confidence eventually.

Goodman and Gilman[2] have suggested an antispasmodic function for calcium, while Wright has suggested that calcium gluconate tended to regulate the excitability of nerve fibers and nerve centers. What is most important is the rapidity with which pain of withdrawal disappears with the administration of calcium gluconate, suggesting therefore a possible effect on the pain center—in the thalamus.

We believe much more work needs to be done in this sphere, and we advocate very highly the use of calcium gluconate (Sandoz) in the further study of this problem that has so forcibly gripped this entire nation.

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Serum calcium levels After the Administration of Some Calcium Salts

Many publications[1] can be obtained concerning the absorption and utilization of the intake of Ca, especially as Harrison[2] etc. (1951) points out, vitamin D has a vital relationship on the utilization of Ca. In this connection, Hansard[3] etc. (1951) points out the deficiency or abundance of the amount of Ca in the organism, while Henry, etc.[4] (1947) stresses the same problems with the amount of P. It is understood, however, that the absorption is effected also by the simultaneous intake of other nutriments. For instance, writings by Mori and Takeuchi[5] (1955), Shimizu[6] (1955), Kuroda[7] (1955), etc., report on the effect on the absorption of Ca by adding amino-acid, dextrose, proteid, fat and organic acid experimenting on dog intestines. However, so far there are no comparative reports available on the differences of the absorption of the various kinds of Ca salts conducted under identical experimental conditions.

Experimental Materials and Methods

A domestic male rabbit weighing 2-3 kgs. raised on the same feed during a certain duration of time was provided for experimentation. The provision of Ca salts was through intravenous and oral administration. For the latter method the rabbit was given 50 g. of bran mash feed mixed with a certain amount of Ca salts, after fasting for twenty-four hours. After feeding, the piercing of the heart to extract 3 cc. of blood was conducted at regular intervals. The serum from the coagulated blood was then used as a base for experiments.

Serum calcium levels were measured (plus the slightly adulterated Ca volume) according to the Yanagisawa method[8-11] (1952-53). Using a photoelectric spectro-photometer[12] the wave length was set at 620 mf and the total Ca volume (Ca^{++}) was measured from the set volume curve line obtained by using the standard melting liquid of $CaCO_3$ and each are indicated by mg %.

Ca salts used in the experiment were $CaCl_2$, $CaCO_3$, pearl calc (Nikimoto Seiyaku), calcium citrate (Sumitomo Chemical), calcium glutamate, calcium succinate (adjusted by Dr. Katsumi Horiguchi).

Results of Experiments

(1) Serum calcium level changes in a domestic rabbit

Figure 1 shows the serum calcium level after feeding it 50 g. of bran mash after fasting for twenty-four hours, measuring it at 30 minutes, 1 hour, 2 hours, 3 hours and 5 hours later thereafter.

(2) Administration of $CaCl_2$

(a) Intravenous injection.

The experimental results of administering 0.5g per domestic rabbit using the solution of 5g/d/$CaCl_2$ is shown in Figure 2. As seen Ca volume was 26.3 mg%, Ca^{++} volume was 11.8 mg%, highest at the half hour after administration with gradual decline showing that after four hours the figure was lower than at the time of injection.

The same administration of 0.5g was made on man with same results as with rabbits, see Figure 3.

(b) Oral administration.

Figure 4 shows the results of serum calcium levels when administering 0.5g and 1.0g of $CaCl_2$ to a rabbit. The serum calcium level showed a maximum after one hour and the past two-hour level declined to a pre-administering level. Thereafter the decline from the pre-administering level was greater in the case when 1.0 g was given rather than when 0.5 g was administered. The Ca^{++} volume reached the maximum after 30 minutes and at two hours it declined to the pre-administering level.

Figure 5 shows the experimental results of administering 10 cc. of 10 g /d/$CaCl_2$ solution to a dog by a stomach tube after fasting for 24 hours. In the case of a dog the serum calcium level before administering is almost at the median (12 mg% $\pm$) of that of man and rabbit. The rise of serum calcium level as the result of administering $CaCl_2$ was not as significant as in the case of the domestic rabbit.

The following are comparative experiments of administering various Ca salts mixed with a volume of Ca corresponding to $CaCl_2$ 0.5 g orally to domestic rabbits.

(3) When administering $CaCO_3$

Figure 6 shows that when administering $CaCO_3$ to a domestic rabbit, the maximum rise is after 30 minutes and one hour and thereafter declining. At the three hour point it levels to the same as it was prior to administering but does not decline further within five hours. The variation in the case of Ca^{++} parallels that of Ca.

(4) Administration of pearl calc: $CaHPO_4 2H_2O$

Figure 7 shows the reaction of oral administration of 0.8 g and 1.6 g of pearl calc (corresponding amount of

1.0 g of $CaCl_2$. Unlike $CaCl_2$ and $CaCO_3$, in the case of Ca the rise is not significant after 30 minutes or one hour after administration but gradually shows a rise after 4,6 and 8 hours and as more time goes by the serum calcium level rises above that of what it was prior to administration.

(5) Administration of calcium glutamate.

Figure 8 shows the experimental results of oral administration of calcium glutamate as an amino acid Ca salts. When comparing with with Figure 1, it can be observed that while it rises slightly at 30 minutes, 1 hour and 2 hours, at 5 hours it declines.

(6) Administration of calcium citrate, calcium succinate.

In Figures 9 and 10, calcium citrate and calcium succinate were used for the Ca salts for di- or tricarboxylic acid. It shows that serum calcium volume increases at 30 minutes and one hour but thereafter decreasing gradually to the point at 5 hours the Ca volume was slightly lower than at the time of administration. This volume, however, cannot necessarily be considered low when compared to a case where no experiment has taken place.

Review

There have been many absorption experiments of nutrition but not many are satisfactory. In this paper, we attempted to make comparisons of the serum calcium level under the most normal physiological conditions.

The extraction of blood was done by using $CaCl_2$ including the radio calcium cited by Harrison and others (1951).

We first determined the timely reactions of the serum calcium levels after administering $CaCl_2$ orally and intravenously. The intravenous method showed that Ca and Ca^{++} both increased momentarily but then decreased to a lower level than the level at the time of injection. The result was the same as reported by Moller (1952) [13] and Yanagisawa (1954)[14]. In the case of oral administration, the serum calcium level increased at 30 minutes and by one hour reached a maximum increase but by two hours showed the same pre-administering level. This is also reported by Yanagisawa (1954) [15] but by measuring the serum calcium level within a shorter time we were able to determine that the serum calcium level increases also after oral administration.

Summary

The serum calcium volume of a domestic rabbit after administering various Ca salts was measured by the Yanagisawa method.

(1) A significant rise of the serum calcium was determined in the cases of $CaCl_2$ and $CaCO_3$.

(2) Compared with other Ca salts, the continuous rise of the serum calcium was more obvious in the case of phosphoric acid.

(3) A conspicuous rise of the serum calcium could not be noted in the cases of calcium succinate, calcium citrate and calcium glutamate. However, the relative rise was in the above order.

Reference Material

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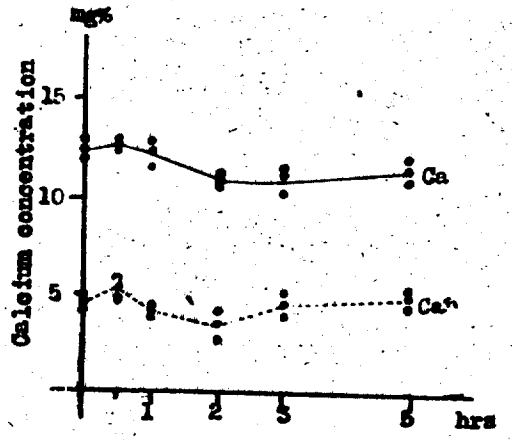

Fig. 1 Serum-calcium levels in untreated rabbit.

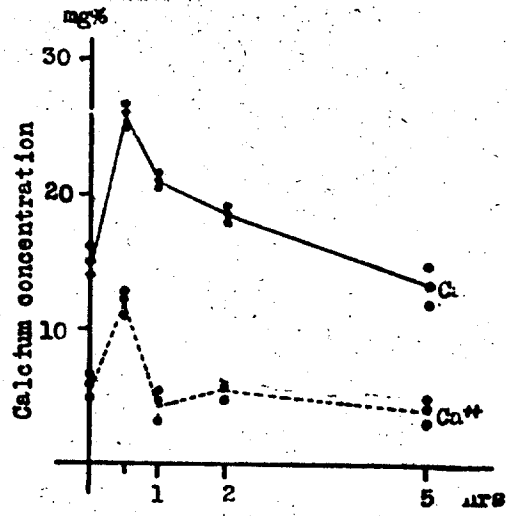

Fig. 2 Serum-calcium levels in rabbit after the intravenous administration of 500 mg $CaCl_2$.

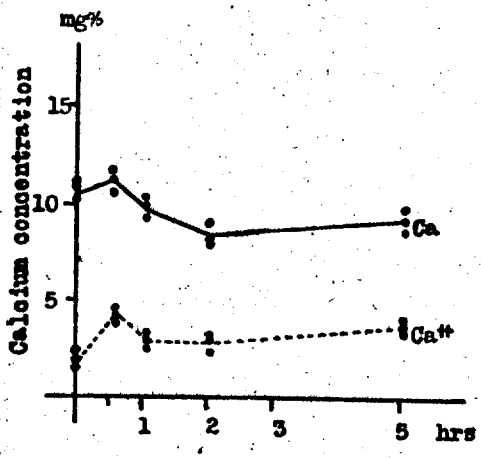

Fig. 3 Serum-calcium levels in man after the intravenous administration of 500 mg $CaCl_2$.

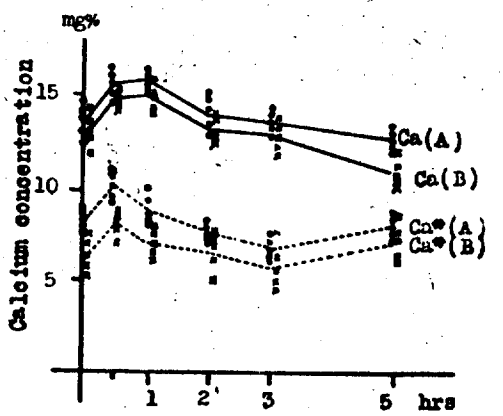

Fig. 4 Serum-calcium levels in rabbit after the oral administration of 0.5 gm(A) and 1.0 gm(B) of $CaCl_2$.

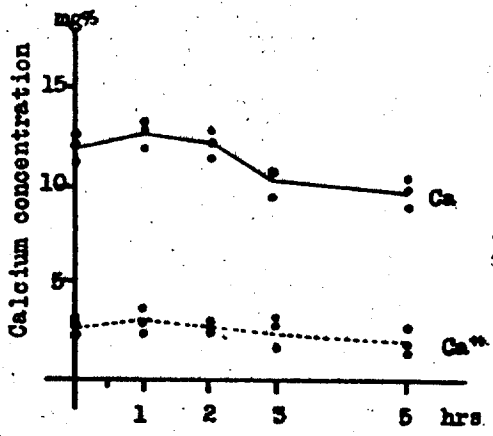

Fig. 5 Serum-calcium levels in dog after the oral administration of 1 gm $CaCl_2$.

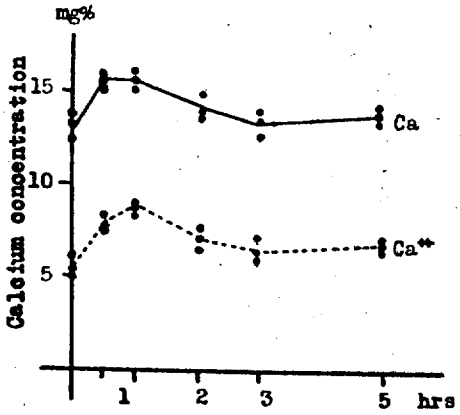

Fig. 6 Serum-calcium levels in rabbit after the oral administration of 0.4 gm $CaCO_3$.

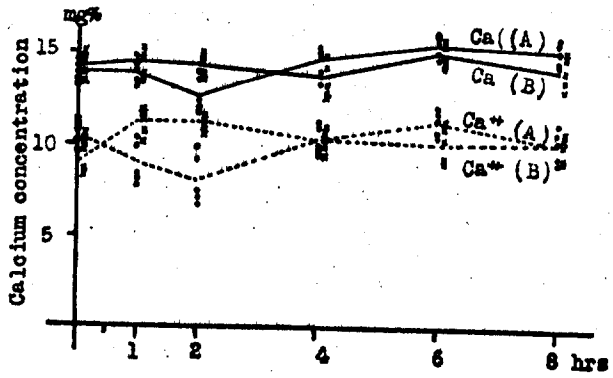

Fig. 7 Serum-calcium levels in rabbit after th oral administration of 0.8 gm(A) and 1.6 gm(B) Pearl Calc.

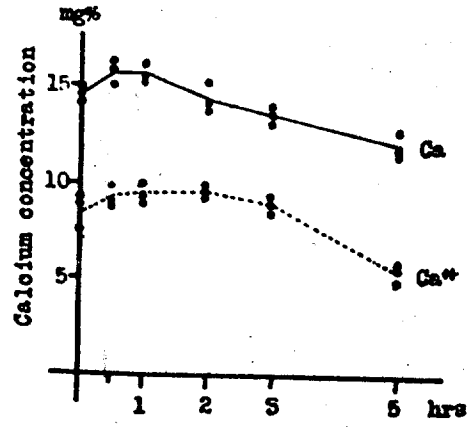

Fig. 8 Serum-calcium levels in rabbit after the oral administration of 0.45 gm Calcium glutamate.

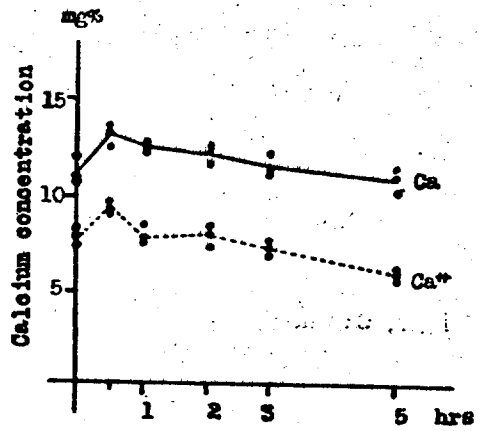

Fig. 9 Serum-calcium levels in rabbit after the oral administration of 0.81 gm Calcium succinate.

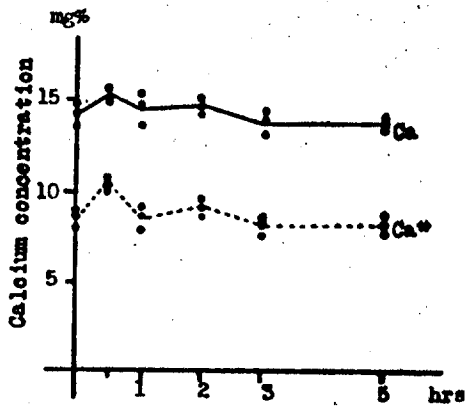

Fig. 10 Serum-calcium levels in rabbit after the oral administration of 0.5 gm Calcium citrate.