PANTOTHENATES #71

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P A N T O T H E N A T E S

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PANTOTHENATES

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PANTOTHENATES

Summary

Pantothenic acid is a member of the B complex of vitamins and has been referred to as vitamin B_5. It appears to be universally present in all plant and animal tissue usually in a conjugated form, and more particularly as a component of an enzyme, coenzyme A. Since its discovery some forty years ago as related to chick dermatitis, it has been synthesized as a raceme; only the natural, dextrorotatory form has vitamin activity. Calcium and sodium salts of pantothenic acid are also synthesized commercially; d-calcium pantothenate, the dextrorotatory isomer, only possesses vitamin activity and is a common form used in nutritional supplements. Solutions of the calcium salt, however, are not stable to autoclaving and require filtration for sterilization. For this reason, the alcohol derivative, d-pantothenyl alcohol or dexpanthenol, which is reasonably stable at sterilizing temperatures and times, is a preferred form of pantothenic acid in practical use, as in multivitamin preparations. It is also widely used in medical practice to increase peristalsis and the intestinal tone following surgery. Other medical uses for therapy in medical practice will be noted in the materials reviewed in this monograph.

The nutritional requirements of pantothenic acid have not been established, but a daily intake of 5-10 mg appears to be adequate; a typical multivitamin preparation of the one-a-day type lists the amount of pantothenic acid (as "Panthenol", or pantothenyl alcohol) as 1 milligram. Comparison of the presumed nutritional requirement of pantothenic acid with the relatively enormous doses given to experimental animals and used in human therapy shows that vitamin B_5 is of a very low order of toxicity. For example, the daily requirement of 10 mg for an individual weighing 70 kilograms (154 lbs) represents a dose of 0.14 mg/kg; a typical postoperative therapeutic dose for paralytic ileus consists of 500 mg given intramuscularly, followed by the same dose in two hours and thereafter every six hours for 48 hours, or longer. The total therapeutic dose may well be 3 or more grams, or 3000 milligrams per day, a dose of 43 mg/kg, roughly 300 times the nutritional requirement. A patient using pantothenic acid in treatment for a skin disease has been reported to have consumed a total of 3.6 kg (8 pounds) over a period of several years (148).

An early (1940) study by Unna and Greslin (311) included determinations of the LD_{50} in g/kg of calcium pantothenate in mice and rats by four different routes: orally, LD_{50} was: mice 10; rat, greater than 10; subcutaneously; mice, 2.7; rat 3.4; intravenously; mice, 0.91; rat -; and intraperitoneally; mice, 0.92; rat, 0.82. Of the 180 mice and 110 rats used, those that died did so within several hours; no late deaths were seen. A single dose of 1 g/kg given 5 dogs and 1 monkey produced no toxic symptoms.

NAS/NRC information (280) gives the LD_{50} for d-pantothenyl given orally to mice as about 15 g/kg; the LD_0 was 10 g/kg and the LD_{100} was

20 g/kg. Intravenous doses in mice showed the LD_{50} to be in excess of 10 g/kg; the LD_0 was 4 g/kg; and the LD_{30} was 10 g/kg. Solutions of calcium pantothenate given mice intravenously showed an LD_{50} of 685 mg/kg, an LD_0 of 500 mg/kg, and an LD_{100} of 800 mg/kg. The LD_{50} for intravenous pantothenyl alcohol in rabbits was in excess of 10 g/kg; in 9 dogs similarly injected, the LD_0 was 10 g/kg. Single, intramuscular injections of 200 mg or 2 g/kg of sodium pantothenate or d-pantothenyl alcohol in young rats produced no significant changes in serum transaminase concentrations or the cytological picture of the liver.

Weiss et al. (193) reported an LD_{50} of 7 g/kg intravenous pantothenyl alcohol in mice, and 4 g/kg in rabbits; the LD_0 for mice was 6.25 g/kg and for rabbits, 3 g/kg.

The effects of large intramuscular doses of sodium pantothenate, d-pantothenyl alcohol and d-pantethine on the liver of rats were studied by Schwartz and Bagdon (186) in 1964. The doses ranged from 100 to 2000 mg/kg; 96 hours after injection, blood samples were taken, the animals sacrificed and livers examined for toxic effects. The results were generally negative; smaller animals showed a slight, but significant, increase in liver lipid from the pantothenyl alcohol.

An early (1940) report by Spies (305) demonstrated that doses of 100 mg of calcium or sodium pantothenate given intravenously to humans produced no significant changes in blood pressure, pulse, temperature or respiration. Similar studies were made using riboflavin (200 micrograms/kg) combined with the pantothenates, resulting in an increased blood concentration of pantothenic acid compared to the pantothenate given alone. An interrelationship between the two vitamins in human nutrition was suggested.

Pantothenyl alcohol given orally to rats at dose levels of 2 mg daily for 6 months, and 20 mg daily for three months was reported by Weiss et al. (193) in 1950 to have produced no histopathological changes; 500 mg fed daily to dogs for 3 months also produced no changes. Hematological determinations were also normal.

The chronic toxicity of calcium pantothenate given in the diet of rats, dogs and monkeys was tested by Unna and Greslin (311) in 1940. Rats given either 50 mg or 200 mg daily for 120 days grew normally. Dogs fed 50 mg daily for 100 days showed normal growth without signs of toxicity. Monkeys were unaffected by 1 g daily for 100 days. A report one year later by the authors (140) further indicated an "extremely low toxicity" for calcium pantothenate. Rabbits were given subcutaneously 1 ml up to 10% solution or had instilled 0.5 ml 10% solution into the conjunctival sac without irritation or effect. Rats given orally doses up to 50 mg showed no effects on oxygen consumption or diuretic action. Cats injected intravenously with 10-50 mg/kg maintained normal blood pressure and respiration.

NAS/NRC (280) information shows that rats and dogs given pantothenyl alcohol at concentrations up to 0.2% for 3-6 months in the diet produced no adverse effects on growth, body tissues or hematology. Rats given pantothenyl alcohol in drinking water in doses of either

100 or 400 mg/kg daily for 13 weeks remained healthy and upon sacrifice showed no gross pathologies. The animals on 400 mg/kg dose were retarded in growth, and animals at both dose-levels showed some slight cytological evidence of toxicity in kidneys; lungs and liver showed slight changes. A similar study, using calcium pantothenate, dl-pantothenyl alcohol and d-pantothenyl alcohol in rats dosed with 20, 50 or 200 mg/kg for 90 days produced no significant toxicological effects. Six male hospital patients given 500 mg sodium pantothenate or pantothenyl alcohol intramuscularly twice daily for 4 days showed no hematological abnormalities.

A longevity study by Pelton and Williams (94) in 1958 was inspired by the long life of the queen bee, whose royal jelly diet contains an abundance of pantothenic acid. Young mice, about 35 days old, were given a daily dose of about 0.3 mg calcium pantothenate in drinking water throughout their lives. The mean life span for the treated mice was 653.1 days; untreated controls, 549.8 days.

Zver'Kova (154) reported in 1966 that calcium pantothenate has a desensitizing effect against the allergen, dinitrochlorobenzene, in guinea pigs. Of doses, 40 and 50 mg/kg pantothenate, given intramuscularly, only 80 mg/kg protected nearly completely the allergic reactions, hyperemia, desquamation and incrustation in the animals sensitized to the allergen which was administered by the drop method. Blood tests of the sensitized animals showed a reduction of acetylation of nearly 50% compared to normals.

Pharmacological studies of calcium pantothenate given intravenously to cats were reported in 1965 by Gabor et al. (39). The increase of blood pressure following injection of 3-10 micrograms of adrenaline was potentiated by doses of the pantothenate ranging from 10-200 mg/kg in direct proportion to the dose of pantothenate, but unrelated to the original adrenaline dose. The depressant effect of acetylcholine on blood pressure was also increased by pantothenate, but not proportionally. Pantothenate had no influence on the blood pressure depression or lowering by injections of histamine.

Ueshima (139) reported in 1958 on the use of therapeutic doses of calcium pantothenate given to rabbits and to man under conditions of acute and chronic liver damage in the rabbit and hepatitis or cirrhosis in man. In general, rabbits with induced liver damage, treated intramuscularly with daily doses of 10 mg calcium pantothenate, showed improvement over nontreated controls with respect to ester quotient, serum pyruvic acid concentrations, total liver pantothenic acid, and liver pathology. Thirty hospital patients with hepatitis or cirrhosis, given 20-40 mg calcium pantothenate daily for a month, when given liver-function tests showed improvement.

In the course of treatment for the skin disease, lupus erythematosus, some patients were treated with pantothenyl alcohol and/or calcium pantothenate (both dextro- and dl) at dosage levels of 10-12 g daily for as long as 3 years, according to a report by Welsh (148) in 1952. One patient, in the course of therapy, took a total of 3.6 kg (8 pounds); 15 patients took over 2 kg (4-5 lbs), all without "untoward effect".

Some dissension concerning the use of pantothenic acid in the relief

of paralytic ileus following surgery is found in the literature. Banerji (11) in 1953 reported several cases of severe paralytic ileus which, having failed to respond to routine treatment, were quickly relieved by several intramuscular doses of 50-150 mg calcium pantothenate. Other cases of successful treatment are included. A clinical evaluation of pantothenyl alcohol in the treatment of ileus was reported in 1964 by Polacek et al. (176). A double-fluid test on 100 elective cholecystectomies and 75 gastric resections was described. After surgery, the patient received either a placebo or 250 mg pantothenyl alcohol, plus a lead marker to be swallowed with the first dose and traced by x-ray for 5 days or until it reached rectum or sigmoid colon. Only in the cases of cholecystectomies was the treatment effective in accelerating the transit time of the lead marker. The authors did not recommend a routine use of pantothenyl alcohol, but rather suggested continued use of standard precautionary measures.

Klykov and Minsker (63) in 1971 reported on studies using calcium pantothenate in therapy of chronic circulatory insufficiency in a number of patients with rheumatic heart defects or atherosclerotic cardiosclerosis. Calcium pantothenate was given, 200 mg three times daily for 5 days. Blood samples taken before and after treatment showed upon analysis for pyruvic acid, sulfhydryl (SH) groups and thermal coagulation time, some improvement, particularly in reduction of pyruvic acid levels and heat coagulation time.

Weiss et al. (193) in 1950 demonstrated that pantothenyl alcohol was not stored unchanged appreciably in the livers of rats fed daily doses of the substance of 2 mg orally for periods of up to 5-6 months. The kidney content of pantothenic acid, however, was increased 42% after pantothenyl alcohol compared with equal doses of calcium pantothenate. Liver coenzyme A content was only slightly increased in short tests of 24-45 days of both compounds, and not at all in longer periods of 5-6 months. Liver pantothenic acid after treatment was shown to be practically entirely in the bound form.

A diet of calcium pantothenate given to rats by Middleton and Morrison (80) in 1965 over a 5-week period with doses of up to 160 micrograms every 4 days followed by a dose of 250 mg/kg sulfanilamide, demonstrated that no change in acetylation abilities were produced by giving pantothenate. The authors suggested that pantothenate was readily absorbed and utilized.

Braekkan (16) in 1955 concluded that pantothenyl alcohol is the form of choice in therapy with pantothenic acid by the oral route, by virtue of its utilization as indicated in studies of the different excretion rates of pantothenyl alcohol and calcium pantothenate. The pantothenate given intraperitoneally showed in rats a urinary excretion of pantothenate about 25% higher than pantothenyl alcohol similarly administered. Orally administered, the pantothenate appeared only 10% excreted in the urine, with 50% excreted unchanged in the feces. Intraperitoneal dosages of the two compounds ranged from 0.35 to 4 mg; oral dosage, 1 to 4 mg.

Loading doses of calcium pantothenate and pantothenyl alcohol were given to rats in the diet at several dosage levels; 10, 5 and about 2 mg/kg

by Rubin et al. (111) in 1949. Urine and fecal examinations showed that pantothenyl alcohol was excreted more rapidly and more completely as pantothenic acid than was a comparable dose of the pantothenate, implying a greater degree of utilization of the pantothenyl alcohol. Further tests, with the doses accompanied, or not, with food showed no differences in urinary excretion, but pantothenate given with food was excreted to a greater degree in the feces at the highest dose-level (10 mg/kg). The authors regarded pantothenyl alcohol as being more available, or at least as available, as calcium pantothenate under any feeding conditions. A similar study by Yamawaki and Ishida (152) in 1961, with the same general treatment in rats by the same two substances, reached similar conclusions: "that pantothenyl alcohol is quite advantageous as a dosage type of the pantothenic acid homologues".

The abnormalities of metabolism associated with the disease, tuberculosis, frequently include indications of a pantothenic acid deficiency and liver damage. Papers by Nakamura et al. (88) in 1961, and by Stepangan and Stukalova (126) in the same year reported the results of treating mice, rats and tubercular human patients with calcium pantothenate, in addition to streptomycin which is used in the therapeutic treatment of tuberculosis. The animals were given streptomycin intraperitoneally in doses of 5-50 mg/kg daily for 6 weeks, plus, in treated animals, 1 mg calcium pantothenate. The gross pathologies of liver and kidneys appearing in the streptomycin-treated animals, as well as the acetylation capacities, were generally ameliorated or maintained in those animals receiving calcium pantothenate in addition to streptomycin.

Intramuscular doses of 20 mg calcium pantothenate given tubercular patients produced improved conditions in some types of exudative and fibro-caseous tuberculosis; infiltrative tuberculosis showed little response. Various side effects of streptomycin therapy, such as tinnitis and deafness, were frequently relieved by intramuscular injections of 40 mg calcium pantothenate. Liver tests of tubercular patients showed in many instances a disturbance of liver function associated with the tubercular condition; amelioration by the use of pantothenate, found by Ueshima (139) was also observed.

Convalescent patients were given intravenous infusions of coenzyme A and calcium pantothenate by Bigler and Tholen (15) in 1966, to test the balance of free and bound pantothenic acid. The dose of coenzymes A, 192 mg, and of 119 mg calcium pantothenate were given at a rate of 1 ml/min. for 4 hours. Blood and urine concentrations of the two substances were analyzed before and after treatment and indicated that the coenzyme is broken down in the body rapidly. Urinary excretion of pantothenic acid is increased after administration of either substance; about 20-30% of the coenzyme A and 16-29% of the pantothenate were excreted in the urine. The fate of the remainder of the doses was not known.

A study of the absorption and excretion of calcium pantothenate in normal and depleted dogs was reported in 1945 by Silber (116). Fasted animals given orally 4 mg/kg, showed a urinary excretion of pantothenate of only 0.13% of the test dose; normal, fed animals excreted 1.7% of the test dose. Intraperitoneal doses showed similar differences in excreted amounts: depleted, 3.3%; normal, 11.7%. Oral doses of panto-

thenate resulted in fecal excretion in both normal and depleted animals of 14-27%. Human subjects given doses orally of 50 mg calcium pantothenate, excreted about 6 mg in the urine in the 4 hours following dosing compared to about 1 mg in the same period previous to dosing. Studies in man's urinary excretion of pantothenic acid by Gourrelle and Richet (162) in 1957 showed that ingestion of 100 mg pantothenyl alcohol raised urinary concentrations of pantothenic acid ten to fifty-fold above normal during a 4-hour period following dosage; within 24 hours, excretion levels were only slightly above normal. One subject showed a total, 24-hour excretion, of 38.4 mg after ingesting 100 mg pantothenyl alcohol.

The influence of doses of calcium pantothenate of 100 micrograms daily or of 1 mg daily, given in the diet of pregnant rats upon the offspring was reported in 1954 by Chung et al. (23). Histological studies of liver, duodenum, adrenals and tibias of sacrificed newborn showed no influence of the pantothenate diet of the parent on the offspring. Histochemical findings were also negative except for the adrenal alkaline phosphatase reaction which was depressed in the offspring of treated mothers who had received no pantothenic acid during pregnancy or only stock diet without pantothenic acid supplement. The same group (essentially) of researchers (Everson et al. (35)) reported that ascorbic acid and alkaline phoshatase serum levels in fetal tissues were not affected by the maternal diet in similar experiments. Pantothenic acid-deficient diets did produce smaller, undersize litters with low serum pantothenic acid concentrations and some excess of pyruvic acid.

CALCIUM PANTOTHENATE

Chemical Information

I. Nomenclature

A. Common Names

1. Calcium Pantothenate
2. Dextro Calcium Pantothenate

B. Chemical Names

1. Calcium Pantothenate
2. Pantothenic acid calcium salt
3. Calcium D(+)-N-(2,4-dihydroxy-3,3-dimethyl butyryl)-B-alaninate

C. Trade Name

1. Pantholin

D. Chemical Abstracts Registry Number

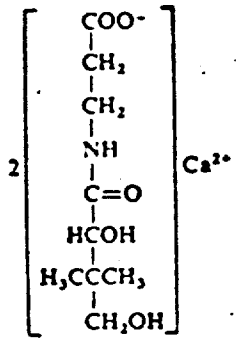
000137-08-6

II. Empirical Formula

$C_{18}H_{32}CaN_2O_{10}$

III. Structural Formula

$(HOCH_2C(CH_3)_2CH(OH)CONH(CH_2)_2COO)_2Ca$



IV. Molecular Weight

476.54

V. Specifications

Food Chemicals Codex:

Assay	Not less than 90.0% and not more than the equivalent of 110.0% of dextrorotatory calcium pantothenate ($C_{18}H_{32}Ca-N_2O_{10}$), calculated on the dried basis.
Calcium content	Not less than 8.2% and not more than 8.6% of Ca after drying
Specific rotation	$[\alpha]_D^{25°}$. Between +25° and +27.5°
Limits of Impurities	
Alkalinity	Passes test
Alkaloids	Passes test
Heavy metals (as Pb)	Not more than 20 parts per million (0.002%)
Loss on drying	Not more than 5%

VI. Description

A. General Characteristics

The calcium salt of the dextrorotary isomer of pantothenic acid; minute needles from methanol, or as a slightly hygroscopic, white powder; odorless; sweetish taste (as crystal) with slightly bitter aftertaste.

B. Physical Properties

Soluble, 1 gram in 2.8 ml H_2O, also soluble in glycerol; slightly soluble in alcohol, acetone; practically insoluble in chloroform or either.

C. Stability

Reasonably stable in air and light; in solution, most stable at pH 5-7. Should be stored in tight containers.

VII. Analytical Methods

From Food Chemicals Codex:

A. A 1 in 20 solution gives positive tests for Calcium, page 926.

B. The infrared absorption spectrum of a potassium bromide

dispersion of the sample, previously dried at 105° for 3 hours, exhibits maxima only at the same wavelengths as that of a similar preparation of U.S.P. Calcium Pantothenate Reference Standard.

C. Boil 50 mg in 5 ml of 1 N sodium hydroxide for 1 minute, cool, and add 5 ml of 1 N hydrochloric acid and 2 drops of ferric chloride T.S. A strong yellow color is produced.

From Methods of Analysis, AOAC (318), page 772-773, see Sections 39.076 through 39.084. Albright (2) has published an automated analysis: see Documents Section. See also, Hubbard and Sheppard (54) of Food and Drug Administration.

VIII. Occurrence

The name "pantothenic" acid suggests the universality of its distribution (from the Green pantothen, "from all sides"); it appears to be found in all living protoplasm, plant and animal. The richest animal sources are the liver and kidney; muscle (meat) contains considerably less. The free acid appears only in very small amounts; for the most part, pantothenic acid is chemically bound to proteins (as coenzyme A), or forms salts. The commonly available salt is calcium pantothenate, the d-form serving principally as the biologically active form; sodium pantothenate is also available to a lesser degree.

SODIUM PANTOTHENATE

Chemical Information

I. Nomenclature

A. Common Name

Sodium Pantothenate

B. Chemical Name

Sodium Pantothenate

C. Trade Name

Panthoject (See Merck Index, pg 781)

D. Chemical Abstracts Registry Number

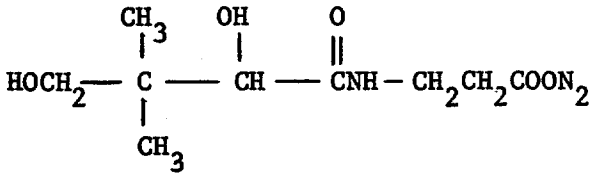
000867-81-2

II. Empirical Formula

$C_9H_{16}NaNO_5$

III. Structural Formula

(See pg 108: Hndbk Food Add.)



IV. Molecular Weight

241.22

V. Specifications

None Found

VI. Description

Very hygroscopic crystals, mp 122-124°. Can be stored in sealed ampules only. (Merck Index, pg. 781).

VII. Analytical Methods

See Calcium Pantothenate (AOAC). Tarli et al. (133) have described a gas-liquid chromatographic technique for quantitative determination of pantothenates (sodium and calcium).

VIII. Occurrence

No Information Available

d-PANTOTHENYL ALCOHOL

Chemical Information

I. Nomenclature

A. Common Names

1. d-Pantothenyl alcohol
2. Dexpanthenol
3. Pantothenylol
4. Pantothenol

B. Chemical Names

1. D(+)-alpha,gamma-Dihydroxy-N-(3-hydroxypropyl)-beta,beta-dimethylbutyramide
2. D-Pantothenyl alcohol

C. Trade Names

1. Panthenol
2. Motilyn
3. Bepanthen
4. Cozyme
5. Ilopan

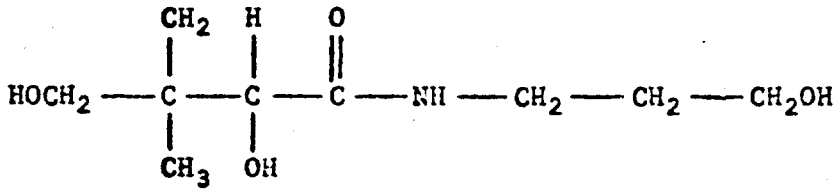
D. Chemical Abstracts Registry Number

000081-13-0

II. Empirical Formula

$C_9H_{19}NO_4$

III. Structural Formula



IV. Molecular Weight

205.25

V. Specifications

(Food Chemical Codex)

Specifications

Assay	Not less than 98.0 percent and not more than 102.0 percent D-panthenol calculated on a dry basis.
Specific Rotation	$[\alpha]_D^{25}$. Between +29.0° and +31.5° on a dry basis.
Refractive Index	n_D^{20}. Between 1.4950 and 1.5020
Limits of Impurities	
Water Content	Not more than 1 percent
Aminopropanol Content	Not more than 0.6 percent
Residue on Ignition	Not more than 0.1 percent
Heavy Metals (as Pb)	Not more than 20 parts per million (0.002 percent).

VI. Description

A. General Characteristics

Clear, viscous, somewhat hygroscopic liquid with slight, characteristic odor; may crystallize some on standing; slightly bitter taste. Physiological activity is in ratio of 1:1.16 of D-calcium pantothenate.

B. Physical Properties

Freely soluble in H_2O, alcohol, methanol; slightly soluble in ether and glycerin; normal pH about 9.5 (alkaline to litmus).

C. Stability

Reasonably stable to usual sterilization time and temperatures in aqueous solutions to pH 3-4; long heating causes racemization. More stable in practical applications, such as multivitamin preparations, than the salts of pantothenic acid (calcium and sodium) particularly in liquid preparations at low pH values.

D-pantothenyl alcohol should be stored in tight containers.

VII. Analytical Methods

Schmall and Wollish (113) published in 1957, "Determination of Panthenol and Pantothenates in Multivitamin Preparations". See also Calcium Pantothenate (318).

VIII. Occurrence

Does not occur naturally; is prepared by addition of propanolamine to optically active alpha,gamma-dihydroxy-beta,beta-dimethylbutyrolactone. It has complete biological activity and superior stability at pH values of 3-5 in solutions.

Biological Data

I. Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage mg/kg Body Weight	Measurement	Ref.
Calcium pantothenate	Mice	-	Oral	10,000	LD_{50}	311
Calcium pantothenate	Mice	-	s.c.	2,700	LD_{50}	311
Calcium pantothenate	Mice	-	i.p.	920	LD_{50}	311
Calcium pantothenate	Mice	-	i.v.	910	LD_{50}	311
Calcium pantothenate	Rats	-	Oral	>10,000	LD_{50}	311
Calcium pantothenate	Rats	-	s.c.	3,400	LD_{50}	311
Calcium pantothenate	Rats	-	i.p.	820	LD_{50}	311
Panthenol	Mice	-	Oral	15,000	LD_{50}	280
Pantothenyl alcohol	Mice	-	i.v.	>10,000	LD_{50}	280
Calcium pantothenate	Mice	-	i.v.	685	LD_{50}	280
Pantothenyl alcohol	Rabbits	-	i.v.	>10,000	LD_{50}	280
Panthenol	Mice	-	i.v.	7,000	LD_{50}	193
Panthenol	Rabbits	-	i.v.	4,000	LD_{50}	193

Unna and Greslin (311) in 1940 tested the toxicity of pantothenic acid (calcium salt) in a number of acute and chronic experiments with various animals; the acute studies are described here.

Local effects of calcium pantothenate were tested in rabbits. Instillation of 0.5 ml 10% solution into the conjunctival sac produced no irritation; subcutaneous injection of 1 ml 10% solution caused no irritation, inflamation or abscess formation.

In mice and rats, the LD_{50} was determined in four methods of administering calcium pantothenate; the following table summarizes the results obtained from a total of 180 mice and 110 rats:

Acute Toxicity of Calcium Pantothenate L.D. 50 (g per kg body wt).

	Oral	Subcutan.	Intraper.	Intrav.
Mice	10.0	2.7	0.92	0.91
Rats	No deaths at 10.0	3.4	0.82	—

The lethal doses in mice and rats produced prostration and respiratory failure; death occurred within an hour following intravenous or intraperitoneal injection, and within 2 hours after oral or subcutaneous administration. No late deaths were seen.

A single dose of 1 g/kg fed to each of 5 dogs and to 1 monkey produced no toxic symptoms; sacrifice of 1 dog and the monkey at 2 weeks showed at autopsy no gross pathologies.

A report from the NAS/NRC Questionnaire files, (280) states without details that the LD_{50} for panthenol given orally to mice was about 15 g/kg. Doses of from 1 to 10 g/kg panthenol resulted in no deaths; a dose of 20 g/kg killed all mice tested.

From the same NAS/NRC files, the intravenous toxicity of pantothenyl alcohol and calcium pantothenate, in mice, rabbits and dogs, is reported:

A 25% solution of pantothenyl alcohol administered intravenously to mice indicated an LD_{50} to be in excess of 10 g/kg; no deaths occurred at a dose of 4 g/kg, and only 33% died at a dose of 10 g/kg. Solutions of calcium pantothenate injected intravenously in mice showed an LD_{50} of 685 mg/kg; no deaths occurred at a dose of 500 mg/kg and all animals died from 800 mg/kg.

Intravenous tests in rabbits given pantothenyl alcohol in doses ranging from 100 mg/kg to 10 g/kg failed to produce a single death among 14 rabbits; the LD_{50} was in excess of 10 g/kg. Nine dogs were similarly treated with doses of from 2 to 10 g/kg; no deaths occurred.

Single intramuscular injections of either 200 mg/kg or 2.0 g/kg of sodium pantothenate or d-pantothenyl alcohol in young Sprague-Dawley rats produced no significant changes in serum transaminase concentrations or in the cytological appearance of liver tissue.

Weiss et al. (193) performed a number of experiments with panthenol

and calcium pantothenate in a variety of animals, and reported on the acute toxicity of panthenol in mice and rabbits; no experimental details were given. The panthenol intravenous LD_{50} for mice was 7 g/kg and for rabbits, 4 g/kg. All mice survived intravenous doses of 6.25 g/kg and all rabbits survived doses of 3 g/kg of panthenol. (Additional studies by these investigators are described in the following sections: Short-Term Studies, and Absorption/Distribution).

Schwartz and Bagdon (186) in 1964 tested several derivatives of pantothenic acid using rats and observing particularly the effects of large intramuscular doses on the liver. Sodium pantothenate, d-panthenol and d-pantethine were administered intramuscularly in single doses of 100, 200 or 2000 mg/kg to Sprague-Dawley rats in 2 series of experiments, the weights of the animals were about 110-120 g in one group, and 225-275 g in another. Ten rats, equally divided as to sex, were dosed as shown in the following table; 96 hours after injection, blood samples for serum transaminase determinations were taken and the animals sacrificed and livers removed for studies of possible toxic effects of the treatment.

THE EFFECT OF SINGLE INJECTIONS OF SODIUM PANTOTHENATE, *d*-PANTHENOL, *d*-PANTETHINE, PANTHOJECT AND BENZYL ALCOHOL ON BODY WEIGHT, LIVER WEIGHT, SGO-T, SGP-T AND HISTOLOGIC FAT IN THE LIVERS OF RATS[a]

Compound	Dose (g/kg)	Average body weight (grams)		Liver weight (% body weight)	SGO-T (units)	SGP-T (units	Number of rats showing fatty change			
		Initial	Final				+	++	+++	++++
				Study No. 1						
Sodium pantothenate	2.0	109	138	4.80	122.2	17.4	4	5	1	0
	0.2	109	137	4.60	115.0	14.0	4	4	2	0
d-Panthenol	2.0	109	136	4.35	100.0	10.0	0	6	4[b]	0
	0.2	109	138	4.35	108.0	12.0	2	4	4[c]	0
d-Pantethine	0.1	109	138	4.40	122.0	14.5	4	6	0	0
Panthoject	2.0	116	145	4.80	113.3	18.5	5	5	0	0
	0.2	117	146	4.65	101 5	20.6	7	3	0	0
Benzyl alcohol (1%)	2.0 eq.[d]	116	146	4.80	101.4	19.3	9	1	0	0
	0.2 eq.[e]	116	142	4.75	111.5	22.4	7	3	0	0
Control	0 25 ml water	117	146	4.55	127.9	18.5	4	6	0	0
				Study No. 2						
Sodium pantothenate	2.0	224	228	3.84	107.0	16.0	3	6	1	0
	0.2	235	239	3.81	99.5	16.0	7	3	0	0
	0.1	244	249	3.84	88.0	15.0	7	3	0	0
d-Panthenol	2.0	266	267	3.71	95.5	13.0	8	2	0	0
	0.2	255	260	3.73	82.5	11.5	7	3	0	0
	0.1	241	246	3.79	104.0	13.0	5	5	0	0
Control	0.25 ml water	237	238	3.49	82.0	11.5	5	4	1	0

[a] Values are means of 10 rats except SGO-T and SGP-T levels of rats given 2.0 g of sodium pantothenate per kilogram in study no. 1, for which values are means of 9 rats.

[b] Significantly different from controls at the 1% level.

[c] Significantly different from controls at the 5% level.

[d] Volume equal to Panthoject 2 g/kg.

[e] Volume equal to Panthoject 0.2 g/kg.

Body weights of the treated animals were not significantly affected, nor did the liver reveal anticipated marked fatty change. The small group of rats (110-120 g) showed a slight but statistically significant increase in liver lipid from d-panthenol, compared to controls, but this effect was not seen in the group of larger animals.

One of the earliest studies of the possible toxicity of pantothenic acid in humans was reported in 1940 by Spies et al. (305). Varying amounts of calcium or sodium pantothenate were given intravenously to 15 persons; 100 mg of either salt so administered produced no reaction or significant changes in blood pressure, pulse, temperature or respiration. Blood and urinary concentrations of pantothenic acid, before and after injection, showed increases up to 50% in blood levels within 3 hours, and more immediate increases in the urinary levels, but in each instance, levels returned to normal within 24 hours.

Similar studies with riboflavin and pantothenic acid were performed; 200 micrograms/kg body weight of riboflavin produced a 45% increase in the blood concentration of pantothenic acid. The authors suggested that from this and other studies, an interrelationship of the two vitamins in human nutrition was indicated.

II. Short-Term Studies

Weiss et al. (193) in 1950 tested the chronic toxicity of panthenol in rats; 24 animals received 2 mg panthenol daily for 6 months without apparent influence on weight gains. A similar 3-month study with a daily dose of 20 mg panthenol given orally to rats produced no histopathological changes; 500 mg fed daily to dogs for 3 months had similar results. White and red blood cell counts, hemoglobin determinations, nonprotein nitrogen, and blood sugar levels were all normal.

Unna and Greslin (311) (see preceding Section) tested calcium pantothenate for chronic toxicity in rats, dogs and monkeys. The report is brief and lacks details.

Two groups of 20 rats, each of 10 males and 10 females were fed 50 mg calcium pantothenate daily in one group; the other 20 animals were fed 200 mg/day. After 120 days, all rats were observed to have grown at a normal rate when compared to untreated controls.

Six dogs were fed 50 mg calcium pantothenate daily for 100 days without showing signs of toxicity or weight changes.

Four monkeys, 2.5-4 kg body weight, were given orally 1 g for 100 days, and failed also to show any effects.

The authors concluded that calcium pantothenate is, like other

members of the vitamin B complex, "a substance of extremely low toxicity".

A report by the same authors (Unna and Greslin (140)) appeared during the year following; additional studies on rabbits were performed by injecting subcutaneously 1 ml of 1, 2, 5 or 10% calcium pantothenate or instilling 0.5 ml, 10% solution into the conjunctival sac. No irritation was evident.

Oral doses of 1 or 50 mg calcium pantothenate given to rats produced no change in oxygen consumption, nor was any evidence of diuresis seen. Blood pressure and respiration in cats, injected intravenously with doses of from 10 to 50 mg/kg, remained unchanged. Isolated smooth muscle from intestine and uterus was unaffected by concentrations up to 1:10,000.

A number of reports from the NAS/NRC Questionnaire files (280) of 1949-63 on studies with d-pantothenyl alcohol, calcium and sodium pantothenates are included in the documents section of this monograph, and are briefly reviewed here.

Chronic toxicity experiments with rats and dogs given pantothenyl alcohol added to a stock diet in concentrations of 0.2 and 0.02% over periods of 3 or 6 months produced no adverse effect on growth, body tissues or blood cell count and hemoglobin level.

Two groups of 10 each Sprague-Dawley, weanling female rats were given pantothenyl alcohol in the drinking water at dose levels of 100 mg/kg in one group and 400 mg/kg in the second for a period of 13 weeks. Daily weights were determined and dosages adjusted to maintain a constant dose level. The animals receiving the 400 mg/kg dose showed a significant retardation of their growth, possibly due to a lower water intake. It could not be determined whether the reduction of fluid intake was due to a disagreeable taste imparted by the pantothenyl alcohol or to a systemic toxicity in the rats. The animals appeared healthy throughout the experiment, and autopsies of the sacrificed animals showed no apparent gross pathologies. Cytological observations noted a slight toxic reaction of the kidneys at the 100 mg/kg dosage level and a more marked reaction of the kidneys at the 400 mg/kg level. Slight changes in lungs and liver were observed.

Subacute toxicity studies of calcium pantothenate, d-pantothenyl alcohol (racemic) and d-pantothenyl alcohol in rats of the CR-strain are described. Three dosage levels of each compound were made available in the drinking water; 6 males and 6 females were used at each dosage level for each compound: 20 mg, 50 mg and 200 mg/kg. Volumes of water consumed were measured and determinations made of drug quantities ingested. The animals were immature (about 100 g weight) at the beginning of the experiments which lasted 90 days; drug concentrations were altered as the animals grew to maintain a constant mg/kg dosage.

Body weights were intially recorded twice weekly during the first two weeks, and weekly thereafter. Hematological studies were conducted before, during and at the conclusion of the 90-day period. Survivors were

sacrificed and organ weights determined; tissues were removed and prepared for histological examination. The toxicological effects of the three compounds were evaluated on the basis of: effect on growth; induced mortality; effect on hemogram; final weights of vital organs; and gross morphology at autopsy.

No significant departures from normal controls with regard to the foregoing criteria for toxicity were observed in any of the three compounds tested. Details of the experiments, results and discussion may be examined in the document included in this monograph.

Tests with isolated rabbit intestine and solutions of pantothenyl alcohol and calcium pantothenate showed no stimulating effect at concentrations of 1 mg/ml.

A study of 6 male hospital patients given 500 mg intramuscularly of either sodium pantothenate or d-pantothenyl alcohol twice daily for 4 days showed no abnormalities in laboratory values for transaminases, cephalin flocculation, serum bilirubin, alkaline phosphatase and thymol turbidity.

III. Long Term Studies

A report by Pelton and Williams (94) in 1958 is introduced by the following: "The disparity between the life span of worker and queen bees suggested the possibility that pantothenic acid which is the most conspicious nutritional constituent of royal jelly might have the effect of prolonging life in other species".

Thirty-four male and 40 female C-57 black mice, 4-5 weeks old, were grouped - 6 or 7 animals of same sex in each cage, and were treated (13 male, 20 females) with supplementary pantothenic acid (calcium salt) or served as controls (21 male, 20 females) without the pantothenate supplement. The average daily dose of calcium pantothenate (added to drinking water) was about 300 micrograms. The mice were about 35 days old at the start of the experiment, and were periodically weighed.

The mean life span for the treated mice was 653.1 days; for the controls, 549.8 days. After 250 days of the experiment, those mice on calcium pantothenate supplement weighed slightly more than controls and continued to maintain this condition throughout the experiment. Negligible differences between sexes in either control or experimental animals were observed; the difference between treated and untreated animals was, however, significant - 18 to 20% greater life span in the treated mice. The following tables summarize the experimental results.

Summary and Statistical Data for Mice Which Received Pantothenic Acid Supplement and Controls.

	Mean life span in days							
	Mice with pantothenic acid supplement			Controls			t test(4)	U test(5)
	No.	Days	S.D.	No.	Days	S.D.	P=	P=
Both sexes	33	653.1	201	41	549.8	233	.05	.01
Male mice	13	660.4	220	21	559.0	167	.15	.04
Female mice	20	648.4	187	20	540.2	285	.18	.05

Analysis by Sex of Mice Given Pantothenic Acid Supplement and Control Mice.

	Mean life span in days				Days difference ♂ & ♀	% difference ♂ & ♀
	No.	♂	No.	♀		
Mice with pantothenic acid supplement	13	660.4	20	648.4	12.0	2
Controls	21	559.0	20	540.2	18.8	3
Days difference treated & controls		101.4		108.2		
% difference treated & controls		18%		20%		

IV. Special Studies

Allergy

Zver'Kova (154) in 1966 reported experiments with guinea pigs demonstrating that calcium pantothenate has a desensitizing effect against dinitrochlorobenzene (an allergen). Three groups of white guinea pigs, weighing 350-500 g were used in the study: group 1 (10 animals) received intramuscularly a 20% solution calcium pantothenate (Ca-PaA) for 2 weeks prior to sensitization with dinitrochlorobenzene and for the 2 weeks during sensitization (5 animals received doses of 40 mg/kg Ca-PaA, 5 received 80 mg/kg); group 2 (10 animals) was given the Ca-PaA for a month, starting 10 days after the last sensitizing dose (in 2 sets of 5 each, 80 and 40 mg/kg); group 3 of 5 animals received only the sensitizing doses of dinitrochlorobenzene by the drop method.

Only those animals in group 1 receiving the smaller dose (40 mg/kg) showed reaction to the allergen, hyperemia, desquamation and incrustation; after a month no reactions with all dilutions of dinitrochlorobenzene were observed. The higher dose of 80 mg/kg protected nearly completely from allergic reaction.

All animals in group 2 that received Ca-PaA only after preliminary sensitization showed a considerable reaction. In the control group, after several months, sensitization was greatly diminished, disappearing almost completely in 3 months.

Determination of the pantothenic acid concentration in the serum of all animals before and after experimentation indicated that all the animals that had been sensitized to dinitrochlorobenzene showed a reduction of acetylation of nearly 50% compared to untreated animals.

Pharmacology

Gabor et al. (39) in 1965 reported some pharmacological actions of pantothenic acid given intravenously to cats. The experimental animals were of both sexes, weighing 1.5-3 kg. Injections were made by cannula inserted in jugular and carotid veins; doses of 3-10 micrograms of acetylcholine, histamine, or adrenalin were given, followed by calcium pantothenate in doses of 10-200 mg/kg. Calcium pantothenate injected by itself had no effect on the blood pressure as determined by an EKG.

Adrenaline, given intravenously to 19 cats, increased blood pressure; calcium pantothenate given 5 minutes later, potentiated the adrenaline effect in proportion to the size of the dose of pantothenate, but not related to the size of the adrenaline dose. The following table demonstrates the experimental results:

No.	Designation of the animal	Adrenalin dose, mcg/kg	Pantothenic acid dose mg/kg	Blood pressure increasing effect of adrenalin, in Hg mm		Blood Pressure variation, %
				before administration	after of P.A.	
1.	12.	3	0.01	25	25	0
2.	22.	3	0.05	25	25	0
3.	24.	3	0.1	17.5	20	12
4.	22.	3	0.2	25	25	0
5.	22.	3	0.2	25	30	20
6.	22.	3	0.2	25	35	40
7.	22.	3	0.2	25	35	40
8.	22.	3	0.5	25	35	40
9.	22.	3	0.5	25	40	60
10.	9.	3	1.0	20	30	50
11.	10.	4	1.0	20	30	50
12.	8.	3	1.0	20	30	50
13.	7.	5	1.0	30	40	30
14.	20.	5	1.0	25	35	40
15.	31.	5	1.0	35	40	15
16.	25.	5	1.0	27	35	30
17.	3.	3	1.0	15	20	30
18.	3.	3	5.0	15	25	60
19.	3.	3	10.0	15	25	60
20.	3.	3	10.0	15	25	60
21.	15.	7	10.0	30	40	30
22.	0.	3	10.0	25	40	60
23.	3.	3	20.0	15	25	60
24.	14.	3	20.0	15	25	60
25.	19.	3	20.0	22.5	30	30
26.	17.	5	20.0	26	45	70
27.	2.	4	25.0	15	30	100
28.	16.	10	40.0	30	40	30
29.	13.	3	50.0	25	35	40
30.	22.	3	100.0	25	40	60

Acetylcholine was given to 17 animals with a depressant effect on the blood pressure; in 11 out of the 17 cats, the calcium pantothenate increased the depressant effect of the acetylcholine. The minimal effective dose of pantothenate was 1 mg/kg, but higher doses did not produce a proportional potentiation. Table II shows the combined effects of acetylcholine and calcium pantothenate:

Table II

No.	Designation of the animal	Acetylcholine dose, mcg/kg	Pantothenic acid dose, mg/kg	Blood pressure lowering effect of acetylcholine in Hg mm before	after	Blood pressure variation, %
				administration of P.A.		
1.	12.	3	0.01	30	30	0
2.	24.	3	0.1	20	20	0
3.	9.	3	1.0	15	20	30
4.	10.	4	1.0	25	30	20
5.	8.	3	1.0	20	30	50
6.	7.	5	1.0	25	35	40
7.	20.	5	1.0	12.5	20	60
8.	1.	4	1.0	25	35	40
9.	25.	10	1.0	40	60	50
10.	11.	5	5.0	30	30	0
11.	4.	6	10.0	30	40	30
12.	0.	3	10.0	40	40	0
13.	19.	3	20.0	30	33	10
14.	18.	3	20.0	20	35	70
15.	17.	4	20.0	38	63	60
16.	2.	5	25.0	27	27	0
17.	16.	10	40.0	37	30	-20

Histamine was given to 14 cats; the doses of calcium pantothenate (up to 40 mg/kg) used had no influence on the depressant effect of the histamine, as can be seen by Table III:

Table III.

No.	Designation of the animal	Histamine dose, mcg/kg	Pantothenic acid dose, mg/kg	Blood pressure lowering effect of histamine in Hg mm before administration	Blood pressure lowering effect of histamine in Hg mm after administration of P.A.	Blood pressure variation in %
1.	12.	3	0.01	29	30	0
2.	24.	3	0.1	30	30	0
3.	9.	3	1.0	10	10	0
4.	10.	4	1.0	15	17.5	10
5.	8.	3	1.0	15	17.5	10
6.	7.	5	1.0	20	20	0
7.	20.	5	1.0	20	20	0
8.	25.	10	1.0	42.5	35	0
9.	0.	10	10.0	50	50	0
10.	19.	5	20.0	20	20	0
11.	18.	5	20.0	15	20	30
12.	17.	6	20.0	40	50	20
13.	2.	5	25.0	22.5	22.5	0
14.	16.	5	40.0	20	22.5	0

Therapy

The metabolism of pantothenic acid under conditions of liver damage in rabbits and in man was studied by Ueshima (139) in 1958. Therapeutic doses of calcium pantothenate were given in the experiments.

Male rabbits weighing about 2 kg with induced acute and chronic liver damage were given 10 mg calcium pantothenate daily intramuscularly; 1 ml saline solution was given similarly to controls. In acute liver damage experiments therapy was initiated 2 days before induction of liver damage and was continued for 5 days; in chronic cases, therapy and damage initiation were simultaneous, and therapy was continued for 70 days. Blood samples were taken and after sacrifice of the animals, livers were removed for histological examination and liver pantothenic acid concentration determined. Serum cholesterol changes were noted (total and esterified), ester quotients and blood pyruvic acid concentrations were determined. The results are shown in the following tables:

Effects of Ca-PaA therapy on the serum cholesterol metabolism in rabbits with acute and chronic CCl_4 liver damages.

		Average per cent change	On the 3rd day after liver damage	On the 7th day after liver damage
Acute Damage	Control group (3 cases)	Total cholesterol ester ratio	+ 138.2	+ 10.7
			- 13.3	- 3.7
	Therapy group (3 cases)	Total cholesterol ester ratio	+ 123.6	+ 43.3
			+ 0.3	+ 1.7
		Average per cent change	On the 30th day after liver damage	On the 60th day after liver damage
Chronic Damage	Control group (4 cases)	Total cholesterol ester ratio	+ 106	+ 317
			- 14.8	- 48.9
	Therapy group (4 cases)	Total cholesterol ester ratio	+ 90	+ 215
			- 10	- 12.3

Effects of Ca-PaA therapy on the blood PyA metabolism in rabbits with acute and chronic CCl_4 liver damage

		Average per cent change	On the 3rd day after damage	
Acute damage	Control group (3 cases)	blood PyA	+ 93	
	Therapy group (3 cases)		+ 49.5	
		Average per cent change	On the 30th day after damage	On the 60th day after damage
Chronic damage	Control group (3 cases)	blood PyA	+ 17	+ 24
	Therapy group (3 cases)		+ 4	+ 9.7

Effects of Ca-PaA therapy on the liver PaA content of rabbits with acute and chronic CCl_4 liver damage

	Days after damage	Ca-PaA administration	Average liver PaA content		
			Free form	Conjugated form	Total
Acute damage	3 days	Control group (4 cases)	3.3	41.7	46.7
		Therapy group (4 cases), received therapy one day before liver damage	12	80.3	92.3
	7 days	Control group (4 cases)	2.1	59.5	61.6
		Therapy group (4 cases), received therapy two days before liver damage	4.8	63.2	68.0
Chronic damage	70 days	Control group (4 cases)	3.0	41.1	44.1
		Therapy group (5 cases), received therapy five days before liver damage	2.2	50.1	52.3
		Healthy group (12 cases)	* 1.5±0.5	65.5±10.5	67±10.7

* Standard deviation

The ester quotient decreased in the control group after acute liver damage, but remained normal in the pantothenate-treated group; some decrease in chronic cases was observed in the control group, but to a lesser degree in the treated group. Increased pyruvic acid concentrations nearly doubled in acute, control animals a few days after liver damage, and to a milder degree in pantothenate treatment. A drop in total liver pantothenic acid occurred in the acute controls, but remained normal in treated animals; the chronic cases showed a marked decrease on the 70th day after liver damage, the treated group showed only a slight decrease in total liver pantothenic acid.

Histological examination of liver tissue in both acute and chronic damage to the liver (by CCl_4) showed typical pathology: lobular central necrosis, hemorrhage and degeneration.

Thirty hospital patients, with hepatitis or cirrhosis, were given 20-40 mg calcium pantothenate for 30 days and various liver-function tests were performed to determine the influence of pantothenate. Serum and urinary concentrations of pantothenic acid were determined, and in general, the pantothenate therapy resulted in improvement. Subjective and objective symptoms such as nervousness were either alleviated or disappeared.

The author devoted a considerable portion of the report to a general discussion of the role of pantothenic acid in coenzyme A formation and

the metabolism of carbohydrates, lipids and proteins, and the importance of the liver in metabolic phenomena. As a storage place for vitamins, the liver, when damaged, can be anticipated to cause abnormalities in various intermediary metabolisms such as those involving vitamins.

A report by Welsh (148) in 1952 on the treatment of the skin disease, lupus erythematosus, is included here principally because of the heavy dosages of Panthenol (d-pantothenyl alcohol) and calcium pantothenate (both racemic and dextrorotatory) employed in the treatment of the disease. Thirty-two patients were variously treated for periods of from a few months to as long as three years with dosage levels of 10-12 g/day of panthenol, and/or d-calcium pantothenate or dl-calcium pantothenate. One patient took a total of 3.6 kg (8 lbs) in the course of treatment and 15 patients took over 2 kg (4-5 lbs), all without untoward effect. The following tables summarize the treatments (some form of vitamin E was also employed):

—*Treatment of Patients Who Had Chronic Discoid Lupus Erythematosus*

Case No. and Initials	Sex	Duration of Disease, Yr.	Previous Treatment	Duration of This Treatment, Mo.	*d*-Calcium Pantothenate, Gm.	*dl*-Calcium Pantothenate, Gm.	Panthenol,* Gm.	Natural Tocopherols, Gm.	Ephynal Acetate,* Gm.	Per Cent of Clearing	Complications of Treatment
1, J. G.	F	3	Yes	29	904.3	100.0	1,799.5	269.5	48.3	95	None
2, T. J. G.	M	9	Yes	21	562.8	.....	3,169.5	89.4	129.4	100	G. I.*
3, E. H.	F	10	Yes	20	1,926.0	68.4	46.5	285.6	138.6	100	None
4, E. H. H.	F	9	Yes	36	431.0	.....	2,073.4	141.5	135.5	99	None
5, H. J.	F	25	Yes	24	76.8	.....	3,122.6	123.9	163.5	85	None
6, L. L.	M	4.8	Yes	10	.....	.....	1,777.0	.....	165.2	85	None
7, R. I. R.	M	11	No	36	477.2	168.0	20.0	.....	53.0	75	G. I.*
8, R. R.	M	29	Yes	24	874.0	200.0	2,412.3	86.8	225.0	90	None
9, L. S.	F	6	Yes	30	1,254.0	.....	1,417.0	8.8	85.2	80	None
10, M. S.	F	2	No	17	1,271.2	.....	1,225.0	80.8	146.4	90	None
11, T. S.	F	10	Yes	12	.....	72.0	873.6	.....	70.2	70	None
12, L. T.	F	8	No	22	799.6	.....	100.7	182.7	.....	100	None
13, L. J. von W.	F	10	Yes	36	1,519.8	150.0	1,166.0	120.6	.....	95	None
14, W. R. W.	M	1.3	No	17	731.7	.....	1,003.0	.....	79.0	80	None
15, D. Z.	F	2	No	30	1,255.8	10.0	.....	153.2	.....	100	None
16, H. W.	M	9	Yes	14	239.4	.....	30.0	75.0	.....	70	None
17, E. H. W.	F	6	Yes	16	336.8	.....	.....	39.9	.....	100	None

* Transient gastric distress from panthenol.*

—*Treatment of Patients Who Had Disseminate Discoid Lupus Erythematosus*

Case No. and Initials	Sex	Duration of Disease, Yr.	Previous Treatment	Duration of This Treatment, Mo.	*d*-Calcium Pantothenate, Gm.	*dl*-Calcium Pantothenate, Gm.	Panthenol,● Gm.	Natural Tocopherols, Gm.	Ephynal Acetate,● Gm.	Per Cent of Clearing	Complications of Treatment
1, F. B.	M	16.5	Yes	20	258.0	11.0	3,566.0	250.5	85.2	95	G. I.*
2, D. McP.	M	3	No	36	126.8	.....	787.0	.....	75.1	100	None
3, J. M.	M	10	Yes	17	421.5	.....	1,927.0	119.8	146.9	75	None
4, A. M.	F	6	Yes	2	.....	385.0	.....	.....	15.8	80	None
5, B. T.	F	7	Yes	34	1,051.6	2,120.0	.....	43.3	215.3	85	None
6, W. T.	M	2	Yes	10	149.0	.....	1,161.0	.....	175.9	90	None
7, J. B.	F	1	No	8	.....	77.0	137.0	.....	11.4	75	G. I.*
8, A. Y.	F	7	Yes	10	.....	.....	1,336.5	126.4	.....	100	None
9, C. B.	F	7	No	18	423.0	.....	.....	72.5	.....	100	None

* Transient gastric distress from panthenol.●

—*Treatment of Patients Who Had Subacute Disseminate Lupus Erythematosus*

Case No. and Initials	Sex	Duration of Disease, Yr.	Previous Treatment	Duration of This Treatment, Mo.	*d*-Calcium Pantothenate, Gm.	*dl*-Calcium Pantothenate, Gm.	Panthenol,● Gm.	Natural Tocopherols, Gm.	Ephynal Acetate,● Gm.	Per Cent of Clearing	Complications of Treatment
1, M. A.	F	0.5	Yes	12	.....	240.0	1,928.0	.....	18.3	100	None
2, M. G.	F	1.5	No	22	.....	.....	1,820.5	.....	149.2	95	None
3, B. H.	F	28	Yes	12	30.0	1,947.0	269.0	14.4	213.4	95	None
4, O. L.	F	6	Yes	27	1,673.0	.....	816.0	254.8	70.4	95	None
5, H. T.	F	0.5	No	8	.....	200.0	1,701.0	.....	130.8	99	None
6, V. D.	F	8	Yes	5	199.8	.....	.....	.....	18.0	100	None

Banerji (11) reported several cases of severe paralytic ileus in 1953 in which routine treatment of every kind had proven ineffective. Intramuscular injection of several doses of 50-150 mg calcium pantothenate in both cases resulted in rapid relief of the ileac failure and the recovery of the patient. Postoperative complications of ileus may be very serious, and Banerji felt that the high incidence of paralytic ileus could be related to pre-existing pantothenic acid deficiency in the patients. A tabular presentation of 19 additional cases treated with calcium pantothenate is included in the report.

A clinical evaluation of d-pantothenyl alcohol in the treatment of ileus, particularly in the amelioration of postoperative ileus was reported by Polacek et al. (176) in 1964. A double-blind clinical evalution was conducted on 100 consecutive elective cholecystectomy and 75 consecutive elective gastric resection cases, as representative of surgical procedures frequently associated with some degree of postoperative paralytic ileus. Several hours after surgery the patient received either a placebo or 250 mg of d-pantothenyl alcohol and several ml of drug or placebo. The injection was repeated in 2 hours, and every 8 hours for 5 consecutive days. A lead marker, swallowed with the first dose was traced by x-ray for 5 days or until it reached the sigmoid colon or rectum. Other clinical observations were recorded: audible peristalsis, flatus and first bowel movements. Only in the cholecystectomies did the pantothenyl alcohol accelerate the bowel transit time of the lead marker; gastric resections were not affected by the treatment. The authors suggest that d-pantothenyl alcohol should not be used routinely or as a replacement for standard precautionary measures (intubation, early ambulation, adequate hydration, drainage of abscesses, etc.); they were less than enthusiastic about the value of d-pantothenyl alcohol in the therapy of paralytic ileus.

Klykov and Minsker (63), in 1971, reported the results of studies utilizing calcium pantothenate in the therapy of chronic circulatory insufficiency. A total of 38 patients, of ages ranging from 40-75 years, with a background of rheumatic heart defects or atherosclerotic cardiosclerosis were treated upon hospital admission with calcium pantothenate only, in a dose of 200 mg three times daily for 5 days. Blood samples were taken before, during and after treatment, and analyzed for pyruvic acid, sulfhydryl (SH) groups, and for thermal coagulation time. Before treatment, elevated pyruvic acid concentrations were observed to be significantly elevated (2.38 plus or minus 0.25 mg % versus a normal 0.71 plus or minus 0.5 mg % (P = less than 0.001)). After treatment with calcium pantothenate, pyruvic acid levels were considerably reduced. Treatment did not influence the SH groups serum levels, which were significantly lower in the patients before treatment, compared to normal levels. Some decrease in heat coagulation time of the serum was observed under the influence of calcium pantothenate, reflecting an improvement of protein metabolism on the whole.

Biochemical Aspects

I. Breakdown

No Information Found

II. Absorption-Distribution

Weiss et al. (193) in 1950 studied acute and chronic toxicities of panthenol in rats, rabbits and dogs (see sections Acute Toxicity and Short-Term Studies) as well as the distribution of both panthenol and calcium pantothenate in the tissues of rats. Daily doses of 2 mg panthenol or its equivalent in calcium pantothenate were given rats by gastric cannula for periods of 24 or 45 days, or were added to stock diet for periods of 5 to 6 months. After sacrifice, the liver, heart, kidney and spleen of the treated animals were removed and total pantothenate determined. The results are shown in the following table:

Rat Tissue Storage of Pantothenic Acid.

Organ	Test period	Total pantothenic acid content (μg/g fresh tissue)							
		Control		Panthenol (2 mg/day)			Ca Pantothenate (2.3 mg/day)		
		No. of organs*	Avg content	No. of organs	Avg content	% incr.	No. of organs	Avg content	% incr.
Liver	24 da.	2 (1)	67	2 (1)	68	2	2 (1)	67	0
	45 "	3 (1)	74	3 (1)	83	12	2 (1)	79	7
	5-6 mo.	7 (5)	100	9 (6)	94	—6	5 (3)	75	—25
Kidney	24 da.	10 (2)	51	10 (2)	55	9	10 (2)	57	13
	45 "	10 (2)	48	10 (2)	61	27	10 (2)	54	12
	5-6 mo.	12 (4)	60	16 (4)	85	42	8 (2)	61	2
Heart	5-6 "	10 (2)	47	14 (2)	56	20	12 (2)	62	32
Spleen	5-6 "	12 (2)	27	14 (2)	27	0	14 (2)	27	0

* The numbers in parentheses indicate the number of analyses performed on separate pools of organs.

Little effect on the total pantothenic acid content of rat livers was observed, nor was a significant increase in liver stores of treated rats seen. In the 5-6 month series, the kidney content of pantothenate after panthenol increased 42%, compared with a 2% increase following administration of calcium pantothenate. The difference could not be explained. Splenic storage was unaffected.

It was determined that no significant amounts of panthenol were stored in the liver unchanged. The normal coenzyme A content of liver tissue was only slightly increased in the 24 and 45 day tests by panthenol and calcium pantothenate; the longer test period of 5-6 months showed no effect.

A comparison of the amounts of free and bound pantothenic acid in the liver of the treated rats showed practically all the pantothenic acid present in the liver in the bound form.

No anti-pantothenate influence by panthenol was observed throughout the experiments and the authors concluded "that panthenol is generally non-toxic to mammals at dosage levels in the therapeutic range".

Middleton and Monison (80) in 1965 described absorption studies of pyridoxine and calcium pantothenate given orally to male weanling Wistar rats at periodic intervals. The experiment with calcium pantothenate consisted of feeding the animals a basal diet without the pantothenate for a 5-week pretest period. Four groups of rats were selected, group A continued the pantothenate-free diet; group B received 40 micrograms calcium pantothenate daily; group C, 80 micrograms every other day; and group D, 160 micrograms every 4 days. The pantothenate was administered by gastric cannula. Weights were measured for each animal periodically during the 16-day growth phase of the experiment, after which the animal's ability to acetylate sulfanilamide was determined. Six animals were selected, fasted for 24 hours (water supplied), and given 250 mg/kg sulfanilanide intraperitoneally, after which an additional 24 hour fasting period was observed. Urine was collected during this period and analyzed for free and total sulfanilamide. Dosing the pantothenate-deficient animals with calcium pantothenate did not affect weight gains or acetylation ability. It was suggested by the authors that pantothenate was readily absorbed and utilized without wastage.

III. Metabolism and Excretion

Experiments on the excretion of pantothenyl alcohol (panthenol) and of calcium pantothenate, administered intraperitoneally or orally to rats, were described in a report by Braekkan (16) in 1955. Twenty experiments, each using 4 groups of 6 rats each (3 males, 3 females) weighing 100-300 g, were performed. Preliminary analysis of diets and of urinary excretion of pantothenic acid established a basal value of urinary excretion of 0.13 mg/day/rat. Dosages of the two compounds tested ranged from 0.35 to 4 mg given intraperitoneally, and from 1 to 4 mg given orally. Urine and feces were analyzed for pantothenic acid excreted per day; results are shown in the following two tables:

Urinary excretion of pantothenic acid after *intraperitoneal injection* of panthenol and calcium pantothenate in single doses.

Dosage	mg pantothenic acid in urine per day				
	1st day	2nd day	3rd day	4th day	5th day
Dist. Water	0.12	0.11	0.15	0.15	0.12
0.35 mg Panthenol	0.25	—	—	—	—
0.50 - -	0.38	0.18	0.13	0.14	0.13
1.00 - -	0.80	0.22	0.16	0.15	0.14
1.50 - -	0.90	0.18	0.16	0.13	—
2.00 - -	1.46	0.27	0.20	0.15	0.14
3.00 - -	2.20	0.31	0.22	0.17	0.14
4.00 - -	2.82	0.35	0.24	0.19	0.11
0.40 mg Ca Pantothenate	0.40	—	—	—	—
0.50 - -	0.49	0.21	0.16	0.19	0.16
1.00 - -	0.83	0.22	0.18	0.17	0.20
1.50 - -	1.20	0.22	0.17	0.15	—
2.00 - -	1.52	0.24	0.17	0.14	0.12
3.00 - -	2.31	0.29	0.17	0.13	—
4.00 - -	3.20	0.32	0.20	0.15	0.13

Urinary and fecal excretion of pantothenic acid after *oral administration* of panthenol and calcium pantothenate in single doses.

Dosage	mg pantothenic acid excreted per day					
	1st day		2nd day		3rd day	
	Urine	Faeces	Urine	Faeces	Urine	Faeces
Dist. Water	0.12	0.23 (25.2)[1])	0.13	0.20 (23.1)	0.13	0.26 (24.0)
1 mg Panthenol	0.80	0.23 (27.0)	0.30	0.24 (30.2)	0.22	0.20 (25.8)
2 - -	1.42	0.26 (26.5)	0.30	0.23 (29.0)	0.22	
3 - -	2.16	0.33 (24.5)	0.39	0.23 (25.8)	0.23	0.17 (25.1)
4 - -	2.57	0.18 (18.2)	0.39	0.17 (22.1)	0.23	
1 mg Ca Pantothenate	0.32	0.33 (30.7)	0.31	0.23 (27.8)	0.22	0.22 (27.0)
2 - -	0.64	0.53 (25.8)	0.24	0.25 (30.2)	0.19	
3 - -	0.77	0.93 (22.7)	0.32	0.18 (25.2)	0.24	0.17 (23.5)
4 - -	0.98	1.33 (25.0)	0.24	0.23 (27.9)	0.19	

[1]) The number in paranteses gives the weight of the faeces in g.

Panthenol and calcium pantothenate are differently excreted in rats after intraperitoneal injections and oral administration; the pantothenate, when injected caused about 25% higher increase in urinary excretion of pantothenic acid than does panthenol and oral administration is the same. Only 10% of the oral calcium pantothenate appeared in the urine; five times this amount appeared, unchanged, in the feces. The author concluded: "for therapeutic purposes by the oral route, panthenol should be used".

The excretion of pantothenic acid in the rat, after ingestion of loading doses of panthenol or calcium pantothenate under various feeding conditions, was the subject of experiments reported by Rubin et al. (111) in 1949. Earlier studies had indicated that panthenol was more available to humans at high doses and as effective in curing pantothenic acid deficiency in the rat, as was calcium pantothenate. This latter discovery lead the authors to the present experimentation as justified in interpreting excretion data in terms of availability.

Adult, male albino, Sprague-Dawley rats, weighing 375-475 g, were dosed with panthenol or calcium pantothenate at 3 dosage levels: 10, 5 and about 2 mg/kg. Ten to 20 animals were used at each dosage level, eating freely up to dosage time and during the following two, 24-hour urine and fecal collection periods. In general, 173, 85 and 7% more pantothenic acid was excreted in the urine after panthenol ingestion at the three levels of dosage (10, 5 and 1.25 mg/kg) than after equivalent calcium pantothenate ingestion. Only the two higher doses gave significantly different results. Panthenol was excreted as pantothenate more rapidly (60% of the dose within 24 hours versus 23-33% of the calcium pantothenate). Little significance was observed during the second 24-hour collection period in the excretion, being about 5% for both compounds.

Controlled feeding experiments in which the rats were starved for 24 hours prior to being given 6 g of stock diet, which was consumed in 45 minutes. The doses of panthenol and calcium pantothenate were given immediately to both fed and unfed controls. Urine and feces were collected and 6 hours later, both groups - fed and unfed controls were placed on the stock diet.

Basal urinary excretion was unrelated to whether the animals had been fed at the beginning of the excretion collection period or not; the basal value (0.14 mg/day) was similar to that found in the ad libitum experiments, nor was the volume of urine influenced. Fecal excretion was directly related to fecal weight.

The presence or absence of food at the time of dosing had no significant effect on urinary excretion for either of the two compounds given. Fecal excretion of the pantothenate given was influenced by the presence of food, being greater with than without (30 versus 16%), at the 10 mg/kg dose level.

The authors compared their experimental results with those found

by others in human studies; panthenol appears to be more available or at least as available as calcium pantothenate under any feeding conditions, at a 10 mg/kg level, and to a lesser degree at a 5 mg/kg level. At lower levels of 1.25-2 mg/kg, no significant differences in urinary excretion of pantothenic acid were found between panthenol and calcium pantothenate, regardless of the immediately previous nutritional status of the animals.

A study of the comparative values of pantothenol (PaOH) and d-calcium pantothenate (Ca-PaA) as pharmaceuticals was reported by Yamawaki and Ishida (152) in 1961. White, Wistar rats weighing 200-300 g, in groups of 3 each, were fed a basic diet for about a month until urine analyses showed a constant excretion of pantothenic acid (about 100 gamma daily). During experimentation, urine and feces were collected daily and analyzed, by microbiological assay (using Lactobacillus arabinosus), for pantothenic acid concentrations (PaA).

Intraperitoneal injection of pantothenol or calcium pantothenate in doses of 10, 5, 3 or 1 mg per rat were given in the morning and urine and feces excreted throughout the day following injection were collected and pantothenic acid determined.

The amounts of urinary PaA over a 5-day period following injection of PaOH or Ca-PaA showed that in the case of PaOH, excretion is somewhat slower or smaller on the first day and recovery to normal levels takes longer than in Ca-PaA treatment. It was considered that PaOH is retained longer and thus its action is more prolonged in the body than is Ca-PaA.

Oral administration of the same quantities of PaOH and Ca-PaA to 4 groups of 3 rats each resulted in for greater urinary excretion of PaOH within 24-hours than of Ca-PaA.

The authors discussed the experimental results at some length. The conclusion "that PaOH is quite advantageous as a dosage type of the PaA homologues" was in agreement with the findings of other studies.

Abnormalities of metabolism and disturbance of the adrenal cortical function are frequently associated with tuberculosis; studies of a close relationship between pantothenic acid (PaA) and the foregoing factors were reported to be few in number by Nakamura et al. (152) in 1961. They reported the results of studies using the rat and human patients (tubercular) administered calcium pantothenate (Ca-PaA) and streptomycin, the latter being used in the therapy of tuberculosis.

White rats of about 100 g weight were used to test the influence of Ca-PaA on animals given prolonged streptomycin treatment; the dose of streptomycin, 5 mg/100 body weight, was given intraperitoneally daily for 6 weeks to a control group. The treated groups received intraperitoneally 1 mg Ca-PaA plus additional compounds daily for 6 weeks, and streptomycin

daily in a dose of 50 mg/100 g for 4 weeks longer. Treatment of the rats receiving streptomycin alone showed that those animals given Ca-PaA maintained near-normal acetylation capacities. Numerous other tests showed that Ca-PaA generally ameliorated abnormalities of metabolism and gross pathologies of liver and kidneys seen in those animals subjected to prolonged streptomycin treatment.

In the studies of 41 cases of pulmonary tuberculosis among hospital cases, urinary excretion of pantothenic acid (PaA) was observed to be somewhat lower than that in normal, healthy individuals. Intramuscular injection of 20 mg Ca-PaA increased the urinary excretion of PaA in tubercular patients to near-normal levels during a 6-hour interval after injection. In general, patients with fibro-caseous or infiltrative tuberculosis showed little response to Ca-PaA treatment; greater response was seen in some types of exudative and fibro-caseous tuberculosis.

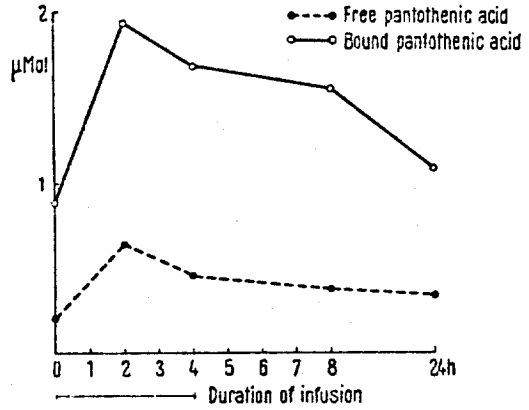
The urinary excretion of PaA in 23 patients who had undergong chemotherapy for less than 6 months and were then treated with Ca-PaA was increased from an average of 4.02 mg to about 6.95 mg; patients under chemotherapy for from 6 months to a year showed increased from 4.78 to 5.47 mg, and those for longerthan a year of chemotherapy, increases from 2.75 to 3.03 mg. The erythrocyte sedimentation rates and the amount of urinary excretion of the Ca-PaA showed that rates of more than 20 min/hour sedimentation lead to a marked increase in urinary excretion of PaA after the patients were loaded with Ca-PaA. Various side effects of streptomycin therapy such as ringing in the ears and poor hearing were accompanied by an average of 3.16 mg urinary excretion of PaA; loading with Ca-PaA increased the excretion to 3.46 mg. In most cases of subjective side effect complaints, Ca-PaA ameliorated the condition. Of 138 tubercular patients under streptomycin treatment, there were 97 cases with side effects in some form or other: numbness of face and lips, headache, tinnitus, hearing loss, etc. Intramuscular injections of 40 mg Ca-PaA relieved many cases.

In summary, tubercular patients often showed indications of PaA deficiency and in serious cases, a drop in PaA utilization capacity was recognized. The similarity to the relationship of PaA metabolism and liver damage (see Ueshima (139) lead the authors to conduct BSP tests on the tubercular patients, with the result that in many of the patients, disturbance of the liver function tended to co-exist with the tubercular condition. The effectiveness of Ca-PaA in mitigating the side effects of streptomycin therapy was discussed.

A second study reported during 1961, by Stepanyan and Stukalova (126) parallels that of Nakamura et al. Mice and human tubercular patients were studied in the same fashion and with the same results.

Bigler and Tholen (15) in 1966 performed balance studies on free and bound pantothenic acid in humans following the administration by intravenous infusion of coenzyme A or calcium pantothenate. The infusions, given to convalescent patients with normal glomerular filtration rates, contained 250 micromoles coenzyme A (192 mg) or 250 micromoles d-calcium pantothenate (119 mg) dissolved in 240 ml isotonic glucose or saline, and delivered at the rate of 1 ml/min for 4 hours.

Blood and urine samples taken before, during and after infusion were analyzed for coenzyme A and for pantothenic acid. The following figure shows the results:



Free and bound (coenzyme A) pantothenic acid in blood before, during, and after intravenous infusion of coenzyme A, α-lipoic acid, diphosphopyridine nucleotide and cocarboxylase. M. S., 1949 ♂; normal glomerular filtration rate.

The data indicate that the injected coenzyme A is broken down in the body (elevated free blood pantothenate) during and after injection; however, the blood level of bound pantothenate is raised. The urinary excretion of pantothenic acid was increased after infusion of calcium pantothenate, accounting for about 16-29% of the administered dose. About 20-30% of the infused coenzyme A was excreted as urinary pantothenate: the fate of the remainder of the dose was not known.

The absorption and excretion of calcium pantothenate by normal and depleted dogs was studied by Silber (116) in 1945. Two adult dogs and 7 pups were used in saturation studies; 3 pups served as controls. A basal diet, containing B vitamins exclusive of pantothenic acid was given in depletion amounts; an overnight fast was followed by a dose of 4 mg/kg calcium pantothenate given either orally or subcutaneously. Food was withheld for 3-4 hours, and urines collected for 24 hours; the depleted animals given oral doses of pantothenate excreted 0.13% of the test dose (4 mg/kg) compared with normal dogs which excreted 1.7%. Parenterally given pantothenate in depleted dogs was excreted to 3.3%; in normal dogs, 11.7%. No significant fraction of the pantothenate was detected in the urine on the second day. In both depleted and normal dogs, 14-27% of the pantothenate dose was excreted in the feces, when the dose was given orally; the subcutaneous doses gave no increase in fecal pantothenic acid.

Ten laboratory workers were given 50 mg calcium pantothenate in 200 ml H_2O, orally, and urines were collected during the 4-hour period following, and assayed for pantothenic acid content. Prior to dosing, urinalyses showed 1.0 plus or minus 0.15 mg pantothenic acid in a 4-hour period; after dosing, 6.0 plus or minus 0.48 mg was measured in a 4-hour period.

Further tests of the effect of food on absorption and excretion of calcium pantothenate in dogs indicated that when food intake was decreased, urinary excretion of the pantothenate was increased by as much as 50% of the supplemental dose; when food was supplied with the vitamin, as much as 50% passed through the intestinal tract unabsorbed.

Gounelle and Richet (162) reported in 1957 a study of the urinary excretion of pantothenic acid in man over a 24-hour period. Variations in amounts of urinary pantothenic acid in normal subjects (6) were of small amplitude; ingestion of 100 mg panthenol raised the urinary concentrations of pantothenic acid ten to fifty-fold above normal during the four hours following the oral dose. By six hours, the excretion tends toward normal levels, but remains slightly above normal for 24 hours. The daily urinary excretion for 4 normal subjects varied from 3.65 to 5.26 mg; one subject given 100 mg panthenol orally showed a total 24-hour excretion of 38.4 mg.

IV. Effects on Enzymes and Other Biochemical Parameters

The influence of pantothenic acid in the diet of pregnant female albino rats of the Wistar strain on the newborn was reported by Chung et al. (23) in 1954. The pregnant females (35) had produced satisfactory first litters; four groups were placed on various diets. Group I received stock ration; Group II received a purified diet supplemented by a vitamin mixture (see Table 1); Group III received the same diet as Group II, plus 100 micrograms calcium pantothenate; and Group IV was given same diet as Group III, except 1 mg calcium pantothenate was added.

TABLE 1

Composition of the purified ration [1]

Vitamin-free casein	25%
Dextrose	55%
Swiftening	15%
Salts [2]	5%

Vitamin mixture fed daily per rat

Choline chloride	10 mg
Inositol	5 mg
p-aminobenzoic acid	100 μg
2-methyl-naphthoquinone	250 μg
Thiamine hydrochloride	150 μg
Riboflavin	100 μg
Niacin	500 μg
Biotin	2.5 μg
Folic acid	6 μg
Pyridoxine hydrochloride	50 μg
Vitamin B_{12}	0.5 μg

Fat soluble vitamins

Cod liver oil:	9 U.S.P. units vitamin D
	90 U.S.P. units vitamin A
Alpha tocopherol	750 μg

[2] Richardson, L. R., and A. G. Hogan — J. Nutrition, *32:* 459, 1946.

The newborn rats were sacrificed and portions of liver, duodenum, and entire adrenals and tibias were prepared for histological examination and for histochemical demonstration of alkaline and acid phosphatase enzymes. No histological evidence of the diet of the females influencing the offspring was found. The only histochemical finding of significance in the newborn was in the alkaline phosphatase reaction in the adrenals, which exhibited a decreased reaction in those offspring born to females that received no pantothenic acid throughout pregnancy, and those that received only the stock diet.

Pantothenic acid-deficient females produced apparently normal offspring insofar as the 4 tissues studied was concerned. Any pathologies that may have been attributable to the diets are obscured by early fetal resorptions.

A paper by much the same study groups (Everson et al. (35)) using the same experimental diets, reported chemical findings: total pantothenic acid content of tissues, serum ascorbic acid, serum alkaline phosphatase and blood pyruvic acid. The results are summarized in the following table:

Chemical findings in rats resulting from differences in pantothenic acid intake of the maternal rat during pregnancy [1]

RATION	LITTER SIZE [2]	BIRTH WEIGHT [2]	PANTOTHENIC ACID CONTENT OF TISSUES			PANTOTHENIC ACID CONTENT OF BLOOD OF YOUNG	ALKALINE PHOSPHATASE OF SERUM OF YOUNG	ASCORBIC ACID OF SERUM OF YOUNG	PYRUVIC ACID OF BLOOD OF YOUNG
			Maternal liver	Maternal carcass	Newborn carcass				
		gm	*µg/gm*	*µg/gm*	*µg/gm*	*µg/100 ml*	*nitrophenol units*	*mg/100 ml*	*mg/100 ml*
Stock diet	11.2	5.2	67.8	7.5	36.9	450	14.6	5.85	1.04
450–600 µg calcium			(64.6–70.9)	(7.0–7.9)	(29.4–45.8)	(342–570)	(9.9–19.5)	(4.13–8.38)	(0.29–1.88)
pantothenate daily	(28)		(10)	(10)	(40)	(50)	(58)	(67)	(18)
Synthetic diet	4.2	3.7	40.7	4.4	6.7	295	12.0	4.12	2.79
No calcium panto-			(38.6–43.6)	(3.2–5.0)	(5.1–7.6)	(108–447)	(7.0–16.9)	(2.47–6.00)	(0.78–6.33)
thenate	(35)		(10)	(10)	(25)	(20)	(20)	(20)	(23)
Synthetic diet	7.8	4.6	54.6	5.5	15.8	502	11.8	4.58	....
100 µg calcium			(45.0–62.4)	(4.8–6.0)	(12.6–24.8)	(320–678)	(7.7–15.8)	(3.19–7.31)	....
pantothenate daily	(17)		(10)	(10)	(25)	(27)	(40)	(42)	....
Synthetic diet	8.0	5.1	72.1	9.2	72.1	2204	12.1	4.22	1.63
1 mg calcium			(64.8–80.5)	(8.5–10.4)	(60.1–75.7)	(1709–2575)	(9.2–15.8)	(3.10–6.08)	(0.42–2.25)
pantothenate daily	(18)		(10)	(10)	(24)	(15)	(42)	(44)	(20)

[1] Average values listed first; range given below; third value indicates number of animals examined in each group.

[2] Number of litters included in litter size and birth weight values.

Serum values for ascorbic acid and alkaline phosphatase in fetal tissues were not affected by maternal diets. Those maternal diets devoid of pantothenic acid produced small litters of undersized young; blood pantothenic acid concentrations were reduced, and some accumulation of pyruvic acid was observed.

V. Drug Interaction

No Information Found

VI. Consumer Exposure Information

From the Handbook of Food Additives (235):

Pantothenic Acid - Pantothenic acid is available in several forms. Panthenol is the most stable in liquid products, but is not recommended for use in foods because of analytical problems. Calcium pantothenate is available as the D-isomer or the d,l racemic mixture. d,l-Calcium pathothenate-calcium chloride complex is less hygroscopic and has superior stability in multivitamin tablets. The l-isomer has no biological activity. Calcium pantothenate has good stability in dry mixes such as grain based complete protein foods. Retention during baking of white bread was 94%.

From NAS/NRC, recommended dietary allowances (Publication #1694, Food and Nutrition Board, 7th Edition (279):

A most important feature of pantothenic acid deficiency in both animals and man is loss of antibody production. Subjects deficient in either pantothenic acid or pyridoxine formed antibodies sluggishly, but men deficient in both failed completely to form antibodies in response to injections of typhoid and tetanus antigens, even though they had previously been immunized with these antigens in military service.

Usual diets of adult Americans furnish about 10 to 15 mg of pantothenic acid daily, with a range of 6 to 20 mg. Diets that meet the other recommended nutrient allowances for children seven to nine years of age provide about 4 or 5 mg of pantothenic acid. Human milk contains approximately 2 mg of pantothenic acid per liter, and cows' milk contains about 3.5 mg/liter. Subjects consuming 5 to 7 mg of pantothenic acid daily excreted 2 to 7 mg/day in their urine and 1 to 2 mg in their feces. A daily intake of 5 to 10 mg is probably adequate for children and adults. There is no evidence for or against a greater requirement during pregnancy or lactation.

Pantothenic acid (vitamin B_5) seems to be universally present in all living protoplasm. The richest animal sources are liver and kidney; other organs are good sources, but the muscle of beef, pork, veal and lamb is rather poor. Various molds and micro-organisms synthesize pantothenic acid, as do green plants. In both plant and animal tissue, the vitamin only slightly appears in the free form but rather is chemically bound to proteins (chiefly as a component of coenzyme A).

Calcium pantothenate is used in the therapy of a number of medical disorders: peripheral neuritis; cramps in the legs during pregnancy; and postoperative ileus. The alcohol derivative, pantothenyl alcohol (dex panthenol) is also given for ileus and for postsurgical improvement of gastric or intestinal atony. The doses of calcium pantothenate range from 10 to 50 mg daily, given orally or intramuscularly; dexpanthenol doses of 250 or 500 mg are injected intramuscularly, with repeated doses every few hours. A 5% ointment of dexpanthenol is widely used in various dermatological disorders. The vitamin has been suggested as a treatment for gray hair or alopecia but tests have failed to demonstrate an effectiveness.

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.

Tables 2 and 3 report the usage of calcium pantothenate in foods; Table 11 reports the annual poundage data. Table 13 shows possible daily intake per food category and total dietary based on food consumption by total sample, Table 14 reports potential daily intakes per food category, based on food consumption by eaters only.

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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 PANTOTHENATE NAS-0047	01	BAKED GOODS(R)	*	.00500	.00500
	05	MILK PRODS(R)	5	.00057	.00157
	10	MEAT PRODS(R)	*	.00600	.00600
	11	POULTRY(R)	*	.00600	.00600
	13	FISH PRODS(R)	*	.00600	.00600
	22	SNACK FOODS(R)	*	.00000	.00000
	23	BEV TYPE I(R)	*	.00000	.00000
	26	RECONST VEG(R)	*	.00250	.00417
	28	IMIT DAIRY(R)	*	.00072	.00072

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TABLE 3 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP II) USED IN INFANT FORMULA PRODUCTS & BABY FOODS(B)

NAS SURVEY NO.	SUBSTANCE NAME	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
0047	CALCIUM PANTOTHENATE	83	FORMULAS(B)	6	.00058	.00262

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

SUBSTANCE NAME (SURVEY 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 FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
CALCIUM PANTOTHENATE NAS 0047	14/ 14	950	6,247	7,996			7,996

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

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
CALCIUM PANTOTHENATE NAS 0047	01	BAKED GOODS(R)	*	0-5 MO.	.170000	.225000	.170000
				6-11 MO.	1.270000	2.590000	1.270000
				12-23 MO.	2.725000	4.490000	2.725000
				2-65+ YR.	6.860000	10.190000	6.860000
CALCIUM PANTOTHENATE NAS 0047	05	MILK PRODS(R)	5	0-5 MO.	.090780	.122800	.084780
				6-11 MO.	.355680	1.710570	.279680
				12-23 MO.	.310650	.994000	.255650
				2-65+ YR.	.225150	.687420	.220150
CALCIUM PANTOTHENATE NAS 0047	10	MEAT PRODS(R)	*	0-5 MO.	.066000	.174000	.066000
				6-11 MO.	1.242000	3.348000	1.242000
				12-23 MO.	1.812000	3.114000	1.812000
				2-65+ YR.	4.704000	7.800000	4.704000
CALCIUM PANTOTHENATE NAS 0047	11	POULTRY(R)	*	0-5 MO.	.030000	.139000	.030000
				6-11 MO.	.234000	.792000	.234000
				12-23 MO.	.396000	1.104000	.396000
				2-65+ YR.	.774000	1.968000	.774000
CALCIUM PANTOTHENATE NAS 0047	13	FISH PRODS(R)	*	0-5 MO.	.006000	.013000	.006000
				6-11 MO.	.078000	.294000	.078000
				12-23 MO.	.324000	.810000	.324000
				2-65+ YR.	.744000	1.854000	.744000
CALCIUM PANTOTHENATE NAS 0047	22	SNACK FOODS(R)	*	0-5 MO.	**********	.000000	**********
				6-11 MO.	.000000	.000000	.000000
				12-23 MO.	.000000	.000000	.000000
				2-65+ YR.	.000000	.000000	.000000
CALCIUM PANTOTHENATE NAS 0047	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

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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

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
CALCIUM PANTOTHENATE NAS 0047	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.	.000000	.015000	.006340
CALCIUM PANTOTHENATE NAS 0047	28	INIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.010080	.010560	.010080
				12-23 MO.	.005760	.024480	.005760
				2-65+ YR.	.006480	.010800	.006480
CALCIUM PANTOTHENATE NAS 0047	83	FORMULAS(B)	6	0-5 MO.	1.947060	3.067000	6.761140
				6-11 MO.	.396720	1.890220	1.381500
				12-23 MO.	.127600	.035960	.444400
CALCIUM PANTOTHENATE NAS 0047	ALL CATEGORIES		14	0-5 MO.	2.240840	4.144800	7.137920
	**********			6-11 MO.	3.586480	10.641360	5.195440
	**********			12-23 MO.	5.701010	10.572960	6.562710
	**********			2-65+ YR.	13.318630	22.531220	12.716970

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TABLE 14, PART A -- POTENTIAL DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUP I & II), PER FOOD CATEGORY, BASED ON FOOD CONSUMPTION BY EATERS ONLY - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

FOOD CATEGORY	SUBSTANCE NAME NO. NAME AGE	NO. OF FIRMS (SURVEY NO.) AVERAGE		POTENTIAL DAILY INTAKE, MB., PER CATEGORY ******* HIGH A	HIGH B	VERY HIGH
83 FORMULAS(E)						
CALCIUM PANTOTHENATE NAS 0047	6	2.336820	0-5 MO.	3.786240	8.138580	13.186560
		1.094180	6-11 MO.	3.536260	5.900420	12.315940
		2.080460	12-23 MO.	4.313460	7.245740	15.022740

CALCIUM PANTOTHENATE

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Albright-'68
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Automated Analysis of Calcium Pantothenate in Multi-Vitamin Preparations

B. E. ALBRIGHT

Research at Miles Laboratories, Inc., has led to the development of an automated fluorometric determination of calcium pantothenate in multiple vitamin preparations, using the Technicon Auto-Analyzer. Most manual determinations are based upon the colorimetric method of Schmall and Wollish (3) while N. E. Dowd (1) has successfully applied a colorimetric method to automated analysis.

The manual method of Panier and Close (2) was successfully adapted to automated analysis. The method involves the fluorometric determination of B-alanine, the product of alkaline hydrolysis of calcium pantothenate. Fluorescence is developed by the reaction of B-alanine with buffered ninhydrin and butyraldehyde. The fluorescent intensity is measured at 465 mμ in the concentration range between 0.1 and 1.0 μg/ml.

An ion-exchange resin column, Fig. 1, consisting of Dowex and Florisil (3) is used to remove any B-alanine that may have been preformed, as well as interfering agents such as dyes and riboflavin.

Apparatus

1. Sampler II
2. Proportioning Pumps
3. Single Coil 95°C. Heating Bath
4. Double Coil 37°C. Time Delay Coil
5. Fluorometer
6. Recorder
7. Nitrogen Tank and Reducing Valve.

Reagents

1. *USP Calcium Pantothenate Reference Standard*—Dissolve 15.0 mg of calcium pantothenate in 100 ml of distilled water. Store in the refrigerator.

   Prepare fresh each week.

2. *Sodium Hydroxide (0.5N)*—Dissolve 20.0 g of sodium hydroxide pellets in 1000 ml of distilled water.
3. *Hydrochloric Acid (0.375N)*
4. *Sodium Bicarbonate (1M)*—Dissolve 84 g of sodium bicarbonate in distilled water and make to 1000 ml.
5. *Sodium Carbonate (1M)*—Dissolve 106 g of anhydrous sodium carbonate in distilled water and make to 1000 ml.
6. *Ninhydrin Reagent*—Dissolve 1.6 g of ninhydrin in a solution containing 100 ml of 1 M sodium carbonate and 300 ml of 1 M sodium bicarbonate. De-aerate in a dark bottle (Fig. 4) with nitrogen gas for at least 30 min prior to use. Maintain the nitrogen atmosphere during the assay.

   Prepare the reagent fresh daily.

7. *n-Butyraldehyde (Butanol) (0.2M)*—Add 9 ml of n-butyraldehyde to 500 ml of water. Use a magnetic stirrer to aid is dissolving. Filter the solution through filter paper and glass wool to remove any undissolved material.

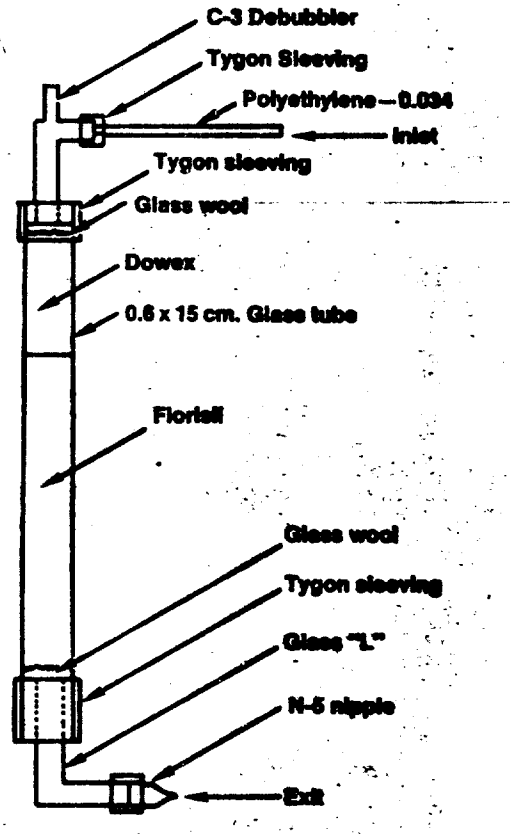

Figure 1

Presented at the Technicon Symposium, "Automation in Analytical Chemistry," Oct. 3, 1967, New York, N.Y., by B. E. Albright, Miles Laboratories, Inc., Elkhart, Ind. Present address, Technicon Corp., Ardsley, N.Y. 10502.

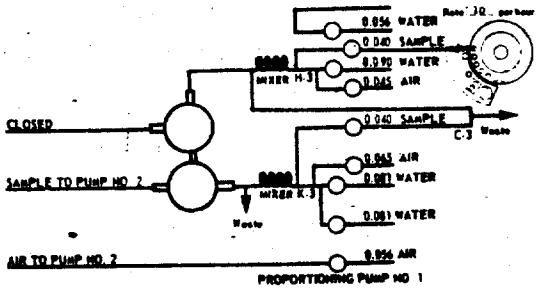

Figure 2

8. *Dowex*—50#—×4(H^+) or 50—×12(H^+), 100 to 200 mesh.
9. *Florisil*—60 to 100 mesh.

Preparation of Ion-Exchange Resin Column

A 0.6 × 15 cm glass tube is used to contain the resins, Fig. 1. Place a glass wool pledget in the bottom of the glass tube. Connect the column in a vertical position to a suitable vacuum source. Prepare slurries of both resins. Fill the column with water and draw Florisil into the column to a depth of 9-10 cm. Fill the remaining column with Dowex. Place a pledget of glass wool over the Dowex. Insert the C-3 connector, being careful not to force any air into the resin layer. By flowing water over the top of the column before the exit is connected to the proportioning pump, inadvertent introduction of air into the column is prevented.

Sample Preparation

Solid samples are disintegrated in water in a Waring Blender for 3 min. A small portion of the sample solution is filtered through Whatman No. 1 filter paper to remove particulate matter. If desired, the samples can be assayed unfiltered; however, a rigid cleansing program must be followed to prevent deposits from building up on the inner

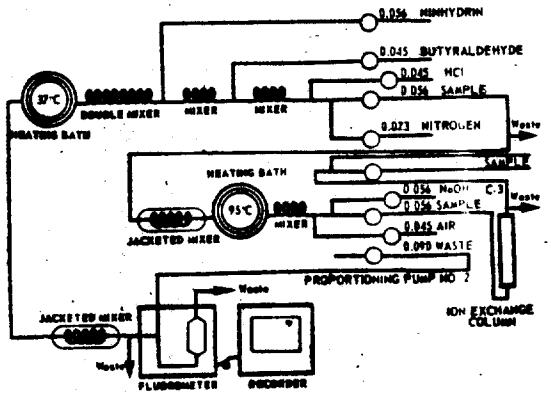

Figure 3

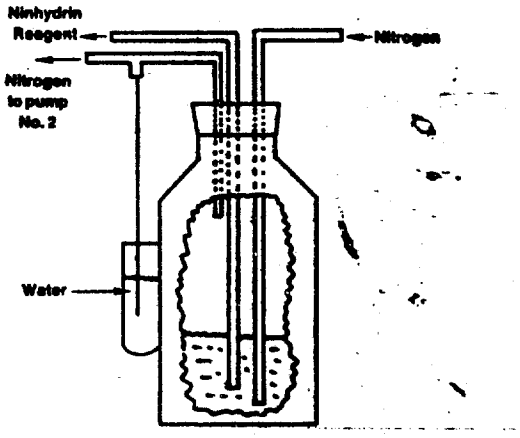

Figure 4

walls of the glass coils. For the flow diagram in this article, all samples contain about 150 μg/ml of calcium pantothenate. If the automatic dilution is not used, the samples should be diluted to contain about 4.0 μg/ml-calcium pantothenate.

Procedure

The automatic dilution flow diagram is shown in Fig. 2, while Fig. 3 depicts the automation of the manual chemical procedure. The sampling rate is 10 samples/hr using a 1:2 sample:wash ratio cam.

After the samples are purified by the ion-exchange column, hydrolysis of the calcium pantothenate to B-alanine is accomplished in a 40-ft coil maintained at 95°C. No improvement in hydrolysis was observed by using the full length of a double coil. After hydrolysis, nitrogen is used for segmentation to further prevent the oxidative degradation of the ninhydrin reagent. Fig. 4 shows the ninhydrin reagent bottle.

The instability of the ninhydrin reagent in air shows up as a continuous upscale baseline drift. As the hydrolyzed sample exits from the heating bath, it is cooled and debubbled to relieve pressure produced inside the heating bath due to thermal expansion. An aliquot of the solution leaving the heating bath is withdrawn and neutralized with hydrochloric acid. The pH of the neutralized solution should be between 6 and 9 to allow maximum buffering capacity by the ninhydrin reagent. Following neutralization, the butyraldehyde and ninhydrin reagent are added to the sample stream. The effect of ninhydrin concentration is shown in Fig. 5.

The combined solutions now pass through a double length time delay coil that is maintained at 37°C. A dialyzer bath may be substituted if a

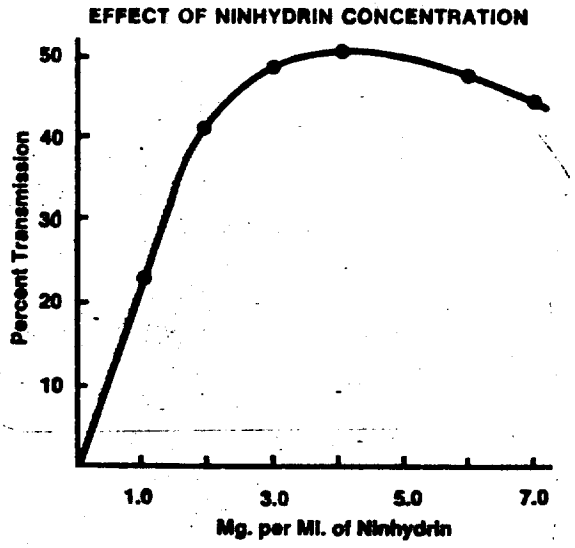

Figure 5

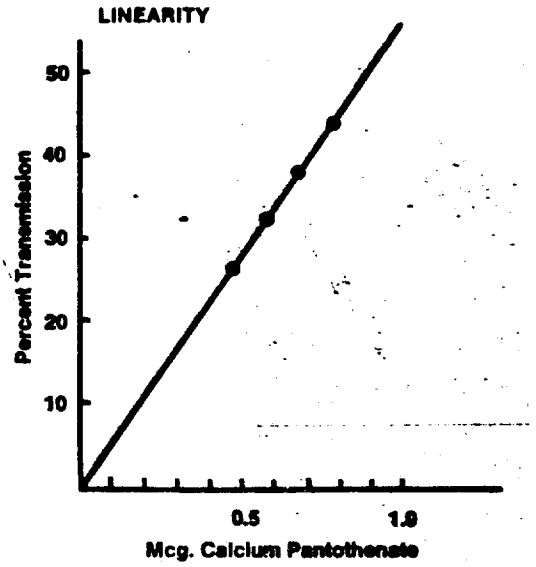

Figure 7

variable temperature heating bath is not available. It has been found that the temperature setting of the time delay coil may be chosen within the range of 37-50°C without adversely affecting the results. At temperatures below 37°C, the coupling reaction proceeds at a reduced rate and at temperatures above 60°C the ninhydrin reagent begins to deteriorate. It has been observed that reducing the length of the time delay coil by one-half reduces the %T by the same amount.

From the time delay coil, the sample passes through another cooling coil to increase the sensitivity, since fluorescence is inversely proportional to temperature. An A-6 connector, to which a 0.020-in. I.D. tube is attached, is placed in line between the time delay coil and the fluorometer to relieve pressures produced inside the time delay coil. The sample now flows into the fluorometer, where it is activated at 360 mμ and the fluorescence measured at 465 mμ. At this concentration the range selector control is set at 30, a No. 3 light shield is used, and the "Blank" knob of the fluorometer is set at 10. With this specific flow diagram, the total assay time is about 25 min.

A series of identical samples was assayed to obtain the reproducibility of the system. Fig. 6 presents a typical series of recordings as well as a steady state diagram. The 95% confidence limit for the assay is ± 6.20%. As shown in Fig. 7, a series of standards of various concentrations were assayed to determine the linear relationship between the fluorescent intensity and the calcium pantothenate concentration. Assays of solutions containing varying amounts of calcium pantothenate were carried out utilizing the AutoAnalyzer, and these results were compared to those obtained on the same samples by the manual method as shown in Table I. The automated procedure should prove to be of significant value for single-tablet assays.

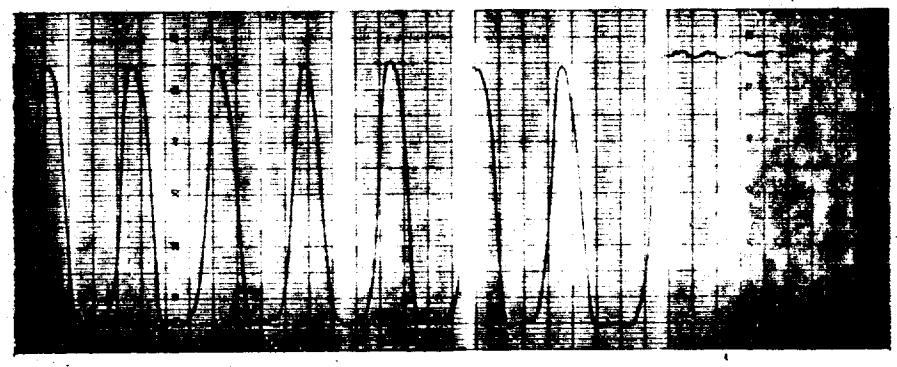

Figure 6

Table I. Comparison of Data: Manual Method vs. AutoAnalyzer Method

Manual Method mg/100 ml	AutoAnalyzer mg/100 ml	Difference mg/100 ml
14.25	14.35	+0.10
12.85	12.35	−0.50
15.30	15.75	+0.45
15.95	16.55	+0.60
14.25	15.05	+0.80
15.50	15.15	−0.35
14.00	13.75	−0.75
14.80	13.55	−1.25
13.05	14.10	+1.05
14.75	14.85	+0.10
13.50	13.00	−0.50
16.15	15.55	−0.60
14.85	14.40	−0.45
14.05	14.50	+0.45

Summary

An AutoAnalyzer procedure for the assay of calcium pantothenate in multivitamin preparations has been developed. This method introduces a new mode of spectroscopy to the automated assay for calcium pantothenate.

Maximum sensitivity of the fluorometer has not been utilized. Therefore, samples of lower concentration could be assayed if desired. Results are available in about 20 min, excluding the automated dilution step.

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CALCIUM PANTOTHENATE IN PARALYTIC ILEUS*

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PANTOTHENIC acid or the "Filtrate factor" of B-complex vitamins, is one of the new, or less well-known components of the family. Some of its known actions have been proved only in animals particularly the rat, in which the syndrome of deficiency of this factor has been worked out. Hæmorrhages in the respiratory tracts, adrenal glands, liver parenchyma, nasal mucous membrane and the intestinal tract have been consistently produced at will by feeding rats on diet devoid of pantothenic acid and the entire picture could be reversed at will by exhibiting the deficient "Filtrate factor" at the proper time. These rats also showed achromotrichia and a great distention of the small bowels with paralysis of the peristaltic movements which regained normal motility if pantothenic acid was exhibited before death (Jurgens and Pfaltz, 1944).

This factor is concerned in the formation of acetylcholine. The therapeutic use of this drug has not been recommended up till now by any responsible authority on the basis of a complete and controlled pharmacological study. We have had clinical reports of its use in "Burning Feet Syndrome" by C. Gopalan (*I.M.G.*, Jan. 1946, p. 22) and G. S. Whitfield (*B.M.J.*, 1947, Aug. 22, p. 165) naming this syndrome as "Gopalan Syndrome" and in postoperative paralytic ileus by J. E. Jacques (*Lancet*, Nov. 10, 1951, p. 861).

As the use of pantothenic acid as a therapeutic agent has not yet become wide, its dosage also is undetermined. There is very little information about the adverse effects of overdosage in man. Its minimum daily human requirements are not known. There is no determined I.U. or dose limit. It occurs naturally in all those foods where B-complex vitamins are found, *e.g.*, liver, wheat and rice bran, peanut meal, brewers' yeast, etc. A synthetic preparation, as a soluble calcium salt for parenteral administration has been marketed by a firm in India. It is stable to heat and to oxidising and reducing agents. It is readily destroyed by alkali.

I came across the report of J. E. Jacques (*Lancet*) mentioned above when I had two cases (Nos. 1 and 2) of severe paralytic ileus on both of whom routine treatment of every kind had proved ineffective. Minim doses of oil Cajaputi, Tincture Carminative, Pitrissin, and Prostigmine had not prevented increase of distention. Continuous gastric suction, intravenous fluid and salt replacement together with subcutaneous fluid, large dosage of anti-gas gangrene serum, oxygen and lastly turpentine and ox-bile enemas had made the patient worse and did not produce any improvement at all. Dilute procaine infiltration of the flanks as recommended by Baily was done in case No. 1 with no result. Both the cases progressed

* Specially contributed to THE ANTISEPTIC.

towards death, with continuous dark-brown regurgitation through Ryles tubes and vomits; they looked extremely ill, the faces pinched, the skin shrivelled, and the radial pulse was uncountable. I read the above mentioned report of Jacques on 3 1-'52 at 3 p.m. and on the same day, Calcium pantothenate (50 mg.) was given intramuscularly to J. (Case 2); and 100 mg. was repeated at 11 p.m. At 5 a.m. on 4-1-'52, *i.e.*, 14 hours after the first injection and after 150 mg. of Calcium pantothenate, he passed flatus. More flatus was passed throughout the whole day and night of 4th January. At 7 a.m. on 5th January a large stool was passed and the patient felt absolutely comfortable. He appeared to have reabsorbed all the fluid from the cesspool of his own paralysed guts and was re-hydrated even when fluid administration by vein and subcutaneous route were stopped before the salt was injected.

At 8 a.m. on 4-1-'52 150 mg. of Calcium pantothenate was injected in a single dose to G H., (Case No. 1). Flatus was passed at 1-30 p.m. the same afternoon, a special nurse being appointed to note the time and report to me. Fluid regurgitation continued. Administration of calcium pantothenate was repeated six hourly in 50 mg. doses; more flatus was passed, regurgitation stopped and all other treatment was suspended except the antibiotic. Copious tarry stool was passed at 11 a.m. on 5th January 1952. The patient was immediately relieved and recovery was thereafter uneventful.

I could never reconcile myself to the use of morphine in ileus in my U.P. patients, though it is strongly advocated by Baily and also by Illingworth.

Baily condemns the use of peristaltic stimulants. My experience with prostigmine has also been unsatisfactory; it probably never did any good to any real case of paralytic ileus. Though I might say that as a prophylactic in early postoperative distentions it acted well, the question remained as to how many of those distentions would or would not have developed into real paralytic ileus.

The success obtained consistently in the dreadful post-operative complication (for any resident surgeon) of ileus by the simple and routine use of calcium pantothenate promises great hopes. After the use of Calcium pantothenate was started in this predominantly surgical hospital of 124 beds this dreadful condition did not recur although the number of abdominal operations have increased. I have now started the prophylactic use of the drug in all post-operative distentions, six hours after the operation. The result has been most gratifying, both to the patient and to the nursing staff.

A school of surgeons condemn the flogging of a tired horse, which is very logical, but the role of pantothenic acid as a component of the co-enzyme for acetylation, being intimately connected with the formation of acetylcholine appears to indicate that the stimulating line of treatment may still be correct, provided the

Results of Treatment with Pantothenic Acid in Paralytic Ileus by Calcium Pantothenate (T.C.F.)

Case No.	Operation	Sex and age	Time between operation and onset ileus in hours.	Time between operation and injection of CPT.	Total C.P.T. in 50 mg. doses.	Time of passing first	
						Flatus	Stool
						after CPT.	
1	Appendicectomy ...	28 MM	24	72	350	5½	27
2	Prostatectomy ..	60 HM	7	76	250	14	40
3	Fracture lumbo-dorsal spine and T.P. of L. 1234 L.	55 HM	after the fracture 36 hrs	—	100	9	12
4	Transplantation ureter in V.V.F.	35 HF	24	50	150	7	—
5	Strangulated Ing. hernia (black bowel.)	36 MM	24	28	50	3	72
6	Strangulated Ing. hernia with operative tear intestine.	30 HM	72	96	150	24	24
7	Strangulated hernia ...	28 HM	34	80	200	3	72
8	Hysterectomy partial and anastomosis of bowels (injury).	25 MF	48	60	100	6	—
9	Gastro-jejunostomy ..	25 HM	24	36	100	4½	7 days
10	Fracture rib 11 and 12 left side.	35 HF	after fract. 12	6th day	50	2	—
11	Prostatectomy ..	60 MM	72	80	200	24	35
12	Cholecystectomy ...	45 MM	50	60	100	2	—
13	Anastomosis ileum (Int. strangulation).	30 HM	18	24	150	18	—
14	Exploratory laparotomy(cancer gall bladder).	45 HF	36	46	50	5	—
15	Rupture liver and injury parietes.	40 HM	72	78	50	3	—
16	Exploratory laparotomy and appendicectomy.	25 HM	24	36	100	8	—
17	Appendicectomy WW ..	22 HF	72	80	300	30	30
18	Nephrolithotomy ..	30 HM	18	24	50	2	—
19	Cholecystectomy ..	25 HF	9	9	100	3	—
			36·5	59·5	138·8	10	51

agent acting on the paralysed guts is not obnoxious. Prostigmine is not the natural stimulant but it prevents the destruction of acetylcholine at the nerve-endings, but that is not fulfilling the deficiency of acetylcholine itself. Again carbachole, which has a similar experimental effect as acetylcholine, is essentially different from the natural compound, because normal cholinesterase cannot destroy it.

The role of the "Filtrate factor" needs to be studied extensively. The average time of passage of flatus in the serious of Jaceues has been 4·25 hours, the minimum being 1½ hours and the maximum being 13 hours. Various doses were tried in the first four cases, 100 mg. being the first dose; 50 mg. was repeated six hourly. The results were not so quick. The average time for passing flatus has been 10 hours, passage of stool was in 51 hours, the minimum for flatus was 2 hours and maximum 30 hours. The average number of 50 mg. ampoules used before complete relief were 2·8. From the fifth case onwards, 50 mg. of Calcium pantothenate, six hourly, was injected as a routine and the results with more frequent doses or larger dosage had no additional advantage. Post-operative distentions which responded to a single injection of Calcium pantothenate are not included in the cases charted below. The results of treatment of paralytic ileus with Calcium pantothenate in 19 cases is given on page 338 in tabular form.

Conclusions.—Calcium pantothenate (T.C.F.) relieved desperate cases of paralytic ileus in a remarkably short time and restoration to normalcy was complete without additional therapeutic aids.

2. The restoration of blood pantothenic acid level reverses various chemical and physical disturbances without the necessity of attending to each of them separately. The fluid is thereby reabsorbed from the gut-lumen, salt balances correct themselves, and B. welchiflora appears to revert to its normal limits. Besides, symptoms of toxicity disappear.

3. The incidence of paralytic ileus appears to be high in this hospital compared to the Western U.P. In my experience, this may point to the fact that the origin of the disease may be a pre-existing pantothenic acid deficiency as the basic food consists of low grade rice and as the average citizen quite commonly shows other B. deficiencies as well.

I wish to thank Dr. T. T. Ratanchandani, M.B, B.S. (Bom.), P.M.S. (I), Civil Surgeon, and Superintendent of the District Hospital, Basti, for permitting me to use the drug in paralytic ileus and for his interest in the work and encouragement given to me and also for according permission to publish this paper.

Bigler-'66
Bib No. 15

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Balance Studies on Free and Bound Pantothenic Acid in Humans after Administration of Calcium Pantothenate or Coenzyme A

Introduction. Infusions containing coenzyme A, α-lipoic acid and diphosphopyridine nucleotide have been administered intravenously to patients with hepatic coma. During therapy the state of consciousness was improved and a decrease of blood pyruvic acid and the fraction acetoin and 2,3-butylene glycol towards normal values could often be observed[1,2]. It is assumed that these effects might be at least partly due to coenzyme A[3], which is involved in the oxidative breakdown of pyruvate (synthesis of acetyl coenzyme A) and acts as an acyl carrier in metabolism (e.g. in vivo formation of acetylcholine).

Nothing is known about the fate of coenzyme A after intravenous infusion (oxidation, breakdown, penetration into cells, excretion). In the present study, blood and urinary concentrations of free and of bound pantothenate (coenzyme A) have been determined before, during, and after administration of coenzyme A. Similar balance studies have been made after infusion of calcium pantothenate.

Methods. (a) Laboratory investigations: In blood and urine, free pantothenic acid and coenzyme A (= bound pantothenate) were determined microbiologically with *Lactobacillus plantarum* (ATCC 17-5 8014) according to the method of SKEGGS and WRIGHT[4], coenzyme A after enzymatic degradation to pantothenic acid with alkaline phosphatase and pigeon liver peptidase as proposed by NOVELLI[5].

(b) Infusions: Coenzyme A or calcium pantothenate was administered intravenously to convalescent patients with normal glomerular filtration rates. The infusions contained 250 μM coenzyme A (Farmochimica Cutolo-Calosi, Naples, Italy) or 250 μM D-pantothenic acid in the form of the calcium salt (F. Hoffmann-La Roche Inc., Basel, Switzerland) dissolved in isotonic glucose or saline (240 ml) and were given at a rate of 1 ml/min for 4 h. The coenzyme A content of each preparation was determined microbiologically. The coenzyme A was further characterized with the phospho-transacetylase-test and the hydroxyacyl-coenzyme-A-dehydrogenase-test[6].

(c) Collection of blood and urine samples: Before, during, and after administration of coenzyme A or calcium pantothenate, the blood level and the daily urinary excretion of coenzyme A and pantothenic acid were checked. Venous blood samples were drawn immediately before and 2, 4, $8\frac{1}{2}$ and 24 h (1 case), 2, 4 and 24 h (1 case), 24 h (1 case) or 24 and 72 h (3 cases) after the beginning of the infusion.

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2 H. THÖLEN, Ergebn. inn. Med. Kinderheilk. *21*, 48 (1964).
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4 H. R. SKEGGS and L. D. WRIGHT, J. biol. Chem. *156*, 21 (1944).
5 G. D. NOVELLI, M. O. KAPLAN, and F. LIPMANN, J. biol. Chem. *177*, 97 (1949).
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Results. The blood levels of free and bound pantothenic acid after infusion of coenzyme A or calcium pantothenate: (a) During the intravenous administration of coenzyme A, blood levels of free and bound pantothenic acid increased. 20 h after the end of the infusion, the blood concentrations of free and bound pantothenate were still found to be elevated (Figure). (b) During the infusion of calcium pantothenate, the free pantothenic acid in blood rose, whereas the bound pantothenic acid remained unchanged or was within the range of experimental error.

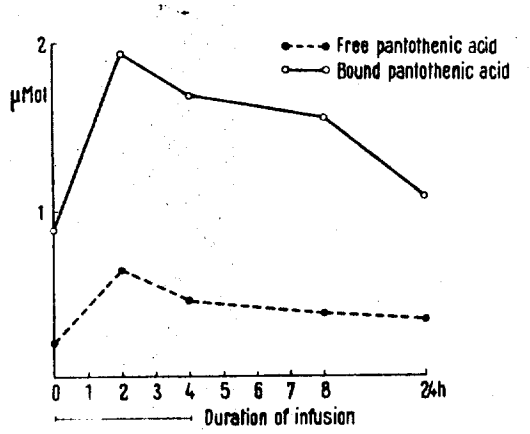
The urinary excretion of pantothenic acid during and after infusion of coenzyme A or calcium pantothenate: (a) The daily urinary excretion of free pantothenic acid was elevated after the administration of coenzyme A and normal again 2 days after the beginning of the infusion. In 3 cases, the extra pantothenate excretion corresponded to 22–24%, and in 1 case to 52% of the infused amount of coenzyme A. No bound pantothenic acid could be detected in urine. (b) For 2 days an extra daily urinary excretion of pantothenic acid was observed after the infusion of calcium pantothenate. It corresponded to 16% and 29% of the calcium pantothenate administered.

Discussion. In humans with normal glomerular filtration rates, an extra urinary excretion of free pantothenic acid is observed after intravenous administration of coenzyme A. This fact and the elevated free blood pantothenate during and after the infusion point to a breakdown of the injected coenzyme A in the organism. On the other hand, the blood level of bound pantothenate is raised after administration of coenzyme A and was found to be still elevated 20 h later.

It is not known whether the enzymatic degradation of coenzyme A to pantothenate by phosphatases and peptidases takes place in the circulating blood in the extravascular extracellular compartment or within the cells.

In 3 out of 4 balance studies with coenzyme A, 20–30% of the dose infused was excreted as pantothenate with the urine. The fate of the remainder is not known. It is assumed that the main part of the coenzyme A infused is adsorbed on the cell surface or is taken up by the cells either as coenzyme A or as one of its breakdown products (dephospho-coenzyme A, phosphopantethein, pantethein, pantothenic acid).

The urinary excretion of infused calcium pantothenate corresponded to 15–30%. This is similar to the results obtained after infusion of coenzyme A and is in good agreement with the observations of SCHMIDT[7,8].



Free and bound (coenzyme A) pantothenic acid in blood before, during, and after intravenous infusion of coenzyme A, α-lipoic acid, diphosphopyridine nucleotide and cocarboxylase. M. S., 1949 ♂; normal glomerular filtration rate.

Zusammenfassung. Bei Versuchspersonen mit normaler glomerulärer Filtration und normaler Leberfunktion wurde nach intravenöser Infusion von Calcium D-Pantothenat oder Coenzym A die Bilanz der freien und der gebundenen Pantothensäure (Coenzym A) ermittelt. Es konnte gezeigt werden, dass infundiertes Coenzym A teilweise zu freier Pantothensäure abgebaut wird. Ungefähr 70% des infundierten Coenzym A konnten weder im Urin (als Pantothenat) noch im Blut wiedergefunden werden.

F. BIGLER and H. THÖLEN

Medizinische Universitätsklinik, Bürgerspital, Basel (Switzerland), December 29, 1965.

[7] V. SCHMIDT, Acta pharmac. tox. *1*, 120 (1945).

[8] The study was aided by grants from Schweizerischer Nationalfonds and from F. Hoffmann-La Roche & Co. AG, Basel.

Braekkan-'55
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From the Governmental Vitamin Laboratory, Norwegian Fisheries Research Institute, Bergen, Norway.

Excretion of Pantothenic Acid by Rats after Administration of Panthenol or Calcium Pantothenate.

By

Olaf R. Brækkan.

(Received February 17, 1955).

Pantothenyl alcohol or panthenol, the alcohol analogue of pantothenic acid, is at the present time used in several vitamin preparations.

PFALTZ (1943) was the first to report that panthenol is as effective as pantothenic acid in promoting growth and in curing achromotrichia of black rats and alopecia in mice.

BURLET (1944) demonstrated that panthenol shows no activity in the microbiological assay with *Lactobacillus casei*, but is oxidized by rat and human subject to pantothenic acid, which is excreted in the urine. He worked only with rats deficient in pantothenic acid and only at doses of 200 μg calcium pantothenate or equivalent doses of panthenol.

RUBIN et al. (1948) found the excretion of pantothenic acid in the urine of human subjects to be lower after a dose of calcium pantothenate than after an equivalent dose of panthenol.

DREKTER et al. (1945) have described a bioassay of panthenol based on the urinary excretion of pantothenic acid by the rat after administration of panthenol. Their method has been successfully used in our laboratory for estimating panthenol in multivitamin preparations, and the results reported for the accuracy of the method have been verified. DREKTER et al. (1948) found equal activities of panthenol and calcium pantothenate in the curative growth test on pantothenic acid deficient rats; further, they compared the curative and excretion bioassays for panthenol, thus demonstrating the specificity of their method. Unfortunately they gave no information about the excretion of pantothenic acid after administration of calcium pantothenate.

SILBER & UNNA (1942) and SILBER (1945) studied the absorption and excretion of calcium pantothenate by normal and depleted dogs. Their results will be further discussed in relation to the findings given below, which are for a comparative study of the excretion of pantothenic acid after intraperitoneal or oral administration of panthenol or calcium pantothenate to rats.

Methods.

The rats were housed in specially designed metabolism cages with separate feeding units, constructed to permit good separation of urine and faeces. They were fed *ad libitum* on the stock diet used in this laboratory, consisting of maize meal 20%, oatmeal 10%, wheat-grindings 15%, rye-grindings 15%, casein 9%, dried milk powder 15%, wheat germ 5%, brewers yeast 5%, arachis oil 5%, sodium chloride 0.5%, calcium carbonate 0.5%, with 1.5 g ferrous chloride and 5 mg riboflavine per kilo diet. The diet was moistened to reduce contamination of the faeces and urine by the food put into the cages.

Analysis showed the diet to contain 17 μg pantothenic acid per g, and a "basal value" for the urinary excretion of 0.13 mg per day was found. By chance this value corresponds with the "basal value" found by DREKTER et al. (1948), and this may be partly responsible for the somewhat close similarity of our response curves.

Each *experiment* involved four groups of six rats each, 3 males and 3 females. The animals were housed separately. The solutions for intraperitoneal and oral administration were freshly prepared by dissolving panthenol or calcium pantothenate in distilled water at concentrations giving uniform volumes of 0.25 ml.

The urines were collected for each twenty-four hours and diluted directly with distilled water for the microbiological determinations. Pantothenic acid in the faeces was determined after digestion of 1 g of the homogenized samples with 20 mg takadiastase + 20 mg papain at pH 4.5 for 24 hrs.

Pantothenic acid was determined microbiologically by the method of SKEGGS & WRIGHT (1944) with *Lactobacillus arabinosus*. The growth was measured turbidimetrically in a Beckman Model B Spectrophotometer at 660 mμ after twenty hours' incubation at 37° in a water-bath.

Analysis of the urine from each cage separately was only carried out in the three first experiments to verify the general findings of DREKTER et al. (1948). Statistical analysis is omitted from the present paper. In the remaining experiments the urine from each group was pooled. When the faeces were to be analysed, they were collected from each group, homogenized in a mortar and stored at — 15° untill the analysis could be completed.

Results.

Twenty experiments, each on 4 groups, were carried out. At the beginning the same rats were used repeatedly after intervals of one week. Control experiments showed that the basal value returned to normal within this time. The groups were changed from panthenol to pantothenic acid and from high to low doses and *vice versa*, thus reducing errors due to individual variations. The groups of rats used had average weights increasing from 100 to 300 g.

Table 1.

Urinary excretion of pantothenic acid after *intraperitoneal injections* of panthenol and calcium pantothenate in single doses.

Dosage	mg pantothenic acid in urine per day				
	1st day	2nd day	3rd day	4th day	5th day
Dist. Water	0.12	0.11	0.15	0.15	0.12
0.35 mg Panthenol	0.25	—	—	—	—
0.50 - -	0.38	0.18	0.13	0.14	0.13
1.00 - -	0.80	0.22	0.16	0.15	0.14
1.50 - -	0.90	0.18	0.16	0.13	—
2.00 - -	1.46	0.27	0.20	0.15	0.14
3.00 - -	2.20	0.31	0.22	0.17	0.14
4.00 - -	2.82	0.35	0.24	0.18	0.11
0.40 mg Ca Pantothenate	0.40	—	—	—	—
0.50 - -	0.49	0.21	0.16	0.19	0.16
1.00 - -	0.83	0.22	0.18	0.17	0.20
1.50 - -	1.20	0.22	0.17	0.15	—
2.00 - -	1.52	0.24	0.17	0.14	0.12
3.00 - -	2.31	0.29	0.17	0.13	—
4.00 - -	3.20	0.32	0.20	0.15	0.13

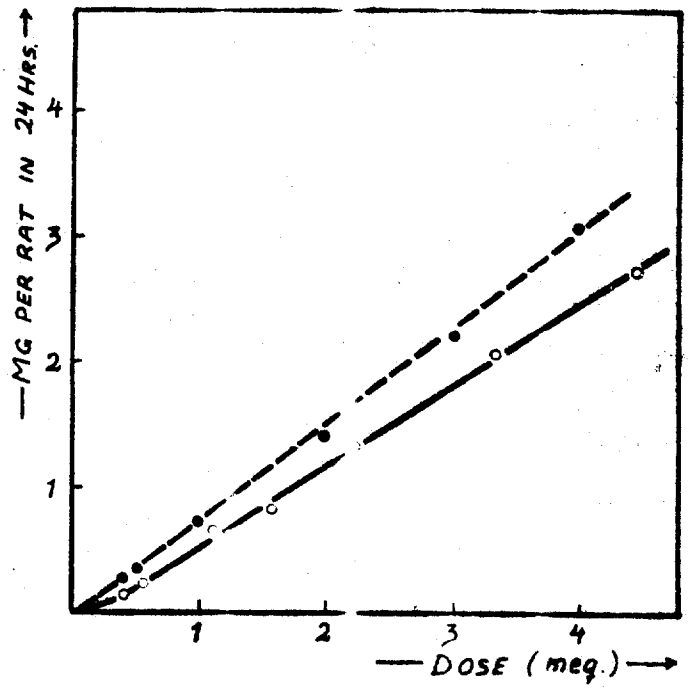

Fig. 1. Extra pantothenic acid in urine after intraperitoneal injections of panthenol and calcium pantothenate.
——○—— after injections of panthenol.
— — ● — — after injections of calcium pantothenate.

After *intraperitoneal injections* the urinary excretion of pantothenic acid was higher when calcium pantothenate was administered than after equivalent doses of panthenol (table 1). The excretions on the next 4 days were low and of approximately the same amounts. The largest doses of panthenol seemed to cause a slight retention, with further urinary excretion on the second and third day. Calculated on an equimolar basis, the twenty-four hours' excretion caused by calcium pantothenate was approx. 25% higher than the corresponding value for panthenol (fig. 1). This result was independent of the weight of the rats, which ranged from 150 to 250 g.

Table 2.

Urinary and fecal excretion of pantothenic acid after *oral administration* of panthenol and calcium pantothenate in single doses.

Dosage	mg pantothenic acid excreted per day					
	1st day		2nd day		3rd day	
	Urine	Faeces	Urine	Faeces	Urine	Faeces
Dist. Water	0.12	0.23 (25.2[1])	0.13	0.20 (23.1)	0.13	0.26 (24.0)
1 mg Panthenol	0.80	0.23 (27.0)	0.30	0.24 (30.2)	0.22	0.20 (25.8)
2 - -	1.42	0.26 (26.5)	0.30	0.23 (29.0)	0.22	
3 - -	2.16	0.33 (24.5)	0.39	0.23 (25.8)	0.23	0.17 (25.1)
4 - -	2.57	0.18 (18.2)	0.39	0.17 (22.1)	0.23	
1 mg Ca Pantothenate	0.32	0.33 (30.7)	0.31	0.23 (27.8)	0.22	0.22 (27.0)
2 - -	0.64	0.53 (25.8)	0.24	0.25 (30.2)	0.19	
3 -	0.77	0.93 (22.7)	0.32	0.18 (25.2)	0.24	0.17 (23.5)
4 - -	0.98	1.33 (25.0	0.24	0.23 (27.9)	0.19	

[1]) The number in paranteses gives the weight of the faeces in g.

After *oral administration* of panthenol urinary excretion was almost equal to the corresponding value after intraperitoneal injections (table 2). There were no differences in the results on rats weighing from 150 to 250 g. Urinary excretion after oral doses of calcium pantothenate showed relatively much lower values, and analysis of the faeces showed increased faecal excretion of pantothenic acid with increased doses of the vitamin. The "basal value" for the faecal excretion on the diet used in the present investigation was approx. 0.23 mg per rat in 24 hrs. Panthenol did not cause any change in the faecal excretion of pantothenic acid (table 2).

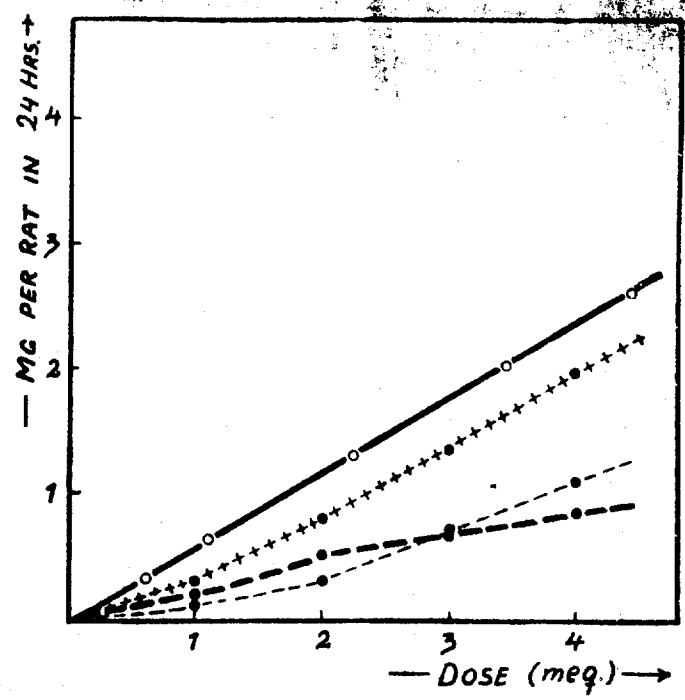

Fig. 2. Extra pantothenic acid in urine and faeces after oral administration of panthenol and calcium pantothenate.
— — ● — — in urine after administration of calcium pantothenate.
— — ● — — in faeces after administration of calcium pantothenate.
+ + + ● + + + total recovered pantothenic acid after administration of calcium pantothenate.
——— ○ ——— in urine after administration of panthenol.

When the results for doses up to 4 mg were plotted graphically (fig. 2,), the curves for the excess urinary and faecal excretion after oral doses of calcium pantothenate crossed each other, indicating a possible maximum value for intestinal absorption. To investigate this possibility, experiments were conducted with higher oral doses of panthenol or calcium pantothenate. The results showed a linearity of urinary and faecal excretion for doses up to 64 mg equivalents calcium pantothenate (fig. 3). Usually a higher total recovery was found for panthenol than for calcium pantothenate (fig. 2 and 3), contrary to the findings after intraperitoneal injections.

Discussion.

The results show that panthenol and calcium pantothenate are differently excreted in rats after intraperitoneal injections and after oral administration.

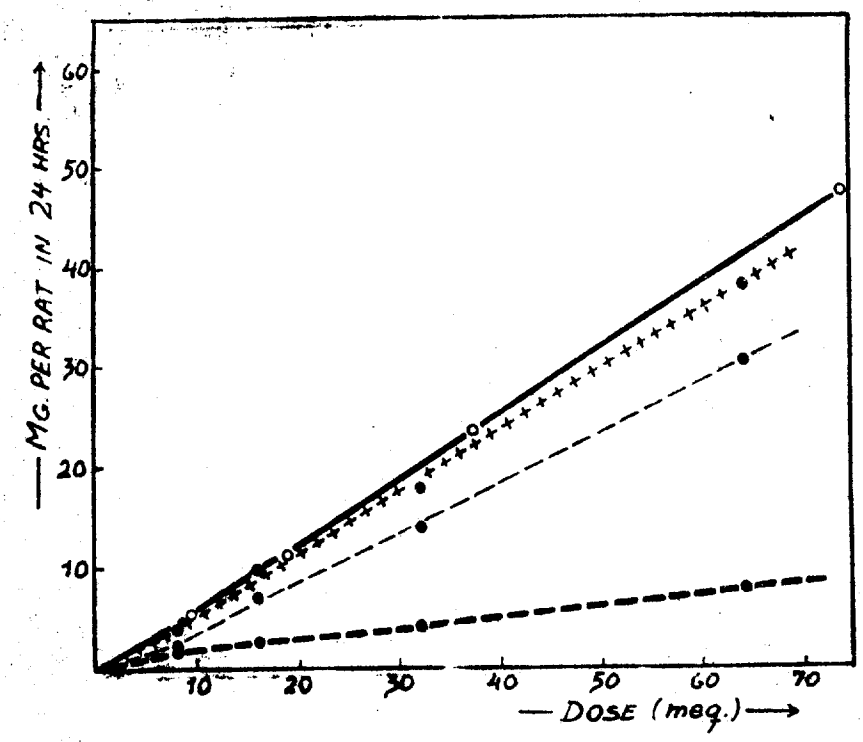

Fig. 3. Extra pantothenic acid in urine and faeces after oral administration of panthenol and calcium pantothenate.

— — ● — — in urine after administration of calcium pantothenate.
— ● — — in faeces after administration of calcium pantothenate.
+ + ● + + + total recovered pantothenic acid after administration of calcium pantothenate.
——○—— in urine after administration of panthenol.

The higher urinary excretion after intraperitoneal injections of calcium pantothenate compared with equimolecular doses of panthenol cannot be readily explained. The likeliest explanation would be a delay in the conversion of panthenol to pantothenic acid in the body. This should result in higher excretions during subsequent days the panthenol groups compared to the corresponding calcium pantothenate groups, but no differences of importance could be observed during the next five days. On the other hand slight but increasing retention of the excess urinary pantothenic acid could be observed with increasing doses of panthenol as well as of calcium pantothenate, but within five days the excretion values always returned to normal (table 1).

The markedly different urinary excretions after oral administration of panthenol and calcium pantothenate may be considered in connection with their absorption in the intestine. The only chemical differences be-

tween the compounds is that panthenol, being the alcohol analogue of pantothenic acid, has a terminal hydroxyl group instead of a terminal carboxyl group. The reason for the striking differences in their absorption may be due to the difference in their physicochemical behaviour, but the possibility of selective absorption cannot be ruled out.

No essential differences were found in the urinary excretion of pantothenic acid in whichever way panthenol was administered. This is in agreement with the findings of DREKTER et al. (1948), and indicates that panthenol has been quantitatively absorbed from the intestine. From calcium pantothenate on equimolecular basis approx. the same recovery was obtained, but four times as much was excreted unchanged in the faeces as could be recovered as excess urinary excretion (fig. 3). URMA & GRESLIN (1940) studied the toxicity of pantothenic acid to mice, rats, dogs and monkeys after oral, subcutanous, intraperitoneal and intravenous administration. No animals died after oral administration of up to 10 g/kg. The present results indicate that the pantothenic acid was probably mainly excreted unchanged in the faeces, thus preventing toxic doses from entering the body by intestinal absorption.

SILBER & UNNA (1942) studied the urinary excretion in dogs after intravenous injection of calcium pantothenate. They collected the urine through a bladder fistula and found a very rapid excretion; for doses of 4 mg/kg 41—57% was excreted within 2 hours. In the present investigation with rats, 75% was excreted within 24 hours. SILBER (1945), in a later study of the absorption and excretion of calcium pantothenate by dogs, found an inverse relation between urinary excretion and food consumption. In the present investigation the rats were fed *ad libitum* on stock diet, and there was fairly good linearity between the doses of calcium pantothenate given and the amounts excreted in the faeces and the urine, the latter being approx. 10% of the dose given (fig. 3). It is interesting to note that SILBER (1942) in an experiment on a group of 10 laboratory assistants found 10% of a 50 mg dose excreted in the urine within four hours.

From the pharmacological aspect our results indicate that for oral pantothenic acid therapy panthenol should be used.

Summary.

When injected intraperitoneally calcium pantothenate caused approx. 25% higher excess urinary excretion of pantothenic acid than equimolecular doses of panthenol.

Panthenol caused the same urinary excretion after intraperitoneal injections as after oral administration, suggesting quantitative absorption in the intestine.

Only approx. 10% of the calcium pantothenate given orally could be recovered as excess urinary pantothenic acid. Five times as much was excreted unchanged in the faeces.

For therapeutic purposes by the oral route panthenol should be used.

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Jour. Nutr. 54(1):97-105. 1954.

EFFECT OF VARYING THE INTAKE OF CALCIUM PANTOTHENATE OF RATS DURING PREGNANCY

II. HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE LIVER, ADRENAL, DUODENUM AND TIBIA OF THE YOUNG AT BIRTH [1, 2]

NAM YOUNG CHUNG, LOIS NORTHROP, ROBERT GETTY AND GLADYS EVERSON [3]

Nutrition Laboratory, Home Economics Research Department, Iowa Agricultural Experiment Station, and Department of Veterinary Anatomy, Iowa State College, Ames

FOUR FIGURES

(Received for publication March 1, 1954)

Numerous histological changes have been observed during advanced pantothenic acid deficiency in the mature rat (Follis, '46). Ashburn ('40) has described the adrenal in pantothenic acid deficiency as including: hemosiderin deposition, fibrosis, "congestion" hemorrhage, cellular atrophy, necrosis and scarring. Nelson and her associates ('50) observed changes in endochondal ossification of the tibia in young rats which were acutely deficient in pantothenic acid. Intestinal changes, particularly of the colon, have been described in some detail by Follis.

While certain chemical changes in newborn rats produced by females receiving different amounts of pantothenic acid were being investigated, the liver, adrenal, duodenum, and

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[2] Financial support from the Department of Health Education and Welfare, Public Health Service, National Institutes of Health (G-3198) is gratefully acknowledged.

[3] Present address: Department of Home Economics, University of California, Davis, California.

tibia were examined simultaneously (Everson, Northrop, Chung, Getty and Pudelkewicz, '54). In addition to investigating the structure of these tissues, the distribution and concentration of acid and alkaline phosphatase of each tissue were also measured. A limited number of studies have been completed in the past regarding the distribution of phosphatase in the organs and tissues of the rat, but as yet little is known of the significance of these findings and of how they are affected by various vitamin deficiencies.

EXPERIMENTAL

Female albino rats of the Wistar strain were used in the study and histological sections of the tissues of the young produced during second pregnancies were prepared and examined. The experimental females were maintained on the customary stock diet from the initiation of the study to the close of first pregnancies. At this time 35 females which had produced satisfactory first litters were transferred to purified rations or were maintained on the stock ration. The composition of the purified diet is given in table 1. The experimental animals were distributed into 4 groups: Group I, stock ration; Group II, purified diet (using vitamin mixture given in table 1); Group III, purified diet (same vitamin mixture plus 100 μg calcium pantothenate); Group IV, purified diet (same vitamin mixture plus 1 mg calcium pantothenate).

The newborn rats were killed with chloroform, and portions of the liver, duodenum, the entire adrenals, and the tibias from each animal were fixed. Tissues fixed in 10% neutral formalin were removed after 48 hours and were dehydrated in three changes of dioxane of one-half hour each. The tissues were then embedded in paraffin (Altman's mixture) after three changes in melted paraffin (53–56°C.) of one-half hour each. Sections were made at 6 μ thickness and the following stain techniques as described by Mallory were routinely used: Harris' hematoxylin and eosin, Heidenhain's hematoxylin, Weigert's elastic tissue stain, and Van Giesen's connective tissue stain.

For the histochemical demonstration of alkaline and acid phosphatase enzymes, the methods of Gomori ('39, '41) as modified by Deane and Dempsey ('45) were followed. Tissues of about 2 mm thickness were fixed in cold 80% alcohol (0°C.) for about 24 hours. These were then changed to 90%, 95% and finally absolute alcohol during the next 24 hours. The

TABLE 1

Composition of the purified ration

Vitamin-free casein	25%
Dextrose	55%
Swiftening	15%
Salts[1]	5%
Vitamin mixture fed daily per rat	
Choline chloride	10 mg
Inositol	5 mg
p-aminobenzoic acid	100 μg
2-methyl-naphthoquinone	250 μg
Thiamine hydrochloride	150 μg
Riboflavin	100 μg
Niacin	500 μg
Biotin	2.5 μg
Folic acid	6 μg
Pyridoxine hydrochloride	50 μg
Vitamin B_{12}	0.5 μg
Fat soluble vitamins	
Cod liver oil:	9 U.S.P. units vitamin D
	90 U.S.P. units vitamin A
Alpha tocopherol	750 μg

[1] Richardson, L. R., and A. G. Hogan — J. Nutrition, *32:* 459, 1946.

tissues were cleared in cedar oil for 24 hours and then in three changes of benzene of one hour each. These preparations were then embedded in melted paraffin as described for tissues fixed in general fixative. The paraffin blocks were sectioned at 6 μ thickness and mounted by use of Mayer's albumin-glycerin mixture. After removal of the paraffin, sections were incubated in buffered solutions containing sodium glycerophosphate and either lead or calcium ions. The solu-

tions were buffered with acetate or sodium barbital depending upon the pH desired. All solutions were examined immediately prior to incubation using a Beckman pH meter and were adjusted to pH 9.4 for alkaline phosphatase and pH 5.0 for acid phosphatase. The sections were incubated routinely for 6, 12, 24, 48 and 96 hours, together with control incubations. Following this treatment the sections were dehydrated, cleared and mounted in the usual manner. The sites of phosphatase activity were made microscopically visible by converting the precipitated phosphatase into visible brown sulfides as described by Gomori ('39). Control sections were prepared by omitting the substrate in the incubation solutions in order to reveal any possibility of preformed insoluble native phosphate. Chilled alcohol was found suitable as a fixative for both acid and alkaline phosphatase. The use of this solvent for the demonstration of both types of phosphatase has been recommended by Gomori ('45).

RESULTS

No structural differences were encountered for the 4 types of tissues examined which could be attributed to differences in dietary treatment of the females.

Histochemical data revealed that none of the tissues examined contained demonstrable acid phosphatase. Alkaline phosphatase concentrations were measured by applying arbitrary values of zero for negative, 1 for slight, 2 for moderate, 3 for intense, and 4 for very intense concentrations.

In the liver sections (fig. 1) all groups of young showed essentially the same distribution and concentrations of alkaline phosphatase as those of the stock young. The cell membrane and cytoplasm showed slight concentrations of alkaline phosphatase; the nuclear membrane and nucleoli were intensely stained. The general distribution from the portal veins and peripheral areas of the lobules to the central veins was about uniform.

Sections of the duodenum (fig. 2) likewise showed no important differences in the concentration or distribution of

phosphatase from those of the normal stock young. The cuticular borders of the villi were very intensely stained, but the crypts were less deeply stained than the upper half of the villi. The nuclear membrane and nucleoli were also very

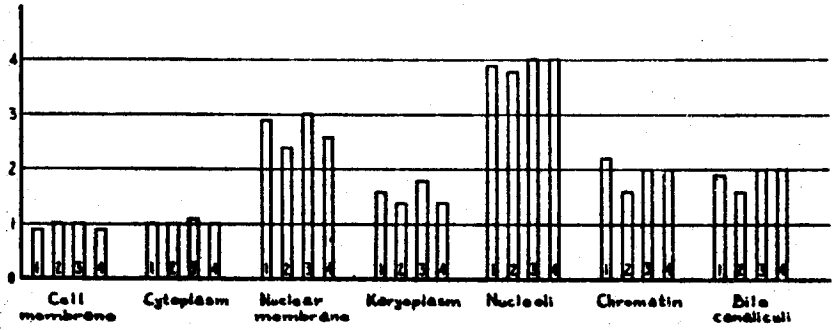

Fig. 1 Distribution and concentration of alkaline phosphatase in the liver.
Diets: 1. Stock, average of 50 samples.
2. No pantothenic acid, average of 14 samples.
3. 100 μg pantothenic acid, average of 36 samples.
4. 1 mg pantothenic acid, average of 42 samples.

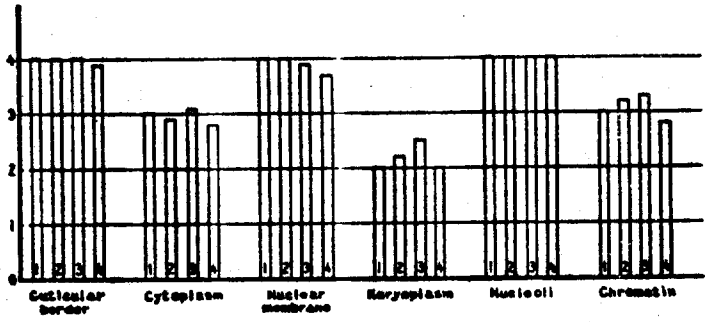

Fig. 2 Distribution and concentration of alkaline phosphatase in the duodenum.
Diets: 1. Stock, average of 49 samples.
2. No pantothenic acid, average of 13 samples.
3. 100 μg pantothenic acid, average of 35 samples.
4. 1 mg pantothenic acid, average of 42 samples.

intense. The cytoplasm was vividly stained as was the chromatin. The karoplasm ranged from moderate to intense.

In sections of the tibia (fig. 3) no real differences were observed in the concentration or distribution of alkaline phosphatase among the various groups. Very marked reactions

of alkaline phosphatase were noted in the perichondrium, periosteum, bone collar, interstitial spaces at the calcifying zones, and the osteoblasts, osteoclasts, and blood cells. The cell membranes of all the hypertrophic cartilage cells in the

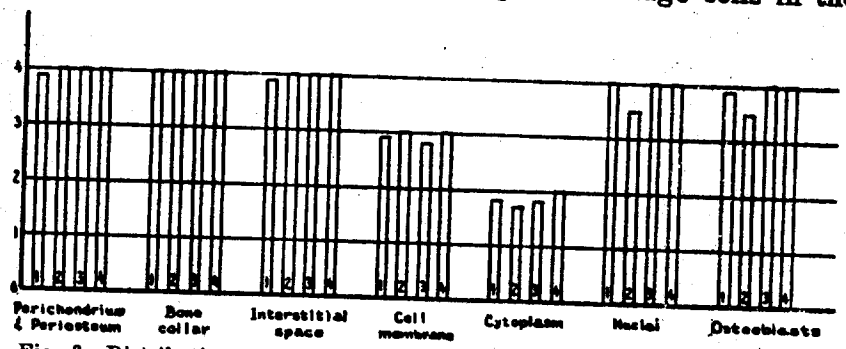

Fig. 3 Distribution and concentration of alkaline phosphatase in the tibia.
Diets: 1. Stock, average of 34 samples.
2. No pantothenic acid, average of 8 samples.
3. 100 μg pantothenic acid, average of 24 samples.
4. 1 mg pantothenic acid, average of 20 samples.

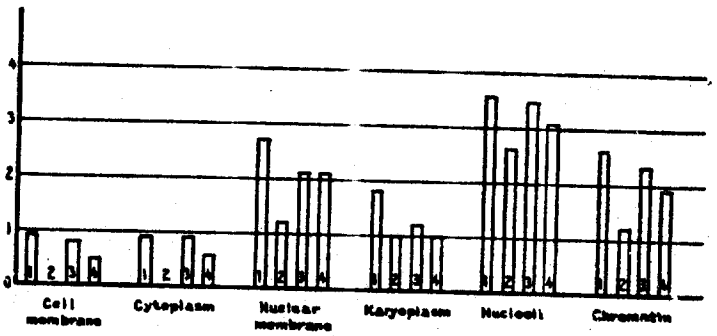

Fig. 4 Distribution and concentration of alkaline phosphatase in the adrenal.
Diets: 1. Stock, average of 49 samples.
2. No pantothenic acid, average of 13 samples.
3. 100 μg pantothenic acid, average of 36 samples.
4. 1 mg pantothenic acid, average of 42 samples.

cartilage cell columns were rich in this enzyme, whereas the cytoplasm showed only moderate amounts. The nuclei of these hypertrophied cells were very deeply stained. The hyaline cartilage cells at the epiphyses showed little or no difference among the 4 groups.

A significant difference (fig. 4) was encountered between the adrenal sections for young born to females receiving no pantothenic acid throughout pregnancy and those receiving the stock diet. Where the controls showed slight amounts of alkaline phosphatase in the cell membrane and cytoplasm, the deficient group showed none. Where the controls showed moderate phosphatase in the nuclear membrane, the deficient young showed only slight amounts. Likewise as the intensity was varied from slight to moderate in the karyoplasm for the controls, the young of animals receiving no pantothenic acid showed only slight activity. Where the controls showed intense reaction in the nucleoli and moderate in the chromatin, the young in the deficient group were correspondingly lower in activity.

Sections of the 4 tissues fixed with formalin showed little or no difference among the groups of young.

DISCUSSION

It will be observed from the histological findings that the demonstration of pantothenic acid dificiency in the rat at birth is difficult to accomplish. Even when the maternal diet contributed no pantothenic acid from approximately one week before conception to parturition, the young born exhibited no structural differences in the liver, adrenal, tibia and duodenum. Such young, however, were known to possess limited concentrations of pantothenic acid in the total body tissue and the pantothenic acid content of the blood was reduced (Everson, Northrop, Chung, Getty and Pudelkewicz, '54). Some rise in blood pyruvic acid concentration was also known to occur in these young. In spite of these changes, the development of the 4 tissues progressed normally histologically. When the distribution and concentration of acid and alkaline phosphatase were measured, it was found that the adrenals of the young with poorest pantothenic acid stores exhibited less demonstrable alkaline phosphatase. Young born of females receiving 100 µg or 1 mg quantities of calcium pantothenate daily throughout pregnancy, or the stock ration,

exhibited increased alkaline phosphatase activity. The fact that the litters of females deprived of pantothenic acid for some period of time were known to resorb in a high proportion of the cases suggests that more vital changes than those being investigated in the present study develop as a result of withdrawal of this vitamin from the maternal diet.

SUMMARY AND CONCLUSIONS

Histological and histochemical studies of the liver, adrenal, duodenum and tibia were made on newborn rats whose dams were fed several levels of pantothenic acid during reproduction. Tissues studied to date reveal little if any evidence of tissue change in the newborn rats except in the case of the adrenals which exhibited a decreased alkaline phosphatase reaction for the most deficient group of animals.

It appeared that the young of pantothenic acid-deficient females were normal at birth insofar as the structure of the 4 tissues studied was concerned. It would appear that whatever pathology may be produced in the fetal tissues by these dietary regimes is evidenced in the prenatal life of the fetuses thus contributing to early fetal resorptions.

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EFFECT OF VARYING THE INTAKE OF CALCIUM PANTOTHENATE OF RATS DURING PREGNANCY

I. CHEMICAL FINDINGS IN THE YOUNG AT BIRTH [1,2]

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The recent review by Lowry ('52) concerned with the biochemical evidence of nutritional status for ascorbic acid, thiamine, and riboflavin points out how meager our information still is with respect to the chemical and structural changes which occur during the onset of nutritional deficiences. This paper also illustrates how different the vitamin intake of the animal must be to accomplish tissue saturation on the one hand and, on the other, to be a handicap to the organism due to chemical or structural changes, resulting from a vitamin shortage.

Earlier studies from this laboratory concerned with the pantothenic acid needs of the rat during reproduction have revealed that there are wide differences in the amount of this vitamin present in the tissues of the rat depending upon the composition of the maternal diet. It was therefore decided

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to investigate the influence of varying the pantothenic acid intake of the rat during pregnancy upon the concentration of additional blood and tissue components of the young at birth. Information has also been sought as to the concentration of pantothenic acid which may be present in the blood and total tissues of the young at a time when chemical or structural changes are detectable.

Total pantothenic acid activity of the tissues of the young and the dams has been measured directly following parturition. Blood pantothenic acid, serum ascorbic acid, serum alkaline phosphatase and blood pyruvic acid have been determined for young rats at birth. Simultaneously, histological examinations of liver, adrenal, intestine and the tibia of the young were carried out. The present paper reports the results of the chemical studies completed to date. Microscopic data will be reported separately (Chung, Northrop, Getty and Everson, '54).

EXPERIMENTAL

Albino rats which were 28 days old were selected from an inbred colony of the Wistar strain. These animals received the customary stock diet consisting of mixed grains and dried whole milk, plus supplements of fresh beef and carrots. Females producing first litters of normal size and of satisfactory appearance and birth weight were distributed into 4 groups: Group I was continued on the stock ration; Group II received a synthetic ration plus a vitamin mix containing all of the known vitamins except pantothenic acid and ascorbic acid; Group III received a ration similar to that of Group II except that the vitamin mix provided 100 µg of calcium pantothenate per day; Group IV received the same ration plus 1 mg of calcium pantothenate daily. The composition of the synthetic diet and of the vitamin mix are given in table 1. First litter young were sacrificed within a few hours after birth. The females were remated for second pregnancies one week following the first parturition. Immediately following the delivery of the second litters and before the young had an op-

portunity to nurse, the newborn were removed from the female's cage. The weight and sex of the rat was recorded.

Total pantothenic acid activity of the blood of young produced by females in each of the 4 groups was determined by microbiological assay using *Lactobacillus arabinosus* as the test organism. The same technique was used in determining

TABLE 1

Composition of the synthetic ration

Vitamin-free casein	25%
Dextrose	55%
Swiftening	15%
Salts[1]	5%
Vitamin mixed daily per rat	
Choline chloride	10 mg
Inositol	5 mg
p-aminobenzoic acid	100 μg
2-methyl-Naphthoquinone	250 μg
Thiamine hydrochloride	150 μg
Riboflavin	100 μg
Niacin	500 μg
Biotin	2.5 μg
Folic acid	6 μg
Pyridoxine hydrochloride	50 μg
B_{12}	0.5 μg
Fat soluble vitamins	
Cod liver oil: 9 U.S.P. units vitamin D 90 U.S.P. units vitamin A	
Alpha tocopherol	750 μg

[1] Richardson and Hogan — J. Nutrition, *32:* 459, 1946.

the total pantothenic acid content of fetal tissues, maternal liver and carcass. In all cases these tissues were incubated with chicken liver enzyme and intestinal phosphatase at pH 8.5.

Total ascorbic acid, determined for serum, was estimated by the Lowry, Lopez and Bessey microadaptation of the dinitrophenylhydrazine method of Roe and Kuether (Lowry, Lopez and Bessey, '45; Roe and Kuether, '43). The alkaline phosphatase activity of the serum of the young was deter-

mined by the Bessey, Lowry and Brock procedure ('46). Pyruvic acid concentrations of the blood were determined by the micromethod of Tsao and Brown ('50).

RESULTS AND DISCUSSION

Transferring young females immediately following the the birth of their first litters from the customary stock ration to synthetic rations containing zero, 100 µg or 1 mg quantities of calcium pantothenate had a marked effect upon the reproductive performance of the females. Animals which received no pantothenic acid from approximately one week prior to pregnancy and throughout the entire gestation period produced inferior litters in all instances. The average number of young born to such females was 4.2 with an average birth weight of 3.7 gm (table 2). In several cases no young were carried to term and in many instances parturition was delayed.

The addition of 100 µg of calcium pantothenate per day to the same ration increased the size of the young and the number of animals per litter. Such females delivered their young on time although an occasional litter was poor. The further addition of calcium pantothenate to furnish 1 mg daily, improved the birth weight of the young slightly but did not alter the litter size. While the majority of young produced by group IV were considered excellent, an occasional animal resorbed her entire litter. The performance of the females on the synthetic ration was not considered equal to that of the stock females although many excellent litters were produced by females receiving the higher intake of calcium pantothenate.

Sufficient blood was obtained from a number of young produced by each of the 4 groups of females to allow for 4 individual checks of the total pantothenic acid activity of this tissue. Results from these assays revealed that the blood of young born to females receiving the stock diet averaged 450 µg of pantothenic acid per 100 ml. Values within the group

TABLE 2

Chemical findings in rats resulting from differences in pantothenic acid intake of the maternal rat during pregnancy [1]

RATION	LITTER SIZE [2]	BIRTH WEIGHT [2]	PANTOTHENIC ACID CONTENT OF TISSUES			PANTOTHENIC ACID CONTENT OF BLOOD OF YOUNG	ALKALINE PHOSPHATASE OF SERUM OF YOUNG	ASCORBIC ACID OF SERUM OF YOUNG	PYRUVIC ACID OF BLOOD OF YOUNG
			Maternal liver	Maternal carcass	Newborn carcass				
		gm	*µg/gm*	*µg/gm*	*µg/gm*	*µg/100 ml*	*nitrophenol units*	*mg/100 ml*	*mg/100 ml*
Stock diet 450–600 µg calcium pantothenate daily	11.2 (28)	5.2	67.8 (64.6–70.9) (10)	7.5 (7.0–7.9) (10)	36.9 (29.1–45.8) (40)	450 (342–570) (50)	14.6 (9.9–19.5) (58)	5.85 (4.13–8.38) (67)	1.04 (0.29–1.88) (18)
Synthetic diet No calcium pantothenate	4.2 (35)	3.7	40.7 (38.6–43.6) (10)	4.4 (3.2–5.0) (10)	6.7 (5.1–7.6) (25)	295 (108–447) (20)	12.0 (7.0–16.9) (20)	4.12 (2.47–6.00) (20)	2.79 (0.78–6.33) (23)
Synthetic diet 100 µg calcium pantothenate daily	7.8 (17)	4.6	54.6 (45.0–62.4) (10)	5.5 (4.8–6.0) (10)	15.8 (12.6–21.8) (25)	502 (320–678) (27)	11.8 (7.7–15.8) (40)	4.58 (3.19–7.31) (12)	....
Synthetic diet 1 mg calcium pantothenate daily	8.0 (18)	5.1	72.1 (61.8–80.5) (10)	9.2 (8.5–10.4) (10)	72.1 (60.1–75.7) (24)	2204 (1709–2575) (15)	12.1 (9.2–15.8) (42)	4.22 (3.10–6.08) (44)	1.63 (0.42–2.25) (20)

[1] Average values listed first; range given below; third value indicates number of animals examined in each group.

[2] Number of litters included in litter size and birth weight values.

varied considerably. The intra-group variation was not found to be due to sex or size differences.

The majority of young born to females deprived of dietary pantothenic acid throughout pregnancy possessed less vitamin per unit of blood than was true for young produced by stock females. In some instances the concentration was as little as 100 µg per 100 ml, while other animals of the group exhibited concentrations equal to those of the control group. Poor condition of the young did not always correlate with a low blood value.

The presence of 100 µg of calcium pantothenate in the vitamin mix allowed for the production of young with blood pantothenic acid values equal to those of the stock animals. When the female's diet was supplemented with as much as 1 mg of calcium pantothenate daily, the concentration was greatly increased. In certain animals this amount was 8 times that of stock animals believed to be well nourished in every respect. This finding suggested that some handicap was being encountered in removing this substance from the blood.

It will be observed from table 2 that the amount of pantothenic acid present in the maternal liver, the carcass and the entire fetus at birth was directly related to the quantity of pantothenic acid present in the maternal diet during pregnancy. The average concentration of the vitamin per gram of fresh fetal tissue for young of females devoid of dietary pantothenic acid was 6.7 µg, or approximately 23 µg per individual animal. If the maternal intake of calcium pantothenate was 100 µg daily throughout the gestation period the young at birth possessed approximately 16 µg of pantothenic acid per gram of fresh tissue. As the maternal diet provided larger amounts of the vitamin, additional quantities were transferred to the young until saturation of the tissues occurred when the maternal diet included 1 mg of the vitamin daily (Bjorck, Lewis and Everson, '45).

While the quantity of pantothenic acid present in the hepatic tissue of the mothers varied with the dietary intake of this factor, the amount of change encountered was consider-

ably smaller than that for fetal tissue. Those females which were unable to produce litters of normal size and birth weight possessed livers averaging 30 μg of pantothenic acid per gram of tissue, an amount which was approximately 56% of the saturation value.

Since earlier work by Nelson and her associates ('50) indicated that numerous changes in the endochondral ossification of the tibia occurred in rats suffering from acute pantothenic acid deficiency, it was thought possible that differences in alkaline phosphatase activity of the serum of the young might exist within the groups of animals being studied. It will be apparent from table 2 that no such differences were encountered. Young born to stock females which appeared in good condition at birth possessed serum concentrations equal to approximately 15 nitrophenol units[4]. Variation within the 58 young examined in this group was large and exceeded that found for the 4 groups under study. It would, therefore, appear that serum alkaline phosphatase activity is of no value in evaluating nutritional status from the standpoint of pantothenic acid stores.

The concentration of ascorbic acid in the serum of the newborn rat was likewise investigated, since it was thought that metabolic changes brought about by the withdrawal of pantothenic acid from the maternal diet might result in variations in the blood ascorbic acid of the young. While the data summarized in table 2 disclosed no effect upon serum ascorbic acid of the young from varying the pantothenic acid intake of the females, the concentrations of this vitamin were interesting. An average concentration of approximately 6 mg% was observed for young of stock females. Considerable variation in blood ascorbic acid was encountered among animals of the same dietary origin. While a small number of low ascorbic acid values were encountered among the animals ex-

[4] One unit is the amount of phosphatase activity per liter of serum required to liberate one millimole of nitrophenol per hour from sodium paranitrophenyl phosphate.

amined, such low concentrations were more common among animals receiving the synthetic diet than the stock ration.

Figures obtained in the present study are of interest when compared with data reported for adult rats by Todhunter and McMillan ('46). The latter workers observed that the ascorbic acid content of the plasma of adult males was 0.87 mg per 100 ml while that of females was only 0.33. In the current study no such difference was encountered between male and female rats at birth. Serum ascorbic acid values apparently are of no assistance in recognizing differences in the nutritional state of animals from the standpoint of pantothenic acid stores.

Blood pyruvic acid values taken at birth have been completed for a small number of young which were produced by females receiving the stock diet and synthetic rations. The results of these analyses suggest that the withdrawal of pantothenic acid from the diet of the female approximately one week before the initiation of pregnancy resulted in some elevation of blood pyruvate in the young at birth. High pyruvic acid values were not observed, however, for all animals in this group. Young born to females receiving the stock diet which provided between 450 and 600 µg of pantothenic acid daily, 150 µg of thiamine, and generous intakes of all other members of the B complex, averaged 1.04 mg of pyruvic acid per 100 ml of blood. There was wide individual variation encountered among the 18 animals tested. Examination of the blood of young produced by females deprived of pantothenic acid revealed an average pyruvic acid concentration of 2.79 mg %, and in 18 of 23 cases the amount of pyruvic acid present exceeded the highest value encountered among the stock animals. While the young produced by females receiving the synthetic diet plus 1 mg of calcium pantothenate daily exhibited lower concentrations of pyruvic acid than when the vitamin was omitted from the female's diet, a few animals included in this group apparently had more pyruvic acid in the blood than any animals fed the stock ration. The fact that the general performance of the females receiving the syn-

thetic diet supplemented with 1 mg of calcium pantothenate per day was not equal to that of the stock ration makes these differences in pyruvic acid especially interesting.

SUMMARY AND CONCLUSIONS

The effect of altering the pantothenic acid intake of the maternal rat during reproduction upon the concentration of several blood constituents of the young at birth and upon the pantothenic acid content of the maternal and fetal tissues at parturition has been studied. The females received a stock diet or synthetic rations furnishing zero, 100 µg or 1 mg amounts of calcium pantothenate daily.

It was observed that if the maternal diet supplied no pantothenic acid from the initiation of pregnancy, small litters of undersized young were produced. The tissues of such young at birth contained approximately 9% as much pantothenic acid as was present in young of mothers receiving 1 mg of the vitamin. Blood concentrations for pantothenic acid were reduced in this group of young and some accumulation of pyruvic acid was encountered.

The presence of 100 µg of calcium pantothenate in the maternal diet supported blood pantothenic acid values of the young equal to those of animals whose dams received the stock ration. Similarly, 100 µg of calcium pantothenate in the maternal diet resulted in normal pyruvic acid values of the young although the amount of pantothenic acid deposited in the fetal tissues at birth was still considerably less than that of rats produced by stock females.

Serum values for ascorbic acid and alkaline phosphatase were not altered by the differences in composition of the maternal diets.

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The Pharmacological Actions of Pantothenic Acid

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Concerning the pharmacological actions of pantothenic acid (P.A. in the following), we have to rely on a very few experimental data (4, 15) - although its clinical importance is steadily increasing (9, 12, 13, 14). The basic research was done by Nassi (4) (blood pressure test) on anesthesized animals, and the testing of the individual actions by Unna and Greslin (15) only on the isolated heart.

Since, as is known, differences may exist regarding affects on anesthesized and on waking animals, it seemed desirable to repeat Nassi's tests on waking animals, supplemented by an investigation of the also highly important relationships between P.A. and acetylcholine (2, 5, 10).

Method

The test animals were cats of both sexes, kept on a normal diet, weighing 1.5 to 3 kg. Under ether anesthesia a median throat cut was made to expose the left Vena jugularis and Arteria carotis communis, and in both vessels a plastic tube was inserted near the aorta to a depth of 8-10 cm, the size of the tube being adapted to the vessel (outside diameter 1-2 mm). The wound was sewn up in layers. One group of cats in the waking state was included in the test 4-5 hours, the other 20-24 hours after the operation; the reactivity was the same in both groups. The arterial cannula was connected by a plastic fitting to an electric pressure gauge whose fluctuations were recorded by a Hellige Multiscriptor. The substances, dissolved in Ringer solution,

were introduced through the venous cannula in quantities of 0.5 ml, in this order: Acetylcholine, histamine, adrenalin, applying each substance three times in succession, always in 0.5 ml Ringer solution, in doses of 3-10 mcg/kg, at intervals of 5 minutes. Before the next drug was given, again an interval of 5 minutes was interposed. The active substances usually resulted in blood pressure variations of 15-40 Hg mm in the various animals. The pressure variations before and after the dose of calcium pantothenate (10-20 mg/kg) given at the beginning of the test were stated in per cent.

In 6 animals under chloralose-urethane anesthesia (0.08 g/kg chloralose + 0.8 g/kg urethane) also EKG's were made in extremity derivation I, II and III ten minutes after the administration of Ca pantothenate and the results recorded with the Multiscriptor.

Test results

1. The pantothenic acid (10 mcg - 200 mg/kg) introduced into the blood stream of the cats had by itself no effect on the blood pressure of the animals.

We then examined whether the P.A. doses, ineffective by themselves, affect the blood pressure increase caused by adrenalin (3-10 mcg i.v.). The tests made in this sense on 19 animals showed (Table 1) that 10-200 mcg/kg Ca pentothenate i.v. does not appreciably change the blood pressure increasing effect of the previously given adrenalin, while P.A. doses exceeding 200 mcg/kg bring about a 30-50%, and doses of 5 mg/kg and more P.A. a still more pronounced potentiation of the adrenalin effect (Fig. 1). The effect, therefore, depends, in linear proportion, on the P.A. dose, but without being influenced by the adrenalin dose - which, incidentally, always varied according to the individual tolerance of the animals.

Table I.

No.	Designation of the animal	Adrenalin dose, mcg/kg	Pantothenic acid dose mg/kg	Blood pressure increasing effect of adrenalin, in Hg mm before administration	Blood pressure increasing effect of adrenalin, in Hg mm after administration of P.A.	Blood Pressure variation, %
1.	12.	3	0.01	25	25	0
2.	22.	3	0.05	25	25	0
3.	24.	3	0.1	17.5	20	12
4.	22.	3	0.2	25	25	0
5.	22.	3	0.2	25	30	20
6.	22.	3	0.2	25	35	40
7.	22.	3	0.2	25	35	40
8.	22.	3	0.5	25	35	40
9.	22.	3	0.5	25	40	60
10.	9.	3	1.0	20	30	50
11.	10.	4	1.0	20	30	50
12.	8.	3	1.0	20	30	50
13.	7.	5	1.0	30	40	30
14.	20.	5	1.0	25	35	40
15.	31.	5	1.0	35	40	15
16.	25.	5	1.0	27	35	30
17.	3.	3	1.0	15	20	30
18.	3.	3	5.0	15	25	60
19.	3.	3	10.0	15	25	60
20.	3.	3	10.0	15	25	60
21.	15.	7	10.0	30	40	30
22.	0.	3	10.0	25	40	60
23.	3.	3	20.0	15	25	60
24.	14.	3	20.0	15	25	60
25.	19.	3	20.0	22.5	30	30
26.	17.	5	20.0	26	45	70
27.	2.	4	25.0	15	30	100
28.	16.	10	40.0	30	40	30
29.	13.	3	50.0	25	35	40
30.	22.	3	100.0	25	40	60

Under analogous test conditions we tried, in 17 animals, to influence with P.A. the blood pressure decrease which occurs upon i.v. injection of different doses of acetylcholine (Table II). In 11 out of 17 animals the blood pressure depressing effect of 3-10 mcg/kg of acetylcholine (the dose was selected individually also in this instance) was increased or enhanced by the previously given P.A. by 10-70% (Fig. 2). The lower limit of the effective P.A. dose was

1 mg/kg (smaller doses had no effect), but higher doses did not necessarily result in a proportional potentiation,that is, the P.A. effect prolonging the acetylcholine action was not dependent on the dose.

Table II

No.	Designation of the animal	Acetylcholine dose, mcg/kg	Pantothenic acid dose, mg/kg	Blood pressure lowering effect of acetylcholine in Hg mm before administration of P.A.	Blood pressure lowering effect of acetylcholine in Hg mm after administration of P.A.	Blood pressure variation, %
1.	12.	3	0.01	30	30	0
2.	24.	3	0.1	20	20	0
3.	9.	3	1.0	15	20	30
4.	10.	4	1.0	25	30	20
5.	8.	3	1.0	20	30	50
6.	7.	5	1.0	25	35	40
7.	20.	5	1.0	12.5	20	60
8.	1.	4	1.0	25	35	40
9.	25.	10	1.0	40	60	50
10.	11.	5	5.0	30	30	0
11.	4.	6	10.0	30	40	30
12.	0.	3	10.0	40	40	0
13.	19.	3	20.0	30	33	10
14.	18.	3	20.0	20	35	70
15.	17.	4	20.0	38	63	60
16.	2.	5	25.0	27	27	0
17.	16.	10	40.0	37	30	-20

In 14 animals we tried to modify by P.A. the blood pressure lowering effect of histamine (3-10 mcg/kg i.v.) (Table III), but at the doses used the P.A. had no influence on the histamine effect.

2. As we were able to establish under the same test conditions, P.A. (10 mcg to 200 mg/kg) did not change the pulse frequency of the animals.

3. Intravenous injection of P.A. (10 mcg - 200 mg/kg) did not cause any change in the EKG curves of the animals.

4. Intravenous administration of P.A. (10 mcg - 200 mg/kg) did not affect the respiration frequency.

Table III.

No.	Designation of the animal	Histamine dose, mcg/kg	Pantothenic acid dose, mg/kg	Blood pressure lowering effect of histamine in Hg mm before administration	Blood pressure lowering effect of histamine in Hg mm after administration of P.A.	Blood pressure variation in %
1.	12.	3	0.01	29	30	0
2.	24.	3	0.1	30	30	0
3.	9.	3	1.0	10	10	0
4.	10.	4	1.0	15	17.5	10
5.	8.	3	1.0	15	17.5	10
6.	7.	5	1.0	20	20	0
7.	20.	5	1.0	20	20	0
8.	25.	10	1.0	42.5	35	0
9.	0.	10	10.0	50	50	0
10.	19.	5	20.0	20	20	0
11.	18.	5	20.0	15	20	30
12.	17.	6	20.0	40	50	20
13.	2.	5	25.0	22.5	22.5	0
14.	16.	5	40.0	20	22.5	0

Discussion of the results

As mentioned above, our knowledge concerning the pharmacological or toxicological effects of P.A. (1, 3, 6, 7, 8, 15) is limited.

Nassi (4) found on the isolated frog heart a negative inotropic and negative chronotropic effect of P.A. (in doses of 50-200 mcg); higher doses (10-80 mg) show a similar effect also on the isolated guinea pig heart. Noteworthy is Nassi's finding that in atropine-containing medium these cardinal effects are not observable. Nassi also studied the effect of large P.A. doses in perfusion tests (on rabbit ear) and found that 5-50 mg of P.A. bring about moderate vasodilatation of the perfused vessel. The blood pressure of rabbits is not influenced by large P.A. doses - in agreement with our own results in the blood pressure test on cats - while the blood pressure increase occurring after i.v. administration of 10 mcg adrenalin was potentiated and even prolonged by high P.A. doses (50 mg) - again in agreement with our own findings. Nassi did not record dose-effect curves.

Unna and Greslin (15) studied the effect of P.A. on circulation on cats anesthesized with chloralose-urethane; 10-30 mg/kg Ca pantothenate i.v.

changed neither the blood pressure of the animals nor their pulse or respiration frequency. These observations, too, therefore, coincide with our own findings.

As is evident, the observations reported earlier - about 20 years ago - by Nassi, Unna and Greslin concerning the use of P.A. alone and its adrenalin effect-potentiating action are confirmed by our results. But our findings are more informative inasmuch as (1) we examined the question in the waking state - corresponding to the physiological state -, (2) took into account a wider range of P.A. dose, and (3) included in our investigation, besides adrenalin, also the interaction of acetylcholine-P.A. and histamine-P.A.

It should be mentioned here that P.A. given intravenously causes no change of blood pressure, pulse or respiration frequency in man either (16).

If we evaluate our experimental results, not from the aspect of the effect on the organs, but from that of the examined important biochemical mediators, we get to some remarkable findings also.

Above all the prolongation of the adrenalin effect by P.A., as found by Nassi, although on the isolated heart.

As to the acetylcholine-P.A. correlation, the respective observations not related to the physiology of the circulatory system are likewise in wide agreement with the observations made in our blood-pressure studies. In earlier studies, in fact, we demonstrated on the isolated intestinal loop that P.A. is able in vitro to potentiate the acetylcholine sensitivity of the intestine (10). The P.A.-acetylcholine synergism has been confirmed on the isolated intestinal loop also by Mosler and Vorherr (5) and recently by Gordonoff (2).

It is not surprising that P.A. did not affect the blood pressure lowering action of histamine in our tests because this had been observed before (11) on the isolated ileum loop.

Summary

In waking cats, intravenously given pantothenic acid is able to potentiate the blood pressure increasing effect of adrenalin and the blood pressure lowering effect of acetylcholine. The potentiation of the adrenalin action is proportional to the P.A. dose used. The blood pressure lowering effect of histamine is not affected by pantothenic acid.

Intravenously injected pantothenic acid alone (10 mcg - 200 mg/kg) causes no changes either in blood pressure or in the pulse or respiration frequency of the animals, and also EKG deviations are not observable.

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Beiträge zu den pharmakologischen Wirkungen der Pantothensäure

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Bezüglich der pharmakologischen Wirkungen der Pantothensäure (im weiteren Ps) sind wir — obwohl ihre klinische Bedeutung stetig zunimmt (9, 12, 13, 14) — auf ganz wenige experimentelle Daten angewiesen (4, 5), wobei die grundlegenden Untersuchungen von Nassi (4) (Blutdruck-Versuch) an narkoti-

sierten Tieren und die Prüfung der einzelnen Wirkungen seitens Unna und Greslin (15) nur am isolierten Herzen ausgeführt wurden.

Da bekanntlich betreffs der Wirkung an narkotisierten und wachen Tieren Abweichungen bestehen können, schien es angebracht, die Versuche von Nassi — ergänzt durch die Untersuchung der ebenfalls hochwichtigen Beziehungen zwischen Ps und Azetylcholin (2, 5, 10) — an wachen Tieren zu wiederholen.

Methodik

Versuchstiere waren 25 bei normaler Kost gehaltene Katzen beiderlei Geschlechts im Gewicht von 1,5 — 3 kg. In Aethernarkose wurden durch einen medianen Halsschnitt die linke Vena jugularis und Arteria carotis communis herauspräpariert und in beide Gefäße nahe der Aorta — bis zu 8—10 cm Tiefe ein Kunststoffrohr eingeführt, dessen Weite der des Gefäßes angepaßt war (äußerer Durchmesser 1—2 mm). Die Wunde wurde schichtweise vernäht. Die eine Gruppe der Katzen wurde in wachem Zustand 4—5 Stunden, und die andere 20—24 Stunden nach der Operation in den Versuch genommen; die Reaktionsbereitschaft war in beiden Gruppen gleich. Die Arterien-Kanüle stand durch einen Kunststoffaufsatz mit einem Elektromanometer in Verbindung, dessen Schwankungen von einem Hellige-Multiskriptor registriert wurden. Durch die Venen-Kanüle wurden die in Ringerlösung gelösten Stoffe in Mengen von 0,5 ml eingeführt, und zwar in der Reihenfolge: Azetylcholin, Histamin, Adrenalin, wobei jedes Mittel in Gaben von 3—10 μg/kg — in Abständen von je 5 Minuten dreimal nacheinander, stets in 0,5 ml Ringerlösung — zur Anwendung gelangte. Vor der Einfuhr des nächsten Pharmakons wurde ebenfalls eine Pause von 5 Minuten eingeschaltet. Die Wirkstoffe hatten an den verschiedenen Tieren gewöhnlich Blutdruckveränderungen von 15—40 Hg-mm zur Folge. Die Tensionsschwankungen vor und nach dem zu Versuchsbeginn gereichten Calcium-Pantothenat (10—20 mg/kg) wurden in Prozent berechnet angegeben.

An 6 Tieren in Chloralose-Urethannarkose (0,08 g/kg Chloralose + 0,8 g/kg Urethan) wurden 10 Minuten nach der Ca-Pantothenatverabfolgung in I., II. und III. Extremitätenableitung auch EKG-Untersuchungen angestellt und die Befunde mit Hilfe des Multiskriptors registriert.

Versuchsergebnisse

1. Die in die Blutbahn der Katzen eingeführte Pantothensäure (10 μg- 200 mg/kg) an sich ließ den *Blutdruck* der Tiere unbeeinflußt.

Anschließend wurde untersucht, ob die an sich unwirksamen Ps-Gaben die durch Adrenalin (3—10 μg i. v.) hervorgerufene Blutdruckerhöhung beeinflussen. Die an 19 Tieren in dieser Richtung vorgenommenen Versuche zeigten (Tabelle 1), daß 10—200 μg/kg Ca-Pentothenat i. v. die blutdrucksteigernde Wirkung des zuvor gegebenen Adrenalins nicht nennenswert verändert, während 200 μg/kg überschreitende Ps-Gaben eine 30—50 %ige, und Dosen von 5

Tabelle 1

Nr.	Bezeichnung des Tieres	Adrenalindosis μg/kg	Pantothensäuredosis mg/kg	Blutdruckerhöhende Wirkung des Adrenalins in Hg-mm vor der Pantothensäureverabreichung	Blutdruckerhöhende Wirkung des Adrenalins in Hg-mm nach der Pantothensäureverabreichung	Blutdruckveränderung in %
1.	12.	3	0.01	25	25	0
2.	22.	3	0.05	25	25	0
3.	24.	3	0.1	17.5	20	12
4.	22.	3	0.2	25	25	0
5.	22.	3	0.2	25	30	20
6.	22.	3	0.2	25	35	40
7.	22.	3	0.2	25	35	40
8.	22.	3	0.5	25	35	40
9.	22.	3	0.5	25	40	60
10.	9.	3	1.0	20	30	50
11.	10.	4	1.0	20	30	50
12.	8.	3	1.0	20	30	50
13.	7.	5	1.0	30	40	30
14.	20.	5	1.0	25	35	40
15.	21.	5	1.0	35	40	15
16.	25.	5	1.0	27	35	30
17.	3.	3	1.0	15	20	30
18.	3.	3	5.0	15	25	60
19.	3.	3	10.0	15	25	60
20.	3.	3	10.0	15	25	60
21.	15.	7	10.0	30	40	30
22.	0.	3	10.0	25	40	60
23.	3,	3	20.0	15	25	60
24.	14.	3	20.0	15	25	60
25.	19.	3	20.0	22.5	30	30
26.	17.	5	20.0	26	45	70
27.	2.	4	25.0	15	30	100
28.	16.	10	40.0	30	40	30
29.	13.	3	50.0	25	35	40
30.	22.	3	100.0	25	40	60

mg/kg und mehr Ps eine noch ausgesprochenere Potenzierung des Adrenalineffektes bewirken. (Abb. 1). Der Effekt hängt also — und zwar in geradem Verhältnis — von der Ps-Dosis ab, ohne jedoch von der Adrenalindosis beeinflußt zu werden — die sich übrigens stets nach der individuellen Toleranz der Tiere richtete.

Bei 17 Tieren wurde — unter analogen Versuchsbedingungen —

versucht, die auf i. v. Einfuhr verschieden hoher Azetylcholingaben auftretende Blutdrucksenkung mit Ps zu beeinflussen (Tabelle II). Bei 11 von 17 Tieren wurde die blutdrucksenkende Wirkung von 3—10 μg/kg Azetylcholin (die Dosis wurde auch hier individuell gewählt) durch die vorher verabreichte Ps um 10—70 % gesteigert bzw. ausgeprägter gestaltet (Abb. 2). Die untere Grenze der wirksamen Ps-Dosis betrug hier 1 mg/kg (niedrigere Dosen blieben wirkungslos), höhere Dosen hatten aber nicht unbedingt eine proportionale Wirkungspotenzierung zur Folge, d. h. der die Azetylcholinwirkung prolongierende Ps-Effekt war nicht Dosis-abhängig.

Tabelle II

Nr.	Bezeichnung des Tieres	Azetylcholindosis μg/kg	Pantothensäuredosis mg/kg	Blutdruckherabsetzende Wirkung des Azetylcholins in Hg-mm vor der Pantothensäureverabreichung	Blutdruckherabsetzende Wirkung des Azetylcholins in Hg-mm *nach* der Pantothensäureverabreichung	Blutdruckveränderung in %
1.	12.	3	0.01	30	30	0
2.	24.	3	0.1	20	20	0
3.	9.	3	1.0	15	20	30
4.	10.	4	1.0	25	30	20
5.	8.	3	1.0	20	30	50
6.	7.	5	1.0	25	35	40
7.	20.	5	1.0	12.5	20	60
8.	1.	4	1.0	25	35	40
9.	25.	10	1.0	40	60	50
10.	11.	5	5.0	30	30	0
11.	4.	6	10.0	30	40	30
12.	0.	3	10.0	40	40	0
13.	19.	3	20.0	30	33	10
14.	18.	3	20.0	20	35	70
15.	17.	4	20.0	38	63	60
16.	2.	5	25.0	27	27	0
17.	16.	10	40.0	37	30	—20

Bei 14 Tieren versuchten wir die blutdrucksenkende Wirkung des Histamins (3—10 μg/kg i. v.) mit Ps zu modifizieren (Tabelle III), doch war die Ps in den angewandten Dosen ohne Einfluß auf den Histamineffekt.

2. Ps (10 μg—200 mg/kg) veränderte — wie unter den gleichen Versuchsbedingungen festgestellt werden konnte — die *Pulszahl* der Tiere nicht.

3. Intravenöse Einfuhr von Ps (10 μg—200 mg/kg) bewirkte auch keine Veränderung der *EKG*-Kurven der Tiere.

4. Unbeeinflußt ließ die i. v. verabreichte Ps (10 μg—200 mg/kg) auch die *Atmungszahl*.

Tabelle III

Nr.	Bezeichnung des Tieres	Histamindosis µg/kg	Pantothensäuredosis mg/kg	Blutdruckherabsetzende Wirkung des Histamins in Hg-mm vor	nach der Pantothensäureverabreichung	Blutdruckveränderung in %
1.	12.	3	0.01	29	30	0
2.	24.	3	0.1	30	30	0
3.	9.	3	1.0	10	10	0
4.	10.	4	1.0	15	17.5	10
5.	8.	3	1.0	15	17.5	10
6.	7.	5	1.0	20	20	0
7.	20.	5	1.0	20	20	0
8.	25.	10	1.0	42.5	35	0
9.	0.	10	10.0	50	50	0
10.	19.	5	20.0	20	20	0
11.	18.	3	20.0	15	20	30
12.	17.	6	20.0	40	50	20
13.	2.	5	25.0	22.5	22.5	0
14.	16.	5	40.0	20	22.5	0

Besprechung der Ergebnisse

Wie schon eingangs erwähnt, sind unsere Kenntnisse bezüglich der pharmakologischen bzw. toxikologischen Wirkungen der Ps (1, 3, 6, 7, 8, 15) gering.

Nassi (4) stellte am isolierten Froschherzen eine negative inotrope und eine negative chronotrope Wirkung der Ps (in Gaben von 50—200 µg) fest; höhere Dosen (10—80 mg) zeigten auch am isolierten Meerschweinchenherzen einen ähnlichen Effekt. Beachtenswert ist die Feststellung Nassis, wonach im atropinhaltigen Milieu diese kardinalen Wirkungen nicht zu beobachten sind. Ebenfalls Nassi hat in Perfusionsversuchen (am Kaninchenohr) die Wirkung großer Ps-Dosen studiert und gefunden, daß 5—50 mg Ps mäßige Vasodilatation am perfundierten Gefäß auslösen. Der Blutdruck von Kaninchen wird durch große Ps-Gaben — in Übereinstimmig mit unseren eigenen Ergebnissen im Blutdruck-Versuch an Katzen — nicht beeinflußt, während die im Anschluß an i. v. Verabreichung von 10 µg Adrenalin eintretende Blutdruckerhöhung durch hohe Ps-Dosen (50 mg) — ebenfalls in Übereinstimmung mit unseren eigenen Befunden — gesteigert, und sogar prolongiert wurde. Dosis-Effektkurven hat Nassi nicht aufgenommen.

Unna und Greslin (15) haben die Kreislaufwirkung der Ps an chloralose-urethannarkotisierten Katzen studiert: 10—30 mg/kg Ca-Pantothenat i. v. veränderten weder den Blutdruck der Tiere, noch ihre Puls- oder Atmungszahl. Auch diese Beobachtungen decken sich also mit unseren eigenen Befunden.

Wie ersichtlich, finden die führ — vor annähernd 20 Jahren — mitgeteilten Beobachtungen von Nassi bzw. Unna und Greslin bezüglich der alleinigen Anwendung der Ps bzw. ihrer den Adrenalineffekt steigernden Wikung durch unsere Ergebnisse eine Bestätigung. Unsere Befunde sagen aber insofern mehr aus, als wir 1. die Frage in — dem physiologischen Zustand entsprechendem — wachen Zustande untersuchten, 2. eine breitere Dosen-Skala der Ps berücksichtigten und 3. außer dem Adrenalin auch die Wechselwirkung von Azetylcholin-Ps und Histamin-Ps zum Gegenstand eingehender Untersuchung machten.

Hier wäre zu erwähnen, daß i. v. verabreichte Ps auch beim Menschen keine Blutdruck-, Pulszahl- oder Atemzahlveränderung bewirkt (16).

Bewerten wir nun unsere experimentellen Ergebnisse nicht vom Gesichtspunkt der Organwirkung sondern von dem der untersuchten wichtigen biochemischen Mediatoren, so kommen wir ebenfalls zu einigen beachtenswerten Feststellungen.

Vor allem die Prolongierung der *Adrenalin*-Wirkung mit Ps, festgestellt von Nassi, allerdings am isolierten Herzen.

Was die *Azetylcholin*-Ps-Korrelation anbelangt, stimmen die einschlägigen, nicht-kreislaufphysiologischen Beobachtungen ebenfalls weitgehend mit den in unseren Blutdruck-Untersuchungen gemachten Beobachtungen überein. In früheren Untersuchungen wiesen wir nämlich an der isolierten Darmschlinge nach, daß die Ps in vitro die Azetylcholinsensibilität des Darmes zu steigern vermag (10). Der Ps-Azetylcholin-Synergismus ist an der isolierten Darmschlinge außer von uns auch von Mosler und Vorherr (5) und neuerdings von Gordonoff (2) bestätigt worden.

Daß Ps die blutdrucksenkende Wirkung des *Histamins* in unseren Untersuchungen nicht beeinflußte ist nicht überraschend, da sie früher (11) an der isolierten Ileumschlinge schon beobachtet wurde.

Zusammenfassung

Bei in wachem Zustand befindlichen Katzen vermag intravenös verabreichte Pantothensäure die blutdrucksteigernde Wirkung des Adrenalins bzw. die blutdrucksenkende Wirkung des Azetylcholins zu potenzieren. Die Potenzierung der Adrenalinwirkung ist proportional der angewandten Ps-Dosis. Die blutdrucksenkende Wirkung des Histamins läßt die Pantothensäure unbeeinflußt.

Intravenös einverleibte Pantothensäure (10 μg –200 mg/kg) allein bewirkt weder im Blutdruck, noch in der Puls- oder Atemzahl der Tiere Veränderungen, und auch EKG-Abweichungen sind nicht zu beobachten.

RESUME

Contribution à l'étude des effets pharmacologiques de l'acide pantothénique. Par M. Gabor, I. Szórady, A. Antal et Z. Dirner.

En injection intraveineuse, l'acide pantothénique n'exerce aucune influence sur la pression sanguine des chats, mais il renforce la hausse de pression due a l'adrénaline, ainsi que la baisse provoquée par l'acétylcholine, cela dans le sens d'une potentialisation. La potentialisation de l'action de l'adrénaline est proportionnelle à la dose d'acide pantothénique injectée.

SUMMARY

Pharmacological actions of pantothenic acid. By M. Gabor, I. Szórády, A. Antal and Z. Dirner.

Intravenous injection of pantothenic acid into cats had no effect on blood pressure, but potentiated both the rise in blood pressure caused by adrenaline and the fall in blood pressure caused by acetylcholine, the extent of the former being proportional to the dose of pantothenic acid.

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Collaborative Study of a Chemical Method for Calcium Pantothenate

By W. D. HUBBARD and A. J. SHEPPARD (Division of Nutrition, Food and Drug Administration, Washington, D.C. 20204)

A chemical method for determining calcium pantothenate in pharmaceutical preparations has been studied collaboratively. The pantothenate molecule is cleaved with acid, and the resulting β-alanine is treated with a chlorinating solution and then with potassium iodide. The free iodine is measured spectrophotometrically at 358 mμ. The average coefficient of variation for all samples studied collaboratively is 8.9%. The method is recommended for adoption as official first action.

This report of the Associate Referee, W. D. Hubbard, was presented at the 82nd Annual Meeting of the Association of Official Analytical Chemists, Oct. 14–17, 1968, at Washington, D.C.

At the present time there is no official chemical method in either the USP or in the *Official Methods of Analysis* for the determination of calcium pantothenate. The method described in this paper is designed for the quantitative determination of calcium pantothenate in pharmaceutical preparations. The method was first reported in 1965 (W. D. Hubbard, *This Journal* 48, 1217–1220 (1965)). During 1967, a comparison study of this method and a method by T. Panalaks and J. A. Campbell (*Anal. Chem.* 34, 64–66 (1962)) was completed by the two laboratories involved. Based on the results of the study, a collaborative study of the Hubbard method was recommended.

Collaborative Study

Each of seven collaborators was sent seven pharmaceutical preparations containing calcium pantothenate along with other vitamins. One of the samples was designated as a practice sample of stated composition. The collaborators were instructed to obtain satisfactory results on the known sample, using the published method, before proceeding with the unknown samples. Some precautionary remarks were included in the instructions. The collaborators were asked to report one determination per sample for all seven samples by the one method only.

METHOD

(Method does not differentiate between *d*- and *l*-isomeric forms. Assuming that racemic Ca pantothenate is 50:50 mixt. of *d*- and *l*-isomeric forms, chemically detd values for total Ca pantothenate are reduced by ½ to give content of active *d*-Ca pantothenate. Similarly, vitamin products, in which isomeric form of pantothenate ingredient is not stated on label, would be reported as contg *d*-pantothenate equiv. to ½ that of detd value.)

Principle

Pantothenate molecule is cleaved with acid and resulting β-alanine is treated with chlorinating soln and then with KI. Free I is measured spectrophtric.

Apparatus

Chromatographic tubes.—Pyrex, 10 mm id × 300 mm long, with coarse fritted disks and tops tooled for rubber stoppers.

Reagents

(a) *Ion exchange resin.*—Dowex 50W-X4 (H^+ form); 100–200 mesh (Dow-Corning Inc., Midland, MI 48640).

(b) *Florisil.*—60–100 mesh (Floridin Co., 2 Gateway Center, Pittsburgh, PA 15222).

(c) *Borate buffer.*—0.05*M*. Dissolve 3.1 g reagent grade boric acid and 3.7 g KCl in ca 900 ml H_2O; adjust pH to precisely 10.5 with 2*N* NaOH and dil. to 1 L.

(d) *Chlorinating soln.*—Dil. 4–6% NaOCl soln 1 + 30 with 0.05*M* borate buffer; prep. daily and protect from light.

(e) *Acidified phenol soln.*—0.5% phenol in 0.1*M* HCl.

(f) *Potassium iodide.*—1% aq. soln. Prep. weekly and protect from light.

(g) *Phenolphthalein.*—0.02% in ethyl or isopropyl alcohol.

(h) *d-Calcium pantothenate reference std.*—USP. Dissolve 50.0 mg in 100.0 ml H_2O. Dil. 10.0 ml to 100.0 ml for std soln (0.05 mg/ml). Store at 10°.

Preparation of Columns

Wash Dowex and Florisil resins with H_2O to remove most of fines and store in H_2O until use. Use 2 columns for each sample to be assayed and one column for std. Add 5 cm Florisil to each column, insert thin layer of glass wool, and add 5 cm Dowex 50W-X4. Wash resins with ca 15 ml 1*M* HCl and rinse with 150 ml H_2O. Prep. new columns before each assay.

Determination

Prep. aq. soln of sample contg 0.05 mg Ca pantothenate/ml based on label claim. Tablets and capsules may be heated in H_2O to facilitate extn. Remove colored outer coating of tablets by carefully washing with H_2O. It may be necessary to cut open and carefully remove contents of capsules with colored coatings.

Add 10 ml aliquots of sample and std to sep. columns and elute each with 70 ml H_2O into 200 ml erlenmeyer. Add 5 ml 2*N* HCl to both eluates and autoclave 30 min at 120°. Cool and dil. to 100 ml. Prep. sample blank by adding 10 ml aliquot of sample soln to sep. column and eluting with 70 ml H_2O. Dil. blank to 100 ml with H_2O. Transfer 10 ml aliquots of sample, sample blank, and std to sep. 50 ml g-s erlenmeyers. Add 2 drops 0.02% phthln soln to each flask. Titr. with 2*N* NaOH until red and then back-titr. to colorless end point with 0.1*N* HCl.

Add 1 ml chlorinating soln to each flask, mix, stopper, and let stand 15 min. Add 1 ml acidified phenol soln to decompose excess hypochlorite and swirl to rinse sides of flasks. Stopper and let stand 5 min. Add 1 ml KI soln and immediately add 20 ml absolute alcohol to each flask in turn. (Add immediately, as I color decreases rapidly in aq. soln.) Transfer solns to 50 ml vol. flasks. Dil. to vol. with absolute alcohol and mix. Let stand not <10 min for max. color to develop; measure *A* of solns at 358 mμ on spectrophtr set at 0 *A* against reagent blank consisting of 10 ml H_2O and each of those reagents added at specified time intervals. Under these conditions I color is constant 24 hr.

Calc. concn Ca pantothenate in original sample = $A_{sample}/A_{std} \times$ declared potency.

Table 1. Collaborative results for chemical determination of calcium pantothenate

Coll.	Calcium Pantothenate Found						
	Sample 1, mg/Tab	Sample 2, mg/Tab	Sample 3, mg/0.6 ml	Sample 4, mg/Cap	Sample 5, mg/Tab	Sample 6, mg/Tab	Sample 7, mg/Tab
A	13.2	7.4	14.6	10.4	7.1	30.1	11.0
B	15.5	6.5	15.6	10.5	9.4	30.5	12.5
C	13.9	6.7	15.3	11.2	8.3	35.8	12.0
D	16.3	7.1	16.2	15.9[b]	10.0	33.0	12.8
E	13.7	5.8	2.3[b]	10.9	6.3	24.7	11.1
F[a]	10.8	2.6	6.3	7.5	3.6	17.2	10.3
G	15.7	6.9	15.9	10.2	10.0	30.0	12.3
Mean	14.7	6.7	15.5	10.6	8.5	30.7	12.0
Std. dev.	1.3	0.6	0.6	0.4	1.6	3.7	0.7
Coeff. of var., %	8.8	9.0	3.9	3.8	18.8	12.1	5.8
Av. coeff. of var., 8.9%							

[a] All results of Collaborator F omitted on basis of ranking test.
[b] Eliminated by Dixon outlier test.

Results and Recommendation

The results obtained by the collaborators for the determination of calcium pantothenate in pharmaceutical preparations are shown in Table 1. All seven laboratories submitted reports. However, Laboratory F reported consistently low values on all samples; those results were omitted on the basis of the ranking test. All the reporting collaborators had difficulty with Sample 5. It was not possible for them to remove all of the colored coating without also removing a portion of the tablet core. Several of the collaborators did not attempt to wash off any of the coating; one stated that he washed off the majority of the coating. The high coefficient of variation for Sample 5 is not surprising in view of these comments. The overall coefficient of variation for all samples is 8.9%.

The mean of the collaborators' results for each sample, using the test method, was subjected to a "t" test comparison against results obtained by the AOAC microbiological method in our laboratories. The test revealed no significant difference between results for the two methods.

It is recommended that the chemical method for calcium pantothenate be adopted as official first action.

Acknowledgments

The Associate Referee wishes to express his thanks to the following collaborators: Miss L. M. Johanson, Canadian Food and Drug Directorate, Toronto, Canada; and George A. Hudson, Washington, D.C.; Gus B. Timpa, Chicago; David W. Baker, San Francisco; Eldon L. Farmer, Los Angeles; Barbara A. Bong, Minneapolis; and Duane E. Hines, Baltimore; all of the Food and Drug Administration.

The recommendation of the Associate Referee was approved by the General Referee and by Subcommittee D and was adopted by the Association. See *This Journal* 52, 334–335 (1969).

Klykov-'71
B&B No. 63

VARIATION OF THE PYRUVIC ACID LEVEL IN THE BLOOD AND THE HEAT COAGULATION TIME OF THE SERUM UNDER THE INFLUENCE OF CALCIUM PANTOTHENATE IN CARDIAC INSUFFICIENCY PATIENTS. [Izmenenie soderzhaniya pirovinogradnoi kisloty v krovi i vremeni termicheskoi svertyvaemosti syvorotki pod vliyaniem pantotenata kal'tsiya u bol'nykh s serdechnoi nedostatochnost'yu]

by

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from

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Translation from Russian

VARIATION OF THE PYRUVIC ACID LEVEL IN THE BLOOD AND THE HEAT COAGULATION TIME OF THE SERUM UNDER THE INFLUENCE OF CALCIUM PANTOTHENATE IN CARDIAC INSUFFICIENCY PATIENTS

N. V. Klykov and I. L. Minsker

Calcium pantothenate--a salt of pantothenic acid--is recommended for use in the complex therapy of patients with chronic circulatory insufficiency (I. B. Likhtsier, R. S. Dedyunona, N. V. Klykov). It has been shown that strophantine treatment in combination with calcium pantothenate has led to better restoration of the contractile capacity of the myocardium and certain parameters of carbohydrates and protein metabolism in comparison with treatment with strophantine alone (N. V. Klykov). This has testified to the favorable effect of calcium pantothenate on the aforementioned types of metabolism. However, for a more complete clarification of the role of calcium pantothenate in the variations of certain metabolic processes, it is necessary to perform direct studies involving the application of this substance without simultaneous treatment by other drugs.

For this purpose, observations were carried out on 38 patients ranging in age from 40 to 75 years with chronic circulatory insufficiency of II-III degree against a background of rheumatic heart defects (12 persons) and atherosclerotic cardiosclerosis (26 persons). There were 15 men and 23 women. All patients upon admission were treated only with calcium pantothenate in a dose of 0.2 g three times daily for a period of five days. Before the start of the calcium pantothenate therapy and after five days of this treatment, certain blood parameters of the patients were studied: the pyruvic acid content by the method using 2,4-dinitrophenylhydrazine, the quantity of sulfhydryl (SH) groups in the serum by the ferric cyanide method in the modification of E. S. Tsiperovich and A. L. Loseva (1948),

the thermal coagulation time of the serum after S. S. Okolov. It was assumed that the variations in these parameters under the influence of the therapy could be conditioned by the action of calcium pantothenate. The results of the studies were in addition compared with data determined in healthy individuals (N. V. Klykov).

The data obtained showed that in all patients, pronounced variations of the blood parameters were noted before the treatment (see Table). Elevated pyruvic acid levels were found in the blood averaging 2.38 ± 0.25 mg% against a normal 0.71 ± 0.5 mg% ($P < 0.001$). This reflects, above all, profound impairments of the carbohydrate metabolism in patients with circulatory insufficiency. After the five-day therapy with calcium pantothenate, a lowering of the pyruvic acid level averaging up to 1.1 ± 0.16 mg% ($P < 0.05$) was observed. No significant difference in the latter magnitude compared to that observed in healthy persons was revealed ($P > 0.05$). Consequently, the above-indicated depression of the pyruvic acid level can be related to the ingestion of the calcium pantothenate.

Variation of the blood parameters of patients with circulatory insufficiency of II-III degree under the influence of calcium pantothenate therapy (average data, M + m)

Parameter	Healthy subjects	Patients		
		Before treatment	After treatment	P
Pyruvic acid (in mg%)	0.71±0.5	2.38±0.25	1.1±0.16	<0.05
Sulfhydryl groups (in mg-val%)	74.9±0.14	66.0±1.9	67±1.2	>0.05
Serum heat coagulation time (in min)	58±2.5	97±6.4	81±3.9	<0.001

In 23 out of 38 patients, a lowering of the SH-group content in the blood serum was observed before the treatment, comprising on the average of the entire group 66.0 ± 1.9 mg-val% against a normal of 74.9 ± 0.14 mg-val%

($P < 0.001$). After a five-day course of therapy with calcium pantothenate, no substantial variation in the SH group level was noted; it averaged 67 ± 1.2 mg-val% ($P > 0.05$) and significantly different from the normal level.

The heat coagulation time of the blood serum, which testifies to the functional state of the serum proteins, was significantly more prolonged in 24 out of 38 patients in the initial state than that of the serum of the healthy subjects, comprising an average of 97 ± 6.4 min against a normal of 58 ± 2.5 min ($P < 0.001$). After a five-day course of calcium pantothenate therapy, there was a noticeable reduction of the heat coagulation time--on the average to 81 ± 3.9 min, which differs significantly from the initial level ($P < 0.001$). In this case, no differences were found in comparison with the parameters of the healthy subjects ($P > 0.05$). The variation of the heat stability of the serum indicates profound qualitative changes in the blood protein with a reduction of their sensitivity to the thermal factor, which reflects substantial disruptions of the protein metabolism. Thus chronic circulatory insufficiency of II and III degree is characterized by a pronounced elevation of the pyruvic acid concentration, a reduction of the SH group content, and by an increase in the heat stability of the serum proteins. As the data cited reveal, the calcium pantothenate treatment for a period of several days leads to a substantial lowering of the blood pyruvic acid. This indicates a role played by calcium pantothenate in the improvement of carbohydrate metabolism in cases of circulatory insufficiency. The reduction of the pyruvic acid level of the blood of the patients may testify to an intensification of oxidative processes.

As is well known, the process of oxidative decarboxylation of pyruvic acid involves the participation of coenzyme A, whose chief component is pantothenic acid. Evidently the pantothenic acid deficit observed in patients with chronic insufficiency of the circulatory system (N. B. Likhtsier) may promote an increase in the pyruvic acid content, while calcium pantothenate makes up this deficit, which causes an intensification of the pyruvic acid decarboxylation processes. The experimental data of Beiglbock and Clotten may speak in favor of this viewpoint, indicating that in cases of pantothenic acid insufficiency in dogs, the blood pyruvic acid level was elevated, while a pantothenic acid load caused a reduction in the blood pyruvate.

The decrease in the heat coagulation time of the serum under the influence of the pantothenate may testify to an improvement in the functional state of the serum proteins, which reflects improvement of the protein metabolism on the whole. The reduction in the SH group level in the blood before the treatment may testify to a lowering of the enzyme activity if one considers that the active centers of the majority of enzymes are represented by SH groups (B. I. Gol'dshtein, Barron, et al.). After five-day administration of calcium pentothenate, the SH group level did not change. The SH group level practically did not increase even after a month of therapy of the patients with circulatory insufficiency of II-III degree involving pantothenate associated with strophantine (N. V. Klykov), although when other biologically active substances were used (vitamin B_{12}, folic acid, dimercaprol), the SH group level was increased.

Conclusions

1. Patients with chronic circulatory insufficiency of II-III degree were observed to have an elevation of the blood pyruvic acid level, a lowering of the serum sulfhydryl group level, and an increase in the serum heat coagulation time.

2. After five days of calcium pantothenate therapy in these patients, a substantial reduction was seen in the blood pyruvic acid and the heat coagulation time, indicating an improvement in the carbohydrate and protein metabolism under the influence of this drug. The sulfhydryl group content did not change noticeably.

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ИЗМЕНЕНИЕ СОДЕРЖАНИЯ ПИРОВИНОГРАДНОЙ КИСЛОТЫ В КРОВИ И ВРЕМЕНИ ТЕРМИЧЕСКОЙ СВЕРТЫВАЕМОСТИ СЫВОРОТКИ ПОД ВЛИЯНИЕМ ПАНТОТЕНАТА КАЛЬЦИЯ У БОЛЬНЫХ С СЕРДЕЧНОЙ НЕДОСТАТОЧНОСТЬЮ

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Пантотенат кальция — соль пантотеновой кислоты — рекомендуется использовать в комплексном лечении больных с хронической недостаточностью кровообращения (И. Б. Лихцинер; Р. С. Дедюшина; Н. В. Клыков). Показано, что лечение строфантином в сочетании с пантотенатом кальция приводило к лучшему восстановлению сократительной способности миокарда и некоторых показателей углеводного и белкового обмена веществ по сравнению с лечением одним строфантином (Н. В. Клыков). Это свидетельствовало о положительном влиянии пантотената кальция на указанные виды обмена. Однако для более полного выяснения роли пантотената кальция в изменениях некоторых процессов обмена необходимы прямые исследования с воздействием этим веществом без одновременного лечения другими лекарственными препаратами.

С указанной целью проведены наблюдения у 38 больных в возрасте 40—75 лет с хронической недостаточностью кровообращения II—III степени на почве ревматических пороков сердца (12 человек) и атеросклеротического кардиосклероза (26 человек). Мужчин было 15, женщин — 23. Всем больным при поступлении назначали лечение только пантотенатом кальция по 0,2 *г* 3 раза в день в течение 5 дней. До начала лечения пантотенатом кальция и через 5 дней этого лечения у больных исследовали некоторые показатели крови: содержание пировиноградной кислоты (ПВК) методом с 2,4-динитрофенилгидразином, количество сульфгидрильных (SH)

групп в сыворотке ферриционидным методом в модификации А. С. Цинеровича и А. Л. Лосевой (1948), время термической свертываемости сыворотки крови по Ф. С. Околову. Предполагалось, что изменения этих показателей под влиянием лечения могли быть обусловлены воздействием пантотената кальция. Результаты исследований, кроме того, сравнивали с данными, определявшимися у здоровых людей (П. В. Клыков).

Полученные данные показали, что у всех больных до лечения отмечались выраженные изменения показателей крови (см. таблицу). Выявлено повышение уровня ПВК в крови, что в среднем составляло 2,38±±0,25 *мг*% при норме 0,71±0,5 *мг*% ($P<0,001$). Это отражает прежде всего глубокие нарушения углеводного обмена у больных с недостаточностью кровообращения. После 5-дневного лечения пантотенатом кальция наблюдалось снижение уровня ПВК в среднем до 1,1±0,16 *мг*% ($P<0,05$). Достоверного различия последней величины по сравнению с наблюдаемой у здоровых лиц не выявлено ($P>0,05$). Следовательно, указанное понижение уровня ПВК можно связать с приемом пантотената кальция.

Изменение показателей крови у больных с недостаточностью кровообращения II—III степени под влиянием лечения пантотенатом кальция (средние данные, $M \pm m$)

Показатель	Здоровые	Больные		
		до лечения	после лечения	P
Пировиноградная кислота (в *мг*%)	0,71±0,5	2,38±0,25	1,1±0,16	<0,05
Сульфгидрильные группы (в *мг-экв*%)	74,9±0,14	66,0±1,9	67±1,2	>0,05
Время термической свертываемости сыворотки (в мин.)	58±2,5	97±6,4	81±3,9	<0,001

У 23 из 38 больных до лечения наблюдалось снижение содержания SH-групп в сыворотке крови, которое в среднем по всей группе составляло 66,0±1,9 *мг-экв*% при норме 74,9±0,14 *мг-экв*% ($P<0,001$). После 5-дневного лечения пантотенатом кальция не отмечалось существенного изменения содержания SH-групп, которое в среднем составило 67±1,2 *мг-экв*% ($P>0,05$) и достоверно отличалось от нормального уровня.

Время термической свертываемости сыворотки крови, свидетельствующее о функциональном состоянии сывороточных белков, у 24 из 38 больных в исходном состоянии было значительно более удлиненным, чем сыворотки здоровых, и составляло в среднем 97±6,4 мин. при норме 58±2,5 мин. ($P<0,001$). После 5-дневного лечения пантотенатом кальция заметно уменьшалось время термосвертываемости — в среднем до 81±3,9 мин., что достоверно отличалось от исходного уровня ($P<0,001$). При этом различий по сравнению с показателями здоровых лиц не выявлено ($P>0,05$). Изменение термической устойчивости сыворотки указывает на глубокие качественные изменения белков крови со снижением их чувствительности к термическому фактору, что отражает существенные нарушения в белковом обмене. Таким образом, хроническая недостаточность кровообращения II и III степени характеризуется выраженным повышением концентрации ПВК, уменьшением содержания SH-групп и увеличением термической устойчивости сывороточных белков. Как видно из приведенных данных, лечение пантотенатом кальция в течение нескольких дней приводит к существенному снижению ПВК в крови. Это указывает на роль пантотената кальция в улучшении углеводного обмена при недостаточности кровообращения. Снижение уровня ПВК в крови больных может свидетельствовать об усилении окислительных процессов.

Как известно, процесс окислительного декарбоксилирования ПВК протекает с участием кофермента А, главной составной частью которого является пантотеновая кислота. Очевидно, наблюдаемый у больных с хронической недостаточностью кровообращения дефицит пантотеновой кислоты (Н. Б. Лихцирер) может способствовать увеличению содержания ПВК, пантотенат же кальция восполняет этот дефицит, что способствует усилению процессов декарбоксилирования ПВК. В пользу этого могут говорить экспериментальные данные Beiglböck и Clotten, указывающих, что при недостаточности пантотеновой кислоты у собак повышалось содержание ПВК в крови, а нагрузка пантотеновой кислотой приводила к уменьшению пирувата в крови.

Уменьшение времени термической свертываемости сыворотки под влиянием пантотената может свидетельствовать об улучшении функционального состояния сывороточных белков, что отражает улучшение белкового обмена в целом. Уменьшенное содержание SH-групп в крови до лечения может свидетельствовать о понижении ферментативной активности, если учесть, что у большинства ферментов активные центры представлены SH-группами (Б. Н. Гольдштейн; Barron, и др.). После 5-дневного приема пантотената кальция содержание SH-групп не изменилось. Практически не увеличивалось содержание SH групп и после месячного лечения больных с недостаточностью кровообращения II—III степени пантотенатом совместно со строфантином (Н. В. Клыков), хотя при использовании других биологически активных веществ (витамин B_{12}, фолиевая кислота, унитиол) уровень SH-групп увеличивался.

Выводы

1. У больных с хронической недостаточностью кровообращения II—III степени наблюдается повышение содержания пировиноградной кислоты в крови, понижение содержания сульфгидрильных групп в сыворотке и увеличение времени термической свертываемости сыворотки.

2. Через 5 дней лечения пантотенатом кальция у данных больных отмечается существенное уменьшение в крови пировиноградной кислоты и времени термической свертываемости, что указывает на улучшение углеводного и белкового обмена под влиянием этого препарата. Содержание сульфгидрильных групп заметно не меняется.

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CHANGES OF THE CONTENT OF PYRORACEMIC ACID IN THE BLOOD AND TIME OF THERMIC COAGULATION OF THE SERUM IN PATIENTS WITH CARDIAC INSUFFICIENCY UNDER THE INFLUENCE OF CALCIUM PANTOTHENATE

N. V. Klykov, I. L. Minsker

Summary

Study of the effect of calcium pantothenate on some indices of the blood in 38 patients with chronic insufficiency of the circulation of the II—III degree showed that after a five-day use of this preparation in a dose of 0.6 g only there was a substantial drop in the blood of the increased amount of pyroracemic acid and reduction of the time of thermic coagulation of the serum. This testifies to improvement of the processes of carbohydrate and protein metabolism. The number of subfibrile groups did not undergo any marked changes. It remained on the same lowered level.

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VITAMIN ABSORPTION STUDIES

V. A NOTE ON THE UTILIZATION OF PYRIDOXINE AND CALCIUM PANTOTHENATE GIVEN ORALLY AT PERIODIC INTERVALS

E. J. Middleton and A. B. Morrison

Sarett and Morrison (1) found that when B vitamins were given to growing rats once a day by stomach tube the utilization of the vitamins was similar to that when the vitamins were given in the diet. In other studies (2), rats given

[2]V. Massey, University of Michigan, Ann Arbor, Michigan, a personal communication.

equivalent amounts of riboflavin at 2- and 4-day intervals showed a stepwise reduction in weight gains, as compared with those given the vitamin daily. In contrast to these findings, animals given thiamine daily or every other day grew at comparable rates, but weight gains were reduced when the vitamin was given at 4-day intervals. Investigations with radioactive riboflavin and thiamine (3, 4) showed that large doses of riboflavin apparently were absorbed inefficiently, whereas those of thiamine were absorbed but not utilized effectively.

The studies reported herein were conducted as a continuation of investigations on the absorption and utilization of vitamins given at periodic intervals. Data on the utilization of pyridoxine and calcium pantothenate given orally to growing rats at 1-, 2-, and 4-day intervals were obtained.

Male weanling Wistar rats of the Food and Drug colony were used in the experiments. In experiment 1, the animals were given a pyridoxine-free diet for a 14-day pretest period. The diet was the same as that of Morrison *et al.* (2), except that it contained adequate riboflavin but no pyridoxine. At the end of the pretest period the rats were distributed into four comparable groups of 10 animals each on the basis of body weight. All groups (A–D) continued to receive the pyridoxine-free diet and water ad libitum. Animals in groups B, C, and D, however, received pyridoxine hydrochloride by stomach tube, commencing the first day of the experiment, according to the following schedule: group B, 5 μg daily; group C, 10 μg every other day; group D, 20 μg every 4 days. The animals were weighed daily and, at the end of the 16-day growth period, blood samples were taken for determination of serum transaminase activity. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) were measured by the method of Reitman and Frankel (5) with commercial reagents.

In experiment 2, the animals were fed the same basal diet as in experiment 1, except that it contained adequate pyridoxine and calcium pantothenate was omitted. At the end of a 5-week pretest period, the rats were divided into four groups (A–D) as in the pyridoxine study. Each group continued to receive the basal diet and water ad libitum. Calcium pantothenate was given by stomach tube to certain groups, commencing the first day of the experiment, according to the following schedule: group B, 40 μg daily; group C, 80 μg every other day; group D, 160 μg every 4 days. The animals were weighed individually at intervals during the 16-day growth phase of the experiment, at the end of which time their ability to acetylate sulfanilamide was determined. Six selected animals from each group were fasted for 24 hours (water supplied) and given sulfanilamide (250 mg/kg body weight) by intraperitoneal injection. They then were placed in individual metabolic cages with free access to water but not food, and urine was collected for 24 hours. Free and total sulfanilamide were determined in the urine by the method of Bratton and Marshall (6).

Weight gains (Fig. 1) and serum transaminase levels (Table I) were higher in animals given pyridoxine than in those given the basal diet, but the dosing schedule had no significant effect on either parameter. Similarly, the frequency

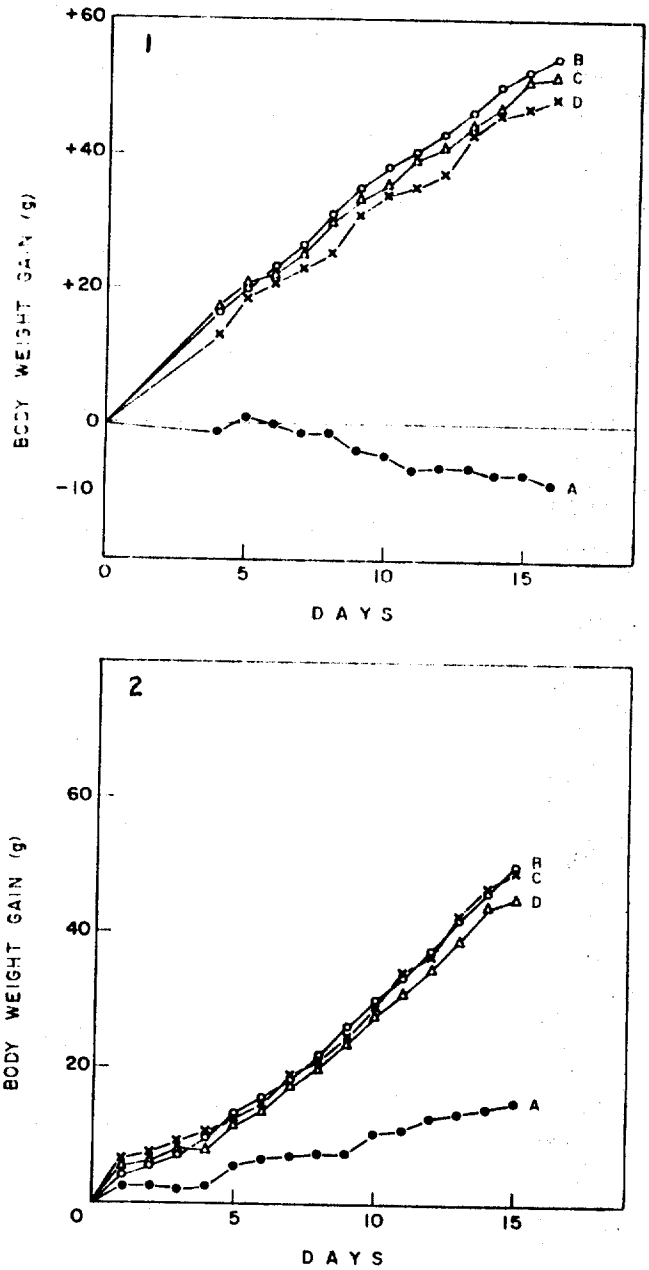

FIG. 1. Effect of pyridoxine given periodically on the growth of pyridoxine-deficient rats. LEGEND: A, no pyridoxine administered; B, 5 μg by stomach tube every day; C, 10 μg by stomach tube every 2 days; D, 20 μg by stomach tube every 4 days.

FIG. 2. Effects of calcium pantothenate given periodically on the growth of pantothenic acid deficient rats. LEGEND: A, no calcium pantothenate administered; B, 40 μg by stomach tube every day; C, 80 μg by stomach tube every 2 days; D, 160 μg by stomach tube every 4 days.

TABLE I

Effect of frequency of administration of pyridoxine on the serum transaminase activities of the rat

Dosage interval (days)	Dose (μg)	SGOT (units/ml serum)	SGPT (units/ml serum)
Depleted	0	111 ± 4*	12 ± 2
1	5	153 ± 7	27 ± 2
2	10	179 ± 27	20 ± 2
4	20	181 ± 27	25 ± 2

*Standard error.

of dosing of pantothenate deficient animals with calcium pantothenate had no effect on weight gains (Fig. 2) or acetylation ability (Table II).

TABLE II

Effect of frequency of administration of calcium pantothenate on the acetylation ability of the rat

Dosage interval (days)	Dose (μg)	Acetylation (%)
Control	0	31.2 ± 1.7*
1	40	38.8 ± 1.4
2	80	38.6 ± 1.6
4	160	38.2 ± 0.6

*Standard error.

The results found with pyridoxine and pantothenate are in marked contrast to those obtained previously with riboflavin and thiamine (1–4). They suggest that both pyridoxine and pantothenate are absorbed readily, and can be utilized efficiently, without wastage.

Acknowledgment

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STUDY OF THE METABOLISM OF PANTOTHENIC ACID IN TUBERCULOSIS AND DURING PROLONGED TREATMENT WITH STREPTOMYCIN

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STUDY OF THE METABOLISM OF PANTOTHENIC ACID IN TUBERCULOSIS AND DURING PROLONGED TREATMENT WITH STREPTOMYCIN

By

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Introduction

It has been well known that the patients with tuberculosis often develop abnormalities of various intermediate metabolism and disturbance of adrenal cortical function as well as numerous side effects during treatment with streptomycin. However, there are few reports on the metabolism of pantothenic acid (PaA) which have close relationships with the factors mentioned above. The authors demonstrated the mechanism of metabolism of pantothenic acid in the patients with tuberculosis and during prolonged treatment with streptomycin. At the same time, they analysed the influence of vitamins on the abnormalities of various intermediate metabolisms and, in addition, attempted to apply the treatment with pantothenic acid to a patient with tuberculosis who developed side effects from streptomycin.

Method of Experiment

For the determination of the capacity of acetylation of the patient with tuberculosis, the patient was prepared by administering 0.5 g of para-aminobenzoic acid (PaBa) per os following discontinuation of chemotherapy for 3 to 5 days, then the patients' urine was collected for a period of 12 hours. To determine the acetylation capacity of animals, the animals were prepared by intraperitoneal injection of 2 mg/100 g body weight of para-aminobenzoic acid; then the animal urine was collected for a period of 24 hours. Both human and animal urine were analyzed by the method of Bratton and Marshall

[1].

The determination of glutathione (GSH) was performed by the Kurosawa's iodine titration method [2]. The α-keto acid was analyzed by Shimizu's method [3]. Shimazonoshi's method was used for the separation and determination of the pyruvic acid (PyA) and α-ketoglutaric acid (KGA). The panthothenic acid (PaA) was determined microbiologically by the use of *Lactobacillus arabinosus* 17-5 following the method of Skeggs-Wright [4]. For this determination, *Lactobacillus arabinosus* 17-5, 10,000 (will be called "resistant bacillus" hereafter), which shows resistance to streptomycin compound 10,000 γ, was grown by the method of Suzuki and Tanaka and used.

Histological examinations of the liver, kidneys, the lungs, the spleen and the adrenals were performed with hemotoxylin-eosin staining. Sudan III stain was also used for the examination of the adrenal glands.

In the animal experiment with a prolonged treatment with streptomycin, white rats weighing an average of 100 g were intraperitoneally injected the streptomycin compound at a dose of 5 mg/100 g body weight daily for a period of 6 weeks. The animals of the treatment group were intraperitoneally injected with 1 mg calcium pantothenate (Ca-PaA), 1 mg chondroitin sulfate, 1 mg lipoic acid (LiA), 1.5 mg cocarboxylase (CoC), 5 mg vitamin C and 5,000 units vitamin A per 100 g body weight, daily for a period of 6 weeks. The animals were also injected with 0.1% mercuric chloride in a dose of 0.1 cc per 100 g body weight every other day, a total of four times, in order to increase the toxicity of the streptomycin. Thereafter the same animals were intraperitoneally injected with the streptomycin compound 50 mg per 100 g body weight, daily for a period of 4 weeks. At the same time, these animals were also intraperitoneally injected with Ca-PaA 2 mg and Rindex, 0.1 cc per 100 g body weight, for a period of 4 weeks.

In a case of experimentally-induced tuberculosis, mice weighing about 20 g were used, injected with *Mycobacterium tuberculosis* var. *hominis* $H_{37}Ry$ group into the tail vein. In this experiment, as treatments beginning one week after the injection of the *Mycobacterium tuberculosis*, a small amount (10 γ/g) of the streptomycin compound and a large amount of the streptomycin compound (30 γ/g), as well as streptomycin compound (10 γ/g) together with Ca-PaA (10 γ/g) were subcutaneously injected in the back of the rats, respectively.

Results of the Experiments

I. Pantothenic Acid Metabolism and Intermediate Metabolism in the Rat Undergoing Prolonged Streptomycin Treatment

1. Treated with streptomycin alone and groups of various treatments

(i) Acetylation capacity: As shown in the Fig. 1, this decreases gradually as the number of intervening days increases to 20 days and to 40. In the group of rats which were treated with PaA, the decrease in acetylation capacity was mild while the group of rats which were treated with LiA showed no significant difference of the acetylation capacity in comparison with the control group.

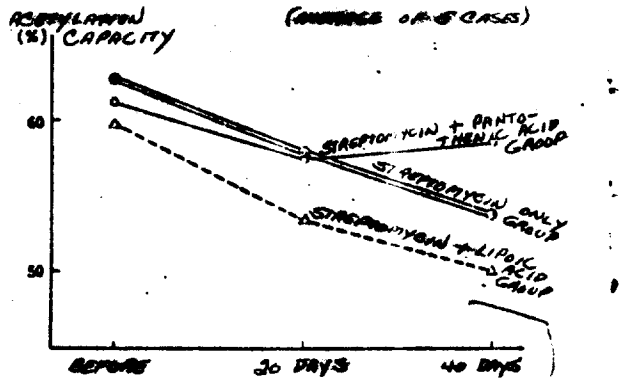


Fig. 1. The acetylation capacity of rats given streptomycin

(ii) α-keto acid metabolism: Both the PyA and KGA of the liver increased in the group of rats given streptomycin, while in the group of rats treated with PaA, they showed only a slight increase. Especially in the group of the rats which were treated with CoC, the tendency to drop to the normal value was most prominent.

(iii) Glutathione (GSH) metabolism: Both total blood glutathione (TSH) and reduced type glutathione (RSH) values were markedly reduced in the group of rats which were given streptomycin, while in the group of the rats which were treated with PaA, they decreased only slightly (Figure 3).

(iv) Amount of PaA in the liver: The amounts of both total and conjugated PaA showed no significant differences in the groups of rats which were given streptomycin alone, and those treated with added vitamin C, vitamin A, and CoC respectively, when these values were compared with those values in the group of normal rats.

(v) Histological findings: The liver and kidneys showed no significant changes. The adrenal glands of the group of rats which were given streptomycin only showed vacuolization of stained cytoplasm of the zona fasciculata. In addition, somewhat increased amounts of lipid granules were seen, but no necrosis was recognized. On the other hand, in the group of rats which were treated with PaA, the above-noted findings were almost absent or very mild if present. And in the group of rats treated with LiA, the histological disturbances of the adrenal glands were relatively mild.

2. *The groups of rats given mercuric chloride (corrosive sublimate: $HgCl_2$) and streptomycin combined and the groups of rats with additional various treatments:*

(i) α-keto acid metabolism: The amount of PyA in the liver markedly increased in the group of rats which were given both mercuric chloride and

streptomycin. In this study, there was no significant difference in the groups of rats treated with PaA and with Rindex respectively. While in the group of rats which were treated with desoxystreptomycin (DOSM) the amount of PyA of the liver showed only mild increase. The amount of KGA of the liver showed a marked increase in the group of rats which were given both mercuric chloride and streptomycin, but it showed a relatively mild increase in the group of rats which were treated with additional PaA (Figure 4).

(ii) Glutathione metabolism: The amounts of TSH and RSH were markedly reduced in the group of rats which were given mercuric chloride and streptomycin but the decrease in the amount of RSH was relatively mild in the group of rats which were treated with desoxystreptomycin (Figure 5).

(iii) The value of pantothenic acid of the liver: In the group of rats which were given mercuric chloride and streptomycin, there were some cases showing marked reduction of the amounts in both total and conjugated pantothenic acid. These animals also showed a significant histological disturbance of the liver tissue. On the other hand, the amount of pantothenic acid was not significantly reduced in the group of rats which were treated with pantothenic acid, while in the group of rats which were treated with Rindex, there was a tendency to increase the amount of pantothenic acid (Figure 6).

(iv) Histological findings: In all groups of rats, the kidney showed cloudy swelling of the lining epithelial cells of the proximal convuluted tubules. The liver revealed fatty degeneration and vacuolization of the cytoplasm of hepatic cells, and yellow atrophy in the central zone of the lobules. However, these changes were most prominent in the group of rats which were treated with mercuric chloride and streptomycin.

The adrenal glands showed increased lipid granules and vacuolization of the cytoplasm of the cortical cells in the zona fasciculata in the group

of rats which were given mercuric chloride and streptomycin. As opposed to this, in the group of rats which were treated with pantothenic acid, the increase of lipid granules was mild. On the other hand, in the group of rats which were treated with Rindex, the increase of lipid granules was prominent when compared with that seen in the control group (histological example).

II. The Metabolism of Pantothenic Acid of Tuberculosis Patients

1. Acetylation Capacity: Many patients with tuberculosis showed a decreased acetylation capacity. A trend of decreased capacity was prominent in the patients who complained of a ringing in the ears.

2. Volume of Pantothenic Acid Urinary Excretion: The patients with pulmonary tuberculosis (41 cases) averaged 3.8 mg urinary excretion of pantothenic acid per day (from 1.2 to 8.1 mg). This is somewhat low in comparison with the average 4.7 mg daily urinary pantothenic acid excretion (from 0.4 to 16.7 mg) in normal healthy individuals. The increased amount of the six-hour urinary excretion of PaA in the patients after intramuscular injection of 20 mg of calcium pantothenate was 4.45 mg (from 2.6 to 9.0 mg). This value did not differ much from the average 4.5 mg or urinary excretion of Ca-PaA (from

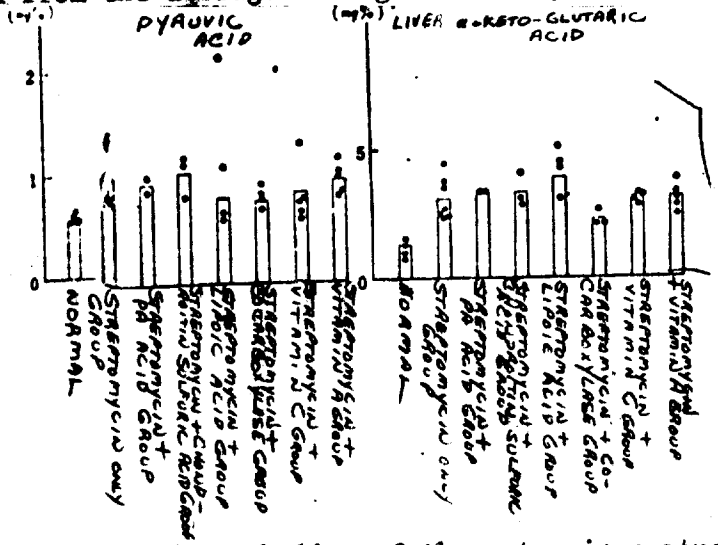


Fig. 2. α-keto acid metabolism of the rats given streptomycin and the effects of various drug compounds on this metabolism

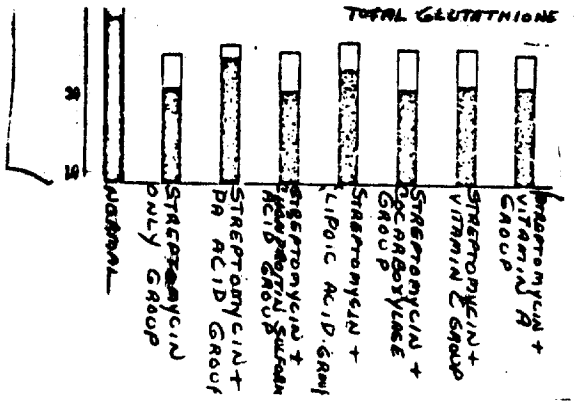


Fig. 3. Blood glutathione metabolism of rats given streptomycin and the effects of the treatment with various drug compounds on metabolism

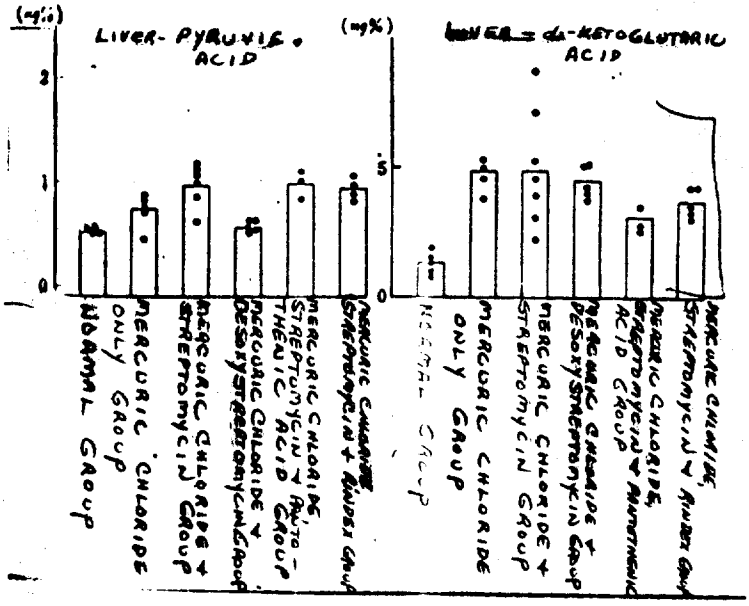


Fig. 4. α-keto acid metabolism of the rats given mercuric chloride and streptomycin and the effects of the treatments with various drug compounds on its metabolism

2.9 to 7.1 mg) in normal healthy individuals; although some patients showed a lower value of the urinary excretion of CaPaA while in some cases showed a markedly increased value of the urinary excretion of this metabolite.

3. Urinary excretion volume of PaA and type of disease. The volume of urinary PaA excretion was low in many patients with the fibro-caseous type of tuberculosis. The urinary excretion volume of PaA obtained after loading

the patients with Ca-PaA tended to be low among patients with both infiltrative and fibro-caseous tuberculosis; although some of the patients with exudative and fibro-caseous tuberculosis displayed markedly increased amounts of urinary excretion of PaA in comparison with those of normal healthy individuals. Those patients who showed increased urinary excretion of PaA were believed to be clinically serious cases with widespread foci of the disease (Figure 7).

4. *Urinary excretion volume of PaA and clinical symptoms:* The average amount of urinary excretion of PaA of 23 patients, who had undergone chemotherapy for a period of less than 6 months, was 4.02 mg; that of 9 patients who had undergone chemotherapy for a period from 6 months to 1 year was 4.78 mg; that of 9 patients who had undergone chemotherapy for a period of more than 1 year was 2.75 mg. The average increase in the urinary excretion volume of PaA after loading with Ca-PaA by patients who had undergone chemotherapy for a period of less than 6 months was 6.95 mg; that of the patients on chemotherapy for a period from 6 months to 1 year was 5.47 mg; and that of the patients on chemotherapy for a period of more than 1 year was 3.03 mg. Both pre- and post-loading amounts of urinary excretion of PaA showed some decrease in the cases in which the patients received chemotherapy for a period of more than 1 year. The relationship between the erythrocyte sedimentation rates and the amounts of urinary excretion of Ca-PaA were as follows: 27 patients, who had an erythrocyte sedimentation rate of less than 10 mm in 1 hour, had an average of 4.4 mg of urinary excretion of PaA; 7 patients, who had an erythrocyte sedimentation rate from 11 mm to 20 mm in 1 hour, had an average of 4.5 mg urinary excretion of PaA; 4 patients, who had an erythrocyte sedimentation rate from 20 mm to 50 mm in 1 hour, had an average of 7.2 mg urinary excretion of PaA; 2 patients, who had an

erythrocyte sedimentation rate of more than 50 mm in 1 hour, had an average of 8 mg urinary excretion of PaA. Therefore, there was a trend for the patients who had an erythrocyte sedimentation rate of more than 20 mm to show a marked increase in urinary excretion of PaA after the patients were loaded with Ca-PaA.

5. *Amount of the urinary excretion and SM side effects:* The 15 patients who complained of ringing in the ears and poor hearing had an average of 3.16 mg urinary excretion of PaA, and an average of 3.46 mg increased urinary excretion of PaA after loading with Ca-PaA. On the other hand, the 14 patients who were free of side effects had an average of 4.09 mg of pre-loading urinary excretion of PaA and an average of 4.24 mg of post-loading urinary excretion of PaA. The 14 patients who complained of side effects other than ringing in the ears, poor hearing, and an obstructed feeling in the ear had average pre- and post-loading urinary excretion of PaA of 4.34 mg and 5.54 mg respectively (Figure 8).

6. *Urinary excretion volume of PaA and the BSP test:* 9 out of 30 patients had an abnormal BSP test result with more than 5% retention at 45 minutes (the disturbance test was 30%). When compared with the amounts of urinary excretion of PaA and BSP retention rate, there was a tendency for the patient with a high abnormal rate of BSP retention to have a decreased amount of urinary excretion of PaA (Figure 9).

III. PaA Metabolism of Mice with Experimental Tuberculosis

The total amount of PaA in the liver of 9 healthy mice was 70 γ/g on the average (54~90), while the total amount of PaA in the liver of 17 rats with experimental tuberculosis averaged 45.9 γ/g (23~64), 3 weeks from the day of the injection of the *Bacillus* tuberculosis showing a remarkably small value relative to the healthy ones. On the other hand, the value for the

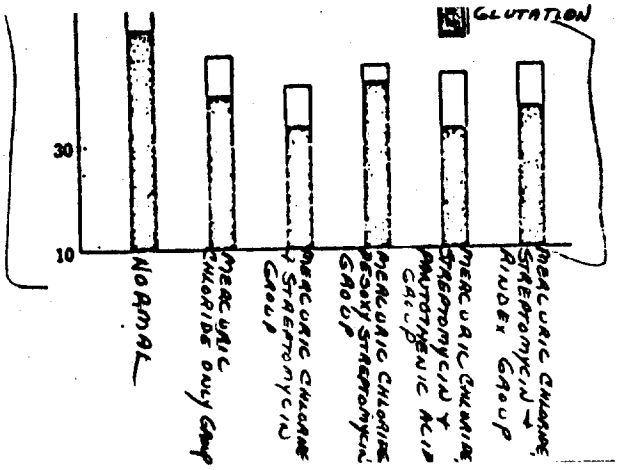


Fig. 5. The (blood) glutathione metabolism of rats given mercuric chloride and streptomycin and the effects of treatment with various drug compounds on metabolism.

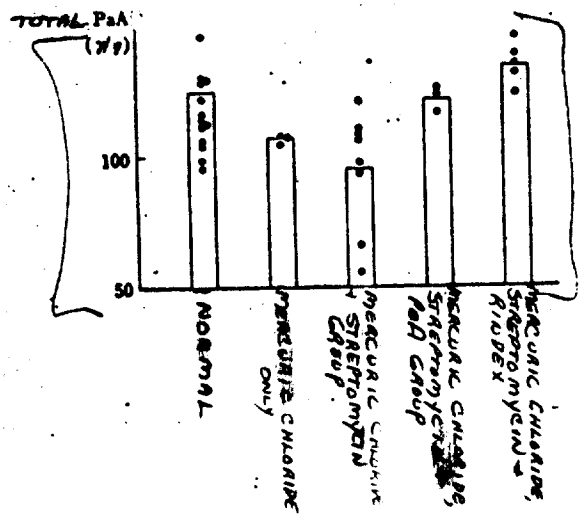


Fig. 6. The amount of pantothenic acid in the liver of rats given mercuric chloride and streptomycin and the effects of various drug compounds.

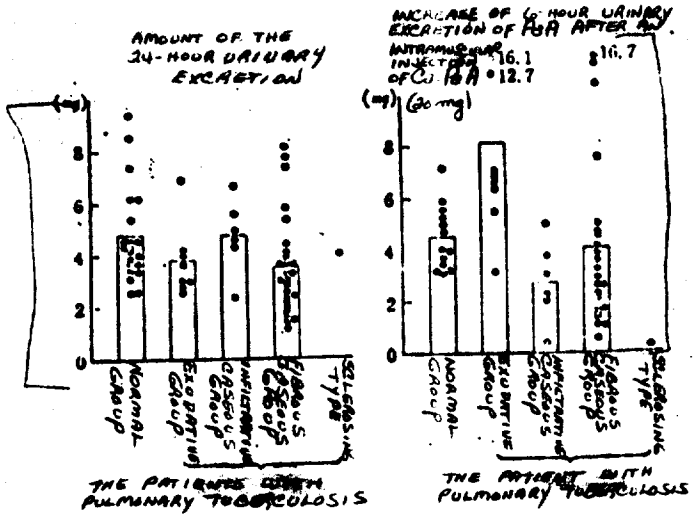
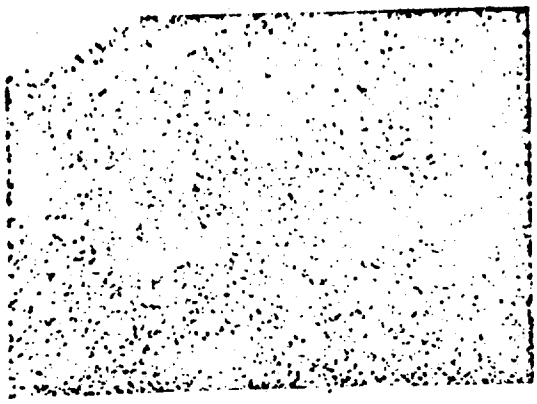
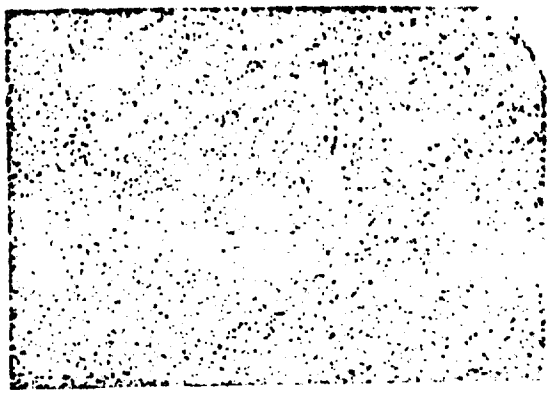


Fig. 7. Amount of urinary excretion of pantothenic acid of patients with pulmonary tuberculosis and the types of the disease.

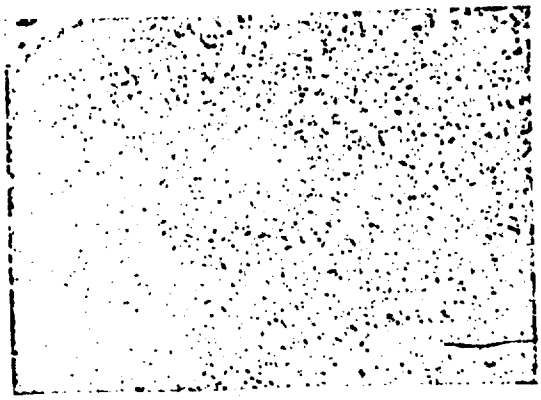
Adrenal Cortex (Sudan III Stain)



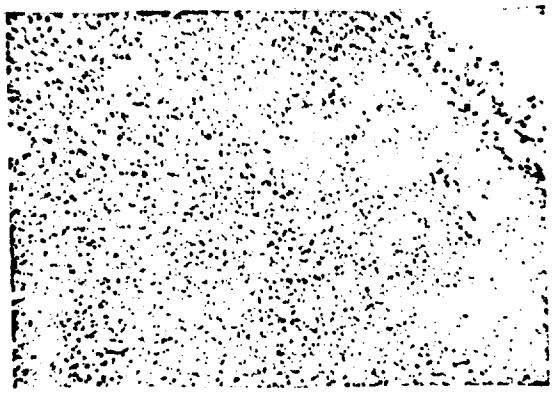
A. *Normal*



B. *Mercuric chloride and streptomycin group*



C. *Mercuric chloride, streptomycin, and PaA group*



D. *Mercuric chloride, streptomycin, and Rindex group*

large dose (30 γ/g) SM treatment group (14 cases) was 41.4 (28-64); that for the small dose (10 γ/g) SM treatment group (4 cases) was 35.9 (32-40); and the value for the SM (10 γ/g) and PaA (10 γ/g) treatment group (8 cases) was 37.4 (31-51), with each group showing only a small difference from the control groups (Figure 10).

IV. Therapeutic Effect of PaA on SM Side Effects

Of the 138 tuberculosis patients under SM treatment, there were 97 cases with side effects in some form or other (including temporary forms - 70%

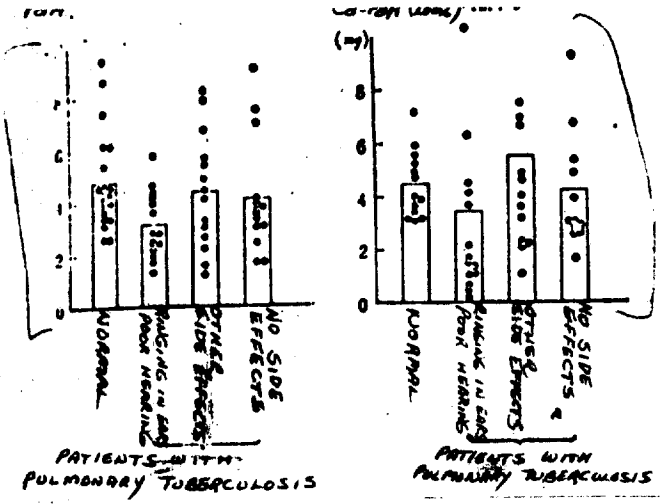


Fig. 8. Amount of the urinary excretion of pantothenic acid of patients with pulmonary tuberculosis and side effects during streptomycin administration

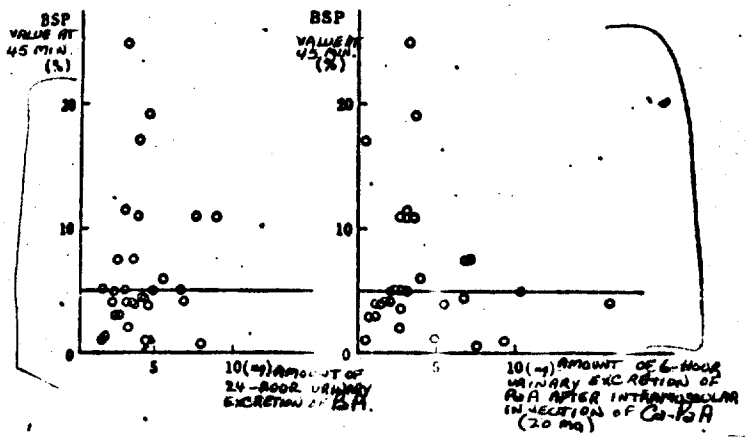


Fig. 9. Correlation between urinary excretion of pantothenic acid and BSP.

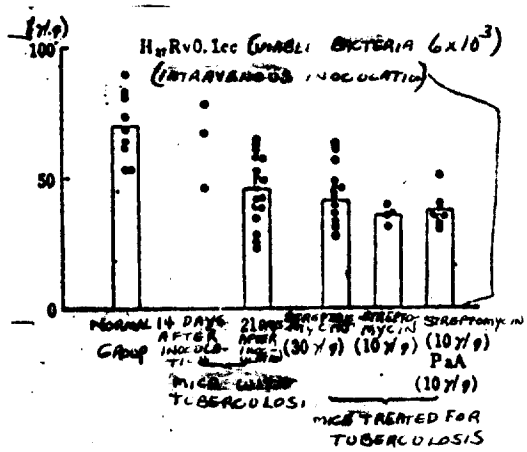


Fig. 10. Amount of pantothenic acid in the liver of mice with experimental tuberculosis.

incidence). The most common side effect was the numbness on the face and lips (27.7%); following this was headache and a heavy feeling in the head (22.5%), then ringing in the ears, hearing difficulty and a blocked-up feeling in the ear (15%). Intramuscular injections of Ca-PaA (40 mg) to these patients relieved these side effects in a large number of cases (Table 1). Dr. Ashida of this hospital gave intramuscular or intravenous injections in a quantity of 50-100 mg for a period of one month to the patients with hearing difficulty and with the ringing in the ears, and saw improvement in 8 cases out of the 10 with ringing in the ears, and in 6 of the 8 cases with hearing difficulty. In the cases involving malfunctioning of the liver, the rate of improvement was better. Furthermore, to the 3 elderly tuberculosis patients in whom disturbances of the adrenal cortical function had been recognized as a result of the Sown test after the ACTH-Z injection and in the urine steroid value, the intravenous injections of dexpanthenol (40 mg) were administered for 2 weeks. As a result, improvements were recognized in 2 cases.

V. Therapeutic Effect on the Side Effect of Desoxystreptomycin

DOSM was used on 44 tuberculosis patients who complained of the side effects from the use of SM. In these, the side effects were found to be reduced in 38 cases. The results of using DOSM along with PaA were as follows: Of the 11 cases with numbness, 4 cases showed a remarkable improvement (disappearance of the side effect); in 7 cases, some effect was recognized (alleviation of the side effect); and there was no difference in the remaining 1 case. Of the 9 cases of headache and heaviness sensation in the head, 4 cases showed a remarkable improvement; some improvement was seen in 4 cases; no difference in 1 case and in 1 case the complaint became worse. For the ringing in the ears, none of the cases showed remarkable improvement;

some improvement was found in 4 cases; there was no difference in 1 case; and there was no case in which the complaint worsened.

Table 1. The effects of the administration of calcium pantothenate on the side effects of streptomycin
(Intramuscular injection of 40 mg streptomycin for 1 month)

Types of side effects	Effects		
Skin rash, headache, nausea, vomiting (vitamin reaction)	11 cases	marked improvement	8 cases
		improvement	3 cases
		no improvement	0 cases
Vestibular and optic nerve disturbance (nervous disturbance)	22 cases	marked improvement	7 cases
		improvement	11 cases
		no improvement	3 cases
		deterioration	2 cases
Fever, arthralgia, asthma, skin change anaphylaxis, blood profile change (allergic reaction)	14 cases	marked improvement	11 cases
		improvement	2 cases
		no improvement	1 case

VI. Effects of PaA on Growth of SM-Resistant Tubercule Bacillus

The SM-resistant tubercule bacillus was cultured for a period of 4 weeks by adding SM and Ca-PaA of various concentrations to the 3% Ogawa culture medium.

As can be seen from the result in the Table, while almost no notable differences were found in 8 cases out of the 10 from the addition of Ca-PaA, a slight tendency of the growth suppression was observed in 2 cases (Table 2).

Table 2. The effects of streptomycin and calcium pantothenate on streptomycin-resistant Mycobacterium tuberculosis

(grown on 3% OGAWA medium for 4 weeks)

Patients	Streptomycin (γ)	Number of colonies	Streptomycin (γ) + Calcium pantothenate (γ)		Number of colonies
A	0						
	10	++	10	10	++
	100	+	100	50	++
			"	100	+
			"	200	++
B	0						
	10					10	10				
	100	++	100	50	|||
			"	100	+
			"	200	|||
C	0						
	10					10	10				
	100					100	50				
			"	100	|||
			"	200	|||
D	0						
	10					10	10				
	100	|||	100	50	++
			"	100	++
			"	200	++
E	0						
	10	++	10	10	++
	100	++	100	50	+
			"	100	+
			"	200	+
F	0						
	10	+	10	10	+
	100	+	100	50	−
			"	100	+
			"	200	−
G	0	++			
	10	++	10	10	++
	100	++	100	50	+
			"	100	+
			"	200	+
H	0						
	10	++	10	10	|||
	100	+	100	50	+
			"	100	−
			"	200	+
I	0						
	10	++	10	10	+
	100	−	100	50	+
			"	100	+
			"	200	−
J	0						
	10					10	10				
	100					100	50				
			"	100	|||
			"	200	|||

Summary and Discussion

There remain many unclear points in the relations between the disturbances of various intermediate metabolisms and of adrenal cortical function on the one hand, and the metabolisms of vitamins, especially of PaA, on the other. At the same time, there are only a few reports on the behavior of PaA when SM is administered for a long period of time. In this research, the metabolism of PaA in the tuberculosis patient and during the period of SM administration were investigated using *Lactobacillus arabinosus 17-5 10,000*; at the same time the role of PaA in the disturbance of various types of metabolisms was examined.

In the measurement of PaA, it can be considered that an error tends to be produced in the obtained values when SM is contained in the sample, due to the microbiological method using mainly *Lactobacillus arabinosus* 17-5. As a result of our investigations from many angles using SM-resistant lactic-acid bacteria grown according to the method developed by Suzuki and Tanaka, we succeeded in obtaining the roughly satisfactory values and confirmed that it is possible to perform measurements with this bacillus.

First, on tuberculosis patients, a PaA loading test was conducted. This proved that there are disturbances of the PaA metabolism in many cases in these patients. In other words, these patients often suffered from PaA deficiency; and, in serious cases, a trend suggesting a drop in PaA utilization capacity was recognized. This can be considered as similar to the condition of PaA metabolism in cases of liver impairment which the authors already had clarified [8]. In view of these results, the BSP test was conducted in order to clarify the role of the liver malfunction in the disturbance of PaA metabolism in the tuberculosis patients. In many tuberculosis patients, disturbance in the liver function tends to co-exist. The BSP test

is said to be most sensitive in such cases [9]. According to our results, the patients with an abnormality in the BSP value (45 minutes) that is higher than 5% comprised the 30% of the total. This result is in approximate agreement with the report by Overrath [9].

In this case, many of the patients with extremely abnormal BSP values were found to be low in the amount of PaA excreted in the urine. On the basis of such results, it is believed that the involvement of impaired liver function should be taken into consideration in the appearance of the disturbance in PaA metabolism.

The amount of PaA in the liver of the experimental tuberculous rats was extremely small relative to that of the healthy group. There was not much difference in the values of the SM treatment group and the SM-PaA treatment group compared with the control group. There is a possibility that SM administration suppresses the synthesis of PaA by intestinal bacteria, and this is considered to be one of the reasons for very small difference compared to the control group. In fact, there is empirical evidence by Lichstein [10] that SM administration tends to induce PaA deficiency.

It is common knowledge that various side effects are produced by the use of SM, and that various drugs are used as countermeasures. We attempted the therapeutic application of PaA on the patients with SM side effects and found improvements in many cases as stated above. These findings are in approximate agreement with the results obtained by Keller [11], Ducrot [12], and Kimmerle [13]. In the rats to which SM was administered for a long period of time, a decrease in the amount of PaA in the liver, disturbances in the α-keto acid metabolism and glutathione metabolism of the liver, a drop in acetylation, and patterns of tissue impairment in the liver and adrenal cortex were recognized. In these cases, the administration of PaA, CoC, etc. were

effective to a certain extent. Morita indicates that the impairment in hearing due to SM is reinforced by the concurrence of impairments of the liver or kidney [14]. The authors also confirmed that when impairment of the kidney is induced beforehand with corrosive sublimate in the animal to which SM is administered, the disturbances of the various metabolisms are reinforced more markedly than in the group to which only SM is administered.

Tachikawa reports histological impairments of the liver and spleen, a decrease in the catalose of the liver, a decrease in the esterase and peri-oxidase of the liver, and the reinforcement of alkaline phosphatase [15].

Nakamura believes the disturbance of carbohydrate metabolism is responsible for the disturbances in the inner ear and acoustic nerve center and holds that the restoration of glycolytic respiration is an appropriate step to take for the prevention and treatment of this symptom, and he recommends the use of vitamins be included in this [16], and he has proven the effectiveness of PaA in such cases. Also, Fukui confirms the effectiveness of PaA in the prevention of impairments of the auditory organs from the experimental result on animals [17]. There are many points yet to be clarified with regard to the etiology of the side effects of SM. Keller [11] and Kimmerle [13] consider SM side effects and Ca-ion deficiency as being related. The authors made it clear that the administration of SM for a long period of time causes the tissue impairment in the cortices of the liver and of the adrenal gland, disturbances in various metabolisms, PaA deficiency, etc. on the basis of the results from animal experiments. It is possible that such functional disturbances play some kind of role in the appearance of the SM side effects. It can be considered that the administration of PaA acts effectively in the prevention or the mitigation of SM toxicity and its side effects by improvement of the various functional disorders mentioned

above. In the meantime, the fact that DOSM causes less side effects than SM has been already indicated by several investigators. As stated above, we confirmed this fact in the tuberculosis patients. This is of interest in that it was also confirmed by our experimental results which showed that disturbances in various metabolisms in rats to which DOSM was administered for a long period were less pronounced than those in rats to which SM was administered.

According to Fernando [18], the use of a drug containing equal amounts of SM-pantothenate and DSM-pantothenate instead of SM on patients in whom the combined use of SM, para-aminosalicylic acid, and hydrazide had produced no favorable effects, resulted in not only in the improvement of the subjective symptoms but also in the objective symptoms, especially in the X-ray picture in more than half the cases. It is also reported that this drug is effective for some strains of *Mycobacterium tuberculosis* that are resistant to SM according to *in vitro* tests. In our investigations similar to this with the 10 cases of SM-resistant tubercular bacilli, no case was found in which clear suppression of growth was achieved with the PaA addition.

Conclusion

1) In rats, which were administered compound streptomycin for a period of 6 weeks, there was a decrease in the acetylation capacity , abnormal metabolisms of the hepatic α-keto acid and glutathione, and a mildly disturbed histology in the cortical tissue of the adrenal glands. The treatment with PaA or CoC produced some improvement in the rats.

2) In rats, which were administered compound streptomycin for a period of 4 weeks, following the injection of mercuric chloride, the abnormalities of the various metabolisms mentioned above, were prominent. There was a decrease in the hepatic total as well as the conjugated PaA. In this

experiment, the treatment with PaA produced some benefit in the rats. In the rats, which were administered oxystreptomycin, the disturbances of the hepatic α-keto acid and glutathione metabolisms were mild in comparison with those in rats which were administered compound streptomycin.

3) Among the tuberculosis patients, many were in a condition which can be considered to be PaA deficiency. And among the serious cases, there were cases which suggest a decrease in the utilization capacity. On the other hand, in many of the patients whose BSP values were abnormal, cases of small amounts of PaA excretion in the urine were found.

4) In many of the tuberculous rats injected with strain $H_{37}Rv$, a drastic decrease in the amount of PaA in the liver was found. In this case, no large difference was found in the group treated with compound SM.

5) PaA was therapeutically applied to tuberculosis patients with SM side effects, and in a large number of cases, the disappearance or mitigation of the side effects was seen.

6) With the DOSM administration, the side effects were reduced compared to those brought about by compound SM. PaA was also found to be effective in the treatment of DOSM side effects.

In closing, our deeply felt gratitude is expressed to Prof. K. Ooji, of the Dept. of Yoshida Internal Medicine, Osaka University, and Dr. S. Tomokuni of Meizi Hospital for their attentive guidance throughout. Also thanks are expressed here to Dr. Nomura of the Dept. of Donomae Internal Medicine, Osaka University, for his assistance in the experimental development of tuberculosis.

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結核症ならびにストマイ長期投与時のパントテン酸代謝に関する研究

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緒言

結核症患者ではしばしば諸種中間代謝異常ならびに副腎皮質機能障害等が招来され，またストマイ（SM）投与時各種副作用のみられることは周知のところである。しかしながらこれらと密接な関連を有するパントテン酸（PaA）代謝に関する報告はきわめて少ない。われわれは結核症ならびにSM長期投与時におけるPaA代謝様相を明らかにするとともに，このさいみられる諸種中間代謝異常に及ぼすビタミン等投与の影響につき検討し，さらにSM副作用を示した結核症患者にPaAの治療的応用を試みた。

実験方法

結核症患者のアセチル化能は化学療法を3～5日間中止してパラアミノ安息香酸（PABA）0.5 g内服後の12時間尿を，また動物のアセチル化能はPABA 2 mg/100g腹腔内注射後の24時間尿をそれぞれ用いBratton-Marschallの方法[1]により測定した。

グルタチオン（GSH）測定は黒岩氏沃度滴定法[2]，α-ケト酸は清水・田中氏法[3]により焦性ブドウ酸（PyA），α-ケトグルタール酸（KGA）を分離測定した。PaA測定はSkeggs-Wright[4]の方法に準じLactobacillus arabinosus 17-5を用い微生物学的に行なつた。このさい鈴木・田中氏法[1]により複合SM 10,000 γに感性を示すLact. arab. 17-5，10000（以下感性菌と略す）を作製しこの菌を用いた。肝，腎，肺，脾の組織学的検索にはヘマトキシリンエオジン染色を，副腎はさらにズダンIII染色もあわせ行なつた。

SM長期投与実験は体重100 g前後の白鼠に複合SM 5 mg/100g，6週間連日腹腔内注射した。治療群として体重100 g宛パントテン酸カルシウム（Ca-PaA）1 mg，コンドロイチン硫酸1 mg，リボ酸(LiA)1 mg，Cocarboxylase (CoC) 1.5 mg，VC 5 mg，VA 5,000単位をそれぞれ6週間腹腔内注射した。さらにSM中毒を増強する目的で0.1 %昇汞0.1 cc/100 g宛隔日に4回腹腔内注射し，その後複合SM 50 mg/100 gを4週間連日腹腔内注射した。このさい治療群として体重100 g宛Ca-PaA 2 mg，Rindex 0.1 ccを同様4週間腹腔内注射した。

結核の実験的発症には体重20 g前後の甘日鼠を用い，人型結核菌 $H_{37}Rv$ 株を尾静脈より注射した。このさい治療として結核菌注射後1週間目より複合SM少量（10 γ/g）および大量（30 γ/g），ならびに複合SM（10 γ/g）Ca-PaA（10 γ/g）の併用をそれぞれ背部皮下に注射した。

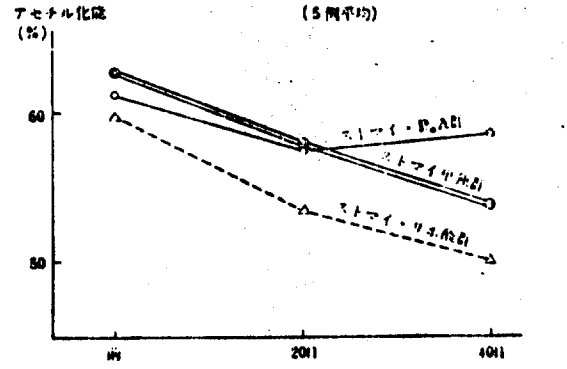
実験成績

I　SM長期投与鼠のPaA代謝ならびに中間代謝

1.　SM単独投与および各種治療群

i）アセチル化能：図1のごとく20日，40日と経日的に漸次下降した。PaA治療群では低下程度であつたがLiA群では対照と大差をみなかつた。

図1　ストマイ投与ネズミのアセチル化能



ii）α-ケト酸代謝：肝PyA，KGAはSM投与でいずれも上昇したが，治療群ではいずれも上昇程度であつた。とくにCoC群で低下正常化傾向がもつとも顕著であつた（図2）。

iii）グルタチオン（GSH）代謝：血液型（TSH）および還元型グルタチオン（RSH）ともにSM群で著し

図 2　ストマイ投与ネズミの α-ケト酸代謝とこれに及ぼす諸種薬剤投与の影響

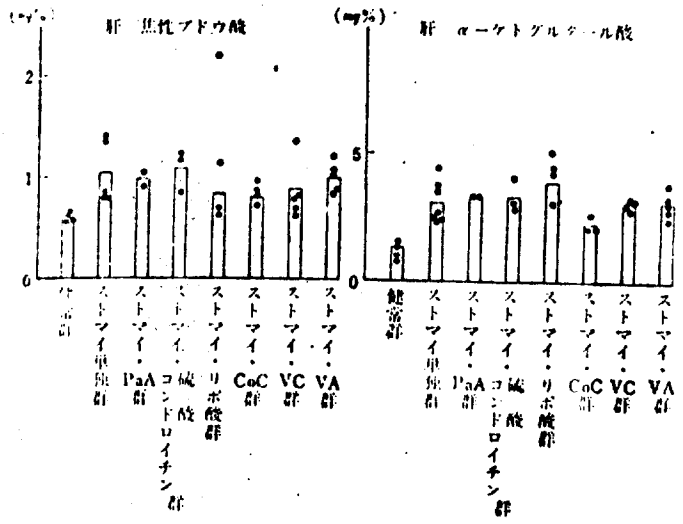


図 3　ストマイ投与ネズミの血液グルタチオン代謝とこれに及ぼす諸種薬剤投与の影響

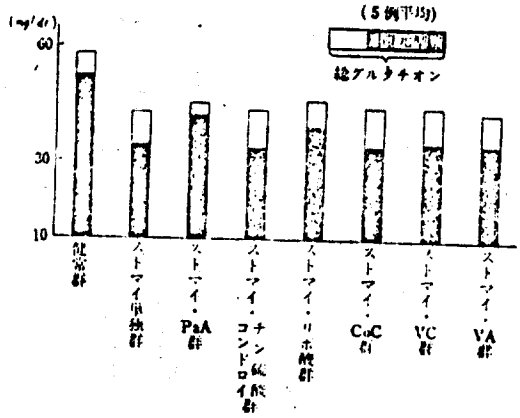
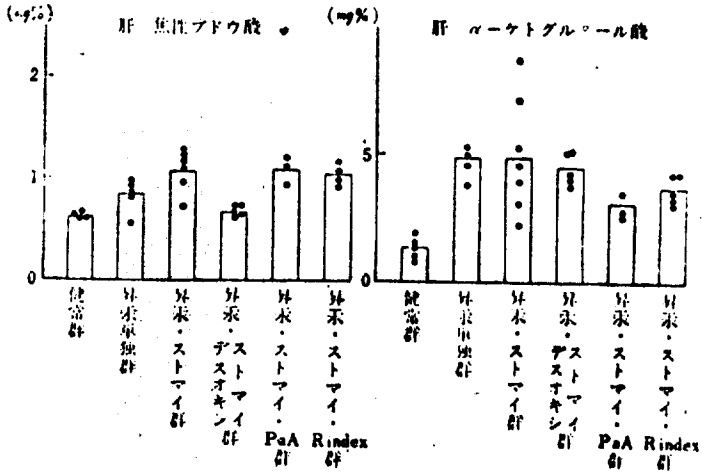


図 4　昇汞・ストマイ投与ネズミの α-ケト酸代謝とこれに及ぼす諸種薬剤投与の影響



く低下したが，PaA 治療群では RSH の低下が比較的軽度であつた（図 3）。

iv）肝 PaA 量：肝臓および結合型PaA量は SM 群および VC，VA，CoC 各治療群でいずれも健常群と大差を認めなかつた。

v）組織所見：肝および腎は著変を認めず，副腎は SM 単独群で束状層の原形質は淡染し空胞化を認め，さらに脂肪顆粒のやや増加を示す例がみられたが壊死は認めなかつた。一方 PaA 治療群では上記障害はほとんど認めないかまたはきわめて軽度であつた。また LiA 治療群でも副腎障害像は比較的軽度であつた。

2. 昇汞・SM 併用投与および各種治療群

i）α-ケト酸代謝：肝 PyA は昇汞・SM 群で著しく上昇した。このさい PaA および Rindex 治療群でもこれと大差をみなかつた。

一方デスオキシストマイ（DOSM）群では上昇はきわめて軽度であつた。肝 KGA も昇汞・SM 群で著明に上昇したが PaA 治療群では上昇が比較的軽度であつた（図 4）。

ii）グルタチオン代謝：昇汞・SM 群では TSH，RSH ともに著しく低下したが，DOSM 群では RSH の低下が比較的軽度であつた（図 5）。

iii）肝 PaA 量：昇汞・SM 群で総および結合型PaA量の著減する例がみられたが，このさい肝組織障害の著しい例に低下顕著であつた。

これに対し PaA 治療群では減少ほとんどみられず，また Rindex 治療群ではかえつて増加傾向を示した（図 6）。

iv）組織所見：腎は各群いずれも細尿管主部の濁腫脹がみられた。肝では原形質の脂肪変性ならびに空胞化，中心帯の萎縮等を認めたが，昇汞・SM 群で変化もつとも顕著であつた。

副腎では皮質束状層の脂肪顆粒の増加，空胞化等がみられた。これに対し PaA 治療群では脂肪顆粒の増加軽度であつた。一方 Rindex 治療群では対照群に比しさらに脂肪顆粒の増加顕著であつた（組織標本）。

II　結核患者の PaA 代謝

1. アセチル化能：肺結核患者のアセチル化能は低値を示す例が少なくなく，とくに喀血を訴える例に低下傾向が著明であつた。

図 5　昇汞・ストマイ投与ネズミのグルタチオン代謝とこれに及ぼす諸種薬剤投与の影響

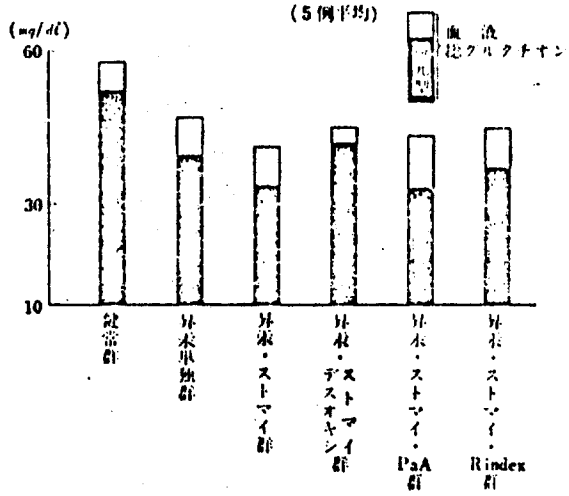


図 6　昇汞・ストマイ投与ネズミの肝パントテン酸ならびにこれに及ぼす諸種薬剤投与の影響

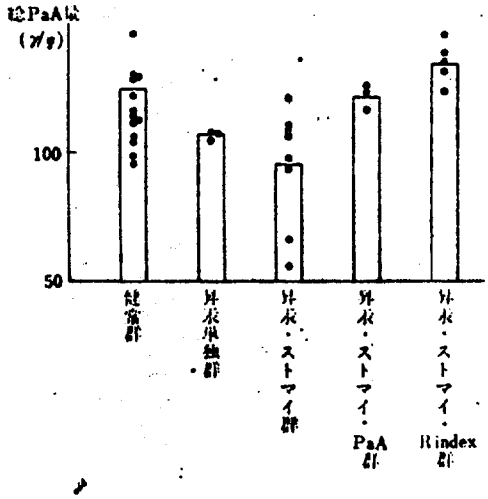
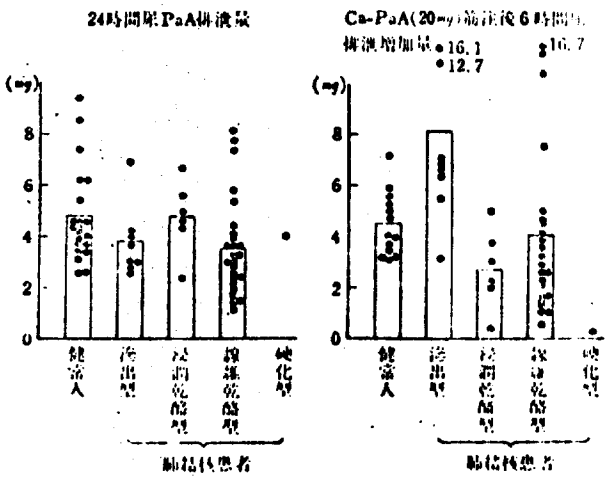


図 7　肺結核患者の尿パントテン酸排出量と病型



2.　尿 PaA 排泄量：肺結核患者（41 例）の 1 日尿 PaA 排泄量は平均 3.8 *mg*（1.2～8.1）で，健常人〔4.7 *mg*（2.6～9.0）〕に比しやや低値を示した。また Ca-PaA 20 *mg* 筋注後 6 時間尿 PaA 排泄増加量は 4.45 *mg*（0.4～16.7）で，健常人〔4.5 *mg*（2.9～7.1）〕と大差をみなかつたが症例により低値を示す例と逆に著しく高値を示す例がみられた。

3.　尿 PaA 排泄量と病型：尿 PaA 排泄量は線維乾酪型患者で低下例が多かつた。また負荷後排泄増加量は浸潤ならびに線維乾酪型患者で低下傾向を認めたが，滲出型および線維乾酪型患者の一部では健常人に比し著増を示す例がみられた。かかる例は病巣が広汎で臨床的に重症と考えられる例に多かつた（図 7）。

胸部レ線写真上空洞の有無と尿 PaA 排泄量との間にはとくに著明な関係はみられなかつた。

4.　尿 PaA 排泄量と臨床症状：尿 PaA 排泄量は化学療法 6 カ月以内（23 例）4.02 *mg*，6 カ月～1 年（9 例）4.78 *mg*，1 年以上（9 例）2.75 *mg*，負荷後排泄増加量は 6 カ月以内 6.95 *mg*，6 カ月～1 年 5.47 *mg*，1 年以上 3.03 *mg* でいずれも 1 年以上持続例にやや低下傾向を認めた。赤沈値と尿 PaA 排泄増加量との関係をみるに赤沈 1 時間値 10 以下（27 例）4.4 *mg*，11～20（7 例）4.5 *mg*，20～50（4 例）7.3 *mg*，50 以上（2 例）8.0 *mg* であつた。すなわち赤沈 20 以上の例では健常人に比し負荷後排泄増加量が著増を示す傾向がみられた。

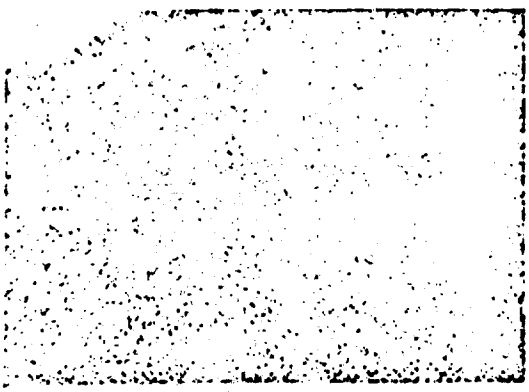
5.　尿 PaA 排泄量と SM 副作用：耳鳴，難聴を認めた 15 例の尿 PaA 排泄量は 3.16 *mg*，負荷後排泄増加量は 3.46 *mg* であつた。他方なんら副作用を認めない 14 例ではそれぞれ 4.09 *mg*，4.24 *mg*，耳鳴，難聴，耳閉塞感以外の副作用を訴えた 14 例では 4.34 *mg*，5.54 *mg* であつた（図 8）。

6.　尿 PaA 排泄量と BSP 試験：BSP（45 分値）5 % 以上の異常者は 30 例中 9 例（陽性率 30 %）であつた。このさい尿 PaA 排泄量と BSP 値との相関関係をみるに，BSP 値の異常著しいものは尿 PaA 排泄量も低い傾向を認めた（図 9）。

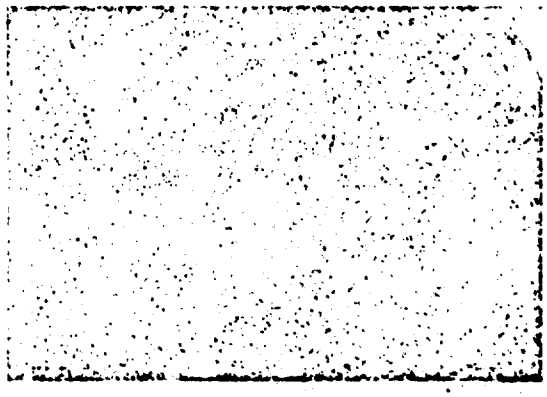
III　実験的結核廿日鼠の PaA 代謝

健常廿日鼠の肝臓 PaA 量（9 例）は平均 70 γ/g（54～90），実験的結核鼠（17 例）の結核菌注射後 3 週間目肝臓 PaA 量は 45.9 γ/g（23～64）で健常鼠に比し著しく低値を示した。他方 SM 大量（30 γ/g）治療群（14 例）41.4（28～64），SM 少量（10 γ/g）治療群（4 例）35.9（32～40），SM（10 γ/g）PaA（10 γ/g）併用治療群（8 例）37.4（31～51）でいずれも対照群と大差をみなかつ

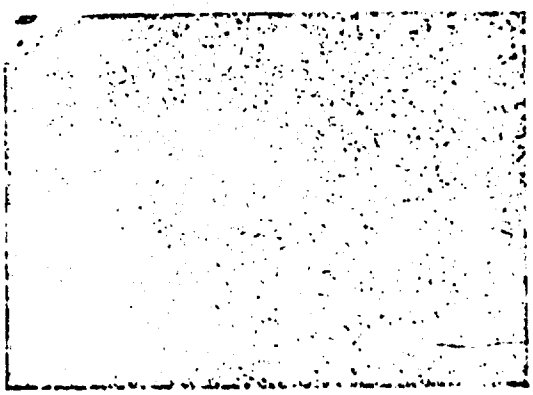
副　腎　皮　質（メダンⅢ染色）



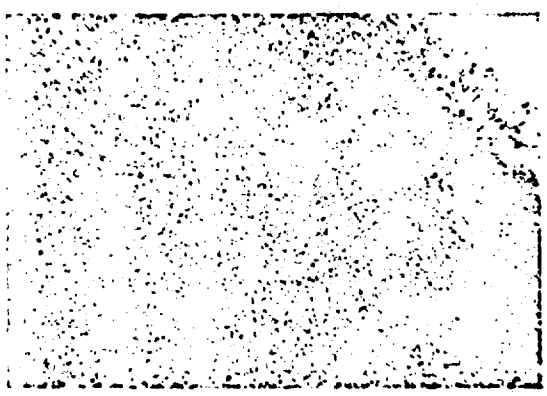
正　常



昇汞・ストマイ群



昇汞・ストマイ・PaA 群



昇汞・ストマイ・Rindex 群

図 8　肺結核患者の尿パントテン酸排泄量とストマイ投与時の副作用

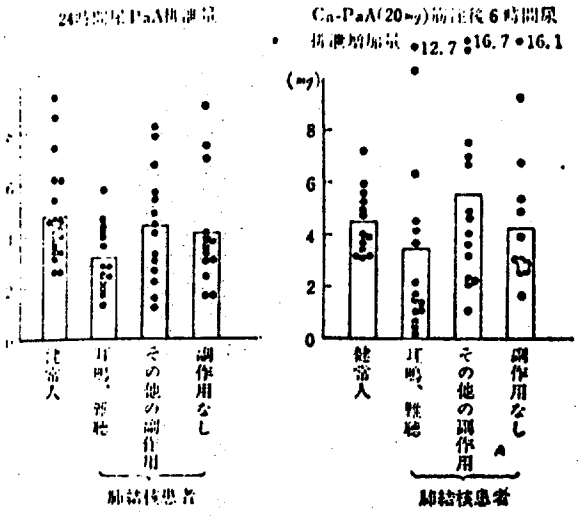


図 9　結核患者の尿パントテン酸排泄量と BSP の相関

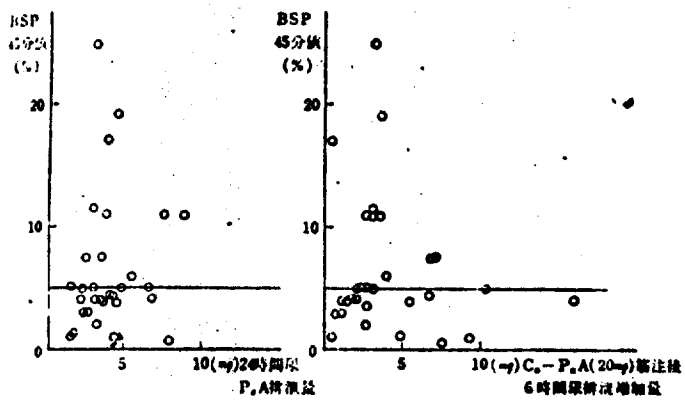
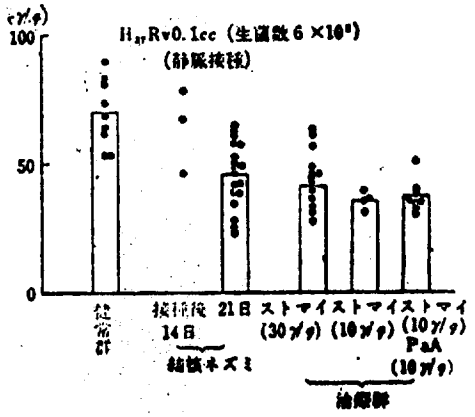


図 10　実験的結核廿日鼠の肝パントテン酸量



た（図 10）。

IV　SM 副作用に及ぼす PaA の治療的効果

SM 治療中の結核症患者138例中，なんらかの副作用を訴えたもの（一過性に出現したものも含む）は 97 例（発現率 70 %）であった。副作用のうちもっとも多いのは注射直後のしびれ感 27.7 %，ついで頭重感，頭痛 22.5 %，耳鳴，難聴，耳閉塞感 15 %等で，これら患者にCa-PaA (40mg) の筋注を行ない多数例に改善傾向を認めた（表 1）。"副作用なし"は耳鳴，難聴例に 50～100 mg 1 カ月間筋注または静脈内注射を行ない耳鳴は 10 例中 8 例に，難聴は 8 例中 6 例にそれぞれ効果を認めている。また肝機能障害を伴う例ではその改善を認める場合が多かった。さらに ACTH-Z 注射後のツーンテストおよび尿ステロイド値より副腎皮質機能異常を認めた 3 例の高令肺結核患者にパンテノール (40 mg) 2 週間筋注し 2 例に改善を認めた。

V　デスオキシストマイ副作用に及ぼす PaA の治療的効果

SM 使用により副作用を訴えた 44 例の肺結核患者にSMの代りに DOSM を用い 38 例において SM に比し副作用の少ない傾向を認めた。このさいDOSM に PaA を併用した結果，しびれ感11例には著効（副作用の消失したもの）を認めたもの 4，有効（軽減したもの）7，不変 1，頭重頭痛 9 例には著効 4，有効 4，不変 0，増悪 1，耳鳴には著効 0，有効 4，不変 1，増悪 0 であった。

VI　SM 耐性結核菌の増殖に及ぼす PaA の影響

3 %小川培地に各種濃度の SM および Ca-PaA を加え，SM 耐性結核菌を

表 1　ストマイ副作用に及ぼすパントテン酸カルシウム投与の影響

(40 mg 1 カ月間筋注)

副作用の種類	効果	
皮膚紅潮・頭痛，悪心，嘔吐（ヒスタミン反応）	11例中	著効 8 例 有効 3 無効 0
聴覚前庭・視神経障害（神経障害）	23例中	著効 7 有効 11 無効 3 増悪 2
発熱，関節痛，喘息，皮膚変化アナフィラキシー，血液像変化（過敏性反応）	14例中	著効 11 有効 2 無効 1

表 2 ストマイ耐性結核菌に対するストマイ・パントテン酸カルシウムの影響
(3% 小川培地 4 週間培養)

姓名	ストマイ (γ)	集落数	ストマイ (γ) + パンカル (γ)	集落数
川○	0	++++		
	10	++	10 10	++
	100	+	100 50	++
			〃 100	+
			〃 200	++
岡○	0	++++		
	10	++++	10 10	++++
	100	++	100 50	++++
			〃 100	+
			〃 200	+++
長○川	0	++++		
	10	++++	10 10	+++
	100	++++	100 50	+++
			〃 100	+++
			〃 200	+++
木○	0	++++		
	10	++++	10 10	++++
	100	++++	100 50	++++
			〃 100	++++
			〃 200	++++
瀬○	0	+++		
	10	++	10 10	++
	100	++	100 50	+
			〃 100	+
			〃 200	+
御○生	0	+++		
	10	+	10 10	+
	100	+	100 50	−
			〃 100	+
			〃 200	−
橋○	0	++		
	10	++	10 10	++
	100	++	100 50	+
			〃 100	+
			〃 200	+
森	0	+++		
	10	++	10 10	+++
	100	+	100 50	+
			〃 100	−
			〃 200	+
梅○	0	+++		
	10	++	10 10	+
	100	−	100 50	+
			〃 100	+
			〃 200	−
吉○	0	+++		
	10	+++	10 10	+++
	100	+++	100 50	+++
			〃 100	+++
			〃 200	+++

4 週間培養した。

その結果表示のごとく Ca-PaA 添加により 10 例中 8 例はほとんど対照と大差をみなかつたが，2 例ではやや発育の抑制される傾向を認めた（表 2）。

総括ならびに考案

結核症患者にしばしばみられる諸種中間代謝ならびに副腎皮質機能障害とビタミン代謝ことに PaA との関連についてはなお不明の点が少なくない。一方 SM 長期投与時の PaA の動向についてもまだ報告はきわめて少ない。われわれは Lactobacillus arabinosus 17-5 10000 を用い結核時ならびに SM 長期投与時の PaA 代謝を検索するとともに諸種代謝障害に占める PaA の役割につき検討した。

PaA 測定は主として Lact. arab. 17-5 を用いる微生物学的方法によるため試料に SM が含まれる場合測定値に誤差を生じやすいと考えられる。われわれは鈴木・田中氏の方法に準じて作製した SM 耐性乳酸菌 Lact. arab. 17-5, 10000 を用いて種々検討した結果，ほぼ満足すべき測定値を得，本菌にて測定可能なることを確認した[7]。

まず肺結核患者で PaA 負荷試験を行ないこれら患者ではしばしば PaA 代謝異常の存することを立証した。すなわち本症患者では往々 PaA 欠乏状態にありまた重症例では PaA 利用能低下を思わせる傾向を認めた。これはわれわれがすでに明らかにした肝障害時の PaA 代謝様相とほぼ軌を一にするものと思われる[8]。かかる成績に鑑み本症患者の PaA 代謝異常に占める肝機能障害の役割を明らかにする目的で BSP 試験を行なつた。結核患者では肝機能障害の存することが少なくなく，この

BSP 試験がもっとも鋭敏といわれる[9]。われわれの成績でも BSP (45分) 値5%以上の異常者は30%にみられ Overrath[9] の報告とほぼ一致した。

このさい BSP 値の異常著しいものは尿 PaA 排泄量も低い傾向を認めた。かかる成績より本症患者にみられる PaA 代謝異常の発現には肝機能障害の関与も考慮すべきことが思推された。

また結核患者の肝 PaA 量は健常者に比し著しく減少を示したが SM 治療群，SM・PaA 併用治療群でこれと大差をみなかつた。SM 投与は腸内菌による PaA 合成を抑制する可能性が考えられ，これが対照と大差をみなかつた一因と考えられる。事実 Lichstein[10] は SM 投与時 PaA 欠乏の起こりやすいことを実証している。

SM 使用時しばしば各種の副作用が出現しその対策として諸種薬剤が使用されていることは周知のところである。われわれは SM 副作用を訴えた患者に PaA を治療的に応用し前述のごとく多数例に効果を認め，Keller[11]，Ducrot[12]，Kimmerle[13] らの報告とほぼ一致をみた。また SM 長期投与鼠で肝 PaA 量の減少，肝 α-ケト酸およびグルタチオン代謝異常，アセチル化能低下，肝および副腎皮質の組織変性像を認めたが，このさい PaA, CoC 等の投与はある程度有効であつた。宮田[14]氏によれば SM による聴器障害の発現は肝ないし腎障害の併発により増強されることを指摘している。われわれも SM 投与動物においてあらかじめ昇汞による腎障害を惹起せしめた場合，SM 単独群に比し明らかに諸代謝障害の増強されることを認めた。

立用氏[15]は SM 長期投与鼠で肝，脾の組織学的障害，肝カタラーゼの低下，肝エステラーゼおよびペルオキシダーゼの減少，アルカリ性フォスファターゼの増強を報告している。

中村氏[16]は SM 難聴の病因として内耳および聴神経中枢の含水炭素代謝異常をあげ，本症の治療予防には糖呼吸系の復調をはかるべきで，これに関与するビタミンの作用が合理的であるという。このさい PaA の有効なことを立証している。福井氏[17]も PaA が SM による聴器障害の防止に有効なことを動物実験成績より証明している。SM による副作用の発現機序に関してはなおその詳細は不明の点が少なくない。Keller[11]，Kimmerle ら[13]は Ca- イオン欠乏と SM 副作用を関係づけて考えている。われわれは動物実験成績より SM 長期投与が肝および副腎皮質の組織変性，諸種代謝異常，PaA 欠乏等を招来することを明らかにした。かかる機能異常が本症患者にみられる SM 副作用の発現になんらかの役割を演ずる可能性も考えられる。PaA 投与は上記諸機能異常の改善を介し，SMの志性軽減，副作用の防止に有効的に働くものと考えられる。一方DOSM が SM に比し副作用の少ないことはすでに諸家により指摘されているところである。われわれも前述のごとく結核患者において副作用の少ないことを実証したが，これは DOSM 長期投与鼠の諸代謝異常が SM 投与群に比し軽度であつた成績からも裏付けされ興味深い。

Fernando ら[18]は SM，パス，ヒドラ併用で効果のなかつた患者に SM の代りに SM-Pantothenate と DSM-Pantothenate を等分に含む製剤を用い，単に自覚症状の好転のみならず他覚所見とくにレントゲン像の改善も半数以上に認められたという。またこの製剤は in vitro の実験で SM に抵抗性を示す Mycobacterium tuberculosis の Strain に対してもその一部に有効であつたと述べている。われわれが 10 例の SM 耐性結核菌で同様の検索を行なつた結果，PaA 添加により著明に発育の抑制をみる例は認められなかつた。

結　論

1) 複合ストマイ 6 週間投与鼠ではアセチル化能低下，肝 α-ケト酸およびグルタチオン代謝異常，副腎皮質組織変性障害像を認めたが，このさい PaA ないし CoC 治療はある程度有効であつた。

2) 昇汞注射後複合ストマイ 4 週間投与鼠では上記諸代謝異常はさらに強度であり肝線および結合型 PaA 量の低下もみられた。このさい PaA 治療はある程度有効であつた。またデスオキシストマイ投与は複合ストマイに比し肝 α-ケト酸およびグルタチオン代謝障害が軽度であつた。

3) 結核患者では PaA 欠乏状態と考えられるものが少なくなく，また重症例ではしばしば利用能低下を思わせる例がみられた。一方 BSP 値の異常者は尿 PaA 排泄量も低値を示す例が多かつた。

4) $H_{37}Rv$ 株注射結核鼠では肝 PaA 量の著減を示す例多く，このさい複合ストマイ治療群でも大差をみなかつた。

5) ストマイ副作用を訴える結核患者に PaA を治療的に応用し多数例に副作用の消失ないし軽減を認めた。

6) デスオキシストマイ投与は複合ストマイに比し副作用が軽度であつた。また PaA はデスオキシストマイ副作用の治療にも有効であつた。

終りにのぞみ終始御懇篤な御指導ならびに御校閲を賜わつた愚師阪大吉田内科王子喜一助教授，明和病院友国院長に深く感謝の意を表します。また結核の実験的発症に種々御援助頂いた阪大堂野前内科野村博士に感謝いたします。

本論文の要旨は昭和 35 年 4 月第 35 回日本結核病学会総会にて発表した。

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NAS/NRC Questionnaire
1949-1963
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D-PANTOTHENYL alcohol

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Report from Lowell O. Randall

Scientific Department Pharmacology to Management

The Acute and Chronic Toxicity of Panthenol (Ni-623).

The Acute and Chronic Toxicity of Panthenol

(Hu-623 : d (+) [N'-Hydroxypropyl] α, γ'-dihydroxy-β,β-dimethyl butyramide.)

The acute toxicity of Panthenol, Hu-623, was determined in mice on oral administration. The LD50 was found to be about 15 gms/kg (see table 1).

Chronic toxicity tests in rats were carried out at two dose levels. Panthenol was added to pulverized rat food to make diets containing concentrations of 0.2% and 0.02%. The diet containing the low concentration was fed to 24 young rats for a 6 months' period. Twelve rats on a normal diet served as controls. The average total intake of Panthenol per rat was 0.64 grams. Another set of 12 young rats received the 0.2% Panthenol diet for 3 months, while 6 control rats received a normal diet. The average total intake of Panthenol for rats in this group was 2.650 grams.

The growth rate of the rats on the 0.02% diet (see figure 1) was nearly identical with that of the control animals. There was a definite lag in growth of the rats on the 0.2% Panthenol diet (see figure 2) as compared to the control animals. The difference, which is statistically significant, is thought to be due to the lower food intake (see table 2).

Blood counts done before the administration of Panthenol and repeated after 3 months indicate that the 0.2% diet had no effect on the number of red or white cells or on the hemoglobin level (see table 3). Similar data are not available for the 6 months' experiments.

Tissue sections from the rats which received the 0.2% Panthenol diet for 12 weeks were examined by Dr. Homer D. Kesten. His attached statement indicates that no lesions were present. Tissue sections from the longer experiment were not examined by Dr. Kesten.

A 3 months experiment was also carried out on 2 one year old dogs. Each dog received one 500 mg tablet of Panthenol each day. Table 5 shows that both dogs lost weight, but the loss is too small to be significant. Nor were there any significant changes in red and white cell counts or hemoglobin, blood sugar and non-protein nitrogen levels (see table 6).

Dr. Kesten's report on tissue sections from these animals states that dog #2 had a patchy edema of the lung, but that all other tissues were normal.

Summary: The LD50 of Panthenol administered orally to mice was about 15 gm/kg. The chronic oral administration of Panthenol to rats and dogs had no adverse effect on growth, body tissues or blood picture.

Margaret D. Roe

Lowell O. Randall

mjp

Table 1

Acute Toxicity in Mice

Dose in mg/kg	No. of dead animals / No. of animals used
20	6/6
10	0/6
5	0/6
2	0/6
1	0/6

Table 2

Average Weekly Weights in Grams and Average Total Food Intake in Kilograms

Number of weeks on experiment	12 Control Rats	Rats on 0.02% Panthenol diet
0	65	63
1	94	96
2	128	127
3	151	159
4	179	181
5	209	213
6	237	240
7	258	259
8	272	274
9	282	285
10	298	301
11	302	299
12	319	310
13	321	314
14	328	320
15	336	322
16	332	327
17	339	325
18	341	327
19	339	326
20	345	330
21	355	342
22	358	343
23	367	362
24	371	364
25	370	360
26	375	363
Average total food intake per rat	3.2 Kg	2.9 Kg

Table 3

Average Weekly Weights in Grams and Average Total Food Intake in Kilograms.

Number of weeks on experiment	6 Control Rats	12 Rats on 0.2% Panthenol Diet
0	68	68
1	91	88
2	123	114
3	146	135
4	172	162
5	215	193
6	245	219
7	264	233
8	282	248
9	294	259
10	309	274
11	313	275
12	320	281
Average total food intake per rat	1.7 kg.	1.3 kg.

Table 4

Average Hemoglobin Levels and Red & White Cell Counts.

	6 Control Rats			12 Rats which received 0.2% Panthenol in diet		
	WBC in thousands	RBC in thousands	Hemoglobin in gms/100 ml	WBC in thousands	RBC in thousands	Hemoglobin in gms/100 ml
at beginning of experiment	13.9	6.3	12.7	11.0	6.4	12.4
after 3 months	20.4	8.0	14.9	16.0	8.0	14.9

L. O. Randall

Table 5

Body Weight in Kilograms

No. of weeks on experiment	Dog #1	Dog #2
0	7.5	9.8
1	7.2	9.6
2	7.3	10.2
3	7.4	9.6
4	7.1	9.3
5	7.3	9.1
6	7.0	9.2
7	6.6	8.7
8	7.3	9.2
9	6.8	9.4
10	7.0	9.0

Table 6

Blood Work Done on Dogs Which Received Orally 500 mg of Lanthanol Per Day

	WBC in thousands	RBC in millions	Hemoglobin in gms/100 ml	Blood sugar in mg/100 ml	NPN in mg/100 ml
			Dog #1		
0 weeks	11.3	5.8	15.4	86	38
after 10 weeks	12.8	7.0	15.6	92	27
			Dog #2		
0 weeks	13.4	4.8	15.6	90	43
After 10 weeks	9.3	7.1	14.4	96	38

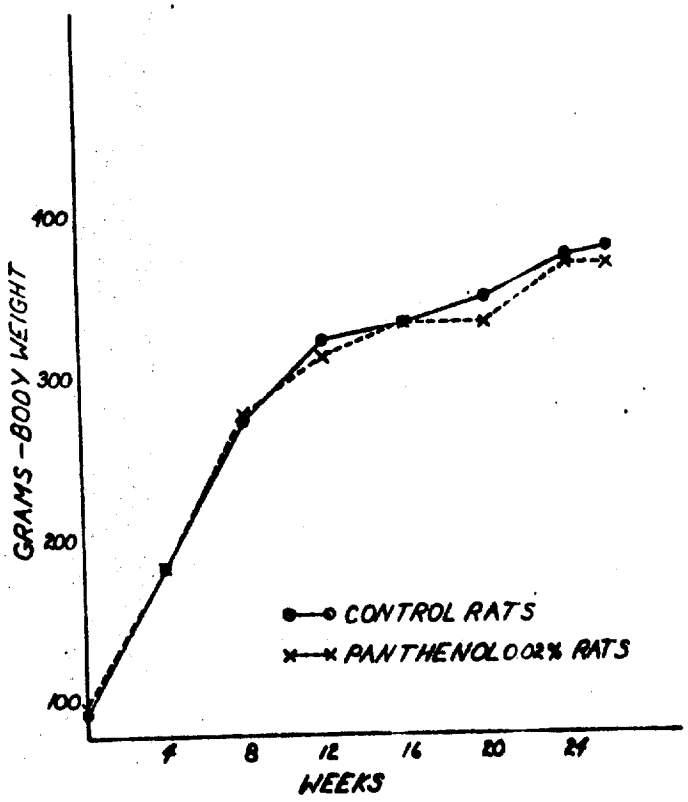

Figure 1: Growth rate of control rats and rats which received a diet containing 0.02% Panthenol for six months.

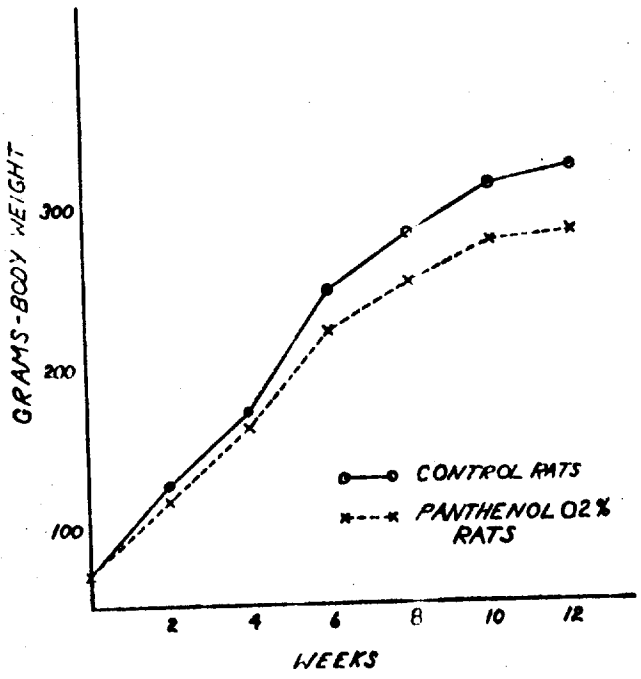

Figure 2: Growth rate of control rats and rats which received a diet containing 0.2% Panthenol for three months.

Columbia University
College of Physicians and Surgeons
630 WEST 168TH STREET
NEW YORK 32, N. Y.
DEPARTMENT OF PATHOLOGY

December 23, 1948

MEMO TO: Hoffmann-La Roche, Inc.

ATTENTION: Dr. Lowell Randall

1. Sections of heart, lung, spleen, liver, pancreas, adrenal, kidney, jejunum, ileum, colon, stomach, duodenum, ovary, thyroid, uterus, and femoral bone marrow have been examined from two dogs that received Panthenol-Nu-623, 500 mg daily orally for 10 weeks. The sections of both dogs are normal except for patchy edema of the lung in dog No. 2. Hemosiderin is abundant in the bone marrow and spleen of this dog, but not beyond normal limits.

2. Sections of heart, liver, pancreas, adrenal, kidney, stomach, duodenum and femoral bone marrow from rats Nos. 1-12 that received 0.2% Panthenol-Nu-623 in the diet for 12 weeks have also been examined. No lesions are present in any of the tissues.

Homer D. Keston, M.D.
Associate Professor of
Pathology

HDK:s

10

April 13, 1951 [redacted]

Report from W. M. Benson

Scientific Department Pharmacology

to Management

Chronic Toxicity in Rats of Panthenol Capsule Mixture Lot No. A-3-105

Chronic Toxicity in Rats of Panthenol Capsule Mixture Lot No. A-3-105

The Panthenol capsule mixture containing Panthenol Roche and Polyethylene Glycol-400 in a ratio of 0.500 grams to 0.570 grams, respectively, was fed orally to weanling Sprague-Dawley female rats for a period of thirteen weeks (October 23, 1950 to January 22, 1951). One group of ten rats received daily in their drinking water approximately 100 mgm/kgm of the mixture. A similar group of rats received 400 mgm/kgm. A third group of ten rats serving as controls received plain tap water. The rats were weighed weekly and the average daily fluid intake for each group determined. Each week the concentration of the Panthenol capsule mixture was changed in accordance with the weight of the rats and their fluid intake so that the dose remained constant. The solutions were made up from a ten percent stock solution which was diluted with tap water to the desired concentration. The rats' water bottles were filled only when required. The same schedule was followed for the control animals. The diet consisted of Friskie's Dog Food Cubes.

Results

As noted in Figure 1, the treated animals gained weight more slowly than the controls. For those on the higher concentration the lag was noticeable after one week and continued throughout the thirteen-week period. The growth lag on the lower concentration was not significant until the fourth or fifth week.

Table 1 indicates that both groups of treated rats drank less fluid than the controls. Those on the higher dose ingested an average

of 2.7 ml less than the controls during the average twenty-four hour period. Those on the lower dose ingested 0.8 ml less than the controls. It cannot be ascertained from the available data whether the reduction of fluid intake was due to a disagreeable taste imparted by the Panthenol capsule mixture or to a systemic toxicity in the animals. The rats appeared healthy throughout the experimental period and when autopsied, exhibited no apparent gross pathology. Tissues are being processed for microscopic studies.

Conclusions

Rats receiving 400 mgm/kgm daily for thirteen weeks of Panthenol capsule mixture Lot No. A-3-105, in their drinking water showed a significant retardation of their growth. At a level of 100 mgm/kgm daily, the effect on growth was slight. The treated animals ingested less fluid than the control animals.

Wilbur M. Benson

Wilbur M. Benson

Experimental Work By:

H. Roo
C. Kunst
M. Paasch

W.B:da

Table 1

Average Daily Fluid Intake of Rats on Pentbonal Capsule Mixtures

No. of Weeks on Experiment	Twenty-four hour intake of rats on 100 mgm/kgm	400 mgm/kgm	Tap Water
0	15 (predicted)	15 (predicted)	15 (predicted)
1	9	8	8
2	20	15	20
3	21	18	21
4	17	16	20
5	21	23	28
6	23	21	24
7	20	18	19
8	20	17	19
9	24	21	23
10	20	19	21
11	25	21	25
12	18	18	23
13	23	20	21
Average*	20.0 ml.	18.1 ml.	20.8 ml.

* Omitting 0 week.

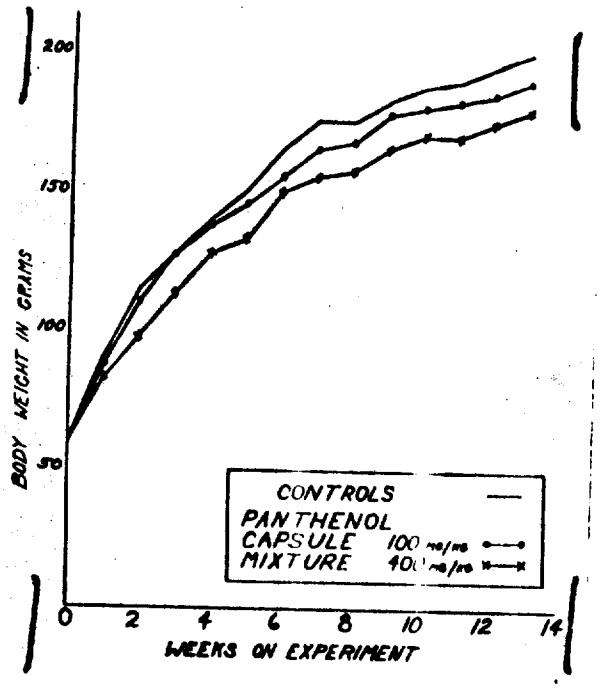

Figure 1

Growth Curves of Rats on Panthenol Capsule Mixture

Feburuary 13, 1952

[redacted]

Report from Wilbur M. Benson

Scientific Department Pharmacology to Management

Microscopic Report on Tissues of Rats Having Received Panthenol Capsule Mixture Lot No. A-3-105

Microscopic Report on Tissues of Rats Having Received Panthenol Capsule Mixture Lot No. A-3-105

The chronic toxicity in rats of Panthenol Capsule Mixture Lot No. A-3-105 containing Panthenol 'Roche' and Polyethylene Glycol-400 in a ratio of 0.500 grams to 0.570 grams, respectively, was reported in Red Corner Report No. 7433. Rats receiving 400 mgm/kgm of the mixture daily for thirteen weeks showed a significant retardation of their growth. At a level of 100 mgm/kgm daily, the effect on growth was slight. When the rats were sacrificed and autopsied, the tissues exhibited no apparent gross pathology.

The attached microscopic report by Dr. Mermod on the tissues of these animals indicates that at the 100 mgm/kgm level only a slight toxic reaction of the kidneys occurred. At the 400 mgm/kgm level this effect was more marked and in addition slight changes in the lungs and liver were noted.

W. M. Benson

Tissue Technician:
Marie Marshall

WMB:da

CAMILLE MERMOD, M. D.
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Hoffman LaRoche, Inc.
Nutley, N.J.

Report of Pathological changes in Rats receiving Panthenol Capsule Mixture

Lungs

Most of the rats used this test had small areas of chronic inflammation in the lungs. These were composed of masses of foam cells surrounded by fibrous tissue and lymphocytes. 7/10 of the control animals showed these changes, as compared to 10/10 of the rats receiving 100 mg/kg of the mixture and 9/10 of those on the 400 mg/kg group.

6/10 of the rats receiving 400 mg/kg showed a moderate dilatation of the pulmonary capillaries. This was not found either in the control animals or in those receiving 100 mg/kg.

Myocardium and Muscle

1/3 of the rats receiving 100 mg/kg of the mixture showed a scattering of the lymphocytes perivascularly in the myocardium and the striated muscle. Similar findings were present in 9/10 of the rats in the 400 mg/kg group and 7/10 of the control animals. No destruction of muscle fibres was found in any case.

Kidneys

2/10 of rats in the 100 mg/kg group showed a slight cloudy swelling in the middle portion of the renal cortex. 8/10 of the rats receiving 400 mg/kg showed similar changes, but only 1/10 of the control animals.

CAMILLE MERMOD, M. D.
18 WASHINGTON STREET
NEWARK 2, NEW JERSEY

Hoffman La Roche, Inc. continued

Liver

No abnormality of the liver cells was found in the 100 mg/kg animals. 8/10 of the 400 mg/kg group had a slight parenchymatous degeneration, which was also found in 5/10 of the controls.

The other organs were normal.

Summary of the pathological changes

(1) The lungs of the animals receiving 400 mg/kg of the Panthenol mixture were more hyperemic than those of the other two groups. The chronic inflammatory changes were of the same degree throughout.

(2) The changes myocardium and muscles were approximately the same in all animals.

(3) Rats receiving the mixture showed a slight parenchymatous degeneration of the middle portion of the renal cortex. This was more marked in those in the 400 mg/kg group, than in the 100 mg/kg or the control animals.

(4) The liver changes were slightly more marked in the 400 mg/kg animals than in the control animals.

CAMILLE MERMOD, M. D.
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NEWARK 2, NEW JERSEY

Hoffman LaRoche, Inc. continued

Discussion

The evidences of chronic inflammation in the lungs, myocardium and muscle was so nearly similar in degree in all the animals, that it is very doubtful whether any of them could be attributed to the effect of the mixture. The pulmonary hyperemia was more marked at the higher dosages. The changes in the kidneys were slight in degree, but definitely more marked in the 400 mg/kg group than in either of the other two groups. They were probably due to the effect of the test mixture. No nuclear or marked cytoplasmic damage was noted in any area. Similarly, the liver damage was very slight but more prominent in the 400 mg/kg group than in the other two groups.

In dosages of 400 mg/kg, the panthenol mixture causes a pulmonary hyperemia and is very slightly toxic to kidneys and liver.

Camille Mermod

Camille Mermod M.D.

Dec.10, 1951

DATE: February 12, 1953

REPORT FROM Lowell O. Randall

Research Department Pharmacology

Subacute Toxicity Studies in Rats Which Received Ca Pantothenate (D-), DL-Panthenol and Panthenol (D-Pantothenyl Alcohol)

Subacute Toxicity Studies in Rats Which Received Ca Pantothenate (D-), Dl-Panthenol and Panthenol (D-Pantothenyl Alcohol)

The report by Mr. Albert R. Lutton of Pharmacology Research, Inc., on, "Subacute Toxicity Studies in Rats Which Received Ca Pantothenate (D-), Dl-Panthenol and Panthenol (D-Pantothenyl Alcohol)", is herewith submitted.

The pathological report will be submitted subsequently.

Lowell O. Randall

LOR:djm

Lowell O. Randall

REPORT ON

Ca PANTOTHENATE (D-)
DL-PANTHENOL
PANTHENOL (D-PANTOTHENYL ALCOHOL)

SUBACUTE TOXICITY STUDIES IN RATS

SUBMITTED AUGUST 31, 1953

TO

HOFFMANN-LAROCHE, INC.
NUTLEY 10, N.J.

BY

PHARMACOLOGY RESEARCH, INC.
DARBY, PENNA.

Ca Pantothenate (d-)
dl-Panthenol
Panthenol (d-Pantothenyl alcohol)

Subacute Toxicity Studies in Rats

This report presents the results obtained in the subacute toxicity investigation of (1) Ca Pantothenate, (2) dl-Panthenol, and (3) d-Panthenol following voluntary consumption at three dosage levels by rats of both sexes for a period of ninety days.

Experimental Design and General Procedure

The three compounds presently studied were investigated simultaneously in both male and female rats of the CR strain; experiments were initiated when the animals were still immature (approximately 100 grams) in order to observe more readily any effects on the normal growth pattern. The compounds were offered to the experimental animals for voluntary consumption following solution at appropriate concentrations in the drinking-water; such solutions served as the sole water supply of the animals. This procedure was adopted in place of the usual dietary method because of two fundamental virtues: (1) Daily measurements could be made of the volumes consumed, thus permitting accurate determinations of the quantities of drug ingested, and (2) the thirst drive is stronger than the hunger drive, thus tending to eliminate the variations in growth pattern which result from impalatability or poor acceptance of products when incorporated in the diet. Six male and six female animals were used at each of three dosage levels with each of the compounds: Two hundred mg/kg, fifty mg/kg, and twenty mg/kg, and a similar group of animals received no drug for control purposes.

THE DRUG CONCENTRATIONS IN THE DRINKING-WATER WERE AT SUCH LEVELS AS TO APPROXIMATE THE INGESTION OF THE DESIRED DOSES; AS THE ANIMALS GAINED IN BODY WEIGHT IT WAS NECESSARY TO ALTER THESE CONCENTRATIONS ACCORDINGLY IN ORDER TO MAINTAIN THE MG/KG BASIS OF DRUG CONSUMPTION. EVERY PRECAUTION WAS APPLIED TO ASSURE STABILITY OF THE SOLUTIONS PREPARED. THE DETAILS OF MATTERS RELATING TO DRUG CONSUMPTION VIA DRINKING-WATER ARE SET FORTH IN APPENDIX B.

BODY WEIGHTS WERE RECORDED TWICE WEEKLY DURING THE INITIAL TWO-WEEK PERIOD, AND THEN WEEKLY UNTIL INVESTIGATIONS WERE TERMINATED. DETAILS AND DATA ARE SET FORTH IN APPENDIX C.

HEMOTOLOGICAL STUDIES WERE CONDUCTED IMMEDIATELY PRECEDING START OF THE INVESTIGATION, AGAIN AT THE MID-POINT OF THE STUDY, AND FINALLY AT TERMINATION. DETAILS AND RESULTS ARE SET FORTH IN APPENDIX D.

AFTER NINETY EXPERIMENTAL DAYS, ALL SURVIVING ANIMALS WERE SACRIFICED AND INDIVIDUAL WEIGHTS OF THE VITAL ORGANS WERE RECORDED; THESE DATA ARE PRESENTED IN APPENDIX E. GROSS EXAMINATIONS OF THE VISCERA WERE MADE AT THIS TIME TO NOTE ANY DEVIATIONS FROM NORMAL APPEARANCE OR STRUCTURE AND REPRESENTATIVE SECTIONS OF VARIOUS ORGANS AND TISSUES WERE REMOVED FOR HISTOLOGICAL EXAMINATION; DETAILS ARE PRESENTED IN APPENDIX F.

A COMPLETE DESCRIPTION OF THE GENERAL OUTLINE PRESENTED ABOVE IS SET FORTH IN APPENDIX A; IN THE LATTER ARE ALSO INCLUDED THE SPECIFIC CHRONOLOGICAL RELATIONSHIPS INVOLVED IN THE INVESTIGATION AND THE SPECIFIC IDENTITY OF THE THREE COMPOUNDS USED.

RESULTS

THE TOXICOLOGICAL EFFECTS OF THE THREE COMPOUNDS INVESTIGATED UNDER THE EXPERIMENTAL CONDITIONS DESCRIBED HAVE BEEN EVALUATED ON THE BASIS OF THE FOLLOWING CRITERIA: (1) EFFECT ON THE NORMAL GROWTH CURVE; (2) INDUCED MORTALITY; (3) EFFECT ON THE HEMOGRAM; (4) EFFECT ON THE FINAL WEIGHTS OF THE VITAL ORGANS; AND (5) GENERAL EFFECT ON VISCERAL ORGANS AS OBSERVED BY GROSS EXAMINATIONS AT AUTOPSY. THE EVALUATIONS WILL BE FURTHER SUPPLEMENTED BY THE FINDINGS OBTAINED IN THE HISTOLOGICAL EXAMINATIONS OF THE VARIOUS TISSUE SECTIONS REMOVED FROM THE EXPERIMENTAL ANIMALS. ALTHOUGH THE EVALUATIONS DID NOT REVEAL ANY SIGNIFICANT DIFFERENCES AMONG THE THREE COMPOUNDS STUDIED, THE LATTER WILL BE CONSIDERED SEPARATELY FOR REFERENCE PURPOSES.

Ca Pantothenate: THE RECORDED DATA (SEE APPENDIX B) INDICATES THAT THE INTENDED DOSAGE LEVELS HAVE BEEN CLOSELY APPROXIMATED. THE ACTUAL VALUES OBTAINED ARE PRESENTED AS FOLLOWS AS AVERAGES THROUGHOUT THE STUDY:

	Intended Dose MG/KG	Actual Dose MG/KG	Difference	Total Drug Consumed MG/RAT
Males	200	177	-12%	4050
	50	44	-12%	1100
	20	19	- 5%	440
Females	200	206	+ 3%	3200
	50	53	+ 6%	850
	20	18	-10%	280

SINCE THE SIX ANIMALS COMPOSING EACH GROUP WERE HOUSED TOGETHER IN A SINGLE CAGE, THE FOREGOING VALUES REPRESENT AVERAGES OF THE ENTIRE GROUP IN EACH INSTANCE.

THE CONSUMPTION OF Ca PANTOTHENATE DID NOT SEEM TO ALTER THE GROWTH PATTERN GREATLY IN ANY OF THE EXPERIMENTAL GROUPS, AS SEEN IN FIGURE 1 AND

IN COMPARISON WITH THE CONTROLS SHOWN IN FIGURE 4. FINAL WEIGHTS OF THE MALE RATS RECEIVING THE HIGH DOSE AND OF THOSE RECEIVING THE LOW DOSE WERE SLIGHTLY LESS THAN THE CONTROLS; HOWEVER, THESE DIFFERENCES ARE NOT CONSIDERED TO BE SIGNIFICANT. THE MALE RATS RECEIVING THE MEDIAN DOSE AND THE FEMALE RATS RECEIVING ANY OF THE THREE DOSAGE LEVELS SHOWED GROWTH CURVES WHICH CLOSELY PARALLELED THE GROWTH CURVES OF THE CONTROLS.

TWO OF THE MALE RATS RECEIVING THE MEDIAN DOSAGE LEVEL AND ONE FEMALE RAT RECEIVING THE MEDIAN DOSAGE LEVEL SUCCUMBED BEFORE THE TEST WAS CONCLUDED. THESE FEW MORTALITIES ARE NOT CONSIDERED TO BE SIGNIFICANT AND DO NOT SEEM TO BE RELATED TO DRUG CONSUMPTION IN ANY WAY. (AMONG THE CONTROLS 4/10 MALE RATS AND 1/14 FEMALE RATS DIED BEFORE THE TEST WAS TERMINATED.)

THE HEMOLOGICAL DATA HAVE BEEN CAREFULLY STUDIED ON THE BASIS OF TOTAL, DIFFERENTIAL, AND ABSOLUTE COUNTS OF THE LEUKOCYTIC ELEMENTS AND ON THE BASIS OF TOTAL ERYTHROCYTE COUNTS AND HEMOGLOBIN CONCENTRATIONS (SEE TABLES 5, 6, AND 9 OF APPENDIX D). COMPARED WITH SIMILAR VALUES OBTAINED WITH THE CONTROL ANIMALS, NO SIGNIFICANT DEPARTURES WERE OBSERVED WITH THE POSSIBLE EXCEPTION OF THE EOSINOPHILS. THE LATTER APPEARED TO INCREASE IN NUMBER DURING THE INVESTIGATION; HOWEVER, IT SHOULD BE POINTED OUT THAT BECAUSE OF THE LIMITED NUMBER OF INDIVIDUAL ANIMAL OBSERVATIONS, IN VIEW OF THE RELATIVE PAUCITY OF THESE PARTICULAR CELLS IN THE CIRCULATING BLOOD, AND IN VIEW OF THE SMALL DEGREE OF THE OBSERVED INCREASE, IT CANNOT BE CONCLUDED WITH ANY DEGREE OF CERTAINTY THAT A MILD EOSINOPHILIA HAD BEEN PRODUCED. IT IS OUR OPINION THAT THE OBSERVATION REQUIRES SUBSTANTIATION BEFORE ANY DEFINITE CONCLUSIONS CAN BE REACHED AND THAT THE DATA ARE ONLY SUGGESTIVE OF FURTHER CONSIDERATION.

THE WEIGHTS OF THE SPLEEN, LIVER, KIDNEYS AT THE TIME STUDIES WERE CONCLUDED DID NOT DIFFER IN ANY EXTENT FROM THE WEIGHTS OF THE CONTROL ANIMALS. THE ADRENAL GLANDS OF THE MALES RECEIVING THE THREE DOSAGE LEVELS WEIGHED APPROXIMATELY 30% MORE THAN THOSE OF THE CONTROLS ; AMONG THE FEMALES, THE FINAL WEIGHTS OF THESE ORGANS WERE ABOUT 15% LESS THAN THEIR CONTROLS, THUS MAKING INTERPRETATION OF THE SIGNIFICANCE OF THE OBSERVED CHANGES DIFFICULT. IT IS CONCLUDED, THEREFORE, THAT THE FINAL WEIGHTS OF THE VITAL ORGANS WERE NOT FOUND TO DIFFER SIGNIFICANTLY FROM THOSE OF THE CONTROL ANIMALS. (SEE TABLE 5 OF APPENDIX E.)

GROSS EXAMINATION OF THE VARIOUS ORGANS AND TISSUES AT THE TIME OF AUTOPSY DID NOT REVEAL ANY DEVIATIONS FROM THE NORMAL APPEARANCE AND STRUCTURE IN ANY INSTANCE.

DL-PANTHENOL. QUANTITATIVELY, THE ACTUAL DOSAGE LEVELS CONSUMED DID NOT DIFFER GREATLY FROM THE INTENDED DOSAGE LEVELS AS SHOWN BY THE FOLLOWING:

	Intended Dose mg/kg	Actual Dose mg/kg	Difference	Total Drug Consumed mg/rat
Males	200	188	- 6%	4400
	50	47	- 6%	1170
	20	20	0	460
Females	200	190	- 5%	2940
	50	47	- 6%	760
	20	20	0	300

THE FOREGOING VALUES REPRESENT AVERAGES OF THE ENTIRE GROUP OF ANIMALS IN EACH INSTANCE.

THE GROWTH PATTERN OF THE MALE RATS RECEIVING THE HIGH DOSE AND RECEIVING THE LOW DOSE FELL BELOW THE CONTROL GROUP BEFORE THE EXPERIMENT WAS TERMINATED; HOWEVER, THE DIFFERENCE WAS SLIGHT AND IS NOT CONSIDERED

TO BE SIGNIFICANT. THE MALES AT THE MEDIAN DOSAGE LEVEL AND THE FEMALES AT ALL THREE DOSAGE LEVELS SHOWED GROWTH CURVES ESSENTIALLY IDENTICALWITH THEIR CONTROLS. (SEE FIGURE 2, COMPARED WITH FIGURE 4.)

NO MORTALITIES AMONG ANY OF THE EXPERIMENTAL GROUPS OCCURRED BEFORE THE INVESTIGATION WAS CONCLUDED.

THE HEMOTOLOGICAL STUDIES DID NOT REVEAL ANY ALTERATIONS WHICH COULD BE ATTRIBUTED TO THE AGENT CONSUMED. HOWEVER, A VERY MILD DEGREE OF EOSINOPHILIA MAY HAVE BEEN PRESENT AMONG THE FEMALE RATS, BUT NOT AMONG THE MALES. THE SAME QUALIFICATIONS AND LIMITATIONS OF ANALYSIS ARE APPLICABLE IN THIS INSTANCE AS DESCRIBED FOR Ca PANTOTHENATE, ABOVE. (SUMMARIES OF THE HEMOTOLOGICAL DATA ARE PRESENTED IN TABLES 5,6, AND 9 OF APPENDIX D.)

THE WEIGHTS OF THE SPLEEN, LIVER, KIDNEYS, AND ADRENAL GLANDS AT THE TIME THE STUDY WAS TERMINATED DID NOT SHOW ANY SIGNIFICANT DEVIATIONS FROM SIMILAR WEIGHTS OF THE CONTROL ANIMALS. ALTHOUGH MINOR DIFFERENCES WERE OBSERVED IN SOME INSTANCES, THESE WERE OF LOW DEGREE AND DID NOT DEMONSTRATE ANY SPECIFIC PATTERN WHICH WOULD PERMIT REFERENCE TO THE ADMINISTERED AGENT. (SEE TABLE 5 OF APPENDIX E.)

GROSS EXAMINATION OF THE VARIOUS ORGANS AT AUTOPSY DID NOT REVEAL ANY DEVIATIONS FROM NORMAL APPEARANCE OR STRUCTURE.

D-PANTHENOL. THE DOSAGE LEVELS ACTUALLY CONSUMED BY THE EXPERIMENTAL ANIMALS CLOSELY APPROXIMATED THE INTENDED DOSAGE LEVELS:

	Intended Dose MG/KG	Actual Dose MG/KG	Difference	Total Drug Consumed MG/RAT
Males	200	187	- 7%	4480
	50	49	- 2%	1300
	20	18	-10%	450
Females	200	183	- 8%	2780
	50	51	+ 2%	840
	20	21	+ 5%	340

THE FOREGOING VALUES REPRESENT AVERAGES OF THE ENTIRE GROUP OF ANIMALS IN EACH INSTANCE.

THE GROWTH PATTERN OF ALL EXPERIMENTAL GROUPS DID NOT DIFFER SIGNIFICANTLY FROM THEIR RESPECTIVE CONTROL GROUPS AS CAN BEEN SEEN IN FIGURE 3 COMPARED TO FIGURE 4.

MORTALITIES WERE OBSERVED ONLY AMONG THE MALES DURING THE COURSE OF THE EXPERIMENT; THESE WERE ONE MALE AT THE HIGH DOSE, TWO MALES AT THE MEDIAN DOSE, AND ONE MALE AT THE LOW DOSE. BECAUSE OF FACTORS ALREADY DISCUSSED, THESE ARE NOT CONSIDERED TO BE SIGNIFICANT AND DO NOT APPEAR TO BE RELATED TO DRUG CONSUMPTION IN ANY INSTANCE.

AS WITH THE TWO PRECEDING COMPOUNDS, NO SIGNIFICANT ALTERATIONS IN THE HEMOGRAM WERE OBSERVED WITH THE POSSIBLE EXCEPTION OF A MILD EOSINOPHILIA WHICH WAS PRESENT AMONG THE FEMALES AT EACH OF THE THREE DOSAGE LEVELS AND AMONG THE MALES AT THE HIGH DOSAGE LEVEL ONLY. THIS MILD EOSINOPHILIA MAY NOT BE SIGNIFICANT AND IS QUALIFIED BY THE COMMENTS ALREADY MADE. (HEMOTOLOGICAL SUMMARIES ARE PRESENTED IN TABLES 5, 6, AND 9 OF APPENDIX D.)

OF THE ORGANS WEIGHED AT THE TERMINATION OF THE EXPERIMENT, THE ONLY MARKED DEVIATIONS OBSERVED WERE IN REFERENCE TO THE LIVERS OF THE TREATED ANIMALS! THE AVERAGE WEIGHT OF THIS ORGAN WAS LESS THAN THE CONTROLS AMONG THE MALE RATS AT THE HIGH DOSAGE LEVEL AND ALSO AT THE LOW DOSAGE LEVEL. THE DIFFERENCE DOES NOT APPEAR TO BE OF SUFFICIENT MAGNITUDE TO BESIGNIFICANT. (SEE TABLE 5 OF APPENDIX E.)

GROSS EXAMINATION OF THE VARIOUS ORGANS AT AUTOPSY DID NOT REVEAL ANY DEVIATIONS FROM NORMAL APPEARANCE OR STRUCTURE.

DISCUSSION

CONSIDERATION OF ALL THE DATA RECORDED AND PRESENTED HEREIN DOES NOT PERMIT ANY CLEAR-CUT CONCLUSIONS IN REFERENCE TO UNTOWARD EFFECTS RESULTING FROM THE VOLUNTARY CONSUMPTION BY RATS OF ANY OF THE THREE COMPOUNDS IN DOSES AS HIGH AS APPROXIMATELY 200 MG/KG PER DAY FOR NINETY DAYS. IN ESSENCE, THE RESULTS OBTAINED WERE NEGATIVE.

A NUMBER OF FACTORS HAVE BEEN USED AS CRITERIA IN THE EVALUATION: GROWTH, MORTALITY, HEMOGRAMS, TERMINAL ORGAN WEIGHTS, AND GROSS CONDITIONS AT AUTOPSY. INSOFAR AS THESE CRITERIA ARE CONCERNED, NONE OF THE THREE COMPOUNDS COULD BE DISTINGUISHED FROM ONE ANOTHER. MINOR ALTERATIONS IN ONE OR SEVERAL OF THESE CRITERIA HAVE BEEN NOTED; HOWEVER, IN EACH INSTANCE THE ALTERATIONS WERE OF SUCH LOW MAGNITUDE AS TO RAISE SERIOUS QUESTION REGARDING THEIR SIGNIFICANCE. WITHOUT CONSIDERATION OF OTHER EXPERIMENTAL DATA WHICH MIGHT BE AVAILABLE, DEFINITE CONCLUSIONS REGARDING SUCH ALTERATIONS CANNOT BE MADE JUSTIFIABLY.

THE ONLY CHARACTERISTIC ATTRIBUTABLE TO DRUG CONSUMPTION UNDER THE CONDITIONS OF STUDY REPORTED HEREIN MAY BE A VERY MILD EOSINOPHILIA. IF REAL, THE CONDITION WAS PRODUCED BY EACH OF THE THREE COMPOUNDS AMONG THE FEMALE ANIMALS; AMONG THE MALES, CA PANTOTHENATE APPEARED TO BE MOST EFFECTIVE AND LITTLE OR NO EFFECT WAS PRODUCED BY THE OTHER TWO COMPOUNDS. IT SHOULD BE POINTED OUT HOWEVER, THAT THE OBSERVATIONS MERELY SUGGEST SUCH A CONDITION AND DO NOT ESTABLISH IT AS A DEFINITE RESULT. FLUCTUATIONS IN THE NUMBER OF CIRCULATING EOSINOPHILS ARE A COMMON OBSERVATION AND FREQUENTLY SUCH FLUCTUATIONS MAY APPEAR TO BE "GREAT" SIMPLY BECAUSE OF THE RELATIVE SCARCITY OF THIS PARTICULAR CELL AMONG THE LEUKOCYTIC POPULATION. THE SUGGESTION HAS BEEN THE RESULT OF CALCULATIONS MADE FROM THE TOTAL WBC AND THE DIFFERENTIAL COUNT, NOT ON THE BASIS OF ACTUAL EOSINOPHIL-

COUNTS, THUS LENDING FURTHER DOUBT IN REGARD TO THEIR VALIDITY. (THE ALTERATION BECAME APPARENT ONLY AFTER AUTOPSIES WERE IN PROGRESS, OBVIATING ANY POSSIBILITY OF CONDUCTING DIRECT COUNTS.) IT IS OUR OPINION, THEREFORE, THAT THESE CHANGES SHOULD BE CONSIDERED WITH A GREAT DEAL OF RESERVATION AND THAT CONFIRMATION THROUGH ALLIED STUDIES WILL BE REQUIRED TO LEND CREDANCE TO THE OBSERVED FINDINGS.

CONCLUSIONS

(1) THE SUBACUTE TOXICITY FOLLOWING VOLUNTARY CONSUMPTION BY MALE AND FEMALE RATS OF Ca PANTOTHENATE, DL-PANTHENOL, AND D-PANTHENOL AT THREE DOSAGE LEVELS (200, 50, AND 20 MG/KG) FOR NINETY DAYS HAS BEEN DETERMINED AND REPORTED HEREIN.

(2) EACH OF THE COMPOUNDS WAS EVALUATED ON THE BASIS OF THE FOLLOWING EXPERIMENTAL CRITIERIA: EFFECTIVE GROWTH PATTERN, INCIDENCE OF MORTALITY, HEMOTOLOGICAL CHANGE, AND FINAL WEIGHTS OF THE VITAL ORGANS; THESE WILL BE SUPPLEMENTED BY HISTOLOGICAL STUDY OF VARIOUS TISSUE SECTIONS.

(3) THE RESULTS OBTAINED WERE ESSENTIALLY NEGATIVE, INDICATING EACH OF THE THREE COMPOUNDS TO BE RELATIVELY INNOCUOUS UNDER THE CONDITIONS OF THE EXPERIMENT.

(4) SIGNS OF A MILD EOSINOPHILA APPEARED TO BE PRESENT AMONG THE EXPERIMENTAL ANIMALS, BUT THIS HAS BEEN SERIOUSLY QUESTIONED.

(5) BASED UPON DATA REPORTED HEREIN EACH OF THE THREE COMPOUNDS INVESTIGATED PRODUCED QUALLY NEGATIVE RESULTS TOXICOLOGICALLY.

PHARMACOLOGY RESEARCH, INC.

ALBERT R. LATVEN
AUGUST 31, 1953

Appendix A

Experimental Outline

Experimental Design:

Voluntary consumption of (1) Ca Pantothenate Dextrorotary, (2) dl-Panthenol, and (3) d-Panthenol when dissolved in the drinking water at concentrations resulting in the daily consumption of 0, 20, 50 and 200 mg/kg; twelve rats (six males and six females) at each level. Consumption for a period of 90 days. Body weights throughout. Hemograms pre-test, mid-test and end-test. Organ weights and tissue section at autopsy.

Group A:	Ca Pantothenate,	200 mg/kg,	6 male and 6 female rats
B:	Ca Pantothenate,	50 mg/kg,	6 male and 6 female rats
C:	Ca Pantothenate,	20 mg/kg,	6 male and 6 female rats
D:	dl-Panthenol,	200 mg/kg,	6 male and 6 female rats
E:	dl-Panthenol,	50 mg/kg,	6 male and 6 female rats
F:	dl-Panthenol,	20 mg/kg,	6 male and 6 female rats
G:	d-Panthenol,	200 mg/kg,	6 male and 6 female rats
H:	d-Panthenol,	50 mg/kg,	6 male and 6 female rats
I:	d-Panthenol,	20 mg/kg,	6 male and 6 female rats
J:	Controls	0 mg/kg,	6 male and 6 female rats

Experimental Observations

(1) Body weights recorded weekly.
(2) Hemograms: RBC, WBC, Hg and differential 3 male and 3 female rats from each group.
(3) Organ weights: Liver, kidneys, spleen and adrenals.
(4) Tissue sections for histopathology: Liver, kidney, spleen, adrenal, heart, stomach, intestine, gonad, thyroid, femoral bone marrow and brain; in addition, sections of the lung and mesentaric lymph node were taken from the female animals.

Experimental Chronology

Expt. Day	Date 1953	Program
-7	5/8	Rats received
-4	5/11	First body weights recorded. Twice weekly until 11th expt'l. day, then weekly.
-2	5/13	Hemograms, male rats (pre-test).
-1	5/14	Hemograms, female rats (pre-test).
0	5/15	Experiment started: Ad lib consumption of drugs added to the drinking water for duration of study. Volumes consumed recorded daily throughout.
41	5/25	Hemograms, male rats (mid-test).
42	5/26	Hemograms, female rats (mid-test).
88	8/11	Hemograms, male rats (end-test).
89	8/12	Hemograms, female rats (end-test).
90	8/13	Male rats sacrificed and autopsied; organ weights recorded and tissue sections taken.
91	8/14	Female rats sacrificed and autopsied; organ weights recorded and tissue sections taken.

(APPENDIX A CONTINUED)

IDENTITY OF COMPOUNDS

EACH OF THE THREE COMPOUNDS WAS RECEIVED ON APRIL 1, 1953 AND IDENTIFIED WITH OUR CODE NUMBER. THE LABELED IDENITIES, AS WELL AS THE CODE NUMBERS APPLIED, WERE AS FOLLOWS:

(1) CALCIUM PANTOTHENATE DEXTROROTATORY ROCHE, LOT #51497 (IDENTIFIED BY US AS PR#53031); SUPPLEMENTED BY LOT#51538, RECEIVED JULY 13, 1953.

(2) CRYSTALLINE DL-PANTHENOL L OT 63-B+C (IDENTIFIED BY US AS PR#53032); SUPPLEMENTED BY LOT PA-EXP.-64, RECEIVED JULY 13, 1953.

(3) PANTHENOL (D-PANTOTHENYL ALCOHOL) ROCHE, LOT#90 (IDENTIFIED BY US AS PR#53033); SUPPLEMENTED BY LOT#507093, RECEIVED JULY 13, 1953.

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Appendix B

Drug Consumption via Drinking-Water

The compounds investigated were offered to the experimental animals for voluntary consumption via the drinking-water. This procedure had been suggested because it possessed certain characteristics which conferred superiority over the customary drug-dietary method; in essence, these characteristics were the result of (1) the fact that the thirst drive is stronger than the hunger drive, thus resulting in considerably smaller fluctuations in consumption as well as body weight when these are due to poor acceptability of the compounds in question; and (2) the fact that the volume of fluid can be accurately measured throughout an investigation through the use of suitable feeding devices (at best, dietary consumption can only be guessed at since the problem of scattering still remains to be solved). Of course, the water-consumption method requires certain precautions such as means of assuring stability of the dissolved agents; also, a greater amount of work is required since daily measurements of consumption must be made, although these are justified on the basis of far greater accuracy and usefulness of recorded data. These factors will be considered separately.

Preparation of Drinking-Water Solutions. Stock solutions of each of the compounds were prepared weekly in 8.0% concentrations; each was acidified with hydrochloric acid so that when added to the drinking-water, the latter possessed a pH not less than 5.0 nor greater than 6.0. Because of the apparent "buffering capacity" of the compounds, at least 24 hours was allowed after addition of the HCl before pH determinations were made. All stock solutions were kept constantly refrigerated. Dilutions were prepared from the stock solutions in such concentrations as to result in the consumption of intended dosage levels; the dilutions served as the sole drinking-water supply of the experimental animals throughout the investigation. These dilutions were freshly prepared every day and the remainder of the previous day's dilutions was discarded.

Manner of Drug-Solution Consumption. Inverted amber 2-oz. bottles with drinking tubes were attached to the outside surface of the cage, the tube entering the latter at an angle. A sufficient quantity of the drinking-water was made constantly available so that consumption was ad lib in all instances.

First, a measured volume was offered to each group of animals; 24-hours later, the volume remaining was measured, discarded, and replaced with freshly prepared solution. Thus, the daily consumption by each group of six rats was determined throughout the 90-day experiment.

Suitable concentrations of each of the three agents investigated were used in order to approximate consumption of each agent at three dosage levels. To facilitate this, the proper concentration for the high dose was estimated, and a 1:4 dilution was used as the intermediate dose concentration, and 1:10 dilution was used as the the low dose concentration.

Since the volume of water consumed in terms of body weight changed as the animals grew to maturity, the drug concentrations were altered so that the average amount drunk by each group approximated the intended dose. Also, since the male animals exceeded the female animals in body weight and in fluid consumption, drug concentrations were adjusted accordingly.

(APPENDIX B CONTINUED)

DATA AND DOSE CALCULATION. THE FLUID VOLUMES CONSUMED DAILY BY EACH GROUP OF ANIMALS HAVE BEEN AVERAGED ON A WEEKLY BASIS AND DIVIDED BY THE NUMBER OF ANIMALS COMPOSING THE GROUP; THESE DATA, IN TERMS OF FLUID CONSUMPTION IN CC/RAT, ARE PRESENTED IN TABLE 1. IT WILL BE NOTED THAT AS THE EXPERIMENT PROGRESSED GREATER VOLUMES OF FLUID WERE CONSUMED; THIS, OF COURSE, WAS DUE IN GREAT MEASURE TO THE GROWTH OF THE ANIMALS AND INFLUENCED ALSO BY THE AMBIENT TEMPERATURE.

THE VALUES REFERRED TO ABOVE HAVE BEEN CONVERTED IN TERMS OF BODY WEIGHT, CC/KG. AND ARE PRESENTED IN TABLE 2; THE DRUG CONCENTRATIONS USED ARE INCLUDED ALSO. IT WILLBE NOTED THAT ON A CC/KG BASIS, FLUID CONSUMPTION TENDED TO DECREASE THROUGHOUT THE STUDY, NOT WITHSTANDING THE INCREASED VOLUMES ACTUALLY CONSUMED AS SHOWN IN TABLE 1.

THE FOREGOING VALUES HAVE BEEN CONVERTED IN TERMS OF THE AMOUNT OF DRUG CONSUMED ON A MG/KG BASIS; THESE VALUES ARE PRESENTED IN TABLE 3. IT WILL BE SEEN THAT THE INTENDED DOSES HAVE BEEN CLOSELY APPROXIMATED IN EVERY INSTANCE, THE MAXIMUM DEVIATION BEING NO GREATER 12%. IT SHOULD BE EMPHASIZED THAT THESE VALUES ARE ACTUAL VALUES OF DRUG CONSUMPTION AND ARE NOT SIMPLE APPROXIMATIONS.

IT MAY BE OF INTEREST TO NOTE THE TOTAL QUANTITIES OF EACH DRUG CONSUMED THROUGHOUT THE STUDY. THESE HAVE BEEN CALCULATED IN TERMS OF MILLIGRAMS PER RAT AND ARE PRESENTED AS FOLLOWS:

	Ca PANTOTHENATE			DL-PANTHENOL			D-PANTHENOL		
	A	B	C	D	E	F	G	H	I
MALES	4050	1100	440	4400	1170	460	4490	1300	450
FEMALES	3200	850	280	2940	760	300	2780	840	340

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Appendix B.

Table 1. Average Daily Fluid Consumption: cc/rat (weekly averages).

Exptl Week	Ca Pantothenate A	B	C	dl-Panthenol D	E	F	d-Panthenol G	H	I	Control J
MALES										
0	22.0	22.1	23.1	24.8	21.8	23.3	24.6	24.0	22.5	22.2
1	22.9	25.0	25.9	25.2	27.8	25.6	22.0	27.1	27.1	27.4
2	25.3	27.8	30.9	30.5	29.8	30.1	26.3	34.3	32.5	28.3
3	31.4	35.8	38.3	39.2	37.9	34.5	36.7	42.4	41.6	34.8
4	43.4	48.1	46.7	51.8	49.4	49.2	51.2	53.3	40.9	44.4
5	40.0	43.1	43.0	43.0	43.6	43.1	45.6	48.1	39.2	40.4
6	55.4	58.2	58.3	59.5	61.2	62.4	62.7	70.9	59.7	55.2
7	63.8	65.2	71.2	67.8	72.7	71.9	73.2	90.4	71.3	86.7
8	51.6	58.1	56.0	56.2	61.4	61.4	55.0	64.0	58.7	56.6
9	50.9	53.1	54.6	55.5	62.2	57.9	55.5	64.0	55.3	63.5
10	65.3	67.0	67.0	69.3	78.7	77.9	67.5	81.7	80.3	69.8
11	48.9	51.2	47.1	49.6	56.3	54.9	53.7	62.1	48.3	53.1
12	44.4	49.5	43.6	46.0	49.3	49.7	51.1	60.8	48.1	51.0
13	49.4	52.6	56.9	52.5	53.2	53.3	56.5	61.7	52.6	47.7
FEMALES										
0	21.6	23.3	18.0	20.3	22.3	21.4	23.1	19.9	23.2	21.5
1	22.1	19.8	21.5	19.4	22.4	20.9	22.2	22.3	22.7	21.7
2	25.3	25.6	21.3	21.9	25.0	24.0	22.7	24.8	25.6	24.1
3	28.6	30.3	24.3	25.5	26.4	26.2	27.4	28.6	29.3	26.1
4	35.4	36.5	28.8	31.1	32.1	36.0	31.7	34.1	37.2	34.5
5	31.1	32.0	24.8	28.5	30.5	31.1	27.7	33.4	32.9	28.5
6	44.0	45.5	35.4	39.8	37.6	41.2	36.1	44.0	45.2	37.2
7	51.8	57.1	44.7	33.8	47.4	47.8	41.8	52.1	50.6	45.3
8	39.5	43.3	36.1	30.5	34.2	36.1	31.3	41.4	42.0	37.6
9	37.1	42.5	32.7	30.7	35.9	35.9	30.7	39.8	37.8	37.6
10	49.1	55.1	40.6	41.8	45.3	44.9	33.4	53.7	50.3	50.8
11	35.0	34.9	33.5	31.4	34.4	31.2	32.2	33.6	39.2	37.2
12	31.7	32.7	31.0	29.7	32.4	29.9	29.9	33.8	38.0	34.8
13	33.1	34.0	32.3	29.3	32.3	32.1	29.9	41.7	36.3	33.7
Total Fluid Consumption During Entire Experimental Period: cc/rat (average)										
MALES	4100	4460	4420	4463	4731	4678	4543	5265	4497	4565
FEMALES	3247	3425	2862	2978	3051	3061	2813	3383	3410	3144

Note: Experimental period = 90 days for the males, and = 91 days for females

Appendix D.

Table 2. Average Daily Fluid Consumption: cc/kg (Weekly Averages)

Expt'l Week	Ca Pentothenate A	B	C	DL-Panthenol D	E	F	D-Panthenol G	H	I	Control J	Drug Conc.* mg/cc
MALES											
0	242	231	249	267	251	256	267	258	242	223	0
1	210	216	221	208	236	217	198	228	231	234	0.96
2	165	166	191	186	176	185	179	196	196	198	1.00
3	164	170	193	198	185	194	187	196	203	191	1.00
4	198	199	207	220	207	214	223	213	169	205	1.06
5	159	154	169	165	161	163	174	165	122	164	1.10
6	208	193	224	216	213	232	218	229	210	199	1.04
7	240	214	274	245	249	262	253	284	244	305	1.04
8	186	191	199	194	200	215	184	194	191	191	1.04
9	166	162	176	178	185	186	172	177	165	195	1.10
10	207	199	214	217	235	253	205	223	238	201	0.96
11	144	143	141	144	154	163	153	160	133	143	0.84
12	124	130	125	130	129	138	138	148	126	131	0.84
13	138	132	168	148	139	151	153	151	140	122	0.84
FEMALES											
0	254	283	217	249	263	263	270	237	271	274	0
1	201	200	215	202	211	211	238	233	239	229	0.96
2	181	194	159	173	182	179	167	173	187	191	1.00
3	179	189	159	176	164	172	181	173	182	195	1.00
4	200	206	169	205	183	215	188	183	213	214	1.14
5	166	169	139	163	161	171	153	174	177	164	1.25
6	256	241	183	215	190	217	191	219	232	203	1.18
7	258	220	223	234	234	249	220	251	253	241	1.18
8	195	205	155	159	162	185	160	193	206	194	1.18
9	173	191	155	150	162	173	143	177	173	178	1.25
10	228	247	189	203	206	212	188	237	232	239	1.07
11	154	140	149	145	147	141	147	144	168	166	0.90
12	135	135	132	131	135	131	131	139	158	149	0.90
13	138	137	134	126	132	136	130	130	150	152	0.90

*Drug Conc. values represent high-dose concentrations (A, D, G); one-quarter of these values represent the medium-dose concentrations (B, E, H); and one-tenth of these values represent the low-dose concentrations (C, F, I); the Control group (J) received no drug, of course.

Appendix D

Table 3. Average Daily Drug Consumption: mg/kg (weekly averages)

Exptl Week	Ca Pantothenate A	B	C	dl-Panthenol D	E	F	d-Panthenol G	H	I
MALES									
1	202	52	21	200	57	21	190	55	23
2	165	42	19	186	44	19	179	49	20
3	164	43	19	198	46	19	187	49	20
4	210	53	22	233	55	23	236	56	18
5	175	42	19	182	44	18	192	45	13
6	216	50	23	225	56	24	227	60	22
7	250	56	29	255	65	27	263	74	25
8	194	50	21	202	52	22	191	50	20
9	183	45	19	196	51	20	189	49	18
10	199	48	21	208	56	24	197	54	23
11	121	30	12	122	32	14	129	34	11
12	108	27	11	109	27	12	116	31	11
13	116	28	14	124	29	13	129	32	12
FEMALES									
1	193	48	21	194	56	20	203	50	20
2	181	49	16	173	46	18	167	43	19
3	179	47	16	176	41	17	181	43	18
4	228	59	19	234	52	25	214	54	24
5	208	53	17	204	50	21	192	54	22
6	314	71	22	254	56	26	226	65	27
7	305	83	26	276	69	29	260	74	30
8	230	61	18	188	48	22	189	58	24
9	216	60	19	188	51	22	179	55	22
10	244	66	20	218	55	23	201	63	25
11	139	32	13	131	33	13	132	32	15
12	122	30	12	118	30	12	118	31	14
13	124	31	12	113	30	12	117	38	14
Average Daily Drug Consumption During Entire Experimental Period: mg/kg/day									
MALES	177	44	19	188	47	20	187	49	18
FEMALES	206	53	18	190	47	20	183	51	21
Percentage Difference of Drug-Dose Received from Drug-Dose Intended									
MALES	-12%	-12%	-5%	-6%	-6%	0	-7%	-2%	-10%
FEMALES	+3%	+6%	-10%	-5%	-6%	0	-8%	+2%	+5%

Appendix C

Animal Identity and Body-Weight Data

Animals. Albino rats of both sexes, of the Charles River strain, and obtained from a single shipment, were used in these studies. The individual animals were identified and housed in groups of six, both sexes separately. The animals were kept for a period of seven days after receipt and prior to start of the experiment in order to insure acclimatization. During the latter interval, individual body weights were recorded and daily water consumptions were determined to permit approximation of the appropriate drug concentrations required to begin the study.

Body-Weight Data. During the initial two weeks after beginning the experiments, body-weights were recorded twice weekly; after this, they were recorded weekly until the experiment was concluded. In originally grouping the animals, those individuals which were of questionable health were transferred to the control group; a surplus of animals justified such division without jeopardizing the value of the data subsequently obtained. During the course of the study, eleven animals died. As is usually the case under such circumstances, the majority of these individuals succumbed during the night, thus preventing the removal of representative tissue sections. Since no particular pattern was evident among these mortalities, it was concluded that they were the result of factors extraneous to the specific medication applied. Therefore, the individual body-weights of these animals have been excluded from the general body-weight data and are presented in Table 2, separately.

The average body-weight values of all surviving animals in each of the twenty experimental groups are set forth in Table 1; in addition, these values have been plotted in Figures 1, 2, 3, and 4 to facilitate examination.

Inhibition of growth at the time the experiment was started was not seen in any instance. Among the male animals receiving Ca Pantothenate, terminal growth at 200 mg/kg and at 20 mg/kg was slightly less than that of the controls; this difference was too small however, to be significant; the group receiving 50 mg/kg demonstrated a growth pattern identical to that of the controls. The growth curves of the female animals at all three dosage levels of Ca Pantothenate paralleled the growth curve of the female control group.

Identical results were obtained with dl-Panthenol. With d-Panthenol, the growth curves of all groups paralleled their respective controls.

It is concluded therefore, that the voluntary consumption of each of the compounds investigated at dosage levels of 20, 50, and 200 mg/kg for a period of 90 days did not influence the normal growth pattern of either the male or female rat.

Raymond J. Brooke, Jr.
Raymond J. Brooke, Jr.

Aaron B. Sloane
Aaron B. Sloane

Appendix C

Table 1. Average Body Weights (in Grams) of Rats Surviving Entire Experiment

Exptl Day	Ca Pantothenate 200 A	Ca Pantothenate 50 B	Ca Pantothenate 20 C	dl-Panthenol 200 D	dl-Panthenol 50 E	dl-Panthenol 20 F	n-Panthenol 200 G	n-Panthenol 50 H	n-Panthenol 20 I	Control 0 J	(mg/kg) (group)
MALES	6	4	6	6	6	6	5	4	5	6	(number)
-4	82	81	82	81	81	81	79	86	80	85	
0	100	105	104	105	102	102	104	106	103	104	
3	114	117	118	121	118	118	114	124	119	122	
5	124	132	128	130	131	130	124	139	132	132	
7	134	140	137	139	141	137	130	150	143	135	
11	153	170	162	164	169	163	153	180	171	151	
18	192	210	198	198	205	198	194	220	211	193	
25	219	240	226	235	238	230	229	253	247	227	
32	252	279	255	261	270	264	261	291	269	261	
39	264	298	246	276	287	269	288	310	289	284	
46	266	300	259	277	292	274	290	319	296	291	
52	277	304	282	289	307	286	299	330	307	304	
59	306	327	310	312	337	312	322	358	336	336	
66	315	337	313	319	336	316	330	366	341	348	
74	339	360	334	338	367	337	351	389	363	372	
81	358	381	348	353	383	361	371	412	382	389	
87	358	399	340	354	384	342	369	402	373	391	
90	364	397	335	360	381	353	368	410	377	396	
FEMALES	6	5	6	6	6	6	6	6	6	13	(number)
-4	75	75	76	75	75	75	78	74	75	69	
0	94	93	90	88	95	88	93	94	96	87	
3	110	104	100	96	106	99	105	107	108	95	
5	111	110	107	101	111	108	112	116	115	102	
7	120	115	117	110	119	117	119	126	120	111	
11	140	136	134	126	137	134	136	143	137	127	
18	160	160	153	145	159	152	156	165	161	144	
25	177	177	170	161	175	167	168	181	175	161	
32	187	190	178	175	189	182	181	192	186	174	
39	197	189	194	185	198	190	189	201	195	183	
46	201	204	200	187	202	192	190	204	200	188	
52	202	211	201	192	211	196	196	209	204	194	
59	215	223	211	205	221	208	215	225	218	211	
66	215	223	215	205	219	212	204	226	217	212	
74	223	249	225	217	234	222	218	233	233	224	
81	234	243	235	226	240	229	229	244	241	233	
87	239	249	241	232	245	237	231	248	243	237	
91	238	249	240	230	244	236	234	248	245	239	

Appendix C

Table 2. Individual Body Weights (in Grams) of Rats Dying During the Experiment.

Group	No.	Experimental Day -4	0	3	5	7	11	18	25	32	39	46	52	59	Day of Death
Males															
B	1	76	100	112	128	136	164	-	-	-	-	-	-	-	18th
B	4	84	104	118	132	140	168	210	250	290	322	320	-	-	51st
G	5	88	98	96	92	90	90	-	-	-	-	-	-	-	20th
H	2	76	104	120	130	144	166	200	236	-	-	-	-	-	27th
H	6	76	100	116	126	136	166	-	-	-	-	-	-	-	17th
I	5	86	110	128	142	146	140	176	218	246	260	266	-	-	51st
J	2	86	104	122	138	142	150	186	-	-	-	-	-	-	20th
J	4	76	92	114	110	114	130	144	122	150	-	-	-	-	35th
J	6	74	94	110	124	130	136	152	182	212	234	240	248	266	63rd
J	8	68	80	86	100	114	110	152	180	184	-	-	-	-	36th
Females															
B	6	74	74	70	74	88	82	-	-	-	-	-	-	-	11th

Appendix D

Hematological Studies

Hematological studies were conducted with three male and three female animals from each of the experimental groups; these were done immediately before the investigation was started (pre-test), half-way through the experiment (mid-test), and immediately prior to termination of the study (end-test).

Methods. Blood samples were obtained directly from the incised tail of each experimental animal and analyzed immediately. Standard procedures were applied in obtaining red cell and white cell counts; Wright's stain was used for the differential count and the hemoglobin concentration was determined colorimetrically by the acid-hematin method.

Data and Results. Individual leukograms are presented in Tables 1, 2, 3, and 4; the average values of the foregoing are presented in Table 5; and the average absolute values in Table 6. These data have been carefully studied for the presence of any significant abnormalities and for the presence of any specific trends of change. Compared with control values, no deviations from the latter norm have been found with the possible exception of the number of circulating eosinophils. In this instance, changes were not at all definite and were not of sufficient extent to be grossly apparent without scrutiny; the observed values were only slightly greater than normal but the trend toward increase seemed to be present with increasing dosage. In view of the relatively small number of animals studied, it is admittedly somewhat hazardous to draw any definite conclusions, especially when such data are in reference to small numbers of a total population. Based upon the number of eosinophils found in the blood of the control animals, the following arbitrary scale was observed, each plus representing an increase in the total number of circulating eosinophilic cells;

Group	Males	Females
A (200 mg/kg)	+	++
B (50 mg/kg)	±	+
C (20 mg/kg)	±	+
D (200 mg/kg)	0	++
E (50 mg/kg)	++	++
F (20 mg/kg)	0	+
G (200 mg/kg)	+	++
H (50 mg/kg)	0	++
I (20 mg/kg)	0	+

Perhaps it should be emphasized again that the foregoing is based upon changes which were a very low order and upon but a small number of animals; the observed trend may therefore not be significant. (In an attempt to substantiate the possibility of significance, sections of the lung and of the mesentaric lymph node were taken from the female animals during autopsy; in this instance it was felt that hyperconcentration of eosinophilic cells would be found in lung tissue if, indeed, a low degree of circulating eosinophilia had been present.).

(APPENDIX D CONTINUED)

INSOFAR AS THE OTHER LEUKOCYTIC ELEMENTS ARE CONCERNED, NO SIGNIFICANT CHANGES OR TRENDS OF CHANGE WERE OBSERVED. THE NUMBER OF CIRCULATING WHITE CELLS INCREASED GREATLY DURING THE COURSE OF THE EXPERIMENT AMONG ALL EXPERIMENTAL GROUPS OF ANIMALS; HOWEVER, THESE CHANGES DID NOT EXCEED IN EXTENT AS SIMILAR CHANGES AMONG THE CONTROL ANIMALS.

THE INDIVIDUAL ERYTHROCYTE COUNTS AND HEMOGLOBIN CONCENTRATIONS ARE PRESENTED IN TABLES 7 AND 8; AVERAGE VALUES ARE SET FORTH IN TABLE 9. BOTH THE TOTAL RBC AND HEMOGLOBIN CONCENTRATION WERE OBSERVED TO INCREASE SIGNIFICANTLY DURING THE STUDY; HOWEVER, THE DEGREE OF CHANGE WAS OBSERVED IN SIMILAR ORDER AMONG THE CONTROL ANIMALS AND IT IS THEREFORE NECESSARY TO CONCLUDE THAT THE CHANGES NOTED WERE NOT RELATED TO AGENTS CONSUMED.

FRANK J. LORZOLA

ALBERT R. LATVEN

Appendix D

Table 1. Leukograms: Ca Pantothenate

Group	Rat	MALES WBC, th	Differential, % L	St	Nr	Eo	Ba	Mon	Rat	FEMALES WBC, th	Differential, % L	St	Nr	Eo	Ba	Mon
Pre-Test																
A	#1	7.25	11	70	17	0	0	2	#1	8.55	18	68	14	0	0	0
	2	12.05	27	60	11	0	0	2	2	6.05	16	57	23	2	1	1
	3	6.25	23	52	23	1	0	1	3	5.60	22	59	19	0	0	0
B	#2	7.95	43	36	17	1	0	3	#1	10.75	21	58	20	1	0	0
	3	5.90	19	69	12	0	0	0	2	9.85	21	61	15	2	0	1
	4	9.45	10	78	9	3	0	0	3	7.75	17	69	13	0	0	1
C	#1	8.90	18	45	35	2	0	0	#1	6.25	17	64	15	3	0	1
	2	6.05	23	60	16	0	0	1	2	5.65	18	57	21	1	0	3
	3	5.85	11	62	26	1	0	0	3	10.10	21	61	15	0	0	3
Mid-Test																
A	#1	14.35	13	63	23	1	0	0	#1	12.60	17	68	14	1	0	0
	2	21.15	26	61	10	0	0	3	2	11.55	15	71	14	0	0	0
	3	12.25	17	61	17	3	0	2	3	8.15	20	65	13	1	0	1
B	#2	15.10	21	60	18	0	0	1	#1	11.30	23	62	11	3	0	1
	3	16.10	24	55	14	2	0	5	2	14.25	20	58	19	2	0	1
	4	15.85	18	60	19	1	0	2	3	5.50	19	56	21	3	0	1
C	#1	12.00	22	62	14	1	0	1	#1	12.45	21	72	6	0	0	1
	2	7.85	8	70	21	1	0	0	2	11.25	20	69	9	1	0	1
	3	10.15	14	61	23	1	0	1	3	13.45	21	66	13	0	0	0
End-Test																
A	#1	13.48	22	56	20	1	0	1	#1	9.60	12	68	11	8	0	1
	2	13.16	20	59	15	5	0	1	2	14.68	13	69	10	7	0	1
	3	13.48	18	46	27	6	0	3	3	9.24	7	66	17	9	0	1
B	#2	13.84	14	60	21	4	0	1	#1	9.84	18	68	11	1	1	1
	3	13.68	20	53	19	4	0	4	2	20.64	15	62	18	5	0	0
	5	11.20	14	57	27	0	0	2	3	7.60	6	65	22	6	0	1
C	#1	13.40	27	60	7	3	1	2	#1	8.16	15	66	13	2	0	4
	2	8.28	7	65	22	3	1	2	2	8.60	12	64	14	10	0	0
	3	10.40	10	71	14	4	0	1	3	8.52	11	56	26	6	0	1

TABLE 2. LEUKOGRAMS DL-PANTHENOL

		MALES								FEMALES							
			DIFFERENTIAL, %								DIFFERENTIAL, %						
GROUP	RAT	WBC, TH	LL	SL	MY	EO	BA	MON	RAT	WBC, TH	LL	SL	MY	EO	BA	MON
PRE-TEST																
D	#3	11.00	22	65	12	0	0	1	#1	7.35	16	67	16	1	0	0
	4	5.20	13	62	22	1	1	1	2	11.90	11	71	18	0	0	0
	5	8.25	23	51	23	2	0	1	3	13.60	22	58	18	0	0	2
E	#2	7.10	18	61	19	1	0	1	#1	16.90	18	55	22	3	0	2
	3	12.25	14	73	11	1	0	1	2	13.00	26	56	17	0	0	1
	5	12.00	13	64	20	1	0	2	4	4.50	18	65	17	0	0	0
F	#4	7.20	25	68	7	0	0	0	#4	11.00	23	51	23	2	0	1
	5	6.35	15	77	7	0	0	1	5	9.70	18	74	6	0	0	2
	6	8.55	13	78	6	2	0	1	6	9.50	24	61	12	0	0	3
MID-TEST																
D	#1	11.45	19	62	17	0	0	2	#1	16.05	26	62	10	1	0	1
	3	18.40	25	58	13	0	0	4	2	10.75	12	66	21	1	0	0
	4	14.55	26	58	13	0	0	3	3	12.70	11	74	9	6	0	0
	5	9.20	22	55	22	0	0	1								
E	#2	16.80	26	60	13	1	0	0	#1	13.05	23	63	13	1	0	0
	3	17.60	22	63	7	6	0	2	2	13.05	21	62	12	2	0	3
	5	13.85	17	66	16	1	0	0	4	6.85	28	56	15	0	0	1
F	#4	22.10	20	63	15	1	0	1	#4	6.60	20	68	8	2	0	2
	5	14.80	16	763	20	1	0	0	5	14.70	19	71	8	1	0	1
	6	10.85	13	66	19	2	0	0	6	13.50	26	59	14	0	0	1
END-TEST																
D	#3	13.60	11	65	23	0	0	1	#2	12.16	13	57	24	5	0	1
	4	8.00	15	63	18	2	0	2	3	16.40	9	59	24	7	0	1
	5	12.04	13	70	15	1	0	1	4	12.04	10	72	10	5	0	3
E	#2	15.72	13	62	19	5	0	1	#1	13.08	12	68	13	6	0	1
	3	9.40	12	71	10	6	0	1	2	14.32	9	59	23	7	0	2
	5	15.44	15	70	9	6	0	0	4	15.48	10	74	13	2	0	1
F	#4	6.20	13	68	16	2	0	1	#4	10.60	10	70	14	5	0	1
	5	10.24	16	63	16	4	0	1	5	16.64	16	70	10	2	0	2
	6	6.24	13	65	21	1	0	0	6	12.28	9	49	35	3	1	3

TABLE 3. LEUKOGRAMS: D-PANTHENOL

	MALES								FEMALES							
			DIFFERENTIAL, %								DIFFERENTIAL, %					
GROUP	RAT	WBC, TH	LL	SL	NT	EO	BA	MON	RAT	WBC, TH	LL	SL	NT	EO	BA	MON
PRE-TEST																
G	#2	4.60	14	74	12	0	0	0	#4	6.30	12	72	14	1	1	0
	3	6.50	14	63	23	0	0	0	5	8.75	14	76	9	0	0	1
	4	8.20	12	52	36	0	0	0	6	8.40	11	68	19	1	0	1
H	#1	5.90	21	71	8	0	0	0	#1	3.45	15	71	13	0	0	0
	8	6.00	21	59	19	0	0	1	2	9.15	12	63	20	2	1	2
	5	4.40	22	52	25	0	0	1	3	4.65	13	77	7	1	0	2
I	#4	6.15	27	61	9	0	0	3	#4	7.75	14	67	16	3	0	0
	5	6.10	22	69	9	0	0	0	5	12.05	18	66	14	2	0	0
	6	4.65	17	68	12	0	0	3	6	7.70	13	62	21	1	1	2
MID-TEST																
G	#2	12.75	20	63	14	1	0	2	#3	14.20	19	64	13	3	1	0
	3	14.35	31	57	10	0	0	2	4	15.80	20	67	11	1	0	1
	4	23.10	20	69	9	2	0	0	5	10.15	19	61	14	5	0	1
H	#1	15.85	18	66	15	0	0	1	#1	12.10	26	53	16	4	0	1
	4	15.80	19	68	13	0	0	0	2	6.60	21	63	13	3	0	0
	5	13.60	18	66	15	0	0	1	3	7.65	21	65	12	0	1	1
I	#4	15.25	18	74	7	1	0	0	#4	14.00	13	72	14	0	0	1
	5	17.70	10	69	20	0	0	1	5	12.05	17	65	12	2	1	3
	6	14.55	13	67	20	0	0	0	6	10.85	9	74	17	0	0	0
END-TEST																
G	#2	13.52	15	54	26	3	0	2	#3	18.84	9	55	25	10	0	1
	3	9.40	11	67	18	2	0	2	4	15.24	12	64	18	4	0	2
	4	8.56	14	65	13	7	0	1	5	8.12	15	60	19	4	0	2
H	#1	9.80	16	70	11	2	0	1	#1	12.96	10	64	17	8	0	1
	4	9.12	18	60	17	2	0	3	2	11.12	13	62	13	9	1	2
	5	7.08	11	63	20	2	1	3	3	9.12	10	69	10	6	1	4
I	#3	4.92	13	74	11	0	0	2	#4	20.88	14	79	4	1	0	2
	4	14.80	17	66	14	1	0	2	5	9.60	19	66	6	6	0	3
	6	9.80	12	59	21	5	1	2	6	12.32	15	67	15	2	0	1

Appendix D

Table 4. Leukograms: Controls

		MALES								FEMALES							
			Differential, %								Differential, %						
Group	Rat	WBC, th	LL	sL	Nt	Eo	Ba	Mon		Rat	WBC, th	LL	sL	Nt	Eo	Ba	Mon
Pre-Test																	
J	#1	5.85	18	56	23	1	0	2		#7	9.75	17	48	33	0	1	1
	2	6.35	17	75	5	0	0	3		8	10.30	16	73	11	0	0	0
	3	5.75	20	64	15	0	0	1		9	9.20	16	72	11	0	0	1
Mid-Test																	
J	#1	15.40	17	64	18	1	0	0		#7	14.20	19	68	12	0	0	1
	3	8.65	14	69	17	0	0	0		8	15.00	14	80	5	1	0	0
	7	14.10	19	63	15	1	0	2		9	18.50	23	61	11	2	0	3
End-Test																	
J	#1	12.72	6	58	34	1	0	1		#7	9.52	18	69	10	2	0	1
	3	8.48	15	65	16	3	0	1		8	12.84	13	68	15	2	0	2
	7	9.96	22	54	15	5	0	4		9	16.04	15	72	10	2	0	1

TABLE 5. LEUKOGRAMS: AVERAGE VALUES.

		MALES								FEMALES							
		TEST		DIFFERENTIAL, %						TEST		DIFFERENTIAL, %					
GROUP	TIME	WBC, TH	LL	SL	NT	EO	BA	MON		TIME	WBC, TH	LL	SL	NT	EO	BA	MON
CA PANTOTHENATE																	
A	PRE-	8.52	20	61	17	0	0	2		PRE-	6.73	19	61	19	1	0	0
	MID-	15.92	18	62	17	1	0	2		MID-	10.77	17	68	14	1	0	0
	END-	13.37	20	54	20	4	0	2		END-	11.17	10	68	13	8	0	1
B	PRE-	7.77	24	61	13	1	0	1		PRE-	9.45	18	63	17	1	0	1
	MID-	15.68	21	58	17	1	0	3		MID-	10.35	20	59	17	3	0	1
	END-	12.91	16	57	22	3	0	2		END-	12.69	13	65	17	4	0	1
C	PRE-	6.93	17	56	26	1	0	0		PRE-	7.33	19	61	17	1	0	2
	MID-	10.00	15	64	19	1	0	1		MID-	12.38	21	69	9	0	0	1
	END-	10.83	15	64	15	3	1	2		END-	8.43	13	63	16	6	0	2
DL-PANTHENOL																	
D	PRE-	8.15	19	60	19	1	0	1		PRE-	10.95	17	65	17	0	0	1
	MID-	13.40	23	58	16	0	0	3		MID-	13.17	17	67	13	3	0	0
	END-	11.21	13	66	19	1	0	1		END-	13.53	11	62	19	6	0	2
E	PRE-	10.45	15	66	17	1	0	1		PRE-	11.47	20	59	19	1	0	1
	MID-	16.08	22	63	11	3	0	1		MID-	10.98	24	60	14	1	0	1
	END-	13,52	13	67	13	6	0	1		END-	15.96	10	67	17	5	0	1
F	PRE-	7.37	17	74	7	1	0	1		PRE-	9.90	21	62	14	1	0	2
	MID-	15.92	17	64	18	1	0	0		MID-	11.60	22	66	10	1	0	1
	END-	7.56	14	65	18	2	0	1		END-	13.84	12	63	20	3	0	2
D-PANTHENOL																	
G	PRE-	6.43	13	63	24	0	0	0		PRE-	7.82	12	72	14	1	0	1
	MID-	16.73	24	63	11	1	0	1		MID-	13.38	19	64	13	3	0	1
	END-	10.49	13	62	19	4	0	2		END-	14.07	12	60	20	6	0	2
H	PRE-	5.10	21	61	17	0	0	1		PRE-	5.75	14	70	14	1	0	1
	MID-	15.08	18	67	14	0	0	1		MID-	8.78	23	60	14	2	0	1
	END-	8.67	15	64	16	2	0	3		END-	11.07	11	65	13	8	1	2
I	PRE-	5.63	22	66	10	0	0	2		PRE-	9.17	15	65	17	2	0	1
	MID-	15.83	14	70	16	0	0	0		MID-	12.30	14	70	14	1	0	1
	END-	12.51	14	66	16	2	0	2		END-	14.27	16	71	8	3	0	2
CONTROLS																	
J	PRE-	5.98	19	65	14	0	0	2		PRE-	9.75	16	64	18	0	0	1
	MID-	12.72	17	65	16	1	0	1		MID-	15.90	19	70	9	1	0	1
	END-	10.39	14	59	22	3	0	2		END-	12.80	15	70	12	2	0	1

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Table 6. Leukograms: Average Absolute Values. Cells/cu mm.

Group	Test Time	Males: Lymphocytes	Males: Granulocytes	Males: Monocytes	Males: Eosinophils	Females: Lymphocytes	Females: Granulocytes	Females: Monocytes	Females: Eosinophils
Ca Pantothenate									
A	Pre-	6900	1450	170	26	5380	1350	0	45
	Mid-	12730	2870	320	248	9170	1620	0	72
	End-	9900	3210	270	550	8710	2350	110	893
B	Pre-	6600	1090	80	100	7650	1700	90	95
	Mid-	12390	2820	470	157	8180	2070	100	276
	End-	9430	3230	260	345	9900	2670	130	508
C	Pre-	5060	1870	0	69	5860	1320	150	98
	Mid-	7900	2000	100	100	9900	1110	120	41
	End-	8560	2060	220	358	6410	1860	170	506
dl-Panthenol									
D	Pre-	6440	1630	80	82	8980	1860	110	36
	Mid-	10850	2150	400	0	11050	2110	0	352
	End-	8860	2420	110	112	9880	3380	270	770
E	Pre-	8470	1880	100	105	9060	2290	110	115
	Mid-	13670	2250	160	434	9230	1650	110	110
	End-	10820	2570	140	772	12280	3510	110	800
F	Pre-	6710	590	70	52	8020	1490	200	66
	Mid-	12900	3020	0	207	10210	1280	120	116
	End-	5970	1510	80	174	10380	3180	280	460
d-Panthenol									
G	Pre-	4890	1540	0	0	6560	1170	80	52
	Mid-	14550	2010	170	167	11100	2140	140	402
	End-	8220	2520	220	420	10120	3660	280	835
H	Pre-	4180	870	50	0	4830	860	60	76
	Mid-	12800	2110	150	0	7290	1410	90	204
	End-	6850	1560	260	173	8410	2440	220	850
I	Pre-	4950	560	110	0	7330	1740	90	183
	Mid-	13290	2540	0	53	10330	1850	120	82
	End-	10020	2250	250	250	12400	1570	290	428
Controls									
J	Pre-	5020	840	120	20	7800	1750	100	0
	Mid-	10430	2170	130	89	14140	1530	160	159
	End-	7590	2600	210	313	10880	1790	130	256

TABLE 7. ERYTHROGRAMS

Ca PANTOTHENATE

Group	Rat	MALES RBC,M	MALES Hb,GM	Rat	FEMALES RBC,M	FEMALES Hb,GM
PRE-TEST						
A	#1	4.28	14.9	#1	5.21	13.2
	2	5.13	13.4	2	5.84	14.0
	3	5.51	13.1	3	5.68	14.9
B	#2	4.49	11.4	#1	4.86	15.9
	3	5.61	13.7	2	6.03	15.9
	4	4.66	14.4	3	5.49	14.9
C	#1	4.59	14.8	#1	4.69	14.7
	2	5.35	14.0	2	5.12	14.9
	3	4.48	13.2	3	5.13	13.3
MID-TEST						
A	#1	6.81	18.8	#1	8.26	18.7
	2	7.46	17.0	2	7.39	17.7
	3	7.24	17.0	3	7.63	17.3
B	#2	6.32	17.7	#1	8.85	17.2
	3	6.38	17.3	2	7.05	17.8
	4	7.40*	16.5	3	7.40	19.0
C	#1	7.37	17.8	#1	7.30	16.7
	2	5.12	18.2	2	7.03	17.2
	3	7.04	16.7	3	7.54	18.3
END-TEST						
A	#1	7.48	19.1	#1	8.24	16.3
	2	6.58	17.6	2	7.06	16.6
	3	7.36	17.6	3	7.53	15.8
B	#2	7.29	17.0	#1	6.99	16.6
	3	8.05	18.3	2	7.17	16.9
	5	7.59	17.7	3	8.26	16.6
C	#1	7.62	17.6	#1	9.04	17.3
	2	7.09	18.0	2	7.86	15.8
	3	7.79	17.0	3	7.10	16.7

*MICROCYTOSIS

DL-PANTHENOL

Group	Rat	MALES RBC,M	MALES Hb,GM	Rat	FEMALES RBC,M	FEMALES Hb,GM
PRE-TEST						
D	#3	5.52	14.9	#1	4.93	15.4
	4	4.25	11.7	2	5.18	15.4
	5	5.51	14.5	3	5.30	13.2
E	#2	5.63	13.4	#1	6.19	16.2
	3	4.65	13.9	2	6.12	15.1
	5	4.64	12.2	4	5.36	13.6
F	#4	4.62	13.2	#4	5.06	14.0
	5	5.28	14.3	5	5.49	14.9
	6	4.99	12.8	6	5.50	14.0
MID-TEST						
D	#1	6.60	17.4	#1	6.48	17.2
	4	6.23	17.1	2	7.78	18.0
	#5	7.89	19.1	3	7.53	17.2
	3	7.89	18.2			
E	#2	6.93	17.6	#1	7.83	17.8
	3	7.02	16.7	2	6.50	17.7
	5	5.99	17.3	4	5.89	17.3
F	#4	7.71	18.4	#4	6.14	14.5
	5	7.94	17.4	5	6.29	15.1
	6	6.95	18.6	6	7.52	14.9
END-TEST						
D	#3	8.21	17.6	#2	8.03	15.7
	4	6.67	16.3	3	8.17	16.9
	5	7.56	19.5	4	6.91	16.3
E	#2	8.12	17.3	#1	9.08	16.3
	3	5.97	17.6	2	7.75	17.3
	5	7.35	18.0	4	7.39	16.0
F	#4	8.17	17.6	#4	6.61	17.3
	5	8.04	17.5	5	7.53	17.3
	6	6.91	18.7	6	8.09	15.7

Appendix D

Table 8. Erythrograms.

D-Panthenol

Group	Rat	Males RBC,M	Males Hb,gm	Rat	Females RBC,M	Females Hb,gm
Pre-Test						
G	#2	4.53	12.3	#4	4.83	15.2
	3	5.07	13.6	5	5.46	14.7
	4	4.37	12.7	6	6.22	17.0
H	#1	5.13	15.0	#1	5.03	15.4
	8	5.73	12.7	2	5.32	14.0
	5	5.11	15.4	3	5.67	17.0
I	#4	5.24	13.5	#4	5.15	14.0
	5	5.48	14.1	5	5.34	14.2
	6	5.50	14.0	6	5.00	14.3
Mid-Test						
G	#2	7.37	18.6	#3	6.25	18.3
	3	6.71	15.8	4	6.96	18.4
	4	7.16	17.1	5	8.07	18.0
H	#1	5.54	15.4	#1	7.61	17.1
	4	7.28	18.2	2	5.90	16.2
	5	6.37	16.5	3	6.99	17.2
I	#4	7.51	18.8	#4	8.11	17.7
	5	7.07	17.2	5	7.41	17.5
	6	6.92	18.2	6	8.03	18.8
End-Test						
G	#2	9.14	18.2	#3	7.90	16.9
	3	7.64	16.9	4	9.30	17.6
	4	7.24	16.9	5	7.02	16.3
H	#1	9.64	18.3	#1	7.07	16.9
	4	8.30	17.5	2	7.55	16.6
	5	7.51	17.6	3	9.20	17.6
I	#3	8.05	17.8	#4	6.69	14.7
	4	8.29	17.5	5	7.52	16.3
	6	8.97	17.6	6	6.67	15.7

Controls

Group	Rat	Males RBC,M	Males Hb,gm	Rat	Females RBC,M	Females Hb,gm
Pre-Test						
C	#1	4.84	13.2	#7	5.89	15.2
7	2	4.68	14.2	8	5.67	13.8
	3	4.96	13.4	9	4.90	15.2
Mid-Test						
J	#1	6.96	17.4	#7	7.54	16.0
	3	5.78	16.8	8	6.43	16.5
	7	6.70	16.7	9	6.58	15.7
End-Test						
J	#1	7.48	17.6	#7	6.66	16.9
	3	7.32	18.1	8	6.88	16.0
	7	8.50	17.0	9	5.41	16.3

TABLE 9. ERYTHROGRAMS AVERAGE VALUES.

GROUP	TEST TIME	MALES RBC.M	MALES Hb.GM	FEMALES RBC.M	FEMALES Hb.GM
Ca PANTOTHENATE					
A	PRE-	4.97	13.8	5.58	14.0
	MID-	7.17	17.6	7.76	17.9
	END-	7.14	18.1	7.61	16.2
B	PRE-	4.92	13.2	5.46	15.6
	MID-	6.70	17.2	7.77	18.0
	END-	7.64	17.7	7.47	16.7
C	PRE-	4.81	14.0	4.98	14.3
	MID-	6.51	17.6	7.29	17.4
	END-	7.50	17.5	8.00	16.6
DL-PANTHENOL					
D	PRE-	5.07	13.7	5.15	14.7
	MID-	7.15	17.9	7.26	17.5
	END-	7.48	17.8	7.70	16.3
E	PRE-	4.97	13.2	5.89	15.0
	MID-	6.65	17.2	6.74	17.6
	END-	7.15	17.6	8.07	16.5
F	PRE-	4.96	13.4	5.35	14.3
	MID-	7.53	18.1	6.65	14.8
	END-	7.71	17.9	7.41	16.8
D-PANTHENOL					
G	PRE-	4.80	12.9	5.50	15.6
	MID-	7.08	17.2	7.09	18.2
	END-	8.01	17.3	8.07	16.9
H	PRE-	5.32	14.4	5.34	15.5
	MID-	6.40	16.7	6.83	16.8
	END-	8.48	17.8	7.94	17.0
I	PRE-	5.41	13.9	5.16	14.2
	MID-	7.17	18.1	7.85	18.0
	END-	8.44	17.6	6.96	15.6
CONTROLS					
J	PRE-	4.83	13.6	5.49	14.7
	MID-	6.48	17.0	6.85	16.1
	END-	7.77	17.6	6.32	16.4

Appendix E

Organ Weight Data

The investigation was terminated after 90 experimental days, at which time all animals were sacrificed by decapitation and exsanguination. Following gross examination of the viscera, individual weights of the spleen, liver, kidneys, adrenals, and testes were obtained after freeing these organs from extraneous membranes and tissues. These data are presented in Tables 1, 2, 3, and 4; in addition, the individual weights of the brains removed from one animal of each group are presented in Table 6. For purposes of brevity, as well as accuracy, the average values of the weights of the various organs according to therapy are presented in Table 5.

In the main, the organ weights of the experimental animals did not deviate much more than 10% from the organ weights of the control animals. Exceptions, of course, were noted but these did not appear to reflect any relationship to the drugs consumed nor to the dose consumed. The adrenal glands of the male rats receiving Ca Pantothenate were somewhat heavier than those of the controls but the difference was not sufficiently great to permit any definite conclusions; in contrast, the adrenal glands of the female rats receiving the same compound seem to possess a weight somewhat less than their respective controls.

In view of the low order of observed differences of organ weights it cannot be justifiably concluded that any of the organs examined differed significantly in weight from the comparative weights of the control animals.

Bernard L. Kabacoff

Bernard L. Kabacoff

Appendix E

Table 1. Organ Weights: Ca Pantothenate (Groups A, B, and C)

Group	Rat No.	Organ Weights in Grams					Body Weight
		Spleen	Liver	Testes	Kidneys	Adrenals	
MALES							
A	1	1.36	12.09	3.12	2.31	0.042	340
	2	0.91	13.60	3.37	2.71	0.044	374
	3	1.11	13.02	3.15	2.50	0.050	370
	4	1.00	12.99	2.92	2.48	0.041	366
	5	1.07	12.27	3.16	2.54	0.047	342
	6	1.05	13.01	3.27	2.83	0.028	394
B	2	0.95	12.22	3.33	2.70	0.054	416
	3	1.33	11.93	3.36	2.78	0.040	376
	5	1.45	15.46	3.11	2.63	0.045	418
	6	1.47	13.78	3.45	2.38	0.042	376
C	1	1.09	10.74	3.46	2.21	0.045	330
	2	1.41	15.22	3.47	3.20	0.050	374
	3	1.54	11.29	3.43	2.67	0.033	348
	4	0.94	10.43	2.99	2.00	0.033	274
	5	1.14	12.08	2.86	2.34	0.040	352
	6	1.17	11.49	3.43	2.70	0.052	330
FEMALES							
A	1	0.97	7.70	--	1.56	0.057	230
	2	0.99	8.24	--	1.71	0.052	252
	3	0.90	8.06	--	1.57	0.050	242
	4	0.88	8.55	--	1.69	0.041	230
	5	0.84	7.81	--	1.70	0.054	234
	6	0.80	8.08	--	1.51	0.051	240
B	1	0.99	8.34	--	1.65	0.052	268
	2	0.93	7.88	--	1.50	0.061	212
	3	0.87	9.10	--	1.84	0.053	268
	4	0.95	9.30	--	1.82	0.044	254
	5	0.78	7.92	--	1.58	0.062	242
C	1	0.93	7.95	--	1.58	0.064	228
	2	1.01	7.59	--	1.52	0.055	240
	3	1.10	9.33	--	1.72	0.071	262
	4	0.80	8.17	--	1.56	0.049	228
	5	0.96	8.36	--	1.70	0.061	254
	6	0.75	7.73	--	1.38	0.046	228

TABLE 2. ORGAN WEIGHTS: DL-PANTHENOL (GROUPS D, E, AND F)

Group	Rat No.	Organ Weights in Grams: Spleen	Liver	Testes	Kidneys	Adrenals	Body Weight
MALES							
D	1	1.60	10.81	3.31	2.50	0.031	324
	2	1.01	11.83	2.79	2.50	0.041	352
	3	1.30	9.71	3.07	1.97	0.034	340
	4	1.35	13.40	3.37	3.12	0.039	436
	5	1.14	9.60	2.66	2.11	0.027	318
	6	1.39	11.78	3.41	2.69	0.044	390
E	1	1.42	12.15	3.24	2.63	0.037	400
	2	1.34	10.11	3.63	2.75	0.039	398
	3	1.29	11.87	3.18	3.02	0.038	394
	4	1.54	11.48	3.78	2.82	0.045	378
	5	1.11	10.82	3.89	2.73	0.046	364
	6	0.87	9.00	3.19	2.85	0.042	354
F	1	0.91	8.87	3.26	2.29	0.045	344
	2	1.30	11.48	3.14	3.01	0.052	412
	3	1.18	11.53	3.31	2.31	0.038	348
	4	1.02	9.44	3.26	2.55	0.035	340
	5	1.24	12.80	3.14	2.67	0.045	378
	6	0.94	9.25	2.65	2.16	0.039	296
FEMALES							
D	1*	1.84*	7.68*	--	1.66*	0.053*	242
	2	0.83	6.93	--	1.55	0.051	220
	3	0.85	7.66	--	1.65	0.048	242
	4	0.96	8.83	--	1.68	0.058	244
	5	1.05	7.02	--	1.61	0.052	232
	6	1.30	7.71	--	1.42	0.045	198
E	1	0.93	7.61	--	1.52	0.048	228
	2	0.80	6.99	--	1.46	0.047	228
	3	0.87	8.60	--	1.72	0.053	228
	4	1.23	9.14	--	1.76	0.067	260
	5	1.12	8.92	--	1.89	0.063	258
	6	0.98	8.76	--	1.87	0.070	260
F	1	0.74	7.70	--	1.59	0.050	234
	2	0.82	8.47	--	1.74	0.067	236
	3	0.84	6.63	--	1.53	0.050	210
	4	0.86	7.51	--	1.60	0.055	238
	5	1.18	9.44	--	1.99	0.069	260
	6	1.21	9.21	--	1.77	0.043	240

*RAT NOT EXSANGUINATED BEFORE AUTOPSY.

Appendix E

Table 3. Organ Weights: D-Panthenol (Groups G, H, and I)

Group	Rat No.	Organ Weights in Grams					Body Weight
		Spleen	Liver	Testes	Kidneys	Adrenals	
MALES							
G	1	1.00	12.00	3.39	2.67	0.052	376
	2	1.26	10.53	3.06	2.77	0.036	386
	3	1.18	10.39	3.66	2.73	0.026	378
	4	1.07	9.40	3.25	2.62	0.038	368
	6	1.16	9.70	3.09	2.94	0.033	332
H	1	1.23	13.40	3.17	2.93	0.035	428
	3	1.28	12.80	3.46	2.73	0.034	410
	4	1.53	14.25	3.55	2.96	0.045	410
	5	1.18	13.33	3.04	3.00	0.044	390
I	1	1.01	13.20	3.36	2.75	0.032	376
	2	0.96	10.65	2.96	2.61	0.046	374
	3	1.34	11.13	3.18	3.05	0.047	396
	4	1.14	11.39	3.31	2.86	0.048	390
	6	0.94	8.70	3.18	2.29	0.040	350
FEMALES							
G	1	0.83	8.16	--	1.54	0.053	232
	2	0.70	8.92	--	1.85	0.064	260
	3	0.67	7.54	--	1.66	0.058	232
	4	1.03	8.73	--	1.64	0.051	246
	5	0.60	7.66	--	1.45	0.055	218
	6	0.52	7.67	--	1.55	0.053	216
H	1	1.23	9.18	--	1.83	0.053	270
	2	1.42	8.41	--	1.53	0.048	240
	3	1.02	7.94	--	1.59	0.038	262
	4	0.87	8.68	--	1.85	0.046	254
	5	0.72	7.39	--	1.61	0.051	244
	6	0.80	6.94	--	1.38	0.040	216
I	1	1.27	10.93	--	1.99	0.067	268
	2	0.96	7.69	--	1.55	0.047	220
	3	1.05	9.00	--	1.84	0.053	242
	4	1.30	8.43	--	1.64	0.068	234
	5	0.97	8.51	--	1.77	0.051	224
	6	1.28	10.37	--	1.84	0.073	276

APPENDIX E

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TABLE 4. ORGAN WEIGHTS: CONTROLS (GROUP J)

GROUP	RAT NO.	ORGAN WEIGHTS IN GRAMS					BODY WEIGHT
		SPLEEN	LIVER	TESTES	KIDNEYS	ADRENALS	
MALES							
J	1	1.37	12.85	3.24	2.57	0.035	356
	3	1.31	11.65	3.02	2.61	0.036	364
	5	1.16	10.01	2.90	2.28	0.020	305
	7	1.28	15.84	3.45	3.45	0.043	456
	9	1.88	15.88	3.38	3.14	0.045	472
	10	0.93	11.82	3.56	2.45	0.044	380
FEMALES							
J	1	1.28	7.71	---	1.73	0.074	218
	2	1.15	8.39	---	1.76	0.072	240
	3	0.80	9.09	---	2.02	0.074	278
	7	1.20	8.74	---	1.53	0.047	224
	8	0.94	7.89	---	1.54	0.052	222
	9	1.25	7.08	---	1.50	0.051	222

TABLE 5. ORGAN WEIGHTS, AVERAGE VALUES

DRUG	DOSE MG/KG	GROUP	AV. ORGAN WEIGHTS IN GRAMS SPLEEN	LIVER	TESTES	KIDNEYS	ADRENALS	BODY WEIGHT
MALES								
CA P	200	A	1.08	12.83	3.17	2.56	0.042	364
	50	B	1.30	13.35	3.31	2.62	0.046	397
	20	C	1.22	11.88	3.27	2.52	0.042	335
DL-P	200	D	1.30	11.19	3.10	2.48	0.036	360
	50	E	1.26	10.91	3.49	2.80	0.041	381
	20	F	1.10	10.56	3.13	2.50	0.042	353
D-P	200	G	1.13	10.40	3.29	2.75	0.037	368
	50	H	1.31	13.45	3.31	2.91	0.040	410
	20	I	1.08	11.01	3.20	2.71	0.043	377
CONTROL	0	J	1.32	13.01	3.26	2.75	0.037	396
FEMALES								
CA P	200	A	0.90	8.07	---	1.62	0.051	238
	50	B	0.90	8.51	---	1.68	0.054	249
	20	C	0.93	8.19	---	1.58	0.058	240
DL-P	200	D*	1.00	7.63	---	1.58	0.051	227
	50	E	0.99	8.34	---	1.70	0.058	244
	20	F	0.94	8.16	---	1.70	0.056	236
D-P	200	G	0.73	8.11	---	1.62	0.056	234
	50	H	1.01	8.09	---	1.63	0.046	248
	20	I	1.14	9.14	---	1.77	0.060	245
CONTROL	0	J	1.10	8.15	---	1.68	0.062	234

* THE AVERAGE VALUES DO NOT INCLUDE RAT DF-1 (TABLE2) WHICH WAS NOT EXSANGUINATED BEFORE AUTOPSY.

Appendix E

Table 6. Brain-Weight Data

Group	Males			Females		
	Rat	Brain Wgt	Body Wgt	Rat	Brain Wgt	Body Wgt
A	--	--	--	#3	1.83	242
B	#3	2.04	376	1	1.89	268
C	2	1.96	374	2	1.87	240
D	#6	1.89	390	#3	1.88	242
E	4	2.03	378	1	1.71	228
F	5	1.92	378	6	1.88	240
G	#1	1.89	376	#4	1.72	246
H	5	2.03	390	2	1.81	240
I	1	2.05	376	5	1.65	224
J	#10	1.94	380	#7	1.71	224

Appendix F

Autopsies and Tissue Sections

Immediately after sacrifice of each animal by decapitation (and exsanguination) at the conclusion of the experiment, autopsies were conducted and the various visceral organs examined for deviations from normal appearance or structure. Of the one hundred and twenty animals examined at that time, no abnormalities were observed except in two instances: (1) Rat Gm-6 was found to possess an infected (?) right kidney, and (2) Rat Ff-6 possessed an enlarged, fluid-filled mesenteric lymph node containing large numbers of mature lymphocytes and but a few scattered granulocytes. Obviously, these exceptions do not possess significance.

Representative sections of the following organs were removed and placed in 1:10 formaldehyde: Liver, kidney, spleen, adrenal, gonad, stomach, intestine, heart, thyroid, femoral bone marrow, and sciatic nerve; also, sections of the lung and mesenteric lymph node were taken from all of the female rats. The entire brain, cerebrum, cerebellum and medulla was removed from one male and from one female animal of each of the ten experimental groups and placed individually in properly labeled containers. All of these tissues were submitted to the Hoffmann-LaRoche Laboratories for histological examination on September 18, 1953.

Aaron B. Sloane
Aaron B. Sloane

Frank J. Zurzola
Frank J. Zurzola

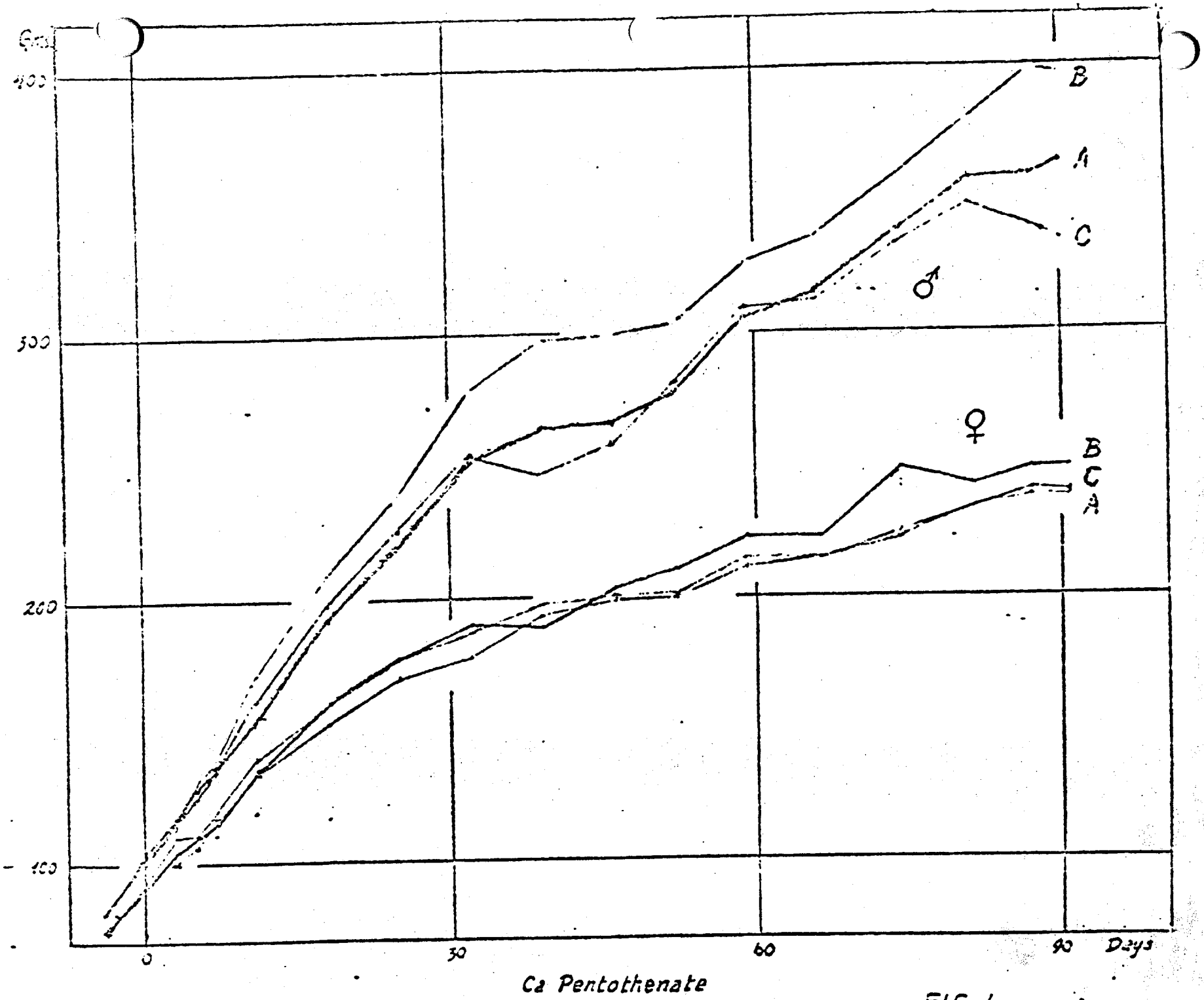


FIG. 1.

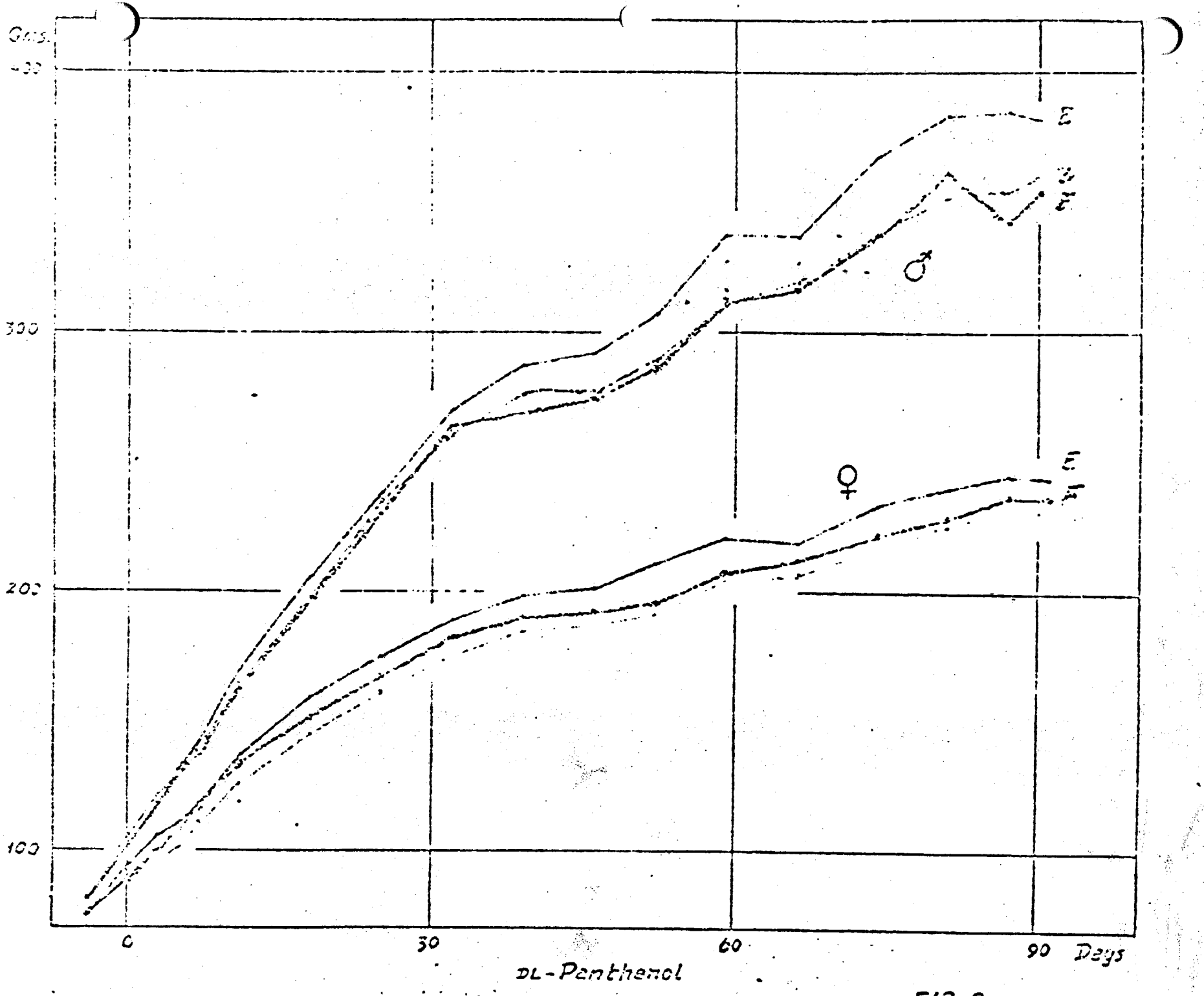
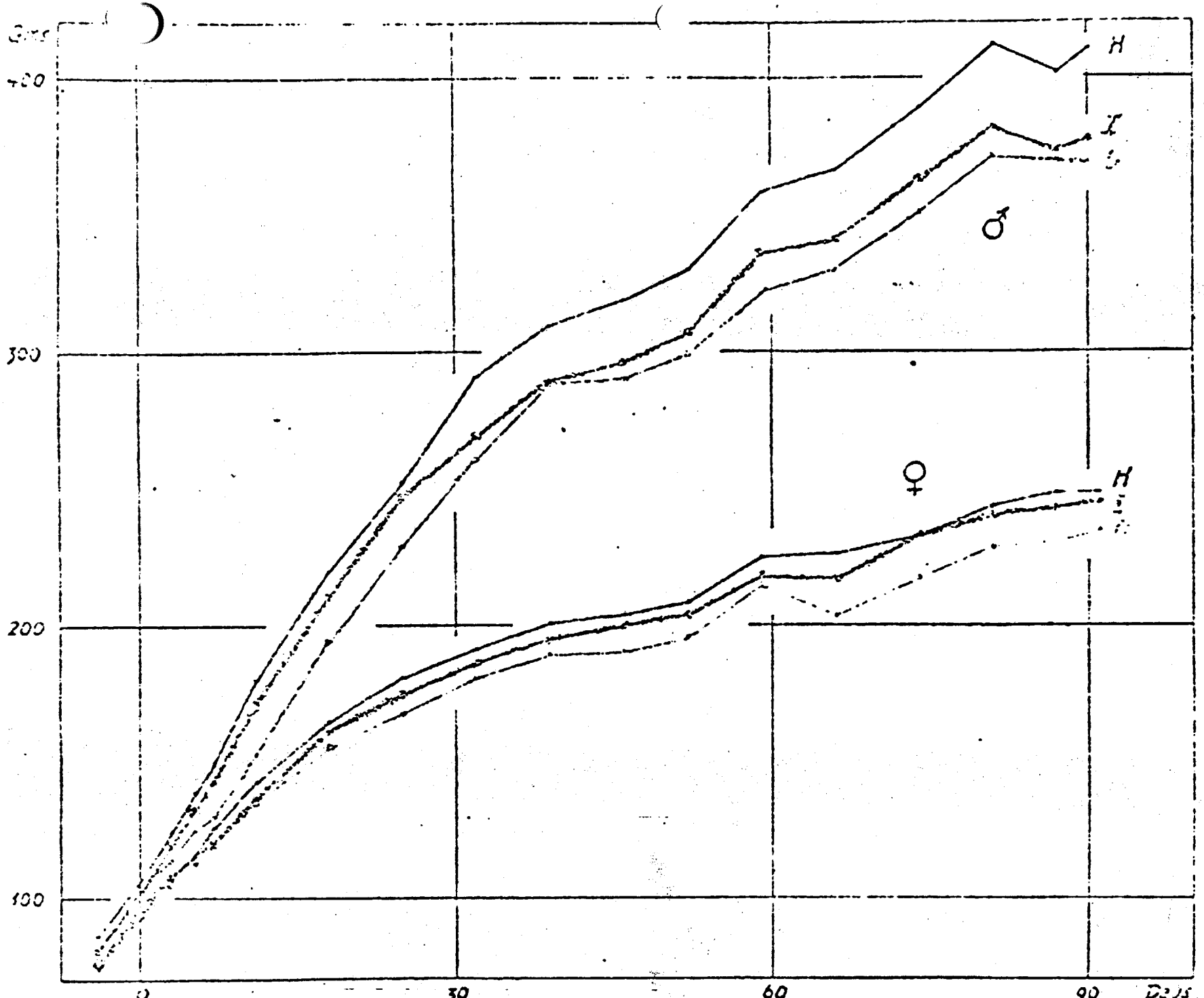


FIG. 2.



D- Panthenol

FIG. 3.

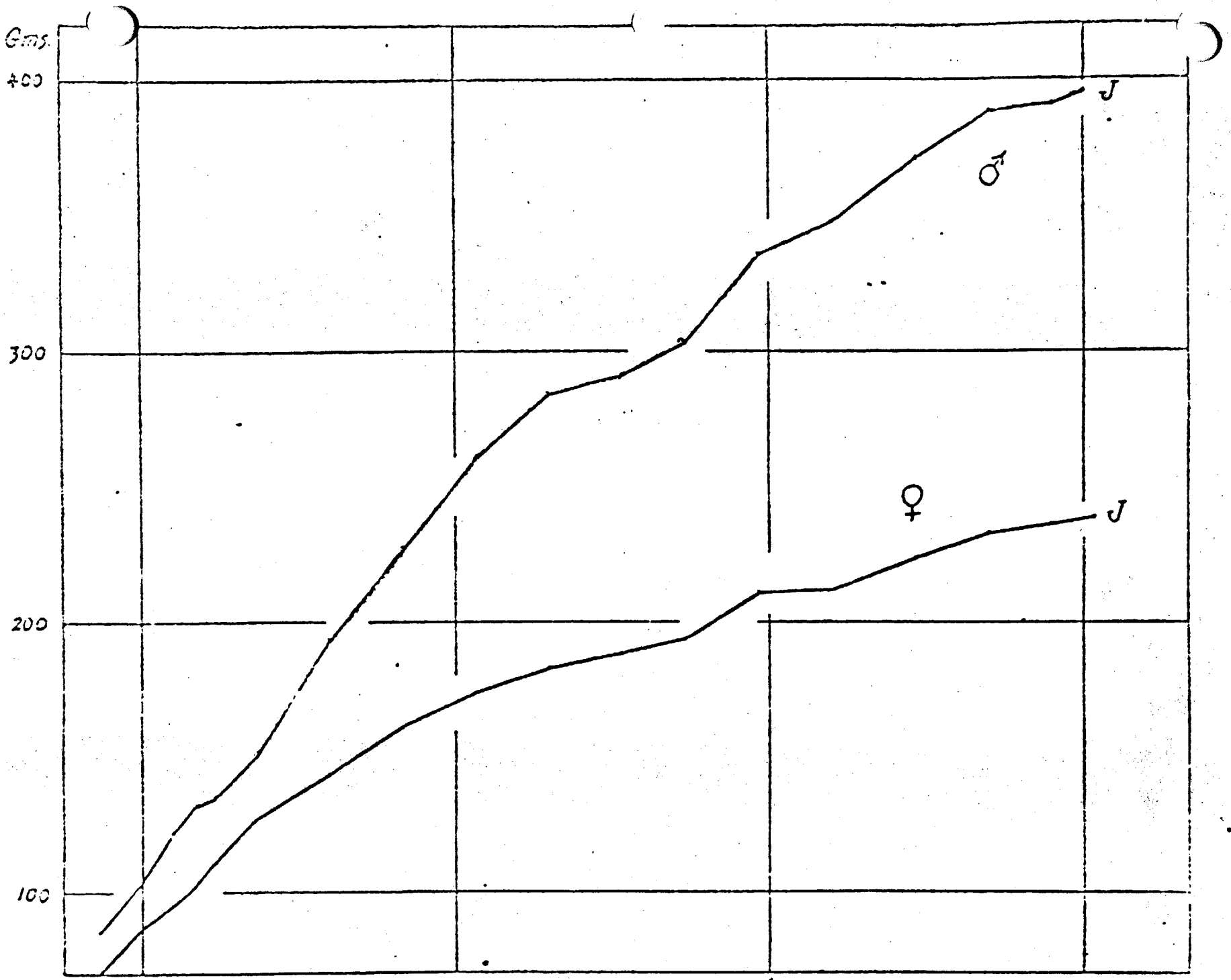


FIG. 4.

August 25, 1954 [redacted] 0141 66

Report from Wilbur M. Benson

Research Department Pharmacology to Management

Toxicity and Activity of d-Panthenol and Calcium d-Pantothenate

Toxicity and Activity of d-Panthenol and Calcium d-Pantothenate

The intravenous toxicity of d-Panthenol (Ro 1-4707/29 and Ro 2-9623) has been investigated in mice, rabbits and dogs. A 25 per cent aqueous solution of the agent (Lot #152) was used. Fresh solutions have a pH of about 9 but these may be buffered with HCl to a pH 7. The same thing may be accomplished by letting solutions stand overnight at room temperature. The tests reported below were carried out on solutions which had stood overnight prior to use. Studies on Calcium d-Pantothenate (Ro 1-4519) also are reported. For additional detailed information on this latter agent one may consult a publication by Unna and Greslin[1].

Results

Acute Intravenous Toxicity in Mice

It may be seen from the following data that d-Panthenol, administered intravenously to mice, is of a very low order of toxicity. No deaths occurred at 4.0 grams/kgm and only 33 per cent mortality occurred at 10.0 grams/kgm. Thus the intravenous LD50 in mice of d-Panthenol may be considered to be over 10 grams/kgm.

Calcium d-Pantothenate, on the other hand, given intravenously to mice was found to have an LD50 of 685 mgm/kgm. No deaths occurred at 500 mgm/kgm and 100 per cent mortality occurred at 800 mgm/kgm. These results essentially agree with those reported by Unna and Greslin.

Agent	Dose mgm/kgm	Vol. in cc of 25% Solution per 20 gm	Number of Mice Injected	Number of Mice Died	% Death	LD50 in mgm/kgm
d-Panthenol	4,000	0.32	6	0	0	> 10,000
	8,000	0.64	6	1	16.7	
	9,000	0.76	6	2	33.3	
	10,000	0.80	9	3	33.3	
Calcium d-Pantothenate	500	0.040	6	0	0	685
	600	0.048	6	1	16.7	
	700	0.056	6	3	50.0	
	800	0.064	6	6	100.0	

(1) Unna and Greslin
J.P.E.T. 73: 85, 1941

Acute Intravenous Toxicity in Rabbits of d-Panthenol

In intravenous tests in rabbits d-Panthenol was given to 14 animals with dosage ranging from 100 mgm/kgm to 10 grams/kgm. The average volume varied from 1.0 cc to 95 cc and the average time for injection varied from 1 to about 70 minutes. The large volume and relatively high viscosity of the solution necessitated a prolonged interval for injection. As can be seen from the table below, no deaths occurred from the acute administration of the material. The animals were observed for a total period of ten days. All of the animals remained alive.

Dose mgm/kgm	Av. Vol. in cc of a 25% Sol./ 2.5 kgm Rabbit	Av. Time in Min. for Inj.	No. of Rabbits Inj.	No. of Rabbits Died	% Death	LD50 in mgm/kgm
100	1.0	1	2	0	0	
500	4.8	2	2	0	0	
1,000	10.5	5	2	0	0	> 10,000
2,000	22.4	15	2	0	0	
4,000	41.3	30	2	0	0	
8,000	88.6	60	2	0	0	
10,000	94.8	70	2	0	0	

Acute Toxicity of Calcium d-Pantothenate As Reported by Unna and Greslin (J.P.E.T. 73: 85, 1941)

Species	LD50 in Grams/Kgm P.O.	S.C.	I.P.	I.V.
Mice	10.0	2.7	0.92	0.91
Rats	> 10.0	3.4	0.82	0.83

Unna and Greslin reported that "lethal doses produced prostration and respiratory failure in rats and mice. Death occurred within 2 hours following intravenous and intraperitoneal injection. No late deaths were observed."

Acute Intravenous Toxicity of d-Panthenol in the Dog

d-Panthenol, also in the form of 25 per cent solutions which were retained overnight before using, was administered intravenously to nine dogs in dosage varying from 2 to 10 grams/kgm. No deaths occurred in these animals (Reference: Stefko memos of 7/23/54 and 8/4/54).

Acute Intravenous Toxicity of d-Panthenol in the Dog (continued)

Dose grams/kgm	No. of Dogs	No. Survived	No. Died	% Death
2	2	2	0	0
5	2	2	0	0
10	5*	5	0	0

No signs of toxicity were evident among the above-injected animals which were observed for a period of seven days. *An additional dog in poor physical condition, which was given 10 grams/kgm, became ataxic within three minutes from the time of injection. This was followed by vomiting, defecation, urination, convulsions with hyperextension of the extremities and death within two hours by respiratory failure.

Effect of d-Panthenol and Calcium d-Pantothenate on Isolated Intestine

d-Panthenol and Calcium d-Pantothenate were tested for their effects on normal motility and against acetylcholine and histamine-induced spasms of isolated rabbit intestine. With d-Panthenol no effect was observed at 10^{-3} grams/cc. The same concentration of Calcium d-Pantothenate produced a slight relaxation of the normally responding segment of intestine but no effect occurred on the acetylcholine-induced spasm.

	d-Panthenol	Calcium d-Pantothenate
Normal Motility	No effect at 10^{-3} gm/cc	Slight relaxation at 10^{-3} gm/cc
vs. Acetylcholine Spasm	" " " " "	No effect at 10^{-3} gm/cc
vs. Histamine Spasm	" " " " "	

Summary

d-Panthenol, administered intravenously as a 25 per cent solution with a pH of 7, was found to possess a low order of toxicity in mice, rabbits and dogs. In these species the LD50 exceeded 10 grams/kgm. Calcium d-Pantothenate, studied in mice, was found to have an LD50 less than 1 gram/kgm. Stimulating activity on isolated rabbit intestine was not observed.

W. H. Benson

Experimental Workers:

D. Hano D. Schoepp P. Stofko

[redacted] 0141 1 70

Date September 9, 1954

Report from Lowell O. Randall

Research Department Pharmacology to Management

Pathological Changes in the Tissues in Rats Receiving Calcium Pantothenate, DL-Panthenol and D-Panthenol, Orally for 90 Days

Pathological Changes in the Tissues in Rats Receiving Calcium Pantothenate, DL-Panthenol and D-Panthenol, Orally for 90 Days

Herewith is submitted the report by Doctor Mermod on the above subject. This completes the toxicity studies submitted in Red Corner Report No. 8805.

The tissue sections were made in the Toxicology Section by Mrs. R. Hechinger.

Lowell O. Randall

LOR:dja

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August 17, 1954

Dr. Lowell Randall
Hoffmann-LaRoche, Inc.
Nutley 10, N.J.

Pathological Changes in the Tissues in Rats Receiving Calcium Pantothenate, D.L. Panthenol and D. Panthenol, orally for 90 days

Control Animals JF1, JF2, JF3, JF7, JF8, JF9, JM1, JM3, JM5, JM7, JM9, JM10

The lungs of one animal in this group showed a slight hyperaemia and focal areas of red blood cells extravasation.
The others were normal
The liver of 3 animals showed a slight accumulation of lymphocytes around the portal vessels and one of them in addition (JF1) showed a slight amount of edema of the cells.
The spleen of 2 animals was slightly hyperaemic.
The kidney of 1 animal (JF1) showed a slight cloudy swelling and lymphocytic infiltration under the mucous membrane cells of the pelvis.
The sections of the other organs examined, heart, adrenals, stomach intestines, pancreas , ovaries, testicles, thyroid, bone marrow, brain and lymph nodes were all within normal limits.

Calcium Panthothenate 200 mg/kg Rat Nos. AF1, AF2, AF4, AF5, AF6, AM1, AM2, AM3, AM4, AM5, AM6

The lung sections examined; AF1 was normal; AF2 showed a slight infiltration of lymphocytes peribronchially.
The liver section showed a slight cloudy swelling in AM5 and occasional small scattering of lymphocytes in AF2. The others were normal.
The spleen, the sections of 6 animals showed a slight hyperaemia, but no abnormal amount of blood pigments or change in the cells.
2 of the kidney sections, AM1 and AM2 showed a slight cloudy swelling of cells and of the tubules.
The bone marrow in AM5 showed a slight increase in eosinophiles.
The other sections, however, were normal as were the rest of the tissues.

Calcium Panthothenate, 50 mg/kg Animals BF1, BF 2, BF3, BF5, BM2, BM3, BM5, BM6

Four sections of lung tissue were submitted and three of them showe a slight hyperaemia and scattering of lymphocytes peribronchially
Of the liver sections, one of BF2 showed a few small lymphocytes, the other BM2 a slight hyperaemia. The liver cells proper were normal.

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Calcium Panthothenate, 50 mg/kg, continued

The sections of spleen BF5 showed a slight increase in blood pigment. BM5 and BM6 showed slight hyperaemia, no abnormality of the cells.
Kidney sections throughout this group were normal, as were all the other sections.

Calcium Pantothenate 20 mg/kg for Rats CF1, CF2, CF3, CF4, CF5, CF6, CM1, CM2, CM3, CM4, CM5, CM6

The sections of lung which were submitted, one of which CF3, showed a slight recent extravasation of blood, but no evidence of inflammation.
The other two sections were normal.
Of the liver sections, CM4 showed a slight cloudy swelling.
The other sections were normal.
Sections of the spleen in case of CF3, there was a slight hyperaemia.
The other sections were normal.
Sections of the kidney, CM4 showed a low grade chronic inflammation, of both of the pelvic mucous membranes and evidence of nephritic changes. This inflammation undoubtedly accounts for the change in the liver cells.
Sections of CM5 showed a slight hyperaemia. The other kidney sections as well as the other organs examined were within normal limits.

D.L. Panthenol 200 mg/kg Rats # DF2, DF3, DF4, DF5, DF6, DM1, DM2, DM3, DM4, DM5, DM6

Lung tissue were normal.
Liver sections. One of them, DF6, showed a slight cloudy swelling of the cells.
The kidney sections of DM2, DM4, DM5, DM6 showed a slight amount of cloudy swelling of the tubular cells and DF6 showed a slight edema throughout. This same animal showed a slight edema and lymphocytic infiltration below the mucous membrane of the stomach. No evidence of ulceration, however, was found.
The muscle tissue of DM1 showed scattered small masses of lymphocytes. The muscle fibers, however, were normal.
The other sections showed no abnormaility.

D.L. Panthenol 50 mg/kg Rats EF1, EF2, EF3, EF4, EF5, EF6, EM1, EM2, EM3, EM4, EM5, EM6

Three sections of lung tissue were submitted in this group, one of which (EF2) showed a slight amount of edema. The others were normal.
Other sections of liver tissue, one of them, EF4, showed a slight infiltration with lymphocytes periportally.

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D.L. Panthenol, 50 mg/kg , continued

The sections of the Splenic tissue throughout were normal.
Sections of the kidney showed a slight edema in EF5 and EM4.
The tubules and glomeruli however, were not abnormal.
The sections of the other tissues in this group were within normal limits.

D.L. Panthenol 20 mg/kg Rats FF1, FF2, FF3, FF4, FF5, FF6, FM1, FM2, FM3, FM4, FM5, FM6

2 sections of lung tissue were submitted in this group, one of which showed foci of edema. The other one was normal. There was no evidence of inflammation.
The sections of liver tissue were normal throughout except for slight amount of hyperaemia in FM1.
Spleen of FM3 and FM4 showed a slight hyperaemia, but all the other sections were normal.
Sections of the kidney tissue throughout were normal.
Sections of the stomach of FM 4 showed a slight edema and lymphocytic infiltration in the submucosa. No evidence of ulceration.
The other sections of the gastro-intestinal tract were normal.

D. Panthenol 200 mg/kg Rats GF1, GF2, GF3, GF4, GF5, GF6, GM1 GM2, GM3, GM4, GM6

4 sections of lung were submitted. All of them were normal.
Sections of liver of GF1 and GM2 showed a slight cloudy swelling of the cells. In addition to that, sections of GF3 and GF4 showed a slight accumulation of lymphocytes around the portal vessels.
Sections of the spleen throughout were normal.
Sections of the kidneys of GM1 and GM2 showed a slight cloudy swelling of the tubular cells, but no change in the nuclei.
The sections taken from GM6 showed a benign leioma, starting from the cortical portion compressing the kidney tissue. There is no definite evidence of invasion or malignancy.
The sections of GM2 showed a slight lymphocytic infiltration and edema in the submucosa of the stomach.
The other sections were within normal limits.

D. Panthenol 50 mg/kg Rats HF1, HF2, HF3, HF4, HF5, HF6, HM1, HM3, HM4, HM5

Three sections of lung tissue were submitted in this group, all of them showed a slight amount of hyperaemia.
The liver sections of HF6 showed scattering of lymphocytes around the portal tissues and that of HM5 showed a slight passive congestion.

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D. Panthenol, 50 mg/kg, continued

Sections of the spleen of all the animals in this group were normal. Sections of the Kidney of HF3 and HM4 showed a slight cloudy swelling of the tubular cells. No evidence of inflammation or cellular destruction was found. HM4, in addition, showed a slight lymphocytic infiltration and edema in the submucosa of the stomach. No evidence of ulceration of the mucous membrane was found. The other organs had normal tissue.

D. Panthenol 20 mg/kg Rats IF1, IF2, IF3, IF4, IF5, IF6, IM1, IM2, IM3, IM4, IM5

Two sections of lungs were submitted with this group. One of them was normal, the other one showed some recent aspiration of red blood cells.
The sections of the liver showed a slight cloudy swelling of the cells in IM3, slight hyperaemia in IM1, and focal infiltration with lymphocytes around the portal tissue in IF6.
Four sections of splenic tissue, IF2, IF5, IM4, IM5 showed a slight hyperaemia. No evidence of increased deposit of blood pigment, however, was found.
The sections of the kidneys of IF2, IF4, IM1, IM2, IM3, IM4 showed a slight amount of cloudy swelling. No increase of exudate was found, either in the glomerular capsules, or in the tubules.
IM2 and IM3 showed a slight evidence of irritation in the submucosa of the stomach. (Lymphocytic infiltration and slight edema).

SUMMARY

The pathological changes found in the tissues of animals receiving D. Panthenol, D.L. Panthenol or Calcium Panthothenate were minimal and irregular. At a dosage of 200 mg/kg of Calcium Panthothenate, the animals showed a slight hyperaemia of the spleen.

With D.L. Panthenol at 200 mg/kg, there was a slight cloudy swelling of the kidney tubular cells.

With D. Panthenol, 20 mg/kg part of the kidney showed a slight cloudy swelling.

In no case were these changes found in every animal in the group and in no case either, were the changes marked.

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CONCLUSIONS

It does not appear that Calcium Panthothenate, D.L. Panthenol or D. Panthenol administered orally to rats for 90 days in dosages of 20 mg/kg, 50 mg/kg or 200 mg/kg caused marked toxic changes. All the changes found were spotty and in no instance did they appear to be irreversible.

Camille Mermod

Camille Mermod, M.D.

October 25, 1962 [redacted] 0141 77

Report from Dr. E. Schwartz

Department PHARMACOLOGY to Management

Studies Of The Possible Hepatotoxicity Induced By Sodium Pantothenate, d-Panthenol And d-Pantethine In Rats

Summary:

Administration of 2.0 Gm/kg and 200 mg/kg of sodium pantothenate both as an aqueous solution and as a trade formulation, Pantoject(R) had no affect upon the SGO-T or SGP-T levels or upon the amount of lipid in the livers of rats. Similar doses of d-pantothenol caused some increase in liver lipid without producing elevations in SGO-T and SGP-T. Treatment with 100 mg/kg of d-pantethine was well tolerated in rats. A summary of clinical studies in man utilizing sodium pantothenate and d-panthenol conducted by Dr. R. Pocelinko is also included.

Studies Of The Possible Hepatotoxicity Induced By Sodium Pantothenate, d-Panthenol And d-Pantethine In Rats

Recently, a publication by Wirtschafter and Walsh entitled: Hepatocellular Lipid Changes Produced By Pantothenic Acid Excess has appeared in the literature (Annals of Surgery, 156, 97, 1962). In this study, the authors used doses of 750 mg and 1250 mg per rat of sodium pantothenate (Pantoject(R)) in addition to doses of 20 mg and 560 mg per rat of "d-Pantethine." Inquiry to California Biochemical Corporation, the supplier named in the paper, has indicated that the material designated by Wirtschafter and Walsh as d-pantethine is commercially unavailable, and that the material which these authors used presumably was the corresponding disulfide d-pantbine, (Letter to California Biochemical Corporation from Dr. W. E. Scott, September 17, 1962).

Wirtschafter and Walsh also state that sodium pantothenate was given in doses of 750 mg in a single injection and 1250 mg in 5 divided doses; however, there is no indication of the body weights of the animals used and therefore, no precise calculation of the dose on a per kilogram basis is possible. Assuming that the mature rats which were used weighed about 200 grams, the rats injected with 750 mg of sodium pantothenate received 3.75 Gms/kg as a single dose. In a comprehensive study by Unna and Greslin on the toxicity and pharmacology of pantothenic acid (J. Pharmacol. Exptl. Therap. 73, 85, 1941), it was determined that the LD_{50} of calcium pantothenate given subcutaneously to rats was 3.4 Gms/kg; it is therefore possible that Wirtschafter and Walsh approached and may even have exceeded the LD_{50} of sodium pantothenate. Using doses which could cause death, it is not surprising that these authors saw the liver changes described. It was also stated by the authors that fat droplets are seldom seen in normal rat liver cells; however, in these laboratories, it has been found that there is often some degree of physiological fat deposition even in normal control rats.

In this communication, the results of toxicity studies of sodium pantothenate, d-panthenol, d-pantethine and sodium pantothenol as Pantoject(R) are reported; the dosage schedule included administration of massive amounts of the substances approximating those employed by Wirtschafter and Walsh as well as doses more nearly comparable to the clinical dose schedule.

Methods:

Young Sprague-Dawley rats of mixed sexes weighing 100-130 grams were used in this experiment. These were divided in ten groups of ten rats each (5 males, 5 females) and were given a single intramuscular injection as follows:

Group	Treatment
1.	2.0 Gm/kg Na Pantothenate in water for injection (500 mg/ml)
2.	200 mg/kg Na Pantothenate in water for injection (500 mg/ml)
3.	2.0 Gm/kg d-Pantothenol in water for injection (500 mg/ml)
4.	200 mg/kg d-pantothenol in water for injection (500 mg/ml)
5.	100 mg/kg d-pantethine in water for injection (100 mg/ml)
6.	2.0 Gm/kg Na pantothenol as Panto ject (R)
7.	200 mg/kg Na pantothenol as Panto ject (R)
8.	Benzyl alcohol (1%) - volumes to equal volumes given in group #6.
9.	Benzyl alcohol (1%) - volumes to equal volumes given in group #7.
10.	Control - water for injection 0.25 ml/rat.

At 96 hours, each rat was weighed, anesthetized with ether, and blood was withdrawn by cardiac puncture for serum glutamic oxaloacetic transaminase (SGO-T) and serum pyruvic transaminase (SGP-T) analyses. The SGO-T analysis was done by the method of Karmen (J. Clin. Invest., 34, 131, 1955) and the SGP-T analysis by the method of Wroblewski and La Due (Proc. Soc. Exptl. Biol. and Med., 91, 569, 1956). The rats were then sacrificed and a gross pathologic examination done; the liver of each animal was removed, weighed, and sections removed for microscopic examination; these sections were stained with hematoxylin-eosin and hematoxylin-sudan IV for visualization of fat.

Results:

The results of a single injection of Na pantothenate, d-pantethenol, d-pantethine, Pantoject(R) and benzyl alcohol upon liver weights and SGO-T and SGP-T levels after 96 hours are seen in Table 1. None of these injections produced significant changes from the control group.

Table 1. Effect Upon Liver Weight, SGO-T and SGP-T Levels

Group	Treatment	Sex	No. Of Rats	Liver Weights (% Of Body Wt.)	SGP-T (units)	SGO-T (units)
1	2 Gm/kg sodium pantothenate in water for injection	M	5	4.9	13.8*	125*
		F	5	4.7	21	119.4
2	200 mg/kg sodium pantothenate in water for injection	M	5	4.6	18	100
		F	5	4.6	10	130
3	2 Gm/kg d-pantothenol in water for injection	M	5	4.3	11	90
		F	5	4.4	9	110
4	200 mg/kg d-pantothenol in water for injection	M	5	4.4	12	103
		F	5	4.3	12	113
5	100 mg/kg d-pantethine in water for injection	M	5	4.5	13	140
		F	5	4.3	16	104
6	2 Gm/kg sodium pantothenate as Pantoject(R)	M	5	4.9	15.4	92.4
		F	5	4.7	21.6	134.2
7	200 mg/kg sodium pantothenate as Pantoject(R)	M	5	4.7	18.2	102
		F	5	4.6	23	101
8	Benzyl alcohol (1%) - volumes equal to group #6	M	5	4.9	18	104.8
		F	5	4.7	21.4	118
9	Benzyl alcohol (1%) - volumes equal to group #7	M	5	4.7	27.2	114.4
		F	5	4.8	17.6	108.6
10.	Control - 0.25 ml/rat water for injection	M	5	4.5	17	118.2
		F	5	4.6	20	137.6

*All figures are means of 5 rats per group except for SGO-T and SGP-T values of males in group one which is a mean of 4 rats.

The gross pathologic examination revealed no marked lesions or changes present in any of the groups. Microscopic examination of the hematoxylin-eosin liver sections revealed little more than some cytoplasmic vacuolization and slight swelling of the liver parenchymal cells except in a few, isolated instances. Rat Nos. 16 and 18 which received 200 mg/kg of Na pantothenate, rat No. 48 given 100 mg/kg of d-pantethine, as well as rat Nos. 92 and 93 of the control group had a few white necrotic lesions in the livers. Histologic examination of these lesions showed a necrotic area well encapsulated by fibrous connective tissue containing many proliferating bile ducts. Since these necrotic foci were found in both control and drug-treated animals, they are judged to be unrelated to administration of either pantothenate or d-pantethine.

With the hematoxylin-sudan IV stain, some fatty change was seen in the liver of all the groups in this experiment. (Table 2). For clarity and also as a means of classifying this data, the fatty changes seen were arbitrarily divided into 3 groups: (1) zero to slight, (2) moderate and (3) severe. When fatty change of the zero to slight category was seen, it was usually in the central area; moderate to severe change was more of a diffuse character.

Table 2. Effect Of Injection On Fat In Liver

Group	Treatment	SHOWING FATTY CHANGE		
		Zero to Slight	Moderate	Severe
1	2 Gm/kg sodium pantothenate in water for injection	4	5	1
2	200 mg/kg sodium pantothenate in water for injection	4	4	2
3	2 Gm/kg d-pantothenol in water for injection	0	6	4
4	200 mg/kg d-pantothenol in water for injection	2	4	4
5	100 mg/kg d-pantethine in water for injection	4	6	0
6	2 Gm/kg sodium pantothenate as Pantoject(R)	5	5	0

Table 2. (cont'd) Effect Of Injection On Fat In Liver

Group	Treatment	SHOWING FATTY CHANGE		
		Zero to Slight	Moderate	Severe
7	200 mg/kg sodium pantothenate as Pantoject(R)	7	3	0
8	Benzyl alcohol (1%) - volumes equal to group #6	9	1	0
9	Benzyl alcohol (1%) - volumes equal to group #7	7	3	0
10	Control - 0.25 ml/rat water for injection	4	6	0

Discussion:

The injection of 100 mg/kg of dexpanthenine to rats produced no alteration in SGO-T or SGP-T levels and no increase in fatty infiltration in the livers of these animals. These findings are in contrast to those of Wirtschafter and Walsh who found that similar doses (20 mg per rat) increased both the SGP-T level and the amount of fat in the liver. Moreover, since this compound is an organic complex of pantothenic acid with cysteamine, possibly a toxic substance in its own right, it does not seem to be valid to include dexpanthenine in a discussion of the toxicity of pantothenic acid and its derivatives.

Sodium pantothenate, when given to rats in doses of 2.0 gm/kg and 250 mg/kg, did not produce any signs of hepatic damage; that is, the serum transaminase levels remained normal and the liver parenchyma maintained its normal integrity. Wirtschafter and Walsh report that comparable doses, both on a single and in repeated injection, produce hepatocellular damage. In any event, since these doses approximate the LD_{50} of the compound, these latter results are of negligible significance. The dose of sodium pantothenate which these authors used (750 mg per weight or 3.75 g/kg), if given to a normal 70 kg man, would be equal to 262.5 Gms in a single injection. In our dose, of 2 Gm/kg, a 70 kg man could receive 140 Gm of Na pantothenate, still a massive dose, and yet not enough to cause liver damage. The usual clinical dosage schedule to man is 250 mg, given q.i.d.

The increase in lipid deposition with d-pantothenol is difficult to explain. However, judging from the normal SGO-T and SGP-T levels, the damage is not severe enought to interfere with liver function even at 2 Gm/kg.

In a study in humans, Dr. R. Focaliuko gave intramuscular doses of 500 mg of d-pantothenol or sodium pantothenate every 12 hours for 4 days to 6 male patients (J.O. Hosp. October 19, 1962). On the 5th day, when liver function studies were done (SGO-T, SGP-T, alkaline phosphatase, serum bilirubin and thymol turbidity), no significant deviations from the normal were noted. In one patient, (O.V.), two 4 day courses of the drugs were administered; a slight increase in alkaline phosphatase was noted in the first course of treatment, and a slight increase in SGO-T to 62 units was observed following the second course of treatment while the alkaline phosphatase returned toward normal levels. However, during this time all the other liver function tests remained normal in this patient.

The studies reported here in both humans and rats with the chronic toxicity study of Unna and Greslin, in which they fed up to 200 mg per rat (approximately 1000 mg/kg, assuming an average body weight of 200 grams) of calcium pantothenate every day for 6 months without any evidence of toxicity, appear to indicate that pantothenic acid and its derivatives are free of hepato toxicity when utilized in the usual clinical dose range.

E. Schwartz

BS:eg

INTEROFFICE CORRESPONDENCE

To: Mrs. E. Garrison October 19, 1962

From: Dr. R. Pocelinko

Subject: Study on Toxicity of Pantothenic Acid and Related Derivatives

METHOD: Six male patients without any laboratory or clinical evidence of liver impairment were chosen from the wards of Northland Medical Center. A control blood was drawn for SGOT, SGPT, alkaline phosphatase, cephalin flocculation, serum bilirubin and thymol turbidity. The patients were then given d-Pantothenyl alcohol or Sodium Pantothenate, 500 mg. I.M. q. 12 h. for four days. On the morning of the fifth day blood was drawn for liver function studies. The results are shown in the attached table.

DISCUSSION: No subjective or objective abnormalities were noted during the course of therapy; no significant abnormalities in the laboratory values were seen in five of the six patients. The sixth patient, O.V., showed an increase in SGOT to 62 and alkaline phosphatase in a second course of treatment. The values for SGOT were borderline, while the alkaline phosphatase was definitely elevated even at the onset of treatment and was lower at the cessation of treatment. The increase in the SGOT values is probably not significant.

R. Pocelinko, M. D.

RP/lmh
Attachment

Patient	Age		Date	SGOT	SGPT	Alkaline Phosphatase	Cephalin Flocculation	Serum Bilirubin	Taysol Turbidity
A.B.	87	Pre	9/13/62	26	45	2.1	0	0.23	1.6
		Post	9/13/62	33	16	2.3	0	0.21	1.4
B.C.	46	Pre	9/13/62	26	23	2.1	0	0.13	2.6
		Post	9/18/62	35	13	1.9	0	0.14	1.6
E.C.	66	Pre	9/18/62	26	20	2.4	0	0.28	6.0
		Post	9/24/62	26	19	2.8	1+	0.26	2.4
C. Mc	64	Pre	9/18/62	35	23	2.3	0	0.37	0.9
		Post	9/24/62	26	13	2.0	0	0.20	0.4
J.H.	53	Pre	9/25/62	29	13	2.4	0	0.26	0.6
		Post	9/29/62	36	26	2.8	0	0.22	1.0
O.F. #1	67	Pre	9/13/62	33	15	3.2	0	0.20	1.4
		Post	9/18/62	23	13	3.4	0	0.20	1.0
O.F. #2		Pre	9/25/62	48	32	4.5	±	0.24	0.8
		Post	9/29/62	62	48	2.8	0	0.44	0.3

Normal Values:

Alkaline Phosphatase - 0.8-2.3 Sigma Units

SGOT - Normal up to 28: Borderline 28-50
SGPT - Normal up to 35: Borderline 35-45

January 7, 1963

Report from E. Schwartz

Department PHARMACOLOGY to Management

Further Studies of the Possible Hepatotoxicity Induced by Sodium Pantothenate and d-Panthenol in Rats

Summary

Single intramuscular injection of 2 gm/kg, 200 mg/kg and 100 mg/kg of d-panthenol and sodium pantothenate had no effect upon SGO-T, SGP-T, liver size, or amount of liver lipid deposition in rats.

E. Schwartz

Further Studies of the Possible Hepatotoxicity Induced by Sodium Pantothenate and d-Panthenol in Rats

In a previous report (Schwartz RCR 15322) which was undertaken to investigate the hepatotoxicity of pantothenic and derivatives, an increase in liver lipid was seen histologically in rats which had received d-panthenol. The study in this communication was performed to reinvestigate this finding with d-panthenol and also to reinvestigate the possible hepatotoxicity of sodium pantothenate.

Methods

Adult Sprague-Dawley rats of mixed sexes were divided into seven groups of 10 rats each (5 male, 5 female) and were given a single intramuscular injection as follows:

1. 2.0 gm/kg Sodium Pantothenate in water for injection (500 mg/ml)
2. 200 mg/kg Sodium Pantothenate in water for injection (500 mg/ml)
3. 100 mg/kg Sodium Pantothenate in water for injection (500 mg/ml)
4. 2.0 gm/kg d-panthenol in water for injection (500 mg/ml)
5. 200 mg/kg d-panthenol in water for injection (500 mg/ml)
6. 100 mg/kg d-panthenol in water for injection (500 mg/ml)
7. Control - given 0.25 ml water for injection

After 96 hours each rat was weighed and then anesthetized with ethyl ether. Blood samples were taken by cardiac puncture for serum glutamic oxaloacetic transaminase (SGO-T) and serum glutamic pyruvic transaminase (SGP-T) determinations. The SGO-T determinations were done by the method of Karmen (J. Clin. Invest. 34, 131, 1955) and the SGO-T by the method of Wroblewski & LaDue (Proc. Soc. Exptl. Biol. Med. 91, 569, 1956). The rats were then sacrificed and a gross pathologic

examination done. The livers of each animal was removed and weighed. Sections of the liver were taken and stained with hematoxylin-eosin and hematoxylin-sudan IV of visualization of fat. The rest of the liver was then utilized in random samples of rats in each group for total fat analyses which was done by the method of Emery & Finch (Arch. of Dis. of Child. 29, 242, 1954).

Results

The effect of a single injection of d-panthenol and sodium pantothenate upon liver weight, SGO-T and SGP-T levels after 96 hours are shown in Table 1. All of these parameters remained within the normal limits seen in this laboratory.

Table 1. Effect of d-Panthenol and Sodium Pantothenate Injections Upon Liver Weight and Serum Transaminase Levels

Treatment	Sex	Liver Weights (% Body Wt.)	SGO-T (units)	SGP-T (units)
Sodium Pantothenate				
2 gm/kg	M	4.09	111	19
	F	3.60	103	13
200 mg/kg	M	4.01	103	16
	F	3.61	96	16
100 mg/kg	M	4.06	88	15
	F	3.63	83	15
d-Panthenol				
2 gm/kg	M	3.63	102	15
	F	3.60	89	11
200 mg/kg	M	3.69	75	10
	F	3.78	90	13
100 mg/kg	M	3.72	119	13
	F	3.86	89	14
Control	M	3.78	78	13
	F	3.20	86	10

All values are averages of 5 animals per group.

The results of the total liver fat analysis in a random number of rats is shown in Table 2. These measurements are much lower than the 5% to 7% usually reported for fat in livers (Hodge et al., J. Biol. Chem. 139, 897, 1941), however, significant differences between treated and control animals were not observed.

Table 2. Total Fat in Livers of Sodium Pantothenate and d-Panthenol Treated Rats

Group	Number	% Total Fat (average)
Sodium Pantothenate		
2 gm/kg	2	0.759
200 mg/kg	3	0.850
100 mg/kg	2	0.939
d-Panthenol		
2 gm/kg	3	0.265
200 mg/kg	3	0.715
100 mg/kg	3	0.800
Control	3	0.800

The gross pathologic examination revealed no marked changes in any of the animals. Several rats had a small yellow focal area on each of the livers which microscopically was necrotic with some connective tissue present; these were rat No. 6 given 2 gm/kg of sodium pantothenate, rats Nos. 18 and 20 given 200 mg/kg of sodium pantothenate, rat No. 26 given 100 mg/kg of sodium pantothenate and rat No. 38 given 2 gm/kg of d-panthenol. These areas are frequently seen in rat livers and are not due to either the sodium pantothenate or d-panthenol administration. Microscopically little was seen aside from varying amounts of slight cytoplasmic vacuolization. The liver of rat No. 19 administered 200 mg/kg of sodium pantothenate microscopically revealed a single focal cirrhotic area and a single focal necrotic nodule. Rat No. 36 treated with 100 mg/kg of d-panthenol had some vacuolization and swelling of the hepatic parenchyma cells resulting in a disappearance of the hepatic sinusoids.

Hematoxylin-sudan IV revealed some fat to be present in all groups (Table 3). The amount of fat in the liver was arbitrarily divided into (1) zero to slight, (2) moderate and (3) severe as a means of classification. There was no increase in the amount of fat in any of the livers of the sodium pantothenate and d-panthenol treated rats over the control group. The reason for the severe fatty change in one of the rats in the control group is uncertain.

Table 3. Effect of Sodium Pantothenate and d-Panthenol Injection On Fatty Change in the Liver

Group	Number Showing Fatty Change		
	Zero to Slight	Moderate	Severe
Sodium Pantothenate			
2 gm/kg	3	6	1
200 mg/kg	7	3	0
100 mg/kg	7	3	0
d-Panthenol			
2 gm/kg	8	2	0
200 mg/kg	7	3	0
100 mg/kg	5	5	0
Control	5	4	1

Discussion

Neither the single intramuscular injection of d-panthenol nor sodium pantothenate in doses up to 2 gm/kg had any effect upon SGO-T, SGP-T, liver size or amount of histologically demonstrable fat in the liver.

A previous report (Schwartz RCR 15(2)) described some increase in the amount of liver lipid deposition seen histologically with 2 gm/kg and 200 mg/kg of d-panthenol, whereas in this study neither of these doses nor 100 mg/kg of d-panthenol produced any increase in lipid deposition. In both studies the serum transaminase levels remained within normal limits.

It is, therefore, concluded that administration of d-panthenol and sodium pantothenate even in massive doses does not have the serious hepatotoxic consequences as forewarned by Wirtschafter & Walsh (Annals of Surgery 156, 97, 1962).

Edward Schwartz

ES:jo

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Ledger - ES 1-289-294
WR - 290
Histology - ES 1 - 29-31

Proc. Soc. Exptl. Biol. and Med. 99(3):632-633. 1958.

Pelton-'58
Bib No. 94

Effect of Pantothenic Acid on the Longevity of Mice. (24442)

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The disparity between the life span of worker and queen bees suggested the possibility that pantothenic acid which is the most conspicuous nutritional constituent of royal jelly(1,2) might have the effect of prolonging life in other species. An additional suggestion came from the finding of Gardner(3) that this vitamin acts as an anti-ageing factor in drosophila.

Method. Thirty-four male and 40 female, C-57 black mice, age 4-5 weeks, were procured from the Jackson Memorial Laboratory. The mice were grouped with either 6 or 7 animals of the same sex in each cage. Thirteen male and 20 female mice were given supplementary pantothenic acid and 21 male and 20 female mice were used for the control group. All mice were fed Purina laboratory pellets throughout the experiment. It was desired to give each of the 33 mice in the experimental group a supplement of approximately 300 μg of calcium pantothenate per day throughout the experiment. The vitamin was added to the drinking water and the amount added was based on the previous week's water consumption, *i.e.*, if average daily water consumption for the group in one cage was 4 ml per mouse, calcium pantothenate was added in the amount of 75 μg/ml. If the consumption was 5 ml then 60 μg of the vitamin were added per ml of water. The age of each mouse was

TABLE I. Summary and Statistical Data for Mice Which Received Pantothenic Acid Supplement and Controls.

	Mean life span in days							
	Mice with pantothenic acid supplement			Controls			t test(4)	U test(5)
	No.	Days	S.D.	No.	Days	S.D.	P =	P =
Both sexes	33	653.1	201	41	549.8	233	.05	.01
Male mice	13	660.4	220	21	559.0	167	.15	.04
Female mice	20	648.4	187	20	540.2	285	.18	.05

TABLE II. Analysis by Sex of Mice Given Pantothenic Acid Supplement and Control Mice.

	Mean life span in days				Days difference	% difference
	No.	♂	No.	♀	♂ & ♀	♂ & ♀
Mice with pantothenic acid supplement	13	660.4	20	648.4	12.0	2
Controls	21	559.0	20	540.2	18.8	3
Days difference treated & controls		101.4		108.2		
% difference treated & controls		18%		20%		

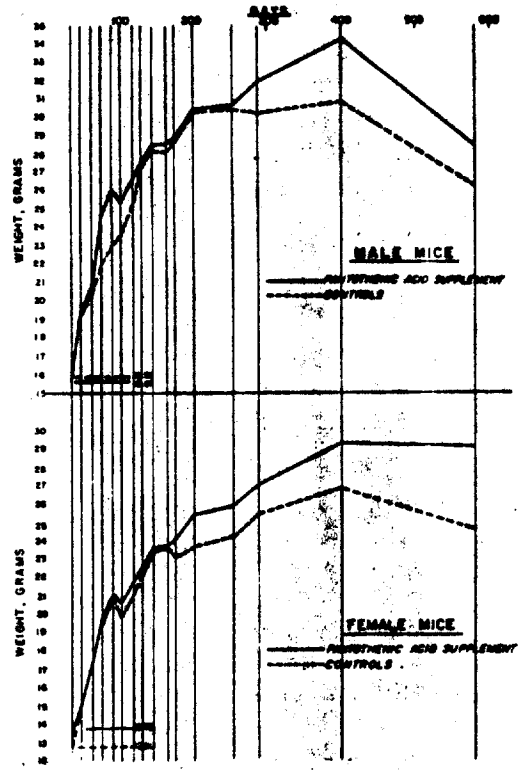

FIG. 1.

taken arbitrarily as 35 days at start of experiment.

Results. The data obtained are given in Tables I and II.

The above analysis (Table II) shows that differences in life span, 2% and 3% between treated males and females and control males and females, respectively, are negligible. There was however, a difference of 18% in life span betwen treated and untreated males and a difference of 20% between treated and untreated females.

The mice were weighed at 2-week intervals for the first 6 months and at greater intervals for the following year (Fig. 1, vertical lines). Both male and female mice which received pantothenic acid supplement gained and maintained slightly greater weight after about 250 days than did control mice. It is speculative as to whether this greater weight should be interpreted as indicative of better general health or as a sign of prolonged pre-senile period. The results of this experiment seem sufficiently significant to suggest further work with other species.

Summary. 1) Thirty-three young male and female C-57 black mice were given approximately 300 μg of calcium pantothenate daily in drinking water. Forty-one control mice did not receive the vitamin supplement. 2) The mean life span for the mice given supplementary calcium pantothenate was 653.1 days and that for the control mice was 549.8 days. The statistical difference between the 2 groups is $P = 0.05$ (T test) 0.01 (U test). 3) The mice which received the vitamin supplement maintained slightly greater body weight after they were approximately 250 days old.

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Rubin-'50
Bib No. 111

Jour. Nutrition 40(2):265-274. 1950.

AVAILABILITY OF PANTHENOL AND CALCIUM PANTOTHENATE IN RELATION TO FOOD INTAKE[1]

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SIX FIGURES

(Received for publication August 3, 1949)

Loading tests with pantothenic acid have been carried out by various workers with human subjects (cf. Burlet, '44; Schmidt, '45; Sarett, '45; Rubin et al., '48) as well as with animals (Silber, '45; Oleese and Pearson, '48). Only a portion of the ingested doses has been found to be excreted as unchanged pantothenate; e.g., urinary excretion in the human accounted for 15–20% of oral doses of Ca pantothenate ranging from 100 to 250 mg (Rubin et al., '48). Similar percentage excretions were reported by Schmidt ('45) after intravenous injection of 25-mg doses of Ca pantothenate or panthenol. Silber ('45), who studied pantothenic acid excretion in dogs, and Oleese and Pearson ('48), working with sheep, found that some of the ingested Ca pantothenate was excreted unchanged in the feces. Silber ('45) demonstrated that the fecal loss was proportional to the amount of food taken with the dose. None of these studies, however, accounted for an excretion (urine and feces) of more than 50% of the ingested Ca pantothenate. In the present study, the rat was used as the test animal to compare excretion of pantothenic acid after the ingestion of loading doses of panthenol or Ca pantothenate under various feeding conditions. Panthenol,

[1] Presented before the American Institute of Nutrition, Detroit, April 21, 1949 (Fed. Proc., *8*: 393).

the alcohol analogue of pantothenic acid, although inactive for the growth of most microorganisms except *Acetobacter suboxydans* (Walter, '46; De Ritter and Rubin, '49), can replace pantothenate in the nutrition of the rat (Pfaltz, '43; Drekter et al., '48) and chick (Hegsted, '48; Milligan and Briggs, '49). Moreover, this compound is of considerable interest because of its greater availability to humans at high doses (Burlet, '44; Rubin et al., '48) and its greater stability *in vitro* as compared to Ca pantothenate, particularly in the pH range of 3 to 5 (Rubin, '48).

The fact that panthenol is as effective as the salts of pantothenic acid in curing pantothenic acid deficiency in the rat (Drekter et al., '48) provides a basis for interpreting excretion data in terms of availability.

EXPERIMENTAL

Adult, male albino rats of the Sprague-Dawley strain, weighing between 375 and 475 gm, were used for these studies. The animals were housed and the test was handled according to the procedure described by Drekter et al. ('48). The microbiological assay method for urinary and fecal pantothenic acid was that of Skeggs and Wright ('44), for panthenol that of De Ritter and Rubin ('49). Panthenol and Ca pantothenate were compared at all levels on an equivalent basis, all doses being administered by stomach tube in 1 ml of distilled water. In calculating extra urinary excretions, a "statistical" basal value (Drekter et al., '48) was subtracted from the observed excretions. Since these studies extended over a period of months, basal values were determined again at the end of that time and were found to check with the "statistical" value. Experiments were run at three dosage levels: 10, 5 and approximately 2 mg/kg; for each dosage level, 10 to 20 rats were used. The results were evaluated according to the formula for the standard error of the difference between two means.[2]

$$\sigma_D = \frac{m_1 - m_2}{\sqrt{\frac{d_1^2}{N_1} \frac{d_2^2}{N_2}}}.$$

Ad libitum feeding

Rats were allowed to eat ad libitum up to the time of ingestion of the dose and throughout the two 24-hour collection periods. On the average, 173, 85 and 7% more pantothenic acid was excreted in the urine in the 24 hours after the ingestion of panthenol at the 10, 5 and 1.25 mg/kg levels, respectively, than after ingestion of equivalent doses of Ca pantothenate (fig. 1). Only the differences at the 10 and 5 mg/kg levels are statistically significant ($P < 0.05$). Approximately 60% of the dose of panthenol was excreted as pantothenate in the urine in the first 24 hours at all three levels, whereas only 23 and 33% of the doses of Ca pantothenate were excreted in a similar interval at the 10 and 5 mg levels, respectively. At the lowest level (1.25 mg/kg), the percentage excretion approximated that which occurred after giving panthenol. In the second 24-hour interval approximately 5% of the dose of both panthenol and Ca pantothenate was found in the urine at the two highest levels; hence, second-day values exert little effect in any of these experiments.

Controlled feeding experiments

In order to produce conditions under which the effect of food on the relative availability of panthenol and Ca pantothenate could be studied, food was withheld from rats for 24 hours prior to dosage. At the end of this starvation period, the test group were given 6 gm of stock diet (approximately two-fifths of the daily food intake); the rats consumed the food within 45 minutes. The doses were administered to these and to the unfed control rats (postabsorptive state) immediately thereafter. The collection of urine and feces was begun, and after 6 hours all rats were given stock diet. Basal urinary and fecal excretions were determined under similar conditions. The basal urinary excretion was found to be the same regardless of whether or not the rats were fed at the start of the collection period. This basal value

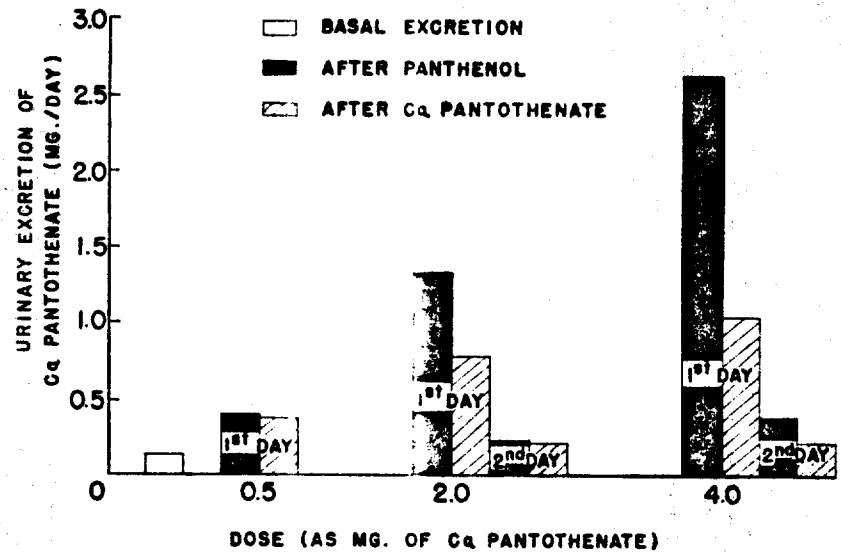

Fig. 1 Urinary excretion of Ca pantothenate after various equivalent doses of panthenol and Ca pantothenate to rats fed ad libitum.

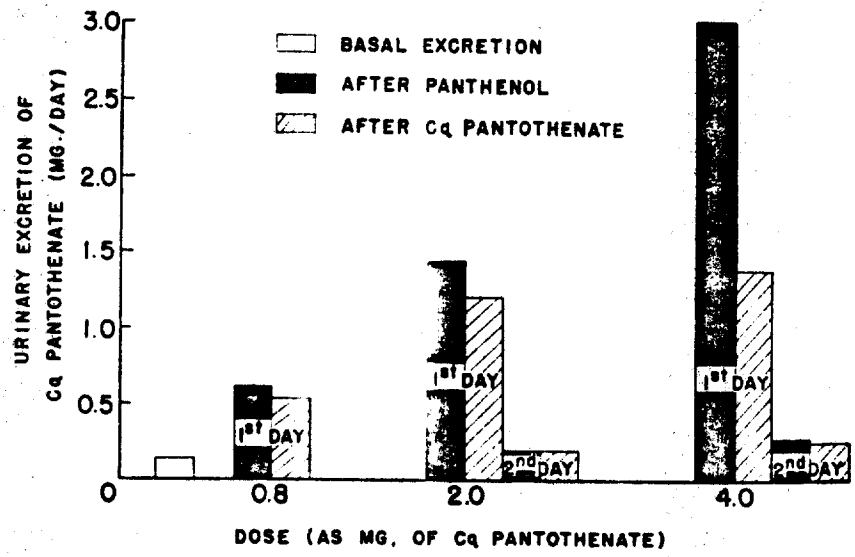

Fig. 2 Urinary excretion of Ca pantothenate after various equivalent doses of panthenol and Ca pantothenate — postabsorptive.

(0.14 mg/day) was similar to that found in the ad libitum experiments. No correlation was noted between the amount of pantothenic acid excreted and the volume of urine. The amount of pantothenic acid in the feces was found to be directly proportional to the weight of the feces. Therefore, to calculate extra fecal excretions, basal values were subtracted in proportion to the amount of feces.

After postabsorptive dosage with panthenol, 131, 24 and 15% more pantothenic acid was excreted in the urine at the 10, 5 and 2 mg/kg levels, respectively, than after dosage with Ca pantothenate (fig. 2). Only the difference at the 10 mg/kg level was significant. Under these feeding conditions, 74, 67 and 59% of the doses of panthenol and 32, 54 and 51% of the doses of Ca pantothenate were excreted at the various levels. When the doses were administered to rats in the postprandial state, the excretion of pantothenic acid after giving panthenol exceeded the excretion after administering Ca pantothenate by 92, 45 and 2% (fig. 3). The differences at the 10 and 5 mg/kg levels were significant. Sixty-seven, 61 and 49% of the doses of panthenol were recovered in the urine as pantothenic acid, as compared to 35, 42 and 48% of the doses of Ca pantothenate.

The presence of food at the time of dosage had no significant effect on the urinary excretion after dosage with either Ca pantothenate (fig. 4) or panthenol (fig. 5). However, fecal excretion of Ca pantothenate was affected by food intake (fig. 6). A significantly greater fecal excretion of pantothenic acid was found when a dose of 10 mg/kg of Ca pantothenate was given simultaneously with food than when food was absent (30 vs. 16%). At the 5 mg/kg level, the presence of food increased the fecal excretion of pantothenic acid slightly, but the effect was not statistically significant. At the lowest level (2 mg/kg), no extra fecal excretion could be demonstrated in either case. At the two higher levels some extra pantothenic acid was excreted in the feces (16 and 7%) even when food was absent at the time of ingestion of the dose. No increase in the fecal excretion of pantothenic

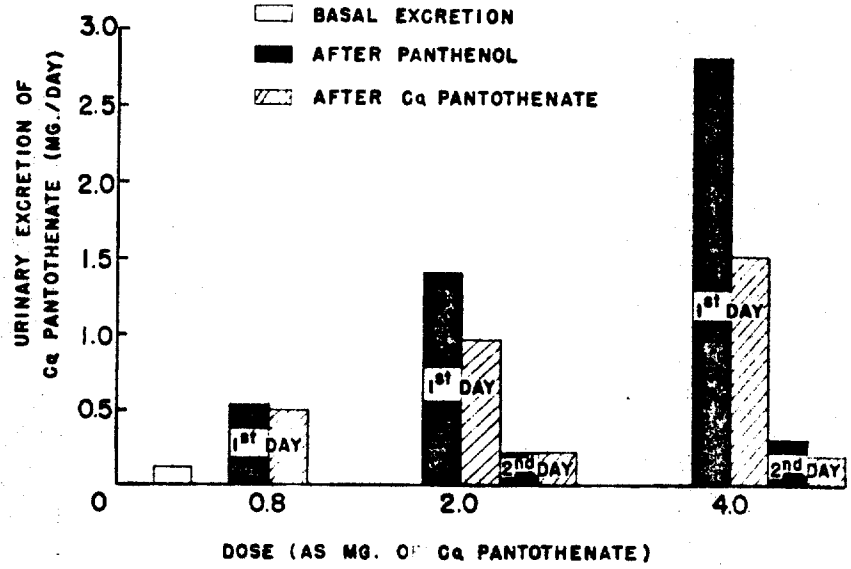

Fig. 3 Urinary excretion of Ca pantothenate after various equivalent doses of panthenol and Ca pantothenate — postprandial.

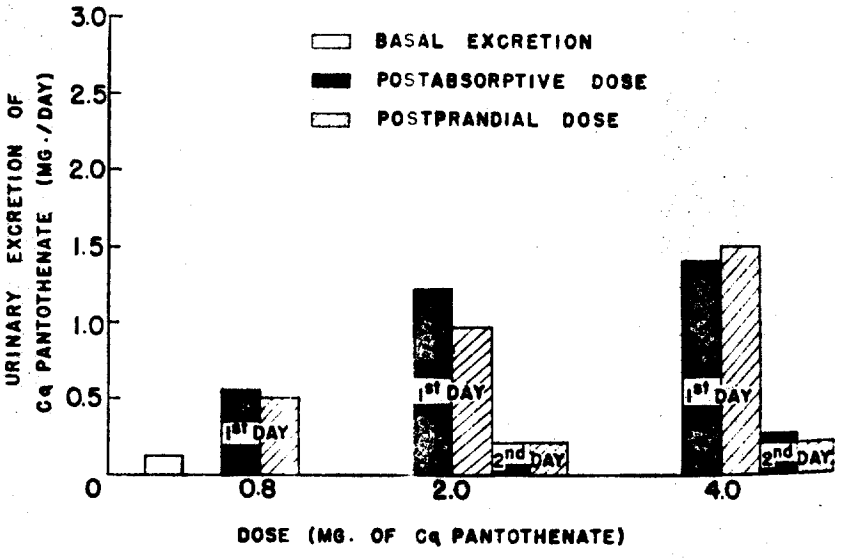

Fig. 4 Urinary excretion of Ca pantothenate after various doses of Ca pantothenate — postabsorptive and postprandial.

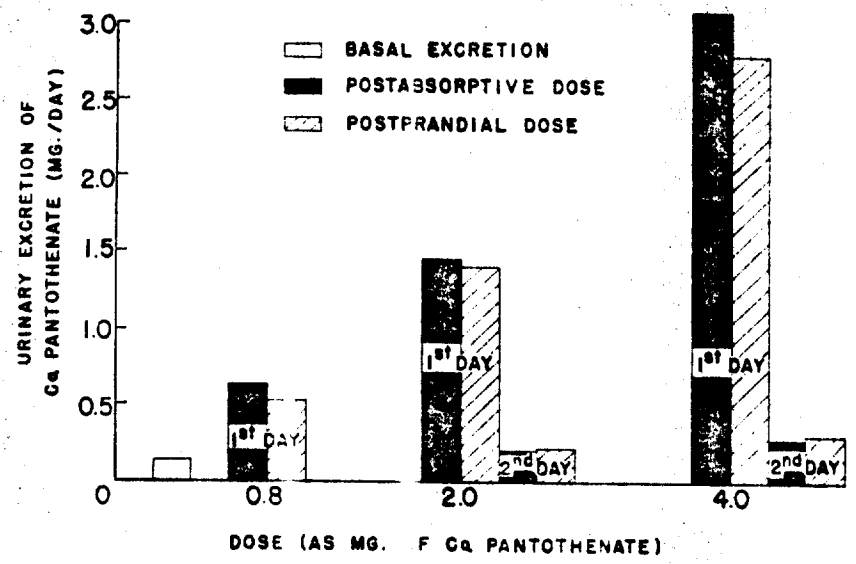

Fig. 5 Urinary excretion of Ca pantothenate after various doses of panthenol — postabsorptive and postprandial.

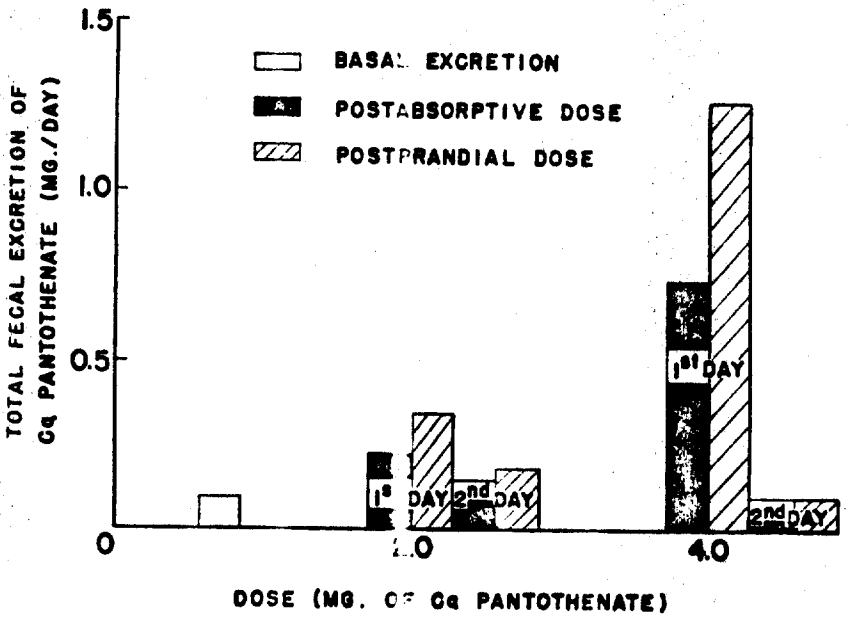

Fig. 6 Effect of food upon fecal excretion after various doses of Ca pantothenate — postabsorptive and postprandial.

acid was observed when panthenol was fed, an indication that no conversion of panthenol to pantothenic acid takes place in the gastrointestinal tract, nor was any panthenol per se found in the feces under any feeding conditions. Second-day excretion values did not change these trends.

DISCUSSION

The excretion of pantothenic acid by the rat after ingestion of equivalent doses of panthenol and Ca pantothenate follows the same pattern as in humans. At the highest level fed (10 mg/kg), which is considerably above the top level used in our human availability tests (Rubin et al., '48), panthenol is more available than Ca pantothenate in all three types of feeding tests. At the intermediate level of 5 mg/kg this difference exists but to a lesser degree, particularly when the dose is given in the absence of food. Similarly, in the human availability tests at approximately the same milligram per kilogram dosage, panthenol was much more available than Ca pantothenate when given postprandially, but equally available when given in the postabsorptive state. At the lowest level fed (1.25 or 2 mg/kg) there was no significant difference in excretion after dosage with panthenol or Ca pantothenate. This parallels the findings with humans at comparable dosage levels as well as those noted in unpublished experiments in which doses as low as 0.4 mg/kg were fed. At the high levels, the greater excretion of pantothenic acid after panthenol administration than after giving Ca pantothenate may be explained by the greater stability of panthenol in acid solutions (Rubin, '48), resulting in better survival of panthenol during the gastrointestinal journey.

Silber ('45) has shown with dogs that when food is given with a dose of Ca pantothenate, urinary excretion is decreased and fecal excretion increased proportionately as compared to values found when the same dose is given postabsorptively. He also found that increasing the amount of food given increased fecal excretion of pantothenic acid. The proportion of fecal to urinary excretion increased from 1.3.

when no food was given, to 20 with the largest amount of food. Olcese and Pearson ('48) found more pantothenic acid in the feces than in the urine when sheep were given 2,000 mg of Ca pantothenate. In the present experiments with rats there was no decrease in the urinary excretion of pantothenic acid in the presence of food, but increased fecal excretion did occur. The amount of pantothenic acid excreted in the feces, even in the presence of food, was never larger than that excreted in the urine. It appears that the rat can absorb considerably more of this vitamin than either the dog or the sheep. Not only is less excreted in the rat feces, but also a larger proportion of a test dose appears in the urine (from 23–58% in the rat as compared to 2.2–16% in the dog and approximately 4% in the sheep), irrespective of whether food is given with the dose or not.

CONCLUSIONS

1. Under all feeding conditions panthenol is more available than Ca pantothenate, as judged by urinary excretion, when fed to rats at a level of 10 mg kg.

2. At an intermediate level (5 mg kg), panthenol has significantly superior availability when administered to rats fed ad libitum or in the postprandial state, the differences being 85 and 45%, respectively. When the doses were administered in the postabsorptive state, the difference was 24%, which is not statistically significant.

3. At the lowest level (2 or 1.25 mg kg), no significant differences in the urinary excretion of pantothenic acid were found between panthenol and Ca pantothenate, regardless of the immediately previous nutritional status of the animals.

4. Recoveries of pantothenic acid in the urine in the 24 hours after dosage range from 49 to 74% of the dose of panthenol and 23 to 58% of the dose of Ca pantothenate.

5. The presence of food at the time of ingestion of either panthenol or Ca pantothenate does not materially affect the urinary excretion of pantothenic acid. However, in the case of Ca pantothenate at the highest level of dosage (10 mg kg),

the presence of food causes a significantly increased excretion of fecal pantothenic acid. At the intermediate level (5 mg kg) there is also an increase in fecal excretion when food is present, but this is not statistically significant. At both these levels some of the ingested Ca pantothenate is excreted in the feces even when food is absent. At the lowest level (2 mg kg) no increase in fecal pantothenic acid was observed.

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Determination of Panthenol and Pantothenates in Multivitamin Preparations

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▶ Crokaert showed that panthenol and pantothenates can be hydrolyzed to β-alanol or β-alanine, respectively, and that these amino nitrogen–containing compounds can then be determined colorimetrically with 1,2-naphthoquinone sulfonate or by the ninhydrin reaction for amino acids. Application of these reactions to multivitamin preparations has been investigated. Other vitamins, minerals, amino acids, and vehicles in pharmaceuticals, which had presented difficulties with other chemical methods, can be eliminated by cation and anion exchange resins and adsorbents. The decomposition products, β-alanol and β-alanine, are removed by the cation exchange resin prior to colorimetry, which renders the method specific for the intact molecule. Quantities down to 2.5 mg. of panthenol or pantothenates can be determined in multivitamin preparations with the naphthoquinone procedure; the more rapid and sensitive ninhydrin method permits determination of as little as 1 mg. of these vitamins with good precision.

PANTHENOL or pantothenates assume an increasingly important role as members of the vitamin B complex in multivitamin preparations.

Several chemical colorimetric methods for the determination of panthenol [2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutyramide] and pantothenates [salts of 2,4-dihydroxy-N-(2-carboxyethyl)-3,3-dimethylbutyramide] have been reported. All require cleavage of the molecule prior to colorimetry. With alkali treatment panthenol will yield β-alanol (3-aminopropanol) and pantoic acid (2,4-dihydroxy-3,3-dimethylbutyric acid) sodium salt, while pantothenic acid will split into β-alanine (3-aminopropionic acid) and pantoic acid sodium salt. Acid hydrolysis will produce β-alanol from panthenol, β-alanine from pantothenic acid, and pantoyl lactone (2,4-dihydroxy-3,3-dimethyl butyrolactone) from both.

The method of Szalkowski, Mader, and Frediani (*13*) is based upon the reaction of β-alanine, one of the cleavage products of pantothenic acid, with 2,4-dinitrophenylhydrazine. Another approach by Szalkowski and Davidson (*12*) led to the reaction of pantoyl lactone with 2,7-naphthalenediol in concentrated sulfuric acid to yield a colored complex. In 1950 the authors (*16*) published a procedure for the determination of panthenol and pantothenates, based on the reaction of pantoyl lactone with hydroxylamine and final development of a purple color with ferric chloride.

Although well suited to the determination of calcium pantothenate in certain feed enrichment mixtures and vitamin preparations, the Szalkowski and Davidson method is subject to interference from sugars and common pharmaceutical vehicles, as well as vitamin C and riboflavin, which can be eliminated only by several purification steps. While the hydroxamic acid method can be applied to the determination of panthenol in certain multivitamin preparations, interferences due to sugars and emulsifiers could not be eliminated. This method could not be used to determine pantothenates in multivitamin preparations.

In 1948 Crokaert (*2, 3*) first described a method for the determination of pantothenates, based upon Folin's (*7*) reaction for amino acids as modified by Frame, Russel, and Wilhelmi (*8*) and applied it to the β-alanine cleavage product of pantothenic acid. The reaction involves acid hydrolysis, followed by coupling of the β-alanine with 1,2-naphthoquinone-4-sulfonate to form a yellowish orange solution at pH 9.3. Subsequently, Crokaert, Moore, and Bigwood (*5*) used the more sensitive ninhydrin reaction, as carried out by Moore and Stein (*10*), for the color formation with β-alanine or β-alanol, particularly for the determination of pantothenates and panthenol in urine. To eliminate some of the interfering substances, these authors passed the sample through the strong cation exchange resin Dowex 50 (H), followed by acid hydrolysis, evaporation, solution, and passage through another Dowex 50 column, (Na^+) form, but were able to obtain a recovery of only 94% with pantothenic acid and 72% with panthenol (*4*).

The object of the present work was to adapt the 1,2-naphthoquinone-4-sulfonate procedure as well as a ninhydrin method to the quantitative determination of panthenol and pantothenates in

multivitamin preparations, particularly to formulations where other methods were not applicable.

INTERFERENCES AND THEIR ELIMINATION

When the basic 1,2-naphthoquinone-4-sulfonate method as described by Crokaert (*4*) was applied to individual components of varied multivitamin preparations, the following caused interference: riboflavin, riboflavin 5′-phosphate sodium, niacinamide, all amino acids, liver extracts, ferrous and ferric iron, copper, tin, zinc, manganese, cobalt, and molybdenum. The same ingredients interfered with the ninhydrin reaction.

Other frequent components of multivitamin preparations, such as sugars, ascorbic acid, and the emulsifiers Tweens (polysorbates) and Spans, which had given rise to troublesome colors with other photometric methods, did not interfere here.

With the exception of riboflavin, riboflavin 5′-phosphate, and molybdates, all of the above-mentioned interferences could be eliminated by passing a solution of the sample through a short column of Dowex 50-X4 (H type). Riboflavin could be completely eliminated by allowing the aqueous sample solution to flow through a double-bed column, consisting of the resin Dowex 50-X4 (H type) and the adsorbent Florisil (60 to 100 mesh).

If riboflavin 5′-phosphate or molybdates were present, an anion exchange resin had to be used. These compounds were held quantitatively by a short column of Dowex 1-X4 (Cl type), 100 to 200 mesh, or Amberlite IRA-400 (OH or Cl type). Panthenol is not held by these anion exchange resins. Although some cleavage may occur, particularly from Amberlite IRA-400 (OH type), this will not affect the determination of panthenol, because the cleavage product β-alanol is not held by the resin.

The above-mentioned anion exchange resins, on the other hand, retain pantothenates and, therefore, cannot be used when assaying pantothenic acid or its salts.

In all cases, where panthenol or pantothenates are to be determined, a Dowex 50 (H) column should be used in order to separate any preformed β-alanol or β-alanine: This renders the methods rather specific and permits their use for stability studies.

A triple-bed column was found advantageous in several particularly troublesome preparations. Such a column consists of the cation exchanger Dowex 50 (H), an anion exchange resin, and an adsorbent, such as Florisil.

Other adsorbents, found useful, were Florex (60 to 90 mesh) (Floridin Co., Warren, Pa.) and Frankonit KL, granulated (Pfirschinger Mineralwerke, Kitzingen/Main, Germany).

METHODS

Naphthoquinone Procedure. APPARATUS. Chromatographic glass tube of about 12-mm. diameter and 20 to 30 cm. length, fitted with a stopcock at the bottom.

REAGENTS. Dowex 50-X4 (H type) 100 to 200 mesh (Dow Chemical Co.).

Anion exchange resin, if necessary. Dowex 1-X4 (Cl type) 100 to 200 mesh, or Amberlite IRA-400 (OH or Cl type) (Rohm & Haas Co., Resinous Products Division).

Adsorbent, if necessary. Florisil (60 to 100 mesh) (Floridin Co., Warren, Pa.).

1,2-Naphthoquinone-4-sulfonate sodium salt, 0.5% aqueous solution, prepared fresh, just prior to use.

Acidic formaldehyde. Three parts of 1.5*M* hydrochloric acid, 1 part of glacial acetic acid, and 1 part of 0.15*M* formaldehyde.

Standards, *d*-Panthenol or crystalline *dl*-panthenol, dried for 4 hours at 56° C. over phosphorus pentoxide in vacuo (Abderhalden). An aqueous solution at a concentration of 1 mg. per ml. is prepared.

d-Calcium pantothenate, U.S.P. reference standard, dried for 3 hours at 105° C. An aqueous solution at a concentration of 1 mg. per ml. is prepared.

PROCEDURE. An aqueous solution of the sample is prepared by dissolving a weighed quantity of a finely powdered solid or by diluting a measured volume of a liquid. An aliquot equivalent to 2.5 to 5 mg. of panthenol or pantothenate in not more than 25 ml. of water is placed on a column of Dowex 50 (H) resin in a layer about 3 cm. long between two small pledgets of glass wool. If other resins or adsorbents are necessary, a length of about 3 cm. is usually adequate, but when a comparatively large quantity of riboflavin is present a Florisil column about 5 cm. long should be added. These resins or adsorbents are placed below the Dowex 50 column, separated from each other by a small layer of glass wool. The entire column is thoroughly washed with about 20 ml. of distilled water. The diluted sample is applied to the wet column and allowed to flow through at a rate of about 0.5 ml. per minute and is collected in a 50-ml. volumetric flask until no liquid remains on the top. The column is then eluted rapidly with enough water to bring the volume in the flask to about 40 to 45 ml.

Three milliliters of 2.5*N* sodium hydroxide are added to the flask, which is then heated in a boiling water bath for 1 hour to assure complete cleavage. After the solution has cooled to room temperature, the volume is brought to the mark with water. A 5-ml. aliquot is pipetted into a 25-ml. volumetric flask. One drop of phenolphthalein reagent is added, followed by about 0.6 ml. of 1*N* sulfuric acid and the mixture is then titrated with 0.1*N* sulfuric acid to a colorless end point.

One milliliter of 1% borax (sodium tetraborate decahydrate) solution is added, whereupon the solution should turn pink (pH 9.3); otherwise, another 5-ml. aliquot is titrated. Then 1 ml. of naphthoquinone reagent is added and the flask is placed in a boiling water bath for 10 minutes, then cooled to room temperature. One ml. of formaldehyde reagent is added, followed by 1 ml. of the thiosulfate. After at least 10 minutes' standing, the volume is brought to the mark with ethyl alcohol (or 3A denatured alcohol). This is particularly useful for samples where turbidities were encountered in the aqueous solution, which almost invariably were thus rendered clear. A reagent blank is prepared in a similar manner with which the photometer is set at 0 absorbance at a wave length of 465 mμ. Readings are taken of the sample solution and an equivalent quantity of the standard, which is subjected to exactly the same procedure, including passage through the type of column as used for the sample.

In some cases the solution may be colored or turbid after hydrolysis. To remove the turbidity, it can be centrifuged and aliquots can be taken from the clear supernatant liquid. To eliminate interference due to color, a sample blank can be prepared with the omission of the naphthoquinone reagent. In this case the photometer is set at 0 absorbance with a blank, containing all reagents except naphthoquinone (Solution A). To another 5-ml. aliquot of the hydrolyzed sample solution all reagents except naphthoquinone are added (Solution B). Another flask contains all reagents, including naphthoquinone (Solution C). The sum of the absorbance readings of Solutions B and C is subtracted from the reading of the sample solution, which will compensate for the color of the hydrolyzed sample solution. The reading obtained with Solution C is subtracted from the reading of the standard.

Ninhydrin Procedure. REAGENTS. Ion exchange resins and adsorbents in a chromatographic tube as described in the naphthoquinone procedure.

Phenol solution. Eighty grams of reagent grade phenol are dissolved in 20 ml. of absolute ethanol, with gentle heating. The solution is shaken with 1 gram of Dowex 50 (H) for 20 minutes to remove any traces of ammonia and then decanted.

Cyanide-pyridine reagent. Two milliliters of a 0.01*M* solution of potassium cyanide (freshly prepared) are diluted to 100 ml. with ammonia-free pyridine, prepared by shaking 100 ml. of CP. pyridine with 1 gram of Dowex 50 (H) for about 20 minutes.

Ninhydrin solution, 0.5 gram of 1,2,3-triketohydrindene in 10 ml. of absolute ethyl alcohol.

Standards, as described in the naphthoquinone procedure.

PROCEDURE. The sample is prepared by dissolving a weighed quantity of a finely powdered solid or by diluting a measured volume of liquid to an appropriate volume. An aliquot of this solution, containing between 1 and 2 mg. of panthenol or pantothenates in not more than 25 ml. of water, is placed on the column, prepared so as to eliminate possible interferences (see discussion of interferences and their elimination).

The sample solution is allowed to flow through the column at a rate of

Table I. Results Obtained with Various Methods

	Sample	Microbio. Assay, Mg./Tab. or Ml.	Naphthoquinone Method	Dev. from Microbio. Assay, %		Ninhydrin Method	Dev. from Microbio. Assay, %		Type of Column[a]
		PANTHENOL							
1	Multivitamin gelatin caps	Gelatin capsules, vitamins A, D, E, C, B complex	4.4	4.2	−0.2	−5	4.1	−0.3	−7	A, D
2	Coated multivitamin-mineral tablets	Coated tablets, vitamins A, D, E, C, B complex, iron calcium, magnesium, manganese, phosphorus	7.0	7.3	+0.3	+4	7.2	+0.2	+3	A, D
3	Multivitamin-mineral tablets	Coated tablets, vitamins A, D, B complex, iron	17.8	18.9	+1.1	+6	19.3	+1.5	+8	A, B, D
4	Multivitamin drops	Drops, vitamins A, D, E, C, B complex, including riboflavin 5′-phosphate sodium	21.3	21.1	−0.2	−1	20.1	−1.2	−6	A, B
5	Multivitamin drops with amino acids	Drops, vitamins A, D, E, C, B complex, including riboflavin 5′-phosphate sodium and several amino acids	19.8	21.2	+1.4	+7	19.6	−0.2	−1	A, B
6	B complex and C ampoule solution	Ampoule solution, vitamins C and B complex, including riboflavin 5′-phosphate sodium	11.5	11.7	+0.2	+2	11.6	+0.1	+1	A, B
7	B complex-liver ampoule solution	Ampoule solution, vitamin B complex, liver injection U. S. P., vitamins A, D, E, and *d*-panthenol	5.0	5.4	+0.4	+8	5.6	+0.6	+11	A, C
8	Multivitamin cream	Coated tablets, vitamins A, D, E, C, B complex with *d*-calcium pantothenate, iron, calcium, magnesium, manganese, phosphorus	56.0	54.0	−2.0	−4	56.0	0	0	A
		PANTOTHENATES								
9	Coated multivitamin-mineral tablets	Coated tablets, vitamins A, D, E, C, B complex with *d*-calcium pantothenate, iron, calcium, magnesium, manganese, phosphorus	4.9	5.0	+0.1	+2	4.8	−0.1	−2	A, D
10	Coated multivitamin-mineral tablets	Drops, vitamins C and B complex, exactly 0.5 mg. *d*-calcium pantothenate added	1.4	...			1.5	+0.1	+8	A, D
11	Multivitamin drops		0.5[b]	0.51	+0.01	+2	0.49	−0.01	−2	A, D

[a] A. Dowex 50-X4 (H).
B. Dowex 1-X4 (Cl).
C. Amberlite IRA-400 (OH).
D. Florisil.
[b] Known quantity added.

about 0.5 ml. per minute until no liquid remains on the top and is collected in a 100-ml. volumetric flask. The column is then eluted rapidly with enough water to bring the volume in the flask to about 40 ml. Five milliliters of sodium hydroxide 1% (about 0.25*N*) are added and the flask is placed in a boiling water bath for 1 hour. After being cooled to room temperature, and after addition of 1 drop of phenolphthalein reagent, the solution is titrated with 1*N* sulfuric acid to a colorless end point, followed with 2 drops in excess. Fifty milliliters of ethyl alcohol (or 3A denatured alcohol) are added, and the solution is mixed and brought to the mark with water. A 2-ml. aliquot is pipetted into a 10-ml. volumetric flask, and 1 ml. of phenol reagent is added, followed by 1 ml. of pyridine-potassium cyanide solution. The flask is placed in a water bath (95° to 100°) for 1 minute and 0.2 ml. of ninhydrin reagent is added. The flask is stoppered and allowed to remain for 5 minutes more in the water bath. (If the stopper is forced out, it should be replaced immediately.) The flask is then removed and brought to room temperature and the volume is adjusted to the mark with 60 volume % ethyl alcohol. A standard, containing a known quantity (1 to 2 mg.) of panthenol or pantothenate, is placed on the same type of column as used for the sample and simultaneously subjected to the same procedure.

A reagent blank is prepared in a 10-ml. volumetric flask, consisting of 2 ml. of 60% ethyl alcohol in place of the sample. It is treated in the same manner as the sample, including addition of all required reagents.

The photometer is set at 0 absorbance at 570 mμ with the blank and readings of sample and standard are taken.

If the sample solution is highly colored after hydrolysis, it may produce a blank reading, which can be compensated for in the following manner.

The photometer is set at 0 absorbance with a blank, containing all reagents except ninhydrin (Solution A). To a 2-ml. aliquot of the hydrolyzed sample solution all reagents with the exception of ninhydrin are added (Solution B). Another 10-ml. flask contains all reagents, including ninhydrin (Solution C). The sum of the absorbance readings of Solutions B and C is subtracted from the reading of the sample solution. The reading obtained with Solution C is subtracted from the reading of the standard.

RESULTS

Accuracy. Results obtained with both methods on a variety of multivitamin preparations are listed in Table I and compared with the results obtained by microbiological procedures. This table also indicates

the type of solvent used for each sample.

Panthenol was determined microbiologically by the method of DeRitter and Rubin (*6*) as modified by Weiss and associates (*15*), while pantothenates were assayed by the microbiological procedure of Skeggs and Wright (*11*). Panthenol and pantothenic acid can be determined satisfactorily by either of the two chemical methods, when present in combination with many other vitamins, minerals, amino acids, liver extract, and vehicles, common in pharmaceutical preparations. As the microbiological methods are claimed to have a precision within ±10%, the results of the chemical methods show good agreement. In sample 11 a known quantity of *d*-calcium pantothenate was added to a multivitamin solution and the recovery, using both chemical methods, was within ± 2%.

Precision. The precision obtainable with both methods, applied to replicate determinations of a multivitamin ampoule solution, is illustrated in Table II. With the naphthoquinone procedure a standard deviation (s) of 0.31 was obtained on six replicate runs, while the ninhydrin procedure resulted in a standard deviation (s) of 0.29, by using the formula:

$$s = \sqrt{\frac{\Sigma (x - \bar{x})^2}{n - 1}}$$

where

x is the individual value
$\bar{x}$ is the mean value
n represents the number of values

Table II. Precision of Methods for Multivitamin Solution

	Naphthoquinone Method		Ninhydrin Method	
Sample	Mg./ml.	Dev. from mean	Mg./ml.	Dev. from mean
1	11.6	−0.2	11.6	+0.1
2	11.7	−0.1	11.7	+0.2
3	12.2	+0.4	11.6	+0.1
4	12.2	+0.4	11.5	0
5	11.7	−0.1	10.9	−0.6
6	11.5	−0.3	11.5	0
Mean	11.8		11.5	
Std. deviation, s	0.31		0.29	

APPLICATION TO STABILITY STUDIES

The specificity of the methods, when applied to stability studies, is demonstrated in Table III. Two ampoule solutions, containing the same B complex vitamins as well as vitamin C, except that sample 12 contained 3.6 mg. per ml. of *d*-panthenol, while sample 13 contained 4.6 mg. of *d*-pantothenate sodium salt, were stored for 2.5 years at room temperature. Both samples were assayed by the microbiological and chemical methods.

The stability of *d*-panthenol (contained in sample 12) is demonstrated by the excellent agreement of all three methods, indicating a loss in potency of approximately only 10% after that time. In sample 13, containing *d*-pantothenate sodium, the potency loss amounted to approximately 75% as proved by the results of all three procedures.

DISCUSSION

The naphthoquinone reaction used was essentially the one described by Crokaert (*2–5*). However, to ensure complete cleavage, alkaline hydrolysis was used in preference to acid treatment. While Crokaert had used the ninhydrin procedure of Moore and Stein (*10*), the more recent method of Troll and Cannan (*14*), which furnishes quantitative color yields is better suited for the present purposes. The reagents are more easily purified than those advocated in another modification of the ninhydrin method, published by Yemm and Cocking (*1, 17*). The necessity of careful purification of methyl Cellosolve, used as the solvent in the Yemm and Cocking method, has been pointed out by Kalant (*9*).

Absorption curves of both color reactions (Figure 1) show the maximum of the naphthoquinone color reaction at 465 mμ and of the ninhydrin-produced color at 570 mμ. For the naphthoquinone procedure Beer's law is obeyed within the range of 12 to 24 γ of panthenol or pantothenate; for the ninhydrin method this holds true within the limits of 2 to 5 γ per ml. of final solution.

Although both methods can be applied satisfactorily to a variety of pharmaceutical preparations, the authors' experience gives preference to the ninhydrin procedure.

Its greater sensitivity makes it possible to assay preparations with a relatively low potency of panthenol or pantothenates.

Because of the greater dilution of the sample in the final solution, the reading of the sample blank is usually 0 or shows a very low absorbance. In assaying samples 8 and 9 (Table I), relatively high sample blank readings had to be subtracted when the naphthoquinone reaction was applied. Neither sample gave a blank reading with the ninhydrin method.

Because the pH of 9.3 for the naphthoquinone reaction is rather critical, it may be difficult to titrate the 5-ml. aliquot of the hydrolyzed sample solution to a phenolphthalein end point, if the solution is highly colored. In the ninhydrin procedure adjustment of the pH of the hydrolyzed sample solution is not critical. The purpose of the titration is merely to bring the pH of the sample solution into the acidic pH range, while the phenol-pyridine reagent will serve as the buffer.

The time required for the ninhydrin method is somewhat shorter, about 2 hours as against 2.5 hours for the naphthoquinone procedure.

Table III. Application to Stability Studies

Sample, B Complex with Vitamin C (Ampoule Soln.)	Assay, Mg./Ml. Microbio.	Naphthoquinone	Ninhydrin
12. With *d*-panthenol			
Initial	3.6		
After 2.5 years at r.t.	3.3	3.3	3.2
13. With *d*-pantothenate			
Initial	4.6		
After 2.5 years at r.t.	1.2	1.1	1.1

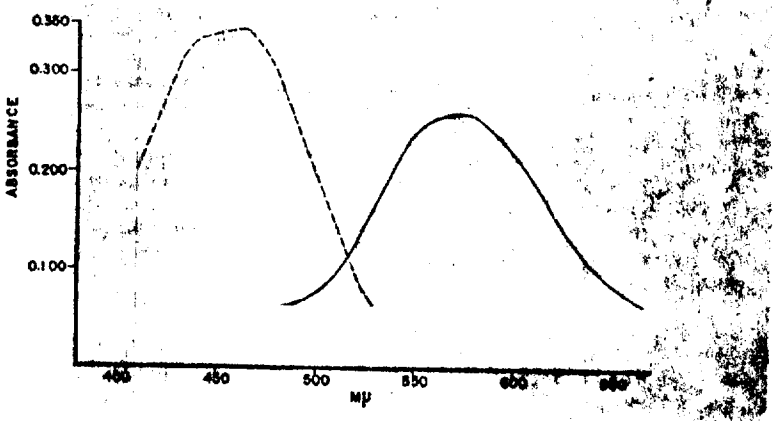

Figure 1. Absorption curves

– – – Naphthoquinone reaction
——— Ninhydrin reaction

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Silber-45
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Absorption and Excretion of Calcium Pantothenate by Normal and Depleted Dogs

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Introduction

Although the urinary excretion of pantothenic acid has been studied in a variety of animal species, only one of the investigators attempted to establish a saturation test for the deficiency. Gordon (1942) was unable to detect any difference in the excretion of a test dose of calcium pantothenate by medical students and patients with general nutritional deficiency. Studies made in this laboratory (Silber, 1943) showed that the pantothenic acid level in tissues of depleted dogs could be increased at least 4 mg. per kg. body weight by calcium pantothenate administration, thus indicating the possibility of developing a saturation test for this species. The results of such a study conducted in the course of an investigation of pantothenic acid depletion in dogs (Seeler and Silber, unpublished; Silber, 1943) are reported in this communication. In connection with the standardization of conditions for oral saturation tests, the effect of food upon absorption and excretion of calcium pantothenate has also been studied.

Experimental

1. Saturation Tests

Two adult dogs and seven pups which received the basal diet (Silber, 1943) and B vitamins, exclusive of calcium pantothenate, were used for saturation studies. All except two pups were depleted twice. Three additional pups which were fed a stock diet consisting of dog pellets plus 20 g. dried beef liver daily served as controls. After a period of depletion in which food consumption, growth, and excretion of pantothenic acid were depressed, a dose of 4 mg. calcium pantothenate per kg. body weight was administered, either orally or subcutaneously, following an overnight fast. Food was withheld for 3 to 4 hours after the test dose and 24 hour urines were collected under toluene for pantothenic acid assay with *L. casei* as the test organism

(Silber and Mushett, 1942). The saturation test data are summarized in Tables I and II.

In the 24 hours following oral administration of 4 mg. calcium pantothenate per kg., depleted dogs excreted 0.13%, and normal dogs 1.7% of the test dose. After parenteral administration of the same dose, depleted dogs excreted 3.3%, and their litter mate controls 11.7%. In none of the tests was a significant fraction of the test dose detected in the urine on the second day.

When 10 adult, non-depleted dogs, selected at random, were given 4 mg. calcium pantothenate per kg. orally, an average of 1.4% of the dose was excreted in the urine in 24 hours. Two of these dogs which showed signs of malnutrition, excreted less than 0.1% of the dose. Three healthy dogs from our stock colony excreted 18, 25, and 27% of a subcutaneous dose of 4 mg. per kg.

TABLE I

Excretion of an Oral Test Dose of Calcium Pantothenate (4 mg. per kg.) by Depleted Dogs and Dogs Fed an Adequate Diet

Dog No. and sex	Weight	Months of depletion	Urinary P.A., per 24 hours		Per cent of dose found
			Before dose	After dose	
	kg.		γ	γ	
Adults					
103 F	7.7	8	4	50	0.15
103 F	7.7	3	17	115	0.32
113 F	7.0	6	3	15	0.04
113 F	6.2	3	9	23	0.06
Pups					
206 M	4.1	2	8	66	0.35*
213 M	4.2	2	13	20	0.04
280 M	4.2	1	—	3	0.0
296 F	5.4	1	11	110	0.46*
301 M	5.0	1	2	5	0.0
302 M	3.2	1	1	3	0.0
304 M	3.7	1	25	25	0.0
					Average 0.13%
Controls					
282 M	11.5	0	90	500	0.9
299 F	8.1	0	350	850	1.5
303 M	9.5	0	135	1200	2.5
					Average 1.7

* It is possible that slight contamination from loose stools occurred.

TABLE II

Excretion of a Subcutaneous Test Dose of Calcium Pantothenate (4 mg. per kg.) by Depleted Dogs and Dogs Fed an Adequate Diet

Dog No. and sex	Weight	Months of depletion	Urinary P.A., per 24 hours		Per cent of dose found
			Before dose	After dose	
	kg.		γ	γ	
280 M	3.9	1.5	1	540	3.5
296 F	4.3	1.5	5	640	3.7
301 M	4.1	1.5	2	230	1.4
302 M	3.0	1.5	1	600	5.0
304 M	3.5	1.5	3	420	3.0
					Average 3.3
Controls					
282 M	9.5	0	60	3300	8.5
299 F	6.4	0	150	3000	11.1
303 M	7.5	0	70	4700	15.4
					Average 11.7

Fourteen to twenty-seven per cent of the administered pantothenic acid was recovered in the 24 hour feces of dogs, whether depleted or normal, when the test dose was given by mouth. However, when it was injected subcutaneously, no increase in fecal pantothenic acid was found.

Evidence of intestinal absorption by the depleted dogs was obtained by determining the urinary excretion of an oral dose of riboflavin. Thus, four pantothenic-acid-depleted pups excreted 8.2% (5.2–11.6) of a 4 mg. per kg. dose of riboflavin whereas 3 control dogs excreted 5.6% (5.0–6.0) of the dose in 24 hours.

That the dogs were actually in a state of pantothenic acid deficiency was borne out by the growth response when calcium pantothenate was supplied daily after the last test dose had been administered. Three pups (No. 280, 296, 302) showed weight increases of 10.2, 7.5, and 7.9 kg., respectively, within 3 months after initiation of calcium pantothenate therapy, whereas in the previous 2½ months they had lost an average of 0.8 kg. each. Similar weight responses resulted when two depleted adult dogs received daily oral therapy. When the 4 mg. per kg. dose was administered daily, the urinary excretion of pantothenic acid gradually increased from 0.2% to 20–30% of the dose within 2 weeks.

In order to obtain information regarding the excretion of oral doses of calcium pantothenate by men, tests were performed on a group of 10 laboratory workers. Since the peak excretion of pantothenic acid after oral administration occurs within the first 4 hours, this was employed as the test period. Two and one half hours after

a light breakfast, the bladder was emptied and 50 mg. of calcium pantothenate were given with 200 cc. of water. Four hour urines were assayed for their pantothenic acid content along with control urines collected in the same manner the previous day. Prior to dosing, 1.0 ± 0.15 * mg. pantothenic acid was excreted in the 4 hour period; after the test dose, this figure was raised to 6.0 ± 0.48 mg.*

2. *Effect of Food on Absorption and Excretion of Vitamins*

In a study of riboflavin and pantothenic acid deficiency in dogs, it was found that as food consumption was depressed in the advanced stages of riboflavin deficiency, the excretion of orally administered calcium pantothenate increased. Furthermore, when pantothenic acid deficiency was induced, again with a decrease in food consumption, the excretion of orally administered riboflavin increased. These results suggested a relationship between the two vitamins similar to that observed by Sure and Ford (1942) for thiamine and riboflavin in thiamine-depleted rats. However, on analysis, the effect was seen to be solely a result of enhanced absorption from the intestinal tract in the absence of food, as demonstrated in the following experiments.

Four dogs which for 6 months had been receiving the basal diet plus a daily supplement of B vitamins (including 40 mg. calcium pantothenate and 1 mg. riboflavin) were given their vitamins in the morning with graded amounts (0–100 g.) of basal diet. Additional diet was supplied *ad libitum* 4 hours later and 24 hour urine and feces samples were assayed for the vitamins. The urinary excretion of both vitamins increased when no food was given with the vitamins and decreased when food was supplied (Table III). Fecal assays showed that the decrease in urinary excretion of pantothenic acid could be correlated with an increase in the fraction of the dose which passed through the intestinal tract unabsorbed. Excretion of pantothenic acid into the intestinal tract did not appear to be involved since parenterally administered calcium pantothenate was not excreted in the feces.

Furthermore, when two dogs had their food intake restricted to 15 g. per day for one week and the B vitamins were administered orally as above, 35 to 65% of the 40 mg. dose of calcium pantothenate was excreted in the urine in 24 hours. When food was then supplied *ad libitum* with the vitamin supplement, the urinary excretion decreased rapidly until one month later it was only 1 to 2.5% of the dose. The food consumption at this time averaged 370 g. per day.

Thus, when the food intake was decreased (either by a vitamin deficiency or by restricting food intake), the urinary excretion of calcium pantothenate was increased by as much as 50% of the amount fed as a dietary supplement and when food was supplied with the vitamins, as much as 50% passed through the intestinal tract unabsorbed.

* Standard Error.

TABLE III

Influence of Food Consumption on the 24 Hour Excretion of Pantothenic Acid (40 mg.) and Riboflavin (1 mg.)

Dog	Diet given with the vitamins	Total diet consumed	Urinary excretion		Fecal excretion	
			P.A.	Ribo.	P.A.	Ribo.
No.	*g.*	*g.*		*mg.*		*mg.*
202	0	140	7.2	0.45	2.0	0.05
203	0	135	1.7	0.86	10.5	0.13
204	0	220	0.2	0.70	9.5	0.12
205	0	65	1.7	0.84	—	—
Averages			0.7	0.7	7.3	0.10
202	25	155	2.8	0.45	23.0	0.27
203	25	225	2.0	0.40	11.0	0.18
204	25	145	5.5	0.37	11.5	0.25
205	25	135	4.5	0.45	7.5	0.10
Averages			3.7	0.42	13.0	0.25
202	50	130	2.4	0.27	11.0	0.13
203	50	135	0.25	0.08	12.5	0.18
204	50	150	1.25	0.45	15.0	0.14
205	50	225	4.7	0.52	7.0	0.08
Averages			2.15	0.33	11.4	0.13
202	100	210	0.6	0.30	—	—
203	100	240	0.15	0.05	22.0	0.34
204	100	130	0.70	0.60	12.5	0.14
205	100	135	2.0	0.35	19.0	0.25
Averages			0.86	0.30	17.8	0.18

Discussion

Gordon (1942), in his study conducted with medical students and patients, did not find any significant difference in the excretion of test doses (10 or 20 mg.) of calcium pantothenate. In our experiments, however, which were conducted on severely depleted dogs, a marked difference was found between the controls and the pantothenic-acid-deficient animals. Normal dogs excreted 13 times as much of an oral

dose of 4 mg. calcium pantothenate per kg. as depleted dogs. After a similar dose was administered subcutaneously, more of the vitamin appeared in the urines of both groups, and the control dogs excreted only 3.5 times as much as the depleted animals. In terms of total urinary excretion, after oral administration, normal dogs excreted 1.6% more of the dose than depleted dogs; and after subcutaneous administration, excretion by the normal dogs exceeded that of the depleted dogs by 8.5% of the dose. Faulty intestinal absorption did not appear to be a complicating factor in the oral test because the pantothenic-acid-depleted dogs excreted as much of a test dose of riboflavin as the control dogs.

An increased urinary excretion of riboflavin as a result of thiamine deficiency (Sure and Ford, 1942) or as a result of thiamine administration (Klopp, Abels, and Rhoads, 1943) has been interpreted as evidence of a vitamin interrelationship. It has also been reported that in thiamine-deficient rats the riboflavin content of tissues decreases (Sure and Ford, 1942) and the riboflavin level of the liver increases (Singher, *et al.*, 1944), that thiamine or pantothenic-acid-depleted rats are limited in their ability to mobilize riboflavin in the liver after feeding (Supplee, *et al.*, 1942), and that pantothenic acid and riboflavin blood levels in man are related because an increase in one was accompanied by an increase in the other (Spies, *et al.*, 1940). Ferrebee and Weissman (1943) also found lower levels of riboflavin in the livers of rats depleted of thiamine. They felt, however, that it was not significant because the levels of riboflavin were essentially the same throughout the depletion period. They also regarded the increased excretion of riboflavin in terminal thiamine deficiency as a non-specific effect due to tissue catabolism. In a 2 hour excretion experiment in dogs (Silber and Unna, 1942), no effect of the intravenous administration of calcium pantothenate on the riboflavin levels in the blood or urine was observed and similarly, when sodium riboflavinate was administered, no effect on the pantothenic acid levels was found.

The present experiments indicate that when excretion data are used to evaluate the status of a deficiency or to demonstrate vitamin interrelationships, extreme care must be taken to make valid comparisons. Not only must food intake be controlled but the vitamin supplement must be administered to the test animals and their controls in exactly the same manner, preferably orally after overnight fasting or parenterally, so that the presence of food in the stomach will not

confuse the test. Since the administration of pantothenic acid with food may result in the loss of a large fraction in the feces, it appears likely that tissue levels may also be higher or lower depending upon the way in which the vitamin supplement is supplied.

Summary

(1) Significant differences in the excretion of oral or subcutaneous test doses of calcium pantothenate by normal and depleted dogs have been found. Intestinal absorption did not appear to be impaired by pantothenic acid depletion as judged by excretion of oral doses of riboflavin.

(2) The urinary excretion of an oral dose of calcium pantothenate by dogs may be as much as 50% or as little as 1% of the dose, in inverse relation to the amount of diet consumed with the vitamin. When food was supplied *ad libitum*, a large fraction of the dose passed through the intestinal tract unabsorbed. It is suggested that this factor be considered in experiments designed to demonstrate vitamin interrelationships on the basis of vitamin levels.

(3) After parenteral administration of calcium pantothenate, no increase in fecal pantothenic acid was detected.

(4) In a four hour period, a group of 10 laboratory workers excreted 10% of a 50 mg. dose of calcium pantothenate.

Acknowledgment

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Stepanyan-'61
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Subject: Calcium Pantothenate in Clinical Tuberculosis Connected with the Side Effect of Streptomycin

Species: Human beings; white mice

Strain: Not stated

Sex: Not stated

Age at start of experiment: Not stated

Number of animals per group: Humans -- 55 adult patients; White mice -- 5 groups; 20 in each

Duration of study: Humans --5 months; White mice -- 1 month

Vehicle: Calcium pantothenate in conjunction with streptomycin; also in combination with isoniazid and PAS (para-aminosalicylic acid). Effect studied on vestibular and allergic reactions.

Dose schedule: Humans -- 100 - 800 mg of calcium pantothenate; 2 intakes internally; in 20% aqueous solution when administered intramuscularly;

White mice -- 1 - 100 gamma to 1 ml of medium

Route of administration- Humans -- internally or intramuscularly: White mice -- Experiments in vitro; infected with tuberculous culture

Number of animals per level: Not stated

Observations

Side effects eliminated in 30 out of 43 patients (humans) by calcium pantothenate. No effect where side effects produced by isoniazid or PAS.

Calcium pantothenate most effective in vestibular disturbances; Negligible effect in the case of allergic reactions.

No pronounced toxicity on part of calcium pantothenate where administered to those ill with tuberculosis.

Addition of calcium pantothenate did not chanbe gacteriostatic effect of streptomycin on tuberculosis cultures in vitro, nor the chemotherapeutic activity in tuberculosis of white mice

Patients ill with tuberculosis sided by calcium pantothenate.

Where side effects of streptomycin, clinical administration of calcium pantothenate recommended. Mechanism of its effect not yet clarified.

Calcium Pantothenate in Clinical Tuberculosis Connected with the Side Effect of Streptomycin[1]

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The treatment of patients ill with lung tuberculosis is effective only in the case of a prolonged application of specific antibacterial preparations. At the same time, in a number of cases, there are observed side effects, and sometimes -- an absence of a therapeutic effect. This is explained, in conformity with the data in the literature, by the antagonistic interrelationship of the antitubercular preparations and of some vitamins in the B group, in particular streptomycin and pantothenic acid.

The percent of side effects in treatment with streptomycin fluctuates within the limits of 2 - 30; it becomes necessary to interrupt the treatment, in 2 - 3% of the cases, because of its intolerability (U.A. Askarov, E.M. Grinshpunt, I.A.Kassirskii, M.A. Klebanov and R. O. Drabkina, A.E. Rabukhin, D. D. Yablokov, et al.).

In order to weaken the toxic effect of streptomycin a number of medicinal agents have been proposed; antihistamine preparations, calcium salts,glycine, cysteine, vitamins B_1 and B_2, PP, pantothenic acid, aminazin, corticosteroid hormones, and so forth. However, only part of them possess a definite toxic effect, and others are poorly effective.

It is interesting to make a study of calcium pantothenate in view of its very low toxicity, and also, the possibilities of its influence both as a vitamin as well as a calcium preparation.

Data existing in the literature concerned with the effect of pantothenic

acid on the toxic action of streptomycin are contradictory. The absence of its effect has been noted, basically, in experiments on animals in cases where streptomycin was designated in admittedly highly toxic doses (Child, Hawkins, Osterberg, et al.). In clinical testing, however, of pantothenic acid, the toxic effect of streptomycin, dihydrostreptomycin, and biomycin, in the majority of cases, was prevented or reduced (Weber, Ducrot, Kuntz, Penman, Stark, et al.).

In the therapeutic clinic of the Tuberculosis Institute of the Academy of Medical Sciences of the U.S.S.R., calcium pantothenate was utilized in 55 adult patients with various forms of tuberculosis of the lungs, in the case of a marked side effect on the part of streptomycin (43), isoniazid (5) and PAS (para-aminosalicylic acid) (7 patients).

Calcium pantothenate was prescribed in a daily dose ranging from 100 to 800 mg internally (in 2 intakes) or intramuscularly in a 20% aqueous solution. No side effects, associated with its prescription, were noted both following a single dose, as well as following a daily intake, over a course of 5 months.

In the case of a prolonged treatment with streptomycin, where it has a side effect, it is constantly necessary to prescribe calcium pantothenate also. Only 6 patients out of 43 were able to continue treatment with streptomycin without calcium pantothenate following their joint application over a period of 20 - 30 days. The application of calcium pantothenate made it possible to continue treatment with streptomycin in 30 out of 43 patients in those cases where it should have been discontinued because of the side effect of the latter.

The prescription, however, of calcium pantothenate for 12 patients, with a pronounced side effect from isoniazid and PAS, in all cases, proved to be ineffective. It should be specified that these were patients with a serious course of tuberculosis. Further observations may solve the problem concerned with the favorable effect of pantothenic acid in the case of a side effect on the part of other antitubercular preparations (see table).

The most rapid and perceptible clinical action of the preparation was observed in manifestations of coronary spasm, giddiness, headaches, and paresthesia. Nevertheless, it didnot have any effect on temperature reactions, hypereosinophilia, and also, in the majority of cases -- on skin eruptions, itching, and exacerbation of eczema, i.e., on reactions of an allergic character which are customarily readily eliminated by antihistamine preparations. Of 13 side reactions that could not be eliminated 10 bore an allergic character.

Effect of Calcium Pantothenate Depending on the Character of the Side Effects of Streptomycin

Character of Side Reactions	Number of Patients	Favorable Effect of Calcium Panththenate		
		Pronounced	Moderate	Absent
Giddiness with Impairment of Movement Coordination	21	15	3	3
Headache with Sleep Deterioration and that of "Self-Sensation:	3	3	-	-
Manifestations of Coronary Spasm	5	5	-	-
Paresthesia	2	2	-	-
Skin Manifestations	7	2	-	5
Temperature Reaction	4	-	-	4
Hypereosinophilia	1	-	-	1
Total.....	43	27	3	13

Proceeding from the data obtained, it is possible to assume that calcium pantothenate is poorly effective in the case of side reactions of streptomycin of an allergic character. This attests to the fact that one cannot explain the reason for its favorable effect on the organism by the antihistamine action of calcium pantothenate (M. L. Rokhlina, T.V. Zubareva).

Nor can one explain the mechanism of the favorable effect of calcium

pantothenate only by the limitation in the binding of ionized calcium with streptomycin, as has been done by Keller and his co-workers.

One may assume a similarity in the mechanism of the favorable effect of corticosteroid hormones and in that of calcium pantothenate in the side effect of streptomycin to the extent that the endogenous synthesis of corticosteroids, which assures a sufficient function on the part of the adrenal cortex, takes place, to a certain degree, from pantothenic acid (Yu. A. Serebrovskaya, Olivi and his co-workers, et al.).

The most probable is the assumption that the administration of calcium pantothenate increases the content of coenzyme A in the tissues; and this is instrumental in eliminating fine impairments in the intermediate metabolism that are sometimes manifested clinically in the form of side reactions.

The experimental data of A. N. Ilimov point out such a possibility to the extent that pantothenic acid is capable of normalizing an impairment in the aerobic respiration and in the oxidative phosphorylation that are due to streptomycin as a result of impairing the synthesis of coenzyme A.

At the same time, we were faced with the problem that an increased formation of coenzyme A, with an excess administration of pantothenic acid, may improve the tolerance to streptomycin as a result of its greater inactivation. At the same time, one could expect a deterioration in the course of illness in patients receiving a combination of streptomycin and pantothenic acid. However, both our data, as well as the data in the literature, attest to the reverse -- to an improvement in the course of illness.

For a definitive solution of the problem concerned with the influence of calcium pantothenate on the becteriostatic and chromotherapeutic effect of streptomycin, the microbiological laboratory at the Institute of Tuberculosis of the Academy of Medical Sciences of the U.S.S.R. has carried out experimental investigations to the extent that it was not possible to find any definite literature data on this problem.

A study of the influence of calcium pantothenate on the bacteriostatic activity of streptomycin was carried out in experiments in vitro. Tuberculosis cultures, which were sensitive and resistant to streptomycin, were inoculated on a synthetic medium containing various concentrations of streptomycin (depending on the sensitivity of the culture to it) and of calcium pantothenate. Concentrations of the latter fluctuated from 1 to 100 gamma to 1 ml of the medium.

In experiments it was disclosed that calcium pantothenate did not reduce and did not increase the bacteriostatic activity of streptomycin.

In order to clarify the action of calcium pantothenate on the chemotherapeutic activity of streptomycin experiments were carried out on white mice infected with a tuberculosis culture. The animals were distributed in 5 groups with 10 animals in each. The first group received only streptomycin, the second and third -- streptomycin in combination with various doses of calcium pantothenate (1 - 10 mg), and the fourth one -- only pantothenate; the fifth one served as a control. Following a month of treatment it was indicated that the therapeutic effect of streptomycin was similar in groups of animals that received only the antibiotic and the antibiotic in combination with various doses of calcium pantothenate.

The results obtained indicated that calcium pantothenate in dosages equivalent to clinical ones do not reduce the bacteriostatic and chemotherapeutic activity of streptomycin.

In clinical literature, the point of view is widespread that streptomycin considerably inhibits the vital activity of the intestinal microflora, and in connection with this -- the synthesis of vitamins of the B group, including pantothenic acid. For this reason, it is also assumed that the clinically observed side effects from streptomycin are due, to a considerable extent, to B_5 hypovitaminosis, all the more because the prescription of pantothenic acid manifests, at the same time, a favorable effect.

Luntz, Manthei are inclined to believe that apantothenosis is a basic side effect on using not only streptomycin, but also isoniazid.

However, if the matter under consideration was concerned with simple B_5 hypovitaminosis, then, in order to eliminate the side effect of streptomycin, it would not be necessary to have recourse to a prescription of pantothenic acid in dosages approximately 100 times in excess of the daily requirement of the human being. We are constrained to doubt the presence of insufficiency of pantothenic acid in treatment with streptomycin in considering, also, the papers of Kallin and Meyer, with their co-workers. These authors have shown that in streptomycin therapy the amount of pantothenic acid in the blood of patients not only did not decrease, but even increased both in fresh, as well as in chronic, forms of tuberculosis. This is explained by the fact that the coli-bacillary flora of the intestine is rapidly adapted to the prescription of streptomycin. Following this, it continues its proliferation and production of vitamins in the same manner as prior to the prescription of streptomycin.

The above-enumerated, highly contradictory points of view relative to the problem concerned with the mechanism of the favorable effect of calcium pantothenate, in the case of the side effect of streptomycin, attest to the fact that this problem has not yet been definitively clarified. Its resolution will be aided by a study of the content of pantothenic acid in the blood and urine in the process of treatment with streptomycin.

Conclusions

1. Clacium pantothenate has eliminated pronounced and persistent side effects that are due to the prescription of streptomycin in 30 out of 43 patients. In the case of side reactions brought about by the application of isoniazid or PAS, it indicated no effect.

2. Calcium pantothenate proved to be highly effective in the most serious side reactions -- vestibular disturbances that, basically, do not limit the possibility of continuing treatment with streptomycin. However, it had a very

negligible effect on lighter -- allergic -- reactions.

3. Calcium pantothenate did not indicate any clinically pronounced toxic effect on those ill with tuberculosis.

4. The bacteriostatic effect of streptomycin on tuberculosis cultures in vitro and its chemotherapeutic activity in the tuberculosis of white mice did not change from the addition of calcium pantothenate.

5. The course of illness in patients suffering from tuberculosis improved where calcium pantothenate was prescribed.

6. The data obtained make it possible to recommend the application of calcium pantothenate in clinical practice in the case of a side effect of streptomycin. The mechanism involved in the effect of calcium pantothenate has not yet been clarified and requires further study.

Footnote

(a) Presented at the Scientific Conference of the Institute of Tuberculosis of the Academy of Medical Sciences of the U.S.S.R. on 29/I 1960 (January 29, 1960) and at a session of the Institute of Vitaminology of the Ministry of Public Health of the U.S.S.R. on 5/I 1960 (January 5, 1960).

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ПАНТОТЕНАТ КАЛЬЦИЯ В КЛИНИКЕ ТУБЕРКУЛЕЗА ПРИ ПОБОЧНОМ ДЕЙСТВИИ СТРЕПТОМИЦИНА[1]

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Лечение больных туберкулезом легких эффективно только при длительном применении специфических антибактериальных препаратов. При этом в ряде случаев наблюдаются побочные реакции, а иногда — отсутствие терапевтического действия. Объясняется это, согласно литературным данным, антагонистическим взаимоотношением противотуберкулезных препаратов и некоторых витаминов группы В, в частности, стрептомицина и пантотеновой кислоты.

Процент побочных реакций при лечении стрептомицином колеблется в пределах 2—30; прерывать лечение из-за его непереносимости приходится примерно в 2—3% случаев (У. А. Аскаров, Е. М. Гриншпун, И. А. Кассирский, М. А. Клебанов и Р. О. Драбкина, А. Е. Рабухин, Д. Д. Яблоков и др.).

Для ослабления токсического действия стрептомицина предложено много средств: антигистаминные препараты, соли кальция, глицин, цистеин, витамины B_1 и B_2, PP, пантотеновая кислота, аминазин, кортикостероидные гормоны и др. Однако только часть их обладает определенным токсическим действием, другие — малоэффективны.

Представляют интерес испытания пантотената кальция ввиду его весьма низкой токсичности, а также возможности воздействия им и как витаминным и как кальциевым препаратом.

Имеющиеся в литературе данные о влиянии пантотеновой кислоты на токсическое действие стрептомицина противоречивы. Отсутствие ее влияния отмечено в основном в экспериментах на животных, в случаях, когда стрептомицин назначался в заведомо высокотоксичных дозах (Child, Hawkins, Osterberg и др.). При клиническом же испытании пантотеновой кислоты токсическое действие стрептомицина, дигидро-

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стрептомицина и биомицина в большинстве случаев предупреждалось или снижалось (Weber, Ducrot, Kuntz, Penman, Stärk и др.).

В терапевтической клинике Института туберкулеза АМН СССР пантотенат кальция применен у 55 взрослых больных с различными формами туберкулеза легких при выраженном побочном действии стрептомицина (43), фтивазида (5) и ПАСК (7 больных).

Пантотенат кальция назначался в суточной дозе от 100 до 800 мг внутрь (в 2 приема) или внутримышечно в 20% водном растворе. Каких-либо побочных реакций, связанных с его назначением, не отмечено как после однократной дозы, так и после ежедневного приема в течение 5 месяцев.

При длительном лечении стрептомицином в случаях его побочного действия приходится постоянно назначать и пантотенат кальция. Только 6 больным из 43 удалось продолжить лечение стрептомицином без пантотената кальция после их совместного применения в течение 20—30 дней. Применение пантотената кальция позволило продолжить лечение стрептомицином у 30 из 43 больных в тех случаях, где оно должно было быть прекращено из-за побочного действия последнего.

Назначение же пантотената кальция 12 больным с выраженными побочными явлениями от фтивазида или ПАСК во всех случаях оказалось безрезультатным. Следует оговориться, что это были больные с тяжелым течением туберкулеза. Дальнейшие наблюдения помогут решить вопрос о благоприятном действии пантотеновой кислоты при побочном влиянии других противотуберкулезных препаратов (см. таблицу).

Действие пантотената кальция в зависимости от характера побочных явлений стрептомицина

Характер побочных реакций	Число больных	Благоприятное действие пантотената кальция		
		выраженное	умеренное	отсутствовало
Головокружение с нарушением координации движений	21	15	3	3
Головная боль с ухудшением сна и самочувствия	3	3	—	—
Явления коронароспазма	5	5	—	—
Парестезии	2	2	—	—
Кожные проявления	7	2	—	5
Температурная реакция	4	—	—	4
Гиперэозинофилия	1	—	—	1
Всего	43	27	3	13

Наиболее быстрое и ощутимое клиническое действие препарата наблюдалось при явлениях коронароспазма, головокружениях, головных болях и парестезиях. Вместе с тем он не повлиял на температурные реакции, гиперэозинофилию, а также в большинстве случаев — на кожные высыпания, зуд и обострение экземы, т. е. на реакции аллергического характера, которые обычно легко устраняются противогистаминными препаратами. Из 13 побочных реакций, которые не удалось устранить, 10 носили характер аллергических.

Исходя из полученных данных, можно полагать, что пантотенат кальция малоэффективен при побочных реакциях стрептомицина аллергического характера. Это свидетельствует о том, что антигистаминным действием пантотената кальция (М. Л. Рохлина, Т. В. Зубарева) нельзя объяснить причину его благоприятного действия на организм.

Механизм благоприятного действия пантотеновой кислоты нельзя также объяснить только ограничением связывания ионизированного кальция стрептомицином, как это делают Keller с сотрудниками.

Можно предположить сходство в механизмах благоприятного действия кортикостероидных гормонов и пантотената кальция при побоч-

ном влиянии стрептомицина, поскольку эндогенный синтез кортикостероидов, обеспечивающий достаточную функцию коры надпочечников, происходит в известной мере из пантотеновой кислоты (Ю. А. Серебровская, Olivi с сотрудниками и др.).

Наиболее вероятным является предположение, что введение пантотената кальция увеличивает содержание коэнзима А в тканях, что и способствует устранению тонких нарушений межуточного обмена, которые иногда клинически проявляются в виде побочных реакций.

Экспериментальные данные А. Н. Климова указывают на такую возможность, поскольку пантотеновая кислота способна нормализовать нарушение аэробного дыхания и окислительного фосфорилирования, обусловленные стрептомицином в результате нарушения синтеза коэнзима А.

Одновременно перед нами встал и вопрос о том, что повышенное образование коэнзима А при избыточном введении пантотеновой кислоты может улучшить переносимость стрептомицина вследствие его большей инактивации. При этом следовало ожидать ухудшения течения заболевания у больных, получающих сочетанно стрептомицин и пантотеновую кислоту. Однако наши и литературные данные свидетельствуют об обратном — об улучшении в течении заболевания.

Для окончательного решения вопроса о влиянии пантотената кальция на бактериостатический и химиотерапевтический эффект стрептомицина микробиологическая лаборатория Института туберкулеза АМН СССР провела экспериментальные исследования, поскольку определенных литературных данных по этому вопросу найти не удалось.

Изучение влияния пантотената кальция на бактериостатическую активность стрептомицина проводили в опытах in vitro. Туберкулезные культуры, чувствительные и устойчивые к стрептомицину, засевали на синтетическую среду, содержащую различные концентрации стрептомицина (в зависимости от чувствительности к нему культуры) и пантотената кальция. Концентрации последнего колебались от 1 до 100 γ на 1 мл среды.

В опытах было обнаружено, что пантотенат кальция не снижал и не увеличивал бактериостатической активности стрептомицина.

Для выяснения действия пантотената кальция на химиотерапевтическую активность стрептомицина были проведены эксперименты на белых мышах, зараженных туберкулезной культурой. Животные были распределены на 5 групп по 20 животных в каждой. Первая группа получала только стрептомицин, вторая и третья — стрептомицин в комбинации с различными дозами пантотената кальция (1—10 мг), четвертая — только пантотенат, пятая служила контролем. После месячного лечения оказалось, что терапевтический эффект стрептомицина был одинаков в группах животных, получавших только антибиотик и антибиотик в комбинации с разными дозами пантотената кальция.

Полученные результаты показывают, что пантотенат кальция в дозировках, эквивалентных клиническим, не снижает бактериостатической и химиотерапевтической активности стрептомицина.

В клинической литературе широко распространена точка зрения, что стрептомицин значительно угнетает жизнедеятельность кишечной микрофлоры и в связи с этим — синтез витаминов группы В, в том числе и пантотеновой кислоты. Поэтому и предполагается, что наблюдаемые в клинике побочные реакции от стрептомицина в значительной мере обусловливаются гиповитаминозом B_5, тем более, что назначение пантотеновой кислоты оказывает при этом благоприятное действие.

Kuntz, Manthei склонны считать апантотеноз основой побочных реакций при применении не только стрептомицина, но и изониазида.

Однако если бы речь шла о простом гиповитаминозе B_5, то для устранения побочного действия стрептомицина не пришлось бы при-

дать к назначению пантотеновой кислоты в дозировках, примерно в ... раз превышающих суточную потребность человека. Заставляют сомневаться в наличии недостаточности пантотеновой кислоты при лечении стрептомицином также работы Karlin и Meyer с сотрудниками. Эти авторы показали, что при стрептомицинотерапии количество пантотеновой кислоты в крови у больных не только не снижается, но даже возрастает как при свежих, так и хронических формах туберкулеза. Это объясняется тем, что колибациллярная флора кишечника быстро адаптируется к назначению стрептомицина. После этого она продолжает свое размножение и продукцию витаминов так же, как и до назначения стрептомицина.

Перечисленные весьма разноречивые точки зрения по вопросу о механизме благоприятного действия пантотената кальция при побочном действии стрептомицина свидетельствуют о том, что вопрос этот окончательно еще не выяснен. Разрешению его поможет исследование содержания пантотеновой кислоты в крови и моче в процессе лечения стрептомицином.

Выводы

1. Пантотенат кальция устранил выраженные и стойкие побочные реакции, обусловленные назначением стрептомицина у 30 из 43 больных. При побочных реакциях, вызванных применением фтивазида или ПАСК, он не оказал действия.

2. Пантотенат кальция оказался высокоэффективным при наиболее тяжелых побочных реакциях — вестибулярных расстройствах, которые в основном и ограничивают возможность продолжения лечения стрептомицином. Однако он очень мало повлиял на более легкие — аллергические — реакции.

3. Пантотенат кальция не оказал клинически выраженного токсического действия на больных туберкулезом.

4. Бактериостатическое действие стрептомицина на туберкулезные культуры in vitro и его химиотерапевтическая активность при туберкулезе белых мышей не изменились от присоединения пантотената кальция.

5. Течение заболевания у больных туберкулезом при назначении пантотената кальция улучшалось.

6. Полученные данные позволяют рекомендовать применение пантотената кальция в клинической практике при побочном влиянии стрептомицина. Механизм действия пантотената кальция еще не выяснен и требует дальнейшего изучения.

ЛИТЕРАТУРА

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CALCIUM PANTOTHENATE IN THE TREATMENT OF TUBERCULOSIS IN UNTOWARD EFFECT OF STREPTOMYCIN

E. S. Stepanyan and *B. Ya. Stukalova*

Summary

Calcium pantothenate (in a daily dose of 400—800 mg) in the majority of patients eliminates the untoward reactions provoked by the administration of streptomycin. Calcium pantothenate is highly effective in vestibular disorders, its action is considerably weaker in the elimination of allergic reactions. Potassium pantothenate does not reduce the bacteriostatic effect of streptomycin on the tuberculous culture and does not decrease its chemotherapeutic activity in tuberculosis of white mice.

The data obtained give ample grounds to recommend the use of potassium pantothenate in the most frequent and serious complications of streptomycin — vestibular disorders. However, the mechanism of its therapeutic action is not quite clear and therefore requires further studies.

Gounelle-'57
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French publication, Societe de Biologie, Seance du 12 Janvier, 1957, pages 24-26

Nyctohemeral distribution of urinary excretion of pantothenic acid, and influence of an oral charge test

by H. Gounelle and F. Richet

Previous works agree in fixing, for man, the daily excretion of pantothenic acid in the vicinity of a few milligrams per day: Sarett (1), 3.7 mg for Rubin (2), 3 mg for Jannes (3), 5.7 mg for Fitzpatrick (4), 2.9 mg for Schmidt (5). And our dosages, made under the direction of Mlle. C. Marnay, using the previously indicated technique (6), yielded figures on the same order.

The urine specimens are collected under acid 10% acetic and stored in the refrigerator. In 6 control subjects the concentration in the urine comes to 2.34, 5.26, 2.81, 4.85, 1.51 and 5.[illegible]9 gamma per cc, insuring a daily urinary excretion, for 4 of them, of 4.56 m[illegible], 5.26 mg, 3.65 mg and 4.32 mg, which are highly comparable quantities.

We studied the nyctohemeral variations, of both the concentration and the excretion of pantothenic acid. The variations are small in amplitude, as demonstrated by the following results, obtained in different 24-hour samples of urine.

Times of micturition	Concentration in gamma/cc	Excretion in mg	Volume of urine in cc
8 a.m.	5.7	1.02	180
12 noon	4.51	0.67	150
5 p.m.	3.87	0.67	175
11 p.m.	5.13	0.89	175
8 a.m.	5.11	1.07	210

Table I

Charge Test

In man, Rubin et coll. (2) find, for 100 mg of calcium pentothenate ingested, a urinary excretion of 18% in 24 hours, which is slightly below that found with panthenol. We thought that such a test charge should receive more profound study in man, looking at the incidences on the various micturitions during the day.

The graph reproduces the results obtained in concentration and excretion in the case of 4 normal subjects, after ingestion of 100 mg of pantothenol.

See graph, page 25)

Urinary excretion (mg) of pantothenic acid after ingestion of a product (0.10 g).

Abscissa: excretion in mg; Ordinate: Time in hours following ingestion

It is easy to note that the rise in the urinary reading is particularly high in the sample of urine emitted in the 4 hours consecutive to ingestion. The concentration rises 10 to 50 times, according to the subject, and a digestive absorption factor must probably intervene. After 6 hours the readings tend to become normal again, but for 24 hours afterwards they remain slightly higher than the starting concentrations. The figures obtained in the course of one of these tests are presented in the table below.

Times of micturition	Concentration gamma/cc	Excretion in mg	Volume of urine in cc
9 a.m. (before charge)	5.79	1.15	200
1 a.m. (4 hrs after charge)	181	21.72	120
3;45 p.m. (6;45 hrs " ")	38.8	5.82	150
5;30 p.m. (8;30 hrs " ")	18.8	1.88	100
7;30 p.m. (10;30 hrs " ")	13.76	1.71	125
Midnight (15 hrs " ")	10.04	2.51	250

Table II continued.

8;45 p.m. (23;45 hrs after charge)	14.48	3.62	250

Table II

Conclusions

The nyctohemeral variations in pantothenuria are small in amplitude, the urine concentration oscillating in the neighborhood of 3 to 5 gamma per cc. A charge test of 0.10 g of panthenol per os raises the urinary excretion 10 to 50 times in the 4 hours consecutive upon ingestion *

(Centre de Recherches Foch).

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Comp. Rend. Soc. Biol. Paris, 24-26. 1957.

Répartition nycthémérale de l'excrétion urinaire d'acide pantothénique et influence d'une épreuve orale de charge.

par H. GOUNELLE et F. RICHET.

Les travaux antérieurs concordent pour fixer, chez l'Homme, aux environs de quelques milligrammes par jour l'excrétion d'acide pantothénique : Sarett (1), 3,7 mg pour Rubin (2), 3 mg pour Jannes (3), 5,7 mg pour Fitzpatrick (4), 2,9 mg pour Schmidt (5). Ce sont des valeurs de même ordre que nos dosages effectués sous la direction de Mme C. Marnay selon la technique précédemment indiquée (6) ont apportées.

Les urines sont recueillies sous acide acétique à 10 % avec conservation à la glacière. Chez 6 sujets témoins la concentration dans l'urine atteint 2,34, 5,26, 2,81, 4,85, 1,51 et 5,79 γ par cm³, assurant une excrétion urinaire quotidienne pour 4 d'entre eux de 4,56 mg, 5,26 mg, 3,65 mg et 4,32 mg, quantités fort comparables.

Nous avons recherché les variations nycthémérales tant de la concentration que de l'excrétion de l'acide pantothénique. Les variations sont de faible amplitude comme le démontrent les résultats suivants obtenus dans les différents échantillons de l'urine de 24 heures.

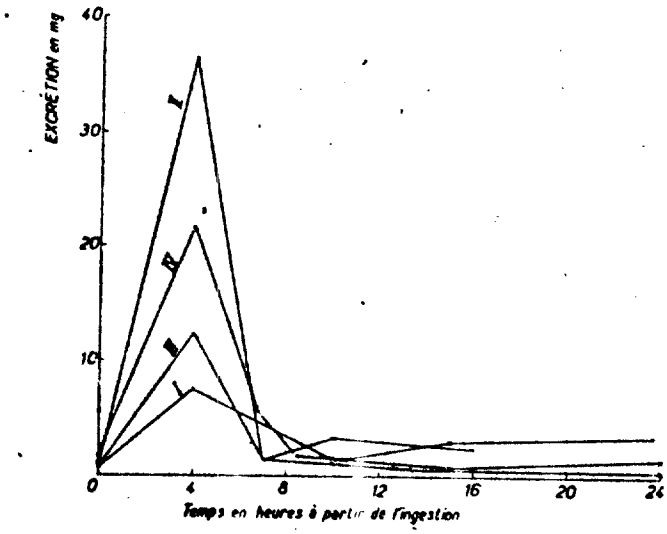
Heures des mictions	Concentration en γ cm³	Excrétion en mg	Volume d'urine en cm³
8 heures.....	5,7	1,02	180
12 heures.....	4,51	0,67	150
17 heures.....	3,87	0,67	175
23 heures.....	5,13	0,89	175
8 heures.....	5,11	1,07	210

Tableau I.

ÉPREUVE DE CHARGE. — Chez l'Homme, Rubin et coll. (2) retrouvent pour 100 mg de pantothénate de calcium ingéré une excrétion urinaire de 18 % dans les 24 heures, légèrement inférieure à celle que l'on trouve avec le panthénol. Nous avons pensé qu'une telle épreuve de charge devait être étudiée de façon plus approfondie chez l'Homme, en recherchant les incidences sur les différentes mictions de la journée.

(1) H. P. Sarett, *J. Biol. Chem.*, 1945, t. *159*, p. 321.
(2) S. H. Rubin, J. M. Cooperman, M. E. Moore et J. Scheiner, *J. Nutr.*, 1948, t. 35, p. 499.
(3) J. Jannes, *Acta. Med. Scandinavica*, 1950, Supp. 249, p. 61.
(4) J. Fitzpatrick et S. L. Tompsett, *J. Clin. Path.*, 1950, t. 3, p. 69.
(5) V. Schmidt, *Acta Med. Scandinavica*, 1951, t. *139*, p. 185.
(6) H. Gounelle et F. Richet, *C. R. Soc. Biol.*, 1956, t. *150*, p. 2167.

Le graphique reproduit les résultats obtenus en concentration et excrétion chez 4 sujets normaux après ingestion de 100 mg de panthénol.



Excrétion urinaire (mg) d'acide pantothénique après ingestion du produit (0,10 g).
Abscisses : excrétion en mg. Ordonnées : temps en heures.

Il est aisé de se rendre compte que l'augmentation du taux urinaire est particulièrement élevée dans l'échantillon d'urine émise dans les 4 heures consécutives à l'ingestion. La concentration augmente de 10 à 50 fois selon les sujets, un facteur d'absorption digestive doit

Heures des mictions	Concentration en γ/cm³	Excrétion en mg	Volume d'urine en cm³
9 h (avant charge)	5,79	1,15	200
13 h (4 h après charge)....	181	21,72	120
15,45 h (6,45 h » »).....	38,8	5,82	150
17,30 h (8,30 h » »).....	18,8	1,88	100
19,30 h (10,30 h » »).....	13,76	1,71	125
24 h (15 h » »).....	10,04	2,51	250
8,45 h (23,45 h » »).....	14,48	3,62	250

Tableau II.

vraisemblablement intervenir. Après 6 heures les taux tendent à redevenir normaux tout en restant cependant 24 heures après légèrement supérieurs aux concentrations de départ. Les chiffres obtenus au cours d'une de ces épreuves sont rapportés dans le tableau ci-dessus.

Conclusions. — Les variations nycthémérales de la pantothénurie sont de faible amplitude, la concentration dans l'urine oscillant aux alentours de 3 à 5 γ par cm^3. Une épreuve de charge de 0,10 g de panthénol par voie buccale élève 10 à 50 fois l'excrétion dans l'urine des 4 heures consécutives à l'ingestion (*).

(*Centre de Recherches Foch*).

Polacek-'64
Bib No. 176

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Clinical Evaluation of D-Pantothenyl Alcohol in Prevention of Postoperative Ileus

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D-PANTOTHENYL ALCOHOL is a B complex vitamin which has been found to be an essential component of coenzyme A, which acts as an acetate carrier and donor for the acetylation of choline in the production of acetylcholine [1]. Acetylcholine, as the transmitter substance at ganglionic and postganglionic parasympathetic nerve endings, is required for normal intestinal motility. On the basis of this requirement of adequate levels of acetylcholine for intestinal motility, large doses of D-pantothenyl alcohol have been advocated in the treatment for paralytic ileus.

In recent years there have been clinical reports on the efficacy of D-pantothenyl alcohol in the treatment of postoperative adynamic ileus [2-6]. Most of these studies were conducted in a double-blind fashion and, in general, the conclusions have been favorable. However, in reported clinical trials of D-pantothenyl alcohol all types of surgical procedures were included, and patients with previous abdominal surgery were not excluded. Also, the methods employed to determine the efficacy of D-pantothenyl alcohol in prevention or amelioration of postoperative ileus were either subjective or unreliably objective, such as time of onset of bowel sounds, flatus or feces, discomfort of patient or length of hospital stay. Of the aforementioned, bowel sounds are probably the most accurate index of return of bowel tone, but McPhail et al. have shown experimental evidence that audibility of bowel sounds does not closely parallel effective bowel motility [7].

A double-blind laboratory study showed a much earlier return of effective peristalsis when D-pantothenyl alcohol was given to dogs postoperatively [8]. The animals underwent laparotomy and a 4 or 5 mm. lead marker was placed in the stomach through a small gastrostomy. To produce a significant ileus, the small intestine was then stripped twice through the gloved fingers of the surgeon, and exposed to air and operating lights for twenty minutes prior to close of the abdomen. The duration of postoperative ileus was then inferred from the evacuation time of the lead marker as determined by daily fluoroscopy. This experimental animal study was then modified for a clinical investigation of the effect of D-pantothenyl alcohol in postoperative ileus.

METHOD

In an attempt to limit the rather significant variable of type of operation in this clinical study, two specific groups of surgical patients were selected. A double-blind clinical evaluation was conducted on one hundred consecutive elective cholecystectomy and seventy-five consecutive elective gastric resection cases. These two common surgical procedures were chosen for this study because they are frequently associated with some degree of postoperative paralytic ileus. The only criterion that the patients were required to meet was that they have no history of previous abdominal surgery to rule out the possible variable of preexisting peritoneal adhesions. No attempt was made to standardize the particular operation or incision specifically. All patients received a general anesthesia and were on Levin tube suction postoperatively.

At the conclusion of each operation a blank envelope was drawn which contained a 3 to 4 mm.

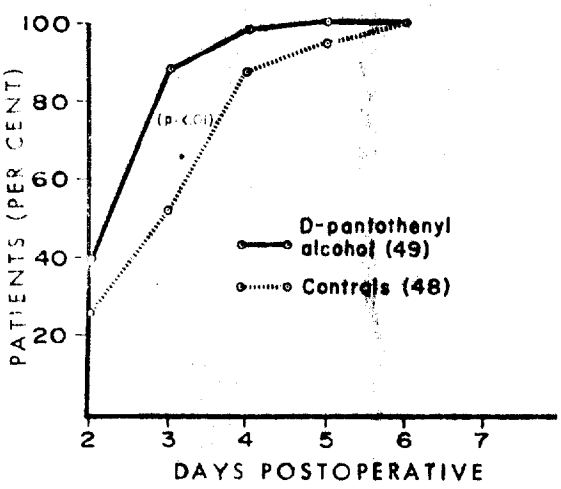

FIG. 1. Effect of D-pantothenyl alcohol in cholecystectomy patients. Cumulative bowel transit time of lead marker was significantly more rapid in the first three days following surgery in those patients receiving the drug ($p = <.01$).

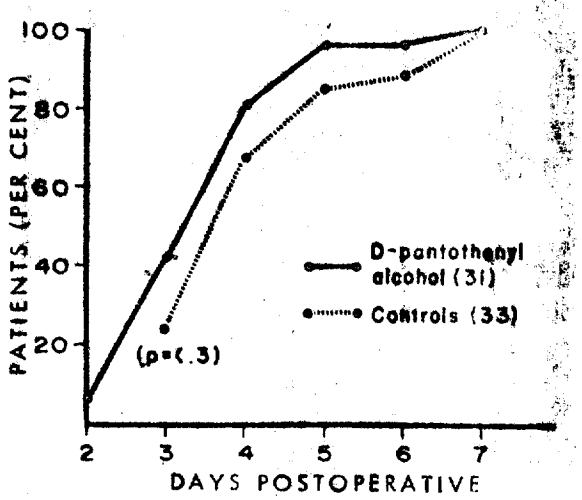

FIG. 2. Effect of D-pantothenyl alcohol in gastric resection patients. Although those patients receiving the drug had a more rapid cumulative bowel transit time of the lead marker, this difference was not statistically significant ($p = <.3$).

lead marker, a red or blue ampule (according to random selection) and a data sheet for the patient's postoperative course. Either the red or the blue ampules contained 250 mg./cc. of D-pantothenyl alcohol* and the other color contained saline. As soon as the patient was alert, usually one or two hours after surgery, he was encouraged to swallow the marker and 1 cc. of the drug or placebo was given intramuscularly. This injection was repeated in two hours and every eight hours thereafter for five consecutive days. The patients did not receive any parenteral vitamins postoperatively which might contain D-pantothenyl alcohol or any of its derivatives.

Beginning on the morning of the second postoperative day, each patient had a flat film of the abdomen taken for five consecutive days or until the lead marker reached the sigmoid colon or rectum. The progress time of the marker to the sigmoid or rectum marked the end point of the study. Concurrently, clinical observations of complications, audible peristalsis, flatus and first bowel movements were made. Any significant complications such as pneumonia, wound dehiscence, infection or major cardiovascular problems automatically eliminated the patient from the study. At the conclusion of the study the results were analyzed before the code was broken.

RESULTS

Figure 1 summarizes the results of this study in ninety-seven of one hundred cholecystectomy patients. Three patients were deleted from the series because of complications. During the first two days after cholecystectomy 40 per cent of the forty-eight patients receiving D-pantothenyl alcohol successfully passed their markers, as compared to 27 per cent of the forty-nine patients receiving saline. By the third day 88 per cent of the drug-treated group had passed the markers compared to 53 per cent of the control group. At four days, 97 per cent of the treated patients had passed their markers compared to 88 per cent of those receiving the placebo. These differences are statistically significant ($p = <.01$) for the third postoperative day.

In the gastrectomy series, eleven cases were deleted because of complications. The remaining sixty-four cases are illustrated in Figure 2. By the fourth postoperative day 80 per cent of the thirty-one patients receiving the drug had successfully progressed their markers, in comparison to 67 per cent of the thirty-three patients receiving saline. However, in this group the statistical differences are not significant ($p = <.3$).

An attempt was made to correlate the onset of peristalsis, flatus and bowel movements with the transit time of the lead markers. There was some correlation, especially in the onset of audible peristalsis; however, closer investigation found this to be more apparent than real. In many cases (twenty-two) the onset of peristalsis was evident before any movement

* Hopan, supplied by Warren-Teed Products Co., Columbus, Ohio.

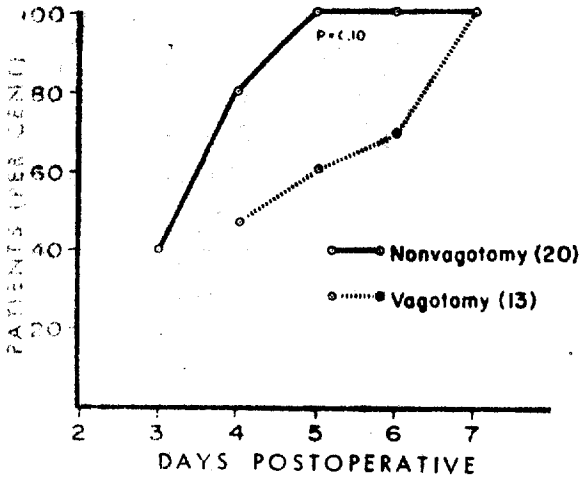

FIG. 3. Effect of vagotomy on cumulative bowel transit time of lead marker in those patients undergoing gastric resections. It is apparent there was a delay in the movement of the marker in the vagotomy group ($p = <.10$). These patients did not receive any drug as they were in the control group. The effect of the vagus on gastric tone and ileus is graphically illustrated in this group.

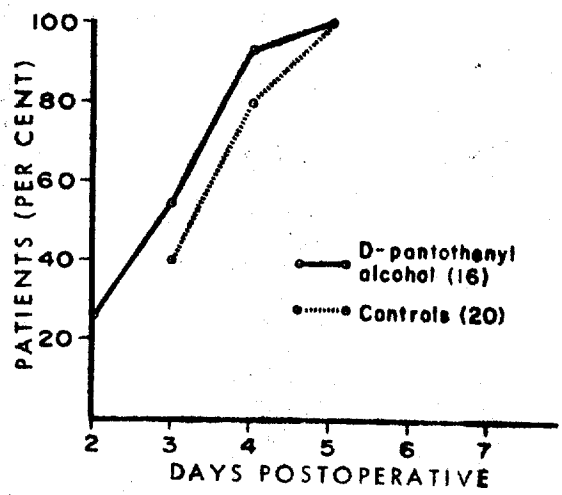

FIG. 4. Effect of D-pantothenyl alcohol in gastric resection patients without vagotomy. The cumulative bowel transit time is more rapid in the group receiving the drug, but the difference is not statistically significant.

of the lead marker occurred and even in the presence of roentgenographic evidence of ileus. In many of these instances of early audible peristalsis, the bowel sounds subsequently disappeared for a variable period. This lack of correlation between audible bowel sounds and other signs of intestinal activity was noted in both the control and drug groups. These observations strengthened our dependence on more objective criteria than onset of peristalsis in evaluating postoperative ileus.

Because of the pronounced effect of D-pantothenyl alcohol on transit time of the lead marker in the cholecystectomy study as compared to the gastrectomy study, further analysis of this latter group was undertaken. As was previously mentioned, no attempt had been made to standardize the type of operation performed. After partial gastrectomy there is usually at least transient atony of the gastric remnant, and in the group with gastric resection the markers were frequently held up along the lesser curvature even after food and fluids were going through the stoma; this paradox did not occur in the cholecystectomy patients. Of the sixty-four gastrectomy patients 56 per cent had subtotal resections with Billroth II anastomosis and the remaining 44 per cent had hemigastrectomy with vagotomy and a Billroth I reconstruction. It is doubtful that the difference in magnitude of resection between a hemigastrectomy (50 per cent) and a subtotal resection (70 to 75 per cent) would influence the progress of the lead marker. However, the stomal difference between a Billroth I and a Billroth II anastomosis may have influenced gastric emptying, and vagotomy certainly does have an influence on resumption of gastric tone. All the vagotomies were performed in the hemigastrectomy-Billroth I group. None of the patients with subtotal gastrectomies had vagotomy. Figure 3 compares the vagotomy patients and the nonvagotomy patients in the control group. It is apparent that the vagotomy controls had a significant delayed marker transit time compared to the nonvagotomy controls. The nonvagotomy patients started moving their marker much sooner (third postoperative day) and reached their end point two days earlier (fifth postoperative day) than the vagotomy group ($p = <.10$). This finding supports the conclusion that the early effect of vagotomy on bowel motility is considerably more important than the frequency or degree of stress-induced pantothenic acid deficiency.

An attempt was made to eliminate the vagotomy variable by separately studying the subtotal resection-Billroth II patients who did not have a vagotomy. Figure 4 graphically illustrates this group and shows that those receiving D-pantothenyl alcohol had a slightly

more rapid progress time of their lead markers; however, this series did not provide a sufficient number of patients (thirty-six) for any real statistical significance. These may be but a few of the reasons accounting for the differences in marker transit time in the gastric resection series as compared to those in the cholecystectomy group receiving D-pantothenyl alcohol.

COMMENTS

A double-blind clinical analysis of the effect of D-pantothenyl alcohol on postoperative ileus in two standard operative groups, cholecystectomy and gastric resections, has been done. It has been shown in the cholecystectomy series that D-pantothenyl alcohol had a significant effect on the bowel transit time of a lead marker as compared to an identical control group of cholecystectomy patients. However, such an effect could not be demonstrated in a series of gastric resection patients. Variable surgical technics employed in this latter operative group, and especially the choice of a vagotomy and its effect on bowel tone, could explain the difference in these results.

We believe that D-pantothenyl alcohol may have a place in the surgeon's armamentarium for the treatment of particularly persistent postoperative adynamic ileus. However, we are in accord with Garvey, Wilson and other investigators who do not consider it an agent that should be used routinely or in place of standard precautionary fundamentals, such as intubation, early ambulation, adequate hydration, drainage of abscesses, etc. [9–11] One of the important features in the use of D-pantothenyl alcohol was that no untoward side effects were noted. If the patient's ileus was not improved, his postoperative course was in no way complicated from the use of the agent. We can find no evidence that its use shortened hospital stay or decreased complication rate; neither did it appear to contribute significantly to patient comfort.

Some investigators have suggested that D-pantothenyl alcohol may be beneficial in postoperative urinary retention [12]. In our series, only two patients required postoperative catheterization for this problem, one in the control and one in the drug group. There was no evidence that D-pantothenyl alcohol had an effect on urinary retention.

SUMMARY

1. A double-blind clinical study of the effect of D-pantothenyl alcohol on postoperative peristalsis was conducted on two standard operative groups.

2. Objective evaluation of the resumption of bowel tone was made by noting the roentgenographic bowel transit time of a lead marker which was introduced orally into the stomach immediately after surgery.

3. The transit time of the markers was significantly decreased in cholecystectomy patients receiving D-pantothenyl alcohol.

4. Significant improvement in bowel transit time was not seen in the gastric resection patients receiving the D-pantothenyl alcohol; however, in these patients early gastric remnant paralysis, type of anastomosis and especially vagotomy were undoubtedly strong variables affecting the end results.

5. There is no justification for routine use of large doses of parenteral D-pantothenyl alcohol postoperatively. Since there is at least presumptive evidence that pantothenic acid deficiency can result from surgical stress, and since it is a harmless vitamin, one should consider its use in patients with prolonged malnutrition either before or after surgery.

6. Our clinical experience tells us that there is usually a simple explanation for prolonged postoperative ileus, such as infection, dehiscence or obstruction.

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Bib No. 186

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Toxicity Studies of Some Derivatives of Pantothenic Acid

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Received May 15, 1963

The toxicity of calcium pantothenate in various species was studied by Unna and Greslin (1941); adverse effects referable to this salt of pantothenic acid were not found. More recently, experiments conducted in rats employing sodium pantothenate and *d*-pantetheine indicated that administration of relatively large doses of these derivatives of pantothenic acid resulted in an accumulation of hepatic lipid and an elevation of SGO-T (Wirtschafter and Walsh, 1962). In this communication, the results of further toxicity measurements in rats of sodium pantothenate, *d*-panthenol, and *d*-pantethine are reported. *d*-Pantethine was used in place of *d*-pantetheine as the latter compound was not available from commercial sources.

METHODS

Two series of experiments were carried out employing Sprague-Dawley rats weighing approximately 110–120 g and 225–275 g, respectively. Rats were distributed into groups of 10 animals equally divided as to sex and were maintained on a diet of Purina Laboratory Chow ad libitum. Body weights were obtained prior to the administration of the drugs and also immediately before sacrifice.

All injections were given intramuscularly following the dosage schedule indicated in Table 1. Sodium pantothenate, *d*-panthenol, and *d*-pantethine were prepared as aqueous solutions containing 500 mg/ml. Panthoject®[1] was administered as the available formulation containing 250 mg of sodium pantothenate per milliliter in an aqueous solution containing 1% benzyl alcohol.

Ninety-six hours after injection, the rats were anesthetized with ethyl ether and blood specimens were obtained by cardiac puncture for measurements of serum glutamic oxalacetic transaminase (SGO-T) and serum glutamic pyruvic transaminase (SGP-T) levels; the SGO-T determinations were done by the method of Karmen (1955) and the SGP-T by the method of Wroblewski and LaDue (1956). The rats were sacrificed and the livers were removed, weighed, and placed in 10% formalin. Sections of liver were stained with hematoxylin-eosin and, for the visualization of fat, frozen sections were stained with hematoxylin-sudan IV.

The amount of liver deposition was graded histologically using an arbitrary rating scale; + designated that little or no fat was seen in either the hematoxylin-eosin or the hematoxylin-sudan IV sections while ++++ indicated that discrete fat droplets were present in the parenchymal cells of sections whether stained with hematoxylin-eosin or hematoxylin-sudan IV. The ++ and +++ ratings were based primarily on the hematoxylin-sudan IV stained sections and designated the deposition

[1] Sodium pantothenate. U. S. Vitamin and Pharmaceutical Corporation, New York, New York

TABLE 1

THE EFFECT OF SINGLE INJECTIONS OF SODIUM PANTOTHENATE, *d*-PANTHENOL, *d*-PANTETHINE, PANTHOJECT AND BENZYL ALCOHOL ON BODY WEIGHT, LIVER WEIGHT, SGO-T, SGP-T AND HISTOLOGIC FAT IN THE LIVERS OF RATS[a]

Compound	Dose (g/kg)	Average body weight (grams) Initial	Final	Liver weight (% body weight)	SGO-T (units)	SGP-T (units	Number of rats showing fatty change +	++	+++	++++
				Study No. 1						
Sodium pantothenate	2.0	109	138	4.80	122.2	17.4	4	5	1	0
	0.2	109	137	4.60	115.0	14.0	4	4	2	0
d-Panthenol	2.0	109	136	4.35	100.0	10.0	0	6	4[b]	0
	0.2	109	138	4.35	108.0	12.0	2	4	4[c]	0
d-Pantethine	0.1	109	138	4.40	122.0	14.5	4	6	0	0
Panthoject	2.0	116	145	4.80	113.3	18.5	5	5	0	0
	0.2	117	146	4.65	101.5	20.6	7	3	0	0
Benzyl alcohol (1%)	2.0 eq.[d]	116	146	4.80	101.4	19.3	9	1	0	0
	0.2 eq.[e]	116	142	4.75	111.5	22.4	7	3	0	0
Control	0.25 ml water	117	146	4.55	127.9	18.5	4	6	0	0
				Study No. 2						
Sodium pantothenate	2.0	224	228	3.84	107.0	16.0	3	6	1	0
	0.2	235	239	3.81	99.5	16.0	7	3	0	0
	0.1	244	249	3.84	88.0	15.0	7	3	0	0
d-Panthenol	2.0	266	267	3.71	95.5	13.0	8	2	0	0
	0.2	255	260	3.73	82.5	11.5	7	3	0	0
	0.1	241	246	3.79	104.0	13.0	5	5	0	0
Control	0.25 ml water	237	238	3.49	82.0	11.5	5	4	1	0

[a] Values are means of 10 rats except SGO-T and SGP-T levels of rats given 2.0 g of sodium pantothenate per kilogram in study no. 1, for which values are means of 9 rats.

[b] Significantly different from controls at the 1% level.

[c] Significantly different from controls at the 5% level.

[d] Volume equal to Panthoject 2 g/kg.

[e] Volume equal to Panthoject 0.2 g/kg.

at slight and moderate amounts of lipid, respectively. The degree of liver lipid deposition was analyzed statistically by the methods of the analysis of variance and the Student *t* test.[2]

RESULTS

The results of these studies are shown in Table 1. Administration of sodium pantothenate, *d*-panthenol, or *d*-pantethine did not significantly affect body weight, liver weight, SGO-T or SGP-T as compared to the controls. Histologic examination of the hematoxylin-eosin and hematoxylin-sudan IV stained sections did not reveal marked fatty change to be present in either the control or treated groups. Rats weighing 110–120 g and given 2.0 g/kg or 0.2 g/kg of *d*-panthenol showed a slight but statistically significant increase in liver lipid as compared to the controls, but this hepatic lipid accumulation was not observed in the group of rats weighing 225–275 g and given similar doses of *d*-panthenol.

DISCUSSION

In these experiments, evidence of hepatotoxicity produced by large intramuscular doses of sodium pantothenate, *d*-panthenol, and *d*-pantethine was not observed in rats sacrificed 96 hours after administration. Unna and Greslin (1941) have reported that the LD_{50} of calcium pantothenate to rats given subcutaneously is 3.4 g/kg and 0.82 g/kg when administered intraperitoneally; data regarding intramuscular administration were not given. In addition, these authors were able to give doses of 0.2 g of calcium pantothenate orally to rats initially weighing approximately 45 g for 190 days without any evidence of untoward effects.

Wirtschafter and Walsh (1962) administered intramuscularly the sodium salt of pantothenic acid in a single dose of 750 mg per rat and also 5 daily doses of 250 mg per rat; they reported that hepatotoxicity characterized as fatty changes in the liver accompanied by elevated SGO-T levels was produced. In these latter experiments, the body weights of the rats were not stipulated, but assuming the weights to be approximately 200 g, sodium pantothenate was given as a single dose of 3.75 g/kg and 5 daily doses of 1.25 g/kg; similarly, the doses of *d*-pantethine would be equal to 2.8 g/kg and 0.1 g/kg of body weight, respectively. Clinically, the total daily dose of *d*-panthenol to man employed in the prevention or treatment of paralytic ileus is 1.5 g given intramuscularly equaling about 20 mg/kg of body weight (Stone *et al.* 1959).

Several differences exist between the methods used in the present experiments and those employed in the investigations conducted by Wirtschafter and Walsh (1962). In this study, Sprague-Dawley rats were used whereas rats of the Long-Evans strain were employed by Wirtschafter and Walsh. The determination of serum transaminase levels were done colorimetrically[3] by the latter authors, but in these studies, spectrophotometric methods were used. The colorimetric method has been reported to yield results about 10% higher than those obtained with the spectrophotometric procedure (Sall *et al.*, 1957). Wirtschafter and Walsh sacrificed their rats at intervals varying from 24 to 168 hours after injection, while in the experiments described herein all

[2] We are indebted to Dr. J. Chassan for performing the statistical analysis of these data.

[3] A Simplified Method for the Determination of SGO-T and SGP-T (Sigma-Frankel method). Technical Bulletin No. 505, Sigma Chemical Co.

the rats were sacrificed 96 hours after administration. It is possible that the hepatocellular damage could have occurred after the 96-hour period and thus was not detected in this investigation; however, Wirtschafter and Walsh (1962) observed hepatic fat deposition and elevated SGO-T levels at this time interval. Finally, differences between the methods for staining for lipids exist; Wirtschafter and Walsh utilized the oil red O stain while hematoxylin-sudan IV was employed in this study.

SUMMARY

Evidence of hepatotoxicity was not found in Sprague-Dawley rats given sodium pantothenate, *d*-panthenol, or *d*-pantethine intramuscularly in doses ranging from 0.2 g/kg to 2.0 g/kg. A slight increase in the amount of liver lipid deposition was observed in the rats weighing 110–120 g which were given 0.2 g/kg and 2.0 g/kg of *d*-panthenol, but not in rats weighing 220–275 g.

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PANTOTHENIC ACID IN HUMAN NUTRITION

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The syndrome resulting from a lack of pantothenic acid in the diet of the chick and the rat has been described [1] and the recent synthesis of pantothenic acid [2] has made this substance available in pure form for clinical studies. Since there was no information regarding the possible toxicity of this substance in human beings, we administered varying amounts of calcium or sodium pantothenate to fifteen people and observed that 100 mg. of either salt may be injected intravenously without causing a reaction and without significant change in blood pressure, pulse, temperature and respiration.

In making these observations we assayed the blood and urine for pantothenic acid [3] before and at intervals after injection. We found that the pantothenic acid content of the blood increases up to 50 per cent above the preinjection level and that this increase is noted within the first three hours after the injection but that in all instances the value returns to its previous level within twenty-four hours. Urine specimens showed an increased pantothenic acid concentration immediately following the injection, but this also returned to normal within twenty-four hours. The blood pantothenic acid concentration of twenty-eight patients with pellagra, beriberi and riboflavin deficiency was decreased from 23 to 50 per cent, as compared with the levels of eighteen normal persons.

Using a similar technic [4] we have assayed this same blood for riboflavin and have found that following the injection of

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pantothenic acid there likewise is a rise of from 20 to 30 per cent in the riboflavin level of the blood. The administration of 20 mg. of calcium pantothenate daily, for four days, to four persons with cheilosis and typical ocular manifestations diagnostic of riboflavin deficiency caused similar temporary increases in the blood pantothenic acid and riboflavin. These values, however, returned to the previous levels when therapy was discontinued.

We have found that the injection of 200 micrograms of riboflavin per kilogram of body weight caused an increase of 80 per cent in the blood flavin concentration and of 45 per cent in the pantothenic acid level. While these values remained high for from three to four hours, they returned in each instance to the former level by the next day.

These observations indicate that pantothenic acid is essential to human nutrition and that its function is probably associated with that of riboflavin.

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QUANTITATIVE DETERMINATION OF PANTOTHENATES BY GAS-LIQUID CHROMATOGRAPHY

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SUMMARY. — *A method has been developed for the gas chromatographic determination of pantothenic acid and its salts. This procedure comprises hydrolysis of pantothenates and quantitative analysis of the relative freed β-alanine as N-trifluoroacetyl methyl ester (or N-trifluoroacetyl n.butyl ester). The method is simple and suitable for analysis of pharmaceutical preparations also containing substances usually interfering with chemical methods.*

RIASSUNTO. — *Viene descritto un metodo per la determinazione gascromatografica dell'acido pantotenico e dei suoi sali. Il procedimento consiste nel dosaggio della β-alanina liberata per idrolisi dai pantotenati, come N-trifluoroacetil metilestere (oppure come N-trifluoroacetil n.butilestere). Il metodo è semplice ed applicabile anche a formulazioni farmaceutiche contenenti sostanze interferenti che impediscono l'applicazione di normali metodi chimici di analisi della vitamina.*

Calcium or sodium salts of pantothenic acid (Vitamin B_5) are importantes components of many pharmaceutical preparations containing vitamin B complex.

Due to the wide occurrence of this vitamin, many methods for its quantitative determination have been reported.

Determinations are usually carried out with biological, microbiological or chemical methods.

Biological methods are based on the use of rats or chicks fed with suitable deficient diets (1), but are of little practical use, as well as requiring a considerable time to carry out.

Microbiological methods use *Saccharomyces carlsbergensis* (2), *Lactobacillus plantarum* (*arabinosus*) (3,4), *Lactobacillus casei* (5, 6) or *Acetobacter suboxydans* (5), the latter being specific for pantoic acid.

However, these methods too, are time-consuming and impractical, although offering great advantages for their sensitivity and specificity.

In turn, chemical methods are simpler and quicker, but are not specific, being based essentially on colorimetric determination of the products obtained on acid or alkaline hydrolysis of the vitamin, i.e. β-alanine and pantoyl-lactone.

In this way, Wollish and Schmall (8) determined the pantoyl-lactone liberated by acid hydrolysis of pantothenates, transforming it into the corresponding hydroxamic acid by reaction with hydroxylamine; this is then determined colorimetrically after development of a characteristic red coloration with ferric ions.

In another method (9) the same authors used the reaction between 1,2-naphthoquinone-4-sulphonate and the other hydrolysis product, β-alanine in the case of pantothenates and 3-amino-1-propanol in the case of panthenol.

However, when pantothenate is found in the presence of ascorbic acid or soluble carbohydrates, determination with the hydroxylamine method is impossible. Moreover in the 1,2-naphthoquinone-4-sulphonate method, sugars and riboflavine (which during hydrolysis give rise to a yellow-brown colour) interfere and must be separated, or else their part in the determination must be calculated through a blank determination. The same is true for the possible presence of other amino acids and niacinamide.

In all these cases, and also when the sample is intensely coloured or contains biological matter, the advantages of spectrophotometric methods are lessened, because of the diminished degree of accuracy and the time required to extract or separate pantothenates from interfering substances.

The recent development of gas chromatography of amino acids has made possible a rapid, accurate and selective determination of β-alanine, thus offering an excellent alternative method to the usual spectrophotometric techniques for the chemical determination of pantothenates.

The purpose of the present work is to describe a method which allows quantitative gas chromatographic determination of pantothenic acid and its salts by means of β-alanine, a product of their hydrolysis, also in cases where the presence of one or more interfering substances would make analysis by the usual chemical procedures more difficult or even impossible.

The procedure consists of the hydrolysis of pantothenates; then an aliquot of the solution is evaporated to dryness; the β-alanine present in the residue is transformed into the N-trifluoroacetyl (TFA) methyl or *n*.butyl ester, and as such can be analysed by gas chromatography in suitable operative conditions.

Experimental

Chromatographic apparatus and conditions

A "Pye-panchromatograph" gas chromatograph fitted with a hydrogen-flame ionization detector (FID) and equipped with a gas pressure and flow controller, was used for the experimental work. The glass column (210 cm x 4 mm i.d.) was U-shaped and packed with 1% Carbowax 20 M on 100/120 mesh acid-washed Gas-Chrom P. The column was operated isothermally at a temperature of 115°C. The carrier gas was nitrogen flowing at 100 ml per minute. Hydrogen and oxygen flow rates (to the FID) were 70 and 140 ml/min, respectively. In all the experiments, an attenuator setting of 10 x and a electrometer output of 3×10^{-8} Amp were used. The chromatograph was equipped with a Honeywell 1 mV strip chart recorder. The chart speed could be varied from 2 to 8.4 mm/min to facilitate quantitation depending on the calculating method adopted. A Hamilton 1-µl syringe with a 11 cm (4.5 in) needle was employed for injections.

Materials

Pharmaceutical products, vitamins and all other reagents and chemicals were reagent grade, obtained from E. Merck AG, Darmstad, Germany, and required no further purification prior to use. Carbowax 20 M and Gas-Chrom P were obtained from Applied Science Laboratories, Inc., State College, Pa. Methanol or *n*.butanol were refluxed before use in all-glass distillation apparatus with magnesium turnings for 2-3 hours. They were then distilled in the same distillation apparatus and brought to 1.25 mM with slow bubbling of dry HCl from a cylinder fitted with a drying tube containing $CaCl_2$ to protect from atmospheric moisture. The alcohol solution was then titrated with N NaOH using methyl red as indicator and diluted, if necessary, to the concentration reported above.

Standard solutions

For the construction of the calibration curve, β-alanine was dissolved in acid methanol in order to obtain a concentration of 1.5 mg/ml. The solution was stirred for 30' at room temperature with a magnetic stirrer; 1-2-3-4-5 ml samples were removed (the quantity of β-alanine therefore fell within the range of 1.5 to 7.5 mg) and all the samples were evaporated just to dryness on an all-glass vacuum rotary evaporator (water pump) with the aid of a water bath at 60°C. 1 ml of methylene chloride containing 0.3% (v/v) of o-toluidine (used as internal standard) and 0.1 ml of trifluoroacetic anhydride were added to the oily residue thus obtained. The solutions were kept for 30' at room temperature, after which they were ready to be analysed. A volume (preferably constant) of 0.5 µl of each of these solutions was injected into the gas chromatograph.

The same procedure could be applied for the preparation of N-TFA *n*.butyl esters, the only variation consisting in using acid *n*.butanol and prolonging the stirring time for 60' at 50°C. Fig. 1 illustrates two typical gas chromatograms showing the separation obtained between β-alanine N-TFA methyl ester (or butyl ester) and o-toluidine. The retention times

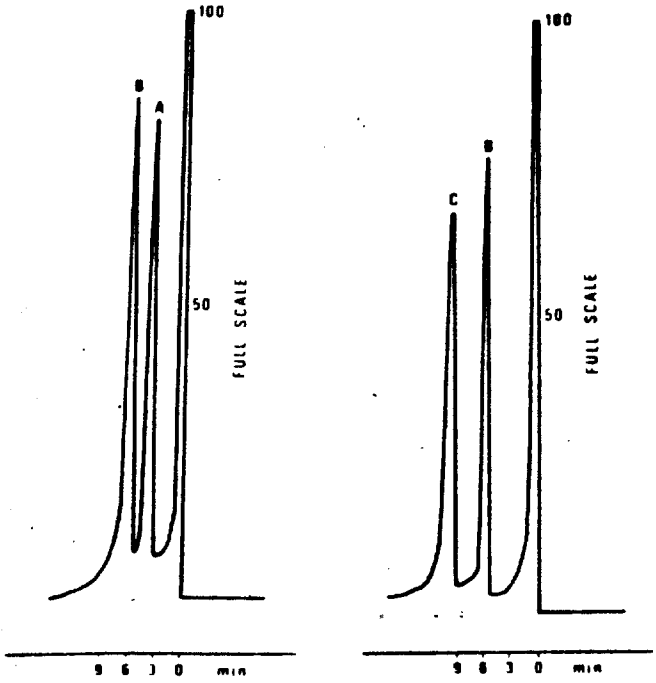

Fig. 1

Gas chromatograms of:
A = β-alanine N-TFA methyl ester
B = o-toluidine (internal standard)
C = β-alanine N-TFA *n*.butyl ester.

are respectively 3'20" for N-TFA methyl ester, 8'50" for N-TFA butyl ester and 5'25" for o-toluidine.

Quantitation

The peak height ratios (PHR) of β-alanine derivatives to o-toluidine were calculated and plotted against concentrations of β-alanine in the final solution. This procedure, based on the measure of peak heights only, has shown itself particularly useful in the case of routine analyses to be carried out on a large number of samples, due to its great simplicity and rapidity. Practically equivalent results were obtained adopting the method of the peak areas ratio, calculated by triangulation (peak areas obtained by multiplying the height by the width at half height). The relationship between the quantities injected and detector responses was linear over the whole range considered for both types of derivatives (0.68-3.41 μg of β-alanine injected). Typical plots are shown in Fig. 2.

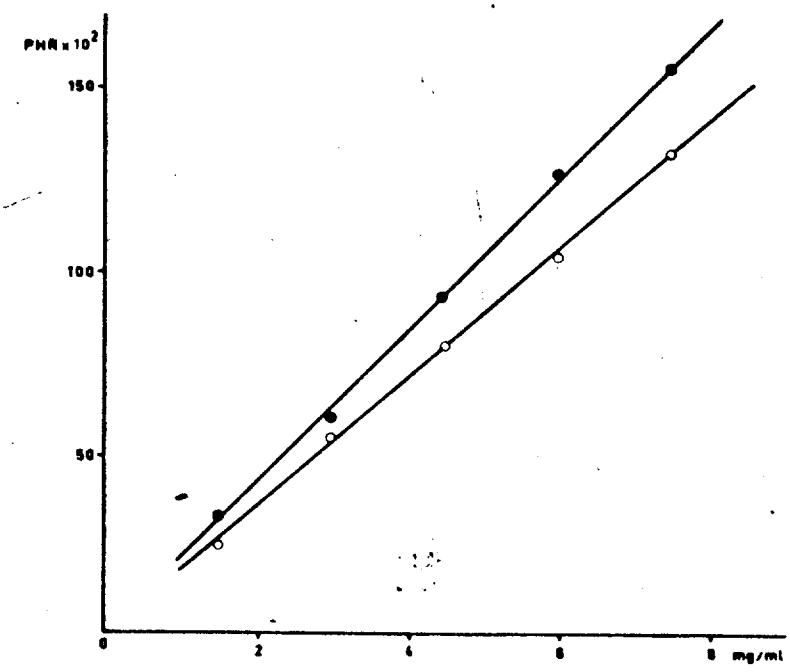

Fig. 2

Typical calibration curves for β-alanine. N-TFA methyl ester (●) and β-alanine N-TFA n.butyl ester (○).

Procedure

The first step in gas chromatographic assay of pantothenic acid and its salts is acid or alkaline hydrolysis to β-alanine. We started with about 20 mg of the vitamin, which were hydrolysed in a closed vial at 100°C for 1 hour (or else at 80°C for 3 hours) with 2 ml of 2N HCl. The vial was then opened and 1 ml of solution was evaporated in an oven at 90°C, if necessary with the aid of a stream of dry nitrogen gas. This temperature can be lowered adequately in the case of pharmaceutical preparations containing biological material.

5 ml of acid methanol were added to the residue and the solution was stirred with a magnetic stirrer for 30' at room temperature (in the case of the sodium salt it is advisable to prolong the stirring time for 1 hour). The sample was then evaporated to dryness using a rotary evaporator and a water bath set at 60°C. 1 ml of methylene chloride containing 0.3% (v/v) of o-toluidine and 0.1 ml of trifluoroacetic anhydride were added to the residue. The solution was kept at room temperature for 30', after which about 0.5 µl was injected into the gas chromatograph.

Should one wish to prepare the N-trifluoroacetyl *n*.butyl esters, the same procedure is followed as that indicated for standard solutions.

The peak height ratios of the sample solutions were calculated and the

concentrations of β-alanine in the final solution were determined from the calibration curve. The factors to obtain the quantity of pantothenic acid or its calcium or sodium salts, are 2.461, 2.674 and 2.708 respectively.

Results and Discussion

The formation of suitable volatile derivatives of amino acids has been studied recently by several authors (10, 11, 12).

We preferred to use the N-TFA methyl ester for β-alanine, principally because of its easy and fast preparation; on the other hand, these derivatives are the most volatile in the class of trifluoroacetylated ester

TABLE I

Pantothenate recoveries from pharmaceutical preparations and synthetic mixtures.

No.	Mixtures	Theoretical mg	Found mg	% of theoretical (*)
1 (**)	Calcium pantothenate	25	25.9	103.6
	Vitamin B_1	5		
	Vitamin B_2 (5'-phosphate Na salt)	5		
	Vitamin B_6	2.5		
	Vitamin B_{12}	1		
	Vitamin PP	125		
2	Mixt. No. 1 (ml 2.5) containing Calcium pantothenate	25	25.6	102.4
	Liver extract	125		
3	Calcium pantothenate	12.5	12.7	101.5
	Vitamin B_1	5		
	Vitamin B_2 (5'-phosphate Na salt)	5		
	Vitamin B_6	5		
	Vitamin C	12.5		
	Glucose	12.5		
	Glycine	5		
	Placental extract (phenolic)	1,250		
	Tween 80	14.5		
	Water (to 2.5 ml)			

(*) Mean of five analyses.

(**) ml 2.5 ampoules commercially obtainable.

derivatives of amino acids and therefore the danger of losses in quantitative analysis may exist. These losses can be avoided by interrupting rotary evaporation as soon as the sample is dried, and by accurately regulating the temperature of the bath and the vacuum generated by the water pump. Higher esters can be used conveniently, however, to reduce the volatility of β-alanine derivatives and we prepared the N-TFA *n*.butyl esters with only small modifications of the methyl esters preparation method. As shown in Figs. 1 and 2, it is also possible in this case to carry out the analysis under the same gas chromatographic operative conditions always keeping o-toluidine as internal standard. The yields obtained from the calcium or sodium pantothenate solutions are in practice the same in both cases.

The elution peaks of both β-alanine derivatives on Carbowax 20 M stationary phase, are nearly symmetrical and therefore very useful for quantitative analysis.

The pantothenates can be determined specifically also in the presence of B-group vitamins, as shown in Table I. The proposed method offers advantages over the usual chemical procedures based on the spectrophotometric determination of their hydrolytic products; as shown in Table I, in fact, there are good recoveries of the vitamin even in the presence of substances interfering with these chemical methods.

Table II shows results obtained from several determinations carried out on accurately weighed samples of calcium or sodium pantothenate. From these data the precision of the method was calculated to be 98.10 ± 2.95 (mean of 10 analyses $\pm$ standard deviation).

TABLE II

Pantothenate recoveries.

Sample	Salt	Theoretical mg	Found mg	% of theoretical
1	Calcium	20.00	19.72	98.6
2	Calcium	20.00	20.28	101.4
3	Calcium	20.00	18.88	94.4
4	Calcium	20.00	19.42	97.1
5	Calcium	20.00	18.60	93.0
6	Calcium	20.00	19.78	98.9
7	Sodium	20.00	20.28	101.4
8	Sodium	20.00	19.64	98.2
9	Sodium	20.00	19.28	96.4
10	Sodium	20.00	20.32	101.6

Conclusions

It can be concluded from the data reported above that our method is suitable for the quantitative determination of pantothenic acid and its salts by gas-liquid chromatography, also in the presence of substances interfering with the usual chemical methods. As with chemical methods, it is obviously impossible to distinguish the D-form from the DL-form, and the procedure is not specific for pantothenates in the presence of their degradation products. This last drawback can be overcome, however, with a preventive cleanup of the sample carried out by passing it through a column of strongly acid resin cation-exchanger, such as Dowex 50X4 (H type) with water as eluent, which will remove any preformed β-alanine (9, 13). This procedure renders the method specific for the intact pantothenate molecule. In addition, using the gas chromatographic method, sugars and riboflavine, which are not removed by the resin, do not interfere with the determination. Another advantage of the proposed method lies in the fact that it is possible to assay niacinamide and lidocaine (which are often present in pharmaceutical preparations together with pantothenates), under the same gas chromatographic conditions, with the only difference of a higher column temperature (14).

Recently a paper was published which described another method for the gas chromatographic determination of pantothenates in bulk (15), but the differences in comparison with our method are quite marked, both as regards the gas chromatographic conditions and the type of derivatives.

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STUDIES OF PANTOTHENIC ACID METABOLISM IN LIVER DAMAGE. IV. PANTOTHENIC ACID TREATMENT IN PATIENTS AND ANIMALS WITH LIVER DAMAGE

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STUDIES OF PANTOTHENIC ACID METABOLISM IN LIVER DAMAGE.

IV. PANTOTHENIC ACID TREATMENT IN PATIENTS AND ANIMALS WITH LIVER DAMAGE

By

Tatsuyuki Ueshima

ABSTRACT: Previous studies concerning the metabolism of pantothenic acid in patients and animals with liver damage indicated that the deficiency of pantothenic acid frequently exists.
(1) Patients: 20 mg of calcium pantothenate was administered intramuscularly daily for 1-4 weeks to patients with various liver diseases. After treatment, improvement in various liver function tests was observed. The improvement in hippuric acid synthesis, serum cholesterol ester ratio, and blood pyruvate level were most remarkable.
(2) Animals: Characteristic metabolic defects previously described seen in rabbits poisoned with CCl_4, were found to be improved in these animals treated with 10 mg of calcium pantothenate daily.
These data suggest that pantothenic acid may play an important role in the disturbance of various liver functions.

It is well known that the absorption, metabolism and storage of various vitamins in animals with liver damage are frequently impaired. However, pantothenic acid (PaA) metabolism in animals with liver damage is not yet clarified and, consequently, no specific method is available to cope with its metabolic abnormality. Previously, we reported that abnormality in PaA metabolism is frequently present in patients with liver diseases and rabbits with liver damage, and this abnormality is closely related to the decreased liver function and abnormality in various intermediary metabolisms [1,2,3]. Based on these findings, we administered therapeutic doses of Ca-PaA to patients and rabbits with liver damage and the results obtained are described and discussed in this paper.

Animal Experiments

(1) Experimental method

The experimental animals used were male rabbits weighing about 2 kg. Acute and chronic liver damages were induced by the techniques as described previously [3]. For PaA therapy, a dose of 10 mg of Ca-PaA per day was injected into the back muscle. For the rabbits with acute liver damage, the therapy was begun 2 days before liver damage was induced and was continued for 5 days. For the rabbits with chronic liver damage, the therapy was started on the day liver damage was induced and was continued for 70 days. Rabbits with liver damage receiving 1 cc of physiological saline solution according to the schedules mentioned above served as controls. Blood samples were collected from ear veins under ether-anesthesia early in the morning following overnight fasting. The liver was removed immediately after decapitation of the rabbit. Determinations of the serum cholesterol level, blood pyruvic acid (PyA) [2] concentration and the liver PaA content, and a histological examination of the liver were carried out according to the procedures described in an earlier report [3]. Changes in serum total cholesterol (T-Ch) [3], esterified cholesterol (E-Ch) [4], ester quotient (E-Q) [5], and blood PyA concentrations were expressed as per cent change with respect to the values obtained before liver damage.

(2) Experimental results

(i) Effects on intermediary metabolisms

a) Serum cholesterol: E-Q measured at the 3rd and 7th days after acute liver damage showed a decrease in the control group while the E-Q was normal in the PaA therapy group. For chronic liver damage, the E-Q decreased gradually in the control group while the decrease in E-Q was milder in the

therapy group.

b) Blood pyruvic acid: Three days after acute liver damage, the blood PyA level was almost doubled in the control group while the increase was milder in the therapy group. In chronic liver damage, the PyA level increased gradually over a 2-month period after liver damage in the control group, while the increase was more gradual in the therapy group.

(ii) Effects on liver PaA content

In acute liver damage, the control group gave a total liver PaA content, especially of conjugated PaA, lower than the normal value on the 3rd day after liver damage, and the value returned to almost normal on the 7th day, whereas in the therapy group no decrease was noted. The total and conjugated PaA contents both decreased markedly in the control group on the 70th day after chronic liver damage, while only a slight decrease was noted in the therapy group.

Table 1. Effects of Ca-PaA therapy on the serum cholesterol metabolism in rabbits with acute and chronic CCl_4 liver damages.

		Average per cent change	On the 3rd day after liver damage	On the 7th day after liver damage
Acute Damage	Control group (3 cases)	Total cholesterol ester ratio	+ 138.2	+ 10.7
			- 13.3	- 3.7
	Therapy group (3 cases)	Total cholesterol ester ratio	+ 123.6	+ 43.3
			+ 0.3	+ 1.7
		Average per cent change	On the 30th day after liver damage	On the 60th day after liver damage
Chronic Damage	Control group (4 cases)	Total cholesterol ester ratio	+ 106	+ 317
			- 14.8	- 48.9
	Therapy group (4 cases)	Total cholesterol ester ratio	+ 90	+ 215
			- 10	- 12.3

Table 2. Effects of Ca-PaA therapy on the blood PyA metabolism in rabbits with acute and chronic CCl_4 liver damage

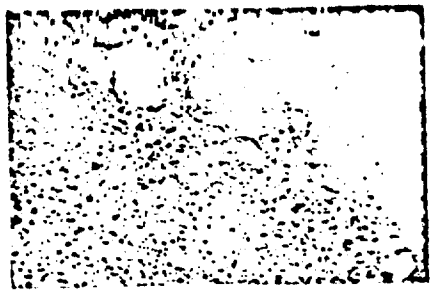
		Average per cent change	On the 3rd day after damage	
Acute damage	Control group (3 cases)	blood PyA	+ 93	
	Therapy group (3 cases)		+ 49.5	
		Average per cent change	On the 30th day after damage	On the 60th day after damage
Chronic damage	Control group (3 cases)	blood PyA	+ 17	+ 24
	Therapy group (3 cases)		+ 4	+ 9.7

Table 3. Effects of Ca-PaA therapy on the liver PaA content of rabbits with acute and chronic CCl_4 liver damage

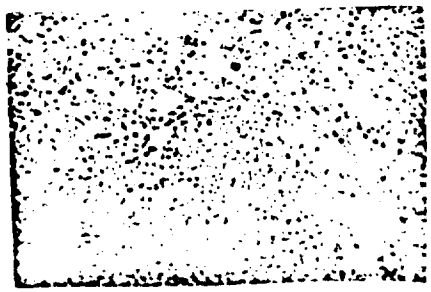
	Days after damage	Ca-PaA administration	Average liver PaA content		
			Free form	Conjugated form	Total
Acute damage	3 days	Control group (4 cases)	3.3	41.7	46.7
		Therapy group (4 cases), received therapy one day before liver damage	12	80.3	92.3
	7 days	Control group (4 cases)	2.1	59.5	61.6
		Therapy group (4 cases), received therapy two days before liver damage	4.8	63.2	68.0
Chronic damage	70 days	Control group (4 cases)	3.0	41.1	44.1
		Therapy group (5 cases), received therapy five days before liver damage	2.2	50.1	52.3
		Healthy group (12 cases)	* 1.5±0.5	65.5±10.5	67±10.7

* Standard deviation

1. CCl_4 - only group without therapy (Haematoxylin-eosin staining)



2. PaA therapy group (Mallory-azan staining)



3. CCl_4 - only group without therapy (Mallory-azan staining)

4. PaA therapy group (Mallory-azan staining)

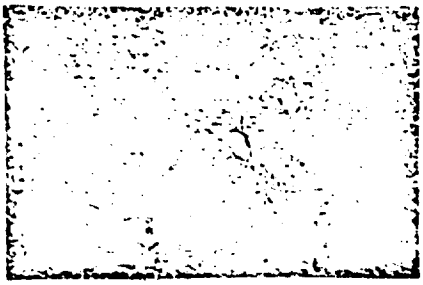


Fig. 1. Picture of rat liver tissues with chronic CCl_4 liver damage and effects of Ca-PaA therapy on the liver tissue picture.

(iii) Effects on liver tissue

Tissue changes observed in the liver of rabbits with acute and chronic CCl_4 liver damage are the same as those reported in our previous paper, i.e. lobular central necrosis, hemorrhage, and degeneration. Fibrosis of the stroma was laso observed by the Mallory-azan staining in the chronic damage group. Liver cell damage, lobular central necrosis, hemorrhage, fatty infiltration and fibrosis of the stroma were much milder in the therapy groups, especially those with chronic damage, than in the control group. The difference was not so distinct between the therapy and control groups with acute liver damage.

Clinical Experiments

(1) Experimental methods

The patients studied were mainly 30 in-patients of this hospital with liver diseases. Diagnosis was based on liver function tests and also liver biopsy. For PaA therapy, 20 mg/day of Ca-PaA were injected intramuscularly for 7-30 days. During therapy, all other therapeutic drugs for liver diseases were discontinued with the exception of glucose. In some patients, B_1, C and methionine (Met) [6] were also used as shown in the Tables. Several healthy subjects receiving 40 mg of Ca-PaA/day for 10 days were used as control. Various liver function tests and intermediary metabolisms were examined before and several days after the therapy. The results were compared with those obtained from control subjects and subjected to statistical analysis.

(2) Experimental results

(i) Effects on subjective and objective symptoms

Subjective symptoms such as loss of appetite, general fatigue and

proneness to tire easily, are improved. The therapy was especially effective in improving the sensory abnormalities such as numbness of the limbs. The therapy was also found capable of alleviating nervous symptoms such as headache, hypersensitivity, hypochondria, and impatience etc. frequently observed in patients with chronic liver diseases. As for objective symptoms, alleviation of liver pain due to pressure or percussion was noted and the tendon reflex became normal in many patients. Alleviation of the symptoms described above was mostly observed in those patients with hepatitis or mild liver cirrhosis and the therapeutic effect was less in those patients with severe liver cirrhosis accompanied by ascites (utilization of PaA was probably impaired in these patients). Administration of 20-40 mg of Ca-PaA for 30 days did not alter the mineral metabolisms of the urine Na, Cl, K and Ca, and no unpleasant side effects were noted in healthy subjects or in patients with liver diseases.

(ii) Effects on the amount of PaA excreted into urine and on serum PaA level

An average serum PaA level is 260 ± 95 mγ/cc for healthy subjects, 173 ± 54 mγ/cc for patients with acute hepatitis, 221 ± 87 for chronic hepatitis and 159 ± 33 mγ/cc for liver cirrhosis; after the PaA therapy, their levels all increased to 617 ± 45, 540 ± 36, 256 ± 24 and 233 ± mγ/cc, respectively. Similar tendency was also observed in the urinary excretion of PaA. However, some patients with severe liver cirrhosis showed a marked increase in the excretion rate; in this group of patients, the improvement of liver function and metabolic abnormality was usually either very slight or not present.

(iii) Effects on various liver functions

(a) Various serum colloid reactions were all abnormal before therapy. After PaA therapy, the thymol turbidity test value for acute hepatitis

patients and the Kunkel lead sulfate test value for chronic hepatitis patients showed significant improvement over those before therapy, but other test results showed no conclusive tendency. No significant change could be seen in healthy subjects or patients with liver cirrhosis.

(b) Icterus index: some degree of improvement was noted in both hepatitis and liver cirrhosis following PaA therapy.

(c) Emperiments on hippuric acid synthesis: In most liver disease patients, hippuric acid synthesis was either decreased or delayed, but the synthesis was significantly improved after PaA therapy in almost all patients studied. This improvement was most remarkable in patients with acute or chronic hepatitis, or mild liver cirrhosis, but was not so distinct in patients with severe liver cirrhosis. The improvement was especially distinct for those patients with low pre-therapy values and their rate of synthesis increased.

(iv) Effects on various intermediary metabolisms

(a) Serum cholesterol metabolism: Most liver disease patients showed a decrease in T-Ch, especially E-Ch and E-Q. After PaA therapy, T-Ch and E-Ch both increased, especially the E-Ch increase was remarkable. Improvement in E-Q was seen in patients with hepatitis, especially chronic hepatitis.

(b) Blood pyruvic acid metabolism. Patients with liver cirrhosis showed relatively high serum PyA concentration. A high concentration was also noted in many patients with hepatitis. The PaA level decreased and tended to become normal following PaA therapy. The extent of the PaA concentration decrease was rather remarkable in patients with chronic and acute hepatitis but less distinct in patients with liver cirrhosis.

Table 4. Effects of Ca-PaA therapy on liver functions of patients with acute hepatitis.

Name	Age/Sex	Name of Diseases	No. of days in which 20 mg Ca-PaA were admin.	Quantity of PaA excretion (12 hours) (γ)		Protein metabolism: Total protein		Gros reaction		Kunkel reaction		Thymol turbidity reaction	
				Before	After	Before	After	Before	After	Before	After	Before	After
Oka	49	Acute hepatitis	20 days	1064	3000	7.0	7.3	1.1	1.3	12	8	8.8	4
Miya	32	"	14	220	2300	7.4	7.8	1.36	0.93	16	16	7	5
Ka	16	"	20		1005	7.0	7.2	1.18	1.47	10	8	3	3
Minami	22	"	20	1800	4500	7.1	7.4	1.45	1.59	4	4	1	1
Toku	28	"	20	1975	2640	7.2	7.4	1.48	1.51	6	14	2	2
Hoso	23	Acute hepatitis accompanied by amyloid infiltration of the lungs	23	1488	2400	7.3	7.4	1.06	1.45	12	8	4	2
Suga	25	Subacute hepatitis	23	1430	2620	7.1	7.2	1.25	1.42	4	8	1	1
Changes in values due to therapy		Average (difference)		1330 → 2638 (+ 1308)		7.2 → 7.4 (+ 0.2)		1.27 → 1.36 (+0.09)		9 → 9.4 (+ 0.4)		3.8 → 2.6 (- 1.2)	
		F_o		24		32		1.2		0.3		3.3	
		Significant level		0.1<P<1		0.1<P<1		P>20		P>20		5<P<20	

Table 4. -Continued

Icterus index		Experiments on hippuric acid synthesis				Serum cholesterol				Blood PyA		Urine				Remar
		One hour value		Four hour combined value		Total		Ester ratio				Ubg* reaction		Brn** reaction		
Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	
54	10	0	25	15	45							+	-	+	-	
81	40	0	20	35	40	168	227	68	68	1.26	1.1	++	+	+	±	used togeth with Met (1 mg)
12	5	65	101	78	121	124	160	75	76	1.0	1.0	++	±	-	-	used togeth with M (1 mg)
117	24	50	52	77	89	122	175	54	70	1.5	1.2	+	-	+	-	
25	72	23	64	77	89	102	137	57	60	1.5	1.3	-	-	+	+	
36	6	30	21	67	51	205	196	74	74			+	±	+	-	used togeth with M (1 mg)
30	18	42	40	73	70	148	125	69	74	1.1	1.0	-	-	+	-	PAS (1
51→25 (-26)		30→46 (+16)		60→72 (+12)		145→170 (+25)		66→70 (+4)		1.27→1.12 (-0.15)						
2.5		4.7		2.48		3.3		2.78		8.6						
5<P<20		5<P<20		5<P<20		5<P<20		5<P<20		1<P<5						

*Ubg = Urobilinogen
**Brn = Bilirubin

Table 5. Effects of Ca-PaA therapy on liver functions of patients with chronic hepatitis

Name	Age/Sex	Name of Disease	No. of days in which 20 mg Ca-PaA were admin.	Quantity of PaA excretion γ (12 hours)		Protein metabolism							
						Total Protein		Gros reaction		Kunkel reaction		Thymol turbidity reaction	
				Before	After	Before	After	Before	After	Before	After	Before	After
Ue	36	Chronic hepatitis	7 days			6.8	6.8	1.32	1.31	4	4	1	1
Yasu	32	"	"	980	1320	7.2	7.4	1.76	1.8	2	2	1	1
Oo	43	"	"					1.2	1.21	6	6	2	2
Ko	48	"	"	1150	3650	7.9	8.0	1.08	0.9	16	14	4	1
Fuka	49	"	"					1.48	1.82	4	4	1	1
Yasu	54	"	"	900	3230	7.6	7.8	0.92	1.3	22	14	4	3
Kame	34	"	"			6.8	6.6	1.34	1.4	10	9	3	3
Tsuji	67	"	"	1258	1308	8.0	8.1	1.18	1.24	12	10	4	5
Iwa*	41	"	"	1512	3400	6.6	6.6	0.92	0.77	14	14	4	3
		active form											
Furu	46	"	"	1032	2220	8.1	8.0	1.2	1.08	12	12	3	2
		active form											
Uchi	25	"	20	598	2512			1.62	1.48	8	8	1	2
Naka	30	"	27	1100	3340			1.15	1.07	18	16	5	6
Yoshi	29	"	30	1040	4888	6.3	5.5	1.14	1.3	12	10	2	1
Jyo	59	"	"			8.0	7.9	1.17	0.9	16	16	5	7
Ma	31	"	24			7.3	7.5	1.53	1.53	6	6	2	2
Minami	61	"	"	1295	7268	6.3	6.3	0.85	0.87	16	16	4	4
		active form											
Kua	27	"	"	555	10400	7.8	7.0	0.72	1.08	24	22	11	10
Changes in values due to therapy		active form Average (difference)		1038→ 3958 (+2920)		7.3 → 7.2 (-0.1)		1.21 → 1.24 (+0.03)		11.8→ 10.7 (-1.1)		3.4→ 3.2 (-0.2)	
		F_o		12		0.9		0.18		5.14		0.02	
		Significant level		0.1<P<1		P>20		P>20		1<P<5		P>20	

* Utilization inhibited

Table 5. -Continued

Icterus index		Experiments on hippuric acid synthesis				Serum cholesterol				Serum PyA		Urine				Remarks
		One hour value		Four hour combined values		Total		Ester ratio				Ubg reaction		Brn reaction		
Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	
7	4	19	22	61	62	159	165	75	76	0.85	0.08	+	+	-	-	
6	5	0	53	30	53	135	167	72	71			±	-	-	-	
5	5	61	71	61	93	130	140	70	78			+	±	-	-	
4	5	0	22	46	40	158	187	78	73			+	+	-	-	
5	5	0	0	20	55	167	167	70	73	1.2	1.0	+	±	-	-	
11	6	0	0	15	56	167	160	78	76	1.2	0.75	++	+	-	-	
6	6	60	74	60	74	128	102	70	74			+	±	-	-	
13	6	44	51	55	61	167	167	76	77			+	-	-	-	
15	15	0	0	37	47	105	120	53	62	0.85	0.89	+++	++	-	-	
11	12	0	0	24	38	148	142	49	76	0.8	0.65	+	+	-	-	
5	5					201	201	65	75	1.05	1.0	±	-	-	-	
9	9	0	16	36	95	94	195	77	80			+	+	-	-	
6	8	34	77	58	77	130	104	70	68	1.7	1.4	+	-	-	-	used with P (20 mg)
6	5	41	53	66	80	143	167	67	64			+	+	-	-	
6	5	38	55	64	70	149	176	57	75			±	-	-	-	used with B (10mg)+ C(10C
7	7	0	31	58	62	131	126	69	67	1.5	1.36	+	+	-	-	
6	7	11	29	11	87	155	203	48	75	2.25	1.85	++	+	-	-	used with M (1 mg)
7.6	6.8	19 → 35		44 → 66		146 → 153		67 → 73		1.27 → 1.08						
(-0.8)		(+16)		(+22)		(+12)		(+6)		(-0.19)						
1.8		15.1		15.3		4.16		5.4		5.9						
5<P<20		0.1<P<1		0.1<P<1		5<P<20		1<P<5		1<P<5						

Table 6. Effects of Ca-PaA therapy on liver functions of patients with liver cirrhosis.

Name	Age/Sex	Name of Disease	No. of days in which 20 mg Ca-PaA were Admin.	Quantity of PaA excretion (12 hours) (γ)		Protein metabolism							
						Total protein		Gros reaction		Kunkel reaction		Thymol turbidity reaction	
				Before	After	Before	After	Before	After	Before	After	Before	After
Mori	50	early stage of liver cirrhosis	26 days	1700	2500	7.8	7.8	1.08	1.13	16	18	6	4
Ki	37	"	14	1339		7.0	6.7	0.95	1.0	30	26	7	7
Naga	49	Hanot's cirrhosis of the liver	10	2960	1703	6.5	6.0	0.64	0.72	14	24	5	10
Naka	53	"	14	430	2000	8.2	8.7	1.05	1.24	20	16	7	3
Yama*	49	severe liver cirrhosis	26	1416		6.5	6.7	0.6	0.6	22	24	10	10
Yoshi*	56	"	26	937	16500	7.0	6.8	0.53	0.5	28	30	10	10
Change in values due to therapy		Average (difference)		1464 → 5176 (+3712)		7.2 → 7.1 (-0.1)		0.81 → 0.87 (+0.06)		22 → 23 (+1)		7.5 → 7.3 (-0.2)	
		F_0		0.8		0.1		1.2		0.37		0.02	
		Significant level		P<20		P<20		P<20		P<20		P<20	

* Utilization inhibited

Table 6. -continued

Icterus index		Experiments on hippuric acid synthesis				Serum cholesterol				Blood PyA		Urine				Remarks
		One hour value		Four hour combined value		Total		Ester ratio				Ubg reaction		Brn reaction		
Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	
7	8	36	50	70	75	142	112	71	71			+	+	-	-	Used togeth with B_1 (10m
27	20	10	20	30	40	160	170	60	65	1.4	1.2	++	+	-	-	
11	6	0	30	31	65	167	150	54	57	1.65	1.35	+	+	-	-	
7	3	29	32	54	82	126	167	65	70	1.5	1.4	+	+	-	-	
8	7	12	15	12	25	130	130	48	50	1.8	1.6	++	++	-	-	Ascites (+) used togeth with B_1 (10m
6	7	0	0	15	15	139	140	46	45	2.0	2.1	++	+++	-	-	Ascites (++ used togeth with B_1 (20m
11 → 8.5 (-2.5)		14.5→ 24.5 (+10)		35.3 →50.3 (+15)		144 → 145 (+1)		57 → 60 (+3)		1.67 →1.53 (-0.14)						
3.3 5<P<20		4.8 5<P<20		7.6 1<P<5		0.07 P>20		5.1 5<P<20		4.3 5<P<20						

Discussion

Most B vitamins are activated in the liver and serve as various coenzymes in a common metabolic path. Activation of PaA, namely formation of CoA, is also carried out in the liver. It has been demonstrated by Lipmann and Ochoa that CoA plays important roles in the metabolic processes of carbohydrates, lipides and proteins. The liver not only plays a central role in the various metabolisms in the living body but also a main storage place for various vitamins. Therefore, it can be anticipated that liver damage can cause abnormalities in various intermediary metabolisms and can damage vitamin metabolisms. Exogenic deficiency of B_1, B_2 and nicotinic acid due to insufficient intake of these vitamins and endogenous metabolic abnormality due to increased requirement and inhibition of utilization of these vitamins are well known, and these abnormalities play important roles in the progress of various diseases and appearance of various symptoms. In has been shown by many investigators [4,5] that administration of vitamins is effective for treatment of liver diseases.

Not many reports concerning metabolism of PaA in liver diseases and its clinical significance and therapeutic application are available. We have previously reported [2,3] that PaA deficiency and inhibition of its utilization may be present in liver diseases, which may accelerate liver damage further by impairment of α-keto acid oxidation, lowering of the detoxication function and abnormality in lipid metabolism and may result in formation of a *circulus vitiosus*. In this study, Ca-PaA was administered to liver disease patients and rabbits with liver damage in an attempt to correct metabolic impairment and to improve general status in liver diseases.

Takaoka [6] of this department has already pointed out that various nervous symptoms observed in chronic hepatitis patients are closely related

to abnormal oxidation mechanisms and that administration of B_1, especially CoC (cocarboxylase) may alleviate these symptoms. We found that the symptoms were alleviated in some patients by PaA administration, but not by B_1 administration. It was also reported that marked nervous impairment was present in animals with experimentally induced PaA deficiency [7], that Ca-PaA was effective for the treatment of nutritional melalgia (reported by Gopalan [8], that Ca-PaA was effective in the treatment of sensory abnormality in patients with experimentally induced PaA (reported by Bean et al.) [9] and that CoA was required in transmission of nervous stimulation. These observations, together with our own findings, suggest that PaA is also closely related to the appearance of nervous symptoms in patients with liver diseases.

PaA is known to be present in plasma as a free acid, while in red blood cells it is in a conjugated form, mainly as CoA [10]. According to Pearson [11], the plasma PaA concentration in healthy persons is 247±57 mγ/cc. Spies et al. [12] reported a normal blood PaA concentration of 190-320, an average of 225 mγ/cc; this value is closer to our own value. Spies et al. [12] also reported that blood PaA concentration in patients with pellayra, beriberi and riboflavin deficiency was 23 to 50% lower than the normal level. Low values were also found in our patients with liver diseases, and the value was especially low in patients with liver cirrhosis. This finding is in agreement with that reported in our previous paper in which PaA deficiency in the body was predicted from the amounts of urinary excretion of PaA before and after loading with Ca-PaA. The serum PaA level returned to normal in all patients after PaA therapy, but the increase was much milder in patients with severe liver cirrhosis who showed a marked increase in urinary excretion of PaA, suggesting impairment of PaA utilization in the body.

Participation of CoA in hippuric acid synthesis (one of the liver detoxication function tests) has been demonstrated by Chantrenne [13] and Schachter et al. [14]. Previously, we demonstrated a clear correlation between the amount of urinary PaA excretion and hippuric acid biosynthesis. In our present study involving PaA therapy, marked improvement in the detoxication function preceeded the recovery of other liver functions and metabolisms. However, in the patients with severe liver cirrhosis accompanied by marked ascites, the improvement of the symptoms was very minor, and prognosis of those patients without improvement in the synthetic function was, in many cases, extremely poor. These findings are in agreement with the report by Kawada of our department [15] that successful therapy with Met-protein hydrolyzate is evidenced by rapid recovery of the detoxication function, while failure in the improvement of the detoxication function usually would result in poor prognosis.

As for lipid metabolism in patients with liver diseases, abnormality in Ch metabolism such as decreases in E-Ch and E-Q has long been recognized. The impairment in Ch metabolism is also demonstrated in rabbits with CCl_4 induced liver damage [16-19]. For their treatment, in addition to Met [20,21] or Met-protein hydrolyzate [15], administration of B vitamins such as B_2, FAD and B_{12} [22,23] is also found to be effective.

Many of our patients showed decreased E-Q, and the decrease was most remarkable in patients with acute hepatitis, liver cirrhosis, and chronic hepatitis diagnosed as active by liver tissue examination. After PaA therapy, most of these patients showed increases in T-Ch, E-Ch, and E-Q. However, patients with impaired PaA utilization failed to improve their E-Q value. Similar results were also observed in rabbits with CCl_4 induced liver damage.

Amatuzio [24], Davis [25], Smith [26], Kipping [27] and Okada et al.

[28] of our department reported that liver damage was frequently accompanied by abnormal PyA metabolism and that B_1, especially its active form CoC, was ineffective in improving this abnormality. In our studies, abnormal PyA metabolism was observed in both patients with liver diseases and rabbits with liver damage. Nitani [29] and Miyano et al. [30] demonstrated that mixed amino acids, or Met and B_1 was effective in treatment of impaired PyA metabolism in aminals with liver damage. Participation of CoC, CoA, diphosphopyridine nucleotide and lipoic acid in the PyA oxidation enzyme system has been demonstrated. In many cases, the use of Ca-PaA in patients with a marked increase in the blood PyA level resulted in lowering of the blood PyA level, and a similar result was also found in rabbits with liver damage. These findings, together with our previous observation that the blood PyA value was closely related to the amount of PaA excreted into the urine suggest that abnormal PyA oxidation in liver diseases plays a role in abnormal PaA metabolism. Amano and Shigeta [31] of our department demonstrated that the decreased ability of acetylation and impairment of α-keto acid oxidation during the glucose tolerance test observed in patients with liver diseases were frequently alleviated by PaA administration. Therefore, it is of value to use PaA together with CoC and lipoic acid for improvement of impaired oxidation function which may be one of the factors causing prolonged hepatitis symptoms.

As mentioned before, many of our patients, who showed marked improvement in their Ch and PyA metabolisms and hippuric acid synthesis after PaA therapy later also showed improvement in the serum colloid tests and BST (bromsulphalein test). In other words, impaired function involving direct participation of CoA was improved by PaA administration within a relatively short period of time while the effect of PaA therapy on other functions was somewhat

delayed.

PaA therapy was also found to be able to improve the liver tissue picture of rabbits with liver damage, as shown in the Figure; the improvement by PaA therapy was most remarkable in rabbits with chronic liver damage. Benda et al. [32] also stated that PaA could protect rats from allyl-formate-induced liver damage (necrosis).

As for side effects of PaA, Welsh [33] reported several cases of mild and transient nausea out of several hundred patients receiving an oral dose of 0.3 to 3 g of PaA per day for a prolonged period of time. No other side effects were mentioned. Gershberg [34] administered 10 to 20 g of PaA for a prolonged period of time and reported diarrhea in some patients and mild edema in patients with rheumatism; no other change was observed. Spies [12] administered 100 mg of Ca-PaA intravenously to healthy volunteers and reported no abnormality in body temperature, blood pressure, pulse and respiration. Kobbori et al. [35] reported no change in adrenal function when they administered 300 mg of Ca-PaA subcutaneously. In our studies, intramuscular administration of 20 mg/day of Ca-PaA into patients with liver diseases did not show any side effect.

So far, we discussed the effect of PaA therapy. For determination of the therapeutic effectiveness of PaA on chronic liver diseases, spontaneous cures of the disease during the therapy period can be ignored, but this problem has to be taken into consideration for acute hepatitis. In the case of hippuric acid synthesis, improvement by PaA therapy was much more remarkable than that observed with other conventional therapy and in some patients the synthetic function became greater than normal. In the severe liver disease patients with possible impairment of PaA utilization, prolonged PaA therapy resulted in metabolic improvement in some patients, but the improvement

was usually very minor. As pointed out by Inoue, liver function damage and endogenous metabolic impairment such as impairment of vitamin utilization in liver diseases are closely related and may interact each other to enhance the diseased state. In severe liver diseases, this interaction may already become irreversible. Therefore, it is understandable that the therapeutic effect of PaA in our patients with severe liver diseases was much less, because these patients clearly showed the picture of impairment of PaA utilization. We reported previously that synthesis of CoA from PaA was not easily impaired even in advanced liver diseases. However, impairment of this synthetic function may be present in severe liver cirrhosis, and the administration of conjugated PaA such as CoA may be of value. Since CoA is not readily available, PaA administration should be attempted first. In some cases, combined use of Met was found to be very effective. Dinning [36] and Umino et al. [31] have demonstrated that Met, through conversion to cysteine, can enhance the formation of β-mercaptoethylamine and thus can also enhance PaA utilization through CoA synthesis. Yamada et al. [37] showed the involvement of B_6 in CoA synthesis, and thus factors other than PaA should also be taken into consideration in CoA metabolism.

PaA is distributed widely in foods, and it is biosynthesized by intestinal micro-organisms and by other mechanisms. Therefore, clear-cut PaA deficiency is very rare. However, in liver damage PaA deficiency may result because of reduced PaA intake due to anorexia and improper restriction of food intake, because of impairment of digestion and absorption and because of increased demand for PaA in the liver; sometimes, impairment of PaA utilization may develop. Frequently, deficiency of other vitamins is also present. Spies [12] et al. reported that patients with low blood PaA level also showed marked B_1 and B_2 deficiency, and administration of PaA or B_2

alone would raise blood concentrations of other vitamins and thus suggested that PaA is metabolically closely related to other B vitamins. Therefore, vitamin therapy for liver diseases requires a large dose of multiple vitamins or vitamin B complex which should include PaA. In our studies, administration of CoC, B_1, B_2 or C etc. did not markedly improve the tired feeling, detoxication function, and metabolic impairment of PyA and Ch in many patients with mild liver cirrhosis but the symptoms were alleviated markedly by combined use of PaA. Of course it is not possible that PaA administration alone can rapidly correct all the very complicated metabolic abnormalities in liver diseases, but PaA administration may correct abnormal metabolisms involving direct participation of CoA and thus may bring about recovery of other liver functions and eventually restore normal status.

Conclusion

Ca-PaA was administered for therapy or prophylaxis to 30 patients with various liver diseases and also to rabbits with acute and chronic CCl_4-induced liver damages. The results obtained are as follows:

1) The depressed liver total PaA content, especially of the conjugated form, and metabolic abnormalities of Ch and PyA in rabbits with CCl_4-induced liver damage are improved by PaA administration. Liver tissue examinations revealed that degree of liver damage was mild in the therapy group; this was especially so in the therapy group with chronic liver damage.

2) PaA treatment improved the serum PaA level and urinary excretion of PaA in patients with various liver diseases. Depressed hippuric acid synthesis and abnormal Ch and PyA metabolisms were also improved. Subjective and objective symptoms such as nervous symptoms were either alleviated or disappeared.

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肝障害時におけるパントテン酸代謝

(IV) 肝疾患のパントテン酸治療

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STUDIES ON PANTOTHENIC ACID METABOLISM IN LIVER DAMAGE (IV) PANTOTHENIC ACID TREATMENT IN PATENTS AND ANIMALS WITH LIVER DAMAGE

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Previous studies concerning the metabolism of pantothenic acid in patients and animals with liver damage indicated that the deficiency of pantothenic acid frequently exists.

(1) Patients: 20mg of calcium pantothenate was administered intramuscularly daily for 1-4 weeks to patients with various liver diseases. After treatment, improvement in various liver function tests was observed. The improvement in hippuric acid synthesis, serum cholesterol ester ratio, and blood pyruvate level were most remarkable.

(2) Animals: Characteristic metabolic defects previously described seen in rabbits poisoned with CCl_4, were found to be improved in these animals treated with 10mg of calcium pantothenate daily.

These data suggest that pantothenic acid may play an important role in the disturbance of various liver functions.

肝障害時に往々種々なビタミンの吸収，代謝ないし貯蔵がおかされることは以前から知られている．しかし肝障害時のPaA*1) 代謝に関しては未だほとんど不明で従がつてその対策にもみるべきものがなかつた．

私は前回肝疾患々者ならびに肝障害ウサギではしばしばPaA代謝異常が存在しこれが肝機能低下，諸中間代謝異常と密接な関連を有することを報告した[1][2][3]．かゝる成績に鑑み今回はこれら患者ならびにウサギにCa-PaAを治療的に応用しその効果につき種々検討した．

動物実験

(1) 実験方法

実験動物としては2kg前後の雄ウサギを用い急性および慢性肝障害は前回と同一方法により惹起せしめた[3]．PaA治療はCa-PaA 1日10mgずつ，急性障害治療群は障害2日前より5日間，慢性障害治療群は障害第1日より70日間それぞれ連日背筋内に注射した．一方対照として障害単独群には生理的食塩水 1cc を同様の方法で投与した．なお採血は早朝空腹時エーテル麻酔下で耳静脈より行い肝臓は断頭後直ちに開腹採取した．

血清コレステロールならびに血液 PyA*2) 濃度の測定，肝PaA定量，肝の組織学的検索はそれぞれ前回の報告に準じて行つた[3]．血清 T-Ch*3), E-Ch*4) E-Q*5) および血液 PyA 濃度の推移は障害前値に対する変化率をもつて表示した．

(2) 実験成績

(1) 中間代謝におよぼす影響

a) 血清コレステロール：急性障害3日目および7日目の変化をみるに障害群ではいずれもE-Qの低下がみられこれに対しPaA治療群ではE-Qはほぼ正常であつた．慢性障害群でもE-Qは減少し，治療群ではその低下が軽度であつた．

*1) PaA = Pantothenic acid　*2) PyA = Pyruvic acid　*3) T-Ch = total Cholesterol
*4) E-Ch = esterified Cholesterol　*5) E-Q = Ester quotient

b) 血液ビルビン酸：急性障害群では障害3日目に血液PyAは前値の約2倍に上昇したが治療群では上昇軽度であつた．また慢性障害群でも1および2ヵ月ともに PyA は増加したが治療群は上昇軽度であつた．

表1 急性および慢性CCl_4肝障害ウサギの血清コレステロール代謝におよぼす Ca-PaA 治療の影響

			急性障害	
		変化率平均(%)	障害3日目	障害7日目
急性障害	対照群(3例)	総コレステロール	+138.2	+10.7
		エステル比	−13.3	−3.7
	治療群(3例)	総コレステロール	+123.6	+43.3
		エステル比	+0.3	+1.7
		変化率平均(%)	障害30日目	60日目
慢性障害	対照群(4例)	総コレステロール	+106	+317
		エステル比	−14.8	−48.9
	治療群(4例)	総コレステロール	+90	+215
		エステル比	−10	−12.3

表2 急性および慢性CCl_4肝障害ウサギの血液 PyA 代謝におよぼす Ca-PaA 治療の影響

		変化率平均(%)	障害3日目	
急性障害	対照群(3例)	血液 PyA	+93	
	治療群(3例)		+49.5	
		変化率平均(%)	障害30日目	60日目
慢性障害	対照群(3例)	血液 PyA	+17	+24
	治療群(3例)		+4	+9.7

(ii) 肝臓PaA量におよぼす影響

急性障害3日目では健常群に比し肝総PaA量，とくに結合型の減少を認め7日目にはほぼ前値に復したが，治療群では減少をみなかつた．また慢性障害70日目では総および結合型PaA量ともに著減し治療群ではいずれも減少軽度であつた．

表3 急性および慢性CCl_4肝障害ウサギの肝PaA 量におよぼす Ca-PaA 治療の影響

	障害後の日数	Ca-PaA 投与状況	肝 PaA 量平均 (γ/g) 遊離型	結合型	総
急性障害	3日	対照群(4例)	3.3	41.7	46.7
		治療群(4例) 前日まで注	12	80.3	92.3
	7日	対照群(4例)	2.1	59.5	61.6
		治療群(4例) 2日前まで注	4.8	63.2	68.0
慢性障害	70日	対照群(4例)	3.0	41.1	44.1
		治療群(5例) 5日前まで注	2.2	50.1	52.3
健常群(12例)			*1.5±0.5	65.5±10.5	67±10.7

*標準偏差

(iii) 肝組織変化におよぼす影響

急性および慢性CCl_4肝障害ウサギの肝組織変化は前回の報告と同様小葉中心性壊死，出血，変性などで慢性群ではマロリー染色で間質の線維化もみられた．治療群，とくに慢性障害治療群ではこれら肝細胞障害，小葉中心性壊死，出血，脂肪浸潤および間質の線維化などいずれも対照群に比し著しく軽度であつた．急性障害治療群と対照群との差は慢性群ほど明らかでなかつた．

臨 床 実 験

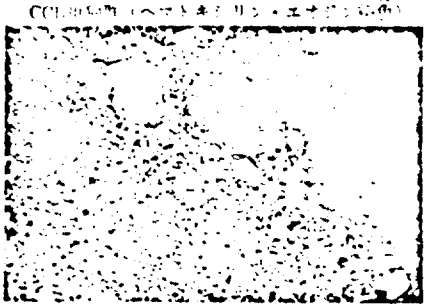
(1) 実験方法

肝疾患々者は主として当院の入院患者30名で臨床的肝機能検査とともに肝生検を行い診断を確定した．PaA治療にはCa-PaAを1日20mgずつ7～30日間静内に注射した．なおこの期間中はブドウ糖の他は肝疾患に対する特別治療は原則として一切中止し，ただ表示の如く一部症例でB群，C，Met[*6]などを併用した．また対照としては健常人数名に1日40mgずつ10日間投与した．しかして治療前および治療終了後数日を経て各種肝機能ならびに中間代謝検索を行い健常人の成績と比較し推計学的に検討した．

[*6] Met = Methionine

図1　慢性CCl_4肝血害ウサギの肝組織像とこれにおよぼすCa-PaA治療の影響（70日）

CCl_4単独群（ヘマトキシリン・エオジン染色）



2. PaA治療群（ヘマトキシリン・エオジン染色）

3. CCl_4単独群（マロニー染色）

4. PaA治療群（マロリー染色）

(2) 実験成績

(i) 自他覚症状におよぼす影響

肝疾患々者にみられた食思不振，全身倦怠，易疲労などの自覚症も好転したが，とくに四肢の知覚鈍麻，しびれ感などの知覚異常には著効を呈した．また慢性肝炎患者にしばしば存した頭痛，神経過敏，憂鬱，根気がないなどの不定の神経症状の軽快にも役だつた．他覚的には肝の圧痛や叩打痛の軽減，腱反射の正常化などをきたす例が多かつた．これら自他覚症状の改善は主として肝炎，軽中等症肝硬変で認められ利用障害型と考えられる腹水貯溜の著しい重症肝硬変症では改善軽度であつた．なおCa-PaA 20～40mg 30日間投与では血清および尿Na, Cl, K, Caなどの鉱質代謝にも異常なく，また健常人肝疾患々者ともにこの際なんら不快な副作用は認めなかつた．

(ii) 尿PaA排泄量および血清PaA量におよぼす影響

血清PaA量は健常人平均2 60±95mγ/ccに対し急性肝炎173±54，慢性肝炎221±87，肝硬変症159±33mγ/ccであつたが，PaA治療によりそれぞれ617±45，540±136，256±24，233±8mγ/ccといずれも上昇正常化ないし正常以上に達した．尿PaA排泄量も同傾向であつたが重症肝硬変の一部では治療後排泄量の著増する者あり，かゝる例では肝機能および代謝異常の改善は軽度ないしほとんどみられなかつた．

(iii) 諸肝機能におよぼす影響

a) 血清膠質反応：諸種血清膠質反応は治療前いずれも異常を示した．PaA治療後は急性肝炎ではチモール濁独反応，慢性肝炎ではKunkel硫酸亜鉛反応が治療前値と有意の差を示したがその他のものは明らかでなく，また健常人および肝硬変では有意の変動を認めなかつた．

(b) 負値指数：PaA治療後肝炎，肝硬度ともある程度の改善がみられた．

c) 馬尿酸合成試験：肝疾患々者では大多数例に早期より合成能の低下および遅延があつたがPaA治療によりほとんど全例に合成能の有意の上昇正常化を認めた．この傾向は急性および慢性肝炎，軽中等症肝硬変とくに慢性肝炎患者で著しかつたが重症肝硬変ではそれほど著明でなかつた．この際治療前値の低下せる例ほどその改善著しく同時に合成能の迅速化も認められた．

表 4　急性肝炎患者の肝機能に

姓名	年令	性	病名	Ca-PaA 20mg/日 投与日数	PaA排泄量 (12時間 μg) 前	後	血清	代謝 総蛋白 (g/dl) 前	後	Gros反応 (cc) 前	後	Kunkel反応 (U) 前	後	チモール 濁反応 (U) 前	後
岡	○	49	含	急性肝炎	20日	1064	3000	7.0	7.3	1.1	1.3	12	8	8.8	4
宮	○	32	含	〃	14	220	2300	7.4	7.8	1.36	0.93	16	16	7	5
加	○	16	含	〃	20		1005	7.0	7.2	1.18	1.47	10	8	3	3
南		22	含	〃	20	1800	4500	7.1	7.4	1.45	1.59	4	4	1	1
徳	○	28	含	〃	20	1975	2640	7.2	7.4	1.48	1.51	6	14	2	2
細	○	23	含	急性肝炎 兼肺浸潤	23	1488	2400	7.3	7.4	1.06	1.45	12	8	4	2
菅	○	25	含	亜急性肝炎	23	1430	2620	7.1	7.2	1.25	1.42	4	8	1	1
治療による動き				平均 (差)		1330→2638 (+1308)		7.2 → 7.4 (+0.2)		1.27→1.36 (+0.09)		9→ 9.4 (+0.4)		3.8→2.6 (−1.2)	
				F_0 危険率 (%)		24 0.1<P<1		32 0.1<P<1		1.2 P>20		0.03 P>20		3.3 5<P<20	

(iv) 諸中間代謝におよぼす影響

a) 血清コレステロール代謝：肝疾患々者ではT-ChとくにE-ChおよびE-Qの低下を示す者が多かつた．PaA治療によりT-Ch, E-Chは治療前低下せる例ではいずれも増加したがとくにE-Chの増加が著しかつた．E-Qの改善も肝炎とくに慢性肝炎に最も著明であつた．

b) 血液ピルビン酸代謝：血液PyA濃度は肝硬変で相当高値を，また肝炎でも上昇例が少なくなかつたがPaA治療でいずれも低下し正常化傾向がみられた．またその程度は慢性および急性肝炎で著しく肝硬変では軽度であつた．

総括ならびに考察

B群ビタミンの多くは肝において活性化され共通代謝系路の種々な補酵素としての役割を果している．PaAの活性化すなわちCoA形成も主として肝で行われCoAは糖質，脂肪，蛋白の各代謝過程に重要な地位を占めていることはLipmann, Ochoa等の明らかにしたところである．肝はかゝる生体内諸代謝に中心的役割を演ずるとともに諸種ビタミンの主な貯蔵所でもあるからその障害に際し各種の中間代謝異常とともにビタミン代謝障害の起る可能性も充分考えられる．事実B_1, B_2, ニコチン酸などについては摂取不足による外因性欠乏症のほか需要増大ないし利用障害による内因性代謝異常がありこれが疾病の進行や種々の症候の発現に重要な役割を演ずることが少なくなく，またこれらビタミンの投与が肝疾患の治療に際ししばしば好影響を与えることはすでにいくたの報告がある[4)5)]．

しかるに肝障害時におけるPaA代謝様相，ことにその臨床的意義ならびに治療的応用などに関しての報告はきわめて少ない．私はさきに肝障害時にはPaAの欠乏ないし利用障害などの代謝異常がありこれがα-ケト酸々化障害，解毒能低下，脂質代謝異常の面より一層肝障害を増強せしめかくして悪循環を形成する可能性のあることを指摘した[2)3)]．本編においては肝障害時にみられるかゝる代謝障害を是正し一般状態の改善を促がす目的でCa-PaAを肝疾患々者ならびに肝障害ウサギに投与しその影響につき検討した．

まず自他覚症状について数家高岡[6)]はすでに慢性肝炎患者にみられる各種の種々症状は酸化機転異常と密接な関連を有しB_1，とくにCoC[*7)]投与でしばしば回復することを報告した．私はこれらの症状がB_1などで奏効せずPaA投与で軽快する例のあることを認めた．こ

*7) CoC = Cocarboxylase

及ぼす Ca-PaA 治療の影響

黄疸指数		尿酸合成能 1時間値 (%)		尿酸合成能 4時間合計値 (%)		血清コレステロール 総 (mg/dl)		血清コレステロール エステル比 (%)		血液 PyA (mg/dl)		尿 Ubg* 反応		尿 Brn** 反応		備考
前	後	前	後	前	後	前	後	前	後	前	後	前	後	前	後	
54	10	0	25	15	45							+	−	+	−	
81	40	0	20	35	40	168	227	68	68	1.26	1.1	++	+	+	±	Met(1mg)と併用
12	5	65	101	78	121	124	160	75	76	1.0	1.0	++	±	−	−	Met(1mg)と併用
117	24	50	52	77	89	122	175	54	70	1.5	1.2	+	−	+	−	
25	72	23	64	77	89	102	137	57	60	1.5	1.3	−	−	+	+	
36	6	30	21	67	51	205	196	74	74			+	±	+	−	Met(1mg) PAS(1mg)と併用
30	18	42	40	73	70	148	125	69	74	1.1	1.0	−	−	+	−	
51→25 (−26)		30→46 (+16)		60→72 (+12)		145→170 (+25)		66→70 (+4)		1.27→1.12 (−0.15)		*Ubg = Urobilinogen **Brn = Bilirubin				
2.5		4.7		2.48		3.3		2.78		8.6						
5<P<20		5<P<20		5<P<20		5<P<20		5<P<20		1<P<5						

れは実験的PaA欠乏動物で著しい神経障害がみられること[7]，Nutritional melalgia にCa-PaAが有効であつたという Gopalan[8]の報告，さらにBean等[9]の人間のPaA欠乏実験の際に生じた知覚異常に Ca-PaA 投与が奏効したという報告および神経刺戟伝導にCoAが必要なことなどとあわせ考え本剤は肝疾患々者にみられる神経症状の発現とも密接な関連を有するものと思われる．

PaA は血漿中には遊離型で，赤血球中では結合型主としてCoAとして存在するとされている[10]．健常人の血漿PaA量は Pearson[11] によれば 347±57mγ/cc，Spies 等[12]によれば血液PaA量は190～320.平均225mγ/ccで私の成績もほぼこれと一致した．Spies 等[12]はペラグラ，脚気，リボフラビン欠乏症患者でも血中PaA量が健常人の23～50%低いと報じている．私の肝疾患々者の成績ではいずれも低値を認め，とくに肝硬変症で低下著明で，これはCa-PaA負荷前後における尿PaA排泄量より体内PaA欠乏の存在が予想された前回の報告と同傾向を示すものである．この際PaA治療により血清PaA量はいずれも上昇正常化したが重症肝硬変では上昇軽度で逆に尿中排泄量が著増し利用障害像を示す例を認めた．

肝解毒能試験の1つである馬尿酸合成能にCoAが関与することはすでに述べた如く Chantrenne[13], Schachter 等[14]により明らかにされた．私は前回尿PaA排泄量と馬尿酸合成能との間に明らかな相関を認めたがPaA治療では多くの場合解毒能が他の肝機能，代謝成績の回復にさきだち著しく改善された．たゞ腹水貯溜の著しい重症肝硬変においては改善軽度でこのように低下した合成能の好転がほとんどみられない場合の経過および予後は多くの場合極めて不良であつた．このことは Met 加蛋白水解物療法に際しとくにその治療が奏効した場合解毒能が最も速やかに回復し一方改善されない者に予後不良が多いという教室河田[15]の報告とも一致する．

脂質代謝をみるに肝疾患々者ではE-Chの減少，E-Q低下などのCh代謝異常のあることおよび CCl_4 肝障害ウサギでも同様の障害をみることは従来より認められている[16～19]．この際Met[20][21]または Met 加蛋白水解物[15]のほかB_2, FAD, B_{12}[22][23] などのB群ビタミンの投与も有効といわれる．

私の成績でも肝疾患々者ではE-Qの低下せる者多くその程度は急性肝炎，肝硬変および肝組織所見で活動型と診断された慢性肝炎患者に著しかつた．これら患者にPaA療法を行つた場合治療前T-Ch, E-Ch, E-Q の低かつた例ではほとんど全例に上昇正常化を来したがPaA 利用障害型を示した重症例では E-Q の改善はほとんどみられなかつた．またその効果はCCl_4肝障害ウ

表 5 慢性肝炎患者の肝機能に

姓名	年令	性	病名	Ca-PaA 20mg/日 投与日数	PaA 排泄量 (12時間)(γ) 前	PaA 排泄量 (12時間)(γ) 後	血液	血液	血液	血液	血液	血液	血液	血液
							総蛋白 (g/dl) 前	総蛋白 (g/dl) 後	Gros 反応 (cc) 前	Gros 反応 (cc) 後	Kunkel 反応 (U) 前	Kunkel 反応 (U) 後	チモール 濁反応 (U) 前	チモール 濁反応 (U) 後
上 ○	36	合	慢性肝炎	7日			6.8	6.8	1.32	1.31	4	4	1	1
龜	32	合	〃	〃	980	1320	7.2	7.4	1.76	1.8	2	2	1	1
大 ○	43	合	〃	〃					1.2	1.21	6	6	2	2
江 ○	48	合	〃	〃	1150	3650	7.9	8.0	1.08	0.9	16	14	4	1
深 ○	49	合	〃	〃					1.48	1.82	4	4	1	1
安 ○	54	合	〃	〃	900	3230	7.6	7.8	0.92	1.3	22	14	4	3
亀 ○	34	早	〃	〃			6.8	6.6	1.34	1.4	10	9	3	3
辻 ○	67	早	〃	〃	1258	1308	8.0	8.1	1.18	1.24	12	10	4	5
岩 ○*	41	合	〃 (活動型)	〃	1512	3400	6.6	6.6	0.92	0.77	14	14	4	3
古 ○	46	合	〃 (活動型)	〃	1032	2220	8.1	8.0	1.2	1.08	12	12	3	2
内	25	合	〃	20	598	2512			1.62	1.48	8	8	1	2
仲	30	合	〃	27	1100	3340			1.15	1.07	18	16	5	6
吉 ○	29	早	〃	30	1040	4888	6.3	5.5	1.14	1.3	12	10	2	1
上 ○	59	合	〃	〃			8.0	7.9	1.17	0.9	16	16	5	7
真 ○	31	合	〃	24			7.3	7.5	1.53	1.53	6	6	2	2
南 ○	61	早	〃 (活動型)	〃	1295	7268	6.3	6.3	0.85	0.87	16	16	4	4
桑 ○	27	合	〃 (活動型)	〃	555	10400	7.8	7.0	0.72	1.08	24	22	11	10
治療による動き			平均 (差)		1038→3958 (+2920)		7.3→7.2 (−0.1)		1.21→1.24 (+0.03)		11.8→10.7 (−1.1)		3.4→3.2 (−0.2)	
			F_0		12		0.9		0.18		5.14		0.02	
			危険率 (%)		0.1<P<1		P>20		P>20		1<P<5		P>20	

* 利用障害型

サギでも同様認められた。

肝障害時には往々PyA代謝異常がありB₁とくにその活性型であるCoC投与で改善をみることはAmatuzio[24], Davis[25], Smith[26], Kipping[27]および教室高岡等[6][28]の報告が示すところである。私の成績でも肝疾患々者ならびに肝障害ウサギでいずれもPyA代謝異常がみられた。また新谷[29], 宮野等[30]は肝障害動物のPyA代謝障害に混合アミノ酸, MetあるいはB₁, Cまたはインテレニンの作用が効果のあることを証明している。しかしながらPyA酸化酵素系にはCoCとともにCoA, Diphosphopyridinenucleotide, リポ酸などの関与することが明らかにされており, 血液PyA上昇著明な患者にCa-PaAを用い多数例に低下正常化を来たしたこと, および肝障害ウサギでも同様有効であつた事実は血液PyA値と尿PaA排泄量との間に有意の相関々係を認めた前回の成績とともに肝障害時のPyA酸化異常にはPaA代謝異常も主要な一役を演ずることを立証し得たものと考えられる。さらに教室海野, 鯖田等[31]は肝疾患々者にみられたアセチル化能の減退および果糖負荷時のα-ケト酸々化障害がPaA投与でしばしば軽快することを認めている。従がつて肝炎遷延化の一因をなすとみなされるかゝる酸化機転障害の改善対策としてCoC, リポ酸などとともにPaAも用いる価値あるものと思われる。

およぼす Ca-PaA 治療の影響

試験開始		ブロム試験(BST) 1時間値 (%)		ブロム試験(BST) 4時間合計値 (%)		血清コレステロール 量 (mg/dl)		血清コレステロール エステル比 (%)		血液 PyA (mg/dl)		尿 Ubg 反応		尿 Brn 反応		備考
前	後	前	後	前	後	前	後	前	後	前	後	前	後	前	後	
7	4	19	22	61	62	159	165	75	76	0.85	0.8	+	+	−	−	
6	5	0	53	30	53	135	167	72	71			±	−	−	−	
5	5	61	71	61	93	130	140	70	78			+	±	−	−	
4	5	0	22	46	40	158	187	78	73			+	+	−	−	
5	5	0	0	20	55	167	167	70	73	1.2	1.0	+	±	−	−	
11	6	0	0	15	56	167	160	78	76	1.2	0.75	++	+	−	−	
6	6	60	74	60	74	128	102	70	74			+	±	−	−	
13	6	44	51	55	61	167	167	76	77			+	−	−	−	
15	15	0	0	37	47	105	120	53	62	0.85	0.89	++	++	−	−	
11	12	0	0	24	38	148	142	49	76	0.8	0.65	+	+	−	−	
5	5					201	201	65	75	1.05	1.0	±	−	−	−	
9	9	0	16	36	95	94	195	77	80			+	+	−	−	
6	8	34	77	58	77	130	104	70	68	1.7	1.4	+	−	−	−	B$_1$(20mg) 併用
6	5	41	53	66	80	143	167	67	64			+	+	−	−	
6	5	38	55	64	70	149	176	57	75			±	−	−	−	B$_1$(10mg) +C (100 mg) 併用
7	7	0	31	58	62	131	126	69	67	1.5	1.36	+	+	−	−	
6	7	11	29	11	87	155	203	48	75	2.25	1.85	++	+	−	−	Met(1mg) と併用
7.6→6.8 (−0.8)		**19→35** (+16)		**44→66** (+22)		**146→153** (+12)		**67→73** (+6)		**1.27→1.08** (−0.19)						
1.8		15.1		15.3		4.16		5.4		5.9						
5<P<20		0.1<P<1		0.1<P<1		5<P<20		1<P<5		1<P<5						

前述の如くPaA治療によりCh, PyA代謝，馬尿酸合成能などの著しく改善された患者ではつづいて二硫質反応，BST[*6]も正常化するものが少なくなかつた．すなわちCoAの直接関与する機能はPaA投与により比較的短時日に改善されるがその他のものの改善はやゝ遅れる傾向を認めた．

一方肝障害ウサギの肝組織所見に対しても好影響をおよぼすことは前述の如くでとくに慢性肝障害群に顕著であつた．Benda等[31]もPaAはネズミのAllylformateによる肝障害（壊死など）を予防できると報じている．

本剤の副作用についてWelsh[32]は数百例に1日0.3～3g長期間経口投与し少数例に軽い一時的悪心を認めた他は何ら副作用はなかつたと述べ，Gershberg[34]は人間に10～20gの大量を長期間与え時に下痢およびリウマチ患者で軽度浮腫を認めたがその他のものはほとんど変化をみなかつたと述べている．またSpies[12]は健常人にCa-PaA100mgを静注したが体温，血圧，脈搏，呼吸などに変化なく小堀等[29]はCa-PaA 300mg皮注では肝腎機能にも変化を認めなかつたと報告，私の肝疾患々者における1日20mg, 30日間筋肉内投与でも何ら副作用は認めなかつた．

[*6] BST = Bromsulphalein test

表 6　肝硬変患者の肝機能に

姓名	年令	性	病名	Ca-PaA 20mg/日 投与日数	PaA排泄量 (12時間) 前	PaA排泄量 (12時間) 後	蛋白代謝 総蛋白 (g/dl) 前	総蛋白 (g/dl) 後	Gros反応 (cc) 前	Gros反応 (cc) 後	Kunkel反応 (U) 前	Kunkel反応 (U) 後	チモール混濁反応 (U) 前	チモール混濁反応 (U) 後
森 ○	50	合	肝硬変前期	26日	1700	2500	7.8	7.8	1.08	1.13	16	18	6	4
木 ○	37	合	〃	14	1339		7.0	6.7	0.95	1.0	30	26	7	7
長 ○	49	早	脾腫性肝硬変	10	2960	1703	6.5	6.0	0.64	0.72	14	24	5	10
中 ○	53	早	〃	14	430	2000	8.2	8.7	1.05	1.24	20	16	7	3
○ 山*	49	合	重症肝硬変	26	1416		6.5	6.7	0.6	0.6	22	24	10	10
吉 ○*	56	合	〃	26	937	16500	7.0	6.8	0.53	0.5	28	30	10	10
治療による動き			平均 (差)		1464→5176 (+3712)		7.2→7.1 (−0.1)		0.81→0.87 (+0.06)		22→23 (+1)		7.5→7.3 (−0.2)	
			F_0		0.8		0.1		1.2		0.37		0.02	
			危険率 (%)		P<20		P<20		P<20		P<20		P<20	

* 利用障害型

以上PaA治療の影響につき検討してきたが本剤の治療効果判定に当つて慢性肝疾患ではその治療期間中における自然治癒による影響をほとんど無視できるが急性肝炎の場合これの関与にも留意すべきである．しかしたとえば馬尿酸合成能などの正常化は在来の治療時に比し著しく速やかで一部かえつて機能充進を示した者もみられた．たゞPaAの利用障害型を示す重症肝疾患々者では長期間使用により多少代謝改善を認めた者もあるが一般にその効果は軽度であつた．これは井上も指摘せる如く肝疾患では利用障害などのビタミンの内因性代謝障害と肝機能障害との間に因果の悪環路を作りとくに重症時にはすでに非可逆性の状態に陥いつている可能性が考えられる．従がつて私の重症者の一部で効果の少なかつたことはこれら患者では明らかなPaA利用障害像を認めた事実に照しても首肯せられる．なおPaAよりCoAへの合成能は肝疾患時でも相当末期まで障害されないと思われることは前回報告したが重症肝硬変などで合成障害の予想される場合CoAなどの結合型の投与が望ましいことは言をまたない．しかしCoAの入手困難な現在PaA投与も一応試むべきものと思われる．なおこの際少数例ながらMet併用で一層著明な効果を示すものがあつた．Metはシステインを経てβ-メルカプトエチルアミン形成を促進することよりCoA合成のためのPaA利用を増進することがDinning[36]，教室海野等[31]により明らかにされている．さらにCoA合成にはその他B_6なども関与することは山田等[37]の報告でも明らかで，従がつてCoA代謝に関してはかゝるPaA以外の因子の関与ももちろん考慮すべきものと思われる．

PaAは広く食品中に分布し，さらに腸内細菌その他による生合成も行われ普通の状態では明らかなPaA欠乏は認めがたいと考えられる．しかし肝臓の障害された場合既述せる如く食思不振，不適当な食餌制限などによるPaA摂取の減退，消化吸収障害あるいは肝における需要増大などによりしばしばPaA欠乏，時に利用障害の状態に陥いる場合が少なくない．この際同時に他のビタミンも欠乏する場合が多い．Spies[12]らは血中PaAが低い人では同時に著しいB_1，B_2欠乏症を生じ，この際PaAやB_2の単独投与で他のビタミンの血中濃度も高めうることからこれらビタミンの間には密接な関係があると述べている．従がつて肝疾患のビタミン治療に際して充分量の綜合ビタミンないしB複合体の投与が合理的であることはいうまでもないがその一員としてPaAも不可欠である．事実中等症肝硬変患者でCoC, B_1, B_2, Cなどを投与してもなお倦怠感，肝解毒能およびPyA, Ch代謝障害の改善は軽度でこれに

およぼす Ca-PaA 治療の影響

黄疸指数		1時間値 (%)		4時間合計値 (%)		総 (mg/dl)		エステル比 (%)		血液 PyA (mg/dl)		尿 Ubg 反応		尿 Brn 反応		備考
前	後	前	後	前	後	前	後	前	後	前	後	前	後	前	後	
7	8	36	50	70	75	142	112	71	71			+	+	−	−	Bi(10mg)と併用
27	20	10	20	30	40	160	170	60	65	1.4	1.2	++	+	−	−	
11	6	0	30	31	65	167	150	54	57	1.65	1.35	+	+	−	−	
7	3	29	32	54	82	126	167	65	70	1.5	1.4	+	+	−	−	
8	7	12	15	12	25	130	130	48	50	1.8	1.6	++	++	−	−	腹水(+) Bi(10mg)と併用
6	7	0	0	15	15	139	140	46	45	2.0	2.1	++	++	−	−	腹水(++) Bi(20mg)と併用
11→8.5 (−2.5)		**14.5→24.5** (+10)		**35.3→50.3** (+15)		**144→145** (+1)		**57→60** (+3)		**1.67→1.53** (−0.14)						
3.3		4.8		7.6		0.07		5.1		4.3						
5<P<20		5<P<20		1<P<5		P>20		5<P<20		5<P<20						

PaAを併用して効果の増強を認める例が少なくなかつた．もちろんPaAのみにて肝疾患々者の複雑な代謝異常のすべてを速やかに是正し得ると期待するのはむりであるが，PaA治療によりCoAの直接関与する諸代謝をまず正常軌道にのせることはひいては他の肝機能および一般状態の回復をもたらす要因となり得るものと思われる．

結　　論

急性および慢性CCl_4肝障害ウサギならびに各種肝疾患々者30名にCa-PaA を予防的ないし治療的に応用しつぎの如き結果を得た．

(1) CCl_4肝障害ウサギにみられる肝組PaA量，ことに結合型の減少ならびにChおよびPyA代謝異常はPaA投与により軽減ないし正常化するを認めた．また肝組織所見でも治療群の障害度は軽度であつたがこれらの傾向はいずれも慢性肝障害治療群に顕著であつた．

(2) PaA治療は各種肝疾患々者の血清PaA 量および尿中 PaA 排泄量の正常化を来たすとともに低下する馬尿酸合成能の増強ならびにCh, PyA代謝異常の改善などに有効で，この際同時に神経症状など自他覚症状の軽快ないし消失を認めた．

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Toxicity of Pantothenic Acid.

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From the Merck Institute for Therapeutic Research, Rahway, N. J.

Pantothenic acid, first discovered as a growth factor for yeast,[1] has been established as a member of the vitamin B complex.[2] The vitamin was recently identified[3, 4] as *N*-(α,γ-dihydroxy-β, β-dimethylbutyryl)-β'-alanine and has been synthesized.[5]

In continuation of our studies of the toxicology of the vitamins of the B complex,[6, 7] the following investigation of the toxicity of pantothenic acid was carried out on animals maintained on completely adequate diets. Synthetic dextrorotatory calcium pantothenate was used in all experiments. Calcium pantothenate is readily water-soluble and almost neutral in reaction, the pH of a 10% solution being approximately 8.

Local effects of calcium pantothenate were studied by instillation into the eye and by subcutaneous injection. Instillation of 0.5 cc of a 10% solution into the conjunctival sac of 3 rabbits did not produce any irritation. Likewise no irritation, inflammation or abscess formation was observed in 4 rabbits following the subcutaneous injection of 1.0 cc of a 10% solution. The infiltration of the subcutaneous tissues subsided about as rapidly as following a control injection of 10 cc of saline.

Acute toxicity was studied in mice, rats, dogs, and monkeys. In mice and rats the L.D. 50 was determined following oral, subcu-

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TABLE I.
Acute Toxicity of Calcium Pantothenate L.D. 50 (g per kg body wt).

	Oral	Subcutan.	Intraper.	Intrav.
Mice	10.0	2.7	0.92	0.91
Rats	No deaths at 10.0	3.4	0.82	—

taneous, intraperitoneal and intravenous injection, 10 animals being used for each dose level. Table I summarizes the results obtained from a total of 180 mice and 110 rats.

All 10 rats dosed with 10 g of calcium pantothenate per kg, when administered by mouth, survived without showing toxic symptoms. Lethal doses in mice and rats produced prostration and respiratory failure. Death occurred within one hour following intravenous or intraperitoneal injection and within 2 hours following oral or subcutaneous administration. No late deaths were observed.

A dose of 1 g of calcium pantothenate per kg was fed to each of 5 dogs and to 1 monkey. No toxic symptoms were observed in these animals. After 2 weeks the monkey and one dog were sacrificed; gross examination of the organs failed to show any pathologic changes.

Chronic toxicity was studied in rats, dogs, and monkeys. Two groups of 20 young rats, 10 males and 10 females, were fed 50 mg and 200 mg respectively of calcium pantothenate per rat per day over a period of 120 days. The animals developed normally, their growth rate did not differ significantly from that of the control group.

A group of 6 adult dogs was fed 50 mg of calcium pantothenate daily over a period of 100 days, and 4 monkeys of 2.5 to 4 kg body weight received 1 g of calcium pantothenate by mouth daily over the same period of time. Both dogs and monkeys failed to show any toxic symptoms, and their weights remained constant.

Our findings, which will be extended later by histological examinations, demonstrate that calcium pantothenate is, like other members of the vitamin B complex, a substance of extremely low toxicity. Excessively large doses (0.8 g to 10 g per kg, depending on the mode of administration) were found to produce death due to respiratory failure in mice and rats. Prolonged feeding of sublethal doses failed to produce toxic manifestations, indicating that an excess of pantothenic acid is either rapidly destroyed or excreted. Studies on this problem are now in progress.

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STUDIES ON THE TOXICITY AND PHARMACOLOGY OF PANTOTHENIC ACID

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The group of chemically identified B-vitamins which comprises thiamine, riboflavin, nicotinic acid and pyridoxine, has recently been augmented by a new member, pantothenic acid. This substance has been identified as a derivative of β-alanine (1), namely, *N*- (α-γ-dihydroxy-β,β-dimethyl-butyryl) -β'-alanine (2); it has since been synthesized (3) and made available in the form of its dextrorotatory calcium salt.

Pantothenic acid was first recognized as a growth factor for yeast by R. J. Williams (4), who coined the name pantothenic acid (meaning ubiquitous) because it was found to be present in a great variety of foodstuffs. The vitamin nature of this substance was discovered simultaneously by Jukes (5) and Woolley *et al.* (6), who found that "chick dermatitis," an experimental deficiency disease, can be cured by the addition of pantothenic acid to the diet. The rôle of pantothenic acid in nutrition of rats has been studied by several investigators (7-12). In young rats, lack of pantothenic acid causes failure to grow, impairment of the fur, inflammation of the nose and hemorrhages, particularly into the adrenal cortex. Black rats maintained on diets free from pantothenic acid develop a conspicuous greying of the hair; the depigmentation is prevented or cured by the administration of pantothenic acid (13, 14). Rats which receive pantothenic acid in addition to the other crystalline factors of the B-complex reach maturity and are reproductive (15, 16).

In this study crystalline dextrorotatory calcium pantothenate has been used. This substance is readily water soluble and almost neutral in reaction, the pH of a 10 per cent solution being approximately 8. All experiments reported here were performed on animals maintained on the usual laboratory diets.

ACUTE TOXICITY

Acute toxicity was studied in mice, rats, dogs and monkeys.[1] A dose of 1 gram of calcium pantothenate per kgm. body weight was fed to each of 5

[1] A preliminary report on the toxicity of pantothenic acid has appeared in the Proc. Soc. Exp. Biol. & Med., 45: 311, 1940.

dogs and to one monkey. No toxic symptoms were observed in these animals. After 2 weeks the monkey and one dog were sacrificed; examination of the organs failed to show any pathologic changes.

In mice and rats the L.D.50 was determined following oral, subcutaneous, intraperitoneal and intravenous administration, 10 animals being used for each dose level. Table 1 summarizes the results obtained from a total of 190 rats and 230 mice.

Rats dosed with 10 grams per kilogram of calcium pantothenate by mouth survived without showing toxic symptoms. Lethal doses produced prostration and respiratory failure in rats and mice. Death occurred within 2 hours

TABLE 1

Acute toxicity of calcium pantothenate

L.D. 50. (Gram per kilogram body weight)

	ORAL	SUBCUTANEOUS	INTRAPERITONEAL	INTRAVENOUS
Mice (20–22 grams)	10.0	2.7	0.92	0.91
Rats (150–200 grams)	>10.0	3.4	0.82	0.83

TABLE 2

Comparison of the acute toxicity of nicotinic acid, pyridoxine and pantothenic acid in rats following oral and subcutaneous administration

L.D. 50 (Gram per kilogram body weight)

	ORAL	SUBCUTANEOUS
Sodium nicotinate	7.0	5.0
Pyridoxine hydrochloride	5.5	3.7
Calcium pantothenate	>10.0	3.4

following intravenous and intraperitoneal injection, and within 6 to 12 hours following oral and subcutaneous administration. No late deaths were observed.

The low toxicity of pantothenic acid agrees well with the low toxicity of other members of the vitamin B-complex. As shown in table 2, its acute toxicity is of the same order of magnitude as that found for nicotinic acid (17) and pyridoxine (18).

CHRONIC TOXICITY

Chronic toxicity was studied in rats, dogs and monkeys. Daily doses of 50 and 200 mgm. respectively of calcium pantothenate were fed to young

male and female rats over a period of 190 days. The animals showed normal development and their growth did not differ significantly from that of a control group (table 3). Autopsies at the end of the feeding period did not reveal any gross or microscopic changes in the organs. Offspring of the group receiving 50 mgm. of calcium pantothenate daily were given the same daily dose of pantothenic acid as soon as they were weaned. These animals (2nd generation) likewise developed normally and their weights increased at the same rate as those of the control group (table 3).

Six adult dogs were fed 50 mgm. per kilogram of calcium pantothenate daily over a period of 6 months, and 4 monkeys of 4 to 5 kgm. body weight received 1 gram of calcium pantothenate daily over the same period of time. Both dogs and monkeys failed to show any toxic symptoms, their weight

TABLE 3

Growth of young rats receiving calcium pantothenate daily by mouth

	AVERAGE WEIGHT—GRAMS							
	At start		30 days		60 days		120 days	
	Males	Females	Males	Females	Males	Females	Males	Females
Control. Stock diet only (10 males, 10 females)	46	44	104	98	208	168	314	228
100 mgm. calcium pantothenate daily (10 males, 10 females)	45	44	96	87	202	154	308	210
50 mgm. calcium pantothenate daily (10 males, 10 females)	44	44	98	95	203	158	312	210
50 mgm. calcium pantothenate daily, 2nd generation (4 males, 4 females)	46	44	120	97	210	152	304	212

other remained constant or increased. All animals were sacrificed at the end of the feeding period. Histopathological examination failed to reveal any changes.

LOCAL EFFECTS

Local effects of calcium pantothenate were studied on rabbits by subcutaneous injection and instillation into the conjunctival sac. No irritation, inflammation or abscess formation were observed in 8 rabbits following the subcutaneous injection of 1.0 cc. of 1, 2, 5 or 10 per cent solutions of the vitamin. The infiltration of the subcutaneous tissues subsided about as rapidly as that following an injection of 1.0 cc. of saline. Instillation of 0.5 cc. of a 10 per cent solution into the conjunctival sac of 3 rabbits did not produce any irritation.

Effect on metabolism

The action of pantothenic acid on the metabolism of rats was studied by the method of Richards and Collison (19). In 16 experiments in which calcium pantothenate was given orally in doses of 1 and 50 mgm. respectively, no change in oxygen consumption was found on observing the animals for three hours after the administration of the vitamin, although it is rapidly absorbed. In studies on the absorption and excretion of pantothenic acid (20) the peak of its concentration in the blood was observed within 2 hours following oral administration.

The effect of pantothenic acid on water metabolism was studied in 30 rats of 180 gram average body weight. The diuresis of groups of 5 rats was fol-

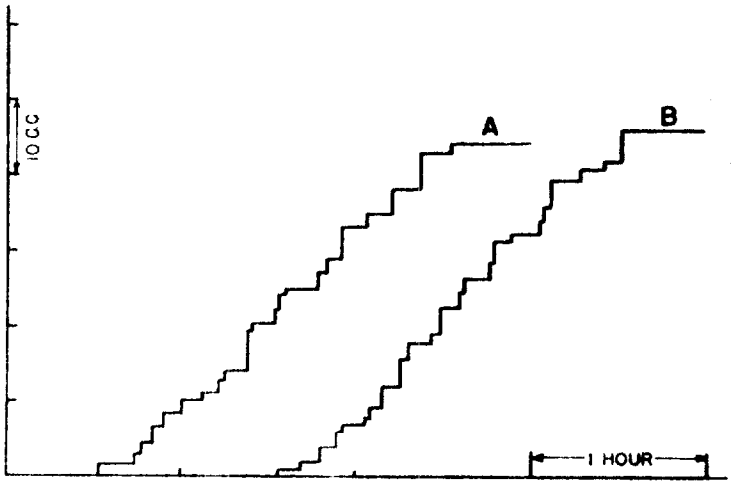

FIG. 1. CONTINUOUS RECORDING OF THE DIURESIS OF 5 RATS FOLLOWING A DOSE OF 5% OF TAP WATER PER 100 GRAMS BODY WEIGHT
A. Water alone. B. Water and 50 mgm. calcium pantothenate per rat

lowed using the diuresis recorder of Kniazuk (21). The normal excretion following 5 cc. of tap water per 100 grams body weight was first established for each group. A dose of 50 mgm. of calcium pantothenate given simultaneously with the water by mouth or by intraperitoneal injection did not influence either the rate or the volume of urine excreted (fig. 1).

Effect on circulation

The blood pressure and the respiration of cats under urethane and chloralose anesthesia were not influenced by intravenous injections of calcium pantothenate in doses ranging from 10 to 50 mgm. per kilogram. The heart rate as measured by a new recording device (Kniazuk and Unna, 23), remained unchanged (fig. 2).

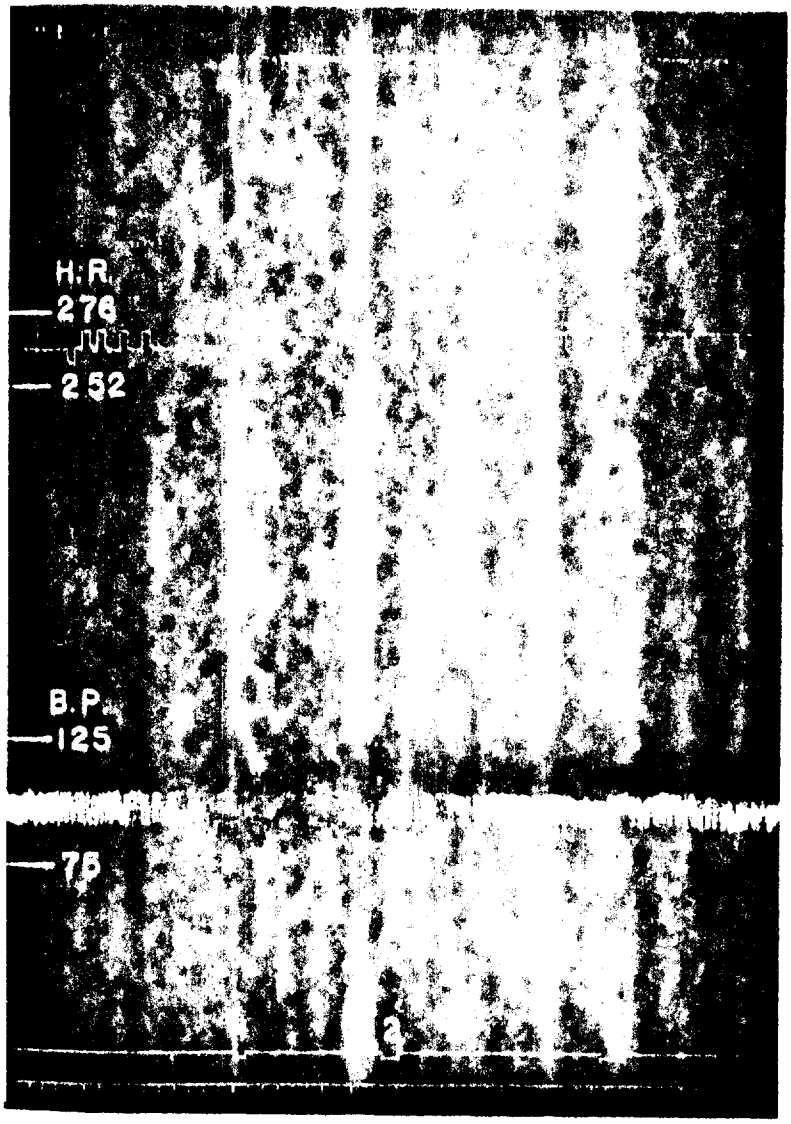

FIG. 2 EFFECT OF PANTOTHENIC ACID ON RESPIRATION, HEART RATE AND BLOOD PRESSURE

Cat 3.5 kgm. body weight. 0.8 gram per kilogram urethane and 0.06 gram per kilogram chloralose. Resp. = Respiration, H.R. = Heart rate, beats per minute; B.P. = carotid blood pressure mm. Hg; time in 30 seconds.

1, 10 mgm. of calcium pantothenate per kilogram; 2, 20 mgm. of calcium pantothenate per kilogram; 3, 30 mgm. of calcium pantothenate per kilogram.

Effect on Smooth Muscles

On the isolated intestine and isolated uterus of the rabbit, calcium pantothenate in concentrations up to 1:10,000 had no visible effect. Spasms of the intestine induced either by barium chloride or acetyl choline were not influenced by pantothenic acid.

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SUMMARY

1. The acute toxicity of calcium pantothenate has been determined in mice and rats following intravenous, intraperitoneal, subcutaneous, and oral administration. The L.D.50 following subcutaneous injection is 2.7 grams per kilogram in mice and 3.4 grams per kilogram in rats.

2. Daily administration of calcium pantothenate over a period of 6 months to monkeys (1 gram per monkey), dogs (50 mgm. per kilogram) and rats (50 and 200 mgm. resp. per rat) failed to produce any toxic manifestations or pathological changes in the organs.

3. The metabolism, the circulatory and respiratory systems, and the smooth muscle organs of normal animals are not influenced by calcium pantothenate.

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Effect of Long-Term Panthenol Administration on the Pantothenate and Coenzyme A Contents of Rat Tissues.* (17659)

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Numerous studies(1-8) have shown that panthenol, the alcohol analog of pantothenic acid, exhibits quantitatively the known biological effects of pantothenic acid, and, moreover, has the advantage of greater stability *in vitro*(9) as well as better availability in mammals at therapeutic dosages(2-5). On the other hand, panthenol was reported by Snell and Shive(10) to have an *in vitro* antipantothenate effect on certain microorganisms, *e.g.*, antibacterial indices of 300, 1000 and 5000 against *Leuconostoc mesenteroides*, *Streptococcus lactis* 125, and *Lactobacillus arabinosus*, respectively. Panthenol had no effect, however, against microorganisms such as *Escherichia coli* and *Staphylococcus aureus*, which are capable of synthesizing pantothenic acid. In all cases where panthenol competitively inhibited the microbiological growth effects of pantothenic acid, the inhibition was overcome by the presence of sufficient pantothenate. It is clear therefore that this antipantothenate effect stems from the circumstance that these microorganisms are incapable of oxidizing panthenol to the acid, a function which mammals perform efficiently.

The present experiments were designed to test whether any anti-pantothenate effect might be detected when high doses of panthenol are administered to rats, mice, dogs and rabbits receiving a stock diet adequate with regard to pantothenic acid content. As a measure of possible anti-pantothenate action, consideration has been given to (1) acute toxicity, (2) chronic toxicity, as evidenced by the production of histological or hematological changes and alteration of the normal rate of growth, and (3) influence of long term feeding upon tissue stores of pantothenic acid. In regard to the last Novelli *et al.*(11) have established that practically all the pantothenic acid found in mammalian tissues may be accounted for as the coenzyme of acetylation, coenzyme A. For this reason, both coenzyme A and free pantothenate as well as total tissue stores were determined in testing for panthenol-induced changes in the storage of pantothenic acid by the rat.

Experimental. (1) *Acute Toxicity.* In acute toxicity studies of panthenol, the intravenous LD_{50} for mice was 7 g per kilo and for rabbits 4 g per kilo. All the rabbits survived 3 g per kilo intravenously and the mice 6.25 g per kilo.

(2) *Chronic Toxicity.* The rate of growth of 24 rats receiving 2 mg of panthenol daily for 6 months was compared to that of 12 control rats receiving the same stock diet, which supplied an adequate amount of pantothenic acid.† As the curves (Fig. 1) relating weight gains to duration of supplementation were practically superimposable over the entire period, it is evident that long term administration of panthenol to growing rats had no

* Presented in part at the 116th Meeting of the American Chemical Society, Abstracts p. 65C, September 23, 1949.

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2. Burlet, E., *Z. Vitaminforsch.*, 1944, v14, 318.
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† This dog chow contains 10 μg of pantothenic acid per gram, i.e., sufficient to provide a daily intake of 100 to 150 μg per rat per day.

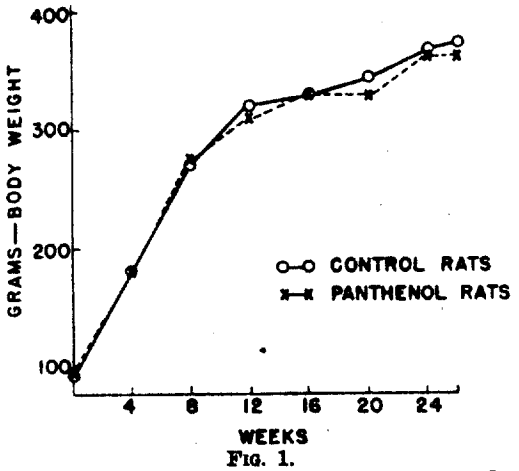

FIG. 1.

Effect of feeding 2 mg of panthenol per day upon the rate of growth of normal rats.

deleterious effect. In a similar study lasting 3 months, 20 mg of panthenol fed daily to rats and 500 mg to dogs produced no histopathological changes. White and red blood cell counts, hemoglobin determinations, nonprotein nitrogen and blood sugar levels showed no deviation from normal.

(3) *Influence of Long Term Feeding upon Tissue Stores of Pantothenic Acid.* Daily doses of 2 mg of panthenol or an equivalent amount (2.3 mg) of calcium pantothenate were fed to rats maintained on the above stock ration for test periods of 24 and 45 days and for 5 to 6 months. Undosed control rats were given the same diet. In the case of the 24 and 45 day test animals, the panthenol or calcium pantothenate was administered by stomach tube and all groups were parallel. For the 5 to 6 months tests, the rats received the same supplements as a part of their ration. Although in these longer experiments, the 5-month test with the calcium pantothenate group was not run simultaneously with the 6-month test of panthenol and control groups, conditions were otherwise controlled so that all three groups are comparable.

(a) *Total pantothenate.* The total pantothenate content of liver, heart, kidney, and spleen tissues from these rats was estimated (Table I) after complete liberation of the pantothenic acid from its naturally occurring bound form as coenzyme A by means of the liver enzyme, alkaline phosphatase procedure described by Novelli *et al.*(11). The liberated vitamin was assayed microbiologically with *L. arabinosus (17-5)* by the method of Skeggs and Wright(12). In the early experiments, the assays were read turbidimetrically with color blanks, while in the later tests, titrimetric measurements were employed. Assays of the same samples by both procedures showed close agreement.

Pooled livers from each of the 3 groups in the 24- and 45-day tests were analyzed simultaneously as soon as the rats were sacrificed. It is evident (Table I) that in these tests neither panthenol nor pantothenate administration appreciably affects the total pantothenic acid content of rat livers. Similarly, the data obtained after 5 to 6 months show no significant increase of liver stores in the dosed rats. Parallel analyses of pools of kidneys from the control, panthenol and pantothenate groups after 24 and 45 days and hearts from the 5 to 6 months series indicate only slightly increased stores of pantothenate after both substances. In the 5 to 6 months series, the 42% increase in kidney content after panthenol compared to only 2% after calcium pantothenate cannot be explained at this time. In contrast, splenic storage was unaffected under these conditions.

(b) *Test for unconverted panthenol.* It was also of interest to determine whether panthenol *per se* is stored in the tissues of these rats. To test this possibility, the microbiological assay procedure of De Ritter and Rubin(13), using *Acetobacter suboxydans* (A.T.C.C. 621 H), was applied to an enzyme-treated suspension of 3 livers from the panthenol-dosed rats of the 6 months test. Following complete alkaline hydrolysis of an aliquot of this suspension, the pantoic acid found was equivalent to 90 γ of pantothenic acid per gram of liver. Direct determination of pantothenic acid before hydrolysis on the same day and with the same organism indicated a content of 79 γ/g. The pantothenic acid content by *L. arabinosus* assay was 93 γ/g. From the agreement of those 3 values it is evident that, under these

12. Skeggs, H. R., and Wright, L. D., *J. Biol. Chem.*, 1944, v156, 21.

13. De Ritter, E., and Rubin, S. H., *Anal. Chem.*, 1949, v21, 823.

TABLE I.
Rat Tissue Storage of Pantothenic Acid.

Organ	Test period	Total pantothenic acid content (μg/g fresh tissue)								
		Control		Panthenol (2 mg/day)			Ca Pantothenate (2.3 mg/day)			
		No. of organs*	Avg content	No. of organs	Avg content	% incr.	No. of organs	Avg content	% incr.
Liver	24 da.	2 (1)	67	2 (1)	68	2	2 (1)	67	0
	45 "	3 (1)	74	3 (1)	83	12	2 (1)	79	7
	5-6 mo.	7 (5)	100	9 (6)	94	—6	5 (3)	75	—25
Kidney	24 da.	10 (2)	51	10 (2)	55	9	10 (2)	57	13
	45 "	10 (2)	48	10 (2)	61	27	10 (2)	54	12
	5-6 mo.	12 (4)	60	16 (4)	85	42	8 (2)	61	2
Heart	5-6 "	10 (2)	47	14 (2)	56	20	12 (2)	62	32
Spleen	5-6 "	12 (2)	27	14 (2)	27	0	14 (2)	27	0

* The numbers in parentheses indicate the number of analyses performed on separate pools of organs.

conditions, no significant amounts of panthenol or pantoic acid are stored in the liver.

(c) *Relative coenzyme A content of livers.* To test whether panthenol or pantothenate administration affects the normal coenzyme A content of liver tissue, organs from each of the various groups in the 24 and 45 tests were pooled and extracts prepared according to the procedure described by Kaplan and Lipman(14). Such extracts were reported to contain intact the coenzyme A, the integrity of which these authors proved by its activity in the enzymatic acetylation of sulfanilamide. The activity of our extracts was determined with *Acetobacter suboxydans*, which we find in agreement with Novelli *et al.*(15), utilizes the intact coenzyme. The response of this organism, grown on the medium of De Ritter and Rubin(13), was measured with reference to a pantothenic acid standard to provide an estimate of relative rather than absolute coenzyme A potencies of the livers from the various groups. A summary of the comparative data is given in Table II, all potencies being expressed in terms of "apparent pantothenate." In both the 24 and 45 day tests, panthenol and pantothenate cause a slight and equal increase in the relative activity of about 20% over the controls. In the longer test period of 5 to 6 months, neither substance affects the activity.

To demonstrate that this activity is due primarily to coenzyme A rather than to free pantothenate, which also has activity in this assay, it was necessary to determine the amount of free pantothenate present in each case.

(d) *Free pantothenate in livers.* Novelli *et al.*(11) have found that normally no more than 3% of the total pantothenate of rat heart, liver, and kidney tissue is unbound. To compare the amounts of free and bound pantothenic acid in livers from the present control and dosed groups, the coenzyme A extracts were analyzed with *L. arabinosus* both directly and after enzymatic liberation of the bound

14. Kaplan, N. O., and Lipmann, F., *J. Biol. Chem.*, 1948, v174, 37.

15. Novelli, G. D., Flynn, R. M., and Lipmann, F., *J. Biol. Chem.*, 1949, v177, 493.

TABLE II.
Coenzyme A Activity of Liver

Test period	Average Coenzyme A activity* (μg apparent pantothenic acid/g of fresh tissue)		
	Control	Panthenol (2 mg/day)	Ca pantothenate (2.3 mg/day)
24 days	134	165	162
45 "	161	190	190
5-6 mo.	199	193	183

* Determined with *Acetobacter suboxydans*.

TABLE III.
Free Pantothenate in Coenzyme A Extracts of Rat Liver.

Test period, days	Pantothenic acid content* (μg/g fresh tissue)								
	Control			Panthenol (2 mg/day)			Ca pantothenate (2.3 mg/day)		
	Free	Bound	% Free	Free	Bound	% Free	Free	Bound	% Free
24	1.4	40	3.5	2.8	67	4.2	1.7	41	4.2
45	1.6	50	3.2	2.5	55	4.5	2.8	55	5.1

* Assay with *L. arabinosus* to measure free and total pantothenate, respectively, *before* and *after* enzymatic liberation; difference equals bound pantothenate.

vitamin. As this organism is insensitive to the intact coenzyme but responds quantitatively to the free vitamin, the concentration of free pantothenic acid in the extracts is measured by the direct assays. It is evident (Table III) that practically all of the pantothenic acid present in livers of rats chronically administered either panthenol or calcium pantothenate is also in the bound form.

Discussion. In view of the anti-pantothenate activity exhibited by panthenol towards certain microorganisms(10), the present experiments have tested for such effects in mammals. Previously reported panthenol availability studies(1,2,4) have demonstrated that the rat readily converts panthenol to pantothenic acid as shown by the urinary excretion of pantothenate. As a consequence, for panthenol to exert an *in vivo* anti-pantothenate effect, it must do so before its conversion by displacing and thereby decreasing normal tissue stores of pantothenate. Comparison of the effect of chronic administration of panthenol or pantothenate upon total tissue stores have tested this possibility. In no case do we find any appreciable decrease attributable to panthenol. In fact, the total kidney and heart stores are increased slightly by both panthenol and pantothenate, whereas splenic and liver stores remain unaffected. On this basis, it is concluded that panthenol does not displace normal tissue stores of pantothenic acid.

In determining the mode of storage of pantothenic acid, the microbiological procedures employed yield only a relative estimate of the coenzyme A content of the livers from the various groups. However, these values (Table II) in terms of apparent pantothenic acid activity per gram of tissue are in direct proportion to the metabolically active coenzyme A present in the extracts(15). Preparation of our extracts by procedures reported by Kaplan and Lipmann(14) to protect the integrity of the coenzyme A molecule and to preserve its acetylation activity precludes the presence of degradation products of coenzyme A such as the pantothenic acid conjugase, reported by King *et al.*(16) to support the growth of *Acetobacter suboxydans*. The small percentages of free pantothenate and the lack of significant amounts of pantoic acid indicate that the *Acetobacter suboxydans* assay measured chiefly coenzyme A.

It is clear that panthenol and pantothenate have practically identical effects upon the

16. King, T. E., Locher, L. M., and Cheldelin, V. H., *Arch. Biochem.*, 1948, v17, 483.

levels of both pantothenate and coenzyme A in rat tissues. There was no appreciable accumulation of free pantothenate or panthenol during these tests. The lack of inhibitory effect of panthenol upon the rate of growth, the histopathological and hematological picture, as well as the high intravenous LD_{50} found for mice and rabbits provide further evidence that panthenol has no *in vivo* antipantothenate effect. Furthermore, in clinical tests of panthenol(6,7) no untoward or toxic effects were observed. For these reasons, it must be concluded that panthenol is generally non-toxic to mammals at dosage levels in the therapeutic range.

Summary. Toxicity tests of panthenol show that this biologically active, hydroxy analogue of pantothenic acid has no *in vivo* antivitamin effect in mammals. In acute toxicity tests, mice tolerated up to 6.25 g per kilo and rabbits up to 3 g per kilo. In chronic feeding tests, 2 mg per day of panthenol had no inhibitory effect upon the growth rate of normal rats on an adequate stock diet and 20 or 500 mg daily produced no histopathological or hematological changes in rats or dogs, respectively. When fed as above at the 2 mg per day level for periods up to 6 months, panthenol did not differ significantly from an equivalent supplement of calcium pantothenate in its effect on rat tissue storage of pantothenate. Total pantothenate in the liver, spleen, kidney and heart, and coenzyme A in the liver were equal or slightly greater in the dosed groups than in the controls. Liver storage in all groups was almost entirely as coenzyme A, and no appreciable amounts of panthenol or free pantothenate accumulated.

We wish to thank Mrs. Irma Sonnenfeld and Mr. Jacob Scheiner for assistance with the tissue studies, and Miss Margaret D. Roe and Mr. Carmen Mangieri for carrying out many of the toxicity tests.

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LUPUS ERYTHEMATOSUS

Treatment by Combined Use of Massive Amounts of Calcium Pantothenate or Panthenol® with Synthetic Vitamin E

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CINCINNATI

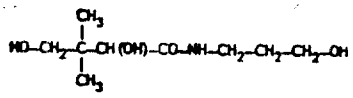
THIS REPORT deals with the results obtained in the treatment of certain phases of lupus erythematosus by the administration of combinations of various forms of vitamin E with either panthenol[4] (the alcohol of pantothenic acid), racemic (*dl*-) calcium pantothenate or dextrorotatory (*d*-) calcium pantothenate. Patients who had chronic discoid, disseminate discoid, or subacute disseminate lupus erythematosus were studied. Patients with acute disseminate lupus erythematosus were excluded.

Racemic calcium pantothenate has been used alone, in doses of 200 to 400 mg. daily for short periods, with benefit in the treatment of chronic discoid, disseminate discoid, and subacute disseminate lupus erythematosus.[1] Panthenol® alone, in doses varying from 0.5 to 6.0 gm. daily for periods varying from two weeks to three months has been used with improvement in all types of lupus erythematosus.[2] Vitamin E alone has been used with improvement in the treatment of lupus erythematosus.[3]

There is no report in the literature of the use of the combination of the drugs employed in this study in the treatment of patients with lupus erythematosus. In addition we could find no record of the use of these drugs in the heavy dosage or for the long intervals here employed.

DRUGS EMPLOYED

Panthenol,[4] the alcohol corresponding to pantothenic acid, which is chemically d-alpha, gamma-dihydroxy-N-(3-hydroxypropyl)beta, beta-dimethylbutyramide, has the following structural formula:



Read before the Seventy-First Annual Meeting of the American Dermatological Association, Hot Springs, Va., May 25, 1951.

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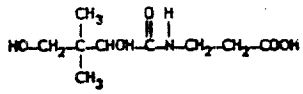
3. Burgess, J. F., and Pritchard, J. E.: Tocopherols (Vitamin E): Treatment of Lupus Erythematosus, Arch. Dermat. & Syph. **57**:953 (June) 1948. O'Leary, P. A.: Communication to Abbott Laboratories, 1948. Burgess, J. F.: Vitamin E (Tocopherols) in the Collagenoses, Lancet **2**:215, 1948.

4. The panthenol® used in this study was supplied by Hoffman-LaRoche, Inc.

It is considerably more stable than pantothenic acid in the pH range 2 to 5.[5] It is the most active analogue of pantothenic acid hitherto described which displays vitamin activity. The medial lethal dose of panthenol[9] for mice upon oral administration was found to be approximately 15 gm. per kilogram. Panthenol,[9] given orally, is converted to pantothenic acid, as judged by the urinary excretion of the latter compound. It was administered in 0.5-gm. capsules. Calcium pantothenate was used as the dextrorotatory salt [6] and as the combination of the dextrorotatory and the levorotatory salts.[7] The formula of pantothenic acid, which is formed from beta, beta-dimethyl-alpha-hydroxybutyrolactone condensed with beta-alanine, is as follows:



The calcium salt is simply this formula with the last hydroxyl radical replaced by calcium. The dextrorotatory calcium salt of pantothenic acid, $(C_9H_{16}NO_5)_2Ca$, is the biologically active form. The levorotatory form is biologically inactive. The biological activity of the *dl*-calcium pantothenate used here was 49% that of the dextrorotatory form, as shown by the microbiological test for pantothenic acid.[8] The medial lethal dose of the synthetic dextrorotatory calcium pantothenate for mice, when given orally, was found to be approximately 10 gm. per kilogram.[9]

Pantothenic acid is essential for the nutrition of many species of animals. It is not known whether it is essential for human nutrition, although this is assumed.[10] The pantothenic acid content of normal human blood varies from 3 to 19.4 γ per 100 cc. In persons suffering from pellagra, beriberi, and riboflavin deficiency, blood levels of pantothenic acid are from 23 to 50% lower than in normal persons.[11] The requirements are increased in experimental hyperthyroidism.[12] There is some evidence that pantothenic acid may be concerned with fat and carbohydrate metabolism, particularly the rate of pyruvate oxidation in the liver.[13]

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Jürgens and Pfaltz [14] demonstrated that in rats pantothenic acid is an essential factor for the normal function of epithelial tissue. Among the effects attributed to pantothenic acid deficiency are nutritional achromotrichia,[15] dermatitis,[16] gastritis, enteritis, and ulcers of the intestinal tract,[17] hemorrhagic necrosis of the adrenal glands,[18] hemorrhagic degeneration of the testes [18] and kidneys,[17a] nerve degeneration,[19] and inhibition of growth.[20] In humans pantothenic acid seems to be combined with riboflavin [21] and inositol metabolism.[22] Folic acid and biotin may be essential for the utilization of pantothenic acid.[23] Pantothenic acid deficiency in experimental animals reduces considerably the phagocytic power of the blood.[24] Pantothenic acid analogues have an antibacterial action.[25]

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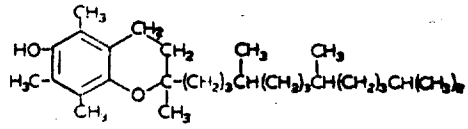
Spies and his associates [10b] administered varying amounts of sodium and calcium pantothenate to 15 subjects, and observed that 100 mg. of either salt may be injected intravenously without causing any significant change in blood pressure, pulse, temperature, or respiration, or any other noticeable reaction.

Studies by Unna and Greslin [9] on the toxicity of synthetic dextrorotatory calcium pantothenate caused the authors to conclude that calcium pantothenate, like the other members of the vitamin B complex, is a substance of extremely low toxicity. Excessive large doses produce death by respiratory failure in mice and rats, but prolonged feeding of sublethal doses fails to produce toxic manifestations. These facts indicate that an excess of pantothenic acid is either rapidly destroyed or excreted. In view of the foregoing investigations and many others, it will be seen that there is no basis for stating a proper dose of pantothenic acid or its salts.

Vitamin E was used as the natural mixed tocopherols and as the synthetic *dl*-alpha-tocopherol acetate (ephynal acetate*).[26] The structural formula of the alpha-tocopherol is



Beta-, gamma- and delta-tocopherols are isomers, possessing one less methyl group than alpha-tocopherol in the aromatic nucleus. Alpha-tocopherol has proved to be most effective, biologically. The dextrorotatory form of the tocopherols and the tocopherol acetate esters are much more active biologically than the racemic forms. Dextrorotatory alpha-tocopherol acetate is the most active form now known. Ephynal acetate* is the acetate ester of racemic alpha-tocopherol.

One of the striking chemical properties of vitamin E is its activity as an antioxidant.[27] It has a protective or "sparing" action in preventing the oxidation of vitamin A and carotene.[28] It also has an antidystrophic action on muscle tissue.[29]

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26. The ephynal acetate* used in this study was supplied by Hoffmann-LaRoche, Inc.

27. Vitamin E as a Physiological Antioxidant, Nutrition Rev. **3**:17, 1945.

28. Krakower, C., and Axtmayer, J. H.: Effect of Alpha-Tocopherol on Lesions of Skeletal Muscles in Rats on Vitamin A Deficient Diets, Proc. Soc. Exper. Biol. & Med. **45**:583, 1940. Moore, T., and Davis, A. W.: Interaction of Vitamins A and E, Nature **147**:794, 1941. Shantz, E. M.; Cawley, J. D., and Embree, N. D.: Anhydro ("Cyclized") Vitamin A, J. Am. Chem. Soc. **65**:901, 1943. Hickman, K. C. D.; Kaley, M. W., and Harris, P. L.: Convitamin Studies: II. The Sparing Action of Natural Tocopherol Concentrates on Vitamin A; III. The Sparing Equivalence of the Tocopherols and Mode of Action, J. Biol. Chem. **152**:303, 313, and 321, 1944.

29. (*a*) Pappenheimer, A. M.: Certain Nutritional Disorders of Laboratory Animals Due to Vitamin E Deficiency, J. Mt. Sinai Hosp. **7**:65, 1940. (*b*) Martin, A. J. P., and Moore, T.: Some Effects of Prolonged Vitamin E Deficiency in the Rat, J. Hyg. **39**:643, 1939. (*c*) Goettsch, M., and Pappenheimer, A. M.: Nutritional Muscular Dystrophy in Guinea Pig and Rabbit, J. Exper. Med. **54**:145, 1931. (*d*) Evans, H. M., and Burr, G. O.: Development of Paralysis in the Suckling Young of Mothers Deprived of Vitamin E, J. Biol. Chem. **76**:273, 1928. (*e*) Moore, T., and Wang, Y. L.: The Fluorescence of the Tissues in Avitaminosis E, Proc. Biochem. Soc., Biochem. J., 1943, vol. 37, p. 1. (*f*) Kaunitz, H., and Pappenheimer, A. M.: Oxygen Consumption in Vitamin E Deficiency, Am. J. Physiol. **138**:328, 1943.

Vitamin E has been reported to have some action on both male and female gonads,[30] on other endocrine glands,[31] on nervous tissues,[32] on muscles,[33] on vessels,[34] and on growth.[35] The recommended dosage of vitamin E varies from 10 mg. to 600 mg. daily for the multiple purposes for which it has been used. There is some slight and indirect evidence that vitamin E can be toxic.[36] Vitamin B increases tolerance for vitamin E.[37] Rancid fats destroy vitamin E.[38]

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30. Martin and Moore.[29b] Evans and Burr.[29d] Kaunitz and Pappenheimer.[29f] Evans, H. M.; Burr, G. O., and Althausen, T. L.: The Antisterility Vitamin Fat Soluble E, Memoirs of the University of California, Vol. 8, Berkeley, University of California Press, 1927. Pappenheimer, A. M., and Schogoleff, C.: The Testis in Vitamin E-Deficient Guinea Pigs, Am. J. Path. **20**:239, 1944. Barrie, M. M. O.: The Effect of Vitamin E Deficiency in the Rat: I. Duration of Gestation; II. Lactation; III. Fertility in the Female, Biochem. J. **32**:1467, 1474, and 2134, 1938; V. Uterine Changes in Chronic Deficiency, ibid. **37**:523, 1943. Bacharach, A. L., and Allchorne, E.: Investigations into the Method of Estimating Vitamin E: Further Observations on Vitamin E Deficiency and Implantation, ibid. **32**:ii, and 1298, 1938. Mason, K. E.: Differences in Testis Injury and Repair After Vitamin A Deficiency and Inanition, Am. J. Anat. **52**:153, 1933.

31. (*a*) Drummond, J. C.; Noble, R. L., and Wright, M. D.: Studies in the Relationship of Vitamin E (Tocopherols) to the Endocrine System, J. Endocrinol. **1**:275, 1939. (*b*) Barrie, M. M. O.: The Relation of Vitamin E to the Anterior Lobe of the Pituitary Gland, Lancet, **2**:251, 1937. (*c*) Nelson, W. O.: Studies on Anterior Hypophysis: The Anterior Hypophysis in Vitamin E-Deficient Rats, Anat. Rec. **56**:241, 1933. (*d*) Kepinov, L.: Les vitamines A et E, agents excitants de la sécrétion de l'hormone glycogénotrope du lobe antérieur de l'hypophyse, Compt. rend. Acad. sc. **210**:188, 1940. (*e*) Tonutti, Von E.: Über die Nebennierenrinde bei Vitamin E-freier Ernährung, Ztschr. Vitaminforsch. **13**:1, 1943.

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33. Burr, G. O.; Brown, W. R., and Moseley, R. L.: Paralysis in Old Age in Rats on a Diet Deficient in Vitamin E, Proc. Soc. Exper. Biol. & Med. **36**:780, 1937. Mackenzie, C. G.: Cure of Repeated Attacks of Nutritional Muscular Dystrophy in the Rabbit by Alpha-Tocopherol, ibid. **49**:313, 1942. Gullickson, T. W., and Calverley, C. E.: Cardiac Failure in Cattle on Vitamin E-Free Rations as Revealed by Electrocardiograms, Science, **104**:312, Oct. 4, 1946. Martin, G. J., and Faust, F. B.: The Heart in Avitaminosis E, Exper. Med. & Surg. **5**:405, 1947.

34. Shute, E. V.; Vogelsang, A. B.; Skelton, F. R., and Shute, W. E.: The Influence of Vitamin E on Vascular Disease, Surg., Gynec. & Obst. **86**:1, 1948.

35. Barrie.[31b] Nelson, W. O., and others: Growth Stimulating Activity of Alpha-Tocopherol, Proc. Soc. Exper. Biol. & Med. **45**:157, 1940. Emerson, G. A., and Evans, H. M.: Successive Generations of Vitamin E-Low Rats, ibid. **45**:149, 1940.

36. Weissberger, L. H., and Harris, P. L.: Effect of Tocopherols on Phosphorus Metabolism, J. Biol. Chem. **151**:543, 1943. Shute, E.: A Reply to Recent Criticisms of the Theory of a Relationship Between Vitamin E and the Oestrogens, J. Endocrinol. **2**:173, 1940. Bicknell, F.: Vitamin E in the Treatment of Neurological Conditions, in The Vitamins in Medicine, ed. 2, New York, Grune & Stratton, Inc., 1946, p. 767.

37. Bicknell, F.: Amyotrophic Lateral Sclerosis and Progressive Muscular Atrophy, in The Vitamins in Medicine, ed. 2, New York, Grune & Stratton, Inc., 1946, p. 768.

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(*Footnote continued on next page*)

TREATMENT PROGRAM

When this study was begun, patients with lupus erythematosus were treated with *d*-calcium pantothenate (100-mg. capsules) alone. The original dosage varied from 0.5 gm. to 2.0 gm. daily. Some improvement was noted. Vitamin E, in the form of natural tocopherols (100-mg. capsules), was then added to the therapeutic program. The original dosage was 300 mg. daily. The dosage of *d*-calcium pantothenate was increased to 3 gm. daily, and the dosage of the natural tocopherols to 400 mg. daily. More rapid improvement was noted in patients treated at once with this higher dosage schedule than in those who started with the lower dosages. Patients who had improved for a time and then remained stationary on the lower dosage schedule began to progress favorably when put on the higher dosage schedule.

Panthenol® was then obtained for use of patients who had lupus erythematosus. At first, this was supplied in 150-mg. capsules, but later in 0.5-gm. capsules. In the early use of this agent, the daily dose was started at 2.0 gm. and slowly increased every two or three weeks to 8 to 12 gm. In later patients, the starting daily dosage was 4.0 gm., and the dosage was increased rapidly over two or three weeks to the 10-to-12-gm. level. *dl*-Calcium pantothenate became available in 0.5-gm. capsules, and we proceeded to use it in the same manner as panthenol.® Ephynal acetate® was then substituted for the natural tocopherols in the treatment schedule. This drug was supplied in 100-mg. capsules. Treatment was started with 400 to 600 mg. of ephynal acetate® daily, and the dosage was rapidly increased to 800 to 1,000 mg. daily.

Many of the patients treated with panthenol® or *dl*-calcium pantothenate with ephynal acetate® had already been taking *d*-calcium pantothenate and natural tocopherols. In order to maintain an uninterrupted treatment program, patients who started treatment with panthenol® were given *d*-calcium pantothenate or *dl*-calcium pantothenate when supplies of panthenol® were short. Conversely patients who began with *dl*-calcium pantothenate or *d*-calcium pantothenate were given panthenol® when supplies of these "starting" drugs were short. Patients were asked to resume natural tocopherols when supplies of ephynal acetate® were short. Consequently, these patients were treated with some source of pantothenic acid and some form of vitamin E for long intervals, with few interruptions.

CLINICAL MATERIAL STUDIED

Forty-two patients[39] who had lupus erythematosus were treated with the above drugs in various doses. Of these patients, 24 had chronic discoid, 11 had disseminate discoid, and 7 had subacute disseminate lupus erythematosus. Ten patients either disappeared from observation before adequate treatment could be

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Nutrition, 1940, Vol. 19, p. 13. Mattill, H. A.: Vitamin E, J. A. M. A. **110**:1831 (May 28) 1938. Cummings, M. J., and Mattill, H. A.: The Auto-Oxidation of Fats with Reference to Their Destructive Effect on Vitamin E, J. Nutrition **3**:421, 1931. Mackenzie, C. G.; Levine, M. D., Rabbit by Alpha-Tocopherol in the Absence of a Water-Soluble Factor, ibid. **20**:399, 1940. and McCollum, E. V.: The Prevention and Cure of Nutritional Muscular Dystrophy in the Mackenzie, C. G., and others: Uncomplicated Vitamin E Deficiency in the Rabbit and Its Relation to the Toxicity of Cod Liver Oil, ibid. **21**:225, 1941.

39. Two of these patients were treated in the department of dermatology and syphilology of the University of Cincinnati at Cincinnati General Hospital.

given, or had so many lengthy interruptions in the program that the treatment course was considered wholly inadequate and unsatisfactory. These 10 patients are therefore not included in the following appraisal of the treatment program outlined above. Of the 32 remaining patients who are included in this report, 17 had chronic discoid, 9 had disseminate discoid, and 6 had subacute disseminate lupus erythematosus. Twenty-three of this group had had previous treatment for lupus erythematosus, including gold preparations, bismuth, liver, oxophenarsine (mapharsen®) hydrochloride and sodium *p*-aminobenzoate. Some of these had had treatment for many years. The remaining nine patients had had no previous treatment for lupus erythematosus.

Of patients with the chronic discoid type many had had the disease from 9 to 29 years. Some already had considerable amounts of disfiguring scar-

TABLE 1.—*Treatment of Patients Who Had Chronic Discoid Lupus Erythematosus*

Case No. and Initials	Sex	Duration of Disease, Yr.	Previous Treatment	Duration of This Treatment, Mo.	*d*-Calcium Pantothenate, Gm.	*dl*-Calcium Pantothenate, Gm.	Panthenol,* Gm.	Natural Tocopherols, Gm.	Ephynal Acetate,* Gm.	Per Cent of Clearing	Complications of Treatment
1, J. G.	F	8	Yes	29	904.8	100.0	1,799.5	209.5	48.8	95	None
2, T. J. G.	M	9	Yes	21	562.8	.....	3,169.5	89.4	129.4	100	G. I.*
3, E. H.	F	10	Yes	20	1,926.0	68.4	46.5	285.6	138.6	100	None
4, E. H. H.	F	9	Yes	36	431.6	.....	2,073.4	141.5	135.5	99	None
5, H. J.	F	25	Yes	24	76.8	.....	3,122.6	123.9	163.5	85	None
6, L. L.	M	4.8	Yes	10	.....	.....	1,777.0	.....	165.2	85	None
7, R. I. R.	M	11	No	36	477.2	168.0	20.0	.....	58.0	75	G. I.*
8, R. R.	M	29	Yes	24	874.0	200.0	2,412.3	86.8	225.0	90	None
9, L. S.	F	6	Yes	30	1,254.0	.....	1,417.0	8.8	85.2	80	None
10, M. S.	F	2	No	17	1,271.2	.....	1,225.0	30.8	146.4	90	None
11, T. S.	F	10	Yes	12	.....	72.0	873.6	.....	70.2	70	None
12, L. T.	F	8	No	22	799.6	.....	100.7	182.7	.....	100	None
13, L. J. von W.	F	10	Yes	36	1,519.8	150.0	1,166.0	120.6	.....	95	None
14, W. R. W.	M	1.3	No	17	731.7	.....	1,003.0	.....	79.0	80	None
15, D. Z.	F	2	No	30	1,255.8	10.0	.....	153.2	.....	100	None
16, H. W.	M	9	Yes	14	289.4	.....	80.0	75.0	.....	70	None
17, E. H. W.	F	6	Yes	16	336.8	.....	.....	89.9	.....	100	None

* Transient gastric distress from panthenol.®

ring. Several had heavy, markedly infiltrated, and hyperkeratotic lesions. Five patients, who had chronic discoid lupus erythematosus when the above treatment was started had experienced in the past episodes of the disseminate discoid type. Four with the disseminate discoid type had subjective systemic symptoms consisting of fatigue, anorexia, arthralgia, and weight loss, as well as objective systemic symptoms consisting of low white blood cell count, relative and absolute lymphocytosis, reversed ratio of albumin to globulin, false-positive serologic reactions, etc. The duration of the disease in patients who had disseminate discoid lupus erythematosus (Table 2) and in patients who had subacute disseminate lupus erythematosus (Table 3) represents the total interval of the disease in these patients, and not just the duration of the attack present when this form of treatment was instituted. Some of the patients in each group had several episodes over a period of years.

The duration of treatment by the combined use of the drugs studied, as indicated in the tables, does not represent continuous treatment interval in some of

these patients. In many instances, a good part of the early course of treatment consisted in the use of very small doses of the drugs used. In some patients the maximum dosage schedule was employed for only the last few months of the treatment course. The shortest treatment period in the series was two months. The longest treatment period in the series was three years.

In patients in whom treatment was interrupted the disease ceased to progress favorably and, if treatment was stopped for too long, relapsed in the old sites, and in some instances new lesions developed. New lesions developed in five patients while they were under treatment with the various combinations of vitamin E and

TABLE 2.—*Treatment of Patients Who Had Disseminate Discoid Lupus Erythematosus*

Case No. and Initials	Sex	Duration of Disease, Yr.	Previous Treatment	Duration of This Treatment, Mo.	*d*-Calcium Pantothenate, Gm.	*dl*-Calcium Pantothenate, Gm.	Panthenol,* Gm.	Natural Tocopherols, Gm.	Ephynal Acetate,* Gm.	Per Cent of Clearing	Complications of Treatment
1, F. B.	M	16.5	Yes	20	258.0	11.0	3,566.0	250.5	85.2	95	G. I.*
2, D. McP.	M	8	No	36	126.8	.....	787.0	.....	75.1	100	None
3, J. M.	M	10	Yes	17	421.5	.....	1,927.0	119.8	146.9	75	None
4, A. M.	F	6	Yes	2	.....	385.0	.....	.....	15.8	80	None
5, B. T.	F	7	Yes	34	1,051.6	2,120.0	.....	43.3	215.3	85	None
6, W. T.	M	2	Yes	10	149.0	.....	1,161.0	.....	175.9	90	None
7, J. R.	F	1	No	8	.....	77.0	137.0	.....	11.4	75	G. I.*
8, A. Y.	F	7	Yes	10	.....	.....	1,336.5	126.4	.....	100	None
9, C. B.	F	7	No	18	423.0	.....	.....	72.5	.....	100	None

* Transient gastric distress from panthenol.*

TABLE 3.—*Treatment of Patients Who Had Subacute Disseminate Lupus Erythematosus*

Case No. and Initials	Sex	Duration of Disease, Yr.	Previous Treatment	Duration of This Treatment, Mo.	*d*-Calcium Pantothenate, Gm.	*dl*-Calcium Pantothenate, Gm.	Panthenol,* Gm.	Natural Tocopherols, Gm.	Ephynal Acetate,* Gm.	Per Cent of Clearing	Complications of Treatment
1, M. A.	F	0.5	Yes	12	.....	240.0	1,928.0	.....	18.3	100	None
2, M. G.	F	1.5	No	22	.....	.....	1,820.5	.....	149.2	95	None
3, B. H.	F	28	Yes	12	30.0	1,947.0	269.0	14.4	213.4	95	None
4, O. L.	F	6	Yes	27	1,673.0	.....	816.0	254.8	70.4	95	None
5, H. T.	F	0.5	No	8	.....	200.0	1,701.0	.....	130.8	90	None
6, V. D.	F	8	Yes	5	199.8	.....	.....	.....	18.0	100	None

one or more of the pantothenic acid radicals. However, such lesions were short-lived and faded out before existing older lesions were completely controlled. Arthralgias developed in six patients while under the above treatment. In each of these patients response of the arthralgia was prompt when sodium salicylate was added to the treatment schedule. In each patient exhibiting increased sedimentation rate, reversed ratio of albumin to globulin, false-positive serologic reactions, leukopenia, relative or absolute lymphocytosis, or albuminuria, reversion of these factors to normal was produced after a variable interval with the above treatment program.

It will be noted in the tables that improvement is recorded in all of the patients who were treated by the combined use of vitamin E and one or more of the pantothenic acid derivatives. Some recovered completely. Many of the others are nearly completely recovered and still under treatment. The response to treat-

ment was best and most rapid in the group with the subacute disseminate type. The group with the disseminate discoid type was next, and that with the chronic discoid type was poorest in response. In the group with the subacute disseminate type improvement was usually noted in three weeks; in the group with the disseminate discoid type, in two months, and in the group with the chronic discoid type, in four months.

Not included in the tables were eight patients with lupus erythematosus (six with chronic discoid, one with disseminate discoid, and one with subacute disseminate lupus erythematosus) who were treated with *d*-calcium pantothenate alone. The dose varied from 2 to 3 gm. daily. There was improvement, but no instance of complete recovery in any patient except the one who had the subacute disseminate type.

COMPLICATIONS ARISING FROM TREATMENT

There were no untoward complications arising from this treatment program except for transient nausea or other slight gastric upset. These symptoms occurred in only a few patients, and usually from the panthenol.* In these patients, either *dl*- or *d*-calcium pantothenate was then used instead of panthenol.* No patient was found who could not take, without reaction, one of the three pantothenic acid derivatives studied. No abnormalities in blood cells, blood chemistry, urine, or blood pressure were noted in patients who were kept on the above treatment program for long intervals. *d*-Calcium pantothenate alone was given to several hundred patients who had various dermatoses, in dosages varying from 0.3 gm. to 3 gm. daily over long intervals without any untoward complications.

Panthenol,* or *dl*-calcium pantothenate, or d-calcium pantothenate together with natural tocopherols or ephynal acetate* was given to 90 patients who had cutaneous diseases other than lupus erythematosus. The number of patients and the dermatoses comprising this group included the following:

40 psoriasis
7 dermatitis herpetiformis
6 lichen planus
6 benign transitory plaques of the tongue
5 chronic infectious eczematoid dermatitis
3 seborrheic dermatitis
3 alopecia areata
2 leukoplakia
2 neurodermatitis

Besides these, one patient was chosen from each of the following disease groups: periarteritis nodosum, vitiligo, contact dermatitis, ichthyosis, morphea, atopic dermatitis, scleroderma, chronic benign pemphigus, prurigo nodularis, epidermolysis bullosum, erythema multiforme, progressive pigmentary dermatosis, erythema perstans, aphthous stomatitis, necrobiosis lipoidica diabeticorum, and lichen sclerosis et atrophicus. These drugs were given to these patients in various combinations and dosages. In no instance was there any complication except for transient gastric distress from panthenol.*

It is evident in the tables that each of 15 patients took over 2,000 gm. (between 4 and 5 lb.), and that Patient 1 in Table 2 took over 8 lb. (3.6 kg.) of these pantothenic acid derivatives without untoward effect. The tables also record 11 patients each of whom took over 200 gm. of vitamin E and Patient 3 in Table 1 who took 424.2 gm. without complication.

SUMMARY

Analysis is recorded of the response to treatment of patients who had chronic discoid lupus erythematosus, disseminate discoid lupus erythematosus, and subacute disseminate lupus erythematosus by the combined use of some forms of vitamin E and one or more derivatives of pantothenic acid. Very favorable results were obtained. There were no harmful effects from treatment noted in this study.

CONCLUSIONS

I feel that the combined use, in adequate dosage, of some form of vitamin E and one or more of the derivatives of pantothenic acid here employed constitutes a safe and effective method of treatment for patients with chronic discoid, disseminate discoid, or subacute disseminate lupus erythematosus.

1219 Carew Tower.

ABSTRACT OF DISCUSSION

DR. ALBERT R. MCFARLAND, Rochester, N. Y.: I would like to discuss Dr. Welsh's very fine paper from the point of view of his concept of the mode of action of these vitamins on lupus erythematosus.

As I think back some 30 years in the past, my first impression was that lupus erythematosus was in the nature of a tuberculid. During my early days of association with Dr. John H. Stokes, he summarized his impression as that of an allergic reaction due either to a tuberculous focus or to a pyogenic focus. Following this concept, we went through a period of cautious but thorough eradication of foci of infection and a search for latent foci of tuberculosis, and also the routine use of gold salts.

Next came the period of stress upon antibacterial agents and the use of arsenic and bismuth. You will recall the paper read before this association by Dr. Richard Weiss on the use of oxophenarsine (mapharsen®) hydrochloride. A little later Dr. Burgess reported improvement in this disease by the use of large doses of vitamin E. Some other investigators were unable to duplicate Dr. Burgess' findings. An unpublished but very interesting group of cases was presented a few years ago before the general staff meeting of the Rochester General Hospital by Dr. Milton Bohrod. Dr. Bohrod presented several cases with autopsy reports in which there was a combination of periarteritis nodosa, scleroderma, and lupus erythematosus. During life all these patients complained of a great variety of symptoms, and physical findings including foci of infection, allergic conditions, disturbances of liver functions, and thyroid dysfunction. He concluded that perhaps all three of the conditions mentioned were related and might be allergic manifestations of a great variety of different pathological conditions.

Then we come to the era of corticotropin (ACTH) and cortisone. Although neither of these drugs is curative in lupus erythematosus, there is no question but that they have some influence on the course of the disease.

This brings us to the question as to what is Dr. Welsh's theory regarding the action of these vitamins. Do they simply increase the body's resistance against infection? Do they in some way influence allergic reaction? Do they perhaps alter the body's reaction to its own metabolic products, or do they have some indirect effect on the glandular system, such as the adrenals and pituitary? Perhaps it would be too much to ask of Dr. Welsh to give a complete answer to these questions, but it would be interesting to know his line of thought, or, in other words, why he picked this particular line of treatment.

The tables showing the percentage of results obtained are a very important part of Dr. Welsh's paper. The percentage of cure is much higher than by any other method we have used in the past. As I recall, many of his patients obtained 98 to 100% improvement, and I believe the lowest percentage was about 75%.

If this work can be corroborated by other investigators, and if this high percentage of cure can be obtained consistently, it will certainly be a big advance in the treatment of lupus erythematosus.

DR. CARL W. LAYMON, Minneapolis: I think that most of us are far from satisfied with our results in the treatment of lupus erythematosus. Dr. Welsh's remarks were directed toward the chronic discoid and subacute forms of the disease, and he did not deal with the acute septic or the protracted systemic types. Most of us will agree that bed rest is of distinct value in all stages of the disease. This, however, is a most difficult thing to carry out when the patient is not particularly ill. Gold, and even bismuth, in the opinion of many of us, has capabilities of doing great harm in some cases. I think that these drugs have been rather overrated in the treatment of lupus erythematosus. The treatment which Dr. Welsh utilizes apparently has safety, which is extremely important. In closing I wish that he would mention the cost of such treatment.

DR. FRANK C. COMBES, New York: Dr. Welsh has taken a new approach to the treatment of lupus erythematosus. I think it is logical and can be applied to other toxic dermatoses, especially those of the bullous type. All the dermatoses in this entire group are merely cutaneous responses to remote bacterial infections, many of which are situated in the gastrointestinal and upper respiratory tracts.

I think Dr. Welsh should be congratulated for persisting in the administration of the vitamin fractions for so long in the absence of any clinical improvement. It is interesting to philosophize on why it is necessary to administer calcium pantothenate and potassium *p*-aminobenzoic acid for two or three months before benefits are evident. I have had similar experiences, and my ideas are as follows: In all our bodies we have friendly bacteria, which we call saprophytes, and those that eventually cause us trouble and which will eventually get us all some day, which we call pathogens. There is normally a balance established between these groups. We call that "good health." We know that all bacteria require vitamins for their growth and well-being. Of course, some organisms are capable of synthesizing vitamins. This is called autosynthesis, and little is known about it. In infectious diseases and bacterial toxemias the pathogens gain ascendency over both the friendly saprophytes and the immunologic abilities of the host. Pantothenic acid as its name indicates, is an all-inclusive vitamin. Its name is derived from the Greek *pas* (all) plus *theos* (god). In other words, it is "all things to all vitamins." *p*-Aminobenzoic acid occupies a similar but not quite so important position in human nutrition. Both are the protectors of all other vitamins and possibly of unknown enzymes and trace substances necessary for life.

Now when we give these large doses of panthenol® or calcium pantothenate, we are aiding vitamin synthesis in the body, necessary for nutrition of the host, and growth of the friendly saprophytes which slowly increase in number until they are predominant. I have done some studies along these lines which are quite significant but not definite enough to draw conclusions. This method of therapy does cause changes in the bacterial flora of the bowel. It is certainly a valuable addition to our therapeutic approach to the toxic bullous dermatoses and lupus erythematosus.

DR. JACOB H. SWARTZ, Boston: My associate, Dr. Glicklich, and I have used large doses of calcium pantothenate and panthenol® in a small series of 13 patients with lupus erythematosus for the past two and one-half years. Nine of the 13 patients showed fair to good improvement. None were cured. Calcium pantothenate was found more effective than panthenol.® The dose required to obtain beneficial therapeutic results was 6 gm. daily. However, 3 gm. a day was sufficient as a maintenance dose. The results were more striking in subacute and disseminate chronic discoid lupus erythematosus.

We have encountered no significant untoward reactions even in those patients who have been treated with the drug for two years. The addition of vitamin E, not synthetic, did not produce any strikingly better therapeutic results. Recently we tried large doses of ascorbic acid, 2 gm. daily, in combination with calcium pantothenate. Ascorbic acid was tried because of the favorable therapeutic results reported by Massell in rheumatic fever (personal communication). Our series is too small to merit evaluation.

One young female patient with acute disseminated lupus erythematosus improved coincidentally with the administration of large doses of calcium pantothenate. Treatment was then reduced to a maintenance dose of 3 to 4 gm. daily. She had two relapses coincidental with the omission of the drug and improved when calcium pantothenate was resumed. Of course, the report of one case is only important when it serves as an addition to a larger series. One must be cautious in such evaluations.

We have tried calcium pantothenate for other dermatoses, including psoriasis, seborrheic dermatitis, atopic dermatitis, and for two patients with lichen planopilaris. Of 25 patients with psoriasis, 13 showed some questionable beneficial therapeutic results. The two patients with lichen planopilaris showed surprising improvement. We have encountered no beneficial effects from calcium pantothenate in the other dermatoses treated, including one patient with dermatomyositis.

DR. ASHTON L. WELSH, Cincinnati: I am grateful for the ideas for further investigations suggested by my discussors. Some of the things they mentioned are already being studied. The work is still going on, as I indicated in saying that the patients were still under treatment.

I cannot explain the mechanism of the action of the combined use of these drugs in patients who have lupus erythematosus. The theory which Dr. Combes just outlined has, in my opinion, great merit. These drugs have no direct antibacterial action; hence their effect must be produced through some action favoring the activity of the normal defense mechanisms of the body.

The work was started because we had a group of patients at the Cincinnati General Hospital and in our office practice who had had lupus erythematosus for a long time in spite of much treatment with gold, bismuth, liver, etc. Many of them had become reactive and could not tolerate these conventional procedures any longer. A search was made for some approach to treatment of this group.

Pantothenic acid, as Dr. Combes stated, seemed, if one intended to employ any vitamin at all, to be the logical choice because of its wide enzyme-protective action and its pansynergetic action on many other vitamins. Vitamin E was credited with so many separate and unrelated actions, on so many tissues, that the combination of these two agents seemed a reasonable starting combination. In addition, there were favorable reports of the use of some derivatives of pantothenic acid in patients who had lupus erythematosus, and also reports of improvement from the use of vitamin E in patients who had this disease.

One drug company was willing to give us enough of these materials to start this experiment. After we had begun the study, because of the cost of the agents used, it became necessary to interest another company also in supplying the materials, in order to get adequate supplies. Patients were switched from one company's materials to materials of another company, depending on the materials currently available. Accordingly, most of the patients had combinations of several types of the two agents used. Later, it was possible to get these materials through our own pharmacist. He was able to supply these agents, at fairly reasonable prices, by purchasing very large quantities from the manufacturers, thereby obtaining a lower price.

The high dosages of the drugs employed and the long treatment intervals were arrived at automatically. As long as the patients had lesions, the drugs were administered to them, and whenever the patients seemed to cease to progress favorably, the dose was elevated. Gradually high dosage levels were reached. It was noted that each time we did elevate the dosage, the patients again showed improvement. As long as these drugs did not produce any sign of toxic reaction, no reason for not using them in this way presented itself.

I was interested in Dr. Swartz's report of his experience with the use of calcium pantothenate and panthenol® for patients who had lupus erythematosus, but I feel that his results would have been much better had he used higher dosages. I feel that his observation that the addition of natural vitamin E did not appreciably improve the therapeutic results was due to the fact that only small quantities of vitamin E were used. I was gratified that his patients improved, as did ours, on calcium pantothenate and panthenol,® and that he attested to the safety of administration of these medications.

Dr. Laymon expressed my own feelings regarding the use of this approach in handling lupus erythematosus over the older, conventional procedures on the basis of safety of the drugs employed.

The work is still continuing. An attempt is being made to find the most effective salt of pantothenic acid in the treatment of lupus erythematosus, and also to find what form of vitamin E produces the best therapeutic result when combined with that salt. It appears, to date, that the dextrorotatory form of the calcium salt of pantothenic acid and the dextrorotatory form of the acetate ester of the alpha-tocopherol constitutes the most effective combination yet employed.

While these agents are expensive, I do not think they are beyond purchase by many patients. Many patients now under treatment are purchasing their own materials. I do not know the exact cost, at the moment.

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STUDIES ON PANTOTHENOL. I. COMPARATIVE STUDY OF THE UTILIZATION OF D-PANTOTHENOL AND D-Ca-PANTOTHENATE BY THE RAT

By

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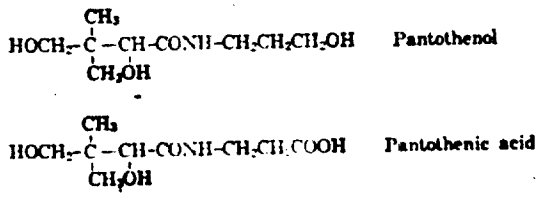
By

Yutaka Yamawaki and Tadashi Ishida

ABSTRACT: The amount of pantothenic acid which appeared in the urine of the rat after the oral and intraperitoneal administration of D-pantothenol or calcium D-pantothenate were determined by means of microbiological assay with Lactobacillus arabinosus. The amount of pantothenic acid excreted in the urine within 24 hours after intraperitoneal injection of D-pantothenate to the rat was larger than that of D-pantothenol injection. However, the total amount of pantothenic acid excreted in the urine during a week after the administration was exactly the same in both cases. It suggests that the utilization of D-pantothenol in vivo continues longer than that of D-pantothenic acid. On the contrary, the amount of pantothenic acid excreted in the urine within 24 hours after the oral administration of D-pantothenol was larger than that of D-pantothenate. This fact may show that the absorption rate of D-pantothenol from the intestine is more rapid than that of the D-pantothenate. Above results suggest that the utilization of D-pantothenol when used as a pharmaceutics in vivo is higher than that of calcium D-pantothenate.



Pantothenic acid (PaA) has been known for a long time as a growth promoting factor of yeast. Its alcohol derivative pantothenol (PaOH) was reported to have a PaA action in warm-blooded animals for the first time by Pfaltz [1]. Burlet [2] reported that PaOH is oxidized in the body, acts as PaA and is excreted in urine as when calcium pantothenate (Ca-PaA) is administered, but in the case of *L. casei* for which PaA is essential, it does not affect its propagation. Subsequently, Snell et al. [3] revised this view, holding that PaOH is a competitive and inhibitive substance for

the micro-organisms needing PaA. Bird et al. [4] applied this characteristic to microbiological quantitative analysis of PaOH. Schmidt [5] confirmed that β-alanine and pantoic acid, which are the components of PaOH, are not synthesized into PaA even if they are administered to humans simultaneously. Rubin [6] et al. found that when PaOH or Ca-PaA is administered to the rat, both of them are excreted in the urine as PaA, and that the ratio of the amount of PaA excreted to the amount of PaOH administered is constant, and utilized this fact in the quantitative analysis of PaOH. Schmidt [7] was of the opinion that when Ca-PaA and PaOH are intravenously injected, there is no difference between their excretion rates in the urine. Rubin [10] et al. and Braekkan [9] report that in oral administration, PaOH is better absorbed and in the intramuscular and intraperitoneal injections, PaOH is slower to be excreted. On the other hand, Rubin [10] reports that while the water solution of PaA is extremely unstable on the acidic side below pH = 5, PaOH is stable relative to PaA in the wide range of pH = 3-7.

In view of these facts, the authors examined the absorption and excretion of these two substances administered orally and non-orally in order to compare the values of these two substances as pharmaceuticals.

Experimental Method

White rats of the Wistar breed weighing 200~300 g were used as experimental animals. Three of them were placed in a cage as a group. The cages were double bottomed for separating the urine from the feces. The feces were collected in a nylon net on the bottom. The urine was quantitatively collected through a large funnel in a flask with a small quantity of toluol placed beforehand, and by washing it with tap water every 24 hours, and this was used as a test solution for a quantitative analysis of PaA.

The basic feed was a paste bait with the components 20% corn starch, 56% wheat flour, 15% casein, 5% yeast, 4% McCollum salt #115. 20 g of this per day per rat was fed to the animals around 3 p.m. The rats were allowed to feed on it freely. By the next morning the feed was completely consumed without leavings. When the white rats were maintained on such a diet, the base quantity of PaA excreted in the urine which varied at first became constant, at an amount around 100 γ, in about a month. At this point, the test substances were administered for the first time. After this, the amount excreted was measured every day. After confirming that it had fully reverted to the base quantity of excretion, the next experiment was conducted.

Preparation of the Solution

The urine and feces were collected in the morning at 9:00. The urine was suitably diluted and then filtered; this was used as the test solution. The test solution of the feces was prepared by treating the excrement with Taka-diastase* in the usual way, and then diluting it suitably with water.

Quantitative Analysis of PaA

The PaA in the urine and feces were quantitatively analysed by means of a microbiological assay using *Lactobacillus arabinosus*, and the acidimetry method according to the *U.S. Pharmacopeia*. As to the measuring range, the final density was set at 0.03~0.3 γ/10 cc as Ca-PaA. The obtained value was expressed as Ca-PaA and was found by subtracting the base quantity of excretion.

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* Diastase produced by Dr. Takamine, Jokichi.

RESULTS

(1) Comparison of volumes excreted in the urine after intraperitoneal injection

The injections of PaOH and Ca-PaA in the quantities of 10, 5, 3 and 1 mg per rat as Ca-PaA were administered intraperitoneally at 9 a.m. immediately after the collection of the excretions.

Figures 1 and 2 show the amount of PaA excreted in the urine throughout the day of the administration of Ca-PaA and PaOH. In the case of Ca-PaA, 70~80% of the amount administered was excreted within 24 hours of administration. From the 2nd day on, the amounts excreted were suddenly reduced, and on the 4th-5th days they reverted to the base quantity of excretion.

In the case of PaOH, although its tendency was roughly the same, the amount excreted on the first day was somewhat smaller relative to the case of Ca-PaA; also, a long period of time was necessary to return to the base excretion volume. Figure 3 shows the result of the comparison of the amounts

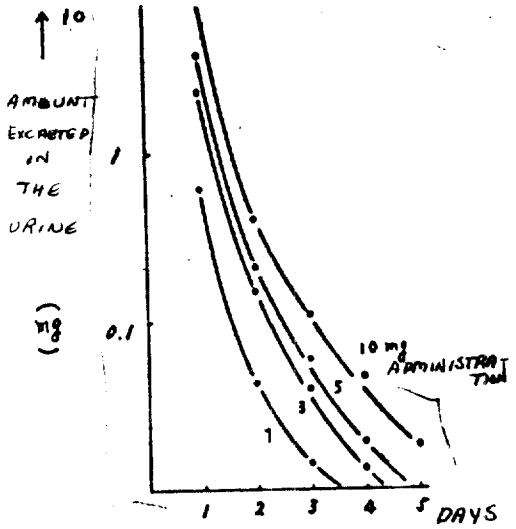

Fig. 1. Excretion in the urine for non-oral administration
(1) Ca-PaA administration

of PaA excreted in 24 hours for the Ca-PaA administration and PaOH administration. It is clear from this that although it is proportionate to the

dosage administered in both cases of Ca-PaA and PaOH, the amount excreted in 24 hours was somewhat less in the case of the PaOH administration than in the case of Ca-PaA. This can be considered as indicating a longer period of retention in the body, thus a longer period of its action in the body, judging by the same rate of collection seen in Figure 4.

(2) Comparison the amounts excreted in the urine after oral administration

The rats were divided into 4 groups with 3 rats in each group. The test substances were administered in the quantities of 10, 5, 3 and 1 mg respectively to these 4 groups as in the case of the intraperitoneal injection. Figure 5 shows the amount excreted within 24 hours after that. In the case of the oral administration (in contrast to the case of the non-oral administration), the amount of excretion in the urine was far larger for the case of the administration of PaOH.

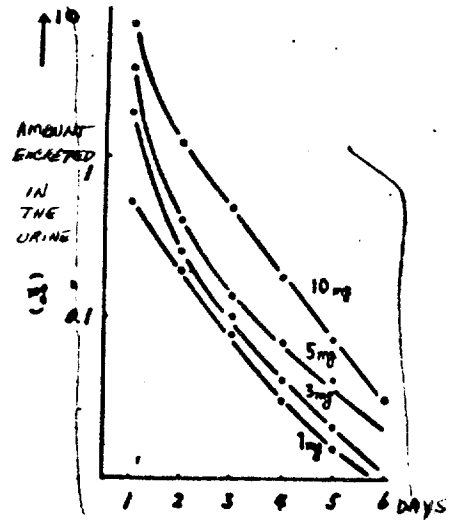

Fig. 2. Excretion in the urine for non-oral administration (2) PaOH administration

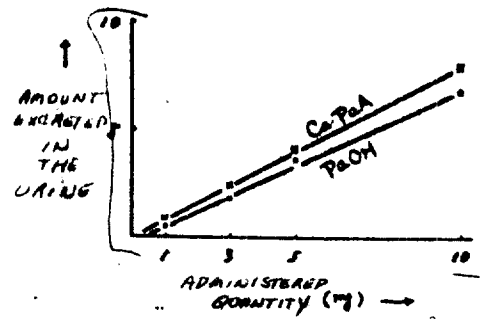

Fig. 3. Amount excreted in the urine within 24 hours for non-oral administration

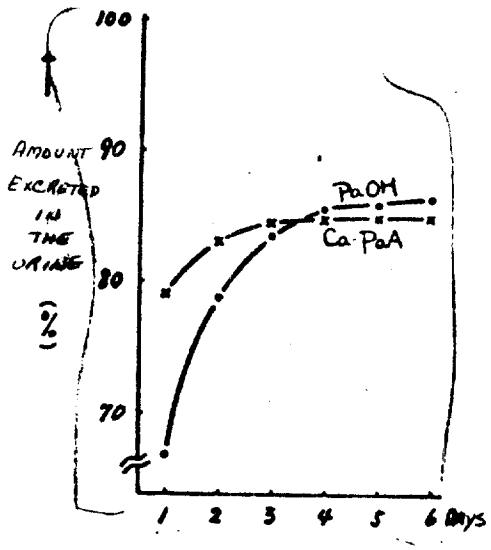

Fig. 4. Comparison of accumulation in the urine after non-oral administration (for 10 mg administration)

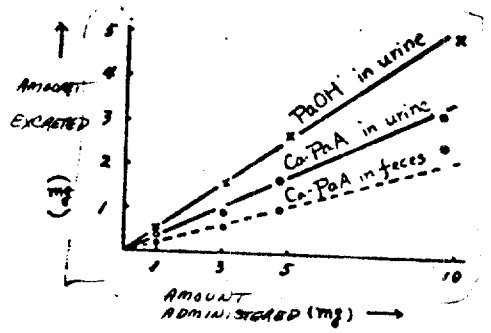

Fig. 5. Amounts excreted in the urine and feces within 24 hours after oral administration

Discussion

The excretion rates in the urine of PaOH and Ca-PaA administered were quite rapid whether the administration was oral or non-oral. The fact that the major parts of these were excreted shows that these two substances are of water-soluble vitamins. Also, on the basis of this, it can be recognized that the danger of toxicity due to the remaining of these in the body is very little. Incidentally, Weiss et al. reported that their chronic and acute toxicity is very weak [11]. However, the fact that the excretion in the urine after the PaOH administration in the case of the non-oral administration, which does not take the absorption rate into consideration, is slower than that after the administration of Ca-PaA can be considered as an indication of its long period of activeness as a vitamin. Therefore it can be said that PaOH is quite advantageous as a dosage type of the PaA homologue.

In the case of oral administration, the problem becomes more complex since the absorption rate from the digestive organ and the absorption rapidity become involved besides the rapidity of excretion. But if the absorption rate is estimated from the amount excreted in the urine, the excretion of PaA in the urine within 24 hours is much better for the group to which PaOH was administered than for the group to which Ca-PaA was administered, in contrast to the case of the non-oral administration. And the portions not detected in the urine in the Ca-PaA administered groups were found in the feces. This shows that the absorption rate in the intestine is better for the PaOH administered group. For this and for the fact that it remains in the body longer, a better evaluation probably can be given to it than to PaA as a vitamin compound.

According to the report by Rubin et al. [12], the absorption rate of Ca-PaA differs according to the presence or absence of the foods in the digestive organs. It is reported that the absorption rate at the time of satiation was quite poor, but was better when the animal was hungry. This difference is negligible in the case of PaOH. Although the condition of a completely empty stomach was not the test condition of this experiment, it can be said that the experimental animals were hungry since they were fed at 3 p.m. and the test substances were administered at 9 a.m. the next morning. Even under such conditions, there was a large difference in the absorption rate between PaOH and Ca-PaA. This difference in the absorption rate is probably due to a higher tissue permeability of the alcohol type relative to the carboxylic acid type, as it was observed in the epidermic absorption.

Conclusion

The absorption and utilization rates of PaOH and Ca-PaA were investigated with the white rat using the amount of excretion of these in the urine as an index. In the case of the intraperitoneal administration, the excretion in the urine was slower for the PaOH administration than for the Ca-PaA administration. This suggests a longer utilization period for PaOH. In the case of the oral administration, the amount excreted in the urine was smaller after Ca-PaA administration than after PaOH by about 10%. This indicates its poor absorption rate. The missing portion was found in the feces. This attests to a higher absorption rate for PaOH.

In closing, the authors wish to express gratitude to Prof. M. Hayashi of Chiba University, who reviewed the manuscript, and to Mr. E. Kikuchi, Department Head, and Mr. Y. Miyazaki, Section Chief, who extended their assistance to this study.

A summary of this paper was delivered at the 12th Convention of the Association for Vitamin Research.

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Vitamin 23:169-172, 1961.

原　著

パントテノールの研究

(I) シロネズミにおけるD-パントテノールおよびD-パントテン酸カルシウムの利用性の比較

東亜栄養化学工業株式会社研究所　山脇　豊・石田　忠

STUDIES ON PANTOTHENOL

(I) COMPARATIVE STUDY OF THE UTILIZATION OF D-PANTOTHENOL AND D-PANTOTHENATE BY THE RAT

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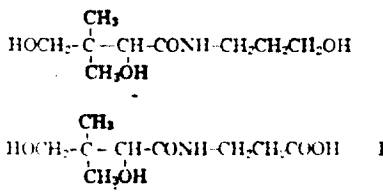
The amount of pantothenic acid which appeared in the urine of the rat after the oral and intraperitoneal administration of D-pantothenol or calcium D-pantothenate were determined by means of microbiological assay with *Lactobacillus arabinosus*. The amount of pantothenic acid excreted in the urine within 24 hours after intraperitoneal injection of D-pantothenate to the rat was larger than that of D-pantothenol injection. However, the total amount of pantothenic acid excreted in the urine during a week after the administration was exactly the same in both cases. It suggests that the utilization of D-pantothenol *in vivo* continues longer than of D-pantothenic acid. On the contrary, the amount of pantothenic acid excreted in the urine within 24 hours after the oral administration of D-pantothenol was larger than that of D-pantothenate. This fact may show that the absorption rate of D-pantothenol from the intestine is more rapid than that of the D-pantothenate. Above results suggest that the utilization of D-pantothenol when used as a pharmaceutics *in vivo* is higher than that of calcium D-pantothenate.



Pantothenol

Pantothenic acid

PaA*1) は古くから鼠類の成長促進因子として知られて来たが，そのアミノール誘導体であるPaOH*2)はPfaltz[1]により初めて溶血性物にたいしてPaA作用の

あることが示された。Burkett[2]はPaOHは体内でPaAに酸化されCa-PaA*3)となるのばあいと同様にPaAとして作用しPaAとして尿中へ排泄されるが，PaAを必

*1) PaA = Pantothenic acid　*2) PaOH = Pantothenol　*3) Ca-PaA = Ca-pantothenate

須とする *L. casei* ではその増殖に影響がないと報告した．その後 Snell ら[3] によりPaAを必要とする微生物にたいしてはPaOHは競合的阻害物質であると訂正されBirdら[4] はこの性質をPaOHの微生物定量に応用した．またSchmidt[5]はPaAの構成単位である β-Alanine と Pantoic acid を人体に同時に投与しても体内ではPaAに合成されないことを確認した．Rubinら[6] はシロネズミにPaOHまたはCa-PaAを投与したとき，いずれも尿中にはPaAとして排泄され，またPaOH投与量にたいする PaA 排泄量の比は一定であることを知り，これを PaOH の定量に用いた．Schmidt[7] は Ca-PaA および PaOHを静注したばあい PaA としての尿中排泄速度は両者の間に差がないといい，Rubinら[8] および Braekkan[9] は経口投与ではPaOH の方が吸収がよく筋肉内注射または腹腔内注射では PaOHの方が排泄のおそいことを報じている．一方 Rubin[10] はCa-PaAの水溶液はpH 5以下の酸性側ではひじように不安定であるのにたいしPaOH は pH3～7の広い範囲で Ca-PaAにくらべて安定であることを明らかにしている．

そこで著者らはこの両物質の製剤としての価値を比較するため非経口および経口投与時の吸収，排泄について検討した．

実　験　方　法

実験動物として体重200～300gのWistar系シロネズミを使用し，3匹を1群として1籠に入れ籠は二重底として尿と糞を分離できるようにした．糞は底のナイロン網に受け，尿は大きな漏斗によりあらかじめトルオールを少量入れたフラスコに受け24時間ごとに水道水にて洗い定量的に採取し PaA 定量のための検液とした．

基礎飼料はトウモロコシ澱粉20%，小麦粉56%，カゼイン15%，酵母5%，McCollum 塩 #115 4%なる組成のものをねり餌として1匹あたり1日20gを午後3時ごろに与え自由に摂取せしめた．翌朝にはほとんど食べつくし，食べ残しはなかつた．このような飼育条件でシロネズミを飼育すると最初はまちまちであつた尿中PaA基礎排泄量は約1ヶ月後には100γ前後と一定値を示すようになつた．そこで初めて検体を投与し連日PaAの排泄量を測定し，ふたたび完全に基礎排泄量に戻つたことを確認してからつぎの実験にうつつた．

検液の調製

尿および糞を毎朝9時に採取し，尿はそのまま適当に希釈し調整して検液とし糞は常法にしたがいタカジアスターゼ処理後水で適当に希釈して検液とした．

PaAの定量法

尿および糞中のPaAは微生物定量法により *L. arabinosus* 17-5を使用し，米国薬局方にしたがい酸滴定法により定量した．測定範囲は終濃度をCa-PaA として 0.03～0.3γ/10ccとなるようにした．また得られた値はCa-PaA として表わし基礎排泄量は差引いて示した．

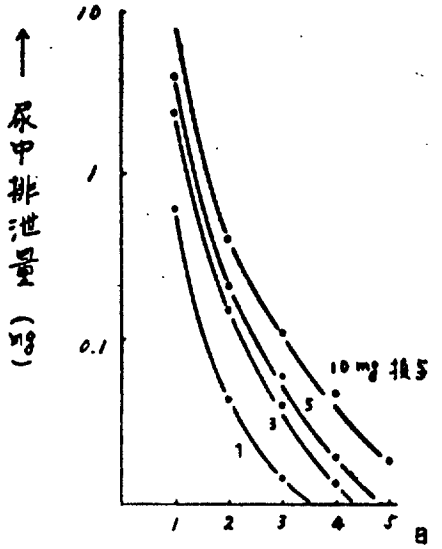
結　　果

（1）　腹腔内注射時の尿中への排泄の比較

各群に1匹あたりCa-PaA として 10, 5, 3, 1 mgのCa-PaA および PaOH を腹腔内に朝9時採便直後注射した．

Ca-PaAおよびPaOHを投与したばあいのPaAの経日尿中排泄量を図1,2に示した．すなわちCa-PaAのばあい投与後24時間以内にすでに投与量の70～80%が排泄され，第2日目より排泄量は急激に減つて4～5日目には基礎排泄量まで戻つた．

図1　非経口投与時の尿中排泄
(1) Ca-PaA 投与



PaOH のばあいはその傾向はほぼ同様であつたがCa-PaA にくらべて第1日目の排泄量がやや少なく，かつ基礎排泄量に達する日時が長くなるのが特徴であつた．また Ca-PaA 投与時と PaOH 投与時の24時間

図2　非経口投与時の尿中排泄
(2) PaOH 投与

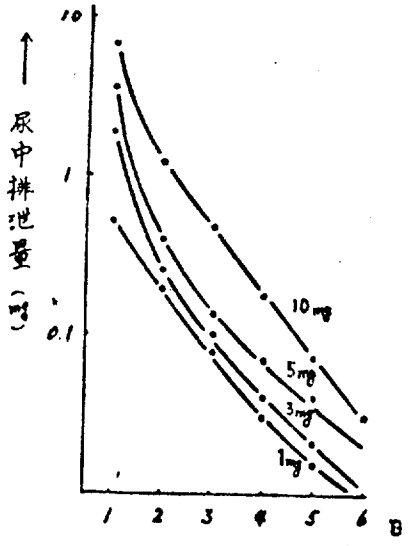


図3　非経口投与時の24時間尿中排泄量

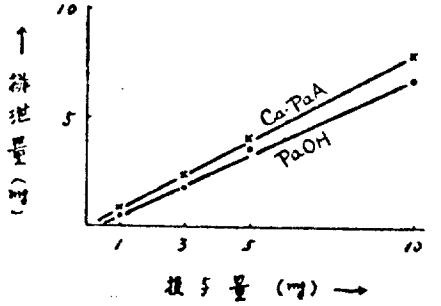


図4　非経口投与時の尿中累積の比較
(10mg投与時)

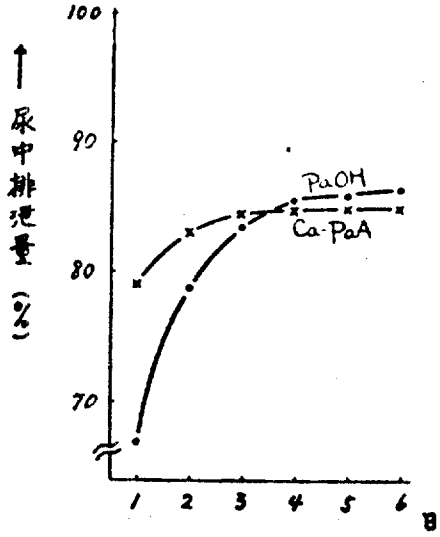
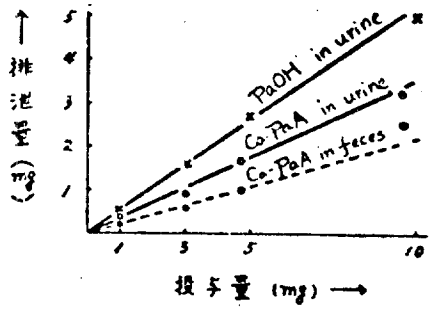


図5　経口投与時の24時間尿中および
糞中排泄量



PaA排泄量を比較すると図3のようになつた．すなわちCa-PaAおよびPaOH投与のばあいいずれも投与量に比例したがPaOH投与時の方がCa-PaA投与時よりも24時間排泄量はやや少ないことが明らかであつた．これは図4に示すごとく回収率が同じであることからみて体内貯留すなわち体内作用時間の長いことを示すものであろう．

(2)　経口投与時の尿中排泄量の比較

実験方法は脱腔内注射時と同様に1群3匹で 10, 5, 3, 1 mg投与の4群に分け胃ゾンデを用いて投与した．その結果の24時間排泄量を図5に示した．この経口投与のばあいは非経口のばあいと異なり尿中への排泄は格段の差をもつてPaOH投与時の方が多い成績を示した．

考　　察

投与されたPaOHおよびCa-PaAは経口，非経口を問わずその尿中への排泄速度はひじように早く24時間内にその大半を排泄することはこの両物質が水溶性ビタミンである特性であり，またこのことからも体内貯留による毒性の危険はきわめて少ないことがうかがわ

れる．ちなみにWeissら[11]によつてその急性および慢性毒性がひじように弱いと報告されている．しかしながら吸収率を考慮に入れない非経口投与時におけるPaOH投与後の尿中排泄がCa-PaA投与時に比して遅いことは体内におけるビタミンとしての作用時間が長いことを示すものと考えられ，PaOHはPaA同族体の投与型としてきわめて有利といえる．

経口投与のばあいは排泄速度のほかに消化管よりの吸収率および吸収速度が関連し，問題はさらに複雑となつてくるが，尿中排泄量から吸収率を推測すれば尿中24時間PaA排泄は非経口時と異なりCa-PaA投与群よりもPaOH投与群の方がはるかによくCa-PaA投与群の尿中に検出されなかつた分は糞中に見出された．このことはPaOH投与群の方が腸管における吸収率がよいことを示し体内貯留が長いことと合わせてPaAに比してビタミン剤としての評価がすぐれているように考えられる．

Ca-PaAの吸収率についてはRubinら[12]の報告によると消化管内にある食物の有無によつて相違するという．すなわち満腹時の吸収率はきわめて悪く空腹時はよいがPaOHのばあいはその相違があまりみられないといわれる．本実験の条件は完全空腹時とまではいえないが午後3時に飼料を与え翌朝9時に検体を投与したので一応空腹時とみてよく，このようなばあいにおいてもPaOHとCa-PaAとの間には大きな吸収率の差を示した．この吸収率の差は経度的吸収[13]のばあいにも認められるごとく，おそらくカルボン酸塩よりもアルコール型の方が組織透過性の強いためであろうと考えられる．

結　　論

PaOHとCa-PaAの吸収利用率をシロネズミを用いその尿中排泄量を指標として検べたところ腹腔内投与ではCa-PaA投与時よりもPaOH投与時の方が尿への排泄がやや遅く利用時間の延長がうかがわれた．また経口投与ではCa-PaA投与時はPaOH投与時に比して尿への排泄が約40%少なく吸収率が悪いことを示し，その不足分は糞中に見出されPaOHの吸収利用度の高いことを観察した．

終りに本論文のご校閲を賜わつた千葉大学林誠助教授，ご指導，ご鞭撻を賜わつた菊地英作部長，宮崎米雄課長に感謝いたします．

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Patol. Fiziol. Eksp. Terap. 10(2):85-86, 1966.
Desensitizing Effect of Calcium Pantothenate

Zver'kova-'66
Bib NO. 154

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The favorable effect of pantothenic acid in the treatment of those ill with allergic dermatoses (hives, eczema, dermatitis) is tied in with its antihistamine effect.

We carried out an investigation of the desensitizing effect of various doses of calcium pantothenate depending on the beginning of development of sensitization to dinitrochlorobenzene.

For sensitization, there was employed a chemically pure dinitrochlorobenzene (isomer 1-2-4), with the utilization of the drop method of N.S. Vedrov and A.P.Dolgov (1) in the modification of S.T. Pavlov (2).

Experiments were carried out on 25 white guinea pigs weighing 350 - 500 grams. 3 groups of animals were isolated. The pigs of the 1st group (10) received, intramuscularly, a 20% solution of calcium pantothenate over a period of 2 weeks prior to sensitization, and for 2 weeks in the process of sensitization. The animals of the 2nd group started to receive, intramuscularly, a 20% solution of calcium pantothenate after a lapse of 10 days following the application of the last sensitizing dose of dinitrochlorobenzene, i.e., already after a complete development of sensitization. The calcium pantothenate was administered over a period of a month.

Depending on the dose of calcium pantothenate, each group of animals was divided into 2 subgroups (each having 5 pigs). In subgroup A, the calcium pantothenate was administered in amounts of 4 mg/100 g, and in subgroup B -- in amounts of 8 mg/100 g.

The 3rd group (control)included 5 pigs that were only sensitized (there was administered, intramuscularly, instead of calcium pantothenate, a physilogical solution).

In the pigs of the 1st group (subgroup A), at the basic focus of sensitization, a reaction made its appearnce on the next day following the 2nd application of an allergen, and it was characterized by a moderate hyperemia and desquamation (scaling). Following a 3rd application of the solution, there appeared fine serous incrustations in the center of the focus. At the area of application of resolving doses of the allergen, there was noted a moderate hyperemia and an edema after 24 - 48 hours. After 1 month, sensitization, for all dilutions of dinitrochlorobenzene, was absent.

In the pigs of the 1st group (subgroup B), at the basic focus of sansitization, there was observed a scarcely noticeable hyperemia and a negligible furfureceous desquamation. Apparently, this should be considered as phenomena not of a sensitization, but of primarily toxic, dermatitis. Upon the application of resolving doses of the allergen, a moderate reaction to a dilution of 1 : 500 was obtained only in one quinea pig.

In the 2nd group of guinea pigs (subgroup A) that had received calcium pantothenate following a preliminary sensitization, control of the latter, after 3 days following the termination of treatment, disclosed a considerable degree of sensitization in all the animals. The same phenomena were noted in the animals of the 2nd group of subgroup B.

In the 3rd group of control animals, after 2 months, there was noted a certain extinction of sensitization, which was expressed in a diminution in the force of the reaction. After 3 months, the sensitization was retained but was weakly pronounced.

Prior to sensitization, and at the end of the experiment, a determination was made, in the animals, of the pantothenic acid content by an indirect method consisting in a reaction of sulfanilamide acetylation according to the method of Bratton and Marshall (3). In all the guinea pigs that had been sensitized to dinitrochlorobenzene there was noted a considerable reduction in the process of acetylation in comparison with the initial level by almost 50%,

in spite of the saturation with calcium pantothenate. The application of large doses of the latter (8 mg/100 g), prior to sensitization, protected the animals from suppression, in them, of acetylation, in comparison with the control (P 0.01).

Conclusions

1. Sensitization with dinitrochlorobenzene produces, in guinea pigs, a noticeable suppression in the process of acetylation; this indirectly points to the reduction in the content of pantothenic acid in the organism.

2. Preliminary saturation with calcium pantothenate (up to 8 mg/100 g in the course of 2 weeks prior to, and 2 weeks subsequent to, sensitization) markedly reduces the sensitivity of the pigs to their sensitization with dinitrochlorobenzene. Small doses of calcium pantothenate are less effective.

3. Calcium pantothenate, when administered to guinea pigs that have been sensitized to dinitrochlorobenzene, brings about the extinction in the increased sensitivity to this irritant.

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О ДЕСЕНСИБИЛИЗИРУЮЩЕМ ВЛИЯНИИ ПАНТОТЕНАТА КАЛЬЦИЯ

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Благоприятное влияние пантотеновой кислоты при лечении больных аллергическими дерматозами (крапивница, экзема, дерматиты) связывают с ее антигистаминным действием.

Мы исследовали десенсибилизирующее действие различных доз пантотената кальция в зависимости от начала возникновения сенсибилизации к динитрохлорбензолу.

Для сенсибилизации применяли химически чистый динитрохлорбензол (изомер 1—2—4) с использованием капельного метода Н. С. Ведрова и А. П. Долгова [1] в модификации С. Т. Павлова [2].

Опыты проведены на 25 белых морских свинках весом 350—600 г. Были выделены 3 группы животных. Свинки 1-й группы (10) получали внутримышечно 20% раствор пантотената кальция в течение 2 недель до сенсибилизации и 2 недели в процессе сенсибилизации. Животные 2-й группы начинали получать внутримышечно 20% раствор пантотената кальция спустя 10 дней после нанесения последней сенсибилизирующей дозы динитрохлорбензола, т. е. уже после полного развития сенсибилизации. Пантотенат кальция вводили в течение месяца.

В зависимости от дозы пантотената кальция каждая группа животных была разделена на 2 подгруппы (по 5 свинок). В подгруппе А пантотенат кальция вводили по 4 мг/100 г, в подгруппе Б — по 8 мг/100 г.

В 3-ю группу (контроль) входили 5 свинок, которых только сенсибилизировали (внутримышечно вводили вместо пантотената кальция физиологический раствор).

У свинок 1-й группы (подгруппа А) в основном очаге сенсибилизации реакция появилась на следующий день после 2-го нанесения аллергена и характеризовалась умеренной гиперемией и шелушением. После 3-го нанесения раствора появились тонкие серозные корочки в центре очага. На месте нанесения разрешающих доз аллергена отмечалась умеренная гиперемия и отек через 24—48 часов. Через 1 месяц сенсибилизация на все разведения динитрохлорбензола отсутствовала.

У свинок 1-й группы (подгруппа Б) в основном очаге сенсибилизации отмечалась едва заметная гиперемия и незначительное отрубевидное шелушение. По-видимому, это следует рассматривать как явления не сенсибилизационного, а первично токсического дерматита. При нанесении разрешающих доз аллергена умеренная реакция на разведение 1 : 500 была получена лишь у одной морской свинки.

Во 2-й группе морских свинок (подгруппа А), получавших пантотенат кальция после предварительной сенсибилизации, контроль последней через 3 дня после окончания лечения выявил значительную степень сенсибилизации у всех животных. Такие же явления отмечены у животных 2-й группы подгруппы Б.

У 3-й группы контрольных животных через 2 месяца отмечалось некоторое угасание сенсибилизации, выражавшееся в уменьшении силы реакции. Через 3 месяца сенсибилизация сохранялась, но была выражена слабо.

До сенсибилизации и в конце опыта у животных определяли содержание пантотеновой кислоты косвенным методом, сводящимся к реакции ацетилирования сульфаниламида по методу Браттона и Маршалла [3]. У всех сенсибилизированных к динитрохлорбензолу морских свинок отмечено значительное снижение процесса ацетилирования по сравнению с исходным уровнем почти на 50%, несмотря на насыщение пантотенатом кальция. Применение больших доз последнего (8 мг/100 г) до сенсибилизации предохраняло животных от угнетения у них ацетилирования по сравнению с контролем (P 0,01).

Выводы

1. Сенсибилизация динитрохлорбензолом вызывает у морских свинок заметное угнетение процесса ацетилирования, что косвенно указывает на снижение содержания пантотеновой кислоты в организме.

2. Предварительное насыщение пантотенатом кальция (по 8 мг/100 г в течение 2 недель до и 2 недели после сенсибилизации) резко снижает чувствительность свинок к сенсибилизации их динитрохлорбензолом. Малые дозы пантотената кальция менее эффективны.

3. Пантотенат кальция, введенный морским свинкам, сенсибилизированным к динитрохлорбензолу, вызывает угасание повышенной чувствительности к этому раздражителю.

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