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Hemorrhagic Encephalopathy Induced by Hypernatremia

9095.

I. Clinical, Laboratory, and Pathological Observations

CHARLES N. LUTTRELL, M.D., and LAURENCE FINBERG, M.D., Baltimore

Hyperosmolarity is characterized by an increase in the solute concentration of body fluids brought about by loss of water, inadequate water intake, or administration of large amounts of hypertonic solutions. When the osmolar concentration of the extracellular fluid is increased, transfer of water from cells to extracellular fluid exceeds that in the opposite direction. Net water movement is thus out of the cells, with resultant dilution of extracellular fluid and concentration of the fluid within cells themselves. If the solute which increases the extracellular fluid osmolarity is one which is in the main excluded from intracellular fluid, such as sodium and chloride ions, this disturbance in water distribution cannot be mitigated by movement of solute into cells. Hence, the ultimate effect of hypernatremia is dehydration of cells. This physicochemical disorder is most frequently encountered in infants and children with severe diarrhea and vomiting, but it also occurs in adults.

Schmidt,[1] in 1850, reported high chloride concentration in the serum of some infants with diarrhea, and the first description of this state is generally attributed to him. In the United States, clinical interest in hyperosmolarity was aroused by Rapoport[2] in the late 1940's. Several authors[3-8] have emphasized central nervous system disturbances in patients with severe dehydration and hypernatremia. The question is raised, therefore, whether central nervous system lesions cause hyperosmolarity, or whether hypernatremia produces central nervous system lesions. In these patients, the neurological symptoms were particularly evident when the biochemical disorder developed rapidly. Subarachnoid and subdural effusions certainly occurred in several infants,[5,7] suggesting that hemorrhagic lesions may be of primary importance in the encephalopathy associated with hypernatremia. Our purpose is to describe the clinical, laboratory, and pathological findings in three infants with neurological disorders due to severe dehydration and hypernatremia. This provides certain basic information for experimental studies on hyperosmolarity in cats, to be described in a subsequent paper.

Accepted for publication Nov. 6, 1958.

Kenny Foundation Scholar (Dr. Luttrell).

This investigation was supported by research grants B1409 and E1230 from the National Institutes of Health, U. S. Public Health Service.

From the Division of Neurological Medicine, The Johns Hopkins Hospital; the Pediatric Division, Baltimore City Hospital, and the Department of Pediatrics, The Johns Hopkins University School of Medicine.

Report of Cases

CASE 1.—Patient admitted Nov. 7, 1957; died Nov. 11, 1957.

An 8-month-old Negro boy was seen in the outpatient department with a history of vomiting and diarrhea of three days' duration. He appeared only mildly dehydrated, with signs of an upper respiratory infection and a temperature of 38.9 C (101.1 F). He was given penicillin and streptomycin intramuscularly. Administration of clear fluids by mouth was advised. During the following day, he vomited twice and had two loose stools, but urinary output seemed adequate. He did, however, look sick and dehydrated, and his fever was unchanged. There were no other abnormal physical signs of note. Spinal fluid was normal, and a blood culture subsequently proved sterile. He was given a trisulfapyrimidenes suspension (triple sulfonamides), more penicillin and streptomycin, and a hypodermoclysis of 250 cc. of one-fourth-

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NUTRITION REVIEWS

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SALT IN INFANT FOODS

The Subcommittee on Safety and Suitability of MSG and Other Substances in Baby Foods of the Food Protection Committee, Food and Nutrition Board, NAS-NRC, recently submitted a report entitled "Safety and Suitability of Salt for Use in Baby Foods" to the Food and Drug Administration. The major findings of the Subcommittee are summarized in this article.

In many areas of the world, infants are breast-fed during the first six to nine months of life and receive little or no solid food. This was true in the United States 50 years ago. However, as S. J. Fomon has pointed out, recent trends show a dwindling proportion of infants who are breast-fed, increasing numbers fed commercial formulas, and progressively earlier introduction of solid foods into the diet (INFANT NUTRITION, pp. 1—9. W. B. Saunders Co., Philadelphia, 1967). H. A. Guthrie has demonstrated that infants who begin to ingest solid foods during the first month after birth have consistently higher intakes of sodium in later life than do infants who begin to consume solids at later ages (AM. J. CLIN. NUTRITION 21, 863 (1968)).

Human milk contains 0.5 to 0.6 mEq sodium per 100 kcal, and infants meeting their protein and calorie needs from breast milk obtain about 1 mEq of sodium per kilogram per day. Most infants in the United States are fed on modified cows' milk formulas, with solid foods being introduced into the diet in the first or second month of life. By the fourth month, solid foods provide about 20 per cent and cows' milk 80 per cent of total calorie intake. The average sodium intake on this type of diet is about 30 to 35 mEq per day, or about 5 mEq per kilogram body weight per day. In general, no salt (sodium chloride) is added in commercial preparation of strained fruits and juices. Most strained meats, egg yolks, "dinners," "breakfasts," and soups contain about 0.4 to 0.6 per cent added salt strained vegetables and desserts from 0 up to 0.4 per cent, and dry cereals about 1.0 per cent.

A recent unpublished survey by R. Stewart, made to determine the sodium intake of infants, reveals that the sodium content of the diet (formula and supplementary infant foods) increases during the first year of life from about 2.2 to about 5.7 mEq per 100 kcal (at the fiftieth percentile of intake). The total sodium intake increases from about 9 to 60 mEq per day. In contrast, if the child satisfied all his calorie needs from food with the same sodium-calorie relationship as that of human milk, his total sodium intake would increase only from 2.6 to 6.5 mEq per day during this period. In this survey of 364 infants one to 12 months of age, eight ingested more than 82 mEq of sodium per day. Two of these were less than six months of age. The maximum intake recorded was 101 mEq per day. In another unpublished survey of 2,160 six-month-old infants selected on a random basis to provide a sample compatible with U.S. Vital Statistics, M. Soifer reports that the median daily intake of sodium was 35 mEq with a standard deviation of 15. Thus the upper limit of intake for 97 per cent of the sample does not exceed 65 mEq of sodium per day.

Although it is difficult to define the sodium requirement of the infant, it is

strength lactated Ringer's injection U. S. P. On the following day, fever, diarrhea, and vomiting persisted. He had become irritable and had not voided for six hours. He was therefore admitted to the hospital.

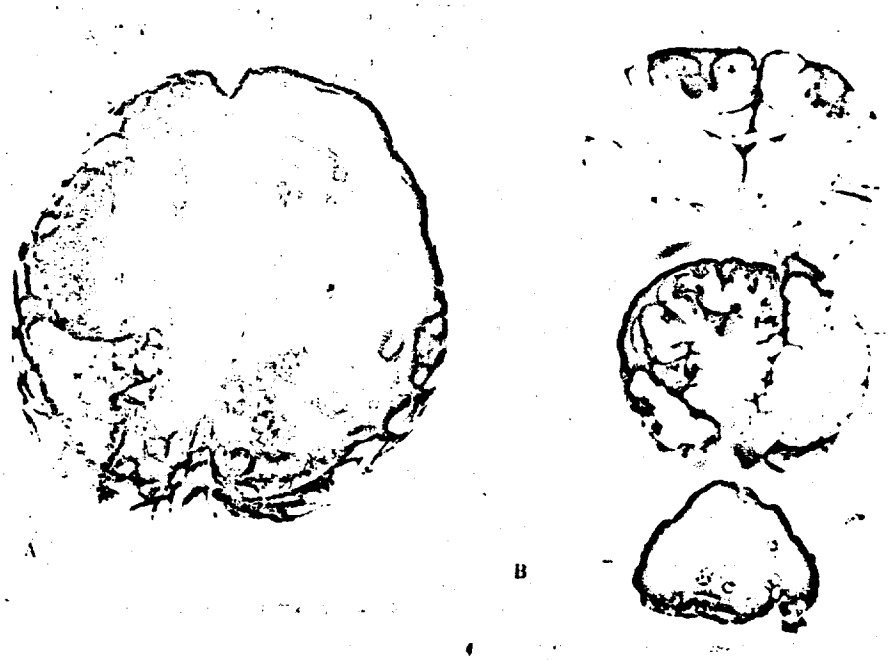
On admission, he was in no distress, but was mildly dehydrated. The vital signs were as follows: temperature 37.5 (99.5 F); pulse 126 and respirations 32, per minute, blood pressure 115/90; weight 8,300 gm. Except for mild dehydration, a depressed fontanel, and a liver edge palpable 2 cm. below the right costal margin, there were no abnormal physical findings. Laboratory studies showed hemoglobin 9.0 gm/ml.; white blood cell count 10,500; polymorphonuclear leukocytes 55%, and lymphocytes 43%. The urine was cloudy; specific gravity, 1.035; pH, 6.0; protein, 3+-4+; sugar, trace. Microscopic study (centrifuged specimen) showed 5-6 rbc per high-power field, and many hyaline and granular casts. Serum analyses showed NPN 70 mg/100 ml., sodium 159 mEq/L., CO_2 13.2 mEq/L., chloride 132 mEq/L., and potassium 5.5 mEq/L.

The infant took a milk formula well during the first 48 hours of hospitalization. He voided four times within the first 24 hours and five times on the second day. The temperature, however, remained around 38-39 C (100.4-102.2 F). Throat, blood, and urine cultures were negative. A nasopharyngeal culture showed a few colonies of hemolytic Staphylococcus aureus. Chest x-rays and an electrocardiogram were normal. The urine continued to show 3+-4+ protein, numerous white blood cells and red blood cells, but fewer casts. On the third hospital day he had a generalized convulsion. Despite intramuscular phenobarbital, he had another convulsion four hours later. Serum analyses at this time were as follows: NPN 80 mg/100 ml.; sodium 174 mEq/L.; chloride 127 mEq/L.; potassium 4.6 mEq/L.; CO_2 11.5 mEq/L.; glucose 240 mg/100 ml.; calcium 11.3 mg/100 ml., and phosphorus 5.7 mg/100 ml. Several hours later he began to breathe more rapidly, with a rate varying from 30 to 50 per minute, and had a pulse rate of 120-160 per minute. Rales were heard in the lungs. He was given one-fourth of the estimated dose of a digitalis glycosides preparation (Digalen) required for full effect. He voided only once that day, and the blood pressure stabilized at 120/60. A few hours later, however, he suddenly became hypotensive and died.

Autopsy.—The body was that of a well-nourished and developed boy, weighing 8,500 gm. There were numerous venipuncture sites in both femoral triangles. The organs were pale. No

Fig. 1 (Case 1).—*A*, dorsal view of the brain, showing bilateral subarachnoid hemorrhage of venous drainage areas. The hemorrhage is more marked on the right and is associated with swelling of the right cerebral hemisphere.

B, coronal sections of the brain, showing bilateral hemorrhagic softening, greater on one side than the other. The lowest section is the frontal poles.



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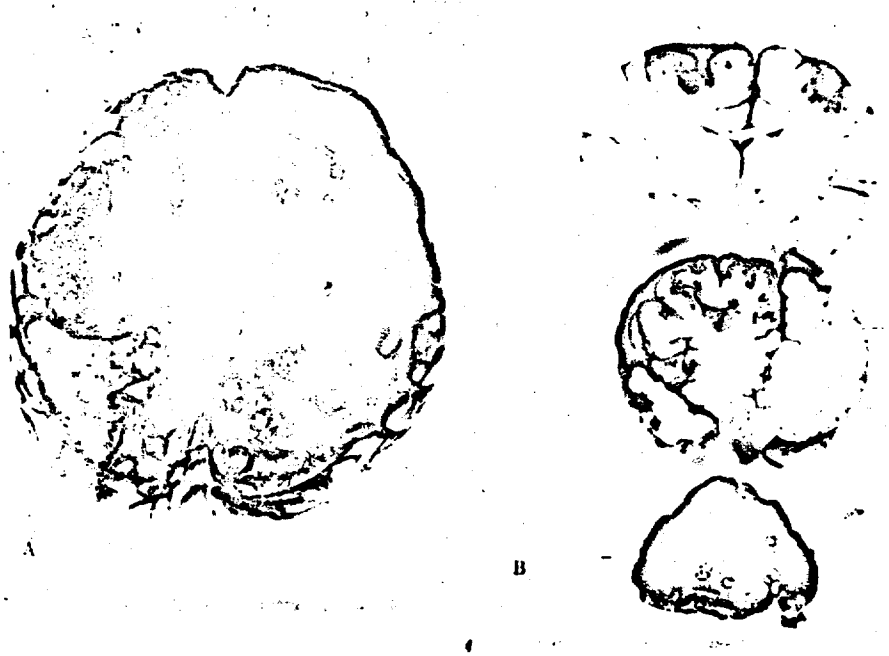

possible to specify with some confidence the needs for growth (1.0 to 1.5 mEq per day) and to assess average losses from the skin and stool (1.0 to 2.0 mEq per day). R. E. Cooke, E. L. Pratt, and D. C. Darrow (YALE J. BIOL. MED. 22, 227 (1950)) showed that concentrations in sweat of normal infants (five to 16 months old) ranged from 1.8 to 7.6 mEq per liter, with an average of 4.8. At an environmental temperature of 91 F., they calculated that the volume of sweat was between 45 and 65 ml. per kilogram per day. The maximum sodium a five-month-old infant (7 kg.) would lose by this route would be 3.5 mEq per day. Under more temperate conditions the loss would be about 1 mEq per day. Total needs, thus, are about 2 to 5 mEq per day.

During minor illnesses the diet is often substantially changed both in quantity and composition. Depending upon the nature of the illness and magnitude of extrarenal losses of sodium and water there will be considerable changes in requirements for both.

With the current patterns of feeding, one must be concerned with the infant's capacity to excrete the sodium load with which he is confronted. The four-month-old infant consuming cows' milk and a combination of commercially prepared infant foods ingests five times as much sodium as the breast-feeding infant; virtually all the excess is excreted in the urine.

The infant can ordinarily handle an acute load of sodium not exceeding 250 mEq per m^2 per day, assuming there is sufficient water available. It would appear that virtually any combination of milk and infant food that would meet the infant's calorie needs could be tolerated from the standpoint of sodium load. The highest levels of intake noted by Fomon, L. N. Thomas, and L. J. Filer, Jr., (J. PEDIAT. 76, 242 (1970)) and by F. A. Puyau and L. P. Hampton (AM. J. DIS CHILD. 111, 370 (1966)) were between 50 and 60 mEq per day. On an acute loading basis, these intakes would amount to no more than 175 mEq per m^2. However, those combinations of foods that impose the highest load of sodium may also impose large loads of other solutes requiring renal excretion.

The evidence associating salt intake and hypertension in man is inconclusive. There are a number of reports correlating average daily salt intake and frequency of hypertension among different cultures (e. g. L. K. Dahl, in ESSENTIAL HYPERTENSION—AN INTERNATIONAL SYMPOSIUM, P. T. Cottier and K. D. Bock, Editors, p. 53. Springer Verlag, Heidelberg, 1960; I. A. M. Prior et al., NEW ENGL. J. MED. 279, 515 (1968)). While these differences are in some measure suggestive, it is possible that dietary, cultural, social, and genetic factors may partially account for them.

The effect of salt intake on hypertension within defined populations has been shown to be quite variable. Dahl and R. A. Love (J. AM. MED. ASSN. 164, 397 (1957)) reported a positive correlation between salt intake and the prevalence of hypertension; N. Sasaki (JAP. HEART J. 3, 313 (1962)) showed a positive correlation between salt intake and the frequency of stroke. On the other hand, O. Paul found indications that hypertensives use less salt than normotensives (in THE EPIDEMIOLOGY OF HYPERTENSION—PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM, J. Stamler, R. Stamler, and T. N. Pullman, Editors, p. 248. Grune and Stratton, Chicago, 1967), and B. I. Ashe and H. O. Mosenthal (J. AM. MED. ASSN. 108, 1160 (1937)) found that distribution patterns of salt use (based on excretion)

among hypertensive and normotensive patients were similar. In the studies of Prior et al. (loc. cit.) and T. R. Dawber et al. (in THE EPIDEMIOLOGY OF HYPERTENSION, p. 255) no significant correlation was demonstrated between salt intake and blood pressure.

Observations, primarily in rats, indicate that progressive increases in sodium intake by the young animal are associated with increasing blood pressure. Dahl, M. M. Heine, and L. Tassinari (J. EXP. MED. 115, 1173 (1962)), by selective inbreeding of rats, developed two strains with opposite genetic susceptibility to development of hypertension. One showed progressive increase in levels of blood pressure with levels of intake of sodium exceeding 1.5 mEq per 100 kcal. The other was resistant at any level of intake. It is of considerable interest that the salt-sensitive strain voluntarily restrict their intake of sodium chloride (O. Wolf, Dahl, and N. E. Miller, PROC. SOC. EXP. BIOL. MED. 120, 301 (1965)).

It is clear that increasing salt intake of young rats can lead to development of measurable hypertension, that genetic factors are important variables in this response, and that the hypertensive effect is greater the earlier in life a continuous salt intake is begun. The mechanism for development of hypertension in animal models is not completely understood.

Some attention may need to be given to the relationship between sodium and potassium intakes of infants, both with respect to acid-base balance, and because there is evidence that potassium exerts a protective or ameliorative effect against hypertension on high sodium intakes.

The ability of the infant to excrete a potassium load (acute) is comparable to his ability to excrete a sodium load: 250 mEq per m^2 per day. In the studies of Steward and Soifer cited previously, the highest intake of potassium by normal infants amounted to no more than 200 mEq per m^2 per day, while the lowest intake was 30 mEq per m^2 per day, and thus they were well within homeostatic limits.

Regulation of sodium and potassium balance is primarily a function of the distal portion of the renal tubule. Potassium and hydrogen ions within the cells of the distal tubule are exchanged for sodium ions within the tubular lumen, thus the rate at which hydrogen and potassium ions are excreted is dependent on the amount of sodium available for exchange. When sodium excretion is at a low level, the capacity to secrete hydrogen and potassium ions becomes limited. Thus sodium ion concentration in tubular fluid can become rate limiting. There is no reason to believe, however, that the feeding of unsalted foods would result in critically low concentrations of sodium within tubular fluid.

In reviewing growth requirements of various mammalian species for sodium and potassium, R. L. Shaw and P. M. Phillips (THE JOURNAL, LANCET 73, 176 (1953)) concluded that the Na:K ratio (w/w) was important and that heavy ingestion of one element raised the requirement of the other. For several species these workers estimated that the ratio of dietary Na:K was between 0.5 and 0.25. Based on available information, the range of dietary Na:K ratios of infants receiving formula, cows' milk, and infant foods extends from 2.0 to 0.25.

A group of 150 normal infants fed human milk as the major source of calories, but with some calories provided as wet packed cereals or fruits, demonstrated normal growth and serum chemistries during the first four months of life (Fomon, Filer, Thomas, and R. R. Rogers, ACTA PAEDIAT. SCANDI-

NAV., Suppl. 202, 1970). This group represents that population exposed to a diet relatively low in Na^+ and relatively high in K^+. The opposite divergent situation, that of high Na^+ and low K^+, would rarely occur in a natural setting, simply because those foods that ordinarily supply Na also provide K.

Using the data of Fomon, Thomas, and Filer (loc. cit.) it can be calculated that the maximum sodium intake for a four-month-old breast-fed infant given solid foods was 36 mEq per day. If cows' milk replaced breast milk, the sodium intake would be 42 mEq per day. If unsalted infant foods were substituted for salted infant foods, the daily sodium intake would be 10 to 16 mEq per day. If infant foods with limited salt addition (0.1 per cent added sodium) were used, daily sodium intake would be reduced by 17 to 34 per cent, depending on the source of milk. The maximum sodium intake for the seven-month-old infants studied by Fomon et al. was 58 mEq per day. If infant foods containing added salt at 0.25 per cent were substituted for infant foods as currently salted, a reduction in sodium intake of 33 to 46 per cent can be calculated.

In essence, the committee has found no evidence that, insofar as healthy infants are concerned, addition of sodium chloride at current levels to infant foods is either harmful or beneficial to the infant. There is no valid scientific evidence available in support of the contention that addition of salt to infant foods contributes to development of hypertension or other disease states in adult life, nor is there valid evidence that the practice is not harmful, or that salt levels now consumed by infants in the United States overburden excretory mechanisms. Present salt intakes, however, provide substantially more sodium than is required by the infant. Thus, there is good reason to limit total intake. If we accept the proposition that infant foods are seasoned primarily to satisfy the mother's taste, then the addition of limited amounts of salt to processed infant foods may well be recommended, even though there is no good basis for recommending any given level.

It seems reasonable, therefore, to recommend that manufacturers add some salt to certain infant foods and that the upper level of added salt in such foods be 0.25 per cent. This level will result in sodium contents ranging from about 1 to 40 mEq per 100 kcal, depending on the food product. No increases should be made in salting those foods, such as fruits, that are now generally acceptable as currently prepared with less than 0.25 per cent added salt.

Iodized salt is not used in the preparation of commerical infant foods except for dry cereals, which are currently salted to the 1.0 per cent level with iodized salt. Limitation of salting to 0.25 per cent would, therefore, lower the iodide content of the cereal to one-fourth of the current level. If this reduction were shown to be undesirable, it could be remedied by direct supplementation of cereal with iodide rather than iodized salt.

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Guest Editorial

Overt and Cryptic Salt Poisoning: Recognition and Prevention

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THE SALT referred to in the title is common table salt, sodium chloride, a substance found in all life and necessary for its existence. It is a substance also capable of lethal toxicity, a fact often overlooked because of its very ubiquity and apparent innocence. Those who have used salt to melt ice on a sidewalk know what it can do to bordering grass and shrubs; veterinarians have described an encephalopathy in piglets who have overdone their stint at the salt lick; [4] finally the tragic substitution of salt for sugar in a hospital formula room during 1962 [1] dramatized the dangers to human infants.

This discussion will review first the physical and physiologic features of sodium chloride metabolism which provide the basis for understanding why poisoning may occur and how homeostatic mechanisms may be overwhelmed with resultant damage. A brief survey of the clinical situations where salt poisonings appear and description of the damage done will precede attention to establishing firm means of prevention.

A look at some of the physical characteristics of sodium chloride makes it clear why errors arise. The close surface resemblance of granulated salt to granulated sucrose can lead to the careless substitution of one for the other in homes and institutions. Pediatricians and infant nutritionists who measure water and sugar in household volumes often fail to realize that, volume for volume, salt has greater weight. The density of crystalline NaCl is 2.165, more than double that of water and appreciably more than that of sucrose. The granulated form of salt is admixed with air so that the standard measuring tablespoonful holds 22.5 Gm. or about 1.5 times a similar volume of water. Household spoons often hold more, and this excess can be magnified further by failure to level the measure. Stated another way, a level tablespoonful of table salt contains more sodium and chloride ions (about 350 mEq.) than does a 5 Kg. baby. A tablespoonful dose for a neonate is, on a weight basis, equivalent to about a pound for an adult.

The physiologic importance of sodium chloride is in large part related to the obligatory position of the bulk of these ions in the extracellular space, the "Millieu Interieur" of Claude Bernard. This sea within us acts as a transport medium for all metabolites, respiratory gases and cellular wastes. It has sodium and chloride ions for its chemical "skeleton," and these together account for over 80 per cent of the solute expressed as milliosmols. In contrast, there is very little sodium and, except for a few tissues, no chloride within the cellular fluid, in spite of the fact that cell membranes are freely permeable to sodium. The best explanation for this peculiar state of affairs, and in accord with the

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evidence thus far available, is that body cells extrude sodium ions actively whenever they enter. This "sodium pump," supplied by energy extruding sodium, paired with chloride by the demands of electroneutrality, and binding water by the demands of osmolar equilibrium, helps to maintain the volume of interstitial fluid between the cells and the plasma. Should a significant change in the total quantity of sodium and chloride within the body occur, their obligatory extracellular position will necessitate a redistribution of the fraction of total water located in each body compartment. This is true whether or not the total quantity of water is decreased (dehydration), increased (edema), or remains unchanged. The toxicity of sodium chloride, so far as is known, is entirely owing to these characteristics.

Under physiologic conditions, sodium metabolism is controlled principally by the kidney and by the adrenal cortex hormones which act upon the kidney and possibly upon other tissues. The secretion of antidiuretic hormone by the posterior pituitary influences renal excretion of water and thus to some extent electrolytes as well. It remains unsettled whether or not the brain has centers which affect directly the renal control of sodium. A very delicate homeostatic mechanism for sodium concentration control does reside in the brain though: thirst, which to be satisfied requires conscious action. Many more mechanisms seem to exist for conservation of sodium than for excretion of a large excess. From a teleologic point of view this is no surprise.

The most severe examples of salt poisoning have come from accidental substitution of salt for sucrose, but there have been and continue to occur many instances of salt overdosage of an iatrogenic nature. Some of these are direct and others are more subtle or even cryptic. An example of a direct instance is the prescription of salt-sugar-water mixtures for infants with diarrhea in which sometimes as much as two tablespoons of salt per quart of water have been recommended. Probably the maximum amount to prescribe should be one-fourth teaspoon per quart; yet even this recommendation may be misunderstood, misinterpreted or disregarded by the maternal "therapist." Accidents such as this are best avoided by not advising this sort of mixture at all. Even when sensible electrolyte mixtures are prescribed and when the instructions for dilution are followed properly, trouble may still ensue if no limit is placed on the quantity of the solution to be given. Hypernatremic babies, not yet seriously ill, are thirsty babies; they will drink salted solutions avidly to their detriment. Even dilute solutions (Na concentration equal 40–50 mEq./L.) can lead to hypernatremia if the stool water output is very dilute for sodium and sufficiently copious. In our experience this phenomenon has occurred more often among children of physicians than in any other group.

A more subtle way of poisoning with salt is the prescription of skimmed milk (cow's milk contains considerable natural sodium and chloride) for infants with diarrheal disease. The virtue of this therapy, if it can be called that, comes solely from the reduction in calories; fat seemingly is benign *per se* even if not digested. When infants are allowed to take a skim milk intake to satiety, the sodium load per calorie is very high. A sick infant is least able to cope with this because he has increased water losses by various routes and often has temporary impairment of renal concentrating ability. The high percentage of calories as protein compounds the insult further, since the excess production of urea will compete for a place under the renal osmotic ceiling. Parenteral fluid therapy may be as injudicious as oral therapy and with more rapidly disastrous results.

Hypernatremia without excess salt ingestion or retention is perhaps not properly called "salt poisoning." Nonetheless, as previously noted, the physiologic defect is much the same. An obvious example of relative water deficit is encountered in diabetes insipidus of either the pituitary or nephrogenic type. Already alluded to are those patients who do not respond to thirst either because of helplessness (normal small infant) or because of disturbance of consciousness as in head in-

juries, central nervous system disease, and psychoses.

Another group of diseases may predispose to hypernatremia as current modes of therapy in some clinics call for the administration of large amounts of sodium salts. Examples are salicylate poisoning treated by vigorous sodium bicarbonate administration, and diabetic coma treated without administration of glucose. It should be pointed out also that repeated infusions of hypertonic urea solutions may also lead to hypernatremia because of a solute-other-than-sodium diuresis. Finally certain symptoms in themselves lead toward a hypernatremic state. These are persistent fever (especially high grade) and hyperventilation.

The clinical signs of hypernatremia have been repeatedly stressed[2,3] but one sign of salt poisoning has only been mentioned in passing, namely, tachypnea and even dyspnea to the point where such infants are often thought to have respiratory disease. The actual damage done by the temporary redistribution of body water is predominantly to the brain. The severely salt poisoned infant has frequently had a very poor prognosis in so far as development is concerned. How much of this is caused by cerebral hemorrhage is not clear. The infants who have died of salt poisoning have all shown extensive hemorrhagic phenomena in the brain.[1] The kidney tubules are also damaged in salt poisoning. Here, however, the process seems reversible; at least survivors have no detectable sequelae.

A number of suggestions for prevention of salt poisoning and iatrogenic hypernatremia come from the foregoing discussion:

1. Sugar used in formula rooms should be dispensed from original packages.
2. When electrolyte mixtures are prescribed for diarrheal disease they should be packaged in such a way that a single sealed unit is dissolved in a standard volume of water to give the desired concentrations. In addition parents are warned to give only the quantity prescribed in 24 hours and that plain sugar water should be given to satisfy any additional thirst.
3. The use of skim milk feedings is to be avoided altogether. Calories can be cut by diluting the regular feedings with water and limiting the total to be ingested.
4. All fluid therapy can be planned to protect against salt excess, including treatment of such conditions as diabetes and salicylism.

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PATHOGENESIS OF LESIONS IN THE NERVOUS SYSTEM IN HYPERNATREMIC STATES

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I. Clinical Observations of Infants

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A RELATIONSHIP between hypernatremia and central nervous system disease has been documented by a number of authors beginning with the report of Allott.[1,2] The occurrence of disturbances of the nervous system during the course of hypernatremic dehydration in infants has been noted and the clinical significance stressed more recently.[3-6] The pathogenesis of the lesions of the nervous system has remained obscure and some confusion has arisen because injury to the central nervous system may give rise to a hypernatremic state. This may occur as a result of specific injury to the brain altering the regulatory mechanisms for homeostasis of water and electrolytes.[7,8] An altered state of consciousness after injury to the brain may lead indirectly to hypernatremia since the normal thirst mechanism cannot operate. Such patients are dependent upon their attendants who may underestimate water requirements; another type of dependent patient, the preverbal infant, may fare similarly.[2,8,9] There is reason to believe that hypernatremia may cause injury to the central nervous system and this aspect of the problem will be discussed here.

The purpose of the present communication is to extend the clinical observations previously made, by demonstration of the occurrence of subdural effusions and hemorrhage in infants with hypernatremic dehydration, and to add to the evidence that hypernatremia may be the cause of severe and irreversible brain damage. A second report[10] will deal with the anatomic and chemical changes in experimentally induced hypernatremia in animals. Seven infants have been observed in whom either subdural hematoma or hygroma was found during the course of an illness marked by the occurrence of hypernatremic dehydration. The salient features of these cases are summarized in Table I. The first case illustrates a number of important points which are detailed in the following case report:

CASE REPORT

History

M.B., a Negro male infant 5 weeks of age, was admitted to Baltimore City Hospitals on April 30, 1956. The parents were in good health as were three older siblings. There were no significant familial illnesses, and the patient had seemed normal before the present illness. He had been born uneventfully at Baltimore City Hospitals, weighing 4,475 gm. On April 28 the mother made the formula, consisting of 13 oz of evaporated milk, 17 oz of water and 2 tablespoons of sugar, while in the home of the patient's grandmother. It was subsequently learned that sugar and salt were kept in identical canisters and that salt had inadvertently been substituted for sugar. The patient took between 360 and 600 ml of this mixture during the next 12 hours before beginning to vomit. The excessive ingestion of salt is estimated to have been 30 to 40 gm or approximately 500 to 700 meq of sodium chloride. After the vomiting he became febrile and on the next day began to have labored breathing. Late in the evening of April 29 he was brought to the accident room of Baltimore City Hospitals because of these symptoms. It was not known at that time that the excessive ingestion of salt had occurred but the infant appeared sick and he was admitted with a diagnosis of respiratory infection.

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21: 180-181

TABLE I

Cases of Subdural Effusion or Hemorrhage Accompanying Hypernatremic Dehydration

Case No. Initials	Age and Sex	Prior Status	Etiology or Underlying Disease Process	Maximum Concentration of Sodium in Serum (mcq/l)	Clinical Features	Concentration of Protein in Cerebrospinal Fluid (mg/100 ml)	Results of Subdural Tap or Exploration	Remarks
1. M.B.	5 wk male	Thought to be normal; no convulsions	Salt poisoning (see text)	193	Stupor, convulsions (details in text)	100	Right 15 ml, left 6 ml; conc. protein 252 mg 100 ml; conc. Na 195 mcq/l	Convulsive disorder requiring medication; permanently and severely damaged (see text)
2. C.T.	11 mo male	Normal development; no convulsions	Diarrhea with dehydration	161	Coma, convulsions	60	4 or 5 ml of grossly bloody fluid; exploration showed no membrane or large hematoma	Convulsive disorder requiring medication; spastic quadriplegia; institutionalized at 7 years of age
3. M.A.	9 mo male	Motor development slow (did not sit up at 9 mo); no convulsions	Fever and anorexia	154	Convulsions	65	Subdural hygroma containing 60 ml. found after tap revealed fluid	Convulsive disorder requiring medication; did not sit up until after 18 months of age; seems probable that damage to central nervous system present before hypernatremic episode and was then aggravated
4. J.P.	8 mo male	Normal development; no convulsions	Diarrhea with dehydration	168	Hyperpyrexia, somnolence, spasticity of muscles; brief convulsion	126	12 ml of clear fluid by tap	Two subsequent convulsions; development "low average" at 2 years of age
5. R.J.	6 mo male	Motor development a little slow; could sit alone for an instant only; no convulsions	Fever and anorexia	154	Hyperpyrexia, convulsion	58	10 to 15 ml of clear fluid; no membrane found on exploration	No convulsions subsequently; slightly retarded but possibly unrelated to hypernatremic episode in view of limited intellectual capacity of others in family
6. M.A.B.	2 mo female	Thought to be normal; no convulsions	Diarrhea with dehydration	156	Somnolence, hemiparesis	72	5 ml of clear fluid by tap bilaterally	No convulsions; hemiparesis persists at 2 years of age
7. M.G.*	6 mo male	Normal development; no convulsions	Diarrhea and vomiting. Received vigorous intravenous therapy with one-sixth molar sodium lactate and saline during first day in hospital	182	Became lethargic and convulsed on third day in hospital	68	15 to 20 ml of xanthochromic fluid; no membrane on exploration	Patient reported to appear normal at time of discharge; no follow-up information can be obtained

* The data concerning this patient were supplied by Dr. Samuel P. Bessman of the University of Maryland who gave permission to include it in this publication. The first six patients were all treated and followed at the Baltimore City Hospitals.

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Physical Findings

The weight was 4,810 gm. Temperature was 39.2°C, pulse rate, 145 min and respiratory rate, 80 min. Respirations were labored and there was inspiratory stridor. He was lethargic but hyperirritable when stimulated and muscle tone was increased. There were bursts of tremors involving the muscles of the extremities and extraocular muscles. The peripheral circulatory status was excellent.

Course and Laboratory Findings

In the absence of an accurate history, the correct diagnosis was not appreciated and the patient was initially considered to have respiratory infection and managed accordingly. About 2 hours later a generalized convulsion occurred which was finally controlled with barbiturates. A lumbar puncture revealed clear fluid which contained no cells and proved sterile. The concentration of protein was 100 mg/100 ml and that of glucose, 50 mg/100 ml. Concentrations of chloride and sodium were 195 and 205 meq/l, respectively. Subdural taps were performed and 15 ml of fluid were obtained on the right side and 6 ml on the left. The fluid was clear, the concentration of protein was 254 mg/100 ml and that of sodium 197 meq/l. Concentrations of electrolytes in the serum are summarized along with subsequent values in Table II. Intravenous administration of fluids of low content of electrolytes was carried out during the next 3 days while the patient remained stuporous. On the fourth day he began to take a solution of glucose with added potassium chloride (3 meq/kg/day) by mouth and the next day he ingested a milk feeding with low content of electrolytes (Lonolac®) which was administered for 4 days before a more usual evaporated milk feeding was given. On May 7 he weighed 5,560 gm, a gain of 750 gm in 8 days. Table II also summarizes the output of urine in terms of volume, concentration of electrolytes and of total solutes. The urinary concentration of sodium remained rather low after collection of the initial specimen despite the persistently high concentrations of sodium in the serum.

The subsequent course has been that of a severely brain-damaged child. There have been recurrent convulsions which necessitate continuous anticonvulsant therapy. At 15 months of age he cannot sit up, muscle tone is markedly hypertonic and he makes no response to people. An electroencephalogram performed on March 22, 1957, was reported as grossly abnormal with continuous generalized seizure discharges consisting of very fast spikes. There were no normal patterns seen on an 18-electrode tracing.

DISCUSSION

The foregoing case illustrates that poisoning with salt with concomitant anorexia produces a state of hypernatremic dehydration with manifestations in the central nervous system similar to those which have been described in hypernatremic dehydration occurring with diarrheal (or other) disease in infants. The development of subdural hygroma in this infant as well as in six others is of particular interest from

TABLE II

SUMMARY OF FINDINGS OF ELECTROLYTES IN SERUM AND URINE OF INFANT M.B. (CASE 1)

Day	Concentrations in Serum					Volume and Concentrations of Urine				
	Na (meq/l)	Cl (meq/l	HCO_3 (meq/l)	K (meq/l)	Urea Nitrogen (mg/100 ml)	Volume (ml/day)	Na (meq/l)	Cl (meq/l)	K (meq/l)	Osmolarity (mosm/l)
1	193	157	23	4.8	—	40	188	155	74	720
2	161	135	17	4.2	48	120	96	84	20	446
3	—	—	—	—	—	285	70	54	6	249
4	161	127	—	—	32	—	—	—	—	—
5	—	—	—	—	—	771	28	19	34	230
6	154	115	20	5.6	26	—	—	—	—	—
8	142	105	—	5.3	16	432	10	8	26	195

both the clinical and theoretic point of view. In six instances the lesion was looked for after a convulsion; the other patient had hemiparesis. A subdural hygroma or hematoma has not invariably occurred in our experience with infants who have hypernatremic dehydration. The precise incidence of this complication is not known since subdural taps have only been performed in a small number of infants upon clinical indication. The very high incidence of abnormal concentrations of protein in cerebrospinal fluid in hypernatremic infants with clinical evidence of involvement of the nervous system has been documented.[4] Approximately half of the known hypernatremic infants have had sufficient manifestations clinically to warrant doing a lumbar puncture. During the period over which the first six cases of Table I were collected, 19 other infants with hypernatremic dehydration had examinations of cerebrospinal fluid which revealed abnormal concentrations of protein, and 22 infants with hypernatremia were seen in whom the cerebrospinal fluid was not examined. Previous experience has shown that dehydrated infants with normal concentrations of sodium in the serum may have abnormal findings in the cerebrospinal fluid but to a much lesser extent than those with hypernatremia.[4] To date we have not observed subdural effusions in dehydration without hypernatremia but no systematic program of subdural taps has been attempted, nor does it seem justified.

In the group of cases reported here neither needle aspiration nor surgical exploration has been shown to have any clear therapeutic benefit. For the present it would seem that each patient should be assessed individually with respect to the proper clinical management of the lesion of the nervous system until such time as criteria for paracentesis and/or surgery can be established. In a previous report[4] two fatalities with subarachnoid hemorrhage were cited as were four cases of permanent neurologic residua in previously normal infants who developed hypernatremic dehydration. The fact that moderate to severe residual damage is noted in six of the seven patients in the present study and that in three of these the infants were previously thought to be normal, further indicates the importance as well as the severity of the damage incurred.

The origin of the symptoms related to the central nervous system and of the findings just described can be conveniently discussed under three headings, the cellular changes or chemical anatomy of the nervous system, changes in concentrations of ions in the extracellular fluid which might affect excitability of nerves and, finally, factors which might predispose to vascular lesions and hemorrhage, i.e., gross anatomic changes. Changes in chemical anatomy at the level of the cell and its immediate environment is a very complex subject, but a few of the factors which are involved in hypernatremia are pertinent here. An increase in the extracellular concentration of an ion such as sodium, which has limited net entry to water within the cell, necessitates osmotic readjustment between the cellular water and the interstitial fluid. There are at least three mechanisms by which this may be accomplished: water may leave the cell; sodium ions may increase in concentration within the cell; there may be a change of cellular constituents to increase osmolar concentration either by dissociation of bound electrolytes or by breakdown of complex polyvalent ions. There is reason to believe that all of these mechanisms take place in the brain, particularly the first and last mentioned. Experimental studies bearing on this point will be presented in the second portion of this work.[10] Hyperosmolarity per se without concomitant shifts of water can be produced by injection of a nontoxic substance which freely permeates the cell (e.g., urea) and does not seem to produce symptoms related to the central nervous system.[10]

Changes in concentrations of specific ions in the extracellular fluid may have a bearing on excitability of nervous tissue. It is difficult to separate any pharmacologic effect of

the sodium and chloride ions from their osmotic roles which have been previously discussed. Changes in extracellular concentrations of potassium are very variable in these patients[1-6] and seem unlikely to be involved in the major symptomatology. An interesting finding has been the frequent hypocalcemia.[3, 4] This disturbance may be aggravated by poor renal excretion of phosphate but has been shown to be independent of hyperphosphatemia.[1, 11] Experimental studies have indicated that loading with sodium in the presence of concomitant cellular deficit of potassium leads to a moderate hypocalcemia.[11] This disturbance, while it may contribute to the manifestations related to the central nervous system in hypernatremic dehydration, is not always or even usually present in patients when the manifestations are the most severe.

Finally, gross anatomic changes in the region of the brain may occur, as has been shown by the case material discussed. Girard[12] produced intracranial hemorrhages in experimental animals by the injection of hypertonic solutions of sodium chloride and these observations have been repeated and extended in our laboratory.[10] The mechanism is incompletely understood. Girard[12] believes that reduction in intracerebral pressure is the key step in the pathogenesis of the hemorrhages. Whatever the mechanism, it is apparent that symptoms and signs often occur before such gross changes are present and, indeed, without having them occur at all. The patients discussed in this report, however, all did have evidence of anatomic lesions and it is perhaps noteworthy that the residual damage was unusually severe. In view of the variety of mechanisms known to produce symptoms referable to the central nervous system in hypernatremic dehydration, it is not surprising that the time of occurrence varies from early in the illness to the point of near recovery from dehydration.

Manifestations in the central nervous system may accompany dehydration because of circulatory disturbances regardless of the concentration of sodium. While it has often been stated that subdural hematoma may complicate dehydration without specification of the type of disturbance of electrolytes, it has been our experience that such dehydrated infants have been hypernatremic. While this may not be invariably true, the knowledge of preponderant occurrence is clinically valuable. Likewise, one does not expect every patient with hypernatremic dehydration to have demonstrable effusions nor is there close correlation with the degree of hypernatremia;[4] the rate of development of the disturbance is probably important and contents of electrolyte and water, as distinguished from concentrations, may be determining factors which are not easily assessed from usual clinical data. The important thing to the clinician at present is the existence of correlation between hypernatremia and lesions of the central nervous system and as much understanding of pathogenesis as present data afford.

SUMMARY

Seven infants with hypernatremic dehydration developed subdural effusions or bleeding. One of these was a previously normal infant accidentally poisoned by excessive ingestion of salt. This infant and five of the others had significant neurologic damage, ranging from moderate to severe. It appears that the hypernatremic dehydration was responsible for the subdural lesions in these patients and responsible for permanent damage in at least three of them. Changes in chemical anatomy and lesions of the cerebral vessels are discussed with regard to producing lesions of the central nervous system in hypernatremic dehydration.

At present no salutary therapeutic approach to the complication of subdural effusions occurring during hypernatremic dehydration has been found.

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Renal Excretion of Sodium During Hypernatremia

Experimental Observations

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Infants with hypernatremic dehydration are sometimes oliguric and as part of the physiologic disturbances of the hypernatremic state, the excretion of a urine with a concentration of sodium in excess of the plasma is not possible. Return to physiologic status requires the addition of water. These observations suggest that there may be impairment of renal ability to excrete sodium. The studies reported herein were undertaken in part to examine the effects of severe experimental hypernatremia upon renal morphology and function. Details of the resultant histologic changes have been reported separately.[1] Results of renal function studies and discussion of their possible significance are presented here.

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Methods

Extracorporeal dialysis for five or more hours with a twin coil Kolff "artificial kidney" was used to create hypernatremia in dogs with functioning intact kidneys. This technique permits study of renal response during periods of constant extracellular electrolyte concentrations with no possibility of even partial correction of the induced abnormality. Large (18-22 kg) female mongrel dogs were anesthetized with sodium pentobarbital, given heparin, and connected to the primed dialysis coil with flow rates in the extracorporeal circulation of 200-300 ml/min. Fig 1 is a diagram of the experimental arrangement. Because half a coil suffices for an animal of this size, it was convenient to do two experiments at once. Throughout the run heparin (1 ml p.r.n.) was added to the circulation. The animals were kept lightly anesthetized with sodium pentobarbital, and in addition, muscular activity was prevented by constant infusion of a curare-like drug, gallamine (Flaxedil) triethiodide.

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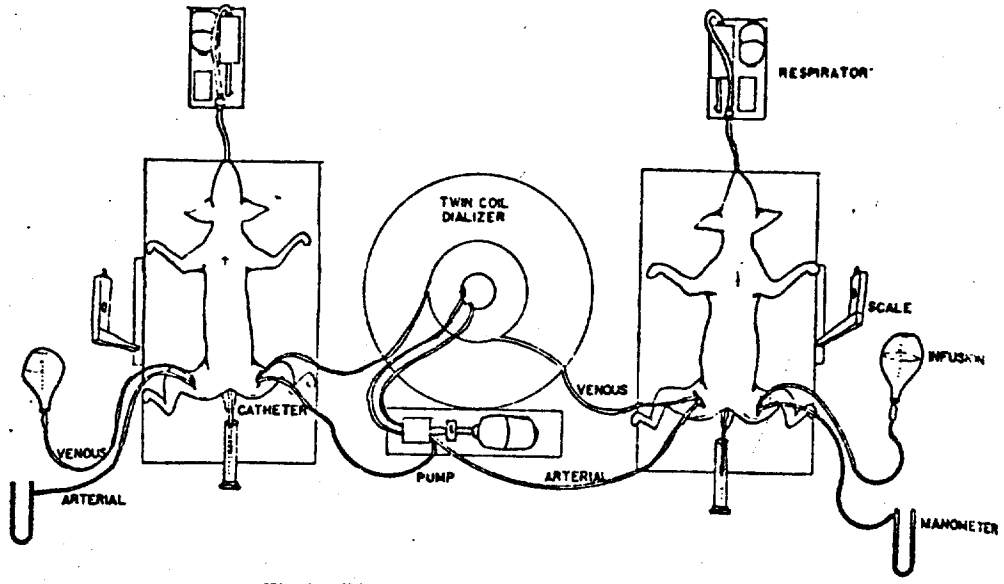

Fig 1.—Diagram of the experimental arrangement.

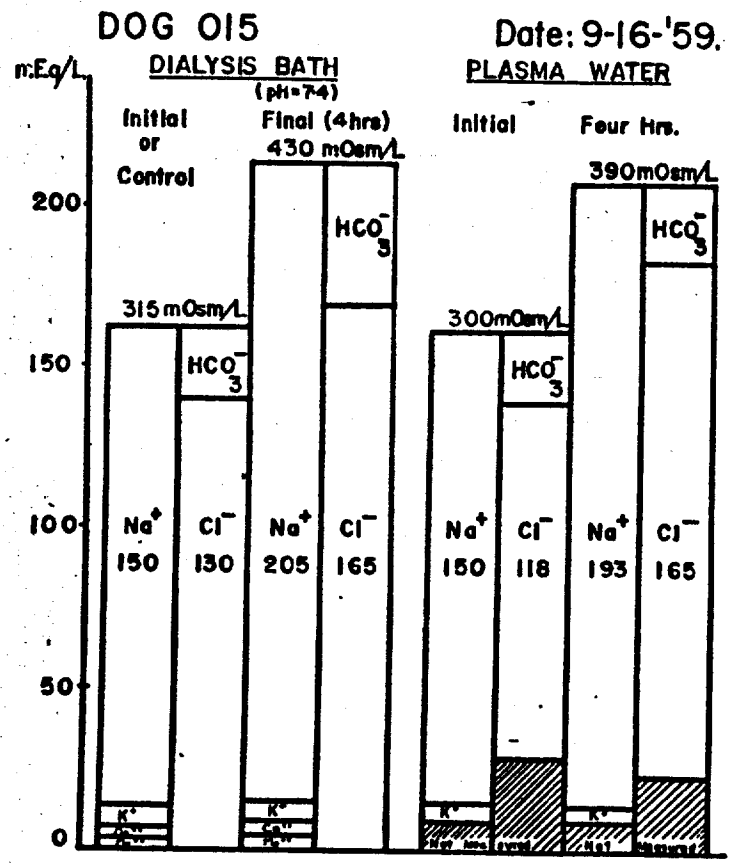

Fig 2.—Diagram of the composition of the dialysis solution and plasma at outset and at achievement of steady state hypernatremia.

Respirations were maintained by a mechanical respirator.

The dialysis bath for control periods had the following ionic composition in mEq/liter: Na 150, K 4, Ca 3.6, Mg 1.1, Cl 136.5, and HCO_3 26.8. To produce hypernatremia 380 gm of NaCl and 186 gm of $NaHCO_3$ were added to the bath, increasing the Na by 87 mEq/liter, the chloride by 65 mEq/liter, and the HCO_3 by 22 mEq/liter. Fig 2 summarizes the composition of the bath fluid for the control period and after dialysis equilibrium had occurred with the high sodium bath. The values in the diagram are based on actual analyses at the times indicated, and therefore represent lower concentrations of sodium than are present immediately after the addition of the supplemental sodium salts. In other words, the excess sodium has been distributed through the water of both the bath and the experimental animals at the time of the analyses shown.

Since this apparatus not only allows for osmosis and diffusion, but also applies hydrostatic pressure within the coil, a net transfer of water from plasma to the dialysis bath results. In one group of nine animals body water volume was maintained constant by the intravenous infusion of a solution similar in composition to the bath (ie, 200 mEq/liter of sodium when the bath contained such a solution and 150 mEq/liter during control periods; chloride and bicarbonate were the anions). Each such animal was paired with another in which body water loss was permitted to proceed without replacement. This permitted comparison by hypernatremia with constant body water volume with hypernatremic dehydration. The animals were suspended on a scale which made it possible to maintain weight or to calculate the degree of water loss.

Urine was collected throughout the experiment by means of an indwelling catheter. Addition of creatinine to the dialysis bath elevated plasma creatinine to a constant level and permitted determination of an exogenous creatinine clearance. Mean arterial blood pressure was continuously measured by means of an arterial cannula connected to a manometer. In all animals serial analyses of plasma and urine were carried out for osmolality, electrolytes, and creatinine. Sodium and potassium were measured by an internal standard flame photometer; osmolality by a Fiske osmometer; chloride by a Cotlove chloridometer; creatinine by the method of Hare and Hare,[2] and urea by the method of Archibald.[3]

Nine additional animals were dialyzed for a similar period against a control bath containing an electrolyte solution resembling plasma water in its composition. These animals were maintained at constant volume by an intravenous infusion of [illegible]tonic sodium bicarbonate-saline solution (Na, [illegible]Cl, 120; HCO_3, 30 mEq/liter). The control [illegible] was always used initially; in the hypernatremic [illegible]ps the additional sodium chloride and bicarbonate was added gradually over the next hour. The pH was kept constant by the addition of lactic acid and by the bubbling of 5% CO_2 in oxygen into the bath. On the other side of Fig 2 are analytic values for the plasma of one of the dogs at the outset and after four hours showing attainment of nearly complete equilibrium with the bath. Intravenous pitressin was given (1.2 units per hour) to three control and three hypernatremic animals to see if an effect could be demonstrated under these conditions.

Results

Limitations in the ability of the kidney to excrete sodium in sufficient quantity to restore physiological levels occurred in both groups of hypernatremic animals. In the constant volume group urine output rose as in an osmotic diuresis and urine osmolality coincidentally fell as expected. Table 1 shows data collected from a representative experiment. The columns showing sodium concentration in plasma and in urine are side by side in the center. It can be seen that sodium concentration in the urine in this experiment always remained less than that of the plasma.

TABLE 1.—*Data From Constant Weight—Hypernatremia Experiment: Dog 019, Oct 21, 1959*

	Plasma		Urine			
Time, Sec	mOsm/Liter	Na mEq/Liter	Na mEq/Liter	mOsm/Liter	Vol Ml/Min	Creatinine Clearance Ml/Min
Start	315	155	48.1	1770	—	—
75	340	180	18.3	1310	0.2	35
255	404	196	110	950	1.8	36
430	445	214	180	780	11.8	42
640	480	215	170	746	4.0	23

The sodium concentrations for plasma are mEq/liter of plasma water corrected by a Donnan factor of 0.96 to give the concentrations in extracellular fluid.

TABLE 2.—*Comparison of Mean Urine Output Between Hypernatremic Groups and Controls: Urine Volume (Ml/Min)*

Dogs (No.)	0-120 Min	120-240 Min	+240 Min
Constant wt (9)	1.0	5.5	7.4
Dehydrated (8)	0.6	1.2	0.4 *
Controls (9)	0.3	0.4	0.5

* All developed severe oliguria terminally.

This occurred in eight out of nine of these experiments. Data on the osmolality of plasma and urine, on urine volume per minute, and exogenous creatinine clearance are also shown in this table.

Table 3 shows data on the nine hypernatremic dogs maintained at constant weight by infusion. The values given are for the one-hour period beginning 30 minutes before the concentration of sodium in the plasma was attained. The urine samples were collected in such a way as to permit this calculation. This arbitrary point was chosen because of interest in severe hypernatremia. Table 4 summarizes the concentrations of total solute and sodium during the period of highest sodium concentration in plasma for all three groups. The mean concentration and the range are given for each category. It can be seen from the concentration in urine and plasma (U/P) ratios for sodium that the constant water volume-hypernatremic group usually excreted a urine with a lower concentration of sodium than that found in the plasma. Thus, the high rate of output of urine while making possible the excretion of a large absolute amount of sodium tends to aggravate the hypernatremia by removing

TABLE 3.—*Renal Function Studies During Period of High Concentration of Sodium in Plasma in Constant Weight Dogs*

Dog	Osmolality, mOsm/kg of Water		Sodium, mEq/Liter			Ml/Min				
	Plasma	Urine	Plasma H_2O	Urine	U/P	Urine Flow	C_{osm}	$C_{x_{20}}$	$Tc_{x_{20}}$	C_{crest}
003	390	485	183	118	0.65	0.70	0.87	—	0.17	45
004	408	520	194	215	1.11	2.45	3.12	—	0.67	34
006	405	352	202	188	0.93	3.77	3.28	0.49	—	40
015	390	304	193	151	0.79	7.0	5.46	1.54	—	34
017	403	504	197	108	0.55	5.1	6.39	—	1.29	60
019	450	746	219	170	0.78	4.0	6.63	—	2.63	23
021	413	668	205	194	0.95	7.53	12.2	—	4.67	33
023	406	614	195	184	0.94	10.0	15.8	—	5.80	40
025	440	408	187	170	0.91	1.43	1.2	0.23	—	17

The dehydrated animals had a much lower urinary output which became vanishingly small after 8% weight loss occurred. Table 2 compares mean urine output at varying time intervals for the three groups of animals. The dehydrated group excreted relatively little sodium simply because of the comparatively low output which becomes close to zero after 8% weight loss.

TABLE 4.—*Comparison of Mean Plasma Water and Urine Osmolality and Sodium*

Dogs (No.)	Osmolality mOsm/Liter		Sodium mEq/Liter		U/P Na
	Plasma	Urine	Plasma Water	Urine	
Hypernatremic (17) (Time of Highest Na)					
Constant wt (9)	412 (390-450)	542 (304-746)	197 (183-219)	166 (108-215)	0.87 (0.55-1.11)
Dehydrated (8)	414 (396-447)	688 (300-860)	198 (192-223)	204 (36-280)	1.20 (0.4-1.6)
Controls, (9) 4-6 hours	300	803 (490-1236)	150	118 (13-165)	0.70 (0.1-1.1)

Sodium concentrations in plasma are corrected by a Donnan factor of 0.96.

proportionately more water. In the dehydrated group the U/P ratios are a little higher on the average but output becomes very small as oliguria supervenes which prevents excretion of significant amounts of sodium.

When pitressin was given to hypernatremic animals no effect on urine osmolality or volume was demonstrated. On the other hand urine osmolality rose significantly in the control animals. In neither group was there a change in mean arterial blood pressure during pitressin administration. This difference was interpreted as indicating maximum endogenous production of antidiuretic hormone (ADH) and maximum response in the hypernatremic animals.

Comment

Hypernatremia without dehydration results in an expanded extracellular fluid volume, decreased cell water, and initiates an osmotic diuresis. Studies on the dog [4-6] and on man [7,8] show that under conditions of osmotic loading with a variety of solutes the urine osmolality progressively falls toward that of the plasma with increased urine flow, if the initial urine was concentrated. The animals studied here exhibited a similar phenomenon. On the other hand it is known that under certain conditions animals [9] and humans [10,11] can excrete urine with concentration of sodium not only greater than that of plasma but greater than those found here. Those conditions, however, involve either hypovolemia with moderate oliguria or sudden expansion of body volume by loading for brief periods. In the present experiments there was a constant body water volume and a rather severe and sustained unphysiologic state.

A separate report has described the histologic changes found in the kidneys of these dogs.[1] Briefly, all but one of the hypernatremic animals whether dehydrated or not showed diffuse necrosis of the proximal and distal convoluted tubules. Inasmuch as the technique utilized prevented systemic hypotension until the terminal moments of each experiment, the changes observed do not appear to be due to generalized circulatory failure. Exogenous creatinine clearances indicate that filtration rate was usually well maintained during the run as shown in the data tables. In experiments in which hyperosmolality of the same magnitude was induced by urea and in which other conditions were the same, no necrosis resulted. The possibility of some degree of renal ischemia occurring cannot be eliminated from consideration but it seems clear that whatever else may be involved, hypernatremia is a potentiator of the lesion.

These studies indicate that during severe hypernatremia of some hours duration, the dog is unable to excrete a urine which might significantly alleviate the physiological disturbance. During severe hypernatremic dehydration the concentration of sodium in urine may tend to reduce the plasma concentration but oliguria prevents significant excretion of sodium. In most instances the hypernatremia would actually be aggravated by the type of urine excreted. These results resemble the observations on infants with salt poisoning who excrete dilute urine in the face of hypernatremia.[12,13] They suggest that the effect of severe hypernatremia on the kidney tends to perpetuate or worsen the level of hypernatremia. This reaction may possibly have immediate survival value but if prolonged may prevent return to physiologic equilibrium.

Present concepts of the renal concentrating mechanism involve an osmotic gradient within the renal papilla maintained by a countercurrent exchanger dependent upon active transport of sodium at loci along the nephron.[14] While it is interesting to speculate on how such a system might be affected by severe hypernatremia, the data presented offer no clue as to the details of the mechanism involved. The data would seem to offer some indication as to why hypernatremic dehydration is difficult to correct by the administration of the usual amounts of parenteral fluid, particularly if some sodium is included. Renal conservation of water may be precluded and extrarenal water losses almost always contain less sodium than plasma. The need for

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exogenous sodium-free water in situations of salt excess is apparent. If renal injury has resulted, the nonadaptive state may be prolonged and the hazards of injudicious fluid therapy are even more pronounced. Questions concerning specific optimal therapy for human infants with more common types of hypernatremic dehydration are not answered by these data.

Summary

By means of extracorporeal dialysis a steady-state severe hypernatremia has been induced in dogs. In one group, body weight was held constant by intravenous infusion of a solution resembling the dialysis bath; in another dehydration was permitted. Renal function studies showed a failure to concentrate sodium in the urine to levels greater than in the plasma water. Since extrarenal water losses are even more dilute with respect to sodium, hypernatremic states, in the absence of exogenous water, may be self-perpetuating.

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SALT AND BLOOD PRESSURE

It has been suspected for several years that excess salt intake may predispose to hypertension. In 1940 Grollman and co-workers showed that certain sterols induced hypertension in animals only when additional salt was given. Meneely and co-workers found that chronic ingestion of salt uniformly produced hypertension in rats which was clinically similar to that in humans.

Modern man's habitual daily salt intake probably is well in excess of normal needs. It has been estimated that most individuals can maintain adequate salt balance with less than 1 gm daily. The salt intake of a population group can be estimated roughly by measuring 24 hour urinary excretion. Some people and some population groups appear to crave salt; such cravings are probably acquired, not innate.

A study of 1,346 people at Brookhaven National Laboratory, Upton, New York, showed direct correlation between salt intake and hypertension. Studies of salt intake in different geographic areas have been even more revealing. Natives of the Marshall Islands eat an average of 7 gm of salt daily, while northern Japanese ingest 26 gm. The incidence of hypertension is 9 percent among the former and 39 percent among the latter.

A group of New Zealand physicians has recently completed a study of the populations of two Polynesian islands whose natives are of the same ethnic origin. They obtained careful household dietary histories, measured blood pressures, and calculated 24 hour urinary outputs. The mean daily dietary intake of sodium in Rarotonga was 130 mEq, and that in Pukapuka 60 mEq. The Rarotongans, accustomed to eating salted canned beef and adding salt to cooked foods, had higher mean blood pressures.

The reason for the apparent relationship between hypertension and salt intake is not known. Ion movements induce acute changes in peripheral vascular tone. Intracellular and extracellular concentration of cations plays an important role in regulating blood pressure. The sodium content of the walls of large arteries and the aorta is increased in experimental hypertension. Abnormally high sodium intake may induce or exacerbate some forms of hypertension in humans.

HOSPITAL UNIT DESIGN

When the Methodist Hospital in Rochester, Minnesota, was developed, the plans provided for a study of alternative designs for nursing units, hospital systems and organization. With the aid of a federal grant, the hospital included units classified as single corridor, double corridor, radial intensive care, and radial pediatric, and a single corridor dermatology unit.

The sociologic studies covered such problems as the subjective feelings of the nursing staff and measurements of fatigue, tension, stress and anxiety. The radial design was judged superior to the single corridor on 85 percent of the comparisons and superior to the double corridor on 60 percent. The double corridor was rated superior to the single corridor on 65 percent of the comparisons. Measurements were made of the distance nurses traveled in their work and the time of travel. Some consideration also was given to use of the time saved, and comparisons were made of the reactions of registered nurses, licensed practical nurses, ward clerks and nurses' aides.

In general the radial units were found superior to other units. Staff absenteeism was definitely less and fewer accidents occurred on the radial services. Patients' relatives and physicians tended to make fewer complaints to the nursing staff on the radial units.

Such studies contribute significantly toward solving problems of hospital design and its effects on personnel and costs.

MORRIS FISHBEIN, M.D.

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THE PERMEABILITY OF THE HUMAN PLACENTA TO SODIUM IN NORMAL AND ABNORMAL PREGNANCIES AND THE SUPPLY OF SODIUM TO THE HUMAN FETUS AS DETERMINED WITH RADIOACTIVE SODIUM

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TO OBTAIN comprehensive information on the permeability of the placenta, one should be in a position, ideally, to measure its permeability to many and diverse test substances. These substances should be closely related chemically and physically to those constituents of the maternal plasma which are normally transferred across the placenta from mother to fetus. The availability of radioactive and stable isotopes, together with exquisitely sensitive and reliable methods for their quantitative measurement, has furnished a method which satisfies this criterion. Because of the chemical identity of these isotopes with their naturally occurring counterparts in the body, we are assured that their biological behavior is normal when the precaution is taken of using radioactive substances in amounts substantially less than that known to produce radiation effects. A molecule may be identified or "marked" with an isotope (e.g., in "heavy water," hydrogen of atomic weight 2 replaces its isotope, hydrogen of mass 1, found in ordinary water) and the molecule subsequently traced by detecting the isotope. Or the isotope may be studied as an ion (e.g., Na^{24} which is radioactive and which is detected by one of several instruments used for measuring radioactivity).

The investigations to be reported here had as their objective the measurement of the permeability of the human placenta to sodium, primarily in normal cases, and, as occasion permitted, in the presence of disease. The observations have been made in twenty-seven women in whom pregnancy was terminated by abdominal hysterotomy before the period of viability or by cesarean section or pelvic delivery at or near term. Ten of these cases were presented in a preliminary report.[1] We entered upon this program with a background of studies on the placentas of animals representing each of Grosser's four morphological types[2]; we are able, consequently, to consider the results on man from the viewpoint of comparative physiology, and to relate them to the results obtained from other members of the hemochorial group. As in the animals studied, we shall also be interested, at various stages of pregnancy, in the amount of sodium supplied to the human fetus as this is related to the fetal need for sodium during growth.

Rationale and Methods

When a radioactive isotope is used in biological research it is mixed with a preponderant quantity of its naturally occurring, nonradioactive isotope; the radioactive substance is employed to tag or label the naturally occurring substance so that the latter may be distinguished from that already present in the body. Thus when radioactive sodium (Na^{24}, i.e., Na with an atomic weight of 24) is used to study the behavior of sodium (Na^{23}) intrinsic to the body, the Na^{24} is present in the sample in an infinitesimally small amount as the chloride together with a weighable amount of ordinary NaCl. The mixture of the two isotopes is referred to as tagged or labeled sodium, and will be designated by the symbol Na.* In practice, a sample of tagged sodium is weighed and the radioactivity measured in terms of the radiation or counts emitted per second; if the radioactivity of any part of the sample is subsequently known, it is then possible to calculate the corresponding weight of the tagged sodium which is present. We shall present our data in terms of tagged sodium; it will be evident that this refers to sodium (Na^{24} plus Na^{23}) introduced into the body and is not to be confused with the sodium intrinsic to the body. The quantities of tagged sodium used in our experiments were kept as small as were compatible with precise measurements of their radioactivity and were so minute in comparison to the normal sodium content of the body that they are properly called tracer quantities (see Table I).

The fundamental assumption in the use of a radioactive isotope to measure placental permeability is that the radioactive substance crosses the membrane precisely as does its naturally occurring isotope, and therefore, the two isotopes cross the placenta in the same proportion as is found in the fluid bathing it. In the case of the placenta this means that:

$$\frac{Na_{mp}}{Na^*_{mp}} = \frac{Na_f}{Na^*_f} \quad (1)$$

where Na_{mp} refers to the concentration of normally occurring sodium in the maternal plasma, Na^*_{mp} to the concentration of tagged sodium in the maternal plasma; and Na_f and Na^*_f are the quantities of the corresponding substances transferred to the fetus during the time of an experiment.

Our experimental data have as a first aim the evaluation of Na_f. This quantity divided by the weight of the placenta will be taken as a measure of placental permeability to sodium. Na_f can be calculated from equation (1) if the other three quantities are known. The concentration of sodium in human plasma, Na_{mp}, has been frequently determined, and has an average value of 3.3 mg. per 100 c.c. of plasma.[3] The evaluation of Na^*_{mp} is less direct. After injection of a solution of Na*Cl into a maternal vein the concentration of Na* in the plasma rapidly diminishes due to its passage into the extracellular fluid of the mother. Since the amount of labeled sodium transferred to the fetus is directly proportional to its concentration in the maternal plasma, only the average concentration in the maternal plasma during the period of transfer is suitable for substitution in the equation. Our first series of experiments, therefore, was designed to measure the rate of disappearance of labeled sodium after its intravenous injection; and from these data the average plasma concentration was obtained. The measure of the quantity of labeled sodium transferred to the fetus (Na^*_f), moreover, is valid for substitution in the equation only if none returns from fetus to mother during the period of observation. It is reasonable to assume that early after introduction of the tagged sodium into the maternal circulation, its concentration in fetal plasma remains so low compared to that of maternal plasma that only a negligible quantity returns from fetus to mother. This condition was taken to be satisfied in the guinea pig[4] when the concentration of the tagged

sodium in the fetus (referred to a unit concentration of tagged sodium in the maternal plasma) increased linearly with time. The period of linear exchange in the guinea pig lasts for from two to three hours after injection; the rate of accumulation in the fetus then decreases until equilibrium is reached at about eight hours. Because of experimental limitations we have not been able to determine the period of linear acquisition by the human fetus. To be as certain as possible about procedure, however, delivery of the fetuses has usually been made approximately thirty minutes after injection of the radioactive material. This procedure gave concentrations of labeled sodium in the fetus as related to unit concentration in the maternal plasma well below the ratio for linearity in the guinea pig.

The umbilical cord was clamped as soon as possible, usually within a minute, after incision of the uterus was begun. Immediately thereafter a sample of maternal blood was taken. The fetus and placenta were weighed, the latter being freed of its membranes, cord, and superficial blood. Nonviable fetuses were ashed as previously described[4]; blood samples were secured from viable fetuses one to three hours after delivery. This time interval was chosen to permit equilibration of plasma sodium with extracellular sodium. The radioactivity of the samples of maternal plasma, fetal ash, and of the fetal plasma was then measured and the quantity of tagged sodium present in these samples calculated from the radioactivity. The total quantity of tagged sodium in a viable fetus was calculated from the concentration found in the plasma and from the known volume of the extracellular space which has been found to average 43.5 per cent of the body weight.[5] The average value for the concentration of tagged sodium in maternal plasma was obtained as will be described below in the presentation of the disappearance curve of tagged sodium from plasma.

The labeled sodium was prepared in the 60-inch cyclotron of the Department of Terrestrial Magnetism, Carnegie Institution of Washington, by deuteron bombardment of metallic sodium. After bombardment, the sodium was dissolved in ethyl alcohol, and Na^*Cl then precipitated with concentrated HCl. Finally an aqueous solution of Na^*Cl was made using distilled water. Appropriate precautions were taken with respect to tonicity, neutrality and sterility of the solution. The amount of tagged sodium injected into an individual averaged about 30 mg. with an activity of 0.3 millicurie. The radioactivity of the samples was measured by a temperature controlled, pressure ionization chamber connected to a string electrometer[4]; these measurements were corrected for self-absorption of radiation by the sample and for the background of the instrument.

Results

The disappearance curve for Na* in plasma after its intravenous injection.—As has been pointed out, the calculation of the placental transfer rate for sodium depends upon an accurate estimation of the average maternal plasma concentration of Na^* present during the period of transfer under observation. This entails the description of the change in concentration of Na^* in maternal plasma from the time of its intravenous injection until the time of delivery of the fetus. Multiple sampling of maternal blood at the time of the transfer studies was avoided by establishing a time-concentration curve in advance (Fig. 1). This was accomplished by injecting a known quantity of Na^* into each of three normal, pregnant women. The curve was standardized so that the average concentration for a given pregnant subject could be determined by measurement of the Na^* concentration of a single plasma sample taken at a known time. This

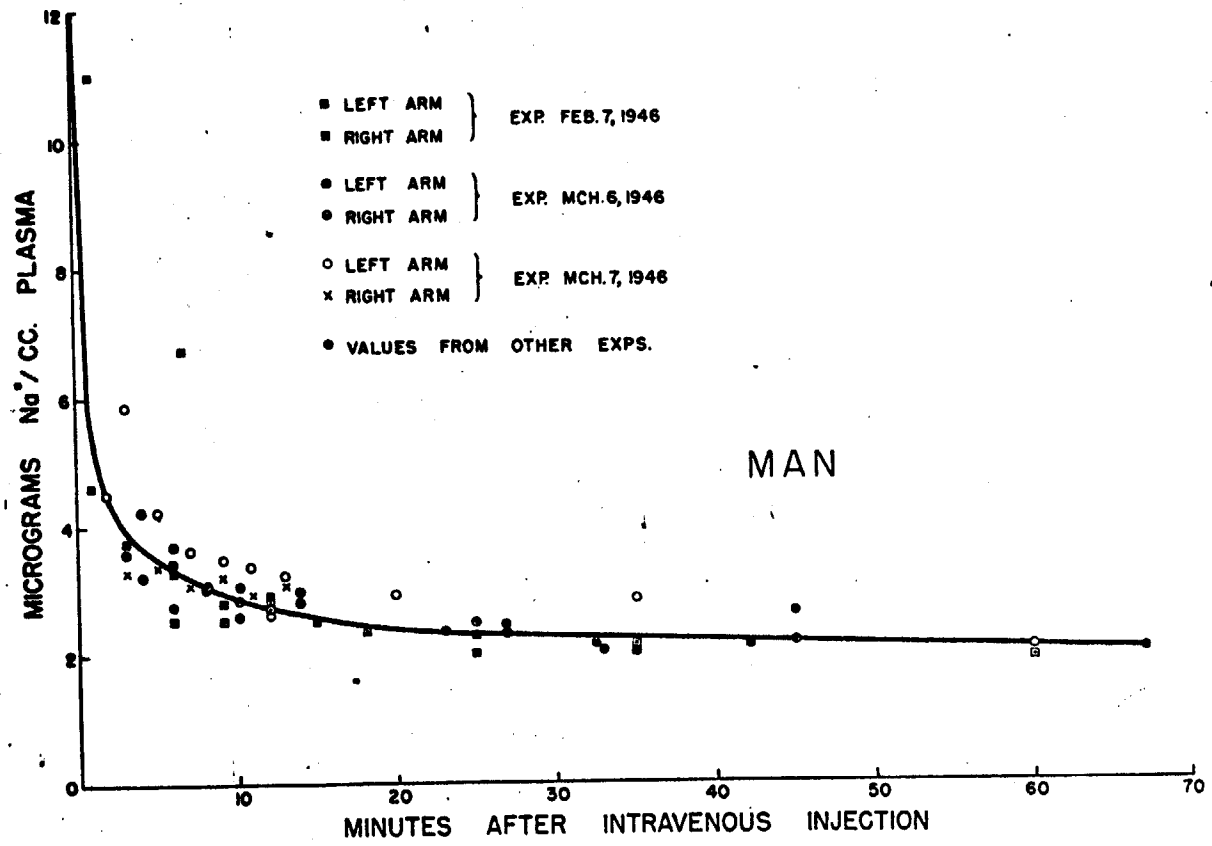

Fig. 1.—Change in concentration of Na* in the plasma with respect to time. The points were adjusted to a standard basis by multiplying them all by a factor which brought the initial concentration to 12 micrograms per c.c. of plasma, as explained in the text.

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was done as follows: For each of the three individuals studied we calculated the plasma concentration of Na* which could have been present immediately after injection if the injected Na* had been distributed uniformly in the plasma. A standard plasma volume of 45 c.c. per kilogram body weight was assumed. This initial concentration (which varied among the three subjects but averaged about 12 micrograms Na* per c.c. of plasma) in each woman was multiplied by whatever factor was necessary to convert it to a standard concentration of 12 micrograms Na* per cubic centimeter of plasma. Subsequent measurements of plasma concentration in each of the three cases were then multiplied by the same factor. Since the standard plasma volume which has been used as a constant, this treatment is equivalent to relating all concentrations to a constant quantity of Na* per unit body weight. The average concentration of Na* in the maternal plasma for a period of thirty minutes after injection was obtained from the standard curve of figure 1 as follows: The area under the curve from zero time to thirty minutes was measured with a planimeter. This area was then divided by the time interval, thirty minutes, to obtain the average altitude, or average concentration. The ratio of this concentration to that found at thirty minutes is the factor, 1.35, by which the concentration of Na* at thirty minutes in a subject must be multiplied to give the average concentration for that subject. Average maternal values calculated in this way are given in the last column of Table I.

As shown in Fig. 1, samples of blood were taken simultaneously from veins of the right and left arms. This provided a measure of mixing time and it was assumed that mixing was essentially complete when the amount of Na* in the two samples was about equal. In one subject, the mixing time estimated in this way amounted to about eight minutes; in the second, to twelve minutes; and in the third, to thirteen minutes. The average concentration of Na* in the maternal plasma will of course be affected by the mixing time. We believe that for our purpose the average concentration in the maternal blood of the placenta differs in no important way from that of the peripheral blood as we have established it.

The normal placental transfer rate for sodium at different gestational ages.—The amount of sodium transferred to a fetus during an experiment was calculated from equation 1. Values for Na^*_f and Na^*_{mp}, the latter averaged for the observed period of transfer, are given in Table I. Na_{mp} was taken to equal 3.3 mg. per c.c. The value of Na_f derived from these data was finally converted to milligrams of sodium transferred across one gram of placenta in one hour. The results are presented in Fig. 2.

The curve of Fig. 2 has been drawn to fit observations made on individuals in which there was no reason to suspect abnormality of placental function. Sixteen normal cases in this series were distributed among individuals who were delivered abdominally because of previous section, mental disease, chorea, tuberculosis, myoma of the uterus, pyelitis, or carcinoma of the abdomen. In addition there are two cases which have been observed during normal labor and vaginal delivery. Six of these cases fell within the ninth to seventeenth week of pregnancy; ten were between the thirty-sixth week and term; only three came between the seventeenth and the thirty-sixth week because of the rarity of interruption of pregnancy during this period.

TABLE I. VALUES FROM WHICH THE DATA OF FIG. 2 AND TABLE II HAVE BEEN DERIVED*

HISTORY NUMBER	INDICATION FOR OPERATION	DELIVERY TIME (MINUTES)	FETAL WEIGHT (GM.)	GESTATION AGE (WEEKS)	PLACENTAL WEIGHT (GM.)	Na* IN TOTAL FETUS (MICROGRAM)	Na*/C.C. OF MATERNAL PLASMA	
							FOUND (MICROGRAM)	AVERAGE (MICROGRAM)
103003	Previous section	33	2700	38	370	700	1.49	1.94
247063	Psychiatric	27	402	16	126	39.0	0.97	1.31
231656	Psychiatric	30	36	10	63	9.5	1.29	1.74
249863	Psychiatric	32	545	20	156	167	1.42	1.84
260095	Previous section	27	2180	37	214	668	4.43	5.98
	Twin pregnancy	28	2030		205	264	4.43	5.98
113669	Previous section	28	3040	40	490	1230	2.67	3.60
128414	Pelvic delivery	55	3340	term	505	1120	1.34	1.60
376433	Previous section	33	3430	37	516	760	1.69	2.20
388120	Chorea	29	92	14	92	19.0	2.40	3.23
311276	Previous section	32	2100	34	370	835	1.79	2.34
254037	Pulmonary tbc.	30	4	9.5	19	0.61	0.88	1.18
386115	Pelvic delivery	30	3680	40	490	1240	1.60	2.16
136080	Myoma uterus	32	2790	36	490	1560	2.35	3.06
398157	Contracted pelvis	31	2630	36	375	1080	1.92	2.59
396381	Chr. pyelitis	46	1500	31	243	1120	3.00	3.80
406823	Ca. abdomen	30	152	17	97	67.0	2.86	3.86
408367	Contracted pelvis	30	2490	40	335	1190	3.86	5.22
257328	Epilepsy	35	19	12	34	10.5	3.58	4.65
240415	Chr. nephritis	31	14	12	34	30.4	4.33	5.84
165446	Hypertension	29	129	16	100	74.3	2.97	4.00
250383	Hypertension	27	614	24	168	49.0	0.45	0.61
259938	Hypertension	28	361	20	140	138	2.97	4.00
389368	Hypertension	31	2780	37.5	455	1190	2.24	3.02
247202	Pre-eclampsia	28	354	18	100	7.3	0.22	0.30
250393	Pre-eclampsia	31	1480	31	220	524	4.70	6.10
317603	Cardiac disease	29	247	18.5	121	278	2.00	5.20
376618	Cardiac disease	34	201	18	107	64.8	1.72	2.25

The average concentration of Na in the maternal plasma for the duration of the experiment has been calculated as explained in the text. Delivery time refers to the interval between i.v. injection of Na* and clamping of the umbilical cord.

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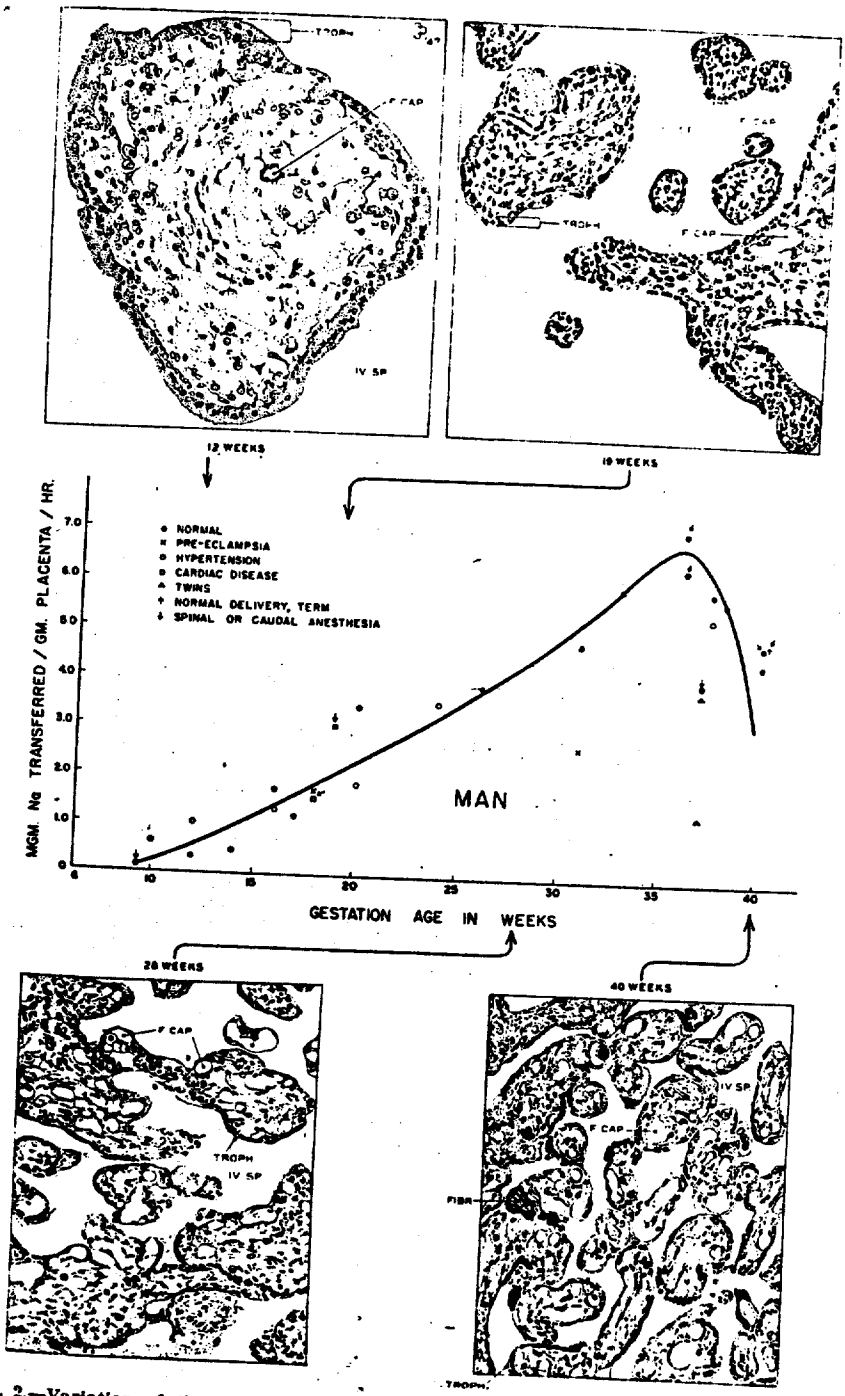

Fig. 2.—Variation of the rate of transfer of sodium with respect to gestation age. The points have been derived from the data of Table I. The camera lucida drawings (×155) are from sections of normal human placentas at the indicated gestational ages and illustrate the histological changes which can be correlated with the change in permeability. Note, as the placenta ages, decrease in thickness of trophoblast (Troph.), increase in surface of villi exposed to intervillous space (Iv. Sp.), and increase in number of fetal capillaries (F. Cap.). Fibr., fibrin.

Several conclusions of major interest to us are to be drawn from the results. The first is that the apparent permeability of the human placenta to sodium increases about 70 times from the ninth to the thirty-sixth week of gestation. The second is that from the thirty-sixth week to term there was in all but one case a rapid decrease in permeability; this decrease was most marked in a single case of twins. The third is that in the two cases where transfer was observed during normal labor for the half hour or hour preceding delivery, there was no decrease in transfer rate. And finally there was no evident difference between the transfer rates when spinal or caudal anesthesia was used and when general anesthesia was obtained with sodium pentothal or gas-oxygen-ether.

The effect of chronic hypertension, cardiac disease and pre-eclampsia on the placental transfer rate.—Five patients with chronic hypertension were studied, one complicated by chronic nephritis. Three of these were between the twelfth and twentieth weeks of pregnancy, one at the twenty-fourth week and the last near term. They varied in severity from mild degrees of hypertension with blood pressures of 140 to 150 over 90, to severe cases with retinal arteriosclerosis and hemorrhages and blood pressures of 180 over 110 or 120. As shown in Fig. 2, there was no evidence of abnormality of placental transfer in any of these cases.

Measurements were made on two patients with cardiac disease, both in the nineteenth week of pregnancy. In one there was complete heart block, diminished cardiac reserve and considerable vasomotor instability; in the other, rheumatic heart disease with mitral stenosis, a bundle branch block and moderate diminution in cardiac reserve. Both of these individuals had normal placental transfer rates.

Finally, observations were made on two cases of pre-eclampsia. One at a gestational age of eighteen weeks had severe hypertension and albuminuria with retinal hemorrhage and partial retinal detachment. The transfer rate in this patient was normal. The other woman, in the thirty-first week of pregnancy, had edema in addition to severe hypertension and albuminuria. The transfer rate in this case was reduced about 50 per cent; this is the single instance in our series where there has been a definite alteration.

Fetal need for sodium relative to its supply across the placenta.—The ratio of the quantity of a substance supplied to the fetus from the maternal plasma to the amount of that substance retained by the fetus in its growth has been called the safety factor for that substance.[4] The quantity of sodium transferred to the fetus across the placenta per hour is calculated from equation (1) as explained above. The amount of sodium retained by the fetus in an hour's growth is equal to the fetal weight multiplied by the hourly per cent weight increase of the fetus multiplied by the total sodium in a unit weight of fetal tissue. The safety factor has been calculated for four fetal ages and is given in Table II. The value of the safety factor varies from 160 at a fetal age of twelve weeks to the remarkably high value of 1130 at forty weeks. This means that of 1,130 parts of sodium delivered to the fetal circulation at forty weeks only one part is retained by the fetus in its growth, and 1,129 parts are returned to the maternal circulation.

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TABLE II. FETAL NEED FOR SODIUM RELATIVE TO SODIUM SUPPLIED ACROSS PLACENTA AT VARIOUS GESTATION AGES*

FETAL AGE (WEEKS)	FETAL WEIGHT (GM.)	TOTAL Na OF FETUS (MG.)	DAILY WEIGHT INCREASE (PER CENT)	TOTAL Na RETAINED DAILY IN GROWTH OF FETUS (MG.)	Na SUPPLIED TO FETUS PER DAY (MG.)	SAFETY FACTOR
12	18.2	21	11.5	2.4	390	160
20	259	620	2.9	18	5600	310
30	960	2250	1.5	34	20600	610
40	2915	5100	1.1	56	63000	1130

*The daily per cent weight increase has been calculated from the data of Streeter,[6] and the sodium content of the fetuses is that found by Iob and Swanson.[1]

Discussion

As stated in the introduction of this paper, we have been primarily concerned with the following problems: (1) measurement of the permeability of the normal human placenta to sodium from early in gestation to term; (2) comparison of the permeability of the human placenta with that of other placentas of the hemochorial group: (3) measurement of the effects of disease on placental permeability and (4) evaluation of the supply of sodium to the fetus as this is related to the requirement for sodium during fetal growth.

The human placenta, as is true for all the placental types which have been studied with the tracer technique,[8] undergoes a very considerable increase in permeability to sodium as gestation proceeds. The peak in transfer rate per unit weight of placenta occurs at about the thirty-sixth week when it is approximately 70 times as great as at the ninth week, the earliest case in our series. This peak is followed by a rapid decline in permeability to term. These changes can be correlated with morphological changes which occur in the placenta during the process of aging. The illustrations of Fig. 2 are typical of placentas of twelve, nineteen, twenty-eight and forty weeks of pregnancy. They are all made at the same magnification (×155) and show clearly some of the important alterations in this organ as gestation progresses. At twelve weeks, the villi are large, relatively few in number and covered with a double layer of cells, the outer, syncytial and the inner, cellular. At nineteen weeks there is an increase in the number of villa, a decrease in their size and Langhans' layer has almost completely disappeared. The increase in total cross sectional area of the villi, together with thinning of their walls, continues through the twenty-eighth week to term. Higher magnification reveals in addition, as pregnancy proceeds, an increase in number of fetal capillaries within the stroma of the villus together with a decrease in the thickness of the capillary walls. The terminal sharp decrease in placental permeability is undoubtedly due in considerable measure to the deposition of fibrinoid over the surface of the villus. All of these morphological changes provide an interpretation of the observed changes in transfer rate per unit weight placenta.

The placenta of man in Grosser's classification[2] belongs to the hemochorial group as do the placentas of rabbit, rat and guinea pig. According to the results of Mossman,[9] the rabbit's placenta in the latter stages of pregnancy becomes

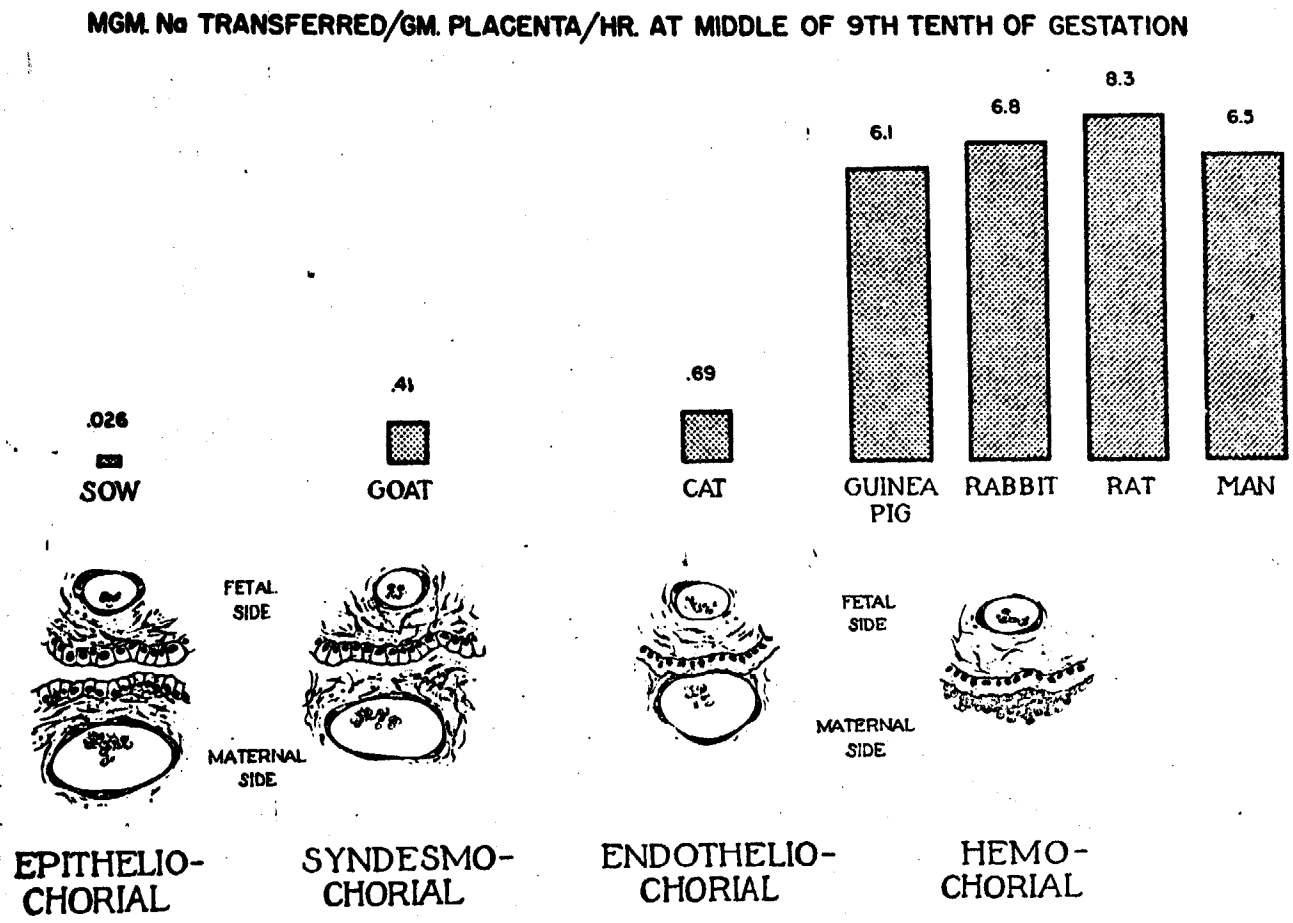

Fig. 3.—Variation of transfer rate of sodium per unit weight of placenta with the morphologic type of placenta. Numerical values give the milligrams of sodium transferred across a gram of placenta per hour as observed in each instance at the middle of the ninth-tenth of pregnancy. The relative magnitudes of the transfer rates are indicated by the relative areas of the dotted rectangles. The diagrams indicate the number and kind of tissue layers interposed between maternal [illegible] in each of Grosser's four groups.

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hemoendothelial and Mossman suggests that the same change may occur in the placentas of other rodents. Hard,[10] however, has obtained evidence using the phosphatase reaction that the placenta of the guinea pig remains hemochorial throughout gestation. The results on transfer of sodium across the human placenta afford a functional test of its morphological similarity to other members of the hemochorial group. Fig. 3 presents schematically the tissue layers interposed between maternal and fetal circulations in Grosser's four groups. Above the diagrams of the placentas there is indicated graphically the amount of sodium transferred per Gm. placenta per hour at the middle of the ninth-tenth of gestation. It is evident that the four placental types can be readily distinguished from one another by the differences in their permeabilities. It is also evident that the permeability of the placenta of man to sodium agrees closely with other members of the hemochorial group. Once again in our studies on placental permeability, Grosser's classification has proved its extraordinary usefulness in the interpretation and integration of our results.

Our experience with the effect of disease, anesthesia and labor on the permeability to sodium is rather meager and suggests only tentative conclusions. The permeability of the placentas from five patients with hypertension and two with cardiac disease was found to be normal. The transfer rate was independent of the type of anesthesia. Two cases in which transfer rates were measured during labor, just prior to the time of delivery, showed normal rates indicating that placental function is not remarkably disturbed during this period. A reduction of transfer rate was noted in one of two cases of pre-eclampsia and the rate was low in the single case of twins which was studied. In our opinion, more experience with sodium and other tracer materials is needed in these several conditions before reliable conclusions about placental function can be drawn.

The use of tracer substances permits the study of that aspect of fetal nutrition which is concerned with the quality of substances supplied to the fetus as this is related to the growth requirements of the fetus. The lowest safety factor (ratio of the amount of a substance supplied to a unit weight of fetus to the amount of that substance retained by the unit weight of fetus in its growth) for sodium has been observed in the sow and has a value of 3.5.[11] The highest safety factor for sodium observed prior to the studies reported here is in the guinea pig and has an average value of 50.[4] Man, in our experience, is unique in the extraordinarily high value of the safety factor which varies from 160 at twelve weeks to over 1100 at term; less than 0.1 per cent of the sodium which reaches the human fetus at term is retained, 99.9 per cent being returned to the maternal circulation. Man is unique also in that sodium is supplied at a constant rate, about 0.9 mg. per Gm. fetus per hour, from the twelfth week of pregnancy when the fetus is reproducing its weight at the rate of about 12 per cent per day to term when the daily per cent weight increase is only about 1 per cent. This is the single exception which has been found to the hypothesis that the fundamental principle underlying placental function is that the rate at which substances are transferred to a unit weight of fetus shall parallel the relative growth rate of the fetus.[9]

Summary

1. Changes in rate of placental transfer per unit weight of placenta have been measured in normal pregnancies, using radioactive sodium, from the ninth week of gestation until term. The permeability of the placenta increases about 70 times during this period. The type of anesthesia was without effect on the rate which was also unaffected during labor immediately preceding birth. The permeability to sodium of the human placenta is like that of other members of the hemochorial group.

2. In a small series of cases, chronic hypertension and cardiac disease were without effect on the rate of transfer. One of two cases of pre-eclampsia showed a marked reduction of permeability.

3. The fetus receives across the placenta at the twelfth week of pregnancy 160 times, and at the fortieth week, 1100 times as much sodium as is incorporated in the growing tissues.

We are grateful to Dr. N. J. Eastman for his interest and generous cooperation.

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Acceptance of unsalted strained foods by normal infants

Consumption of strained foods by normal four-month-old and seven-month-old infants did not appear to be influenced by whether or not sodium chloride had been added. Daily intakes of sodium by infants were in most cases several times greater when the salted foods (as commercially available) were fed. Since the assortments of unsalted strained foods fed to infants nearly always supply more sodium than does human milk, and since most infants by age 4 months are receiving whole cow's milk (which supplies more than 3 times as much sodium as does human milk), there would appear to be no nutritional justification for adding sodium chloride to strained foods for infants.

Samuel J. Fomon, M.D., Lora N. Thomas, R.N., and L. J. Filer, Jr., M.D., Ph.D.

IOWA CITY, IOWA

SEVERAL AUTHORS[1-5] have suggested that amounts of sodium chloride currently present in certain strained foods commercially available for infants may predispose to development of hypertension.* Presumably, salt is added to these foods solely, or at least primarily, for improvement of palatability and not on the basis of nutritional considerations. Strained foods with added salt are generally preferred by mothers, the taste preferences of infants being unknown. The present study was undertaken to compare food consumption of infants receiving salted and unsalted strained foods.

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*At the time of preparation of this manuscript, we were unaware of an additional comment on this topic by Mayer.[9]

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SUBJECTS

Normal full-term infants more than 109 days of age were enrolled in the study. All had served as subjects in another study that terminated at approximately 112 days of age (range 109 to 115 days). As a requirement of this study, the infants were either breast-fed or received one of several experimental formulas; strained foods were introduced into the diet according to the following schedule: 28 days, oatmeal with bananas and applesauce; 56 days, pears; 84 days, applesauce, bananas (two foods).

One group of infants (12 males and 8 females) was enrolled in the present study at 109 to 115 days of age (referred to subsequently as "four-month-old infants") so that information regarding previous feeding history was available. These infants ranged in weight from 5,424 to 7,510 grams at the onset of study. A second group of 5 males

Table I. Composition of strained foods

Variety of food	Constituents in 100 Gm.								
	Calories	Total solids (Gm.)	Protein (Gm.)	Fat (Gm.)	Carbohydrate (Gm.)	Calcium (mg.)	Phosphorus (mg.)	Sodium (mg.)	
								Salted*	Unsalted
Mixed cereal with apples and bananas	89	22.7	1.6	0.4	19.8	16	41	125	7
Peaches	86	22.3	0.4	0.2	20.5	7	14	9	9
Applesauce	59	15.1	0.1	0.4	13.8	2	7	12	12
Bananas	84	20.7	0.5	0.4	19.4	28	9	60	7
Vegetables and beef	53	12.2	2.8	0.2	5.8	34	40	440	130
Carrots	16	7.7	0.8	0.3	2.4	31	15	194	45
Green bean	27	7.7	1.4	0.2	4.8	35	14	223	5
Chicken with vegetables	119	22.3	7.2	7.3	6.0	149	132	353	45

*Salted foods are those commercially available; unsalted foods have no salt added.

and 5 females (referred to subsequently as "seven-month-old infants") ranged from 192 to 209 days of age when enrolled in the present study and had received an unknown assortment of commercially available strained foods purchased by the mother from 112 days of age until enrollment in the present study. Body weights of these infants were not precisely known but most, if not all, weighed between 7 and 8.5 kilograms.

DIETS

Intake of milk or formula was not recorded. Most infants were receiving commercially prepared formulas or whole cow's milk and many were receiving a daily supplement of vitamins and/or minerals. Otherwise the infants received foods* selected by the mothers from those listed in Table I. With the exception of the peaches and applesauce, it may be seen that all of the commercially available strained foods (referred to subsequently as "salted foods") contained added sodium chloride.

EXPERIMENTAL PLAN

The 20 four-month-old infants were offered salted and unsalted foods on alternate weeks. Eleven infants received salted foods during the first and third weeks of study and unsalted foods during the second week; 9 infants received unsalted foods during the first and third weeks of study and salted foods during the second week.

The 10 seven-month-old infants received salted foods during the first week and unsalted foods during each of the next 3 weeks.

Mothers were interviewed by one of us (L. N. T.) at the beginning of the study and were told that we wished to make some observations on consumption of strained foods by infants. The jars were coded inconspicuously to indicate whether they contained salted or unsalted foods; it is unlikely that the mothers noted this coding. Otherwise, the label merely indicated the name of the food.

At the beginning of the first week of study, the family of each infant was supplied with 4 jars (135 Gm. each) of each of the foods listed in Table I. At the end of the week, the emptied or partially used jars as well as any unused jars were returned and a new supply was provided. The weight of full jars was known. At the end of each week, the weight of empty or partially emptied jars was determined and, from the difference, the average daily intake was calculated. Because minor spillage of food undoubtedly

*Generously supplied by the H. J. Heinz Company.

occurred, the estimated intakes of salt by individual infants are not precise. Nevertheless, these estimates are unquestionably better than are likely to be obtained by diet recall studies.

RESULTS AND COMMENT

Four-month-old infants. As may be seen from Table II, consumption of strained foods did not appear to be influenced by the salt content. Total food intake (grams per day) was nearly identical whether foods were salted or unsalted. Since most of the infants were between 5.5 and 7.5 kilograms in weight and probably were receiving approximately 95 kcal. per kilogram of body weight per day, total caloric intakes were likely to have been in the general range of 520 to 710 kcal. per day and the mean contribution from strained foods was probably about 25 per cent of total calories. However, the range was clearly very large.

Table II. Intake of strained foods by four-month-old infants

Type of food and data	*Week of study* 1	2	3
Unsalted foods			
No. of infants	9	11	9
Weight of strained foods (Gm./day)			
Mean	186	200	221
S.D.*	100	100	70
Range	33-304	24-330	83-297
Calories from strained foods (kcal./day)			
Mean	133	142	160
S.D.	71	69	46
Range	21-210	16-214	64-212
Sodium from strained foods (mg./day)			
Mean	56	55	63
S.D.	42	35	33
Range	5-118	7-117	11-111
Salted foods			
No. of infants	11	9	10†
Weight of strained foods (Gm./day)			
Mean	227	221	201
S.D.	100	87	129
Range	26-386	73-313	11-399
Calories from strained foods (kcal./day)			
Mean	128	160	144
S.D.	65	61	87
Range	23-254	54-218	10-274
Sodium from strained foods (mg./day)			
Mean	228	325	278
S.D.	187	186	218
Range	25-702	56-623	19-717

* S.D. = standard deviation.

†Food consumption by one infant during the third week of study could not be determined since the partially emptied jars were inadvertently rinsed out before they had been weighed.

During the various weeks of the study mixed cereal accounted for 22 to 24 per cent of total strained food intake (by weight) and each of the fruits accounted for 14 to 19 per cent. None of the other foods accounted for more than 10 per cent of total intake. Individual variation was great, with at least one infant each week consuming no cereal and another no peaches, while other infants received as much as 62 per cent of strained food from one of these sources. Choices of individual foods were nearly the same whether salted or unsalted foods were given.

Mean intakes of sodium from strained foods (Table II) during the various weeks of study were more than 4 times greater when the salted foods were given than when the unsalted foods were given. The difference was relatively small for those infants whose intakes of strained foods were low; however, consumption of strained foods accounted for more than 100 kcal. per day during most weeks (actually 44 of the 59 weeks*) of observation with the 20 infants. During 23 of these weeks, unsalted foods were given and the mean intake of sodium from strained foods was 39 mg. per 100 kcal. (range 18 to 56); in contrast, during 21 weeks when salted foods were fed, the mean intake of sodium from strained foods was 202 mg. per 100 kcal. (range 93 to 276).

As a point of reference, it may be noted that human milk, a representative prepared formula (Similac), and whole cow's milk provide approximately 24, 52, and 86 mg. of sodium per 100 kcal., respectively.

*See footnote to Table II.

On the basis of animal studies,[6-8] it seems reasonable to consider the possibility that large intakes of sodium may be hazardous for at least some infants. From this point of view, the *average* intake of salt from strained foods is of less interest than the highest intake. In the present study, one four-month-old infant had an average intake of about 717 mg. of sodium from strained foods daily during a week when she was given the salted strained foods. This infant weighed 6 kilograms and received 274 kcal. from strained foods. Assuming that the infant received 95 kcal. per kilogram per day, total intake would be 570 kcal. This infant was breast-fed and received, in addition to human milk and strained foods, an average daily intake of 100 ml. of whole cow's milk. Thus, assuming a daily intake of 67 kcal. from cow's milk (58 mg. sodium) and 229 kcal. from human milk (55 mg. sodium), total intake of sodium may be estimated to have been about 830 mg. per day. Similar calculations suggest that when the infant was receiving unsalted strained foods, her intake of sodium was less than one third this amount (approximately 230 mg. per day).

Seven-month-old infants. Since the seven-month-old infants had been receiving commercially available foods at their mothers' discretion (probably with minimal medical intervention), they had consumed a variety of salted foods. For this reason, salted foods were offered to each infant during the first week of study. Amounts of strained foods consumed may be seen from Table III. During the next 3 weeks, unsalted foods were given and it is apparent that these also were well accepted.

Because body weights of the infants were generally between 7 and 8.5 kilograms, it seems likely that total caloric intakes were generally between 630 and 765 kcal. per day (i.e., approximately 90 kcal. per kilogram per day). Mean caloric intakes from strained foods ranged from 258 kcal. per day during the first week of observation to 302 kcal. per day during the fourth week. Of particular interest are the surprisingly high intakes of some of the infants; one infant received 593 kcal. per day from strained foods during the fourth week of study. This infant probably received 75 per cent or more of total caloric intake from strained foods. It is apparent that some supervision regarding choice of strained foods is desirable for those infants who receive most essential nutrients from this source.

The seven-month-old infants consumed a greater percentage of strained food in the form of vegetables and beef, and chicken and vegetables than did the four-month-old

Table III. Intake of strained foods by seven-month-old infants

Type of food and data	Week of study 1	2	3	4
Weight of strained foods (Gm./day)				
Mean	362	378	394	431
S.D.*	129	164	208	199
Range	165-628	149-676	136-856	213-887
Calories from strained foods (kcal./day)				
Mean	258	262	274	302
S.D.	87	106	138	126
Range	150-462	122-464	105-578	167-593
Sodium from strained foods (mg./day)				
Mean	632	124	125	142
S.D.	234	53	66	70
Range	236-1,075	36-203	30-273	26-283

*S.D. = standard deviation.

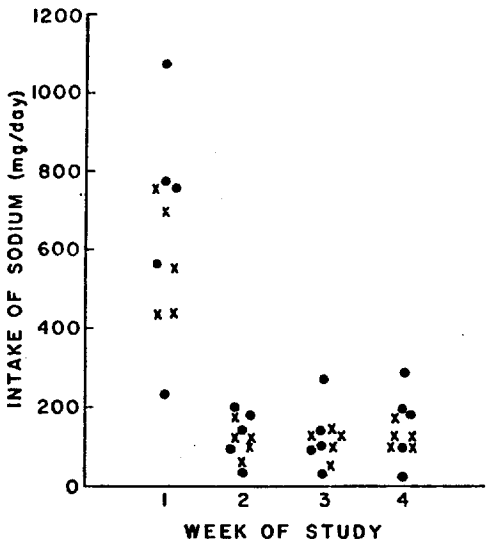

Fig. 1. Average daily intakes of sodium from strained foods by seven-month-old infants during four weeks of observation. Commercially available foods were fed during the first week of study and specially prepared foods with no added salt during the second, third, and fourth weeks. Each x refers to average daily intake of one male infant and each ● to average daily intake of one female infant during the designated weeks of study.

infants. As had been observed with the younger infants, only small amounts of carrots and green beans were consumed.

The assortment of saltetl foods consumed by the infants provided a mean daily intake of about 243 mg. of sodium per 100 kcal. (range 180 to 297), while unsalted foods provided a mean intake of 46 mg. of sodium per 100 kcal. (range 16 to 60). The intake of sodium from strained foods averaged 632 mg. per day during the first week of study (when salted foods were fed), and 124, 125, and 142 mg. per day, respectively, during the second, third, and fourth weeks. The range of intakes was wide (Fig. 1).

It may be seen from Table III that one infant received 1,075 mg. of sodium daily from strained foods during the first week of study. This infant received 460 kcal. per day from strained foods and the remainder from low-fat cow's milk (2 Gm. fat and 54 kcal. per 100 ml.; 106 mg. of sodium per 100 kcal.). Assuming a total intake of 700 kcal. per day with intake of 240 kcal. from low-fat cow's milk, intake of sodium is estimated to have been 1,329 mg. per day, equivalent to 3.4 Gm. of sodium chloride. Because the infant was only about one tenth the body weight (or one fifth the surface area) of an adult, this intake appears extremely high.

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Food Protection Committee. 1972

Comprehensive GRAS Survey—NAS/NRC 1972 Table 2—
Usage Levels Reported for NAS Appendix A
Substances (Group 1) Used in Regular Foods (R)

Natl. Acad. Sci., Natl. Res. Counc., Washington, D.C.

Food Protection Committee. 1972

Comprehensive GRAS Survey—NAS/NRC 1972 Table 3—
Usage Levels Reported for NAS Appendix A
Substances (Group II) Used in Infant Formula Products and Baby Foods (B)

Natl. Acad. Sci., Natl. Res. Counc., Washington, D.C.

Food Protection Committee. 1972

Comprehensive GRAS Survey, NAS/NRC, 1972 Table 11, Part A

Natl. Acad. Sci., Natl. Res. Counc., Washington, D.C.
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Food Protection Committee. 1972

Comprehensive GRAS Survey, NAS/NRC, 1972 Table 11, Part A

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Sodium–Potassium Chloride Mixtures as Table Salt[1]

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SODIUM CHLORIDE is essential for human and animal growth. Although the level needed by human subjects has not been established with any degree of certainty, there is adequate evidence that the daily ingestion of 250–375 mg of sodium chloride (100–150 mg sodium) by adults can be maintained without any apparent signs of abnormalities (1, 2). Despite this low requirement of the body for sodium chloride, the average daily intake has been estimated as ranging from 10 g for Americans to 27 g for inhabitants of certain areas of Japan (1–6).

Concern about the intake of salt stems primarily from the beneficial effects observed following the restriction of its intake by patients with a variety of diseases. The benefits accruing from such therapy have led some investigators to conclude that there exists a causative relationship between a high salt intake and the development of the condition being treated ((1–8) and p. 81 in (9)).

Salt restriction was employed therapeutically as early as 1901 for patients with edematous heart disease (10). Subsequently, this treatment was extended to congestive heart failure (11–14), hypertension (13–20), renal diseases, cirrhosis of the liver, toxemias of pregnancy, and Meniere's disease (13, 14). When salt restriction has been prescribed, it has been stated that one-fourth to one-third of hypertensive patients respond with a reduction in blood pressure (1, 2, 21).

Despite the claim that man's requirement for salt is so low that the endogenous sodium chloride in his unseasoned food should supply his needs, most patients find it a hardship to be subjected to a salt-restricted diet. For this reason, efforts have been made to develop seasoning agents to replace salt. Those most commonly used are based on potassium chloride. To alleviate the unpleasant, rather bitter taste of potassium chloride, it is usually combined with citric or other acids, monopotassium glutamate, choline, ammonium chloride, or spices (13, 14). In general, salt substitutes are little better than no seasoning at all—they do not impart the agreeable, salty flavor of sodium chloride. Lithium chloride, although palatable and salty, cannot be used because of its toxicity (22).

This paper proposes a possible solution to the problem of reducing dietary sodium without depriving the patient of palatable salty seasoning. To this end, mixtures of sodium and potassium chlorides have been studied.

SODIUM-POTASSIUM CHLORIDE MIXTURES

Our experiments with various mixtures of sodium and potassium chlorides have shown that the disagreeable flavor of the latter is masked in such mixtures. This confirms an earlier comment by Ball and Meneely (23). Furthermore, in these mix-

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tures potassium chloride contributes its own saltiness to almost the same extent as ordinary salt so that the mixtures are weight-for-weight about as salty as sodium chloride.

When solutions containing only potassium chloride were tasted by 72 panelists, 67% of them reported a 0.064% solution as tasting bitter (Table I). When an additional concentration of 0.1% sodium chloride was added, the percentage reporting the solution bitter decreased to 11. A solution containing as much as 0.4% potassium chloride but with 0.1% sodium chloride tasted bitter to only 24% of the panelists. This concentration of potassium chloride alone was so bitter to all panelists that it could not be adequately evaluated—the complaint was that the bitter flavor lingered and interfered with subsequent tasting. Other studies with differing ratios of potassium to sodium chlorides have shown similar results.

With regard to saltiness, Table II demonstrates with 72 panelists that aqueous potassium chloride at concentrations above the taste threshold of 0.025–0.050% enhances the saltiness of sodium chloride solutions.

To obtain an evaluation of mixtures of sodium and potassium chlorides as used under actual cooking and eating conditions, 12 graduate students majoring in foods and nutrition were provided a noon meal for 10 days. Each day all panelists received the same meal. The same menu was followed for 5 days. A different "salt" was used on each day of each 5-day period, both for cooking and at the table. This "salt" ranged from pure sodium chloride to mixtures of sodium chloride with increasing concentrations of potassium chloride. Each panelist had an individual shaker, and the weight of "salt" used from each shaker was determined after each meal. The average amount of the various "salts" used by each panelist was: 100% NaCl, 2.30 g; 80% NaCl:20% KCl, 2.25 g; 70% NaCl:30% KCl, 2.25 g; 60% NaCl:40% KCl, 2.25 g; 40% NaCl:60% KCl, 3.05 g.[4] Mixtures containing up to

TABLE I

Bitter taste of potassium chloride solutions with and without added sodium chloride

Concentrations of salts in distilled water, %	Panelists rating solutions bitter, %
0.025 KCl + no NaCl	25
0.064 KCl + no NaCl	67
0.075 KCl + no NaCl	68
0.150 KCl + no NaCl	74
0.256 KCl + no NaCl	83
0.025 KCl + 0.1 NaCl	5
0.064 KCl + 0.1 NaCl	11
0.150 KCl + 0.1 NaCl	12
0.400 KCl + 0.1 NaCl	24

TABLE II

Enhancement of saltiness of NaCl solutions by additions of KCl

Concentrations in distilled water, %		Panelists rating mixture saltier than control, %
NaCl	KCl	
0.10[a]	0.000[a]	
0.10	0.025	30
0.10	0.066	42
0.10	0.150	65
0.10	0.400	93
0.15[a]	0.000[a]	
0.15	0.038	71
0.15	0.099	100
0.15	0.225	71
0.15	0.600	100
0.20[a]	0.000[a]	
0.20	0.050	14
0.20	0.133	86
0.20	0.300	100
0.20	0.800	100

[a] Control.

[4] The lack of variation in the averages suggests that the panelists may have salted their food from habit instead of taste. The individual values for each panelist, however, varied considerably.

about one-half potassium chloride thus appear to be as salty as sodium chloride. This study indicated no unpleasant or untoward reactions of the panelists to foods prepared or seasoned at the table with mixtures of sodium and potassium chlorides. In fact, none of the 12 panelists, when queried about their reactions to the meals, suspected it was the table salt that was being varied.

Potassium chloride is not only a good compound for supplementing the saltiness of sodium chloride, but its physical properties make it technically an ideal substance for admixture with ordinary salt. Mixtures of the two salts are visually indistinguishable from pure sodium chloride. Both salts are colorless, transparent cubic crystals with similar refractive indices. Both are obtainable in any desired particle size. The specific gravity of sodium chloride (2.16) is similar to that of potassium chloride (1.99) so that mixtures will not segregate. Both salts are soluble in water. Critical humidities for sodium and potassium chlorides (76 and 86% at 25 C) are such that mixtures can be protected from caking by the same free-flowing additives as are used with sodium chloride. Mixtures can be readily iodized.

IMPLICATIONS OF EXTRA POTASSIUM

If additional potassium is added to human diets through the adoption of mixtures of sodium and potassium chlorides, what consequences might result therefrom? As a basis for such considerations, it is well to bear in mind that potassium is a normal and essential dietary constituent and that an average individual has more potassium in his body than sodium (24).

The average adult in the United States consumes about 10 g of salt (containing 4.1 g, or 180 mEq, sodium) per day (1–6). There is a great spread in the reported individual intakes. Approximately half the salt intake represents that which is in the food as purchased, while the other half is added during cooking and at the table.[5] Michelson et al. (25) have surveyed teen-age and adult groups in various cities and reported an average intake of potassium of 4.6 g (118 mEq) per day with a range among groups of 2.5–7.7 g. On the basis of these reported average intakes, the dietary gram–atomic Na:K ratio is about 1.53.

Now let us consider the use of a mixture of sodium and potassium chlorides, for example a 1:1 mixture by weight. This ratio may have some advantages since it provides only half as much sodium as does ordinary salt, yet its palatability is favorable to a large percentage of the population. A salt-restricted patient using it at the table instead of salt can achieve twice as much "saltiness" without affecting his total sodium intake. Such a mixture contains 19.65% Na and 26.20% K.

If the estimated average 5 g of salt contributed to the diet by cooking and table use were replaced by a 1:1 mixture by weight of sodium and potassium chlorides, then the average daily intake of sodium would be reduced from 4.1 to 3.1 g, the intake of potassium would be increased from 4.6 to 5.9 g, and the gram–atomic Na:K ratio would change from 1.53 to 0.89. This increase in potassium is well within the normal range cited by Michelson et al., and the new Na:K ratio would approximate the 0.97 ratio as calculated from Sherman's sodium and potassium contents of typical diets as purchased, before being salted in the home (26).

The increase in potassium intake associated with the use of a 1:1 potassium chloride–sodium chloride mixture should pose no problem from a physiological standpoint. The evidence suggests that the dangers from orally ingested potassium salts are remote except in cases of severe kidney disturbances (27–29). Even then, the condition of patients with kidney dis-

[5] Sherman (26) has estimated an average sodium content of 1.94 g/person in typical American dietaries as purchased.

case must be extreme before there is any danger from potassium. This was emphasized by Winkler, Hoff and Smith (30), who stated: "It is unlikely that the conditions necessary for fatal poisoning by oral potassium administration can occur in patients with nephritis so long as urine is being excreted. The relatively slow absorption, the vomiting when large doses are given, the mode of distribution in the body and, most important of all, the continued ability of the most severely damaged nephritic kidney to excrete potassium all combine to make such poisoning very difficult to bring about."

The thiazide drugs, now widely used as diuretics in the treatment of hypertension, frequently produce hypokalemia (31). To counteract this loss, potassium chloride supplements have been administered as enteric-coated tablets. In a few patients these tablets have produced ulcers and stenosis of the small intestine. These abnormalities were caused by high local concentrations of potassium chloride. The enteric-coated capsules reached the intestine in an intact state and became lodged on the mucosal surface where the potassium chloride was liberated. When potassium chloride was given in some form other than as enteric-coated tablets, no difficulties were encountered (32).

The use of a mixture of sodium and potassium chlorides as a salt replacement may aid in replenishing a considerable portion of the potassium lost as a result of thiazide therapy. The maximum dose of potassium chloride in such therapy is 6 g/day with an average of 3 g (33). If a patient were to use 5 g of a 1:1 sodium–potassium chloride mixture, he would reduce by 2.5 g the amount required as a supplement. With the administration of the extra potassium chloride as an integral part of the diet, conditions should also be ideal for its gradual absorption into the blood stream.

There is an additional way whereby patients with hypertension may benefit from the use of sodium–potassium chloride mixtures. The potassium content of a mixture may facilitate a reduction in the incidence of some forms of this disease. This suggestion is based on the ameliorating effects attributed by a number of clinicians to the therapeutic use of potassium. Potassium salts as an adjunct in treating hypertensive patients were suggested by Addison (20) in clinical experiments begun in 1923. He observed a drop in the blood pressure of such patients receiving potassium salts. Priddle (34–36) has routinely treated large numbers of hypertensive patients with a low sodium regimen—supplemented with added potassium salts. He has reported that the increased potassium intake not only leads to a fall in blood pressure beyond that attributable to the low sodium diet, but improves the clinical condition and well-being of the patient.

McQuarrie, Thompson and Anderson (37) observed that some diabetic children have an intense craving for salt, as much as 60–90 g/day, and that these massive intakes have a hypertensive effect. The elevation in blood pressure can be prevented completely when potassium is substituted for sodium in approximately one-third chemically equivalent amounts.

Animal experiments have corroborated these clinical findings. Gordon and Drury (38) reported a decrease in vascular damage as judged by intestinal hemorrhages when hypertensive rabbits were given a 1% potassium chloride solution to drink, although the potassium supplement had no effect on the blood pressure. Bach (39) and Hilker (40) and their co-workers changed the dietary sodium-to-potassium ratio in favor of potassium in hypertensive rats and found the rise in blood pressure to be moderated but not prevented. Similar results with hypertensive dogs have recently been reported in Japan (41).

Meneely and co-workers (42–44) have

demonstrated the protective effects of potassium chloride on rats rendered hypertensive by feeding of high levels of sodium chloride. While potassium chloride reduced blood pressures only in the most hypertensive rats, the survival times of all hypertensive rats were significantly increased. For example, the 50% survival time for 37 rats fed a ration containing 5.6% sodium chloride and 0.66% potassium chloride was 20 months; for 43 rats fed a ration containing 5.6% sodium chloride and 2.9% potassium chloride, the time was 27 months; and for 53 control rats fed a ration containing 1.1% sodium chloride and 0.66% potassium chloride, the time was 22.5 months.[6]

Thus, the incorporation of potassium chloride into ordinary table salt appears worthy of consideration for therapeutic purposes, since such mixtures provide a palatable seasoning with a reduced sodium content while tasting as salty as ordinary salt and since extra potassium for hypertensives not only appears safe but may have a beneficial effect per se. This was emphasized by Tobian (p. 308 in reference 8) when he stated: "An alteration of the amounts of sodium and potassium in the diet of populations susceptible to hypertension may be the most practical way to decrease the incidence of the disease."

USE OF MIXTURES IN FOOD PROCESSING

Sodium–potassium chloride mixtures are also effective replacements for ordinary salt in food processing for dietetic purposes, even where salt has a functional use in addition to its taste effect. This has been demonstrated both in baking bread and curing meat. Loaves of bread made with sodium chloride, with potassium chloride, or with a 1:1 mixture of these two salts were very similar in dough fermentation, carbon dioxide retention, loaf volume, texture, crust, and toasting properties. A panel tasting the fresh and day-old breads could barely distinguish between the breads containing sodium chloride and the mixture; all panelists showed an aversion to the bread containing potassium chloride alone.[7] Similar experiments with hams indicate that potassium chloride is as effective as sodium chloride in producing the visible effects associated with curing. Hams cured with potassium chloride are unpalatable, however, whereas hams cured with ordinary salt or a 1:1 mixture are practically indistinguishable on the basis of both taste and appearance (unpublished observations).

SUMMARY

Mixtures of sodium and potassium chlorides provide a possible replacement for ordinary salt as a therapeutic adjunct in the treatment of hypertension and other conditions requiring a sodium-restricted diet. Such mixtures are palatable and very similar in saltiness to equal weights of sodium chloride. Sodium chloride appears to mask the bitter, disagreeable flavor of potassium chloride. Although the mixtures still contain sodium and for that reason cannot be considered "salt substitutes," they may prove useful as dietary supplements since *a*) present salt substitutes are unsatisfactory from the taste standpoint, and *b*) the introduction of thiazides has obviated the necessity of drastic sodium restriction for most hypertensive patients.

Mixtures of sodium and potassium chlorides are much alike in physical properties and are superficially indistinguishable from common salt. They can be used in industrial food processing as well as in the

[6] The reader may note for comparison that 1 month in the life of a rat corresponds roughly to 3 years in the life of a man and 1% salt in a rat diet to about 5 g/day in a human diet.

[7] This experiment was done by The W. E. Long Co., Independent Bakers' Cooperative, Chicago, Ill., under the direction of Mr. Douglas J. Kirk, Director, Production and Research.

preparation of food in the home and as table salt.

The extra intake of potassium resulting from the use of mixtures of sodium and potassium chlorides should pose no nutritional or physiological problems. The amount of potassium thus added to the diet under ordinary conditions does not bring the total intake beyond normal limits. Potassium chloride, when taken orally, is almost nontoxic since large doses of the compound are absorbed only slowly from the gastrointestinal tract. It is also readily excreted, even by kidneys damaged by nephritis. Additionally, there is mounting evidence that potassium supplements may ameliorate hypertension and that decreasing the dietary sodium-to-potassium ratio may be as important as decreasing the sodium intake.

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Evidence for the Renal Conversion of Angiotensin I in the Dog

10021

By W. G. Franklin, M. J. Peach, and J. P. Gilmore

ABSTRACT

Experiments were carried out on perfused canine kidneys in situ to determine if renal conversion of angiotensin I occurs. Various concentrations of decapeptide produced an immediate increase in renal vascular resistance when injected directly into the renal artery. Since the decapeptide is biologically inactive, the increased resistance was interpreted as indicating generation of angiotensin II. The extent of renal conversion of angiotensin I to angiotensin II was estimated to be approximately 19% in the normal dog. This percent conversion was reduced to about 10% in dogs maintained on high salt and DOCA for 7 to 10 days.

ADDITIONAL KEY WORDS converting enzyme angiotensin II

■ Although it is well documented that the kidney contains renin, there is still some question whether this organ also contains the enzyme which can convert angiotensin I, a decapeptide, to angiotensin II, an octapeptide. Ng and Vane (1) reported that intrarenal injection of angiotensin I in the dog was not associated with changes in renal resistance; however, after a single passage through the lung, renal resistance increased. From these experiments, Ng and Vane concluded that the kidney did not contain converting enzyme and that decapeptide conversion occurred primarily in passage through the pulmonary vasculature. Gocke and associates (2) reported that the concentration of angiotensin II is greater in the renal venous blood of patients with renal arterial stenosis than in arterial blood, suggesting that the kidney may indeed convert the decapeptide to the octapeptide. Oparil et al. (3) observed 20% conversion of angiotensin I across the kidney using ^{3}H-leucine-10-angiotensin I. The findings of these investigators suggested to us a study to compare the influence of the decapeptide on renal resistance at normal blood flows with its effect on renal blood flow following a prolonged cardiovascular intervention, i.e., chronic deoxycorticosterone acetate (DOCA)-salt treatment. We thought that the hemodynamic state of the animal might influence the effect of angiotensin I on renal resistance. This would indicate physiologic or pathophysiologic modulation of converting enzyme activity and would resolve the apparent discrepancy between the findings of Ng and Vane and Gocke and associates.

Methods

Mongrel dogs of either sex anesthetized with pentobarbital sodium (30 mg/kg iv) were used in all the experiments. The trachea was intubated and the required blood vessels exposed. The left kidney was perfused using the technique described by Gilmore (4). Essentially, the lower abdominal aorta was approached retroperitoneally through a left flank incision and the lumbar arteries ligated. After administration of heparin (5 mg/kg iv), a modified Gregg coronary cannula perfused through a rotameter from a common carotid artery was inserted upstream into the aorta approximately 5 cm below the left renal artery. This cannula was then moved cranially and wedged into the left renal artery without interrupting blood flow. During this procedure the kidney was not disturbed; the position of the cannula was determined by palpation. The total length of the renal perfusion shunt from the carotid artery to the renal artery was approximately 110 cm. The internal volume of the perfusion shunt was 35 ml. In most instances, peptide injections were made into the perfusion

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system approximately 20 cm from the kidney. Angiotensin I (1-asp-5-ileu-angiotensin I), 0.75 to 5.0 μg, or 1-asp-5-ileu-angiotensin II, 0.2 to 2.0 μg, or both were administered.[1] In some experiments, the injection site was varied so that injections were made as far as 100 cm from the kidney. Renal arterial pressure was measured between the rotameter and carotid artery. Systemic blood pressure was monitored through a small peripheral artery.

Eight normal dogs and four dogs maintained on a high salt and DOCA regimen for 7 to 10 days were studied. The regimen consisted of 6 g of salt twice a day and 25 mg of DOCA once a day. The percent conversion of angiotensin I to angiotensin II was calculated as the percent change in renal blood flow per dose of angiotensin I divided by the percent change in blood flow per dose of angiotensin II multiplied by 1.25. The latter factor takes into account the fact that if a microgram of angiotensin I is completely converted to angiotensin II, it will yield only about 0.8 μg of octapeptide. In about half the animals, dose-response comparisons were obtained between the peptides, and percent conversion of angiotensin I was based on the dose-response curves.

[1]The peptides (1-asp-5-ileu-angiotensin I and II) were supplied by Dr. F. M. Bumpus of the Research Division of the Cleveland Clinic and assayed by us periodically with the estrogen-treated rat uterine assay.

Results

The mean initial renal blood flow in control dogs was 2.53 ± 0.30 ml/min/g; in DOCA-sal animals, 2.56 ± 0.18. Mean systemic arteri pressure in animals under pentobarbit anesthesia was 128 ± 7.53 mm Hg for contr and 162.85 ± 9.73 for those on the DOCA-s regimen ($P < 0.01$). Pressures in the caroti renal shunt were 114.27 ± 10.9 mm Hg controls and 147.72 ± 6.11 for DOCA-s animals ($P < 0.01$).

All peptide concentrations were made up t a final volume of 1 to 2 ml with saline. Administration of 1 to 2 ml of physiologic saline produced a small injection artifact o renal blood flow which lasted about 15 seconds.

Eighty observations were made in normal dogs comparing the responses of renal blood flow to angiotensin I and II. An example of one such experiment is shown in Figure 1. It can be seen in the figure that doses of angiotensin I (0.75 or 1.50 μg) reduced renal blood flow by about 15 and 40 ml/min, respectively. With a control flow of about 110 ml/min, these represent flow decreases of 14 and 37%. Angiotensin II in a dose of 0.50 μg

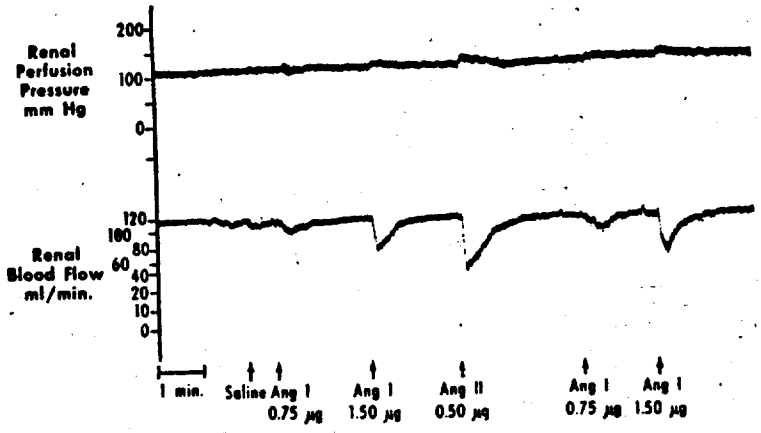

FIGURE 1

A comparison of the effects of angiotensin I (0.75 and 1.50 μg) and angiotensin II (0.50 μg) on renal artery perfusion pressure and left renal blood flow. Rapid injections were made directly into the left renal artery. Onset of response was immediate, and time to peak response was about 10 seconds. Angiotensin I responses were about the same after injection of angiotensin II. The kidney weight was 44.8 g.

TABLE 1

Percent Conversion of Angiotensin I to Angiotensin II

	Normal dogs	Salt and DOCA
No. of dogs	8	4
No. of observations	80	32
Mean ± SE	18.97 ± 2.12	10 ± 1.32*

*Significantly different from control dogs.

reduced renal flow by 68 ml/min (a decrease of 63%). Net conversion of angiotensin I was calculated in the following way:

$$\frac{0.14/0.75}{0.63/0.50} \times 1.25 = 18.5\%.$$

When this calculation was made using the percent change in renal blood flow with the high dose of angiotensin I compared to the dose of angiotensin II the conversion was 24.5%.

The mean calculated net conversion of angiotensin I to angiotensin II in normal dogs was 19% (Table 1). The conversion was independent of the decapeptide concentration. In dogs maintained on high salt and DOCA for 7 to 10 days, the mean conversion for 32 observations was 10%. This percent conversion of angiotensin I was significantly less than found in normal dogs.

The distance from the kidney of the site of injection of the peptides did not significantly alter the magnitude of the renal responses. For a given dose of angiotensin I the same quantitative change in renal blood flow was obtained whether the injection was made 20 cm or 100 cm from the kidney. Injecting the decapeptide 100 cm from the kidney lengthened the time from injection to peak renal blood flow response by 20 to 30 seconds, depending on the existing renal blood flow.

Discussion

These experiments clearly show that injections of angiotensin I directly into the renal arterial blood are associated with an increase in renal vascular resistance. This effect of the decapeptide could indicate that the decapeptide itself is vasoactive or that it is converted to the octapeptide, which is known to be vasoactive. With respect to the former possibility, in vitro experiments using isolated smooth muscle (5, 6) and renal flow in the saline-perfused kidney (7) have demonstrated the inactivity of angiotensin I. Conversion of the decapeptide to the active octapeptide could occur either between the site of injection and the kidney, within the kidney itself through plasma converting enzyme, or via a renal converting enzyme. With respect to this first possibility Ng and Vane (8) determined that "in one circulation time (15 to 20 seconds) there is little or no conversion of angiotensin I to angiotensin II in the blood." Oparil and associates (3) found that with incubation at 37° in heparinized plasma or whole blood the half time for conversion of angiotensin I to II was 15 and 3 minutes, respectively. These findings give direct support to the position that any conversion of the decapeptide observed in the present experiments was not extrarenal, that is, did not occur between the site of injection and the kidney. Further support for this position are the experiments in which we found that if the distance of the decapeptide injection site from the kidney was increased up to fivefold (this increased the extrarenal contact between angiotensin I and plasma converting enzyme from about 5 seconds to 25 seconds), there was no significant increase in the calculated conversion of the decapeptide to the octapeptide.

The onset and duration of responses obtained with angiotensin I in the present study and the $t^{\frac{1}{2}}$ of the decapeptide in whole blood (Oparil et al., 3) argue against significant intrarenal conversion due to plasma converting enzyme. The data indicate the existence of a renal angiotensin I converting enzyme.

In general, the results of these experiments are consistent with the suggestion of Gocke et al. (2) and report by Oparil et al. (3) but not consistent with the general findings of Ng and Vane (1) and Skeggs et al. (7). Of interest was the observation that with bioassay we found a 19% conversion of angiotensin I to angiotensin II, while Oparil et al. (3) found a 20% conversion using labeled angiotensin I. In

the experiments of Ng and Vane, angiotensin II or I were injected into the aorta immediately above the renal arteries. They reported that the injection of 1.33 μg of the decapeptide had essentially no effect on renal blood flow, whereas the intravenous injection of 2.0 μg of angiotensin I produced a substantial reduction in renal blood flow presumably due to recirculation of octapeptide that was formed in the lungs. Skeggs and associates (7) also found no influence of the decapeptide on renal resistance when it was injected into the renal artery. However, in these experiments the isolated rat kidney perfused with physiologic salt solution was employed. Under these conditions it is possible that there was depletion of the converting enzyme. In the present investigation, chronic DOCA and salt treatment appeared to modulate converting enzyme activity. This observation suggests that changes in renal converting enzyme might well influence the major in-vivo sites of the generation of angiotensin II and thus alter the in-vivo distribution of greatly elevated angiotensin II levels.

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ACCIDENTAL SALT POISONING IN A HOSPITAL NURSERY*

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SYNOPSIS

In a suburban hospital in Sydney, salt was accidentally used instead of sugar in the preparation of feeds intended for a number of babies. Five babies were involved in the ensuing epidemic of hypernatraemia: 4 died and one, less severely affected, made a complete recovery. Three of the babies who died were of low birth weight and they presented with symptoms different from those previously described, in that they had diarrhoea, manifested no thirst, and had neither muscular twitchings nor convulsions. Overlapping of the sutures was striking in all cases.

Ingestion of an excessive quantity of salt causes hypertonicity of the extracellular fluid which largely explains the disorders involved in hypernatraemia.

It is suggested that salt should not be stored in an area where infants' formulae are prepared.

At 5 p.m. on 18th February 1967, salt, instead of sugar, was added by accident to the milk formulae intended for the babies in the newborn nursery of a hospital in a suburb of Sydney.

Figure 1 attempts to represent briefly the events of the epidemic of hypernatraemia which resulted. The symbols representing the babies involved have been plotted against the estimated serum sodium levels at the time of presentation, and against the number of hours elapsed between the addition of salt to the feeds and the onset of symptoms. In none of these cases was it possible to determine with certainty the period of time between the ingestion of a salt loaded feed and the development of symptoms. The horizontal lines represent survival in hours after onset of symptoms.

CASE REPORTS

Case 1

The first victim was a baby girl aged 3 weeks. Her weight at birth had been 2.035 Kg. and on 18th February 1967, she weighed 2.065 Kg. After 5 p.m. on that day, she vomited her feeds and by 8 p.m. she had developed peripheral cyanosis and periodic breathing. Peripheral pulses were not palpable; there were no reflexes and the cranial sutures were noticeably overlapping. Before she died, 3 hours later, she passed 2 loose stools.

Case 2

This boy, 4 weeks old, had weighed 1.360 Kg. at birth and weighed 1.695 Kg. when he died. Early in the night he had had some vomiting and later was found dead in his cot.

Chloride estimations were carried out by the Government Analyst on whole blood collected at autopsy, 3 days and 1 day after death, in Cases 1 and 2 respectively. The blood chloride in Case 1 was 245 mEq/L and in Case 2, 209 mEq/L. It is difficult to estimate from this what the serum sodium levels might have been before death, but it is probable that they were not lower than the level of blood chloride found at post mortem.

Case 3

This baby boy was 8 days old, weighed 2.350 Kg. at birth and 2.135 Kg. 3 days before death. During the night following the salt-laden feed, he was listless and disinclined to suck, vomited blood-stained mucus, had a little diarrhoea and developed rapid respirations. Within 2 hours of the first symptoms he became cyanosed and respirations were gasping. On admission to our Department, peripheral pulses were not palpable, there were no spontaneous movements, tone or reflexes, the eyes were slightly sunken and the cranial sutures were markedly over-riding. Aspiration of the somach yielded a small quantity of blood-stained fluid which continued to drain slowly over the next few hours. When blood was taken by heel prick for electrolyte estimation, the puncture bled profusely for about one hour.

Treatment was instituted in the belief that the baby had gastroenteritis and serum electrolyte studies were performed. Salt poisoning was suspected some time later when hypernatraemia was

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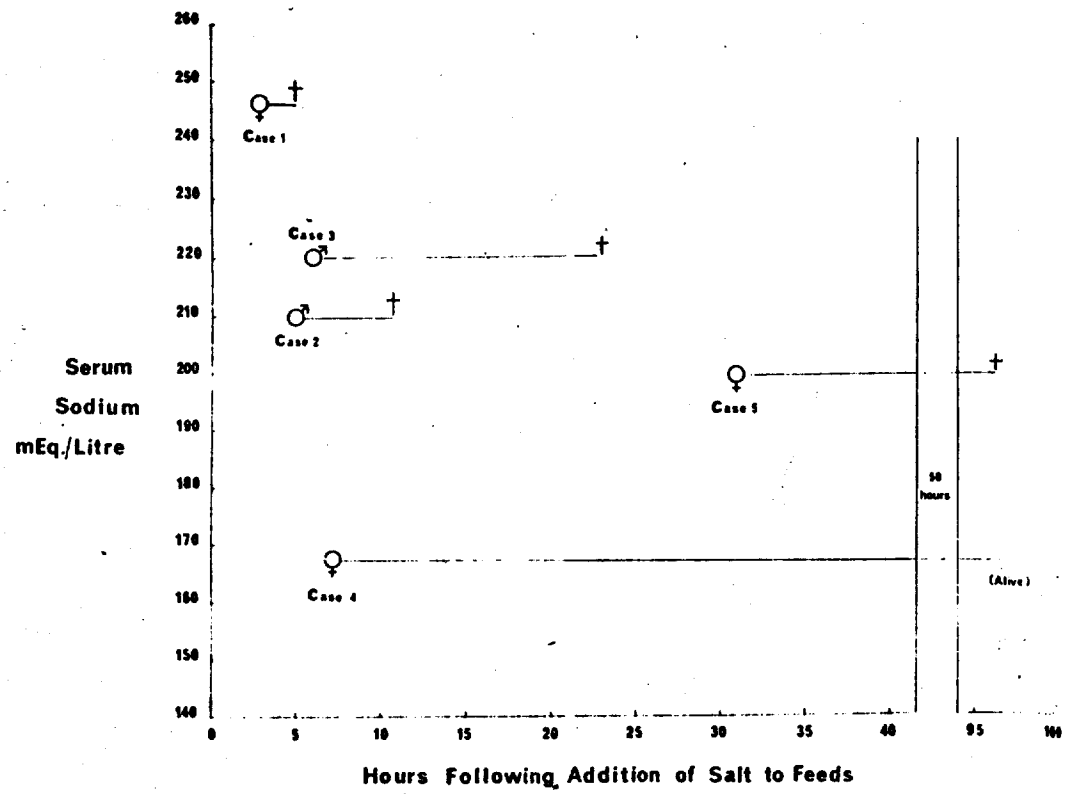

Figure 1. For explanation, see introduction.

found in this baby as well as in Case 4, whose serum electrolytes were estimated at the same time (Table I). The baby died a few minutes later and enquiries then revealed that the milk mixtures prepared for these babies tasted salty.

Case 4

A baby girl, 17 days old, birth weight 2.125 Kgs., weighed 2.100 Kg at the time of the accidental salt poisoning. During the night when it is presumed she was fed a salted formula, she vomited each feed as well as some blood-stained mucus, and passed two greenish yellow offensive stools.

When transferred to our Department, the only abnormal findings were a little wasting and some dryness of the mucous membranes. She, too, was thought initially to have gastroenteritis, an impression further reinforced by the passage of two large liquid stools, followed by more marked dehydration.

The serum electrolytes are shown in Table I.

At first 0.45% (half-strength physiological) saline was given intravenously to correct the dehydration. When hypernatraemia was diagnosed, the infusion was replaced by 0.22% (quarter-strength physiological) saline which was given in a quantity just sufficient to meet maintenance requirements. She was also given 3 mEq of sodium bicarbonate in the first 24 hours and 5 mEq of potassium per day. 30 hours after admission she took a modified milk formula, equivalent to half strength cow's milk with lactose, and subsequently made an uneventful recovery.

Thirst and muscular twitching were observed for the first time 24 hours after admission, when the baby was already recovering. She was very thirsty throughout the second day, but the muscular twitchings were only transient.

She is now well at 21 months of age. Her psychomotor development is a little slow, but her accomplishments are comparable to those of her siblings at the same age.

TABLE I

Biochemical Findings — Cases 3 and 4

	Case 3	Case 4
Serum Sodium (mEq/L)	220	167
Serum Chloride (mEq/L)	195	144
Serum Potassium (mEq/L)	9.1	4.5
Standard Bicarbonate (mEq/L)	5.9	15.4
Blood pH	6.742	7.237
Base deficit	22	12.2

Case 5

This baby girl of 7 days, birth weight 3.950 Kg., was the only mature baby in this series. Following one or more salted feeds she was at first lethargic, feverish and disinclined to feed. When transferred to our Department a few hours later, she had a dry tongue and noticeably over-riding sutures, but the tone of the skin was normal and the eyes were not sunken. She was apathetic and did not cry, but the tendon jerks were brisk and she was very tremulous. Shortly after these observations, irregular clonic fits began.

The initial and subsequent estimations of the serum electrolytes and the acid base are shown in Table II.

At first 0.22% saline was given intravenously, at a rate sufficient for maintenance. A few hours later convulsions began, the serum biochemistry had deteriorated (Table II), and peritoneal dialysis was commenced.

Two dialyses were carried out, using 'Dianeal with 1.5% dextrose', Baxter, (Table III). The first, on the day of admission, consisted of 16 exchanges each of 100 mls. The second, the following day, consisted of 19 exchanges of 150 mls. The biochemical effects are shown in Table II.

During the course of the first dialysis, the baby became apnoeic and life was maintained by artificial respiration until she died approximately 65 hours after the onset of symptoms.

When it was thought that an epidemic of gastroenteritis was the likely cause, all babies who seemed well were sent home from that nursery, and there is little doubt that more deaths would have occurred but for this. When the real nature of the problem was apparent, those babies who had been discharged were reviewed in their homes and were found to be well.

PATHOLOGY

The main pathological findings were not significantly different from those already reported in children who died of hypernatraemia or hypernatraemic dehydration (Finberg and Harrison, 1955; Macaulay and Blackhall, 1961; Elton, Elton and Nazareno, 1963; Macaulay and Watson, 1967). At autopsy all the infants had subarachnoid haemorrhages, and multiple small intracerebral haemorrhages, and the fifth baby also had a moderate degree of cerebral oedema. Subdural haematomas and sinus thromboses described in some cases (Finberg, 1959; Luttrell and Finberg, 1959; Elton, et. al., 1963; Macaulay and Watson, 1967), were not present.

In the kidneys there was, in addition, shrinkage of the convoluted tubular cells from the basement membrane, a feature described by Rush, Finberg, Daviglus and Cheung (1951), and Elton et al. (1963). The stomach in all cases, and the small intestine of the fifth baby, showed diffuse reddening of the mucosa. In the stomach a well demarcated line suggested that the reddening was due to the direct effect of the salt.

TABLE III

Composition of 'Dianeal with 1.5% dextrose'

	mEq/L
Sodium	142
Chloride	101.0
Calcium	3.5
Magnesium	1.5
Lactate	45.0
Bisulphite	1.0
Dextrose	15.0

DISCUSSION

Clinical Features

In 1963, Finberg, Kiley and Luttrell described an epidemic of salt poisoning in a hospital nursery (Binghampton General Hospital,

TABLE II

Biochemical Findings — Case 5

	On Admission	8 Hours later	After 1st dialysis	12 Hours later	After 2nd dialysis	12 Hours later
Serum Sodium (mEq/L)	199	195	175	175	150	158
Serum Chloride (mEq/L)	173	173		145		122
Serum Potassium (mEq/L)	3.8	7.0	5.2	7.0	4.6	5.7
Standard Bicarbonate (mEq/L)	13.9	11.7		15.2		18.8
Blood pH	7.236	7.188		7.011		7.118
Base deficit (mEq/L)	14.2	18.9		12.2		7.0

New York), and reported a high incidence of convulsions and muscular twitchings. There was also fever and tachypnoea suggesting respiratory distress, and the affected babies displayed avid thirst until they became quite ill, and then refused feedings. All but one vomited. Case 5, the only mature baby, fitted this clinical pattern quite well.

However, our premature babies (Cases 1 to 4) behaved very differently; all vomited, but they also had diarrhoea. One died too quickly for detailed observations to be made, but the other 3 had slightly sunken eyes, and 2 had peripheral circulatory failure and respiratory depression. Both of the premature babies whom we observed at length showed a tendency to bleed from the gastrointestinal tract and from heel pricks. None of them had convulsions or muscular twitchings, except Case 4, in which muscular twitching appeared only when she had almost recovered.

Pathogenesis: (Figure 2). It is often not realised how little salt is too much. One level tablespoonful of salt contains approximately 350 mEq of sodium and 350 mEq of chloride, amounts greater than the total content in a 5 Kg. baby. The feeds which were offered accidentally contained from 2,000 to 2,500 meq of sodium per litre, and 100 mls of such a mixture would have been more than sufficient to double the total body sodium of a small newborn baby.

Excess salt results in hypertonicity of the extracellular fluid which leads to cellular dehydration and expansion of the extracellular compartment (Finberg, 1959; Finberg, Luttrell and Redd, 1959; Elton et al., 1963). This produces dilution of the bicarbonate buffer and metabolic acidosis (Winters, Scaglione, Mahas and Verosky, 1964) to which an important contribution is also made by the release of hydrogen ions from the damaged cells (Cotos, Dodge and Talbot, 1962). Dehydration of the brain cells, causing shrinkage of the brain with over-riding of the sutures, is probably the main cause of the cerebral symptoms.

The mechanism of the intracranial haemorrhages remains uncertain. It has been suggested that cerebral collapse and reduction of intracranial pressure are important factors (Girard 1956; Finberg, Luttrell and Redd, 1959; Logothetis, 1966). Of importance also would be the engorgement of intracerebral and meningeal capillaries due, at least in part, to over-expansion of the intravascular compartment (Elton, Elton and Nazareno 1963). It is possible that the bleeding tendency observed in our cases is a contributing factor.

Clinical observations have shown that hypernatraemic infants excrete urine with a concen-

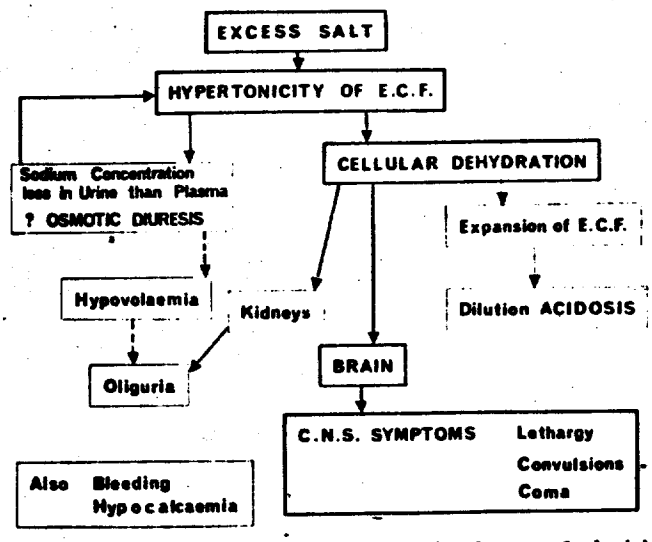

Figure 2. Outline of the pathogenesis of the disturbances of physiology in hypernatraemia.

tration of sodium below that of the plasma, at least until they become oliguric (Weil and Wallace, 1956; Finberg 1959). It appears then, that in the absence of exogenous water, hypernatraemia is likely to be self-aggravating until renal function fails. In addition, experimental evidence obtained with dogs (Finberg, Rush and Cheung, 1964) and kittens (Finberg, et al., 1959), but unconfirmed in humans, suggests that hypernatraemia may induce an osmotic diuresis causing serious fluid loss. If the kidneys of the human infant react in the same fashion, and there is no reason to suppose they might behave differently, an initial diuresis might explain why the smaller infants in our epidemic had clinical signs of dehydration. In that age group, such a diuresis would certainly escape observation.

Thus it would seem that the effects of hypernatraemia can be explained on the basis of an increase in the tonicity of the extracellular fluid. Certainly, any pharmacological effects which might be due to the sodium and chloride ions are overshadowed by their osmotic effect and would be difficult to separate from them.

The frequent occurrence of hypocalcaemia in hypernatraemic states has been an interesting feature (Rapoport, 1947; Finberg and Harrison 1955). The pathogenesis is not known and Finberg and Harrison showed that the level of serum calcium did not correlate with the level of phosphorus. Serum calcium was not estimated in our patients.

COMMENT

Epidemic hypernatraemia must be uncommon for a diligent search of the literature yielded only one other reported instance, mentioned above. It is possible that other unreported epidemics have occurred. However, the symptoms in our small babies were so unlike those previously described, and death was so rapid, that it seems likely that other epidemics could have escaped diagnosis.

To prevent the recurrence of such a tragedy it is recommended that salt should not be stored in a formula room. Should this be unavoidable, for instance because other food stuffs are prepared in the same area, then it is recommended that the salt should be kept in its original container.

ACKNOWLEDGMENT

We thank Dr. C. Corby, Division of Forensic Medicine, Sydney, who carried out the autopsies and made his findings available to us.

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Scientific Articles

Antidiabetic Effect of Acetohexamide

Effect of Potassium Supplements*

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WE HAVE EVALUATED the long-term antidiabetic effect of acetohexamide in diabetic patients who have previously been receiving other forms of treatment. In short-term studies we have compared its effect, in single and divided doses, with that of a placebo. In other studies we have evaluated the effect of adding potassium chloride to the sulfonylurea.

Materials and methods

For the long-term studies, 48 patients were selected from those attending the metabolism clinic of Bellevue Hospital. The patients had been attending the clinic approximately every two months and had been prescribed a diabetic diet designed to obtain optimum body weight. Forty-four had been receiving treatment with tolbutamide and/or phenformin (DBI-TD) for at least six months previously; 4 had been receiving 20 to 30 units of insulin daily. Of those taking tolbutamide, 4 were taking 0.5 to 1 Gm. a day and the others 2 to 3 Gm. a day; those receiving phenformin were taking 50 to 100 mg. a day. The dosage of acetohexamide administered was one half that of the previous tolbutamide dosage, and 0.5 Gm. was substituted for 50 mg. phenformin. The dosage was increased when necessary to a maximum of 1.5 Gm. daily. Phenformin, 50 to 150 mg., was added in several subjects when the control with acetohexamide alone was inadequate.

The patients were seen weekly at first, then monthly, and then at two- to three-month intervals for up to six months. Daily urine records were kept at home, and fasting or postprandial blood glucose levels were obtained on return visits.

Diabetic control was graded in the following way: good control—75 per cent of postprandial blood glucose levels less than 150 mg. per 100 ml. and 75 per cent of urines 0 or 1 plus glycosuria, determined by reagent tablets (Clinitest); fair control—75 per cent of postprandial blood glucose levels less than 200 mg. per 100 ml. and 50 per cent of urines 0 or 1 plus glycosuria; and poor control—all others.

Thirteen other maturity-onset diabetic patients were selected for comparing the acute effect of acetohexamide with that of a placebo. The subjects were diabetic individuals treated with diet alone or those who were responding poorly to other oral hypoglycemic agents. They were seen either weekly or biweekly, and fasting blood glucose levels were determined on each visit. Urine records, fasting and postprandial, were kept by the patients at home. After two to three weeks of observation, prior medication was discontinued, and 1 Gm. of acetohexamide was given every morning. Depending on the effectiveness, the dosage was changed on the next visit to 0.5 Gm. or increased to 1.5 Gm. given once a day or in divided doses, with-

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TABLE I. Patients previously treated with tolbutamide, phenformin, or insulin

Initial Diabetic Control			Diabetic Control During Treatment with Acetohexamide		
Good	Fair	Poor	Good	Fair	Poor
Tolbutamide					
3	...	...	3	...	...
...	11	...	4	7*	...
...	...	6	2	2*	2*
Phenformin					
1	...	...	1	...	...
...	...	1	...	1	...
Insulin					
...	1	3	...	1†	3**

* One with added phenformin because of poor result with acetohexamide alone.
† Alone.
** With phenformin.

TABLE II. Patients previously treated with tolbutamide in combination with phenformin

Initial Diabetic Control			Diabetic Control During Treatment with Acetohexamide					
			Alone			With Phenformin		
Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor
2	...	...	1	...	...	1	...	...
...	6	...	1	1*	1†	3*	1†	1
...	...	14	...	...	1	...	3	10**

* One patient fair alone, good with phenformin.
† One patient poor alone, fair with phenformin.
** Seven of these patients needed insulin subsequently; 3 did poorly with acetohexamide alone.

out fixed order. If the diabetes was still inadequately controlled, 25 mg. of phenformin was added to the acetohexamide and increased to a maximum of 50 mg. three times a day. For one week during the study, the patient was taken off all medication and given a placebo. During the last week of the study, 30 ml. daily of a 25 per cent solution of potassium chloride was added to the previously most effective therapeutic regimen.

Results

LONG-TERM STUDIES. The results are shown in Tables I and II. In those patients previously treated with tolbutamide, diabetic control improved in 4 of 11 who had been under fair control and in 4 of 6 who had been under poor control. Control improved in 1 patient previously treated with phenformin who had been poorly controlled. Those who had been under good control remained well controlled with acetohexamide.

Most of the patients previously treated with tolbutamide and phenformin had been poorly controlled. Of those under fair control, half were better controlled with acetohexamide and phenformin. One did better with acetohexamide alone, and 1 did worse with acetohexamide and phenformin. Of those under poor control, 3 did better with acetohexamide and phenformin and the rest remained poorly controlled.

Three patients treated with insulin and poorly controlled remained poorly controlled with acetohexamide and phenformin (Table I). One patient under fair control with insulin was similarly controlled with acetohexamide alone.

SHORT-TERM STUDIES. The changes in blood glucose paralleled closely the changes in glycosuria. In 11 of the 13 patients, acetohexamide alone and acetohexamide plus phenformin reduced the blood glucose (Fig. 1). The reduction was the same whether acetohexamide was taken the morning of the blood glucose test (6 patients) or twelve or twenty-four hours before the fasting blood glucose level was determined (average levels 127 mg. per 100 ml. and 118 mg. per 100 ml.; probability greater than 0.10).

In 7 of 9 patients a single 0.5-Gm. dose of acetohexamide was less effective than a dose of 1 Gm. in reducing the blood glucose when the latter was given in one dose

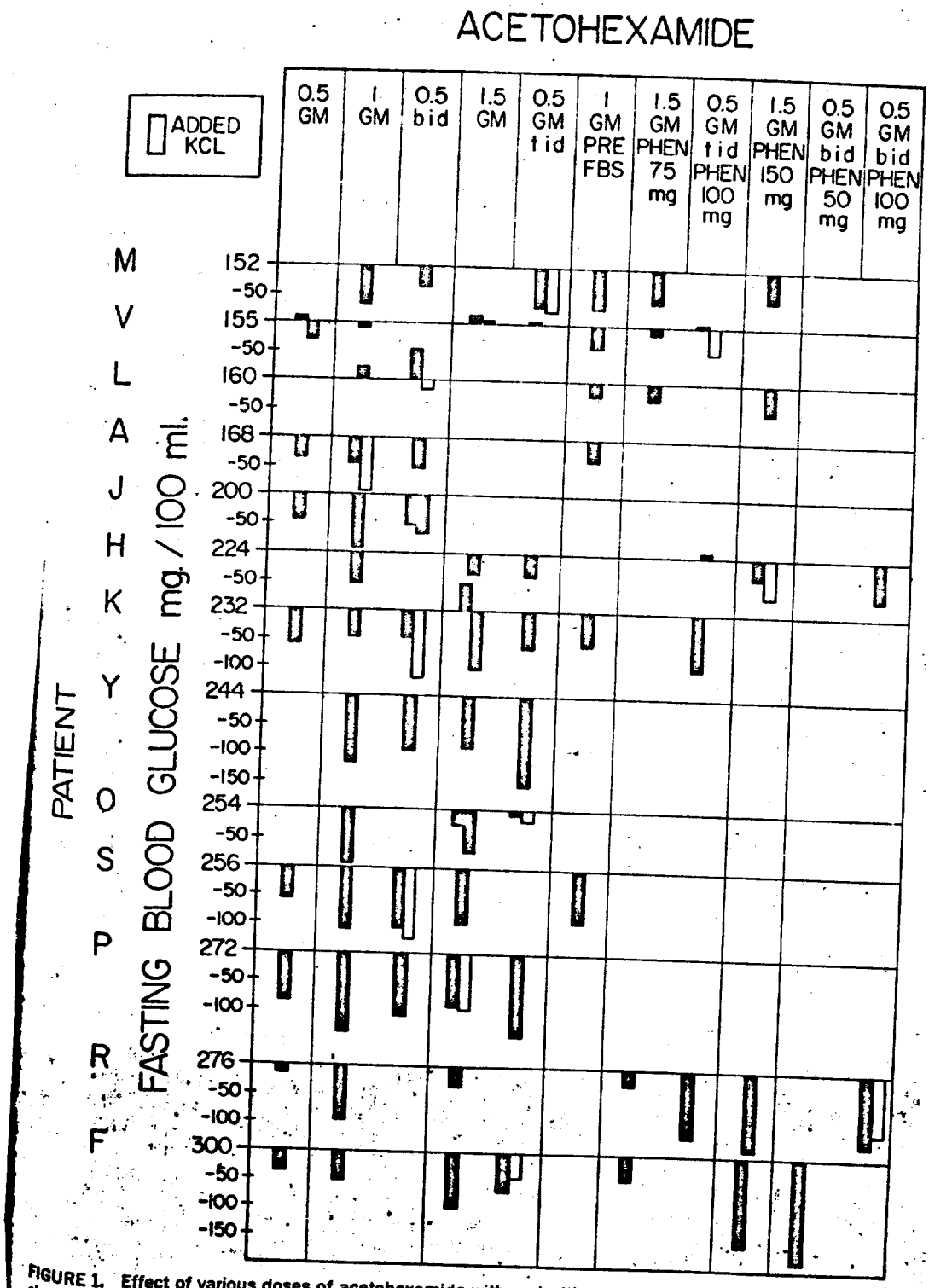

FIGURE 1. Effect of various doses of acetohexamide with and without addition of phenformin or potassium chloride. Each period one or two weeks. Zero line represents fasting blood glucose after one week of placebo therapy. Patient F had 4 plus glycosuria during placebo therapy and took acetohexamide last four days; her fasting blood glucose arbitrarily taken as 320 mg. per 100 ml. Six patients took acetohexamide the morning of fasting blood glucose determination.

TABLE III. Comparison of single and divided doses of acetohexamide; average fasting blood glucose (milligrams per 100 ml.)

Dose and Number of Experiments	Acetohexamide: One Dose	Acetohexamide: Two Doses	Acetohexamide: Change*
1 Gm.			
9	137	143	Plus 11 (p >0.10)
1.5 Gm.			
11	187	169	Minus 11 (p >0.10)

* Change in blood glucose in same patient, using blood glucose on one dose as the control level.

(average levels 175 and 147 mg. per 100 ml., probability less than 0.05). One and 1.5 Gm. were equally effective. Administering the latter in divided doses was not more effective than administering it in one dose (Table III). In poorly controlled patients, the addition of phenformin appeared to aid the reduction in blood glucose.

Acetohexamide was most effective at higher initial blood glucose levels (Fig. 2A). The relationship of the initial blood glucose level to the hypoglycemic effect of the most effective drug treatment is seen in Figure 2B. Two patients with control levels of 155 and 160 mg. per 100 ml. had little effect from the drug.

The addition of potassium chloride potentiated the hypoglycemic effect of the acetohexamide in 8 of 11 patients (Table IV). The change is highly significant when calculated using each subject as his own control.

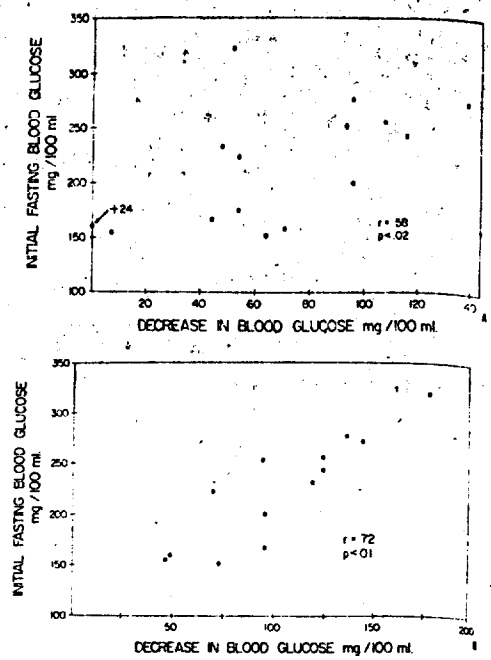

FIGURE 2. Relationship of initial blood glucose level to hypoglycemic effect of (A) 1 Gm. acetohexamide given in single dose. Initial level is fasting level after one week of placebo therapy; and (B) most effective drug treatment as depicted in Figure 1.

TABLE IV. Effect of potassium supplement on fasting blood glucose in 11 diabetic patients taking acetohexamide; decrease in milligrams per 100 ml.

Formula	Before Potassium	During Potassium
Mean ± S.E.M.	50 ± 16	73 ± 12 (p <0.005*)

* Calculated using each patient as his own control.

Comment

It appears that acetohexamide is as effective as tolbutamide in the approximate dosage of 0.5 Gm. acetohexamide to 1 Gm. tolbutamide in the control of maturity-onset diabetic patients and that it is ineffective, as are other sulfonylureas, in insulin-dependent diabetic patients.[1-3] Our results confirm, in addition, that acetohexamide and phenformin may be more effective in patients poorly controlled with tolbutamide and phenformin.[4]

Results of drug treatment are notoriously difficult to evaluate in long-term studies in ambulatory patients since rigid supervision alone of diabetic patients promotes better diabetic control. The effectiveness of acetohexamide was clearly shown in the short-term studies by the close correlation between the initial blood sugar and the effect of treatment and by the deterioration of diabetic control in the more severely diabetic patient when a placebo was substituted. The persistence of control in the milder diabetic patient during placebo treatment is consistent with observations that a placebo is as effective as most oral therapy in some 26 to 31 per cent of diabetic patients.[5,6]

The maximum effective dose of acetohexamide appears to be 1 Gm. administered in a single dose. Its effectiveness when given in a single dose reflects its persistence

in the body in a metabolically active form for a prolonged period and constitutes a practical advantage for the patient who might forget to take divided doses.[7]

The importance of potassium in carbohydrate metabolism has recently been emphasized, and it has been suggested that improvement of glucose tolerance in maturity-onset diabetic patients might indicate the presence of hyperaldosteronism.[8] Our subjects were asymptomatic diabetic individuals, and almost all showed improvement when potassium was added to the antidiabetic drug. This suggests that the potassium aided glucose utilization or provoked insulin secretion. It should be mentioned that potassium deficiency in normal individuals reduces glucose tolerance,[9] and that potassium administration to uremic subjects in whom body potassium is reduced increases insulin secretion and improves glucose tolerance.[10]

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Fish. Technol. 6(2):120-123

TOXICITY OF FOUR THERAPEUTIC COMPOUNDS TO FRY OF INDIAN MAJOR CARPS

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Toxicity of four therapeutic compounds, viz; sodium chloride potassium permanganate, potassium dichromate and acriflavine to fry of *Catla catla, Labeo rohita* and *Cirrhinus mrigala* was tested. Bio-assays were conducted in 5 and 10 litre glass jars in the laboratory for periods of 24 and 48 hours at temperatures of 26° and 32°C. LC_{50} values of the chemicals were in the ranges of 5500 to 7500 ppm, 37.5 to 48 ppm, 92.5 to 125 ppm and 47.5 to 80 ppm respectively. The results obtained are of value in evolving cheap control methods against some common fish diseases. Sodium chloride and acriflavine can be widely used for chemotherapy and potassium dichromate is preferable to potassium permanganate for prophylaxis.

INTRODUCTION

Very little work has so far been done in India on 'chemotherapy' of fishes. The importance of controlling parasites and diseases of cultivated fishes of the country has been recognised in the present context of production-oriented fishery research programmes in the country. In this connection emphasis has naturally to be laid on evolving cheap and efficient disease control methods. Screening of popular chemical compounds used in chemotherapy of fishes is an essential pre-requisite for this purpose. The present study, therefore, was undertaken to determine the toxicity of four therapeutic compounds to fry of Indian major carps viz. *C. catla, L. rohita* and *C. mrigala.*

MATERIALS AND METHODS

Fry of *C. catla, L. rohita* and *C. mrigala* were procured from different sources and those found healthy were quarantined for 15 days prior to experimentation. The length and weight ranges of the experimental fishes were

C. catla	22–47 mm	0.3–0.8 g
L. rohita	25–49 ,,	0.2–0.9 ,,
C. mrigala	24–48 ,,	0.2–1.3 ,,

The tests were conducted in 5 and 10 litre glass jars in the laboratory. Dissolved oxygen, pH, total alkalinity and temperature were within ranges of 4.5–5.5 ppm, 7.8–8.2, 193–322 ppm and 28–32°C respectively. Water was free from permanent hardness.

Each test included different concentrations and number of fish tested varied from 2 to 10 depending on the nature of of trial, viz; initial or final and keeping the total weight of fish within 1g per litre of water.

Concentrated stock solution in distilled water of each chemical was prepared first and added to the test jars in the required dosages.

Observations on survival and mortality of fish were recorded after 24 and 48 hours. Either the total mortality or 50% mortality of fry were recorded depending on the type of the test The data were then analysed to determine the concentration of the chemical producing 50% mortality (LC_{50}) of fry following Litchfield and Wilcoxon (1949).

RESULTS

All the compounds under test were found to be toxic to *C. catla, L. rohita* and *C. mrigala* and the LC_{50} values observed are given in tables I and II.

Effects of sodium chloride and potassium permanganate were found to be uniform in all the species whereas effects of potassium dichromate and acriflavine varied specieswise. Potassium permanganate was found to be most toxic closely followed by acriflavine, where LC_{50} values were within the ranges of 37 5 to 48 ppm and 47.5 to 80 ppm respectively. Effect of potassium permanganate was found to be excellent in controlling the infection of *Gyrodactylus* sp and *Dactylogynus* sp. whereas acriflavine could check the initial infection of tail rot in laboratory experiment. Potassium permanganate at a dose of 2 ppm when applied in rearing ponds thrice in a week could check the infection of monogenetic trematodes and dropsy within a period of one and a half months. In field application acriflavine could not check the infection of tail rot when the fishes were badly affected. LC_{50} value of potassium dichromate was within the range of 92.2 to 125 ppm to fry of Indian major carps. In laboratory condition it was noticed that this chemical could

TABLE I TOXICITY OF FOUR THERAPEUTIC COMPOUNDS TO CATLA, ROHU AND MRIGAL AT 26°C

COMPOUND	Catla LC_{50}		Mrigal LC_{50}		Rohu LC_{50}	
	At 24 hrs	at 48 hrs	at 24 hrs	at 48 hrs	at 24 hrs	at 48 hrs
Sodium chloride	7500 ppm	6000 ppm	7500 ppm	6000 ppm	7500 ppm	6000 ppm
Potassium dichromate	125 ,,	110 ,,	110 ,,	110 ,,	100 ,,	95 ,,
Potassium permanganate	48 ,,	40 ,,	48 ,,	40 ,,	48 ,,	40 ,,
Acriflavine	60 ,,	50 ,,	65 ,,	55 ,,	80 ,,	70 ,,

TABLE II TOXICITY OF FOUR THERAPEUTIC COMPOUNDS TO CATLA, ROHU AND MRIGAL AT 32°C

COMPOUND	Catla LC_{50}		Mrigal LC_{50}		Rohu LC_{50}	
	At 24 hrs	at 48 hrs	at 24 hrs	at 48 hrs	at 24 hrs	at 48 hrs
Sodium chloride	7250 ppm	5500 ppm	7250 ppm	5500 ppm	7250 ppm	5500ppm
Potassium dichromate	120 ,,	105 ,,	105 ,,	97.5 ,,	95 ,,	92.5 ,,
Potassium permaganate	45 ,,	37.5 ,,	45.0 ,,	37.5 ,,	45 ,,	37.5 ,,
Acriflavine	55 ,,	47.5 ,,	60 ,,	52.5 ,,	70 ,,	65 ,,

better act as prophylactic. Sodium chloride was found to be least harmful among the four therapeutic compounds mentioned above. 3% sodium chloride solution could check the infection of *Trichodina* sp. and monogenetic trematodes in both laboratory and field conditions. In this strength *Gyrodactylus* sp. was found dead when the fish was given a bath for 10 minutes. In rearing ponds the infection of monogenetic trematodes and *Trichodina* sp could be completely checked when both dip treatment in 3% followed by group treatment in 0.5% of sodium chloride solution were applied.

LC_{50} values of the four chemical compounds were determined at 26°C and 32°C and it was always observed that the values varied due to differences in temperature.

Discussion

Dip treatment in 2-5% sodium chloride solution for fishes infected with protozoan and monogenetic trematode parasites had been suggested by earlier workers (Davis 1956, Gopalakrishnan 1963, 1964 and 1967, Tripathi 1954 and '57 and Van Duijn 1956). LC_{50} values of this chemical were within the ranges of 5500-7500 ppm for carp fry at temperatures of 26°C and 32°C. In field treatment it was also found that this chemical could be used as prophylactic at a dose of 5000 ppm when fish size was more than 25 mm. As such it may be suggested that dip treatment in 3% followed by group treatment in 0.5% sodium chloride solution would be advantageous in combating the diseases like Trichodiniasis, Gyrodactylosis and Dactylogyrosis.

Potassium permanganate at a dose of 2, 3 and 4 ppm was used as a general prophylactic and its effect on Trichodina and monogenetic trematodes was found to be excellent (Allison 1957). But Van Duijn (1926) was of opinion that "an excessive dose of permanganate will kill fishes, while perfectly safe dose is frequently not strong enough to guarantee a cure". However, LC_{50} values of this chemical were found to be ranging from 40 to 48 ppm when the fish sizes varied from 25 to 49 mm. In rearing ponds 'group' treatment at a dose of 2 ppm applied thrice in a week for a period of one and a half months could check the infection of monogenetic trematodes and dropsy. As such it would be of worth to mention that the cumulative effect of the repeated applications of this chemical at a dose of 2 ppm had no adverse effect on fishes but on the contrary yielded better result.

Van Duijn *(loc. cit)* infers that potassium dichromate is safe at a dose of 50 ppm if it is not used for more than 10 days. In the present set of experiments mortalities were recorded within 48 hours at a dose of 30 ppm though LC_{50} values were within the ranges of 95 to 125 ppm for carp fry. In laboratory condition it was observed that this chemical could act better as prophylactic than any particular cure. This is in agreement with Van Duijn *(loc. cit)* who observed that potassium dichromate has a hardening effect on the skin of the fish which increases its resistance to further outbreaks of infection.

Wilford (1967) found that LC_{50} values of acriflavine were within the ranges of 10-100 ppm for rainbow, brown, brook and lake trout. The present observations showed that LC_{50} values ranged from 47.5 to 80 ppm for Catla, Rohu and Mrigal fry. It was also found that this chemical was having very toxic effect to the fry of Indian major carps and all the fry died within 24 hours when treated with a dose of 20 ppm. In field this chemical could not control the infection of tail rot when fishes were badly affected but in laboratory condition it could check the infection at the initial stage.

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Review Section

Trace Chemical Contaminants in Food: Potential for Harm*†

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Introduction

The organizers of this Symposium are to be congratulated on its timeliness. No one can deny that we are going through a stressful period in the history of food safety evaluation. During three decades of steady progress, immense benefits were conferred on the public through advances in agriculture and food technology, developments that had involved the introduction into food of a variety of chemical additives and contaminants. Considering the state of knowledge at the time when decisions were made on the acceptability of these materials, the record of safety of these chemicals is truly remarkable and stands as a tribute to those on whom the burden fell to make the critical judgements. Despite the fact that some of these men are now being vilified, we honour them and recognize, as History will, the magnitude of their achievements.

The reaction we are witnessing is a well-known social phenomenon, in which established standards in general are in process of demolition. Not only is there a movement in many countries to ban food additives as unnecessary and dangerous, but the harmlessness of trace contaminants is particularly called in question. It is appropriate that we undertake a critical reappraisal. My task, as I see it, is to explore the wider implications of food contaminants in order to assess, in practical terms, the problems and degrees of hazard associated with some of the major categories of these chemical agents.

Scope of the issues considered

When the Food Protection Committee (1959) defined safety as "practical certainty that injury will not result from use of a substance in a proposed quantity or manner" they had in mind a degree of certainty which, while not the theoretically desirable state of perfect assurance, is yet an attainable measure in practice. In the present climate of opinion, questions are raised: Is practical certainty acceptable when the potential hazard, however remote, is one that may be shared unwittingly by vast numbers of people of various ages and in various states of health? Not only is this degree of safety deemed insufficient, but

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equally inadequate is much of the traditional supporting evidence of safety—as, for instance, a long history of safe use of a chemical agent that contributes a trace residue to food.

Bolstering the insistence on 'absolute' safety is the introduction of what one might call 'tenets of uncertainty', many of which would not have been regarded as insurmountable in the recent past. As a reflection of this uncertainty, much of my presentation will be concerned with categories of potential hazard regarding which, in the opinion of some authorities and many would-be experts, no generally-acceptable assessment can be made to-day. The process began with the concept of 'zero tolerance' for chemical carcinogens, and the Delaney Amendment has enshrined this dogma beyond the reach of scientific criticism or the exercise of informed judgement. The underlying reasoning has been set out by Weisburger & Weisburger (1968) and in the Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health, the so-called Mrak Report (Commission on Pesticides, 1969, p. 492).

Almost imperceptibly, the same reasoning has been extended to teratogenic effects of food contaminants; even though, in my view, the scientific basis of zero tolerance for carcinogens is inapplicable to teratogens. The strong emotional reaction stirred up by fears of genetic hazard to future generations has sufficed to stretch the zero tolerance concept yet further, to the area of mutagenic effects. To my knowledge, not a single case exists in which a food contaminant of any sort, even a mycotoxin, is known to have produced mutagenic effects in man. As the crowning pediment of the entire structure of largely hypothetical hazards, we have the thesis that the phenomena of mutagenesis, carcinogenesis and teratogenesis are so closely linked that a positive result in any one of these areas automatically renders the compound suspect on all three counts. On the other hand, should it happen that a chemical agent turns out negative in all the tests applied, it remains under suspicion until such time as someone can discover an organism, devise a route of administration or achieve a sufficiently heroic dose to produce some positive biological result, however bizarre. Thus there is an obvious premium on ingenuity and persistence!

Carried to their logical conclusion, these considerations of carcinogenesis, teratogenesis and mutagenesis strike at the very roots of technological advance in food production, processing and distribution. Hence the practical reality of the potential hazards in these categories merits special discussion in the context of food contaminants.

Carcinogenesis

Numerous factors are known to influence an animal's response to a dietary carcinogen, so that any extrapolation to man of a no-effect level in animals is fraught with great uncertainty. The statisticians have embellished this plain biological fact with mathematical adornments (Commission on Pesticides, 1969, p. 493), but what they have failed to stress are the uncertainties attending the demonstration of weak carcinogenic activity. Even with a compound as extensively studied as DDT, the conclusions based on the Bionetics study (Innes, Ulland, Valerio, Petrucelli, Fishbein, Hart, Pallotta, Bates, Falk, Gart, Klein, Mitchell & Peters, 1969) are subject to dispute both on statistical grounds (Weil, 1969, 1970) and on such questions as the interpretation of liver nodules without antecedent study of their pathogenesis, without looking for possible regression on discontinuing administration of the test compound, without adequate attempts to transplant the hepatic nodules without trying to grow the cells making up the nodules in tissue culture (Slifkin, Merkow, Pardo, Epstein, Leighton & Farber, 1970) and without detailed attention to pulmonary metastases, both in controls and test animals.

The insistence on administration of a 'maximum tolerated dose' may also be misleading if this is the only dose tested, as in the Bionetics study (Innes *et al.* 1969). No justification is forthcoming for abrogating the need to establish a dose–response relationship, which is fundamental to all toxicological endeavour. Furthermore, the route of administration is all-important in tests for carcinogenesis or teratogenesis. We are told that "parenteral administration is an appropriate test route for pesticides to which humans are exposed by inhalation, or for pesticides which are systemically absorbed, following ingestion" (Commission on Pesticides, 1969, p. 660). Alas, the lessons of yesteryear are so readily forgotten! The production of subcutaneous malignant tumours by water, salt, glucose and a host of other common nutrients (Grasso & Golberg, 1966a) seems not to act as a deterrent to undiscriminating experimentation by this route, despite the demonstration that physical properties of a compound, such as the surface activity, lipid solubility, amphipathic character or hyperosmolarity of the solution injected determine the neoplastic outcome in many instances (Grasso & Golberg, 1966b; Gangolli, Grasso & Golberg, 1967). It is safe to predict that, by appropriate choice of dose, concentration of solution and frequency of administration by the subcutaneous route, any chemical agent can be shown to be a carcinogen in the rat and probably also in other species of laboratory rodent.

Teratogenesis

In checking on the potential teratogenicity of trace chemical contaminants in food, the height of absurdity is achieved by the combination of a maximum tolerated dose, a parenteral route of administration and the application of zero tolerance on the basis of the results. In this area we know full well that any one of a host of adverse influences on the mother is reflected in foetal deaths, resorptions and/or abnormalities. Teratogenic effects are elicited by: transport of mice by air on days 12 and 13 of pregnancy (Brown, Johnston & Niswander, 1970); fasting for 24 hr or less at a critical stage of gestation (Kalter & Warkany, 1959), a situation which may be brought about inadvertently or unsuspectingly by inducing somnolence, lethargy, muscle weakness or ataxia; a diet of raisins for 1 day (Peters & Strassburg, 1969); severe limitation of movement or avoidance behaviour (Rosenzweig & Blaustein, 1970); and many other non-specific factors probably acting through a stress mechanism, as well as hyper- or hypothermia and endocrine influences (Kalter & Warkany, 1959). Even subcutaneous sodium chloride is teratogenic in mice (Nishimura & Miyamoto, 1969).

Here above all is a situation that demands the utmost care in selecting doses that do not render the mother sufficiently ill to produce even transient inappetance. The use of a maximum tolerated dose overlooks the possibility of non-specific toxic stress. Apart from artefacts that arise by this means, attention should be directed to the mechanism by which particular teratogenic effects are elicited. The production of even transient deficiency of one of many vitamins or minerals suffices to induce reproductive failure or malformations. An example is the case of EDTA, which is teratogenic in mice (Tuchmann-Duplessis & Mercier-Parot, 1956). The production of congenital malformations by an excess of vitamin A is well known. A tenfold excess of nicotinamide is embryotoxic in the pantothenate-deficient rat (Lefebvres-Boisselot, 1951). Maximum tolerated doses of other B vitamins or of most amino acids have not been studied. A single injection of 2 mg leucine elicited abnormalities in 41% of chick embryos in an experiment in which 4 mg thalidomide brought about abnormalities in 22%, against an incidence of 2% in the control group (Bergström, Erilä & Pirskanen, 1967).

These considerations are provided as a background to recent developments that led to the withdrawal of a petition to establish negligible residue tolerances for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The petition would have set tolerances of 0·2 ppm for 2.4,5-T from the application of the acid form or certain salts or esters in or on a variety of foodstuffs. Subsequently it was announced that the use of 2,4,5-T on food crops would be cancelled.

This interesting case study began with the observation that 2,4,5-T was teratogenic and foetocidal in two strains of mice when administered either subcutaneously or orally and in one strain of rats when administered orally (Courtney, Gaylor, Hogan, Falk, Bates & Mitchell, 1970). Analyses of the specimen of 2,4,5-T that had been tested revealed the presence of approximately 30 ppm 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (referred to henceforth as dioxin). Subsequent study of standard 2,4,5-T containing less than 1 ppm dioxin, given to rats by gavage in doses up to 24 mg/kg daily, failed to reveal evidence of teratogenic or embryotoxic effects (Emerson, Thompson, Gerbig & Robinson, 1970). Under similar conditions, the dioxin produced no discernible effect at a dose of 0·03 μg/kg/daily, while doses of 0·125 μg/kg/day or greater manifested toxicity to the foetus and, at 8·0 μg/kg/day, to the mother also (Sparschu, Dunn & Rowe, 1970).

Finally in evidence presented before Senator Hart's subcommittee, samples of 2,4,5-T containing less than 1 ppm dioxin were reported to have been tested by the oral route in rats at levels up to 150 mg/kg, with negative results. In three strains of mice, levels of 100–150 mg/kg/day, given subcutaneously in dimethyl sulphoxide, proved teratogenic; so did the dioxin in both species tested (*Food Chemical News*, 1970).

Even assuming that doses of 100 mg/kg daily did not serve to introduce sufficient dioxin to account for the observed effects, it should be realized that such quantities of 2,4,5-T are ill-tolerated by mice. They lie within the range of doses studied by Sos & Kertai (1958) and, later, by Florsheim & Velcoff (1962) and Florsheim, Velcoff & Williams (1963) in the case of 2,4-dichlorophenoxyacetic acid (2,4-D). In experiments on rats, 2,4-D was shown to produce a striking reduction in the serum level of protein-bound iodine, as a consequence of the displacement of thyroxine from serum proteins into the liver and to a lesser extent into the kidney. In this way a characteristic 'hyperthyroid' state is created in these organs. Ethyl chlorophenoxyisobutyrate acts in a manner similar to 2,4-D, though some differences in detail may be observed (Ruegamer, Ryan, Richert & Westerfeld, 1969).

The profound effect exercised by 2,4-D, and presumably also by 2,4,5-T, may not be related in any way to the observed teratogenic action of 2,4,5-T in mice. Nevertheless the metabolic and other actions exercised by massive doses on the mother, the foetus, or both, should be taken into consideration in extrapolating from 100 mg/kg/day in mice to residue levels below 0·2 ppm associated with a limited number of foods in the human diet—a ratio possibly exceeding 5000. The possibility of introducing dioxins into the environment has been put forward as a further reason for taking action against 2,4,5-T. This aspect of polychlorinated compounds is dealt with below.

Quite apart from concern with mechanisms of action, or the avoidance of artefacts due to non-specific stress, the metabolism of a test compound is a highly relevant consideration in teratogenesis brought about by drugs or food contaminants. If the metabolic pathway in the test animal differs radically from that in man, the results are unlikely to be useful for the assessment of hazard arising from trace contaminants. The finding of embryotoxicity only has meaning in an appropriate animal model.

There is a further aspect that has been insufficiently stressed in the past: the fact that the

use of a maximum tolerated dose involves risks of artefacts, among them those arising from abnormal metabolism—either qualitatively or quantitatively, or both. There is no metabolic pathway that cannot be overloaded, with consequent diversion into other metabolic routes and/or abnormally prolonged retention or excretion of the unchanged compound. Such situations may be totally unrepresentative of the body's handling of the test compound at levels of exposure approaching more realistically the practical conditions of intended use. While the object of the toxicologist is to seek out target-organ effects elicited by high doses, his purpose in doing so is to provide guidance to informed searching for effects at lower doses. Grossly aberrant pathways and rates of metabolism that may exist at exaggerated doses make it imperative not to assume that effects observed at these doses are necessarily characteristic of the changes occurring at lower levels of exposure.

Mutagenesis

The risk of mutagenic effects to future generations of mankind is now thought to be so great and imminent that no chemical agent may be regarded as acceptable for use in the diet without being subjected to a battery of tests for mutagenicity. We are assured that these tests are "entirely practical and feasible . . . precise, efficient and relatively inexpensive . . . practical, sensitive and relevant" (Commission on Pesticides, 1969, p. 572). Why then the reluctance to apply tests of this sort to all food contaminants? Presumably any positive result in any one test at any dose makes it necessary to consider the compound as dangerous until proved safe—though how such proof of safety can ever be achieved in these circumstances is not clear. On this basis, for example, it becomes necessary to dispose of chelating agents, since all those tested so far are mutagenic (Kihlman, 1966). The problem is that chelators are plentifully present in food and throughout the body. Adenine, a common constituent of food, breaks chromosomes; that is it acts as a 'clastogen' (Shaw, 1970) by chelating certain bivalent metal ions.

It is not my intention to convey the impression that as a toxicologist I am less concerned than the geneticists over the possibility of mutagenic effects of food contaminants. Where we differ is on priorities for testing and possibly on the degree of satisfaction with the tests available at present. The range of compounds tested so far for mutagenic and clastogenic potential is so limited (Table 1) that in effect we know virtually nothing about the response of the test systems to the multitude of common chemicals found in 'natural' food or in the body. I fail to see how, lacking such background experience, we can assess the potential for harm of trace contaminants in food by equating them with carcinogens and with powerful alkylating agents used in cancer chemotherapy.

In the months that have elapsed since the publication of the Mrak Report (Commission on Pesticides, 1969) the view has gained wider acceptance that non-mammalian systems are too far removed from the areas of concern to be adequate test systems for assessing potential mutagenic hazard to man. Unfortunately, for the detection of point mutations, the host-mediated assay still employs micro-organisms; but this situation will no doubt be remedied through the use of mammalian cells maintained in tissue culture and implanted into the host animal treated with the test compound. A variety of interesting approaches are under development, based on *in vivo* cytogenetics, the dominant lethal test and *in vitro* cell-culture systems. All these tests call for careful evaluation and the acquisition of substantial experience and perspective before being applied indiscriminately for predictive purposes to new compounds.

Table 1. *Food clastogens (chromosome-breaking agents; C) and mutagens (M)*

I. C or M in non-mammalian organisms				
Acetate	Cyanide	K^+ (excess)	Ethylene bromohydrin	DDT
Amyl alcohol	Ethanol	Captan	Ethylene chlorohydrin	Nitrite
Acetophenone	Formate	Ethylene oxide	Ethylene dibromide	Pyrophosphates
II. C in mammalian organisms				
Hexamethylenetetramine				
III. C in human cells *in vitro* or *in vivo*				
Adenine	Bisulphite	Cyclohexylamine	Theobromine	
Aflatoxin	Caffeine	Lead (excess)	Theophylline	
Arsenates	Cyclamate	Methylmercury		
IV. Suspected of being M or C				
Acetone	Ethyl acetate	Methyl acetate	Monosodium glutamate	
Benzo[*a*]pyrene	Methanol	Propanol	Toluene	

Mainly from Shaw (1970).

Pollutants derived from environmental sources

Many and varied are the possibilities for the entry into food of chance contaminants whose existence in the environment was unrecognized or whose harmful potential was not fully realized. In recent years contamination of food with persistent chlorine-containing pesticides, principally DDT, aldrin and dieldrin, has commanded increasing attention. The environmental and other problems presented by these compounds have been discussed in numerous publications and reviewed most comprehensively in the report of the Commission on Pesticides (1969). On the basis of this report, action has been taken to eliminate the use of DDT and DDD and, progressively, the uses of aldrin, dieldrin and other compounds that "cause or can cause contamination of the environment and damage to various life forms within it". A price of unknown magnitude, possibly involving serious future repercussions, has thus been paid for the protection of wildlife and the environment. As far as trace contamination of human food is concerned, the report states clearly that no evidence has ever been produced of harm to man stemming from this source. The sole known consequence of human exposure to traces of DDT in food is the acquisition of measurable levels of DDT and DDE in blood, body fat and other tissues. No harmful effect has been found to follow such storage (Hayes, 1967). Essentially similar conclusions were reached in a British report on persistent organochlorine pesticides (Advisory Committee on Pesticides and Other Toxic Chemicals, 1969), and again the recommendations have been followed by restriction of the use of these materials.

By a remarkable coincidence the present age of ferment has witnessed the discovery of the presence of polychlorinated biphenyls in a wide range of organisms (Risebrough, Rieche, Peakall, Herman & Kirven, 1968) as well as further recognition of the evil potentialities of chlorinated naphthalenes and dioxins (Friedman & Shibko, 1969; Bauer, Schulz & Spiegelberg, 1961; Jones & Krizek, 1962; Higginbotham, Huang, Firestone, Verrett, Ress & Campbell, 1968). To eliminate such materials from the food supply or from the environment is a more complex undertaking than might appear at first glance. 2,4-Dichlorophenol, a potential precursor of chlorinated dioxins, is synthesized by a strain of Penicillium from soil (Ando, Kato & Suzuki, 1970). A diligent search will probably reveal a variety of

chlorinated compounds of this sort synthesized in Nature (Hylin, Spenger & Gunther, 1969). 2,4,5-Trichlorophenol, formed as a metabolite of the organophosphate pesticide Ronnel, is stored in body fat and other animal tissues (Menzie, 1969).

The distinction between polychlorinated biphenyls and polychlorinated dioxins is more apparent than real. Thus Vos, Koeman, van der Maas, ten Noever de Brauw & de Vos (1970) have demonstrated that the striking toxicity of commercial polychlorinated biphenyls is associated with the presence of tetrachloro- and pentachlorodibenzofurans. These furans closely mimic the toxic properties of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin but at tenfold dosage. Attention should also be directed to the question of photo-oxidation products, several of which, in the case of pentachlorophenol, are potentially capable of undergoing further cyclization to tricyclic and pentacyclic polychlorinated dioxin derivatives (Munakata & Kuwahara, 1969).

In an era of increasing concern about lead as an atmospheric pollutant as well as a food pollutant, there comes, as a final blow, the recognition that hazards of mercury contamination are not confined to fish from remote Swedish lakes, but, as forecast so accurately by Berglund (1969), are here on the American continent. Each of these problems has its own individual characteristics, yet there is a common thread running through them all: the fact that past ignorance or disregard of ecological implications is only now being overcome as present-day methods of analysis, in all their tremendous sensitivity, specificity and accuracy, are brought to bear on the complex questions at issue.

The mercury problem is typical in this respect. An excellent account of its various facets has been presented by several authors in a volume edited by Miller & Berg (1969). An already difficult environmental situation, stemming from treatment of seed grain with organic mercury compounds, has been confounded by the discovery that inorganic mercury can be methylated by anaerobic micro-organisms (Jensen & Jernelöv, 1969), specifically by methanogenic bacteria (Wood, Scott, Kennedy & Rosen, 1968). The existence of mechanisms by which methylcobalamin can readily transfer its methyl group to mercuric ion introduces a new dimension into the problem, since we are now concerned exclusively, or almost exclusively, with methylmercury residues far more than with inorganic mercury in fish and other foods. Such a transfer of interest has far-reaching implications, in view of allegations of subliminal neuronal damage, potential for teratogenesis and observed mutagenic effects in lower organisms.

Emphasis on the accumulation of methylmercury in the central nervous system, with preferential injury to certain groups of neurones, such as the cerebellar granule cells (Kurland, Faro & Siedler, 1960; Miyakawa & Deshimaru, 1969), has been extended to the peripheral nerves, where the initial impact of methylmercury was found to elicit specific selective damage to sensory fibres (Miyakawa, Deshimaru, Sumiyoshi, Teraoka, Udo, Hattori & Tatetsu, 1970). Accounts of human poisoning with methylmercury also refer to initial peripheral sensory disturbances, ataxia and polyneuritis (Kurland *et al.* 1960; Miyakawa & Deshimaru, 1969; Tsubaki, 1968). The suggestion that accumulated methylmercury has the capacity to "pick off" individual neurones raises three questions that are the core of the issue concerning methylmercury:

1. Is the nature of local binding sites for methylmercury in certain individual neurones such that selective accumulation occurs within these cells, more or less independently of the rate of intake of methylmercury, the resulting total body burden and the nature and extent of redistribution of the methylmercury that occurs

within the body with the passage of time, and, finally, irrespective of the rate of excretion of the methylmercury and the resulting decrease in total body burden?
2. Does localized selective damage to individual neurones or groups of neurones represent a permanent and irreplaceable loss which may not necessarily become apparent, at least initially, as a functional deficit?
3. If such loss does indeed occur, what is the extent of the damage that must be sustained before clinical signs and symptoms are manifest? In other words, how useful is clinical evidence as a means of establishing a threshold or 'no ill-effect' dose for methylmercury in food?

Consideration of the accumulation of methylmercury must also take into account excretion from the brain and from the body (Berglund & Berlin, 1969). The body burden, expressed as the concentration of methylmercury per gram of brain, does bear a direct relationship to intake and to the appearance of neurological signs. The brain content of methylmercury, however, may not reflect adequately the extent of irreversible damage to susceptible neurones or other structures. Despite the elegant studies of distribution of organic mercury in mice (Ostlund, 1969) and men (Aberg, Ekman, Falk, Greitz, Persson & Snihs (1969), we have as yet no means of knowing the amount of methylmercury needed to cause a critical degree of long-term irreversible damage to the foetal nervous system, which is said to be more susceptible than that of an adult (Berglund & Berlin, 1969). Equally, possibilities of effects on germ cells cannot as yet be gauged by measuring body burdens.

The generally-accepted value for the biological half-life of methylmercury is about 70 days. According to Aberg *et al.* (1969), a weekly intake of unit amount of methylmercuric nitrate will result in a total body burden of 15·2 units of mercury at infinite time. The long-term consequences of such storage remain to be determined, particularly in man. The opportunity for epidemiological study of exposed populations must not be missed.

Preoccupation with those problems that are currently the most pressing must not cause us to lose sight of traditional, yet increasingly serious contaminants like lead, or environmental contaminant hazards of uncertain magnitude like cadmium or asbestos. The dimensions of the lead problem in food and drink are still not accurately comprehended. A recent series of analyses of basic foodstuffs in Germany (Lehnert, Stadelmann, Schaller & Szadkowski, 1969) revealed an average intake of lead through food of 0·518 mg/day, with green vegetables contributing a major proportion. Undoubtedly some of this lead originates as an air pollutant (Everett, Day & Reynolds, 1967; Danielson, 1970). Also, unsuspected lead hazards abound, particularly for children, as exemplified by the consequences of intrauterine and neonatal exposure of the offspring of mothers consuming untaxed whiskey in the southeastern United States (Palmisano, Sneed & Cassady, 1969).

The complexities of biological interrelationships of trace minerals, both commonplace and rare, in food and water are attracting increasing attention on the part of those interested in hypertension and cardiovascular disease (Masironi, 1969), cancer (Rose, 1968; Kanisawa & Schroeder, 1969) and dental caries (Losee & Adkins, 1968; Jenkins, 1969). With each passing day it becomes more difficult to disentangle potentially-harmful from potentially-beneficial effects of many trace metal contaminants of food.

Interactions between contaminants

Concern has often been expressed that, among the thousands of chemical contaminants present in food, some subtle forms of interaction with one another or with food components

will be manifested as unexpected toxic effects. Instances of this sort that have arisen in the past (Golberg, 1967; Friedman & Shibko, 1969) indicate that there is no room for complacency. On the other hand, one must be rational in assessing the extent of real hazard that exists in practice.

Thus, in the case of pesticides, a masterly analysis of possible interactions and their significance has been presented in the Mrak report (Commission on Pesticides, 1969). Among many topics covered, this account places in proper perspective the rather miniscule dangers, under current conditions of use, that are presented by interactions between pesticides on the one hand and methylenedioxyphenyl synergists and methylenedioxyphenyl food components on the other. Several attempts to unearth other forms of hazard arising from mixtures of pesticide residues have yielded so little evidence of biological interaction as to discourage further exploration of such possibilities. Suggestive indications of cocarcinogenicity with aflatoxin can be obtained when cyclopropenoid fatty acids are tested (Sinnhuber, Wales & Lee, 1966). The results, however, probably have little relevance to the conditions that obtain in human or animal diets.

Radioisotopes

The issue of "Fallout, Food and Man" was thoroughly aired in a Symposium held in 1963 (Menzel, 1963; Comar, 1963; Eisenbud, 1963). Among more recent developments, the strenuous efforts that have been made to achieve acceptability of irradiated foods will be discussed later in this Symposium in connexion with contaminants arising from food processing. A new controversy has recently arisen over the suggestion put forward by Sternglass (1969a) who states in effect that nuclear fission products and other forms of radiation in food, particularly ^{90}Sr in milk, are the primary cause for the interruption in the decline of foetal and infant mortality at the present time. The possibility exists that gonad–dose calculations made in the past and based largely on ^{137}Cs were grossly in error, by not taking into account ^{90}Sr and its yttrium daughter product as well as radioisotopes such as ^{14}C and ^{3}H that "seek out specific critical sites in the mammalian cell" (Sternglass, 1969b, c). Attempts to refute the allegations concerning foetal and infant mortality (Tamplin, 1969; Sagan, 1969; Lindop & Rotblat, 1969) have only partially succeeded in restoring confidence that both the total level of radioactivity in food, as well as that portion of it that most directly affects the gonads, are well within acceptable limits.

An attack from a different quarter has been launched by Gofman & Tamplin (1969) on the question of tritium levels in water issuing from nuclear-fuelled power stations (Weaver, Harward & Peterson, 1969). Here the substance of the charge is that the existing allowable dose of whole-body ionizing radiation laid down by the Federal Radiation Council (0·17 rads/year), is too high by a factor of 10. The practical implications, in terms of public health and of the design of power stations to conform with these higher safety standards, are of such magnitude as to demand critical consideration of the Gofman & Tamplin proposals (Holcomb, 1970).

Fundamentally the problems that arise over the assessment of hazard from prolonged exposure to low levels of radioactivity in food and water are exactly analogous to those that confront us in the case of carcinogens. They involve extrapolation from known effects at high levels of exposure to presumed potential effects at very low levels, levels that are often below the natural background of radiation or of carcinogens from other sources. The effects on the body are in both instances regarded as strictly additive, despite evidence that natural food stock ration contains one or more factors that protect against X-irradiation

carcinogenesis in mice (Ershoff, Bajwa, Field & Bavetta, 1969) and that anticarcinogenic factors and mechanisms abound (Shamberger, 1970).

Among the few voices to dispute the rigid orthodoxy of the majority, Budinger (1970) has pointed to dose rate, rather than total dose, as the determining factor which, at low values, may permit recovery and repair mechanisms to operate with exquisite sensitivity. From all we know of the systems involved, the likelihood that effective thresholds do exist at low dose rates seems greater than the prophets of doom will admit. This statement applies to cancer, genetic effects and chromosomal aberrations induced by exposure to radiation.

Residues of drugs and feed additives

Trace contaminants present as residues in foods of animal origin constitute another critical area. In an era of increasingly intensive production of animal foods, the use of chemical agents as feed additives serves to improve efficiency of feed utilization directly, or indirectly by preventing disease. Residues derived from these additives are augmented by trace contaminants resulting from the use of chemotherapeutic agents and pesticides applied directly to the animal, as well as the pesticide residues forthcoming indirectly from consumption of feed of plant origin.

Detailed consideration of the implications of the use of drugs in animal feeds has been presented in a Symposium (National Academy of Sciences, 1969). Munro & Morrison (1970) have also dealt with this topic. The most crucial issue at present is that of antibiotic residues, a subject dealt with at length in the report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine (1969). This UK committee (the 'Swann Committee') has arrived at far-reaching conclusions. Despite the fact that present antibiotic residues in food were not considered by the Committee to constitute a toxic or allergic hazard to the consumer, evidence of potential risks resulting from administration of antibiotics to farm livestock was deemed sufficiently great to justify prompt legislative action. This evidence related to a marked increase in the number of strains of enteric bacteria of animal origin that showed resistance to one or more antibiotics and were able to transmit this resistance to other bacteria; to the observation of human infection with enteric bacteria derived from animals as a result of consumption of foods of animal origin; and to the possibility that antibiotic resistance could thereby be introduced into organisms that are highly pathogenic to man.

The sweeping recommendations of the Committee, and the resulting legislative measures applied in the UK have given authorities in other countries much food for thought. Without entering here into the detailed data supporting these actions, one can decide that there are grounds for reasonable suspicion of hazard but as yet little conclusive epidemiological information. In this situation, as in so many others involving food contaminants, one is left with the alternatives of drastic action on a slender basis of fact or the introduction of limited precautions during the period of grace needed to try to establish the reality or otherwise of the presumed hazards. The crucial issue seems to be our increasing doubts as to the usefulness, if any, of antibiotic growth supplements in well-conducted animal husbandry. This, more than any other factor, militates against continued acceptance of the hazards of what has been termed a "potentially explosive situation".

Migrants from food-contact materials

Here we have a vast heterogeneous group of some 5000 chemical agents which for the most part are present in food in small numbers and in minute amounts at any one time.

Determination of the precise nature and quantities of compounds migrating into various foods from any single packaging material often presents formidable obstacles. Nevertheless, such information is necessary if the components of the package extractable into food are regarded in essence as food additives (McCollister, 1964).

Faced with these difficulties, the extent of migration has been assessed by means of food-simulating solvents and various schemes have been devised for broad flexible approval (British Plastics Federation; Golberg, 1963), recipe-type approval (West German Federal Health Office) or approval by end use (US Food and Drug Administration.) A recent report by the UK Food Additives and Contaminants Committee (1970) recommends that a total migration limit should be laid down, based on the use of specified food-simulating solvents. Statutory control would be exercised by drawing up permitted lists of ingredients, presumably similar to the German lists.

In an effort to rationalize various approaches on the basis of likely hazard to the consumer, Frawley (1967) elaborated a general plan, based on experience of no-effect levels of chemicals (other than pesticides and heavy metals) deduced from lifespan studies in animals. The basic conclusion drawn from this experience was that any material suitable for use as a functional component in food packaging at a level of 0·2% or less could not attain an unsafe level in food contained in such packaging material.

A further step in the process of bringing common sense to bear on these problems was taken by the National Academy of Sciences–National Research Council's Food Protection Committee (1969) in a report entitled *Guidelines for Estimating Toxicologically Insignificant Levels of Chemicals in Food.* There was every reason to believe that some logical implementation of these approaches by the US Food and Drug Administration would at long last culminate in a rational method of regulating food-contact materials (Oser, 1969).

At this point, unfortunately, progress has been halted and in the prevailing climate of doubt it is hard to foresee whether any fresh solution will emerge. The need for action is greater than ever, for the development of entirely new forms of food packaging needs to be encouraged if mankind is to solve its problems of waste disposal.

In the meantime, we have to recognize frankly some of the difficulties inherent in this area of food contaminants. Considering the five major classes of food-packaging materials, namely glass, coated metal, paper, regenerated cellulose and plastics, one has to take account of the amounts of the same compound contributed to the diet from several of these sources (Ingle, 1968), or the addition to food-grade oils of what is, in effect, a food additive (BHT, 4,4′-thiobis-6-*tert*-butyl *m*-cresol or other antioxidant) derived from low-pressure or, more particularly, high-pressure polyethylene (Woggon, Uhde & Zydek, 1968).

Another problem often arises: how to dispel notions of hazard based upon the results of administering huge doses to animals, when the amounts migrating from food packaging are minute. Probably nothing can better exemplify this absurdity than the case of ethylene glycol (EG) which, if permitted for use in food packaging at extremely low levels, would serve several important technical purposes. EG suffers from two stigmata. In man, rats and other species given large doses it produces renal calculi, calcification and tubular damage (Rowe, 1963). Diethylene glycol, which is probably metabolized along lines similar to EG, gives rise to bladder stones and bladder tumours. The attendant controversy over this form of 'carcinogenesis' has been resolved by evidence that implantation even of glass beads into the rat bladder produces bladder tumours (Weil, Carpenter & Smyth, 1965). In the monkey, Blood, Elliott & Wright (1962) showed that dietary levels of 0·2 and 0·5% EG could be tolerated for up to 3 years without apparent effect. Recently we have demonstrated the

capacity of rats and monkeys to excrete and metabolize small amounts of EG with great dispatch (McChesney, Golberg, Parekh, Russell & Min, 1971). This facility is shared by man (F. Coulston, J. H. Wills, L. Golberg & J. C. Russell, in preparation, 1970). Finally, it now transpires that the normal mammalian organism has EG among the 'diol lipid' components of its tissues (for references see Bachmann & Golberg, 1971).

The consumer's organoleptic threshold for taint acts as a limiting factor to volatiles transferred from plastics and other packaging to food. With increasing complexity of packaging materials, for instance polyvinylidene chloride–cellophane–polypropylene laminates, traces of residual solvents may be transferred to food. Toluene, a typical example, is probably innocuous at levels well above those that cause rejection of the food because of taint (Wilks & Gilbert, 1969). Similarly, in the case of polyvinyl chloride, the extent of migration of monomers and oligomers is so small as not to influence consumer acceptability. Nevertheless, now that vinyl chloride has been reported to be "highly cancerogenic"—admittedly by inhalation—in rats (Viola, 1970), other considerations enter into the picture. It will be interesting to see how 'zero tolerance' is applied in this instance.

In an age when problems of waste disposal loom large, the toxicity of pyrolysis products of materials like vinyl plastics assumes importance. Studies of polyvinyl chloride homopolymer and vinyl chloride–vinyl acetate copolymer have established the presence of HCl, CO, methyl and ethyl chlorides, benzene, toluene and a variety of short-chain alkanes among the products formed (Le Moan & Chaigneau, 1969; Cornish & Abar, 1969).

A rapidly-increasing and advantageous use of plastics is in the transport of potable water. The five major types of thermoplastics in water pipes all contribute minute amounts of contaminants. In some countries the use of stabilizers containing lead or cadmium is accepted practice; it is doubtful whether the amounts of these elements entering the water supply from this source appreciably increase the total intake into the body from the environment as a whole (Lefaux, 1968).

Conclusion

I could cite many more groups of food contaminants that furnish examples of uncertainty as to what constitutes a safe level in food. Sufficient has been said, however, to establish that the potential for harm is not confined to any one class of compounds or to any one source of contaminants. In the presence of this multitude of trace chemicals entering into our food, what should the regulatory approach be? Should we strive to return food to its 'natural' pristine state? Chemically-uncontaminated food has never existed. In fact, if one includes all forms of contamination, our present diet is the least contaminated in humar history.

There are, as I have indicated, many disturbing features in the present situation. The accumulation of complex issues and scientific imponderables makes it necessary to grapple with urgent practical necessities by adopting more limited objectives than the establishment of levels or other conditions of use that ensure practical certainty of freedom from hazard. In many instances the most we can hope to do is to try to define as clearly as possible the likely hazard associated with particular conditions of exposure; in other words the price, in terms of potential harm, that the consumer may be expected to incur for a certain increment of contaminant level in his diet. We should cease trying to assure the public that all food is safe and nutritious when we recognize that, in clinical terms, the capacity for harm of 'natural' food is virtually unknown. All that the toxicologist can claim to accomplish, or hope to do at present, is to assess the degree to which particular levels of food

contaminants, in designated foods consumed in defined amounts at defined intervals, add to the pre-existing potential for various sorts of harm that the diet already possesses.

Given an indication that the increment in potential harm attributable to the presence of a food contaminant is unacceptably great, how much information is needed before preventive measures are taken? Here we are on a delicate tightrope, balanced between rushing in with ill-considered action before results have been adequately established and, on the contrary, awaiting detailed confirmation of every aspect of an experimental or epidemiological finding, in spite of mounting evidence that calls urgently for decisive intervention. Too many facile solutions to this problem have been proposed. Least heed of all should be given to the demand that every straw in the scientific wind should prompt immediate withdrawal of the product thus impugned—with the rather naïve idea that the material can readily be restored on the market once its safety has been successfully re-established.

We have had ample experience recently of the chaos, public alarm and confusion, waste of food and economic loss that can result when precipitate restrictions are introduced too late and without adequate warning, in place of measured and timely precautions instituted as the data develop. Neither neglect nor panic is the answer. Somewhere between these two extremes lies the course of reasonable action appropriate to each chemical-contaminant contingency. Given good science, good judgement and, above all, freedom from extraneous pressures, the right course can be found.

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The Effect of High Salt Intake on the Blood Pressure of Rabbits

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The blood pressure of the normal, young adult giant female, chinchilla rabbit is insensitive to the presumably hypertensive effect of a high intake of sodium chloride. The ingestion of 5 gm. of sodium chloride daily, for many months, did not induce a significant elevation of the direct mean systemic blood pressure in intact rabbits fed a normal diet. Unilateral nephrectomy did not result in elevation of the blood pressure in rabbits fed the standard diet containing the normal amount of salt. Some unilaterally nephrectomized rabbits on the high salt diet, which was initiated immediately after the operation or two months later, eventually showed a slight elevation of the blood pressure which, however, was not significantly greater than the rise that occurred in some control rabbits of the same age fed only the normal diet for a similar period.

Additional key words: Blood pressure, Hypertension, High salt intake, Renal ischemia.

The effect of high salt intake on the blood pressure of animals has been investigated mainly in rats.[5, 6] All of the investigators are agreed that the daily ingestion of a large amount of salt has no immediate effect on the blood pressure of the intact normal rat, and that the pressure does not begin to rise significantly for a variable period. The amount of salt which has proved effective in the rat is relatively great in comparison with the average intake of normotensive humans. The most consistently hypertensive effect of a high salt diet has been reported in genetically susceptible rats.[1]

There have been few studies on the effect of high salt intake on the blood pressure of rabbits. Fukuda[2] reported that there was a slight rise in normal intact rabbits as the result of the intake of 5 gm. of NaCl daily for several months. He determined only the systolic blood pressure in the central artery of the ear by the indirect method of Grant and Rothschild.[4] The rise began approximately 1 month after the initiation of the high salt intake and persisted for 8 months. When the high salt diet was discontinued, the blood pressure gradually returned to normal over approximately 4 months. Fukuda found that the presence of the adrenals was necessary for the elevation of the blood pressure caused by high salt intake.

In another study[7] on the effect of high salt intake on the blood pressure of the rabbit, no elevation occurred in unilaterally nephrectomized animals that had also received injections of the mineralocorticoid, cortexone trimethylacetate.

MATERIALS AND METHODS

Giant female chinchilla rabbits, 6 months old, weighing from 3.75 to 4.25 kg. each at the beginning of the control period, were used exclusively in this study. All were fed a normal rabbit chow diet *ad libitum*. The high salt intake was effected by restricting the source of fluid to a 1 per cent aqueous solution of sodium chloride. Most of the rabbits refused to drink a 2 per cent solution, and some drank an insufficient quantity of the 1 per cent solution. Only those which drank 500 ml. of the 1 per cent salt solution (5 gm. of NaCl) daily are included in this report. The total amount of salt ingested daily by every rabbit was almost equal to the daily quantity of a normal adult human in this country (United States), and was at least 10 times the amount taken in daily in their food during the control period. The direct, mean systemic blood pressure was determined at least every week by the percutaneous method previously described.[3] The control period for the normal salt intake was never less than 12 weeks, and was much longer for most of the animals (Table 1).

RESULTS

In this study the blood pressure of many rabbits fed the diet with normal salt concentration was determined for a variable period. From these animals, those were chosen with a stabilized blood pressure. In some young growing rabbits, the blood pressure continued to rise slightly for many weeks, so they were not chosen for the test of the effect of high salt intake or were used only after a long control period when the blood pressure had reached a definite plateau. The blood pressure of the animals used for the effect of high salt intake was remarkably uniform within a narrow range during the long control period (Fig. 1).

The Effect of High Salt Intake on the Blood Pressure of Normal Rabbits

This group (Table 1) consisted of six rabbits, all of which, after the control period (16 to 39 weeks), drank 500 ml. of 1 per cent NaCl daily for 17 to 65 weeks. During the control period, the blood pressure of the individual rabbits varied only slightly within a narrow range. The maximal rise in these six animals varied from 4 to 24 mm. In only one of the six rabbits (176, Fig. 1) was there a slight, persistent rise of pressure, which occurred during the last 30 weeks of the high salt intake; however, the maximal rise even in this animal was not as great as that which occurred during a similar period in some rabbits on the normal intake of salt.

The Effect of High Salt Intake on the Blood Pressure of Unilaterally Nephrectomized Rabbits

In one group of six rabbits after a variable control period on the normal diet, unilateral nephrectomy was performed, and after another variable period still on the normal salt intake, the animals were given the 1 per cent salt solution to drink, but only three of the animals drank 500 ml. daily. Figure 2 (rabbit 34) illustrates what happened in all three animals. During the control preoperative period of 9 weeks and during the postnephrectomy period of 9 weeks before the beginning of the high salt intake, the blood pressure remained at a fairly uniform level; during the 18 weeks of high salt intake there was also no significant elevation of the blood pressure (Table 1, rabbits 34, 40, and 45). When the high salt intake was discontinued, the main artery of the right kidney was permanently constricted to a moderate degree in order to test for a hypertensive response

Table 1

Rabbit	Normal salt intake		High salt intake	
	Time	Maximal rise of blood pressure	Time	Maximal rise of blood pressure
	weeks	*mm. Hg*	*weeks*	*mm. Hg*
54	39	16	42	4
176	38	8	65	14
182	26	12	34	12
185	24	10	24	2
197	24	24	31	8
134	16	4	17	0

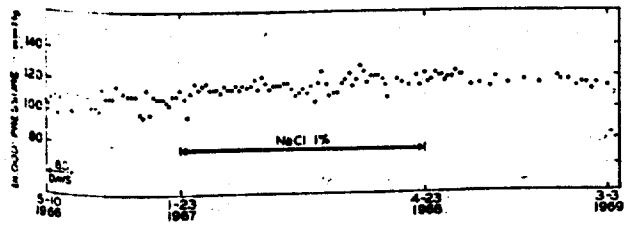

Fig. 1. Rabbit 176. NaCl 1 per cent, 500 ml. ingested daily for 65 weeks.

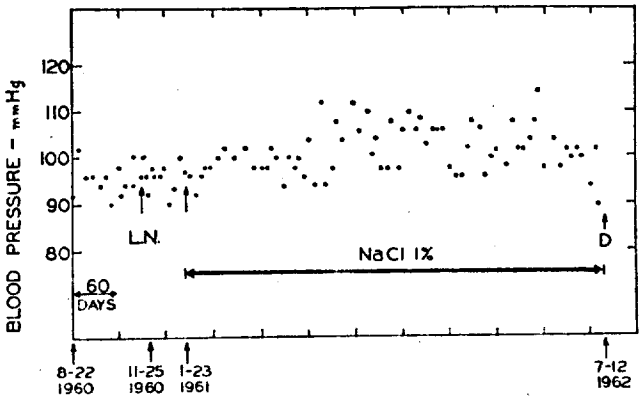

Fig. 2. Rabbit 34. *LN*, Left nephrectomy. NaCl 1 per cent, 500 ml. ingested daily for 76 weeks. *D*, Dead.

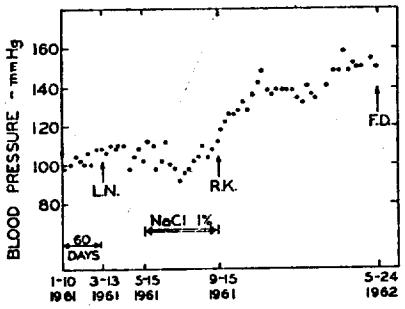

Fig. 3. Rabbit 45. *LN*, Left nephrectomy. NaCl 1 per cent, 500 ml. ingested daily for 18 weeks. *RK*, moderate permanent constriction of right main renal artery; *FD*, found dead. Acute suppurative pneumonitis.

Table 2

Rabbit	Control period: normal salt intake		Period after unilateral nephrectomy: normal salt intake		Period after unilateral nephrectomy: high salt intake	
	Time	Maximal rise of blood pressure	Time	Maximal rise of blood pressure	Time	Maximal rise of blood pressure
	weeks	*mm. Hg*	*weeks*	*mm. Hg*	*weeks*	*mm. Hg*
42	12	2			53	16
44	12	6			94	20
61	28	18			52	12
40	14	16	4	8	82	28
34	12	10	9	12	76	10
45	9	2	18	2	18	10

to renal ischemia.[3] The prompt and persistent elevation of the blood pressure which followed the production of the renal ischemia in rabbit 45 (*RK*, Fig. 3), was in striking contrast to the slight, if any, effect of the high salt intake on the blood pressure of any of the intact or unilaterally nephrectomized rabbits of this entire investigation.

In another group of six rabbits with blood pressure stabilized, after a control period of not less than 12 weeks, a left nephrectomy was performed, and high salt intake was initiated the day after the operation. Only three of the animals (Table 2, rabbits 42, 44, and

61) drank 500 ml. of the 1 per cent salt solution daily. There was no significant change in the blood pressure for at least 12 weeks, after which time it rose slightly and persisted at this elevated level during the entire period of the high salt intake. The maximal rise during the postnephrectomy (unilateral) high salt period was not as great as in some of the rabbits on the normal intake of salt.

DISCUSSION

The giant chinchilla rabbit is a particularly useful laboratory animal for studies of chronic experimental hypertension. It is large, cooperative, and permits determinations of blood pressure by the direct percutaneous method[3] for long periods. Because the blood pressure of this variety of rabbit tends to increase gradually during the early period of rapid growth, it is best to use young adults at least 6 months old, at which age the blood pressure becomes stable within a narrow range. Although the hypertensive effect of the high salt intake proved negligible in this variety of rabbit, it is considered of value to record this especially because the same animals were definitely responsive to the hypertensive effect of renal ischemia. The individual rabbits used in this study were from different litters, obtained at different times during a period of 5 years, and from different sources. It is probable, therefore, that the insusceptibility of this variety to the hypertensive effect of high salt intake is inherent.

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SORBIC ACID AS A FUNGISTATIC AGENT FOR FOODS. IX. PHYSICO-CHEMICAL CONSIDERATIONS IN USING SORBIC ACID TO PROTECT FOODS

Food Res. 20: 639-6
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In preceding papers it was shown that sorbic acid is an effective non-toxic and practical fungistatic agent for foods. Indeed it is unique among food additives, being a metabolizable food *(3, 4)* added for protective purposes to another food. The published reports have dealt with the use of sorbic acid to inhibit mold spoilage in cheese. For a review of the earlier developments in four different laboratories, reference is made to the Smith and Rollin summary paper *(14)*.

EXPERIMENTAL PART

In investigating the application of sorbic acid to other food products, a number of physio-chemical studies were carried out to obtain basic information of value in supplementing foods with this fungistatic agent. Earlier studies in this field have dealt with the spectrophotometric determination of sorbic acid in foods *(5, 6)* and on wrappers *(6)*, the degrees of resistance to oxidative deterioration of sorbic acid as such, on wrappers, and in foods *(8)*, and the importance of maintaining an adequately high ratio of sorbic acid to mold concentration *(9)*. Additional basic information, accumulated since the earlier investigations, is now being reported.

Purity of Commercially Available Sorbic Acid

Studies were conducted on ten lots of commercially available sorbic acid[a] to determine the purity of the material available to investigators for use in both laboratory and plant studies. Most analyses were conducted on the materials as received[b] but with results expressed on a moisture-free basis.

Melting point values were obtained on the sorbic acid samples after drying for 4 hours at 50°C. in a vacuum oven at 20 mm. (Hg) pressure. In determining equivalent weight the sorbic acid was dissolved in neutral isopropanol and then titrated with 0.1 N sodium hydroxide solution to the first end-point with phenolphthalein as the indicator. Sorbic acid concentrations were calculated from the found equivalent weights using 112.12 as the theoretical for pure moisture-free sorbic acid. The ultra-violet

[a] These samples were selected at random from aliquots of production lots retained by Carbide and Carbon Chemicals Company, New York.

[b] Sorbic acid loses moisture very rapidly during weighings at room temperature. For this reason, glass stoppered weighing bottles were employed in taking samples for analyses.

absorption tests were conducted on isopropanol solutions[c] of the sorbic acid samples, and extinction ratios calculated to define the shape of the absorption curves. The color intensities of the commercially available sorbic acid samples were determined spectrophotometrically on isopropanol solutions. The readings in both the ultraviolet and visible regions of the spectrum were made with the Beckman Spectrophotometer. Ashings of the samples were carried out in platinum dishes. Heavy metal contents were determined according to a spectrophotometric adaptation of the U.S.P. method for benzoic acid *(15)*. Moisture analyses of the test samples were conducted according to the toluol distillation procedure *(10)*.

Results of the analyses of the lots of commercially available sorbic acid

TABLE 1

Values obtained in analyzing ten lots of commercially available sorbic acid (s.a.) selected at random

Analysis[1]	Test material	Average value	3σ limits
Melting point	Moisture-free s.a.	134.5°C.	133-136°C.
Equivalent weight	0.5% s.a. in isopropanol	112	110.5-113.5
Sorbic acid content	Calculated value	100%	101.5-98.5%
$E^{1\%}_{1\,cm}$ 254 mμ	0.00025% s.a. in isopropanol	2420	2300-2540
E245 mμ/E254 mμ	0.00025% s.a. in isopropanol	0.83	0.80-0.86
E265 mμ/E254 mμ		0.76	0.73-0.79
Color, light transmission at 400 mμ	3.00% s.a. in isopropanol	92%	86-98%
Ash content	25 gram aliquot of s.a.	0.07%	0.00-0.15%
Heavy metal	4.00% s.a. in acetone	less than 10 p.p.m.	less than 10 p.p.m.
Moisture	10 gram aliquot of s.a.	6.5%	3-10%

[1] All analyses, other than melting point, were conducted on the samples as received. Based upon the moisture figures obtained, all other values were calculated to a dry basis.

are summarized in Table 1. Three sigma limits have been calculated to define the reproducibility of the sorbic acid; 997 batches out of a 1000 might be expected to yield values encompassed by these 3σ limits. The most precise test employed in this study is the titrimetric determination of equivalent weight. The melting point and particularly the spectrophotometric values are important in being more specific for sorbic acid. Based upon the results of these three tests, it has been concluded that the purity of commercially available sorbic acid is 100.0% ± 0.5% (one standard deviation), calculated to a moisture-free basis. The other tests in this series are of practical value to users of sorbic acid and the one for heavy metal content has been included to show that the sorbic acid is regularly free of such undesirable contaminants.

[c] Whereas sorbic acid is in aqueous solution in the spectrophotometric determination of the fungistatic acid in foods *(5, 6)*, isopropanol is the preferred solvent in testing crystalline sorbic acid for purity. Errors due to incomplete solubility of the sorbic acid are avoided when isopropanol is employed as the solvent.

Solubility of Sorbic Acid

The solubility of sorbic acid in various solvents is important in determining (a) expeditious ways for adding the fungistatic agent to foods and (b) the maximal concentrations that can be retained in solution. In conducting tests with these objectives in mind, the sorbic acid was added in obvious excess to the test solvent in a closed volumetric flask. The mixture was heated in a hot-water bath to a temperature of 140°F., agitated at this temperature by mild shaking, and allowed to cool slowly in the dark to the desired temperature. After 16 hours at each of the desired temperatures, the undissolved sorbic acid was filtered off, and the clear solutions analyzed spectrophotometrically *(6)* for sorbic acid content.[4] The results in this series of studies of sorbic acid solubility are summarized in Table 2.

TABLE 2

Solubility of sorbic acid in solvents of potential value in supplementing foods with the fungistatic acid

Solvent	Sorbic acid in solution at 74°F.
	gm. per 100 gm.
Ethanol (95.0%)	14.5
Acetic acid (99.8%)	12.3
Lactic acid (85.5%)	2.26
Citric acid (50.0%)	0.26
Phosphoric acid (85.0%)	0.12
Water (distilled); solution at pH 3.1	0.16
Buffered with phosphate to pH 4.4	0.22
to pH 5.9	1.02
Limpid vegetable oil (soybean oil)	0.52

Ethyl alcohol, the vehicular material for many flavoring compounds in foods, is an excellent solvent for sorbic acid. Among the acids found in foods and employed in the present study, acetic acid is by far the best solvent for sorbic acid. The fungistatic agent is soluble in water at room temperature (74°F.) to the extent of 0.16%. In buffered solutions with pH values well above that of 3.1 (that of sorbic acid in distilled water), more of the fungistatic agent can be dissolved. This is due, of course, to partial conversion of sorbic acid to the highly soluble salts of sorbic acid during solution of the acid in the buffered solutions. Sorbic acid is about three times as soluble in edible oils and fats as in water.

In Figure 1 are plotted curves showing the extent to which sorbic acid solubility in water can be increased with increasing temperature, or with increasing concentration of ethanol or acetic acid in the water. Information supplied by Table 2 and Figure 1 should be of value in determining expeditious ways for adding sorbic acid to food formulations.

[4] In these and in subsequent spectrophotometric analyses of test systems for sorbic acid content, absorbancy readings from 245 to 270 millimicrons were taken. In every case the absorption curve was typical of that of pure sorbic acid, with the position of the absorption maximum dependent upon the pH of the test solution *(5, 6)*.

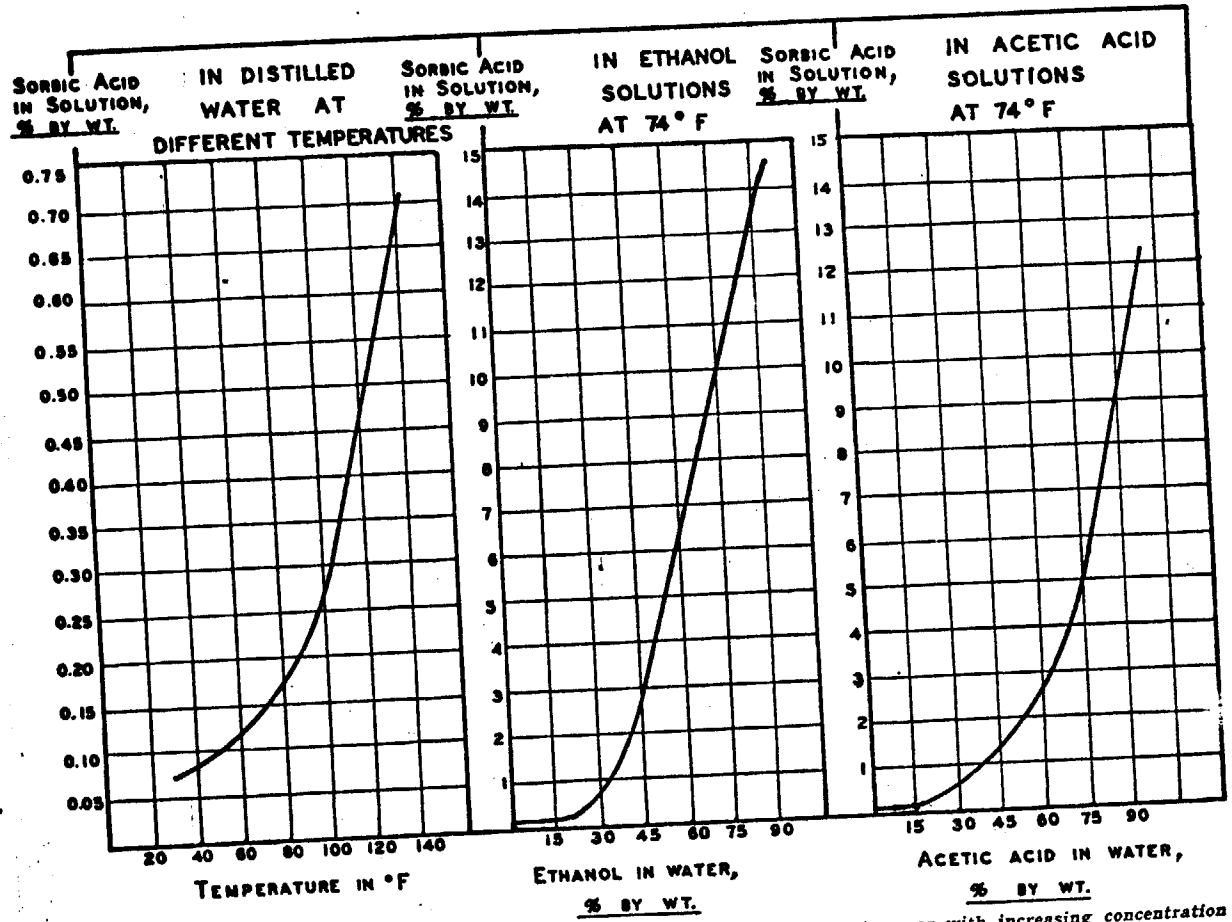

Figure 1. Increase in solubility of sorbic acid in water with increasing temperature, or with increasing concentration of ethanol or acetic acid in water.

Phase Distribution Studies with Sorbic Acid

A series of test solutions, containing sorbic acid in water with other solutes (the latter as single concomitant additives or in combinations), were prepared using the same procedure described in the preceding section to effect maximal solubility of the sorbic acid. To each filtrate was added an equal part by weight of limpid vegetable oil (soybean oil), the mixture shaken mechanically for 30 minutes and then held in the dark for 16 hours at room temperature (74°F.). The aqueous phases before and after extraction with the vegetable oil were analyzed spectrophotometrically *(6)* for sorbic acid content. The results of this study on the influence of (a) other soluble food constituents and (b) the concomitant presence of an edible fat phase on the solubility of sorbic acid in the aqueous phase are plotted in Figure 2.

The distribution ratio reflecting the solubility of sorbic acid in fat to that in water is 3.0. The presence of soluble food components, such as glucose, sucrose or sodium chloride, at high concentrations reduces the solubility of the sorbic acid in water. Phase distribution studies on such

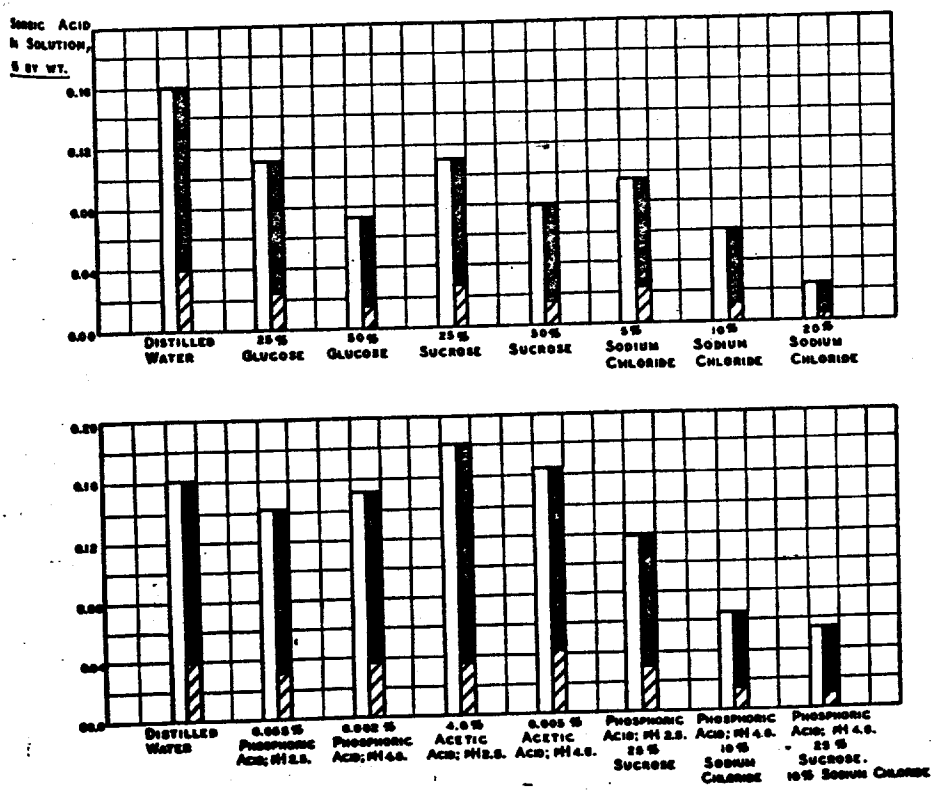

Figure 2. Solubility at room temperature (74°F.) of sorbic acid (a) in the specified aqueous test system, □; (b) in the aqueous phase after equilibration with an equal part by weight of a limpid vegetable oil, ▨; and (c) in the vegetable oil extract, ■.

test systems indicate that the ratio of sorbic acid solubility in oil to water of 3.0 is increased to values as high as 5.0 as a result of the concomitant presence of other water-soluble components in the aqueous phase. However, the acids in concentrations, as found in foods, modify to an insignificant degree the solubility of sorbic acid in the aqueous phase both before and after equilibration with the vegetable oil phase.[e] The influences of the solutes when added in combinations on sorbic acid solubility in water are not additive. Sorbic acid solubility under such circumstances is the same as that for the system containing the one solute which affected sorbic acid solubility to the greatest degree.

In Table 3 are shown the results obtained in comparable phase distri-

TABLE 3

Phase distribution studies[1] with fungistatic acids

Fungistatic acid	Concentration in			Distribution ratio, b/a
	Original aqueous phase	Extracted aqueous phase (a)	Vegetable oil phase (b)	
Sorbic	0.160	0.040	0.120	3.0
Benzoic	0.320	0.045	0.275	6.1
Propionic	0.280[2]	0.240	0.040	0.17

[1] Equal parts by weight of limpid vegetable oil and distilled water solutions of the fungistatic acids shaken together (system at 74 F.).

[2] Whereas the values for sorbic acid and benzoic acid concentrations in the original aqueous phases represent maximal solubilities, this value for the highly water-soluble propionic acid was arbitrarily selected.

bution studies conducted at room temperature (74°F.) with two other fungistatic acids. The latter were determined titrimetrically, the method having in it blank titrations conducted on the control unsupplemented test systems. Whereas propionic acid is predominantly water-soluble and benzoic acid is predominantly fat-soluble, sorbic acid falls in between these two as shown by the values for distribution ratio.

Based upon findings in our own and in other laboratories, it is well known that sorbic acid activity, in protecting foods against mold and yeast spoilage, is increased in an acid environment. Thus, the overall effect of acids including even those that slightly depress sorbic acid solubility in water is in favor of fungistatic activity. Whereas, salt noticeably lowers sorbic acid solubility, the salt has a marked synergistic effect on sorbic acid fungistasis. Therefore, the reduced concentration of sorbic acid in brine solutions is no deterrent to its use. Likewise in systems of high sugar content, sorbic acid fungistasis is increased[f]; this has been noted in systems with pH values of even 6.5 and above.

[e] The same results were obtained in similar studies with citric and lactic acid in concentrations of 1.0 per cent and less in water.

[f] In an aqueous solution, high in sugar content, sorbic acid is an effective fungistatic agent provided the test system remains uniform. Due to refluxing activity within the closed unmolested container, a surface film of pure water may condense on the walls of the container and on the syrup itself. On such surfaces mold growth can occur since sorbic acid migration to these vulnerable areas may not be sufficiently rapid. Mixing the contents of the container prevents this sequence of events.

Fat in the food lowers the concentration of sorbic acid in the aqueous phase—the phase which must be protected in order to prevent mold growth. But even here sorbic acid enjoys a much more favorable distribution ratio than benzoic acid. When tested by the agar-disk-plate method, sorbic acid is at least three times as effective as benzoic acid *(11)*. Similar results have been obtained in comparing the relative effectiveness of the two fungistatic agents in protecting margarine *(12)*, cheese *(13)*, fish *(2)*, bread *(1)*, and cake *(1)* products. The superiority of sorbic acid over propionic acid is independent of distribution ratio, since in this property propionic acid has the advantage. Sorbic acid has been found to be about four times as effective as propionates in protecting cheese *(13)*, bread *(1)*, and cake *(1)* products.

Sublimation of Sorbic Acid

Sorbic acid on a food wrapper is not sufficiently volatile under ordinary circumstances to cross air-pockets between wrapper and food. The fungistatic acid is capable of migrating from wrapper into food in direct contact with it *(7)* and in this way eventually reaching the surface of the food separated by air pocket from the wrapper. Because migration of sorbic acid may not be sufficiently rapid to protect the food at such vulnerable areas, it has been recommended that close contact of food and sorbic acid-treated wrapper be an objective in packaging *(13)*.

Recent studies have demonstrated that sorbic acid sublimes from wrappers so readily at elevated temperatures that air pockets in packaging need no longer be a critical factor in packaging foods in sorbic acid-treated wrappers. In these studies Payne Permeability Cups[5] were employed. A piece of each of the test wrappers was inserted between the two sections of the cup with the sorbic acid-coated surface of the wrapper facing the cup cavity. Each of the clamped assemblies on an asbestos mat was then placed in a large thermostatically controlled electric oven for various periods of time. Sublimed sorbic acid was deposited as fine crystals on the inner surface of the cup cavity. The sublimed material was dissolved, after cooling to room temperature, in 95% ethanol for spectrophotometric determination of quantity of sorbic acid and for positive identification of the sublimed material as pure sorbic acid *(6)*. Typical results obtained in the course of this study are summarized in Table 4.

Based upon the results obtained in the present study, it is concluded that, among those tested cellophane is the preferred wrapping material from which sorbic acid may be sublimed during the flash heating. Exposure of the cellulose acetate or glassine sheets to a temperature of 550°F. for 30 seconds is so severe that some loss of sorbic acid to the atmosphere occurs. This is apparent from the odor of sorbic acid in the surrounding air and from the failure to account for all the sorbic acid originally present in testing for sorbic acid retained on the wrapper and for that sublimed into the cup cavity.

Films of pliofilm and polyethylene are unsatisfactory as the base sheet from which sorbic acid may be sublimed by the flash heating process. These films decompose at the elevated temperatures.

[5] Obtained from the Fisher Scientific Company, New York.

TABLE 4
Heat sublimation of sorbic acid from wrappers

Wrapper[1]	Exposure		Amount of sorbic acid			
	Time	Temp.	Unheated disc	Sublimed	Retained	Recovered
	sec.	°*F.*		*mg. per sq. in.*		
Sorbic acid on cellophane (MSAT 86); binder, 6% by wt. of the sorbic acid.	30	350		0.28	2.07	2.35
	30	450	Avg.	1.37	0.78	2.15
	7	550	2.20	1.45	0.90	2.35
	30	550		1.65	0.37	2.02
Sorbic acid on cellulose acetate sheet of 2 mil thickness ("Lumarith"); binder, 6% by wt. of the sorbic acid.	30	350		0.48	3.90	4.38
	30	450	Avg.	1.64	2.33	3.97
	7	550	4.10	1.42	2.48	3.90
	30	550		1.77	0.79	2.56
Sorbic acid on glassine (25 lb. per ream); binder, 6% by wt. of the sorbic acid.	30	350		0.85	2.95	3.80
	30	450	Avg.	2.05	1.15	3.20
	7	550	4.10	2.53	2.02	4.55
	30	550		2.18	0.32	2.50
Sorbic acid on cellophane (MSAT 86); thermoplastic wax coating, 1400% by wt. of the sorbic acid.	30	350		0.14	3.66	3.80
	30	450	Avg.	0.69	2.60	3.29
	7	550	3.80	0.27	3.66	3.93
	30	550		2.65	1.15	3.80

[1] Wrappers kindly furnished by Milprint, Inc., Milwaukee, Wisconsin.

The practical value of the sublimation procedure has been demonstrated in studies on wrapped foods. Heating such a food under preferred conditions, at 450° or 550°F. for 30 or 7 seconds, respectively, results in sublimation of the sorbic acid from the inner surface of the wrapper onto the surface of the food without heating the latter to elevated temperatures. Indeed, the food supplies a cool condensing surface for the volatilized sorbic acid so that reverse sublimation to the wrapper does not occur. Tests conducted on a variety of foods, such as luncheon meats, fresh meats, processed and natural cheeses (including Swiss cheese with eyes), fruits, cakes, and incompletely baked yeast-raised products (included the so-called Brown 'N Serve rolls) have shown that the foods are not heat-damaged. Slight oiling off is noted after the flash heating of the luncheon meats and cheeses. Crystals of sorbic acid are also readily noted on the surface of these foods. However, after over-night storage free oil is no longer apparent and sublimed crystals of sorbic acid no longer visible, having been absorbed into the foods. The flash-heat treatment is so mild that surface discoloration of raw meats does not occur. The appearance of some products covered with crystals of sorbic acid may be objectionable, particularly when the crystals are not readily absorbed into the food, *viz.*, prunes, strawberries, and chocolate cake, when so processed. Other products may also fail to absorb the sorbic acid crystals but remain acceptable in appearance because the color of the foods permits the sublimed sorbic acid to escape visual detection. The product, Brown 'N Serve rolls, is an excellent example of such an application. During the baking in the home the sublimed sorbic acid on the surface of the rolls is volatilized, but this is of no concern since from this time on there is no further need of fungistatic protection. For practically complete volatilization of sorbic acid from the Brown 'N

Serve rolls, the cellophane overwrap should be removed from the package prior to baking.

A feature of this flash sublimation of sorbic acid from wrapper onto food is that the appearance of the wrapper can serve as a good indicator of sufficient heating in volatilizing the fungistatic acid. Thus, when using cellophane or cellulose acetate wrappers treated with sorbic acid and a minimal amount of binder material, these wrappers change in appearance from a translucent white to a crystal clear film as a result of volatilizing the layer of fungistatic acid crystals. The wrappers with the thermoplastic wax coating in high concentrations also exhibit transparency when hot, but become cloudy on cooling due to the resetting of the non-volatile wax base. Changes in transparency are not visually detectable when the fungistatic glassine wrappers are employed.

In general the flash heating of sorbic acid-treated wrappers has been promoted to protect foods which have irregular external surfaces or configurations such that the wrappers containing the fungistatic acid cannot be conveniently placed in intimate surface contact with the wrapped foods. In the flash-heat treatment of the above-mentioned foods less volatilization of sorbic acid occurs from the areas of the sheets in direct contact with the food surfaces. The areas over the air pockets become clear, while those in direct contact with the food surface remain cloudy. This can be ascertained only on examining the wrapper removed from the food. The failure to sublime sorbic acid at the areas of contact of wrapper with food is of no concern since these areas are less vulnerable to mold spoilage and opportunities exist for migration of sorbic acid from these areas on the wrapper into the foods.

SUMMARY

An important consideration in the control of an additive to a food is the purity of the commercially-available material. Sorbic acid as produced on a commercial scale is 100.0% pure ± 0.5% (one standard deviation), calculated to a moisture-free basis.

In determining expeditious ways for adding sorbic acid to food formulations, advantage should be taken of its greater solubility in certain solvents, such as in ethyl alcohol, in acetic acid, and in aqueous solutions buffered to pH values above 3.1. Values for solubility of sorbic acid in certain systems indicate the maximal quantities which may be added in soluble form to certain foods.

Whereas propionic acid in phase distribution studies is predominantly water soluble and benzoic acid is predominantly fat soluble, sorbic acid falls in between these two as shown by the values for distribution ratio. The ratio of the solubility of sorbic acid in fat to that in water at room temperature (74°F.) is 3.0. The presence of solutes such as sugar and salt, but not acids, is responsible for an increase in this ratio. The lower and more favorable distribution ratio for sorbic acid is responsible in part for the more than the three-fold greater effectiveness of sorbic acid in comparison to benzoic acid in protecting foods against mold spoilage, but this cannot explain the four-fold greater effectiveness of sorbic acid in comparison to the propionates.

Advantage can be taken of the ease with which sorbic acid sublimes at elevated temperatures to augment fungistatic protection of foods. A flash heating method has been described, one that has been specially designed to protect foods which have irregular external surfaces or configurations such that the wrappers containing sorbic acid cannot be conveniently placed in intimate surface contact with the wrapped foods.

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Teratogenic Significance of I. ic and Fluid Imbalances

.Abstract. *Some agents teratogenic to the chick embryo cause serum electrolyte and fluid imbalances which initiate an edema syndrome leading to malformations. Differences in ionic composition of serum and yolk of normal chicks help explain how these imbalances can be produced.*

A study of the effects of moderate hypoxia on young chick embryos has shown that many of the induced malformations can be attributed to a disturbance in fluid balance (*1*). This disturbance, which characterizes the edema syndrome, includes a marked increase in circulating and extracellular fluids, followed by the formation of clear blisters and hematomas which cause malformations mechanically. The edema syndrome can also be induced by Trypan blue (*2*) and injections of 0.01 to 0.02 ml of fresh egg albumen into the allantois of 4- to 5-day chick embryos. The latter can induce malformations in 20 percent (30 out of 149) of embryos treated at 4 days and 9 percent (9 out of 91) at 5 days (*3*). Less than 2 percent malformations were found in 225 controls that were injected with saline. All three agents can induce identical anomalies, indirectly caused by blisters, when applied to embryos of the same age. Because changes in the fluid volume of the embryo initiate the edema syndrome, we decided to study the composition of embryonic fluids in normal and experimental chicks.

We made assays of Na^+ and K^+ in normal extracellular and extra-embryonic fluids of the 5-day chick embryo (Table 1). These fluid compartments can be divided into two groups on the basis of the concentration of these electrolytes. Albumen and yolk, which are separated from the embryo by cellular membranes, are very high in K^+, and low in Na^+ and in total Na^+ and K^+. Blood serum, cerebrospinal, chorionic, and amniotic fluids are low in K^+, and high in Na^+ and in total Na^+ and K^+. The fluid of the allantois, which has an opening into the yolk sac, is intermediate. As might be expected from the data of Table 1, but contrary to the popular assumption that these fluids have equivalent osmotic pressures (*4*), we found that the depression of freezing point of albumen and subgerminal yolk were markedly lower than that of normal serum (*5*). These observations show that the osmotic relationships of the chick embryo to its surrounding fluids are more complex than generally believed.

Because of these differences between the internal and external fluids of the chick embryo we felt that fluid and ionic imbalances could be induced by a variety of physiological stimuli, so we exposed embryos to several teratogenic agents and tested the serum (Table 2). Saline-injected controls showed an increase in the concentration of potassium ions but no changes in the concentration of sodium ions. This change is probably the result of mild trauma from opening the egg. Occasional malformations are also found in these controls. Embryos subjected to hypoxia (*6*) or treated with albumen or Trypan blue, consistently showed a twofold increase in serum potassium and a significant decrease in serum sodium. The decrease in the concentration of sodium ions compared with that in normal sera, when the embryos were subjected to hypoxia or to treatment with albumen, was significant at the 0.1 percent level (*t*-test); when treated with Trypan blue, the decrease was significant at the 3 percent level. The lower total concentration of these two ions in experimental sera helps to interpret the edema which results from these treatments. The depression of freezing point of the serum of embryos exposed to hypoxia is substantially lower than that of normal serum (*5*).

The data clearly demonstrate that fluid and electrolyte imbalances are produced in the embryo by hypoxia, albumen, and Trypan blue. The edema syndrome is a common factor to all of them and leads to the development of similar malformations.

Although these data cannot be assumed to apply directly to the mammal, we feel a similar study would be a profitable approach to problems of mammalian teratology, because effects comparable to the edema syndrome have been described many times in mice and rats, both after the administration of Trypan blue (*2*) and vasopressin (*7*), and in deficiencies of linoleic and pantothenic acids (*8*), as well as in genetic mutants (*9; 10*).

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Table 1. Sodium and potassium in fluids of normal 5-day chick embryos. The results are expressed as milliequivalents per liter. Determinations were made on a Beckman DU spectrophotometer with a flame attachment, subsequent to perchloric acid digestion.

Fluid	No. of assays*	Na^+ Av.	Na^+ S.D.	K^+ Av.	K^+ S.D.	Total Na^+ and K^+
Albumen	7	65	13.0	61.3	7.5	126
Subgerminal yolk†	6	98	1.6	19.4	3.72	117
Allantois	7	115	10.6	9.0	1.14	124
Blood serum	15	136	9.0	3.2	0.55	139
Cerebrospinal fluid	7	131	11.1	4.0	1.38	135
Chorion	6	133	5.5	4.1	0.79	137
Amnion	4	131	6.2	2.2	0.25	133

* Each sample obtained from different chicks except for serum and cerebrospinal fluid which were samples pooled from 2 to 3 embryos. † Clear yolk immediately underneath embryo.

Table 2. Serum sodium and potassium in 5-day chick embryos. The results are expressed as milliequivalents per liter. Experimental treatments: Hypoxia, 10.5 percent O_2 for 6 hours (6); fresh egg albumen, 0.02 ml injected into allantois; Trypan blue, 0.06 ml of a 0.1 percent solution injected into yolk sac; and NaCl, 0.02 ml of 0.85 percent saline injected into allantois. The serum samples from the embryos subjected to hypoxia were obtained within 1 hour after treatment was terminated. Other samples were obtained 5 to 6 hours after injection.

Treatment	No. of assays	Na^+ Av.	Na^+ S.D.	K^+ Av.	K^+ S.D.	Total Na^+ and K^+
None	15	136	9.0	3.2	0.55	139
Hypoxia	9	117	10.7	6.5	1.06	124
Albumen	7	121	6.6	5.7	1.34	127
Trypan blue	5	122	11.6	6.6	1.14	129
NaCl	5	137	10.7	5.1	0.60	142

brief communications

Relationship of sodium and potassium intake to blood pressure[1]

Gerard Gros,[2] M.D., John M. Weller,[3] M.D., and Sibley W. Hoobler,[3] M.D.

The relationship of salt to hypertension is not clear. Dahl's population studies indicate that higher sodium intakes are associated with a greater incidence of hypertension that is not accompanied by gross accumulation of sodium in tissues (1–5). The acute hypertensinogenic effect of sodium chloride in chickens, rabbits, and rats is established (6–8). In man, similar studies are few and are concerned with the effects of large amounts of sodium chloride resulting in weight gain, edema, and blood pressure elevation (9–11).

Further observations of the effect on the blood pressure of a moderately increased salt intake given over a relatively short period of time are indicated. Since potassium has a protective action against sodium-induced hypertension in animals (12), such an effect in man also deserves study.

The following experiments were devised to test the effect of:

1) Increased sodium chloride intake on the blood pressure of normal individuals and patients having mild, untreated, essential hypertension (experiment 1A).

2) Administration of a mixture of potassium and sodium chloride on any alterations in blood pressure resulting from the increased sodium chloride intake in the above group (experiment 1B).

3) Increased sodium chloride intake on the blood pressure of patients receiving thiazide therapy for mild systemic, arterial hypertension (experiment 2).

4) Administration of a mixture of potassium and sodium chloride on the blood pressure of patients receiving thiazide for mild systemic arterial hypertension who became hypokalemic and normotensive by that therapy (experiment 3).

Methods

In all experiments, patients were instructed to take blood pressure determinations on themselves at home in the early morning and late evening, in standing and reclining positions. Because the four blood pressure values bore a quite constant relationship, and because of greater usefulness as base-line values, only early morning lying pressures were utilized.

Experiment 1A

Group 1A consisted of seven patients with labile blood pressures or with mild, untreated, systemic arterial hypertension (mean lying morning diastolic pressure was 89 mm Hg) and three normotensive volunteers. Subjects maintained their usual daily sodium chloride intake and recorded body weight every morning before breakfast. Additional sodium chloride was provided in shakers, each containing 6 g sodium chloride plus 6 g inert filler to be ingested daily, either on food or with beverages, for 2 to 9 weeks. Occasional 24-hr urine sodium determinations were performed to confirm the level of sodium intake in experiments 1 and 2.

Experiment 1B

Group 1B consisted of the same 10 individuals of Group 1A, who at the end of the sodium chloride loading period were given shakers each containing a mixture of 6 g sodium chloride and 6 g potassium chloride, to be ingested daily for a 4-week period.

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Experiment 2

Group 2 consisted of eight patients with mild systemic arterial hypertension (mean lying morning diastolic pressure was 103 mm Hg). Base-line blood pressure readings were obtained on a normal diet and while previous hydrochlorothiazide medication was suspended for 2 weeks or more, during which time the patients received a placebo tablet daily. All patients were then given 50 mg hydrochlorothiazide daily for 4 weeks. During the following 4 weeks, hydrochlorothiazide was continued and 5 g sodium chloride were added daily to the diet.

Experiment 3

Group 3 consisted of five patients from Group 2 who, on thiazide therapy, had normal, or only slightly elevated, blood pressure levels and hypokalemia. These patients were given, while continuing thiazide therapy, 5 g sodium chloride and 5 g potassium chloride for a 2-week period, then 2 weeks of only 5 g added sodium chloride daily. Finally, while still on thiazide, the sodium and potassium chloride mixture of 5 g each was administered daily for an additional 2-week period. At the end of each period, serum potassium was determined.

Results

Experiment 1A

The added sodium chloride intake of 6 g did not cause a significant change in blood pressure (Table 1). The average daily sodium intake, as measured by 24-hr urine sodium excretion, was 191 mEq during the control period and 279 mEq during sodium loading.

TABLE 1
Maximal change in early morning lying diastolic blood pressure values with added NaCl and with added NaCl-KCl mixture

Patient	Change in diastolic blood pressure	
	With 6 g NaCl	With 6 g each NaCl and KCl
	mm Hg	mm Hg
ON	+8	−1
CM	−9	+6
McL	−3	+5
HH	−11	−8
VB	+4	+1
TB	−1	+1
WS	−4	+3
BS	−8	−5
JK	0	0
McG	+2	+6
Mean	**−2.2**	**+0.8**

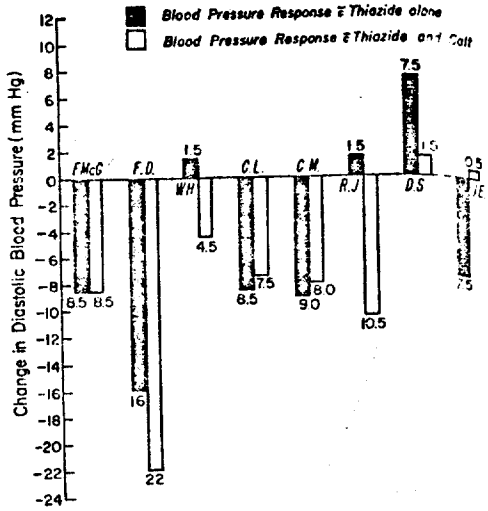

FIG. 1. Change from mean control value of the early morning lying diastolic blood pressure after 4 weeks on hydrochlorothiazide (solid bars) and after an additional 4 weeks on hydrochlorothiazide plus 5 g added sodium chloride (open bars).

Experiment 1B

The administration of a mixture of 6 g sodium chloride and 6 g potassium chloride had no significant effect on blood pressure (Table 1).

Experiment 2

In eight patients treated for 4 weeks with 50 mg hydrochlorothiazide daily, the mean diastolic blood pressure went up in one, down in five, and remained unchanged (less than 2 mm Hg) in two, compared with the mean pressure during the control period (Fig. 1). Adding 5 g sodium chloride resulted in the following (Fig. 1): *1*) In the three patients who had not shown blood pressure lowering during thiazide treatment, the mean diastolic blood pressure went lower. *2*) One of the five patients who had an antihypertensive response to thiazide had further lowering of the mean diastolic blood pressure; three had no change (less than 2 mm Hg), and one had an increase.

Experiment 3

Adding 5 g each sodium chloride and potassium chloride to the daily intake of five patients who were being treated with thiazide

and who had hypokalemia had the following effects: *1*) In four patients the mean diastolic blood pressure did not change, whereas further elevation was noted in one. *2*) In three patients the thiazide-induced hypokalemia was unchanged; in two it was corrected. *3*) In the three patients whose hypokalemia was not corrected, one became more hypertensive. In the two whose hypokalemia was corrected, mean blood pressure was unaffected.

Discussion

The added sodium chloride, which was ingested in beverages or sprinkled over food, was in excess of that which the subjects' tastes required and was at the limit of palatability. The amount of potassium chloride added to the sodium chloride was selected from tests by Frank and Mickelsen (13) indicating that the maximal acceptable mixture of sodium and potassium chlorides was one-half of each by weight.

Previous studies of excessive salt intake in humans include that of McQuarrie et al. (9), in which the administration daily to diabetic children of the adult equivalence by body weight of 70 to 140 g sodium chloride resulted in a gain of 4 to 5% body wt and an increase in both systolic and diastolic blood pressures of 30 to 50% above control levels. McDonough (11) gave 37 g sodium chloride daily above the usual salt intake to a normotensive individual, causing facial puffiness with progressive increase in blood pressure. Our study tested the effect of short-term sodium chloride administration of moderate degree that did not cause gross weight gain.

Herrera (10) reported observations on six hypertensive patients following a base-line period of 4 g sodium chloride daily added to a salt poor diet, then increased to 15 g sodium chloride daily for 8 days. Resting blood pressure rose in five patients within 1 to 4 days. In our short-term study, 5 g sodium chloride added daily failed to influence body weight or blood pressure.

Grieble and Johnston (14) found that supplemental potassium chloride partially counteracts the hypotensive effect of bendroflumethiazide. This effect was not noted in our study.

Summary

1) An added daily sodium chloride intake of six g supplementing a normal salt intake and reaching to the limit of taste tolerance was given for 2 to 9 weeks to seven patients with mild, untreated, essential hypertension and to three normal subjects. It did not cause edema or significant change in body weight or blood pressure.

2) The subsequent administration to the same group of subjects of a mixture of 6 g sodium chloride and 6 g potassium chloride for 4 weeks had no significant effect on body weight or blood pressure.

3) Five g supplementary sodium chloride given daily for 4 weeks failed to antagonize the antihypertensive action of hydrochlorothiazide in the majority of eight patients studied.

4) A mixture of 5 g each sodium chloride and potassium chloride administered for 2 weeks to five patients with mild, controlled, systemic arterial hypertension and thiazide-induced hypokalemia had no effect on the blood pressure of four patients; in one patient the diastolic pressure rose. In two patients hypokalemia was corrected by the salt mixture without affecting blood pressure.

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Editorial

POTASSIUM DEFICIENCY AND HEART FUNCTION

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OUR CURRENT idea of the mechanism of tension development by the heart cell [1, 2] is that depolarization, involving an increase in sodium permeability, travels over the surface and transverse tubular sarcolemmal membranes and in some way releases calcium bound to these membranes into the sarcoplasm. This pulse of calcium raises the concentration of free ionic calcium in the sarcoplasm from less than 10^{-7} M in the resting state to a level which activates the contractile proteins. The calcium initiates contraction by binding to troponin, a protein in the thin filament which in the unbound state inhibits contraction despite the presence of magnesium and ATP. Actin in the thin filament then forms a complex with myosin in the thick filament. This complex, in the presence of magnesium, has a very high ATPase activity. The result is increased ATP breakdown and liberation of energy. An interaction between the heads of the myosin molecules in the thick filaments and sites on the thin filaments leads to force generation.

Repolarization then occurs due to loss of potassium from the cell and, in association with this, the concentration of free ionic calcium in the sarcoplasm falls below 10^{-7} M and calcium is removed from troponin resulting in relaxation. The level of calcium decreases (1) because of termination of influx, (2) because of accumulation in the sarcoplasmic reticulum (both via a calcium activated, magnesium dependent ATPase and via simple binding), and (3) possibly because of rebinding directly to the sarcolemma. Sodium is then pumped out of the cell and potassium is pumped in by a sarcolemmal mechanism which involves a sodium and potassium activated, magnesium dependent ATPase and the cell is ready for another sequence. Clearly cations are involved in the entire sequence of tension development (depolarization, contraction, repolarization, relaxation and the coupling of depolarization to contraction).

It is therefore not surprising that changes in the usual external concentrations of cations have important effects upon cardiac function. It has been known for some time that tension development in isolated cardiac muscle is suppressed by lowering the calcium concentration or by raising the potassium or hydrogen concentration and that the reverse occurs with elevation of calcium concentration or lowering the potassium or hydrogen concentration. The effect of decreasing the extracellular potassium concentration is particularly interesting [3, 4]. Small changes in the bath surrounding isolated cardiac muscle immediately produce substantial increases in contractile tension and associated with this are major changes in the cellular membrane potential, including (1) increase or decrease in the resting membrane potential,

(2) increase in the amplitude of the action potential, (3) increase or decrease in the overshoot, and (4) decrease in the duration of the plateau (accelerated early repolarization) and increase in the total duration of the action potential (prolongation of late repolarization). Some of these changes (increased action potential amplitude and duration), when produced artificially by voltage clamp, cause an increase in contractile tension, presumably by permitting the delivery of more calcium to the contractile elements. The decrease in the resting membrane potential, when present, may result from inhibition of the sarcolemmal sodium, potassium ATPase activity and consequently from inhibition of the electrogenic sodium–potassium pump. Decreased sodium, potassium ATPase activity with impairment of the pump has been suggested to be important in the production of the inotropic effect. In the view of one author, the increased intracellular sodium concentration stimulates a sodium–calcium exchange system leading to increased influx of calcium.

There is also evidence that decreased extracellular potassium concentration increases norepinephrine efflux from sympathetic nerve endings (5). Norepinephrine transport in sympathetic nerve endings is very sensitive to cation concentrations in the extracellular fluid. Like sodium and potassium pumping across the sarcolemma of the muscle cell, norepinephrine uptake into nerve endings is inhibited by ouabain and is dependent on the presence of sodium and potassium. Uptake is decreased by lowering external sodium or potassium concentration. It is increased by raising external sodium concentration and by slightly increasing external potassium concentration. Increased calcium concentration decreases uptake. These changes are parallel to the effects on sodium potassium ATPase and are opposite the effects on tension. This suggests that altered uptake of norepinephrine into nerve endings might participate in the tension responses to changed extracellular cation concentration.

If isolated cardiac muscle is exposed to zero potassium [4], the increased tension eventually gives way to a decreased tension which is associated with marked decrease in the duration and amplitude of the action potential. The electrical abnormalities are reversible but the tension change is not. Apparently prolonged incubation in potassium-free medium is deleterious to contractility. Perhaps relevant to this is the observation that glucose utilization for contractile activity is blocked in rat ventricular strips placed in potassium-free medium [6], possibly resulting in failure of ATP production by the mitochondria. Reduction in the tissue levels of high energy phosphates has been reported. Experimental dietary deficiency of potassium also leads to reduced contractile tension but here it is more difficult to be certain that the effect is the result of the reduced extracellular potassium concentration *per se* (prolonged dietary deficiency also induces other electrolyte abnormalities).

Studies in the essentially intact, blood perfused, working heart reveal an initially inappropriate response of the coronary vascular bed for the change in contractile activity [7]. Reduction of the potassium concentration in coronary arterial blood produces an almost immediate increase in contractile force (on the average, a 50 per cent decrease in inflow potassium concentration produces an 82 per cent increase in contractile force). Coronary resistance, rather than decreasing as it usually does with increased myocardial activity, also increases (on the average by 39 per cent) and associated with this is decreased blood flow and P_{O_2} in the coronary sinus blood. The increased contractile force is sustained for at least 20 min but the increase in resis-

tance wanes with time. Consequently decreased flow soon diasppears. Of interest are the observations that raising the sodium concentration in coronary arterial blood also raises contractile force and that this increase in force is more prominent in the presence of hypokalemia.

It is apparent that cardiac function should be carefully studied when hypokalemia and especially hypokalemia combined with hypernatremia are generalized throughout all of the blood for prolonged periods of time. It is possible that the findings will be relevant to experimental cardiac necrosis [8]. Among the factors which sensitize the myocardium to experimental necrotization are sodium salts, particularly in association with potassium and magnesium deficiency; conversely several types of experimental cardiomyopathies can be partially or completely prevented by potassium and magnesium salts. It is also possible that such studies will be relevant to cardiac function in those chronic diseases in man which produce hypokalemia and hypernatremia, singly and in combination.

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The Mechanism of the Acute Effect of Sodium Chloride on Blood Pressure (35309)

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While it is well established that acute changes in plasma sodium chloride concentration can affect blood pressure, the mechanism of this effect is not clear. Studies in perfused vascular beds indicate that the sodium chloride *per se* is not vasoactive; its effect on resistance seems to result indirectly from osmotic shift of water rather than from some direct action (1). In an attempt to determine whether this also applies to the intact cardiovascular system as a whole, blood pressure was measured in the anesthetized dog during acute selective sodium chloride depletion without water depletion; total osmotic pressure of extracellular fluid was held constant to prevent osmotic shift of water into cells.

Methods. This was accomplished by injecting a potent diuretic intravenously and then exactly replacing the water lost in the urine with an isosomotic modified Ringer's solution in which mannitol was substituted for the sodium chloride. The method has been described in detail elsewhere (2). In brief, dogs having an average weight of 18.5 kg were anesthetized with sodium pentobarbital and ventilated artificially. The femoral artery, femoral vein, and urinary bladder were catheterized for measurement of arterial pressure, infusion of the solution, and measurement of urine flow, respectively. Furosemide, 20 mg/kg, was then injected intravenously and immediately afterward 500 ml of the replacement solution were injected intravenously to stimulate urine flow. When 500 ml urine had been excreted, the replacement solution was infused intravenously exactly at the rate of urine flow. Particular attention was given the depth of anesthesia: supplemental doses of pentobarbital were given whenever a conjunctival reflex appeared. Arterial blood was sampled periodically. The urine was pooled and sampled at the end of the experiment. Plasma and urine osmolality were measured by the freezing point depression method, potassium and sodium by flame photometry, and magnesium and calcium by atomic absorption. Blood pH was measured by the method of Astrup and hematocrit by capillary centrifugation.

In eight control experiments the replacement solution contained (g/liter): NaCl, 7.2; $NaHCO_3$, 1.8; KCl, 0.3; $MgCl_2 \cdot 6H_2O$, 0.5; and Ca gluconate, 1.0. In eight experiments designed to selectively lower the plasma sodium concentration, mannitol was substituted for the NaCl and $NaHCO_3$ in the solution. In both groups, an isotonic solution of KCl was also infused intravenously via a forelimb vein at 1.23 ml/min during the entire post-furosemide period to obviate a fall in the plasma potassium concentration (2). The volume of this fluid as well as that of the blood samples were taken into account in arriving at a precise water balance.

Results. Table I shows that when mannitol was substituted for the sodium chloride and sodium bicarbonate in the replacement fluid, plasma sodium concentration fell 29 mEq/liter in 76 min. Plasma osmolality, $[K^+]$ $[Mg^{2+}]$, $[Ca^{2+}]$, blood pH and hematocrit did not change appreciably. The findings were similar in the control experiments, except that here the sodium concentration also remained constant. Mean arterial pressure rose 14 mm Hg in the experimental group and 12 mm Hg in the control group. The reason for the small blood pressure rise in both groups is unknown but could be, at least in part, related to lightening of the anesthesia and renin release. Heart rate did not change in either group.

TABLE I. Arterial Plasma Osmolality, Plasma Cation Concentration, Blood pH, Hematocrit, and Blood Pressure in the Control and Experimental Groups.*

t (min)	V (ml)	Osm (mOsm/kg)	(mEq/liter) Na	K	Ca	Mg	pH	Hct (%)	(mm Hg) P_M	P_S	P_D
					Control ($n = 8$)						
0	0	309 ± 3	155 ± 2	3.2 ± 0.1	3.9 ± 0.1	1.84 ± 0.07	7.40 ± 0.02	34.5 ± 1.5	111 ± 7	143 ± 10	100 ± 6
65 ± 14	561 ± 10	312 ± 2	150 ± 2	3.4 ± 0.1	3.9 ± 0.1	1.88 ± 0.09	7.40 ± 0.02	39.9 ± 1.9	123 ± 5	155 ± 11	111 ± 4
					Experimental ($n = 8$)						
0	0	300 ± 2	154 ± 2	4.0 ± 0.1	4.2 ± 0.1	1.99 ± 0.05	7.39 ± 0.02	38.8 ± 1.5	98 ± 7	127 ± 8	84 ± 7
48 ± 6	548 ± 10	303 ± 2	139 ± 2	3.7 ± 0.1	4.3 ± 0.1	1.89 ± 0.04	7.36 ± 0.04	42.6 ± 1.4	111 ± 6	141 ± 7	99 ± 6
76 ± 8	1034 ± 69	304 ± 1	125 ± 3	4.0 ± 0.1	4.2 ± 0.1	1.86 ± 0.05	7.35 ± 0.02	40.6 ± 1.5	112 ± 8	140 ± 9	96 ± 7

* Values represent means ± SE; t = time after injection of furosemide; V = volume of replacement solution (and urine); P_M = mean arterial pressure; P_S = systolic arterial pressure; P_D = diastolic arterial pressure.

The osmolality and concentrations of sodium, potassium, calcium, and magnesium in the pooled urine were 335 mOsm/kg, 124 mEq/liter, 31 mEq/liter, 2.7 mEq/liter, and 5.39 mEq/liter in the control group. The corresponding values in the experimental group were 308, 90, 24, 2.2, and 1.96, respectively. The latter animals excreted 1034 ml of urine and received no sodium. Hence they lost 93 mEq of sodium in 76 min. Assuming an extracellular volume of 3700 ml, calculated extracellular sodium in the control state equaled 570 mEq. Seventeen percent of this appeared in the urine. This agrees with the 19% fall in plasma sodium concentration observed.

Discussion. Studies in perfused vascular beds have failed to demonstrate that sodium chloride *per se* affects vascular resistance (1, 3). In the dog forelimb (1), for example, an isosmotic reduction of the sodium chloride concentration in the perfusing blood over ranges which occur naturally has no discernible effect on blood vessel geometry. Constriction becomes manifest only when a fall in osmolality accompanies the fall in sodium chloride concentration. Elevation of the sodium chloride concentration reduces forelimb vascular resistance. This effect, however, cannot be attributed to sodium chloride *per se* since it also occurs when osmolality is equally elevated with other agents, dextrose and urea for example. Thus these effects seem to result from osmotic shift of water rather than from a specific action of sodium chloride.

Data from isolated muscle (4–8) show that hyposmolality causes cell hydration, a reduction in the intracellular potassium concentration, a decrease in the membrane potential and cellular contraction. The opposite is produced by hyperosmolality. Other data suggest that sodium competes with calcium for a carrier in the membrane such that reducing extracellular sodium concentration results in calcium influx and cell contraction (9). The latter mechanism however is not compatible with the absence of a response in perfused vascular beds when extracellular sodium concentration is reduced without changing osmolality or with the similarity of the responses seen when osmolality is raised with sodium chloride, dextrose, or urea (1, 3).

The findings in this study are in agreement with those obtained from perfused vascular beds. Blood pressure failed to change relative to the control series when plasma sodium concentration was selectively reduced over ranges which occur naturally without changing osmolality. Apparently the changes in blood pressure seen with acute manipulation of the total body sodium chloride concentration (10–14) also result via associated changes in extracellular osmolality. These changes in osmolality redistribute total body water such that blood volume and cell hydration are altered (10, 15, 16). These alterations in turn influence cardiac output, total peripheral resistance, and hence blood pressure. Thus acute pure salt depletion lowers osmolality resulting in osmotic shift of extracellular water into cells (16). This lowers blood volume and hydrates vascular and red cells. The fall in blood volume decreases cardiac output via the Starling mechanism and increases resistance via the baroreceptor system (10, 11). Cell hydration also increases resistance (1, 3) through (i) activation of the contractile machinery of the vascular smooth muscle (see above and 4–8), (ii) luminal bulging of endothelial cells, and (iii) swelling of red cells. Hemoconcentration (10, 11) may also play a role in the increased resistance. The fall in cardiac output is proportionately greater than the rise in peripheral resistance and the blood pressure falls (10, 11).

Acute hypernatremia on the other hand raises osmolality resulting in osmotic shift of cellular water into the extracellular compartment (16). This raises blood volume (15) and dehydrates cells (16). The hypervolemia increases cardiac output via the Starling mechanism (13–15) and momentarily decreases resistance via the baroreceptor mechanism; cell dehydration also decreases resistance (1, 3, 6, 12–15) via deactivation of the contractile machinery of the vascular smooth muscle (see above and 4–8) and shrinkage of endothelial and red cells (14). Hemodilution (13–15) may also play a role in the decreased resistance. The fall in resistance is proportionately greater than the rise in cardiac output when the hypernatremia is very acute and the blood pressure falls (12–14). The fall in resistance and rise in cardiac output are more proportionate when the hypernatremia is less acute and consequently blood pressure changes very little (15).

According to this analysis, cell hydration in the absence of a fall in blood volume and hence right heart filling pressure should raise blood pressure. Perhaps this explains the rise in blood pressure seen in hypertensive subjects following intravenous infusion of 5% glucose (17). Such an infusion does not lower blood volume (17) but should reduce extracellular osmolality as the glucose is metabolized.

Summary. Blood pressure was measured in anesthetized dogs during acute selective sodium chloride depletion without water depletion: total osmotic pressure of extracellular fluid was held constant thereby preventing osmotic shift of water into cells. The blood pressure changes seen were not different from those in a control series where sodium concentration remained constant. These studies fail to show that acute sodium chloride depletion *per se* affects blood pressure. Apparently the blood pressure changes seen with acute manipulation of the total body sodium chloride content are produced indirectly via associated changes in extracellular osmolality.

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HYPERNATREMIC DEHYDRATION

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Early recognition of the hypernatremic* state is of great importance in the treatment of this problem, since delay in treatment may lead to an irreversible state resulting in either death or severe brain damage with permanent sequelae. Knowledge of the factors which may predispose to hypernatremic dehydration is of value in the diagnosis, and, perhaps of even more importance, can lead to recommendations for the prevention of this disturbance.

PREDISPOSING FACTORS IN GENESIS OF HYPERNATREMIC DEHYDRATION

Early Infancy and Prematurity

The relatively large evaporative water losses per kilogram of body weight of this group of infants, and the inefficiency of renal conservation of water due to renal immaturity, explain their susceptibility to hypernatremia.[7]

Interference with Oral Intake of Water

This may occur as a result of the anorexia or nausea and vomiting of acute infections. Reduced intake may also be due to neglect of water administration to infants. A special group of hypernatremic patients is accounted for almost solely by this mechanism, namely, those with cerebral defect who either have insufficient access to water or whose

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*We have classified patients as hypernatremic if serum sodium concentrations are 150 mEq. per liter or higher.

cerebral impairment leads to a lack of thirst response in the presence of the usual stimuli.[10] Clinically these patients have to be distinguished from those whose brain damage is secondary to hypernatremic dehydration. At times this distinction may be difficult.

Excessive Losses of Electrolyte-Free Water because of Increased Evaporative Water Loss through Skin and Lungs

This is seen in febrile states, environmental heat stress, and in salicylism and other causes of hyperventilation.[8] Profuse sweating may also contribute, since normal sweat has a concentration of sodium only 10 to 35 per cent that of extracellular water.

Excessive Losses of Water in Stools

The concentration of sodium in the water of normal stools and often in diarrheal stools is considerably less than that in extracellular fluid.[9] However, there is great variation in the concentration of sodium in the water of diarrheal stools, and we have not been able to demonstrate any good correlation between the concentration of sodium in stool water and the development of hypernatremia. This factor, then, appears highly variable in etiologic significance, since no significant difference can be found between the concentrations of sodium in stool water of hypernatremic patients and normonatremic patients who have a similar degree of water loss due to diarrhea. Probably the volume of water lost in the stools is the important factor.

Excessive Administration of Solute (Electrolyte and/or Protein) to Sick Infants[1]

An infant who is being subjected to a dehydrating influence, be it diarrheal disease, anorexia or fever, has a smaller margin of safety for water conservation. The intake of high protein and electrolyte feedings, or large volumes of saline solutions, orally or parenterally, increase the renal water requirement obligatory for excretion of excess electrolyte and urea. This water may have been easily spared in health, but its loss now serves to increase water deficit. The feeding of undiluted and unmodified cow's milk to a two-month-old infant is an example of a high solute load which is easily tolerated by a well infant, but may cause water deficit in a sick infant.[7] In infants with diarrhea the total quantity of sodium intake is important as well as the concentration of sodium in the solution. Even though a fairly dilute electrolyte solution (50 mEq. per liter of sodium) is given orally to infants with diarrhea, hypernatremia may result if it is given in unphysiologically large quantities.[1] Such patients have been noted to produce copious watery stools which

are apparently very dilute in sodium. Only a few actual measurements are available on these infants, but no other explanation is at hand except that of large losses of water in the stool.

Salt Poisoning, Either Inadvertently by Addition of Salt to Feedings, or Iatrogenically by Injudicious Therapy, so that Excessive Amounts of Sodium Salts Are Given to Dehydrated Infants[3, 4, 9]

Excessive intake of salt produces an increased body sodium content, and because of attendant anorexia or vomiting plus the usual evaporative water losses there may be dehydration as well.

Excessive Renal Water Loss as in Diabetes Insipidus and "Nephrogenic" Diabetes Insipidus

Any interference with the necessary large water intake of these patients quickly leads to hypernatremic dehydration.

PHYSIOLOGY AND PATHOLOGY OF HYPERNATREMIA

Hypernatremia can be associated with an increased total sodium content of the extracellular fluid as in patients given excessive amounts of salt. In subjects with severe water deficit, however, total extracellular sodium is reduced despite high concentrations of sodium because of reduction of extracellular fluid volume. All patients with hypernatremia show certain manifestations which are thought to be the result of intracellular dehydration secondary to the increased osmotic concentration of extracellular water.

During experimental hypernatremia the muscle cells take up sodium, as well as lose water. In addition they reach osmotic equilibrium by apparent dissociation of intracellular complex particles into smaller units. This latter reaction appears to occur to a much greater extent in brain cells, where little or no sodium enters.[5] This special behavior of brain cells possibly accounts for the severe functional central nervous system changes seen in hypernatremia.

Intracranial bleeding occurs at times in hypernatremic patients and also in animals made acutely hypernatremic. This is apparently the result of injury or rupture of capillaries and venules secondary to reduction in cerebrospinal fluid pressure.[3, 5, 6] Subdural effusions have also been encountered.[3] These phenomena are probably not responsible for the common symptoms and signs of this disorder, but may in occasional instances cause death or severe residual damage.[3, 4, 5, 9]

The composition of the extracellular fluid may show other derangements because of the underlying disease, and such secondary phenomena as metabolic acidosis and the biochemical changes of renal insufficiency are seen in addition to the effects of the hypernatremia itself. The most

important of the biochemical disorders secondary to hypernatremia are changes in concentration of potassium and calcium. Potassium deficits are often large in these patients because of losses in gastrointestinal secretions and in the urine. At times the serum potassium concentration will be low in the face of evidences of renal insufficiency;[4] this phenomenon may represent an extrarenal adjustment to hypernatremia as well as being a reflection of a reduced body potassium content. If renal impairment persists, serum potassium levels, of course, will rise.

Serum calcium levels are frequently reduced in hypernatremic patients. The equilibrium of this ion is affected by multiple factors, but it seems probable from studies in patients and experimental animals that the calcium level is in some way altered by hypernatremia, particularly if associated with potassium deficiency.[3, 4] In addition, acute renal insufficiency leads to phosphate retention, which may play a contributing role. The metabolic acidosis present in some patients may protect against overt tetany, but when the acidosis is corrected before correcting the hypernatremia, hypocalcemic tetany may occur, as may occasionally be true in untreated hypernatremic dehydration if acidosis is minimal or absent.

CLINICAL RECOGNITION OF HYPERNATREMIC STATES

This topic is of paramount importance in any discussion of therapy, since in many situations there is a considerable time lag in obtaining chemical data from the laboratory. The condition can be deceptive because the dehydration, though marked, may seem inapparent to one looking for signs of classic dehydration.

The first step toward recognition of hypernatremic dehydration begins with the history of the etiologically disposing factors discussed above. An objective criterion is loss of 10 to 15 per cent of the body weight without the signs of classic dehydration such as loss of skin elasticity and turgor, or circulatory impairment. The hypernatremic patient may have lost 10 per cent of his body weight without evidence of circulatory embarrassment and look deceptively well for a time. These infants are often extremely thirsty, and this is one of the reasons why excessively large intakes of sodium may result if they are given electrolyte solutions *ad lib.* As they become sicker, however, nausea and vomiting limit the fluid intake. Fever is commonly associated with hypernatremia, so that it may be both a predisposing factor and a manifestation of this state.

The presence of signs and symptoms referable to the nervous system has been most important in the diagnosis. Disturbances in consciousness ranging from lethargy to coma have been common. Hyperirritability to stimuli despite extreme lethargy when undisturbed has been characteristic. Other signs such as tremors, exaggerated deep tendon reflexes and

muscle rigidity, including nuchal rigidity, have been common, and occasionally convulsions are seen. The concentration of protein in the cerebrospinal fluid is usually elevated.[5]

Hypernatremic dehydration when extreme may also be accompanied by reduction in extracellular fluid volume, with the associated circulatory disturbances. Such a "mixed" clinical picture is difficult or impossible to diagnose by clinical criteria alone, and in such instances the laboratory remains the *sine qua non* of diagnosis. However, this latter type of patient has a greater sodium deficit than the group previously described, so that administration of solutions with the usual concentrations of sodium salts given to dehydrated patients would not be so harmful as in the more common form of hypernatremia.

TREATMENT

The goal of treatment is not the rapid restoration of serum sodium levels to normal, but rather the safe return of the patient to optimum physiologic balance without undue risk of complications. Experience in the treatment of hypernatremic subjects has indicated that administration of water rapidly in large quantities in an effort to bring the sodium concentration into the normal range is dangerous. Such an approach may precipitate a severe convulsive state. It has been pointed out that certain adjustments of brain cells to the increased extracellular sodium concentration have occurred, and rapid reduction of extracellular sodium evidently precipitates another disturbance of equilibrium. Since these patients are usually not in circulatory collapse, the pressure for rapid replacement of fluid volume is removed, and the rehydration process may safely be carried out gradually. It is important, however, that fluid administration be started promptly when the diagnosis is suspected and that it be continued at the proper rate.

The composition of the fluid to be administered should, of course, be hypotonic with respect to sodium and chloride. Theoretically, it might be possible to eliminate these ions completely and give electrolyte-free glucose water. This has two drawbacks: first, many patients do have some extracellular electrolyte deficits, and, secondly, the addition of some sodium makes slightly more rapid administration of fluids possible and safe. Concentrations of sodium above 40 mEq. per liter have been found to be excessive for these patients. Current information suggests that sodium concentrations of 15 to 20 mEq. are the most suitable. Since most patients have a greater deficit of sodium than of chloride (metabolic acidosis), it is customary in our clinic to use 1 part of sixth-molar sodium lactate to 2 parts of 0.85 per cent saline solution in preparing the sodium-containing portion of the solution. The desired concentration indicated above may be reached by combining 1 part of sixth-

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mo. dium lactate, 2 parts of 0.85 per cent sodium chloride and 21 parts of 5 per cent glucose in water. If the acidosis is severe (indicated by carbon dioxide contents of less than 8 mEq. per liter), sixth-molar sodium lactate may be used in place of the saline, and thus 3 parts of sixth-molar sodium lactate and 21 parts of 5 per cent glucose in distilled water are given. (Final concentration of sodium lactate is M/48.) Solutions such as these should be given intravenously. The rate of administration to an infant should be such that 200 ml. per kilogram are given during the first 24 hours (0.14 ml. per kilogram per minute). This provides for the deficit of water of about 10 to 15 per cent of the body weight as well as for the continued water requirements of 70 to 100 ml. per kilogram per day for infants.

Fluid therapy for the second and subsequent days can be reassessed daily. If tolerated, glucose water may be given orally together with the appropriate electrolyte by the end of 12 to 24 hours or even earlier in milder cases. Although potassium deficits may be severe, it is wise to withhold administration of potassium initially, since many of these patients are oliguric or temporarily anuric. Once it is apparent that urinary output is adequate, potassium replacement should be started. The oral route is preferable when feasible, and 3 to 5 mEq. per kilogram per day are advised initially (10 per cent potassium citrate solution provides 1 mEq. of potassium per milliliter and can be given orally diluted with fruit juice or other fluid). If the parenteral route is necessary, the usual precautions for parenteral potassium administration should be carefully followed. The concentration of potassium chloride in the solution given intravenously should not exceed 20 mEq. per liter (0.15 per cent potassium chloride).

A daily weight is important in assessing the previous day's fluid replacement. It is common for these patients to show evidences of overexpanded extracellular fluid with visible edema even before they are completely rehydrated because of the lag in intracellular uptake of water.

Part of the therapy should be directed to controlling nervous system complications. Should convulsions occur, the usual anticonvulsant drugs are indicated. We have used subcutaneous phenobarbital sodium and rectal paraldehyde as agents which seem particularly safe in infants. The dose of phenobarbital sodium used is 5 mg. per kilogram repeated once in 20 minutes if necessary. Subsequent maintenance phenobarbital therapy is continued as in any acute convulsive state. When paraldehyde is required to control convulsions, 2 to 4 ml. are given rectally with an equal volume of cottonseed oil. If instilled through a small catheter and the buttocks taped tightly together with adhesive, the paraldehyde is usually retained. It is disconcerting to have nervous system manifestations develop while the patient is receiving therapy, but present knowledge indicates that this occurrence may be inevitable after a certain

amount of damage has occurred during the hypernatremic phase. Thus some nervous system damage may be irreversible before convulsions occur.

Subdural effusions may be suspected and diagnosed by needle aspiration. Our experience to date has not shown any improvement in these patients from paracentesis or surgical drainage of subdural effusions over simple expectant watching. It would seem wise, until better criteria are established, to regard and assess each case individually. Intracranial bleeding may occur in several different sites, and here again individual consideration of treatment is indicated. Hypocalcemia may be averted or treated when present by the administration of 10 to 30 cc. of 10 per cent calcium gluconate per day in the intravenous fluids.

In summary, the treatment of hypernatremic dehydration consists in administering an appropriate fluid volume of low (15 to 20 mEq. per liter) sodium concentration at a slow rate until water deficit is overcome. At the same time attention should be paid to needs for potassium and calcium. Meanwhile the nervous system complications must be managed symptomatically. In rare instances aspiration of subdural effusion or hematoma may be indicated.

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Evidence is presented to show that in both experimental animals and man dietary manipulations may initiate, modify, and even reverse hypertension, especially when of renal origin. It seems likely that the relation of dietary sodium to most forms of hypertension may involve the juxtaglomerular cells of the kidney, and thus an endocrine system. The significance of these findings is discussed.

THE NUTRITIONAL ASPECTS OF HYPERTENSION AND ITS REVERSIBILITY

W. Stanley Hartroft, M.D., Ph.D.

THE past few years have seen a considerable diminution of interest on the part of clinicians in relations between diet and hypertension, largely because contemporary methods of treatment now more heavily rely on certain groups of pharmacologic agents (adrenergic and/or cholinergic-blocking agents and the thiazide-group) than on therapeutic devices based primarily on regulation of the kind and amount of food consumed by these patients. Optimum control of the hypertensive individual cannot, of course, entirely disregard the nutritional approach, particularly in the case of either obese subjects or those who are accustomed to generous intakes of salt (NaCl). But few clinicians today depend solely upon dietary measures in the treatment of hypertension—as was the case only a decade ago when rather rigid restrictions of salt, protein, and calories not infrequently appeared to be the most effective means available at that time for the treatment of a considerable number of hypertensive patients.[1-5] Even in cases in which sodium balance is a problem, the use of diuretic agents nowadays has proved to be perhaps a more practical approach than a simple dietary restriction of salt alone.

So-called "basic scientists" are often accused by their clinical colleagues of "ivory-tower isolationism," but a perusal of the literature dealing with experimental hypertension during the past ten years, indicates that interest in the research laboratory in the purely dietary approach to hypertension has also fallen off, although perhaps not to the same degree as among clinicians. Apparently then, the basic scientist is influenced by his practical clinical partner. For example, a recent review by one of the leaders in this field, Page,[6] presents "a concept of the various equilibrated facets of blood pressure control . . . called the 'mosaic theory.'" His facets (chemical, reactivity, volume, vascular caliber, viscosity, cardiac output, elasticity, and neural) do not specifically or directly name any nutritional factor, although such might be implied indirectly. Yet, only 10 or 15 years ago, from this same investigator's laboratory, publications concerning the influence of

protein, carbohydrate, or salt on levels of blood pressure were announcing important new insights concerning their interrelationships.[7] Other examples of prominent groups active in the study of hypertension who have changed their focus from diet to hormones (particularly the renin-angiotensin-aldosterone system) or to pharmacologic agents (already referred to) readily could be cited.

When such definitive swings of the pendulum occur in medical science, it is sometimes the result of a degree of overenthusiasm on the part of influential groups. At such times, it is well that not everyone go with the "swing," and I am accordingly appreciative of this opportunity to discuss some nutritional aspects of hypertension and possibly to bring out a few interrelations between the predominately dietary approach of a decade ago and the current pharmacologic emphasis. Perhaps the account to follow may indicate that the two are more closely related than is apparent at first sight.

In the experimental animal there is no question but that under a number of conditions manipulations of the protein, sodium, and caloric content of the diet can enhance and even initiate the hypertensive state. Again, by suitable dietary alterations, usually in the opposite direction, the levels of blood pressure can be reduced and even returned to the normal range. For instance, a high protein diet intensifies the hypertension of rats following subtotal nephrectomy[8,9] or the injection of nephrotoxic sera,[10] although the development of nephrosclerosis and hypertension in rats induced by desoxycorticosterone injections are independent of the level of dietary protein.[11,12] But a reduction of dietary protein and salt, or in caloric intake, lowers the blood pressure of subtotally nephrectomized rats.[13]

The rather classic experiments just cited involve renal or hormonal manipulation plus dietary alterations. Prior to some 15 years ago all attempts to produce sustained elevations of levels of blood pressure (i.e., hypertension) by dietary means alone were unsuccessful. Significant increases in levels of systolic pressure were produced in chicks[14,15] and rats[16] by adding sodium chloride to the drinking water (1.2 to 2.0 per cent), but pressures returned to normal when the salt water was replaced by tap water. In 1948, however, the Friedmans in British Columbia[17] announced the production of hypertension in rats by giving them saline in place of drinking water plus one single injection of desoxycorticosterone acetate. Hypertension persisted for some months even after tap water had replaced the saline. A number of investigators has since then established that salt added to the drinking water will, without any additional surgical or hormonal treatment, produce a sustained and permanent hypertension.[18-21] In some cases withdrawal of the saline and replacement by fresh water will reverse the condition. Months, however, of augmented salt intake were required in all these experiments to produce the hypertension. But recently, Hall and Hall[22] found that incorporation of 5 per cent sucrose in a 1 per cent saline solution induced hypertension within a month in rats so treated: its progression was more rapid and a larger percentage of animals were affected than when saline alone was consumed even if higher concentrations of NaCl were employed without the sucrose. The addition of the sucrose induced the animals to consume large amounts of saline per day and thereby explained the findings, the investigators having taken advantage of the fondness of rats for sucrose.

Our own interest in the relation of diet and hypertension however stems from still another approach, namely choline deficiency. The successful production of a permanent, sustained ele-

vation of blood pressure in rats by a brief period of choline deficiency in their early life clearly showed that dietary manipulation alone (without either surgical intervention or exogenous hormonal administration) could produce hypertension in the later life of these animals. Weanling rats were fed a diet low in choline and other lipotropic agents (methionine, betaine) for periods of less than one week and then given a normal diet (stock laboratory chow) for the remainder of the experimental period of three to five months.[23] This finding has now been confirmed in a number of laboratories[24] using either rats or dogs. In this form of hypertension, high levels of protein or salt also enhance the degree of elevation of blood pressure, and conversely low levels of these dietary ingredients will reduce it.[24]

Pertinent to the subject of this presentation (nutritional aspects of hypertension and its *reversibility*), is the still unexplained fact that following the brief period of choline deficiency, restoration of choline to the diet not only fails to prevent or reverse the development of hypertension but, also and paradoxically, such restoration is actually *essential* to its production. We and others have shown[23,25] that if choline is withheld from the animals' diets following the initial period of deficiency (by continuing them on a choline-deficient diet for several months), hypertension does not develop although renal damage of a degree equally severe to that found in the hypertensive groups does. Moreover, the hypertension present in subtotally nephrectomized rats is abolished by dietary choline deficiency.[26]

Although the hypertension in all these models is clearly of renal origin, careful comparison by conventional means of the type and extent of the damage present in their kidneys failed to provide us with any clue to help explain these apparent paradoxes. Somewhat in desperation, perhaps, we turned our attention to the juxtaglomerular cells (JG-cells) of the kidneys hoping that their study might elucidate the problem. We started these investigations a decade and a half ago. Dr. Phyllis Hartroft at first assisted me with these investigations and eventually took them over. More recently, Dr. L. E. Sutherland in my laboratories in Toronto also has made the juxtaglomerular cells her particular interest. I wish I could now reveal that these JG-cells have given us the answers posed in the foregoing. They have not as you will see. However, our studies have been rewarding in other ways. They soon involved us deeply with dietary manipulations of sodium and so their results are pertinent here. The rest of this account will deal with some of these studies.

During the past several years, the juxtaglomerular cells of the kidney have finally emerged into prominence from the comparative obscurity in which they lay scientifically enshrouded for well over a quarter of a century following their discovery in 1925.[27] Prior to a few years ago, anyone speaking on this subject was forced to review their anatomic and cytologic characteristics. Accordingly, it is only from force of habit today that these cells, present in every mammalian species examined to date* (as well as lower forms to be described later), occupy the media of the afferent glomerular arteriole, replacing its smooth muscle cells in the area next to the glomerulus. In mammals at least, they consistently abut the macula densa of the distal tubule of the same glomerulus, and in hypertrophic states, particularly in man, they increase in size and number most prominently on the side of the arteriole nearest this portion of the tubule. Electron microscopy[28] has confirmed the older observations by light microscopy[29] that

* With one possible exception—see reference 40.

the JG-cells contain secretory granules, probably of an endocrine nature. High magnifications (20,000X or more) have revealed that the granules possess a complex structure suggestive of "granules within granules."[30] Such complexity would be compatible with a current hypothesis held by some that the granules may represent localization of more than one hormone (i.e., renin and erythropoiten). The intriguing anatomical relation between the juxtaglomerular cells within the afferent arteriole and its adjacent macula densa (probably an important site of sodium reabsorption) is still just as mysterious today as when first emphasized by Goormaghtigh[31] and later by McManus.[32]

More than a decade ago, by steps described elsewhere,[30] we were led to suspect that levels of dietary sodium might play an important role in regulating the degree of granulation in JG-cells, and in rats we were able to demonstrate this fact fully.[33] Furthermore, it was found to this correlation (a reverse one) of the amount of dietary salt (or level of serum-Na), with the intensity of JG-granulation, could be added another correlative—namely that the greater the degree of granulation in any particular animal, the wider its zona glomerulosa of the adrenal cortex.[34] We were also able to report that short term (two-three weeks) manipulations of levels of blood pressure by appropriate pharmacologic agents (Apresoline or Privine) administered to rats were accompanied by changes in JG-granulation. The correlation was again of a negative type, i.e., the higher the level of blood pressure the sparser the granulation and vice versa.[34] It was further demonstrated that in unilateral renal hypertension induced in rats (Goldblatt) severe JG-degranulation developed in the contralateral ("untouched") kidney.[35] This phenomenon has been confirmed in cases of unilateral renal hypertension in man.[36] The "empty" appearance of the cytoplasm of these degranulated JG-cells, as they appear in sections of renal biopsies taken from the relatively normal kidney of these human cases, has suggested to us a convenient term for this condition—"clear cell hypertension."

The above sequence of findings naturally made us suspect that renin might be closely associated with juxtaglomerular cells. In 1959, Hartroft and her colleagues Pitcock and Newmark[37] were able to report a highly significant degree of statistical correlation between the renin content of the kidneys of a series of rats fed various levels of dietary sodium and the degree of JG-granulation in the same organs. The lower the amount of dietary sodium and the lower the level of serum sodium, the greater the intensity of juxtaglomerular granulation and the higher the renin content of crude extracts of the same kidneys. Although it is now widely accepted that dietary sodium restriction will increase the renin content of the kidney in a variety of species, this discovery originally came as a surprise at that time to many investigators in the field.

At this point, our all-over interpretation of the role of juxtaglomerular cells, based on the above series of experiments, led us to the concept that they were the site of manufacture and release of renin, which not only acted on angiotensin-precursor to produce angiotensin, but also either directly or through angiotensin served as a tropic hormone aimed at the zona glomerulosa of the adrenal to stimulate the release of aldosterone.

Finally, in 1961, Hartroft and her associates were able to provide relatively direct evidence that renin was localized in granular form within juxtaglomerular cells. The Coons-Kaplan immunofluorescent technic was employed.[38] By fluorescein-labeling of antihog renin produced in dogs and

ultraviolet microscopy, they were able to demonstrate specific binding of JG-granules of rabbit kidneys by the labeled antirenin. In subsequent experiments conducted with Dr. Sutherland in Toronto, it was possible to show that such specific staining was not likely due to any impurities present in their starting antigen, renin. They absorbed the antirenin with extracts of kidneys obtained from pigs fed high-salt diets. These kidneys, of course, contained very few JG-granules and extremely small amounts of renin, but were otherwise histologically identical with the pig-kidneys from which the original starting antigen (renin) had been prepared. This absorption removed all nonspecific "background" staining by fluorescein, but did not impair the brilliance of the fluorescence of the juxtaglomerular cells in sections of the rabbits' kidneys treated with the absorbed and labeled antirenin, when examined under the ultraviolet microscope.

But even this relatively direct approach in support of the notion that renin is localized within juxtaglomerular granules still left unanswered certain objections to the hypothesis. In 1963,[39] Dr. Meyer Friedman, at the annual meeting of the American Heart Association, in discussing the evidence above, presented at that time by P. M. Hartroft, objected that ". . . nor could we find anything that even looked like a juxtaglomerular cell in the 18-mm fetus of the pig. Yet per gram of weight, there was adequate renin in such kidneys (mesonephros). . . . In the metanephros, the renin content increased along with the tubular mass . . . as long as these fetal kidneys assay for renin, and as no one has found juxtaglomerular cells, I think it is difficult to postulate that renin is coming from nothing in the fetal kidney." With Dr. Sutherland, both Phyllis Hartroft and I now believe this challenge has been answered. It has been quite conclusively demonstrated, using both the Bowie stain and the fluorescent antibody technic that granulated juxtaglomerular cells are present in abundance in both the mesonephros and metanephros of a series of pig embryos ranging from 2.4 to 25 mm crown-rump length. Of particular interest was the fact that as long as the mesonephros was fully developed and its JG-cells richly granulated, similar granulation in the metanephros was scant. But as the embryo increased in length and the mesonephros (along with its JG-cells) underwent progressive regression and eventual disappearance, JG-cells became increasingly prominent and granulated in the metanephros—apparently replacing those granules of the mesonephros as the latter faded away with embryonic growth.

During the past year or so, this investigation has been carried much further by Sutherland[40] and she has demonstrated JG-granules in the metanephros of the embryo-rat. These observations on rat and pig embryos finally enable us, as Dr. Sutherland has put it, to reassure Dr. Friedman that it will not be necessary after all "to postulate that renin is coming from nothing in the fetal kidney."

Dr. Sutherland[40] has also found that the possession of JG-cells is not the sole prerogative of Mammalia. She and her students have demonstrated JG-cells in Budgerirgars, Pekin Ducks, and a considerable number of different kinds of fish, including Rainbow Trout, Black Mollies, Neon Tetras, Threespine Sticklebacks, Medaka, Rock Bass, Carp, Labeo Bicolour, and also in Leopard Frogs. Earlier with Dr. P. M. Hartroft, Dr. Sutherland had found juxtaglomerular cells in Guppies, Bull Frogs, Chrysemys Picta (reptilia) and in Cat Fish. One rather well "pickled" Armadillo proved the only mammal examined to date in which JG-cells could not be demonstrated in sections of its kidneys. This mammal is the only armoured species

we have examined to date, but before drawing up any hypothesis involving armour and JG-cells, more and better preserved specimens must be obtained.

Conclusion

Abundant evidence is now available that in both man and experimental animals various dietary manipulations may alter, modify, initiate, and even reverse hypertension, particularly that of renal origin. In this presentation, dietary protein, sodium, caloric content, and choline deficiency have been emphasized, although there is scattered evidence that vitamin A, heat-stable fractions of the vitamin B complex, and vitamin E may also under at least some conditions affect hypertensive states. It is quite likely that the clearly established relation of dietary sodium to most forms of hypertension may involve the juxtaglomerular cells of the kidney and thus the renin-angiotensin-aldosterone system.

These cells, which respond dramatically in both animals and man to fluctuations in dietary and serum sodium levels, have now been shown conclusively by a variety of approaches to contain high concentrations of renin, particularly in sodium-deficient states. Their granule and renin-content also vary with the level of blood pressure in several circumstances. Juxtaglomerular cells are found not only throughout Mammalia but appear in representative members of much of the entire animal kingdom, including birds, reptiles, and a variety of fish. They are present in both the mesonephros and metanephros of embryonic pigs and at least in the metanephros of fetal rats. Although the treatment of hypertension by pharmacologic agents may have taken precedence recently over purely dietary measures, continued investigations by experimental nutritionists should help unravel the many yet unsolved complexities of this serious disease. To the experimental pathologist, the juxtaglomerular cell may be the essential cytologic link between macula densa and zona glomerulosa in the chain of hormonal and dietary interrelationships expressed in Page's "mosaic theory" of hypertension.

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Hawley, G.G.

Condensed Chemical Dictionary, 8th edition

Van Nostrand Reinhold Co., N.Y., N.Y.

BUREAU OF CHEMISTRY AND SOILS,
U. S. DEPARTMENT OF AGRICULTURE,
OCTOBER 9, 1929

SALINE DRINKING WATER

BECAUSE of the ease of modern modes of transportation, man of to-day is exposed to an ever-increasing change in living conditions to which he must adjust himself. Without a doubt, water is the greatest variable which one encounters in passing from one region to another, and it is this variable which is responsible for the greatest physiological changes in the body.

The waters of certain areas of the United States are either quite free from dissolved salt or contain only very small amounts. Such waters are called "soft" waters. In contrast to these practically salt-free waters are those waters which have come into contact with limestone beds and have taken up calcium carbonate in the form of the bicarbonate. Such waters are said to have temporary hardness. Surface waters which have passed through alkaline soils, or well and spring waters which have come into contact with salt beds, will dissolve these salts and will consequently have a brackish taste.

Large sections of the southwestern part of the United States have alkaline soils which impregnate surface waters with salts, and in addition there are in these regions extensive salt beds which contaminate the deep wells and many of the springs. The salt content of the rivers in these sections is often so high that it will kill vegetation along their shores. The salts present in these waters are sodium chloride, calcium chloride, calcium sulphate and magnesium sulphate. Analyses of waters from such sections have shown these salts in certain instances to be present in excess of 200,000 p.p.m., and 5,000 to 50,000 p.p.m. are common.

The question of the possible deleterious effects of such waters upon the animal organism immediately presents itself. Travelers drink such salty waters with considerable hesitation on account of their unpleasant taste and disagreeable physiologic action. Live-stock growers frequently attribute the death or poor condition of their stock to the salty waters which the animals must drink. This is especially true where the cattle drink from small streams which receive waste saline waters from the oil wells.

Strange as it may seem, scientific literature does not answer this vital question. There are volumes of semi-popular articles dealing with the medicinal properties of certain spring water. In the pharmaceutical litera-

ture the physiological action of certain salts is recorded, but practically nothing can be found in regard to the physiological effects of the ingestion of saline drinking water. In a pamphlet published in 1925 by the U. S. Public Health Service, "Drinking Water Standards," an arbitrary standard is set for waters which are to be used on interstate commerce carriers, but it is a matter of common knowledge that much of the water being used continually by people in the southwest contains more saline matter than is permitted by the U. S. Health Service, and, nevertheless, there is no evidence of any deleterious effects arising as a result.

An investigation was begun in an attempt to answer the following questions in regard to saline waters. 1. What is the effect of the presence of considerable quantities of salt in our daily drinking water? 2. At what percentage does salt become deleterious to the organism? 3. Is it possible for the organism to adjust itself in time to saline drinking waters? The experimental animals used in this work were rats and other small animals. They were placed upon well-balanced rations supplemented with drinking water which was prepared by dissolving different amounts of various salts in distilled water. Sodium chloride was added to the water in amounts ranging from 500 p.p.m. to 50,000 p.p.m. Similar series were tried using calcium chloride, magnesium sulphate, sodium carbonate, sodium bicarbonate and calcium sulphate (up to the limits of saturation). The general appearance of the animals, their growth rate, their ability to reproduce and to rear their young and the condition of their organs at death were considered in studying the effects of the various salts upon the animal.

The first point determined was the maximum percentage of a single salt which could be administered in the water without producing harmful effects upon the animal. Mixtures of the salts were also added to the water to determine whether there might possibly be an antagonistic action between the various ions: that is, whether the deleterious effect of one ion might not be offset by the effect of a second ion. Such an antagonistic ion effect has been observed in certain body processes and also in plant nutrient solutions between sodium, calcium and magnesium ions.

From the data obtained, it is possible to postulate that 15,000 p.p.m. of magnesium sulphate has a retarding effect upon growth and that increases the mortality rate of the young. Twenty-five thousand p.p.m. of magnesium sulphate have a decided toxic effect. Calcium chloride produces even more harmful effects. No normal litters were found among rats drinking 10,000 p.p.m. of this salt; 15,000 p.p.m. lessen the growth rate, and 25,000 p.p.m. produce death. Ten thousand p.p.m. of sodium chloride affect reproduction unfavorably; 20,000 p.p.m. cause the growth of the animal to be stunted, and 25,000 p.p.m. produce a rapid decline in weight. Sodium carbonate is decidedly deleterious at a concentration of 20,000 p.p.m., and reproduction was interfered with at quite low levels. Sodium hydrogen carbonate is less injurious to the animals. A combination of 20,000 p.p.m. of sodium chloride and 5,000 p.p.m. of magnesium sulphate or calcium chloride inhibits growth. The results of feeding various other combinations of salts seem to indicate that in animals there is no antagonistic action between the ions such as there is in plants. The chlorine ion appears to be more toxic than either the carbonate or the sulphate ion. The calcium and magnesium ions are more harmful than the sodium ion. This can be explained by certain known physiological actions of ions. It is interesting to note that as the salt concentration increased the amount of water consumed by the animal daily also increased up to the point where the animal preferred to die of thirst rather than drink the necessary amount.

When animals which had been given a salty water were placed on distilled water soon after their first loss in weight, they rapidly regained their weight and appeared not to have been permanently injured by the saline content of the water. Animals which have received the salty water for some time seem better able to stand such water than do animals which are suddenly changed from distilled water to salty water.

The above results indicate that the continued use of saline solutions as drinking water produces injurious effects upon the animal organism. The concentration at which the solution becomes harmful varies with the particular salt used. The animal organism can apparently accustom itself within limits to a saline water, but in all probability it can not become adjusted to the effects of high concentrations.

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Occasional Survey

HYPERTONIC SALINE IN MANAGEMENT OF INTRACTABLE PAIN

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Summary 108 patients with intractable pain from malignant disease or benign conditions were given intrathecal saline (10–15%). Three months later over 50% of cancer patients were still experiencing pain relief as judged by their need for mild analgesics only or none at all. Muscle weakness (3%) and sphincter disorders (8%) were the most important complications.

INTRODUCTION

ALTHOUGH the physiology and anatomical pathways of pain remain controversial a number of procedures, such as cordotomy, have provided worth-while relief from intractable pain. Unfortunately many procedures are not tolerated by patients who are very weak nor is percutaneous or stereotaxic cordotomy suitable for all cases. For these patients there is a tendency to rely on stronger and stronger analgesics more frequently prescribed. Although some of the new analgesics are less addictive than others there may be troublesome side-effects such as nausea and mental confusion. In such situations, and especially where life expectancy is short, some relatively simple procedures have been developed for the relief of pain.

One of the first of these was intrathecal alcohol for the relief of severe lower-limb pain.[1] The diffusion of alcohol is very difficult to control, however, and, although very effective in relieving pain, the risk of paralysis is considerable and the method is only advisable in patients who already have paralysis of the limbs or sphincter disorders.

Maher[2] developed the technique of intrathecal phenol and later combined it with glycerin to slow down diffusion. Subsequently iophendylate ('Myodil') was incorporated so that the droplet could be observed radiologically and the solution manipulated to the desired part. Phenol is effective in about 65% of cases, but most workers report sensory loss, paralysis, and bladder or bowel dysfunction as complications. These complications are less common with the diluted solution of phenol now more often used.

One of us (E. H.) has described the use of cold subarachnoid irrigation for intractable pain and described 12 cases.[3] 9 of these had malignant disease and 3 had benign disorders. Of the patients with malignant conditions 7 obtained worth-while relief lasting for eight to sixty days. However, 2, although experiencing immediate relief, had recurrence of pain within a few hours. The 3 patients with benign conditions obtained no relief.

The method seemed simple, relatively safe, requiring no special technique, and therefore seemed especially indicated for patients in poor general condition with pain as the most important symptom. Another important point was the absence of sensory loss and bladder or bowel dysfunction. At first it was thought that the effect was due to hypothermia, but subsequent patients were injected with the supernatant fluid which was somewhat hypertonic. In view of this further cases were treated by hypertonic solutions at room temperature.[4]

Many reports confirm the efficacy of the procedure in a variety of conditions including neoplasia, neuralgias, and causalgias.[5–14] Some workers[8,13,14] have given repeated injections and most have used the original technique of hypothermia irrigation.

Our technique has changed since June, 1967, when we concluded that the effect might be due to hyperosmolarity of the supernatant fluid rather than the hypothermia. Hypertonic saline has been used ever since in a solution varying from 10 to 15%.

PATIENTS AND METHODS

The patients in this series had intractable pain of variable duration. Many of those with benign disease had been in pain for many years and most of those with malignant disease were not expected to live for more than six months. All were taking powerful analgesics or hypnotics, usually with little or no relief. Most of the cancer patients had pain despite heavy doses of morphine or morphine-like derivatives.

Detailed records were available for only 180 patients, and of these 108 had a complete follow-up until death or discharge more than six months from first attendance. The results are analysed in this paper. 56 were males; 75 had malignant disease; the average age was sixty-five (range twenty-eight to eighty-one). The total number of procedures was 116.

Method

Lumbar puncture was performed in the usual manner, the needle being inserted at the L3/4 or L4/5 interspace. In

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earlier cases cerebrospinal fluid (C.S.F.) was withdrawn, aided by tipping the patient until he complained of headache or, in later cases where general anæsthesia was given, until no further C.S.F. could be obtained. In the last 100 or so patients only a few millilitres of C.S.F. was withdrawn for analysis.

The patient was rapidly tilted—head up for lower-body pain, head down for upper-body pain—and with the affected side dependent, since the saline was slightly denser than C.S.F. Saline was injected before this movement was made, the concentration being chosen according to the patient's size and general condition. For example, a patient who was small or in very poor general condition would have the weaker solution, and those in good general condition and those of larger build would have the higher concentration, varying between 10 and 15% but in general 12·5%. A 15% solution of saline was used and diluted if need be, and 20 ml. of the solution was injected rapidly through the lumbar-puncture needle.

When the procedure was done under local anæsthesia it was followed by severe paræsthesia, especially in the lower limbs, associated with hyperventilation. Several premedications and sedatives, including intravenous diazepam, were tried, but general anæsthesia was used in all later cases. A rise in blood-pressure often followed injection,[9] and fasciculation was often observed together with piloerection and some venostasis and faint cyanosis of the dependent parts of the body. About thirty minutes after the injection the patient was tilted back into the neutral position, and when the reactions had subsided, which was usually about this time, the anæsthetic was stopped and the patient was returned to the ward. Most patients reported immediate pain relief after recovery from the anæsthetic, and pain relief continued as detailed later. Full neurological examination was done before and after injection, and a special note was made of pre-injection bladder function and pre-injection sensory loss attributable to the primary disease.

RESULTS

Pain relief is difficult to assess in patients who have been on strong narcotics. All too often the complaint of pain is in an expression of the patient's despair and desire for cure. Relief can be assessed, however, by the absence of previous complaint of pain and by a reduction in the dose of analgesics required. In earlier cases we tried to use the patient's assessment of the degree of relief afforded, but many subjective factors entered this assessment, especially in dying patients. A more objective system seemed to be one related to the need for analgesics before and after the procedure. The patients were assessed and placed in the following groups:

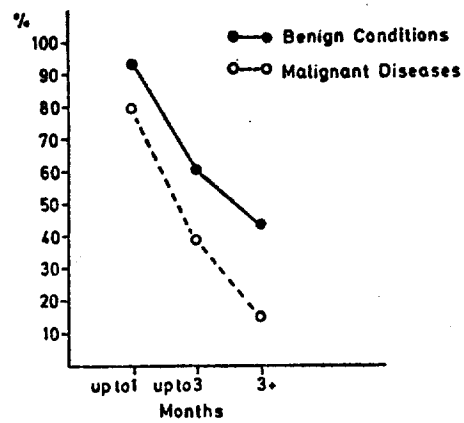
Group 1.—No relief. Strong analgesics do not relieve pain completely.

Group 2.—Partial relief. Strong analgesics relieve pain completely.

Group 3.—Good relief. Mild analgesics relieve pain completely.

Group 4.—Complete relief. No analgesics required.

16 patients were not relieved of pain. Some of them died within a few days. Others got no relief even after subsequent cordotomy. However, these have still been regarded as unsuccessful responses to saline although in many patients the influence of psychic factors was overwhelming. The follow-up was often difficult. Many patients came from distant hospitals and were already being nursed as terminal patients in hospital or at home. The follow-up period was, therefore, limited to six months or so.



Pain relief at follow-up.

The figure compares the results obtained in malignant and benign conditions. 44% of patients with benign conditions experienced "permanent" relief as against 15% for the malignant-disease group. The figure, however, takes no account of patients dying during the period of follow-up, and the percentage of patients with pain relief amongst the survivors of malignant disease is higher than for those with benign disease.

Hypertonic saline seems to be more effective than hypothermic saline (10·5% failure against 14·6% for hypothermic solutions) for periods up to three months. For periods of more than three months a slightly better percentage obtains for hypothermic saline. For the purpose of this paper it is assumed that patients in groups 1 and 2 had no worth-while pain relief and that the technique had failed. Patients in groups 3 and 4 were regarded as successful results. As soon as strong analgesics were prescribed (pethidine, morphine group, pentazocine) we assumed that pain had recurred. In fact, many patients were given morphine during the terminal stages to relieve distress even though they were pain-free. Because of difficulties of assessment, however, we have included all such patients as having pain recurrence.

COMPLICATIONS

Sphincter Disorder

The overall complication-rate for sphincter disorder was 8%. This complication is difficult to assess since many of the patients already had a severe disorder of bladder and bowel dysfunction due to the underlying disease. Patients who already had pelvic malignant disease were more prone to transient bladder and, rarely, bowel dysfunction, although few failed to recover within a week. In patients with no previous history of bladder disorder micturition problems were rare and never permanent, with the exception of the ones presented below.

Muscle Weakness

Muscle weakness was rare (3% of cases); it was transient and not demonstrated objectively, being a complaint by the patient of weakness usually in the leg. 1 patient with periarteritis nodosa had lower-limb weakness for three days which then disappeared. 2 patients had a severe complication. 1 patient with

adhesions due to multiple disc operations was given hypothermic hypertonic (15%) saline. He developed an immediate cauda equina syndrome, and, although muscle function recovered in the next few months, he continued to have severe disorder of bladder function and required a bladder-neck resection. The second patient had severe peripheral vascular disease. She also developed a cauda equina which gradually recovered over several months before she was able to walk and have her catheter removed. Bladder function returned to normal, but the severe peripheral arterial disease still prevented complete recovery. We conclude, therefore, that the technique should not be used for patients with severe arterial disease, nor if there is any evidence of intrathecal adhesions, because this may prevent diffusion and dilution of the injected solution. In patients with pelvic disease the risk of sphincter disorder is probably a little less than with other pain-relieving procedures.

DISCUSSION

Difficult problems face all those dealing with patients with intractable pain. The pain is often the expression of distress or the demonstration of a need for care, experienced mainly in patients who know how ill they are. Some patients were addicted to morphine or other analgesics, and the problem of pain relief was often increased by the need to treat drug addiction.

Injection of hypertonic saline is simple, effective, and there are few complications. It does not require special instruments or trained staff or a specialised centre. If a good result is not achieved the patient's condition will not be worsened by the injection, so further treatment may be given. Compared with other methods, however, the period of pain relief is short. Against this, safety and simplicity seem to be strong advantages. The best indication for its use is in patients who are likely to die within three months (i.e., before pain might be expected to recur). For patients with widespread pain, especially those with pain associated with metastases, intrathecal saline offers a safe, simple, and effective pain-relieving procedure.

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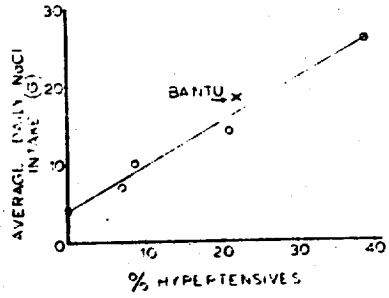
The Lancet

SODIUM INTAKE AND HYPERTENSION

SIR,—The role of sodium intake in the causation of hypertension is still uncertain. Dahl,[1] having studied five population groups throughout the world, suggested that a linear relationship exists between dietary sodium intake and the communal incidence of hypertension. While engaged on an apparently unrelated study,[2] we have encountered data which support this hypothesis.

An accurate method of assessing minimum daily sodium intake, in the absence of excessive sweating, is the estimation of the 24-hour urinary sodium content.[1] We have obtained values of 24-hour urinary sodium output for 100 healthy adult Bantu males; these men were temporarily domiciled in a hospital enclave for routine physical examination before starting work as miners.



They were eating their usual "Bantu type" of diet, high in maize, with salt allowance ad lib. Their mean 24-hour urinary sodium output was 310 mEq. (standard deviation 103 mEq.). This corresponds to a minimum NaCl intake of 18·6 g. per day.

While both clinical and necropsy studies suggest that hypertension is common amongst the South African Bantu,[3-5] the only large-scale survey is that of Ordman.[5] He places the overall prevalence of hypertension, amongst Bantu males of all ages, as 22% (defining hypertension as a blood-pressure reading above 140/90 mm. Hg; Dahl used identical criteria).

We reproduce here Dahl's original graph showing the linear relationship he found. We have added the single point representing the data obtained from the Bantu subjects. It is plain that this fits well on the line illustrating the mean relationship.

More detailed work to assess the possible relationship between salt intake and blood-pressure in the Bantu is under way in the Nutrition Unit of the University of Cape Town.

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MECHANISM OF THE THYROID STIMULATION PRODUCED BY SODIUM CHLORIDE IN THE MOUSE[1]

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ABSTRACT

The mechanism by which prolonged ingestion of sodium chloride induces a stimulation of the thyroid in mice has been investigated.

Mice fed for 2 months on a low iodine diet containing 1% sodium chloride exhibited larger thyroid glands with a smaller iodine content than control mice fed the same diet without sodium chloride. Furthermore, radioiodine measurements performed at intervals revealed that the presence of sodium chloride in the diet increased the fraction of the body iodide excreted into the urine and decreased the fraction taken up by the thyroid.

In mice given radioiodine and receiving a single intragastric dose of 30 mg. sodium chloride, the amount of radioiodine excreted in the urine within the next 2–3 hours was augmented 10–20 times, while the radioiodine level in blood was lowered moderately. (Similar effects were observed in thyroidectomized but not in nephrectomized mice.) Twenty-four hours after sodium chloride administration, the radioiodine content of the thyroid decreased to one half the amount in controls.

These results indicated that sodium chloride increases the urinary loss of iodine and thus lowers the plasma level of this element. As a result, less iodine is available to the thyroid gland and less thyroid hormone is secreted. The pituitary gland responds by releasing more thyrotrophic homone and thus stimulates the thyroid gland.

ADDITION of sodium chloride to the diet of mice induces a stimulation of the thyroid gland characterized by hypertrophy, hyperplasia and hyperemia, with loss of colloid and increased iodine uptake (1). Furthermore, if the diet is low in iodine, sodium chloride enhances the severity of the resulting goiter (1). The present work was undertaken to elucidate the mechanism by which sodium chloride stimulates the thyroid gland.

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MATERIALS AND METHODS

The experiments were carried out with 2.0–6.5 month old C_3H/Jax male mice, fed on a basic low iodine diet containing 89% corn meal, 10% dried brewer's yeast, and 1% calcium carbonate (1). When needed, sodium chloride (reagent grade) was added so as to make up 1% of the diet, and the amount of corn meal was correspondingly reduced to 88%. Iodine was estimated in each batch of the foodstuffs by a modified Chaney procedure (2) following ashing of 5 gm. of food with 30 gm. of potassium carbonate at 1300° F for 30 minutes. A few batches of the foodstuffs have been tested for sodium and chlorine content: sodium by flame photometry after digestion in concentrated nitric

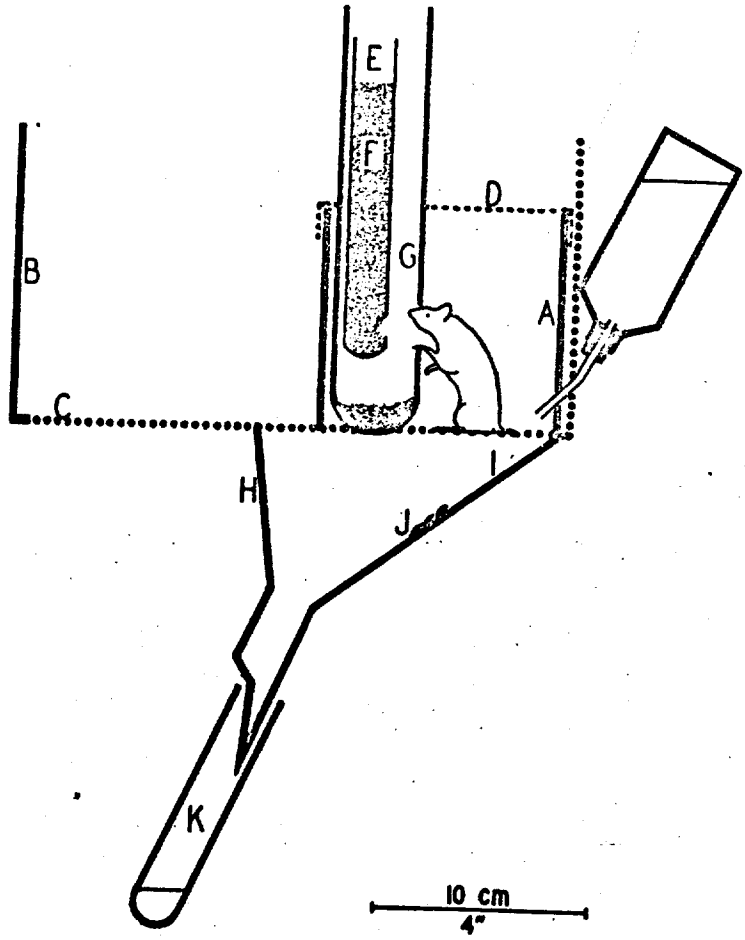

FIG. 1. Mouse cage for urine and feces collection. The cage consists of a cylindrical metal box (A), the bottom of which has been removed. This box is placed in an ordinary metal cage (B) fitted with a wire mesh bottom (C). The top of the box is closed by a wire mesh (D). A glass tube (E) containing the food (F) in powder form is placed in a larger glass tube (G) which collects most of the food spilled. A polyethylene funnel (H), cut obliquely, is placed under the box. A film of silicone grease (I) spread on the surface of the funnel retains the feces (J), hair and the minute amount of food falling down. The urine runs freely into the ashing tube (K) in which 1 ml. 4N Na_2CO_3 had been placed

acid, and chlorine by the method of Wilson and Ball (3). Only yeast contained measurable amounts of sodium, 0.03–0.13%, and of chlorine 0.01%. Therefore, the concentration of these ions in the diet did not exceed 0.013% Na and 0.001% Cl.

The cages were kept in a room where the temperature was maintained at 80° ± 5° F. The room had no window and the artificial light was automatically turned on from 8:00 A.M. to 8:00 P.M.

Most of the experiments were designed to investigate the *acute* effect of sodium chloride on the metabolism of radioiodine. Each mouse received 10 μc. carrierfree I^{131} subcutaneously and was placed in a 250 ml. beaker fitted with a wire mesh floor allowing the urine to settle at the bottom. The animal had access to distilled water but not to food. Two and a half hours later the mouse was anaesthesized with ether in the beaker. Blood, urine (including bladder content) and various organs were homogenized in 2N NaOH solution. Standards were made for each tissue by adding a known amount of radioiodine to the hydrolysate of the corresponding tissue from an animal that had not been given radioiodine. In all cases 1 ml. of the homogenized solution was poured on a watch glass-supported filter disc (lintine discs, 1¼" diameter, obtained from Johnson and Johnson, Montreal), dried at room temperature and counted with a Geiger-Mueller counter. The counts were corrected for background and expressed in percent of the injected dose of radioiodine.

In these acute experiments, the isotope was injected at the same time as the treatment was administered. Prior to the time of treatment, all the animals had been subjected to the same conditions and presumably had the same average level of I^{127} in blood and tissues. Since the same amount of radioiodine was injected into each animal, the specific activity (I^{131}/I^{127}) of the iodide in the body must have been the same in all groups. Thus the I^{131} content of a tissue truly reflected its I^{127} content, and the experimental production of a change in radioiodine content indicates an actual change in I^{127} metabolism.

In an experiment designed to examine the *chronic* effect of dietary sodium chloride on iodine metabolism, some mice were kept in individual "metabolic cages" (Fig. 1) for measurement of urinary and fecal iodine (I^{127}). The design of the food container was such as to minimize the amount of food falling through the wire mesh floor. A plastic funnel coated with iodine-free silicone grease was held under the cage, so that the feces, as well as hair and the minute amount of food falling through, were held by the grease. The urine ran down the funnel walls into a test tube, where it was collected free of feces, hair and food. The feces were removed from the funnel with forceps. The iodine content of thyroid, urine, and feces was estimated according to a modification of the Barker (4) dry ashing method. In this experiment, other mice, used for I^{131} measurement, were kept in non-metabolic cages. These cages, referred to as "ordinary cages," consisted of galvanized iron drawers, 76×14×14 cm., inserted into a rack. The floor and front wall were made of wire mesh. Food was provided in cold-cream jars.

PROCEDURES AND RESULTS

First hypothesis: sodium chloride enhances the output of thyrotrophic hormone by a direct effect on the pituitary

This hypothesis was tested by administering thiouracil—which is known to induce an excessive secretion of thyrotrophic hormone causing an increase in thyroid weight—to two groups of animals fed diets with and without sodium chloride respectively. If the hypothesis were correct, the thyrotrophic hormone output and, therefore, the thyroid weight would be greater in the presence than in the absence of sodium chloride.

Forty mice with a body weight averaging 18–19 gm. were fed on a batch

of the basic diet containing 6.1 μg. iodine per 100 gm. Half of them received 1%, and the other no sodium chloride in the diet. Both groups were divided into two sub-groups, one receiving no additional treatment, the second 0.2% thiouracil mixed in the diet. Twenty-four days later each mouse was sacrificed with ether and the left thyroid lobe was weighed. Thiouracil increased thyroid weight to about the same extent whether or not sodium chloride was administered (from 0.46 to 2.54 mg. without sodium chloride and from 0.76 to 2.80 mg. with sodium chloride). Thus, no evidence was obtained in favor of the hypothesis that the primary effect of sodium chloride is to enhance the secretion of thyrotrophic hormone from the pituitary.

Since an increased amount of thyrotrophic hormone accelerates the release of radioiodine-labelled hormone from the prelabelled thyroid, another approach was to test whether or not sodium chloride had an effect on this release. Forty mice fed on a batch of the basic diet containing 0.6 μg. iodine per 100 gm. for 3 weeks were given a single subcutaneous injection of 10 μc. carrierfree I^{131}. Twenty-four hours later, 30 mg. of sodium chloride were given by intragastric injection and the animals were sacrificed after 10 and 25 hours. The radioiodine content of the prelabelled glands was not significantly different in controls—59.2 and 51.7% of the dose at 10 and 25 hours respectively—and in sodium chloride treated animals—52.9 and 57.8% at 10 and 25 hours respectively. Thus the salt did not influence the release of hormonal I^{131} from the thyroid. These observations again suggested that the primary effect of sodium chloride was not to enhance the release of thyrotrophic hormone from the pituitary.

Second hypothesis: sodium chloride interferes with iodine metabolism in the thyroid in the same manner as antithyroid drugs

The acute effect of sodium chloride on radioiodine metabolism was compared to that of the antithyroid drug, propylthiouracil. Mice were fed on a batch of the basic diet containing 2.1 μg. iodine per 100 gm. for 3 weeks and were then divided into two groups. The first group received 0.2 ml. of water and the second 30 mg. NaCl in 0.2 ml. of water by intragastric injection. Half of the animals in each group had no further treatment, the other half were injected subcutaneously with 2.5 mg. propylthiouracil (5 mg. of 6N-propylthiouracil per ml. in 0.04N NaOH). Simultaneously, all mice received 10 μc. I^{131} subcutaneously. Two and a half hours later they were sacrificed with ether and the radioiodine content of thyroid, blood and urine was measured.

Propylthiouracil, but not sodium chloride, diminished the thyroid I^{131} uptake (Table 1), whereas sodium chloride, but not propylthiouracil, increased the urinary excretion and lowered the I^{131} blood level. Thus, the two substances had an entirely different effect, and it was concluded that sodium chloride did not act as an antithyroid drug. The incidental observation that sodium chloride increased by 20 times the amount of radioiodine

TABLE 1. THE ACUTE EFFECT OF SODIUM CHLORIDE AND PROPYLTHIOURACIL ON RADIOIODINE METABOLISM IN MICE

Propyl-thiouracil injected, mg.	NaCl injected, mg.	Number of animals	% of injected radioiodine in		
			Thyroid*	1 ml. Blood*	Urine*
0	0	7	10.6 ±0.9	4.3 ±0.4	0.5 ±0.2
0	30	7	10.5 ±1.0	*1.9* ±0.4	*11.6* ±2.6
2.5	0	8	*2.6* ±0.3	6.0 ±0.8	0.8 ±0.3
2.5	30	9	*2.0* ±0.3	3.6 ±0.5	*8.3* ±2.9

* Mean ± standard error of the mean.
The italicized values are significantly different from those found in controls injected with water only.

excreted in the urine, while the thyroidal uptake was left unchanged (Table 1) raised the possibility that this salt depleted the body stores of iodine by promoting its urinary excretion. Hence the next hypothesis.

Third hypothesis: sodium chloride increases the excretion of iodine by the kidney and, as a result, deprives the thyroid gland of iodine

The experiment just described was repeated in bilaterally nephrectomized and in thyroidectomized mice. Thyroidectomy was performed according to a modification of the method of Dent, Gadsden and Furth (5). As soon as the operation had been completed, sodium chloride and radioiodine were injected in the same manner as in previous experiments. The results (Table 2) revealed that sodium chloride increased the urinary excretion of radioiodine and decreased the blood radioiodine in thyroidectomized as well as in control animals. Therefore, these two effects of sodium chloride administration were not mediated by the thyroid gland. However, when the kidneys had been removed, the level of radioiodine in blood was no longer affected by sodium chloride, a result indicating that the lowering of the blood radioiodine produced by this salt in intact animals was due to the increased excretion of radioiodine by the kidney.

TABLE 2. THE ACUTE EFFECT OF SODIUM CHLORIDE ON RADIOIODINE METABOLISM IN THYROIDECTOMIZED AND BILATERALLY NEPHRECTOMIZED MICE

Treatment	NaCl injected, mg.	Number of animals	% of injected radioiodine in		
			Thyroid*	1 ml. Blood*	Urine*
Controls	0	7	10.6 ±0.9	4.3 ±0.4	0.5 ±0.2
	30	7	10.5 ±1.0	*1.9* ±0.4	*11.6* ±2.6
Nephrectomy	0	9	10.8 ±2.0	5.3 ±1.1	—
	30	9	11.4 ±2.2	5.9 ±1.2	—
Thyroidectomy			(trachea)†		
	0	10	0.13 ±0.025	10.4 ±0.9	1.7 ±0.3
	30	10	*0.07* ±0.007	*7.3* ±1.0	*11.1* ±3.6

* Mean ± standard error of the mean.
The italicized values are significantly different from those found in animals subjected to the same treatment but receiving no sodium chloride.
† The difference in tracheal I^{131} reflects the difference in blood I^{131}.

More information about the sequence of events was obtained by repeating the experiment and sacrificing the animals at two different time intervals, 3 and 24 hours after radioiodine injection. The urinary excretion of radioiodine was increased ten times after 3 hours and 5 times after 24 hours under the influence of sodium chloride (Table 3). The radioiodine content of the tissues was decreased to a greater extent at 24 hours than at 3 hours after sodium chloride administration. The more pronounced depletion of radioiodine in the blood at 24 than at 3 hours under the influence of sodium chloride clearly indicated that the effect of the salt extended well beyond 3 hours.

The thyroidal uptake of radioiodine was only slightly affected by sodium

TABLE 3. THE ACUTE EFFECT OF SODIUM CHLORIDE ON RADIOIODINE METABOLISM IN MICE, AS MEASURED 3 AND 24 HOURS AFTER ADMINISTRATION

Time interval between radio-iodine injection and sacrifice	NaCl injected mg.	Number of animals	% of injected radioiodine in				
			Thyroid*	1 ml. blood*	Urine*	Gastro-intestine*	Carcass*
3 hours	0	8	16.9±1.4	3.5±0.2	2.0±0.5	28.7±3.0	39.3±1.3
	30	9	13.4±1.2	2.4±0.6	*22.0*±4.6	*13.5*±2.4	35.5±1.8
24 hours	0	8	49.7±2.8	1.5±0.3	8.1±3.8	13.5±2.7	21.3±1.9
	30	9	*28.3*±2.5	*0.3*±0.1	*38.4*±5.8	*4.6*±2.3	17.2±3.0

* Mean ± standard error of the mean.
The italicized values are significantly different from those found in animals sacrificed after the same period of time but receiving no sodium chloride.

chloride at 3 hours ($0.05 > P > 0.1$), but was significantly decreased at 24 hours ($P = 0.001$) to about one half the control value. Thus, the decrease in the radioiodine content of the thyroid appeared at a later time after sodium chloride administration than the increase in urinary iodine excretion.

The immediate increase in radioiodine excretion due to sodium chloride and the later occurrence of a decrease in blood and thyroid radioiodine strongly suggested that sodium chloride increases iodine losses and thus reduces the amount of iodine available to the thyroid. A relative iodine deficiency would thus be created and, as a result, the thyroid would eventually become stimulated.

Thyroid stimulation has actually been observed in animals fed sodium chloride in their diet for many weeks (1); the evidence which has been presented concerning the mechanism of the stimulation was obtained in animals receiving a single injection of the salt. It was, therefore, necessary to determine whether or not *chronic administration of sodium chloride*, under conditions which elicit thyroid stimulation, is associated with the same changes in iodine metabolism as were observed after acute administration

of the salt. Mice were divided into two groups, one fed on a batch of the basic diet containing 2.5 μg. iodine per 100 gm. for 2 months, and the other on the same diet containing 1% sodium chloride. Food and distilled water were given ad libitum. Each group was divided into 6 sub-groups.

Information on I^{127} excretion was obtained in those animals of the first sub-group that were housed in individual metabolic cages (Fig. 1); urine and feces were collected once a week over a 24 hour period and their I^{127} content was measured. The food intake was estimated at intervals. Sixty-three days after the beginning of the experiments, the animals were sacrificed, the weight and the I^{127} content of one lobe of the thyroid was measured, and the other lobe was examined histologically.

Information on I^{131} uptake by the thyroids and excretion in the urine was obtained in the other 5 sub-groups, which were housed in ordinary cages

TABLE 4. THE EFFECT OF DIETARY SODIUM CHLORIDE ON THE THYROID OF MICE FED ON A LOW IODINE DIET FOR 63 DAYS

Housing	% NaCl in diet	Number of animals	Body weight,* gm.	Thyroid weight,* mg.	Thyroid I^{127}* μg.
Metabolic cages†	0	8	27.6 ± 1.5	3.3 ± 0.3	1.06 ± 0.28
	1	8	27.4 ± 0.6	*5.6* ± 0.4	*0.26* ± 0.24
Ordinary cages	0	11	23.1 ± 1.3	2.2 ± 0.3	1.38 ± 0.19
	1	11	22.6 ± 0.9	2.5 ± 0.2	1.33 ± 0.29

* Mean ± standard error of the mean.

† In the animals housed in the metabolic cages, the weight and iodine content of the thyroid have been measured using the left lobe only. The figures obtained were doubled for presentation in this table.

(6–8 animals per cage). These animals were sacrificed after 1, 7, 21, 35 and 63 days respectively. On the evening preceding sacrifice, food was removed from their cages. Two and a half hours before sacrifice, the mice were given access for half an hour to the same diet as before but labelled with 10 μc. I^{131}/gm. diet. After one half hour, during which each animal ate an average of 0.37 gm. of food, it was placed individually in a 250 ml. beaker for urine collection, as in the acute experiments, and was sacrificed 2 hours later. I^{131} was measured in urine and thyroid. In the last sub-group, sacrificed at 63 days, the weight and the I^{127} content of the thyroid were also measured.

The animals kept in metabolic cages, whether fed sodium-chloride or not, had goiters which histologically showed the usual signs of thyroid stimulation. Thyroid weights were higher in the sodium chloride treated than in the control group (Table 4). Histologically, although individual variations were large, there seemed to be on the average less colloid in sodium chloride fed than in control animals. Furthermore, the total iodine content of the thyroid was diminished by sodium chloride (Table 4, last column). The mice receiving sodium chloride excreted more iodine in the urine than controls did (Table 5, Fig. 2), except during the last two weeks,

TABLE 5. THE EFFECT OF DIETARY SODIUM CHLORIDE ON IODINE METABOLISM IN MICE

Days on diet	% NaCl in diet	mμg. I^{127}* excreted daily (mice in metabolic cages)		Radioiodine distribution* (mice in ordinary cages)		mμg. I^{127}† newly accumulated in thyroid
		Urine	Feces	Thyroid I^{131}.100 / Thyr. I^{131} + Ur. I^{131}	Urine I^{131}.100 / Thyr. I^{131} + Ur. I^{131}	
1	0	383 ± 56	952 ± 386	47.7 ± 4.6	52.3 ± 3.6	349
	1	248 ± 60	996 ± 277	*20.3 ± 3.2*	*79.7 ± 3.2*	63
7	0	221 ± 32	233 ± 52	39.4 ± 5.5	60.6 ± 5.5	144
	1	*451 ± 92*	260 ± 26	*13.8 ± 1.5*	*86.2 ± 1.5*	71
14	0	88 ± 17	212 ± 36	—	—	—
	1	108 ± 17	166 ± 37	—	—	—
21	0	44 ± 5	117 ± 16	37.7 ± 4.7	62.3 ± 4.7	27
	1	56 ± 6	120 ± 28	*23.4 ± 3.8*	*76.6 ± 3.8*	17
28	0	12 ± 4	118 ± 7	—	—	—
	1	*32 ± 5*	*83 ± 11*	—	—	—
35	0	19 ± 5	96 ± 12	72.2 ± 5.8	27.8 ± 5.8	49
	1	23 ± 6	*61 ± 7*	*39.3 ± 9.1*	*60.7 ± 9.1*	15
42	0	5 ± 1.7	102 ± 21	—	—	—
	1	13 ± 4.6	71 ± 7	—	—	—
49	0	6.6 ± 2.8	91 ± 21	—	—	—
	1	4.9 ± 2.2	55 ± 9	—	—	—
56	0	4.5 ± 1.3	50 ± 4	—	—	—
	1	4.2 ± 1.6	*66 ± 6*	—	—	—
63	0	6.1 ± 2.6	57 ± 9	88.5 ± 2.6	11.5 ± 2.6	47
	1	3.9 ± 1.7	39 ± 10	*75.1 ± 5.0*	*24.9 ± 5.0*	12

* Mean ± standard error of the mean.

$$\dagger = \text{Urine } I^{127} \cdot \frac{\text{Thyroid } I^{131}/(\text{Thyr. } I^{131} + \text{Ur. } I^{131})}{\text{Urine } I^{131}/(\text{Thyr. } I^{131} + \text{Ur. } I^{131})}$$

The values showing a significant effect of sodium chloride are italicized.

when the amount of iodine excreted was too small to permit significant differences to be detected by the technique. In feces, iodine was excreted in the same amount in the two groups during the first three weeks; but less iodine was found in the feces of sodium chloride fed than of control animals during the next four weeks (Table 5, Fig. 3). At the end of the experiment the total amount of iodine excreted in feces and urine, about 0.06 μg. per day, was close to the amount of iodine ingested, 0.07 ± 0.015 μg. per day (as calculated from the daily food intake, 2.9 ± 0.2 gm., and the iodine content of the diet, 2.5 ± 0.5 μg. per 100 gm.). In the early phase of the experiment the urinary excretion accounted for an important fraction of the total iodine losses; later on this fraction became gradually smaller, and at the end, was only about 10% of the total (Table 5).

The thyroid glands of the mice housed in ordinary cages were not significantly increased in weight by sodium chloride and were, in fact, smaller

than in the mice kept in metabolic cages (Table 4). Furthermore, the mice in ordinary cages lost 0.3 gm. whereas those in metabolic cages gained 3.7 gm. on the average during the course of the experiment. (The uncontrolled factors that interfered with the growth of the mice housed in ordinary cages may also account for the smaller thyroid weights). Nevertheless radioiodine measurements showed that striking differences in iodine metabolism were brought about by sodium chloride. The computation of radioiodine data

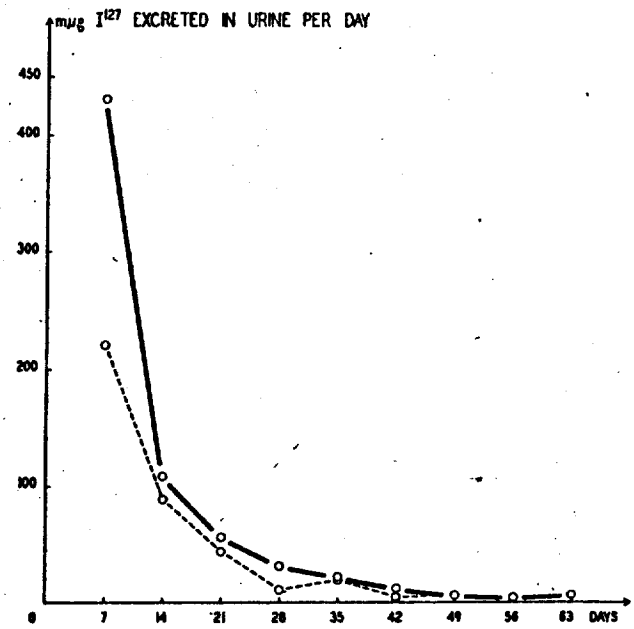

FIG. 2. Effect of dietary sodium chloride on the urinary excretion of iodine in mice fed on a low iodine diet (mice in metabolic cages). Sodium chloride increased the urinary iodine.

required a rather elaborate method since the animals ate variable amounts of I^{131}-labelled food and, as a result, the dose of radioiodine ingested varied from mouse to mouse. Under these conditions, it was unsafe to average the radioiodine contents of tissues or excreta and to compare them in sodium chloride fed and control mice, but it was safe to compare the I^{131} content of the thyroid to that of the urine in any mouse. This was done in each animal by calculating the ratios:

$$\frac{\text{Thyroid } I^{131} \cdot 100}{\text{Thyroid } I^{131} + \text{Urine } I^{131}} \quad \text{and} \quad \frac{\text{Urine } I^{131} \cdot 100}{\text{Thyroid } I^{131} + \text{Urine } I^{131}}.$$

The results (Table 5, Fig. 4) showed that the radioiodine distribution was drastically modified in the animals given sodium chloride over a long period of time. Under the influence of this salt the fraction of the radioiodine lost

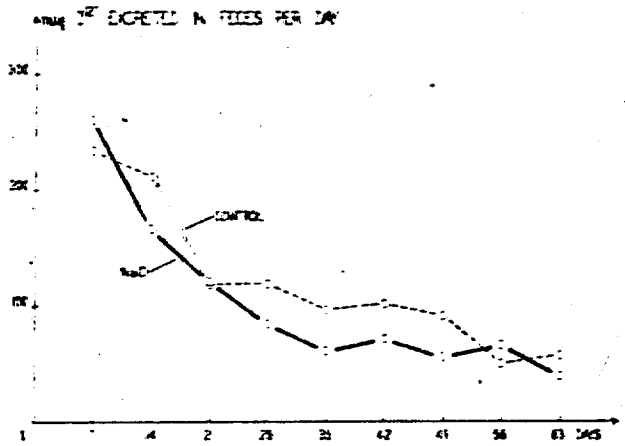

FIG. 3. Effect of dietary sodium chloride on the fecal excretion of iodine in mice fed on a low iodine diet (mice in metabolic cages). Sodium chloride decreased the fecal iodine.

in urine was increased and that taken up by the thyroid was decreased: the proportion retained in the thyroid was cut to one half of the control value at the beginning, but only to five sixths of the control value at the end of the experiment. Apparently, as the thyroid became more active, the effect of sodium chloride on the urinary excretion of iodine decreased.

Since data were available for the relative distribution of iodine between thyroid and urine (mice in ordinary cages, Table 5) as well as the absolute amount of I^{127} excreted daily in the urine (mice in metabolic cages, Table

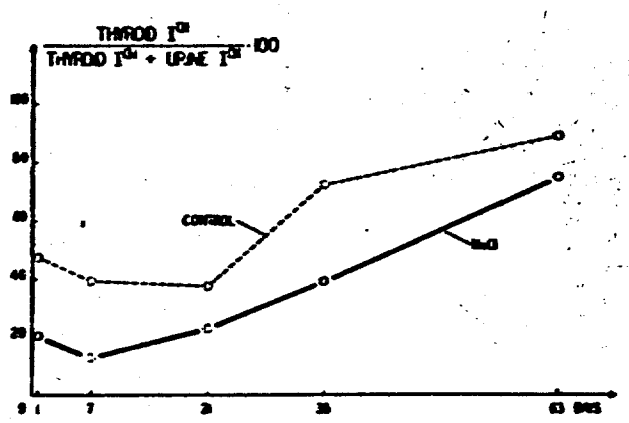

FIG. 4. Effect of dietary sodium chloride on the radioiodine uptake of the thyroid expressed as percentage of the total radioiodide concentrated in both thyroid and urine. (The complement to 100 is lost in the urine.)

If it is assumed that all the iodide in the body ultimately goes either to the thyroid or the urine, the ratio indicates the proportion of the body iodide diverted to the thyroid. It thus appears that the thyroid share of the body iodide is decreased by sodium chloride.

5), it was possible to calculate the amount of I^{127} newly accumulated in the thyroid per day (Table 5, last column). (This calculation assumes that the body inorganic iodine ultimately goes either to the thyroid or into the urine). At the end of the experiment the thyroid weights in the mice used for I^{127} estimation (metabolic cages) were different from those in the mice used for the determination of the relative distribution of iodine (ordinary cages) (Table 4); the thyroid glands must therefore have gradually become different under the two different conditions of housing. Thus only the earlier values in the last column of Table 5 can be considered reliable. Examination of these values shows that the amount of I^{127} accumulated daily in the thyroid was markedly reduced by prolonged ingestion of sodium chloride.

DISCUSSION

The histological picture of hypertrophy, hyperplasia, hyperemia and colloid loss observed in the thyroid gland of the mouse after prolonged ingestion of sodium chloride was identical with that resulting from chronic exposure to cold or to antithyroid drugs. In both of these conditions, it is known that the level of circulating thyrotrophic hormone is raised (6, 7) and in fact, the picture was similar to that obtained soon after injection of thyrotrophic hormone itself. Accordingly, even though no direct measurement of the circulating level of thyrotrophic hormone was made, it was reasonable to conclude that ingestion of sodium chloride ultimately resulted in an increased secretion of this hormone from the pituitary. However, the mechanism by which an over-secretion of thyrotrophic hormone was induced in sodium chloride fed animals remained to be elucidated.

Three hypotheses were examined: 1) a direct effect on the *pituitary* enhancing thyrotrophic hormone release; this possibility was eliminated because, unlike thyrotrophic hormone, sodium chloride did not speed up the discharge of thyroidal iodine, nor did it increase the effect of thiouracil treatment; 2) a direct effect on the *thyroid gland* by which sodium chloride would, like thiourea, inhibit the incorporation of iodine into thyroglobulin, or, like thiocyanate, impair the concentration gradient of iodide between thyroid and blood (8); either possibility should produce an immediate, profound depression of radioiodine uptake (9), but such an effect was not observed (Table 1); 3) the production of a relative iodine deficiency by an increase in the *urinary excretion of iodine*, which was actually shown to happen after *acute* administration of sodium chloride (Tables 1–3). Measurements of the clearance of radioiodide between the 3rd and 24th hour after administration of 30 mg. of sodium chloride[4] revealed that 13 microliters of blood were then cleared per minute, as compared with only 2 microliters in controls. Thus a 6.5 fold increase in the renal clearance of iodide was caused

[4] The radioiodine in blood was assumed to be inorganic only. The clearances were calculated from the radioiodine content of blood and urine at 3 and 24 hours after in-

by the salt (a result in agreement with observations made in the dog, 10). Since the increase in the urinary excretion of radioiodide under the influence of sodium chloride was observed in thyroidectomized animals (Table 2), this phenomenon must have occurred independently of the thyroid gland. Furthermore, the lowering of the blood radioiodide level after sodium chloride administration (Table 1) was not observed in the absence of the kidney (Table 2); it was, therefore, attributed to the urinary loss of radioiodide. Finally, sodium chloride had no significant effect on the radioiodine content of the thyroid measured after 3 hours, but decreased the 24 hour uptake (Table 3). The three hour result was in line with the earlier findings that sodium chloride did not impair the ability of the thyroid to take up iodine (Table 1). Since it was shown earlier than the salt did not influence the release of radioiodine from the gland either, the low thyroid I^{131} at 24 hours could only be explained by a decrease in the availability of radioiodine. The overall sequence of events appears to be that sodium chloride increases the urinary excretion of radioiodine and, as a result, lowers the circulating level of iodide so that less iodide is made available to the thyroid gland.

This conclusion was derived from experiments in which a single, acute administration of sodium chloride was used, but a similar sequence of events was observed in animals *chronically fed on the diet containing 1% sodium chloride*. At the end of the first week, these animals started excreting more iodine (I^{127}) in the urine than did the controls (Fig. 2). Similarly, calculations of the urinary excretion of administered radioiodine[5] indicated

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jection (Table 3) over the interval of 21 hours (1260 minutes) according to the formula:

$$(U_{24} - U_3) \cdot \frac{\ln B_{24} - \ln B_3}{B_{24} - B_3} \cdot \frac{1}{1260} \text{ ml. cleared per minute}$$

$U_3 = I^{131}$ excreted in the urine during the first 3 hours (% of injected dose)
$U_{24} = I^{131}$ excreted in the urine during the first 24 hours (% of injected dose)
$B_3 = I^{131}$ concentration in blood at 3 hours (% of injected dose/ml.)
$B_{24} = I^{131}$ concentration in blood at 24 hours (% of injected dose/ml.)

[5] On the basis of the results reported in Table 3, it was assumed that at the beginning of the experiment the 2 hour radioiodine uptake of the thyroid was the same whether or not the animals ingested sodium chloride. As long as this is the case, it is readily seen that:

$$\frac{\text{Urinary } I^{131} \text{ in NaCl group}}{\text{Urinary } I^{131} \text{ in Control group}} = \frac{U_{NaCl}}{U_{Controls}} \cdot \frac{T_{Controls}}{T_{NaCl}} \qquad (1)$$

in which

$$T = \frac{\text{Thyroid } I^{131}}{\text{Thyroid } I^{131} + \text{Urine } I^{131}} \qquad U = \frac{\text{Urine } I^{131}}{\text{Thyroid } I^{131} + \text{Urine } I^{131}}$$

The formula (1) was used to compare the excretion of radioiodide in sodium chloride fed and control animals, and showed that, after one day treatment, the sodium chloride group excreted 3.6 times more, and after 7 days, 4.0 times more radioiodine than the controls.

a 4-fold increase over that of controls. Presumably the renal clearance had also increased four times, since it is likely that the circulating level of radioiodine was not much affected by sodium chloride under the conditions used. (It was reasoned that since an 11-fold increase in urinary excretion lowered the blood radioiodide by only 30%, as shown in Table 3, the 4-fold increase found here should have had still less effect on the blood radioiodide.)

The plasma level of inorganic iodine (I^{127}) in animals on a low iodine diet was known to become too low for accurate estimation, so that information on the subject had to be obtained by a circuitous route. Although the renal clearance was increased 4 times in the sodium chloride fed animals, the urinary I^{127} was increased only 1.8 times on the average during the first 6 weeks (Table 5). Therefore, the plasma iodide (I^{127}) level in the mice fed the salt diet must have been about half the level in controls. Thus, under chronic conditions too, sodium chloride increased the renal clearance and decreased the blood level of iodide.

In the thyroid gland less iodine was accumulated daily (Table 5, last column) and ultimately less iodine (I^{127}) was found in sodium chloride fed than in control animals (Table 4), a result in agreement with the observation of Remington (11) that, in rats fed a low iodine diet containing 1% sodium chloride, the iodine content of the thyroid gland was 0.8 μg. as compared to 2.5 μg. in animals fed a diet free of the salt. Indeed, the increase in radioiodine excretion and thyroid weight caused by sodium chloride in the mouse was also found to occur in the rat (Isler, unpublished).

A smaller thyroid iodine uptake and content must result in a smaller release of thyroid hormone from the thyroid, and consequently a lower level of circulating hormone in sodium chloride fed than in control animals. An index of the amount of hormone released from the thyroid gland could be derived from the quantity of iodine (I^{127}) excreted via the feces, since the fecal iodine consists almost exclusively of thyroxine and triiodothyronine (12) and its amount is related to the dose of thyroxine administered (13). The smaller amount of fecal iodine found in sodium chloride treated mice (Table 5) suggests that such animals released less thyroid hormone into the circulation than did the controls. The reduced level of this hormone in blood would result in an increased pituitary secretion of thyrotrophic hormone which, in turn, would account for the ultimate increase in thyroid weight.

In conclusion, the data obtained support the concept that the stimulation of the thyroid gland observed after prolonged ingestion of sodium chloride occurs according to the following mechanism:

1) The urinary excretion of iodide is increased, and, as a result, the blood level of iodide is decreased, so that the amount of iodide taken up by the thyroid is reduced.

2) Consequently, the amount of hormone produced and released by the thyroid gland is diminished, and the level of circulating thyroid hormone is reduced.
3) As a result, the pituitary gland increases its secretion of thyrotrophic hormone, which eventually stimulates the thyroid gland.

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J. T. BAKER CHEMICAL CO.

April 4, 1974 1558

150114

Sodium Chloride, USP, Crystal
Comm. No. 3628

Assay (NaCl)	99.5-100.5%
Description, solubility, identification	passes test
acidity or alkalinity	passes test
loss on drying	o.5% max.
Iodide or Bromide	passes test
calcium & magnesium	0.05%
Sulfate (So_4)	0.015% 150 ppm
Sodium Ferrocyanide	0.0013
Arsenic	0.0003% ppm

Potassium Chloride, VSP-FCC
#3046 Crystal of
#3052 Powder

Assay (KCl)	99.0-100.5%
Description, identification. solubility	passes test
acidity or alkalinity	passes test
loss on drying	1% max
Iodide or bromide	passes test
Arsenic	0.0003%
Calcium & Magnesium	passes test
Heavy metals	0.001%
Sodium	passes test

Company Code [illegible]

Sub. No. 0188

PRODUCT LISTING

Comm. No. 3624

Date Issued 8-22-67

Primary Listing: Sodium Chloride, 'Baker Analyzed' REAGENT, Crystal

Secondary Listing:

Formula: NaCl

F W 58.44

Specifications: Meets A. C. S. Specifications

Assay (NaCl)	99.5	%	Calcium, Magnesium and R_2O_3 Precipitate	0.005	%
Insoluble Matter	0.005	%	Heavy Metals (as Pb)	0.0002	%
pH of 5% Solution at 25°C	5.0-7.0		Iron (Fe)	0.0001	%
Iodide (I)	0.002	%	Potassium (K)	0.005	%
Bromide (Br)	0.005	%			
Chlorate and Nitrate (as NO_3)	0.003	%			
Nitrogen Compounds (as N)	0.0005	%			
Phosphate (PO_4)	0.0005	%			
Sulfate (SO_4)	0.001	%			
arium (Ba)	0.001	%			

* Remove Arsenic
GEW/jkk

Company Code [redacted]

Sub. No. 0188

PRODUCT LISTING

Comm. No. 3625

Date Issued 3-17-66

Primary Listing: * Sodium Chloride, C. P., Crystal

Secondary Listing:

Formula: NaCl

F W 58.4428

Specifications:

Assay (NaCl)	99.0	%
Insoluble Matter	0.005	%
pH of 5% Solution at 25°C.	5.0-8.0	
Iodide (I)	0.005	%
Bromide (Br)	0.010	%
Phosphate (PO_4)	0.001	%
Sulfate (SO_4)	0.005	%
Barium (Ba)	0.002	%
Calcium, Magnesium and R_2O_3 Precipitate	0.010	%
Heavy Metals (as Pb)	0.0005	%
Arsenic (As)	0.0004	%

* Change Style Label to show maximum limits of impurities.
GEW/jkk

Biol. Neonat. *11:* 261–272 (1967)

From the Institute for the Care of Mother and Child and from the Institute of Physiology of the Czechoslovak Academy of Sciences, Prague

The Distribution of Sodium, Chloride and Fluid in the Body of Young Infants with Increased Intake of NaCl

M. JANOVSKÝ, J. MARTÍNEK and V. STANINCOVÁ[1]

Water and salt homeostasis concerns the osmolality and volume of extracellular fluid. Osmolality primarily depends on water content, the volume mainly on sodium content (PITTS, 1964). In newborn and older infants both osmo- and volume regulation are realized differently from adults. This is indicated by a difference in the balance and distribution of electrolytes and fluids in the body. Water economy in infants is characterized by a high rate of turn-over of water (GAMBLE, 1958), electrolyte economy by high retention (MCCANCE *et al.*, 1957) and body composition by a large volume of extracellular fluid and a high content of Na and Cl per kg body mass (FRIIS-HANSEN, 1957; FORBES, 1952).

Apparently the regulation of water and salt homeostasis is also less precise than in adults since an osmolar disbalance due to either lack or excess of water in infants is less efficiently compensated for (MCCANCE *et al.*, 1947; HELLER, 1958; BARNETT and VESTERDAL, 1953; VESTERDAL, 1961). MCCANCE and WIDDOWSON (1957) showed that piglets and premature infants fed on milk with added salt retain a considerable part of NaCl and gain excessive weight. The authors assume that water retention leads to expansion of extracellular fluid. This has been confirmed by direct measurement also in full-term infants (MARTÍNEK *et al.*, 1961).

In the present work the distribution of fluid, Na and Cl in the body of young infants given milk with added salt was studied. Some of the results have been presented in a preliminary communication (STANINCOVÁ *et al.*, 1964).

[1] With the technical assistance of V. BALÍKOVÁ and R. BOUČKOVÁ.

Subjects and Methods

Subjects. Six full-term healthy breast fed male infants were examined. Four were studied when aged 14 days, two at the age of 42 days.

Examination. The following general scheme was followed: Infants were weighed at 9 a.m. Their rectal temperature was taken, they were bathed and placed in a balance bed for continuous collection of urine and stools. In 3 sweat was also collected. These were washed 3× with distilled water after the usual bath, and wiped with cloth also soaked in distilled water. All their clothes were boiled in distilled water several times so that no electrolytes were left in them. Urine and stools were collected over 24-h-periods; after each period the infant was washed 3× with distilled water, clothes were changed and eluted in distilled water. All infants were breast fed 6× daily. The rectal temperature was taken at each feeding. Infants were weighed every 12 h (9 a.m. and 9 p.m.). The milk consumed was determined by differential weighing. The first day was the control day with normal breast feeding and normal electrolyte and water balance.

On the next day, after the morning procedure, a sample of venous blood was taken for determination of the dry weight and Na and Cl content of the serum. The concentration of Na, Cl and K was determined in whole blood. Via the same needle a thiosulphate solution was injected. Starting with the 2 p.m. feed infants received 750 mg NaCl for every 100 ml milk consumed. The amount of milk consumed was extrapolated from that drunk on the control day. The salt was dissolved in a small amount of breast milk and given by stomach tube just before breast-feeding. Salt was added until the increase in body weight was at least 170 g. In infants C., S. and M. salt supply was interrupted already on the 2nd day of increased salt intake, in infants K. and R. on the 3rd day. In infant H. salt supply was interrupted at an atypical hour on day 3 since his body temperature increased.

After salt addition was terminated the venous blood was again examined and the thiosulphate distribution space again determined. Urine, stools and sweat were collected for, at least, another day. Details are shown in Table I. The *distribution space of thiosulphate* as an indicator of the volume of extracellular fluid was determined according to FRIIS-HANSEN (1954).

The *Na* and *K concentrations* were determined on a Zeiss III flame photometer in the urine, in the ashes of milk dissolved in 10% HNO_3, in incinerated stools, in evaporated water of the bath and in the evaporated eluate of the clothes and cloths. In the blood the Na and K determinations were performed in H_2SO_4 according to HALD (1947) after deproteinization with picric acid (VAN SLYKE and HILLER, 1947).

Cl concentration was determined in urine, the evaporated water of the bath and clothes, in the serum deproteinised with picric acid, in deproteinised blood filtrate and in the filtrate of incinerated stools by potentiometric titration (SANDERSON, 1952) as modified by DRAHOTA and JELÍNEK (JELÍNEK, 1962).

Osmolarity of breast milk (with and without salt) was determined cryoscopically with a Beckmann thermometer exact to ±5mOsm/l.

Water and electrolyte *balances* were calculated for the 24 h periods (9 a.m. to 9 a.m.) and also for the period between the 1st and 2nd determination of thiosulphate space. The visible water balance was obtained by subtracting the amount of urine from the amount of water consumed in milk. Water content of milk was taken as 87.6% (CLEMENT *et al.*, 1960). For the Na balance the amount of Na in the thiosulphate administered was subtracted. Losses of Na, K and Cl in sweat are small as shown by our results and hence balances in the two infants in which sweat was not collected were calculated without taking these losses into consideration.

Results

Clinical state. No changes were found in the clinical state. Only infant H. showed an increase in rectal temperature to 38°C. The stools were less firm than usual but were passed at normal frequency. Three infants had a slight oedema of the eyelids before the termination of salt application, one showed slight swelling of the subcutaneous region in the ankles and slightly enlarged breasts. The behaviour of both age groups was the same.

The *electrolyte concentration* in milk was 7.0 meq. Na, 12.6 meq. K and 8.5 meq./l Cl. After salt addition this increased to 136 meq. Na and 137 meq. Cl. Average total osmolality was 308 mOsm/l and after salt addition 560 mOsm/l.

Urine flow in all infants was decreased during the period of salt administration. Increased water retention is indicated by the visible water balance (Table I).

The *concentration* of *Na* and *Cl* in the *urine* (U_{Na}, U_{Cl}) was always increased during the period of increased salt intake. With the exception of infant R. U_{Na} on the 2nd and 3rd days of the experiment was always lower than the Na serum concentration. Thus the concentration index of Na was lower than 1.0.

The *K concentration in urine* (U_K) was slightly increased in all infants during the period of increased salt intake, with the exception of infant C.

The consumption of salted milk induced an *increased excretion* of the *total amounts* of *Na* and *Cl* both by the kidneys and via sweat and stools while the total amount of K excreted did not change (Table I). Renal and extrarenal Na and Cl losses were still lower, however, than their simultaneous intake in milk. Hence there was a pronouncedly higher positive balance of Na and Cl during the whole period of increased salt intake (Table I). After termination of salt addition, urine flow was increased, the total amount of Na and Cl excreted was higher than that consumed and the balance became negative (Table I).

The values in Table II show that intake of salted milk *increases body weight* by 185 g in group 1 and by 220 g in group 2. After termination of the experiment body weight again decreased and the weight curve continued about in its original path. The volume of extracellular space was expanded by 174 ml in group 1 and by 189 ml in group 2 on the average. Simultaneously the mean concentration of Na and Cl per kg serum and per litre serum water was also increased and the

Table I. Individual values for water and electrolyte balance

Days	Baby Č. 1	2	3	4	5	Baby H. 1	2	3	4	5	Baby K. 1	2	3	4	5
Milk intake ml	550	495	495	520	560	640	550	550	570	530	510	640	570	580	590
Urine volume ml	260	137	330	315	295	345	139	290	255	375	260	134	330	380	—
U_{Na} meq./l	8.1	95.0	42.6	24.8	15.0	3.7	109.0	80.0	51.0	29.0	4.9	94.0	114.0	54.0	—
U_{Cl} meq./l	15.6	67.0	33.0	29.0	14.1	14.1	106.0	82.0	50.0	35.0	18.9	80.0	116.0	49.0	—
U_K meq./l	6.8	3.1	4.6	6.0	6.5	7.1	28.0	8.6	4.0	7.4	10.7	14.0	7.3	7.3	—
Na intake meq.	3.3	51.7	3.1	3.2	3.4	3.9	60.4	30.1	3.5	3.2	3.1	32.7	65.0	10.9	3.5
Na excretion meq.															
urine	2.1	13.0	14.0	7.8	4.4	1.3	15.2	23.2	13.0	10.8	1.3	10.2	37.6	20.5	—
stools	0.1	1.0	0.7	0.8	0.3	0.1	0.2	0.4	0.2	—	0.1	0.5	0.6	0.3	—
sweat	0.2	1.1	1.3	1.0	0.7	0.2	1.3	1.5	2.0	1.0	—	—	—	—	—
Na balance	+0.9	+36.6	—12.9	—5.4	—2.0	+2.3	+43.7	+5.0	—11.7	—8.7	+1.7	+22.0	+26.8	—9.9	—
Cl intake meq.	4.7	52.9	4.3	4.4	4.8	5.4	62.0	31.2	4.9	4.5	4.4	33.5	66.4	11.1	5.0
Cl excretion meq.															
urine	4.1	9.1	12.2	10.4	8.0	4.9	14.7	23.8	12.8	13.1	4.9	10.7	38.4	18.6	—
stools	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
sweat	0.1	0.8	0.8	0.7	0.4	0.2	1.0	1.3	1.6	0.8	—	—	—	—	—
Cl balance	+0.5	+43.0	—8.7	—6.7	—3.6	+0.3	+46.3	+6.1	—9.5	—9.4	—0.5	+22.8	+28.0	—7.5	—
K intake meq.	6.9	6.3	6.3	6.5	7.0	8.1	7.1	5.7	7.2	6.7	6.4	7.7	7.2	7.2	7.3
K excretion meq.															
urine	1.8	0.4	1.5	1.9	1.9	2.4	3.9	2.6	1.0	2.8	2.8	1.9	2.4	2.8	—
stools	0.2	0.2	0.4	0.1	0.2	0.2	0.6	0.4	0.3	0.5	0.2	0.4	0.3	0.3	—
sweat	0.05	0.1	0.15	0.15	0.1	0.1	0.3	0.5	0.6	—	—	—	—	—	—
K balance	+4.85	+5.6	+4.3	+4.4	+4.8	+5.4	+2.3	+2.2	+5.3	+3.5	+3.4	+5.4	+4.5	+4.1	—
Visible water balance ml	+221	+297	+104	+141	+196	+216	+343	+192	+244	+89	+188	+427	+170	+128	—
C_{Na} ml/1.73 m²	0.08	0.51	0.55	0.31	0.18	0.05	0.60	0.91	0.55	0.45	0.05	0.41	1.51	0.84	—

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(*Table I.* Continued)

Days	Baby R. 1	2	3	4	5	Baby M. 1	2	3	4	Baby Š. 1	2	3	4	5
Milk intake ml	530	530	550	500	570	615	690	760	750	800	890	800	870	890
Urine volume ml	325	175	225	425	350	347	255	350	370	370	280	472	450	400
U_{Na} meq./l	6.0	172.0	290.0	65.0	38.6	6.0	65.4	43.5	28.6	11.4	129.5	49.7	40.0	28.2
U_{Cl} meq./l	9.0	120.0	285.0	66.5	38.0	6.1	40.4	39.8	26.2	17.5	117.5	53.2	44.0	50.4
U_K meq./l	9.0	32.0	40.0	12.8	10.5	16.4	38.0	13.1	24.0	10.9	17.8	14.5	9.9	22.4
Na intake meq.	3.2	67.3	67.3	3.0	3.5	3.7	72.6	4.6	6.6	4.9	91.6	4.9	5.3	5.4
Na excretion meq.														
urine	1.0	30.1	65.3	27.6	13.5	2.1	16.7	15.9	10.6	4.2	36.3	23.5	18.0	11.3
stools	0.1	0.2	0.4	0.2	0.3	0.04	0.1	0.1	0.07	0.08	0.3	0.3	0.4	0.2
sweat	—	—	—	—	—	0.2	1.0	0.1	0.5	—	—	—	—	—
Na balance meq.	+1.1	+37.0	+1.6	—24.8	—10.3	+1.46	+54.8	+11.5	—6.6	—0.6	+55.0	—18.9	—13.1	—5.9
Cl intake meq.	4.5	68.6	68.6	4.2	5.0	5.2	74.3	6.4	6.4	6.8	93.9	6.8	7.4	9.6
Cl excretion														
urine	2.9	21.0	64.1	28.3	13.3	2.1	10.3	13.9	9.7	6.5	32.9	25.1	19.8	20.2
stools	—	—	—	—	—	—	—	—	—	—	—	—	—	—
sweat	—	—	—	—	—	0.3	0.9	0.2	0.3	—	—	—	—	—
Cl balance	+1.6	+47.6	+4.5	—24.1	—8.3	+2.8	+63.1	—7.7	—3.6	+0.3	+61.0	—18.3	—12.4	—10.6
K intake	6.7	6.7	6.9	6.3	7.2	7.7	8.8	9.6	9.6	10.1	10.7	10.1	10.5	10.7
K excretion														
urine	2.9	5.6	9.0	5.4	3.7	5.7	9.7	4.6	8.9	4.0	5.0	6.8	4.5	9.0
stools	0.2	0.3	1.1	0.6	0.7	0.1	0.1	0.1	0.1	0.2	0.4	0.4	0.2	0.3
sweat	—	—	—	—	—	0.03	0.1	0.2	0.1	—	—	—	—	—
K balance	+3.6	+0.8	—3.2	—0.3	+2.8	+1.87	—1.1	+4.7	+0.5	+5.9	+5.3	+2.9	+6.0	+1.4
Visible water balance ml	+139	+289	+256	—13.0	—149	+192	+349	+316	+287	+331	+500	+229	+312	+380
C_{Na} ml/1.73 m²	0.04	1.21	2.62	1.1	0.53	0.07	0.59	0.56	0.38	0.16	1.35	0.89	0.68	0.43

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Table II. The effect of increased intake of NaCl on body weight and extracellular fluid/ECF/volume

Infant	Č.	H.	K.	R.	M.	Š.	Mean 15 days	Mean 42 days	Mean of all infants
Age in days	15	15	15	15	42	42			
Body weight 1 (grams)	3.000	3.200	3.330	3.220	4.340	4.500	3.187	4.420	3.598
Body weight 2	3.170	3.370	3.500	3.450	4.510	4.470	3.372	4.640	3.795
ECF_1 volume in % of body weight	42.6	43.5	39.7	41.2	38.8	38.6	41.7	38.7	40.7
ECF_2 volume in % of body weight	45.0	46.2	42.0	44.3	40.3	41.4	44.4	40.8	43.2
Increment of body weight in grams	170	170	170	230	170	270	185	220	197
Increment of ECF in ml	150	170	176	201	136	242	174	189	179
Period of increased NaCl intake (ECF_1–ECF_2) in hours	24	27.5	48	48	24	24			
Na and Cl serum concentration									
Na_1 meq./kg serum	142.1	136.0	140.0	144.1	141.0	132.0	140.5	136.5	139.2
Na_1 meq./l serum H_2O	152.4	145.6	152.0	154.0	151.8	141.6	151.0	146.7	149.5
Na_2 meq./kg serum	144.3	145.0	142.0	142.0	141.7	134.3	143.3	138.0	141.5
Na_2 meq./l serum H_2O	153.6	153.8	152.5	150.2	151.7	142.9	152.5	147.3	150.7
Cl_1 meq./kg serum	114.4	110.6	107.2	109.8	110.0	104.5	109.7	107.3	108.9
Cl_1 meq./l serum H_2O	119.5	118.4	116.4	117.0	118.5	113.2	117.8	115.9	117.2
Cl_2 meq./kg serum	112.1	119.1	109.0	109.2	112.0	104.5	112.3	108.3	110.9
Cl_2 meq./l serum H_2O	119.4	126.4	117.1	115.5	119.9	111.8	119.6	115.8	118.4
Serum dry weight$_1$	6.76	6.61	7.90	6.23	7.16	6.76	6.87	6.96	6.90
Serum dry weight$_2$	6.10	5.78	6.91	5.50	6.60	6.0	6.07	6.30	6.15
Total ECF_1 Na_1 meq.	194.8	200.9	196.7	200.4	255.5	245.7	199.2	250.6	216.3
Total ECF_2 Na_2 meq.	219.3	238.4	224.2	229.5	275.9	282.5	227.9	279.2	245.6

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amount of dry substance decreased. For calculating the total amount of Na and Cl dissolved in the extracellular fluid values per litre serum water were used. Since both this concentration and the volume of ECF were increased during the period of increased salt intake the actually retained amounts of Na and Cl in the body were also raised. Younger infants retained a mean of 57.7% of Na consumed and 63.0% of Cl, older infants 67.8 and 75.0% respectively (Table III). The amount retained in the body determined by calculating the electrolyte balances was higher than the amount disolved in the expanded ECF. In 14-day-old infants the part disolved in the ECF was 57.0% Na and 40.2% Cl of the total retained in the body. In 42-day-old infants these figures were 51.0% Na and 34.0% Cl. The remainder must thus be outside the ECF. Part of the Na and Cl enter the erythrocytes (Fig. 1). The K concentration in the blood does not change.

Discussion

An analysis of the above results may be limited by the following factors: (1) the possibility to determine exactly the retention of electrolytes from their external balance and (2) the possibility to determine the volume of ECF. Total electrolyte losses should include losses by perspiration. Even with the most careful collection we must expect figures to be somewhat smaller than actual losses. Since most of the excreted electrolytes appear in the urine, sweat losses are not a very important factor. In ECF volume determinations the question arises which substance is distributed in such a way as to give an optimum picture of this volume. In young infants the distribution spaces of Na, Cl, thiocyanate, thiosulphate and inulin are about the same (Kerpel-Fronius, 1961). At what age they begin to differ, evidently because of increased connective tissue appearance, is not known. Hence the distribution space of thiosulphate may be taken as the volume of ECF to the same approximation as that of any other substance.

Since the kidneys cannot rapidly excrete excess Na and Cl these are retained together with water. Changes occur in the volume and the osmolality of the ECF. Considerable increases in the Na and Cl concentrations in ECF are prevented by (1) an expansion of the volume of ECF and (2) by a shift of part of the retained ions out of the ECF. The expansion of ECF cannot be due only to a shift of intracellular water during temporarily increased osmolality of ECF, the main cause

Table III. The distribution of Na and Cl in the body

Infant	Č.	H.	K.	R.	M.	Š	Mean 15 days	Mean 42 days	Mean of all infants
Sodium intake total meq. in period ECF_1 to ECF_2	51.7	82.6	108.6	134.7	72.6	91.6	95.2	82.1	90.8
Sodium excretion total meq. in period ECF_1 to ECF_2	15.1	20.3	48.9	96.0	17.8	36.6	45.1	27.2	39.1
Na retention in the period ECF_1—ECF_2 in meq.	36.6	65.8	59.7	38.7	54.8	55.0	50.2	64.9	51.8
in % of Na intake	70.8	76.4	55.0	28.7	75.5	60.0	57.7	67.8	59.4
Na retained within ECF space in meq.	24.5	37.5	27.5	25.1	20.4	36.8	28.7	28.6	28.6
in % of total Na retained	62.0	55.7	45.9	64.5	36.5	66.5	57.0	51.5	55.2
Na retained outside ECF space in meq.	15.0	29.8	32.4	13.8	35.5	18.2	22.8	26.9	24.5
in % of total Na retained	38.0	44.3	54.1	35.5	63.5	33.5	43.0	44.8	44.8
Cl intake total in meq. in period ECF_1 to ECF_2	52.9	87.6	110.0	137.2	74.3	93.9	97.2	84.1	92.6
Cl excretion total in meq. in period ECF_1 to ECF_2	9.9	19.1	49.1	85.1	11.2	32.9	40.8	22.1	34.5
Cl retention in the period ECF_1—ECF_2 in meq.	43.0	68.5	61.9	52.1	63.1	61.0	56.4	62.1	58.3
in % of Cl intake	81.2	78.2	55.8	38.0	84.9	65.0	63.3	74.0	67.2
Cl retained within ECF space in meq.	17.7	32.5	21.5	21.2	18.7	24.6	23.2	21.7	22.7
in % of total Cl retained	39.7	46.9	33.6	40.8	29.2	40.3	40.2	34.8	38.5
Cl retained outside ECF space in meq.	26.9	37.1	42.5	30.7	45.3	36.4	35.0	40.9	36.9
in % of total Cl retained	60.3	53.1	66.4	59.2	70.8	59.7	59.8	65.3	61.5

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is water retention. This is indicated by simultaneously occurring expansion of the ECF, increase in body weight and decrease in urine flow. Less water, than Na and Cl, however, is retained. If they were retained equally the ECF volume would have to expand by 375 ml for Na isotonicity to be maintained and by 430 ml for Cl isotonicity to be maintained in 14-day-old infants. The inability of the kidneys to excrete an osmolar load is thus partly compensated for by deposition of electrolytes without water. Hence nearly half the Na and more than half the Cl are deposited outside the ECF. More Cl than Na is thus deposited and thus the difference in concentration between the two in the ECF is maintained. Part of the Na and Cl evidently enter the cells (Fig. 1, red blood cells).

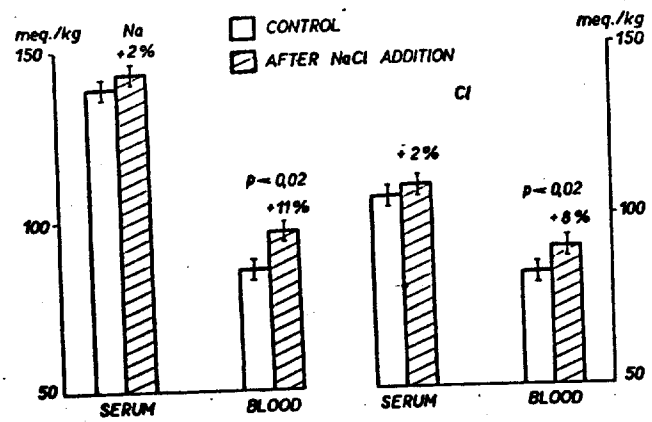

Fig. 1. Sodium and chloride concentrations in serum and whole blood in all infants (mean values and S.D.).

Temporal relationships are difficult to study in infants. In 10-day-old rats fluids are mainly transferred into the gut and the ECF is expanded temporarily only in 23–33 day-old rats (JELÍNEK, 1963).

What are the reasons for the insufficient osmoregulatory function of the kidneys? In adults osmotic diuresis occurs if the tubular concentration of electrolytes exceeds a certain limit. The new-born kidney, however, does not react even to hypertonic NaCl or urea with osmotic diuresis as effectively as the adult (DEAN and MCCANCE, 1949). Evidently this is also due to the fact that the hypertonic solution does not increase the glomerular filtration rate and thus the tubular load as much as in adults. In our experiments urine flow, on the contrary, is

decreased during the salt load. The stimuli for osmotic diuresis, of course, are different from those of hypertonic urea or NaCl. The total osmolality of milk with added salt is a hypertonic solution. The large retention of minerals and protein nitrogen, however, decrease the total osmotic load and thus conditions for osmotic diuresis are not present. The decreased urine flow may be due to: (1) low glomerular filtration rate, (2) increased water reabsorption, (3) a combination of both. The glomerular filtration rate on feeding milk with added salt is increased by 30% (MARTÍNEK *et al.*, 1963). Thus the tubular load is also increased and thus the clearance of Na (Table I). This, however, does not suffice to prevent Na retention in the body. Thus a fall in the glomerular filtration rate is not the cause of the decreased urine flow, which may hence be due to increased reabsorption of water, regulated by ADH. Sufficiently effective stimuli for its release, however, do not seem to be present in this situation. The Na serum concentration is increased only slightly and the expansion of ECF volume should rather depress ADH secretion. In addition ADH activity in the plasma in infants younger than 2.5 months is rarely found even in response to strong osmolar stimuli (JANOVSKÝ *et al.*, 1965). Perhaps the real level of ADH in the plasma is so small that it cannot be titrated biologically, yet may be effective physiologically (DLOUHÁ, 1966). Evidently this problem requires further studies.

A breast fed infant retains about 60% of the consumed Na in its first weeks of life (JANOVSKÝ *et al.*, 1963; STANINCOVÁ, 1966). Surprisingly enough infants fed on salted breast milk retain the same percentage (Table III). This might be due to the intrarenal balance of Na which might be 'set' to ensure high retention. The regulation of this balance would then be less sensitive to stimuli from the ECF and would react later.

The literature does not agree on K excretion during excessive Na intake. LEAF *et al.* (1949) consider the anion accompanying Na as decisive. GAMBLE *et al.* (1952) observed that NaCl and $NaHCO_3$ increase K excretion. MCCANCE and WIDDOWSON (1957) also found a slight rise in the K excretion after NaCl administration. In our experiments K excretion was not increased which agrees with the fact that there is no significant shift of the ICF into ECF and that Na entry into cells is not accompanied by an exchange for K as shown by the fact that the K content of red blood cells remains unchanged.

Our results show that osmoregulation in young infants is imperfect since both the volume and the osmolality of the ECF are changed and

since a large amount of the excess electrolytes consumed is stored outside the ECF.

Summary

The distribution of fluid, sodium and chloride in the body of young infants (4 newborns 14 days old, 2 infants 42 days old) given milk with added NaCl was studied by means of the balance method.

The urine flow after the salt addition was decreased, the excretion of Na and Cl in urine, stool and sweat increased. The gain of body weight simultaneously with the expansion of extracellular volume (by repeated estimation of thiosulphate distribution) was observed.

The retention of Na and Cl in the body (estimated by their balance) being higher than their retention in extracellular fluid shows that the remainder must be retained outside the ECF.

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Antidiuretic Activity in the Plasma of Human Infants After a Load of Sodium Chloride

by M. JANOVSKÝ, J. MARTÍNEK, V. STANINCOVÁ[1]

It is known that under comparable conditions of dehydration newborn and infant animals are unable to form urine of as high an osmotic concentration as adult animals [8, 17]. The difference in concentration capacity in the young and in the adult is more pronounced the less mature the newborn. This low concentration capacity has also been described for human infants, and it has been stated that the mean maximum limits of osmolarity in children in the first weeks after birth are about 800 mOsm/L, which means that they only slightly exceed half the level of urine osmolarity in adults [9, 18]. It is believed that functional and structural immaturity of the hypothalamo-neurohypophyseal system producing antidiuretic hormone (ADH), and insufficient sensitivity of the distal portions of the nephron to ADH are involved. The level of antidiuretic activity (ADA) in the neurohypophyses of newborn rats and puppies is very low [2, 7]. Even in newborn children the ADA content in the neurohypophysis does not reach the level of adults [10]. In addition, the findings of Rodeck [25, 26] indicate undeveloped neurosecretion in the hypothalamus of newborn children. Under normal hydration ADA cannot be identified in the plasma of infant rat before the 23rd day of life [3], and in puppies not before the eleventh day of life [2]. Under dehydration or after a NaCl load ADA can be detected in the plasma already in 10-day-old rats and 5-day-old puppies [2, 4]. Heller & Hradcová [11] have found ADA in the plasma of normally hydrated children only at the beginning of the 5th month.

In the present work it was enquired whether, after an osmolar stimulus, ADA can be detected in the plasma of even younger infants. Part of the findings has been published as a preliminary communication [14, 20].

Methods

Disposal of an NaCl load

NaCl (0.7 g/kg body weight dissolved in two thirds of the child's normal milk intake) was administered to 50 mature children between the ages of 16 days and 6 months. The NaCl dose was chosen in such a way as not to allow the relative water deficit resulting from administration of a hypertonic solution into the gastrointestinal tract to exceed 5% of the body weight. Close be-

[1] Technical assistance V. Balíková and R. Bouřková.

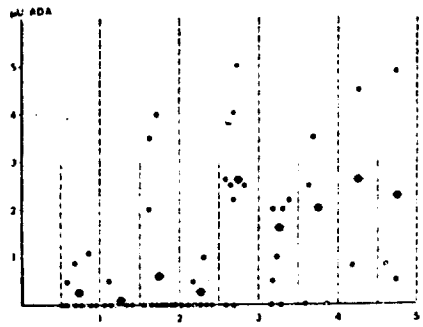

Fig. 1. Antidiuretic activity (ADA) in the plasma of infants before and after administration of a NaCl load. *Abscissa*: Age of infants in months. Months (long vertical lines) divided into 14-day sections (shorter lines). *Ordinate*: ADA in μU of vasopressin 1 ml of plasma. Individual values before administration of NaCl: ● individual values 45 minutes after administration of NaCl; -●- means of individual values in 14-day sections.

fore, and 45 minutes after administration of this osmotic load samples of blood were as a rule taken from some veins in the head into heparinized test tubes, and ADA was determined in the plasma. The concentration of sodium and chlorides in the plasma of some of these infants was also determined. To a further 13 infants aged from 16 days to 5 months a NaCl load was applied in precisely the same way as described above: just before, and 5, 10, 20, 30 and 45 minutes after administration of milk enriched with salt. blood was collected into non-heparinized test-tubes, and the Na and Cl concentration, density and dry substance of the serum were determined. In 7 children of this group all values were determined up to 60 and 120 minutes after administration of the NaCl load. These determinations were made with a group of children different from those used for ADA titration in order to avoid greater losses of blood and repeated nociceptive stimuli.

Chemical determinations

The Na concentration in the plasma and in the serum was determined by means of a flame photometer Zeiss III.

The Cl concentration was determined by potentiometric titration according to Sanderson [27] in the modification of Drahota & Jelínek [5].

Density of the serum was ascertained in 5 decimal fractions in gradient tubes in a mixture of brombenzene and kerosene prepared according to Lowry & Hunter [19].

The dry substance was obtained by drying a sample of the serum to constant weight at 105°C.

Antidiuretic activity (ADA) determinations

ADA in the plasma was determined in hydrated rats under alcohol anesthesia according to Heller et al. [12]. One ml of plasma was injected into the tail vein of an adult rat and the percentage of antidiuresis was compared with the percentage of antidiuresis provoked by intravenous injection of vasopressin Parke-Davis used as a standard preparation. A single lot of pitressin was used throughout.

The statistical significance of the results was estimated by means of Student's *T*-test.

Results

ADA in the plasma

In normally hydrated infants ADA was first demonstrated at an age of 4½ months (Fig. 1, Table 1). At this time, ADA is low,

TABLE 1. *ADA 45 minutes after administration of NaCl in μU of vasopressin/1 ml of plasma and concentration of Na in the plasma before (Na_0) and 45 minutes after administration of NaCl (Na_1).*

No.	Name	Age	ADA μU/ml plasma	Na_0 mEq/L plasma	Na_1 mEq/L plasma
1	R.	20 d.	0.0	141.2	146.0
2	P.	22 d.	0.0	138.1	151.0
3	Pe.	22 d.	0.5	140.2	151.1
4	K.	24 d.	0.0		
5	M.	24 d.	0.0		

Table 1 (*continued*)

No.	Name	Age	ADA μU/ml plasma	Na_e mEq/L plasma	Na_i mEq/L plasma
6	B.	26 d.	0.9		
7	S.	28 d.	0.0		
8	Ro.	30 d.	0.0		
9	Mo.	30 d.	1.1	135.0	145.5
10	Sa.	1 mo. 3 d.	0.5		
11	H.	1 mo. 6 d.	0.0		
12	Z.	1 mo. 9 d.	0.0	138.0	141.0
13	P.	1 mo. 12 d.	0.0	137.5	143.5
14	Pb.	1 mo. 12 d.	0.0		
15	Zd.	1 mo. 15 d.	4.0	141.0	157.0
16	Ki.	1 mo. 18 d.	0.0		
17	Pr.	1 mo. 19 d.	0.0		
18	Sc.	1 mo. 20 d.	2.0		
19	Mo.	1 mo. 21 d.	0.0		
20	B.	1 mo. 22 d.	0.0		
21	Zi.	1 mo. 22 d.	0.0		
22	W.	2 mo.	0.0		
23	Mr.	2 mo.	0.0		
24	Pi.	2 mo. 2 d.	0.0		
25	Pz.	2 mo. 3 d.	0.0		
26	Po.	2 mo. 12 d.	0.0		
27	Pe.	2 mo. 3 d.	0.0		
28	Zd.	2 mo. 7 d.	0.5		
29	J.	2 mo. 15 d.	1.0		
30	Sm.	2 mo. 15 d.	0.0		
31	Ja.	2 mo. 16 d.	5.0		
32	Jo.	2 mo. 19 d.	2.5		
33	Jh.	2 mo. 19 d.	2.3		
34	B.	2 mo. 23 d.	2.5		
35	Ha.	2 mo. 23 d.	4.0		
36	Mc.	2 mo. 27 d.	2.6		
37	Bo.	2 mo. 29 d.	0.0		
38	L.	3 mo.	0.0		
39	Ku.	3 mo. 1 d.	0.0		
40	Ka.	3 mo. 2 d.	0.5		
41	No.	3 mo. 5 d.	1.0	139.0	143.0
42	K.	3 mo. 4 d.	2.0	137.6	148.5
43	A.	3 mo. 11 d.	2.0		
44	Ju.	3 mo. 15 d.	2.3		
45	N.	3 mo. 20 d.	0.0		
46	Mk.	3 mo. 21 d.	2.5	141.0	141.5
47	V.	3 mo. 21 d.	3.5		
48	I.	4 mo. 8 d.	0.8	135.5	146.5
49	Tr.	4 mo. 13 d.	4.5	145.2	155.0
50	Sa.	4 mo. 26 d.	0.5		
51	So.	4 mo. 26 d.	4.0		

attaining a value of about 1 μU/1 ml of plasma. After a NaCl load the presence of ADA can be established only quite exceptionally in infants younger than 2½ months; but ADA appears under these conditions regularly in infants older than 2½ months (Fig. 1, Table 1). The mean of 6 individual values of the Na concentration in the plasma showed a rise by 11.2 mEq/L in 2½-month-old infants, and by 9.2 mEq/L in infants older than 2½ months (Table 1).

Concentration of Na and Cl, density and dry substance values of serum

The values given in Table 2 show that up to 20 minutes following a NaCl load a rise in Na and Cl concentration in the serum is seen in all cases. This increase of electrolyte content persists for 45 minutes in all cases, i.e. until the blood for titration of ADA in the plasma was taken from the second group of infants. With the exception of one case (C.) the concentration remained elevated for 120 minutes. The values of Na and Cl concentration in the serum collected in the 45th minute are

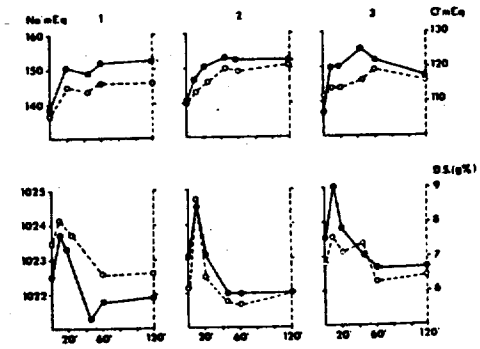

Fig. 2. Concentration of Na and Cl in the serum, density and dry substance of serum in infants before and after administration of NaCl. Infant 16 days old (1), 2 months and 8 days old (2), 4 months and 14 days old (3). *Abscissae*: Time in minutes after administration of NaCl. *Top*: (Ordinate at the left): Concentration of Na in mEq/L of serum ●—●; (Ordinate at right): concentration of Cl in mEq/L of serum ○---○. *Bottom*: (Ordinate at the left): density of serum ●—●; (Ordinate at right): dry substance of serum ○---○.

TABLE 2. *Concentration of Na and Cl before and after administration of NaCl in the serum. Indexes of serum concentration after administration of NaCl: 1 = 5 minutes, 2 = 20 minutes, 3 = 30 minutes, 4 = 45 minutes, 5 = 60 minutes after administration of NaCl, 0 = before administration of NaCl.*

No.	Name	Age (days)	Na_0	Na_1	Na_2	Na_3	Na_4	Na_5	Cl_0	Cl_1	Cl_2	Cl_3	Cl_4	Cl_5
			mEq/L						mEq/L					
1	Ra.	16	137.5	137.5	145.3	144.0	146.5	146.5	108.6	111.7	120.9	119.4	122.4	[illegible]
2	C.	18	143.5	154.0	14.50	146.8	145.5	140.0	107.0	110.1	—	116.2	113.0	[illegible]
3	F.	18	140.3	140.0	143.0	143.0	144.1	143.6	102.4	103.2	107.8	110.8	113.9	[illegible]
4	P.	34	139.3	137.3	141.0	—	145.3	149.5	110.9	103.2	117.8	—	116.3	[illegible]
5	H.	68	140.5	147.0	150.5	153.0	153.0	154.0	110.3	113.2	—	120.5	119.1	[illegible]
6	R.	90	139.6	140.5	—	143.0	—	—	100.0	107.6	—	112.1	—	[illegible]
7	W.	111	139.5	150.0	153.0	152.5	153.0	—	108.6	117.0	121.7	122.4	—	[illegible]
8	Pi.	134	136.5	146.0	150.0	152.0	152.0	147.5	105.7	114.7	115.8	116.1	119.0	[illegible]
9	S.	155	139.0	141.6	—	149.6	—	—	102.9	105.8	114.2	111.3	—	[illegible]
10	Wi.	164	139.0	145.0	138.6	142.2	—	—	104.2	107.1	100.0	105.7	—	[illegible]
11	Z.	164	141.0	141.0	—	146.5	—	—	103.0	104.5	—	107.6	—	[illegible]
12	M.	180	139.5	137.2	149.0	153.0	—	—	110.1	114.0	119.7	120.9	—	[illegible]
13	Zh.	224	141.4	138.6	—	149.4	—	—	100.0	101.4	—	112.8	—	[illegible]

higher, on the average, by 10.0 mEq and 8.2 mEq/L respectively than the initial values (Table 2).

The density of the serum rises within 5–10 minutes after NaCl application by 0.00112 on the average; after 120 minutes there is a return to initial values, and later a decrease. Similar changes can be found in the dry substance (Table 3). Typical changes in 3 infants of different age can be seen in Fig. 2. It follows that in the first stage after administration of a NaCl load a concentration of the plasma takes place, while, in the second, a dilution occurs.

TABLE 3. *Density (D) and dry substance (D. W.) of serum before and after administration of NaCl. The same indexes as in Table 2.*

No.	Name	Age (days)	D_0	D_1	D_2	D_3	D_4	D_5	$D.W._0$	$D.W._1$	$D.W._2$	$D.W._3$	$D.W._4$	$D.W._5$
									g/100 ml					
1	Ra.	16	1.02244	1.02373	1.02325	1.02126	1.02173	1.02190	6.37	7.15	6.93	6.07	5.56	[illegible]
2	C.	18	1.02297	1.02507	1.02385	1.02396	1.02332	1.02366	6.82	7.30	6.31	6.09	7.20	[illegible]
3	F.	18	1.02500	1.02540	1.02570	1.02460	1.02460	1.02450	6.69	7.06	7.47	7.49	6.51	[illegible]
4	P.	34	1.02286	1.02335	1.02218	1.02170	1.02088	1.02254	5.29	6.32	6.23	5.96	6.37	—
5	H.	68	1.02308	1.02560	1.02350	1.02194	1.02194	1.02194	6.10	8.75	6.48	5.77	5.65	[illegible]
6	R.	90	1.02436	1.02560	—	1.02467	1.02400	1.02400	8.12	8.90	—	8.29	8.15	[illegible]
7	W.	111	1.02435	1.02554	1.02435	1.02366	1.02337	1.02337	7.61	8.16	7.55	7.15	7.08	[illegible]
8	Pi.	134	1.02356	1.02514	1.02372	1.02300	1.02260	1.02260	6.75	7.54	6.65	6.93	—	—
9	S.	155	1.02366	1.02511	1.02414	1.02464	—	—	7.16	8.08	7.73	7.39	—	—
10	Wi.	164	1.02510	1.02580	1.02540	1.02444	—	—	8.33	7.94	8.28	7.97	—	—
11	Z.	164	1.02344	1.02458	—	1.02339	—	—	7.92	8.33	—	7.65	—	—
12	M.	180	1.02484	1.02536	1.02620	1.02550	—	—	7.15	5.96	7.59	8.71	—	—
13	Zh.	224	1.02528	1.02546	—	1.02546	—	—	8.64	8.30	—	9.14	—	—

Discussion

The present investigation was rendered possible by the use of a very sensitive method which, according to the authors, should allow the determination of ADA corresponding to 0.625 μU of vasopressin. But this method does not admit of a continual tracing of ADA so that in our considerations we have to limit ourselves to the interpretation of two fixed values. ADA appears in the plasma of normally hydrated infants at 4½ months at the earliest, as found by Heller & Hradcová [11] and in this work. After a NaCl load in infants, on the other hand, the presence of ADA can be demonstrated already in the second half of the 3rd month; in younger infants only quite exceptionally.

Changes in the concentration of Na, Cl, of density, and of serum dry substance have shown that the time course of osmotic and volume stimuli, which affect the release of ADH from the neurohypophysis, is the same in all the investigated groups. The changes in dry matter content of the serum may possibly be due to a shift of body fluids into the gastrointestinal tract where a dilution of the hypertonic solution takes place with subsequent absorption. The intensity of osmotic or volume stimuli being equal in all groups, the absence of ADA in most infants up to 2½ months is caused neither by delayed absorption nor by a different distribution of electrolytes in body fluids.

Assuming that the ADA level in the plasma is an indicator of the presence of ADH, the results seem to lend justification to the conclusion that the participation of ADH in the regulation of water and electrolytes economy develops in 3 periods characterized as follows:

In the *first* period—up to 2½ months—under normal hydration, it is not possible to identify ADA in the plasma of infants. After an osmotic stimulus ADH is found very rarely. In this period, even exogenously administered vasopressin has a statistically lower antidiuretic effect under water diuresis than in older infants, and does not provoke further excretion of hypertonic urine [23]. Under comparable conditions of dehydration provoked by administration of concentrated milk, 2½-months-old infants furthermore excrete hypertonic urine, but of a lower osmolarity than older infants [21].

In the *second* period (approx. up to the beginning of the 5th month) ADA cannot be detected under normal hydration, but is regularly found after a NaCl load.

In the *third* period (beginning with the 5th month) ADA can be demonstrated in the plasma even in normal hydration. It can be assumed that the appearance of ADA is probably an expression of a more mature regulation of water and electrolytes economy which might have gained a new level characterized by a lower intake of fluid [13, 15], decreased hydration of the body [6], and decrease of urine flow in this period [16].

This classification is confirmed by the experiments with young rats [3] and puppies [2]. These seem to show that in the earliest postnatal period in puppies the balance between synthesis and release of ADH from the neurohypophysis is not yet developed. This may be the cause of the quick disappearance of ADA from the plasma during dehydration. Our preliminary examinations have shown that 24 hours after administration of concentrated milk ADA cannot be detected in the plasma

of infants younger than 2½ months. Even in such infants, however, ADA may temporarily appear in the first hours after lowered intake of fluids. This assumption seems to be supported by the findings of Ames [1] and Nikitin [24] who found surprisingly high values of ADA in the urine of children in the first days after birth, dehydrated in Ames' observations for 6 hours, and in those of Nikitin for 6–8 hours.

Though we could not demonstrate the presence of ADA in the plasma in the first period of postnatal development, we are faced with the fact that young infants excrete hypertonic urine of a mean osmolarity of about 600–800 mOsm/L during dehydration [22]. What, then, is the character of the concentration mechanism, and its function? The question is interesting also because participation of a decreased glomerular filtration rate does not seem to be essential in the formation of hypertonic urine [22]. A study of the functional peculiarities and morphology of the infants' kidney, focused especially on the morphologic basis of the countercurrent mechanism is also desirable.

Summary

Antidiuretic activity (ADA) was determined in 50 mature, healthy infants from the age of 16 days up to the end of the 5th month, just before and 45 minutes after administration of a NaCl load (0.7 gr NaCl/kg body weight) by stomach tube. Detection of antidiuretic activity in the plasma of normally hydrated infants was possible only in infants older than 2½ months of age; following the administration of a NaCl load ADA was detected already in the second half of the third month. At the time when the blood was collected for the determination of plasma ADA after a NaCl load, the concentration of Na in the plasma of all age groups increased by 11.2 mEq, and of Cl by 9.2 mEq/L on the average. Relations are being discussed between the development of ADA in the plasma and the development of concentration capacity as well as of the antidiuretic effect of exogenously administered vasopressin. The possibility is considered that hypertonic urine is formed in infants younger than 2½ months with little ADH being available, or even in its absence.

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SOME EFFECTS OF POTASSIUM SALTS IN MAN *

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During the last few years both the physiologist and the clinician have discovered many new facts regarding the metabolism of potassium. Several factors have stimulated recent studies in this subject and among these have been the availability of accurate microchemical methods for the estimation of potassium in body fluids, the application of physiologic principles to the study of certain clinical syndromes, and a desire to establish more clearly the dividing line between the pharmacologic and toxic effects of potassium salts.

The problem of toxicity has been confused in the past by conflicting results obtained in two types of studies. The initial experiments of Blake [1] in 1839 revealed that the rapid intravenous injection of a potassium salt into a dog is quickly fatal. However, 40 years later, in 1881, Feltz and Ritter [2] injected a similar dose slowly, for example in 10 minutes, and no untoward symptoms developed. The latter result gave us a clue as to why relatively large doses of potassium salts have been given by mouth for many years without obvious toxic effects. As much as 40 gm. of potassium chloride have been ingested in one day by patients suffering from myasthenia gravis without toxic symptoms.[3] McQuarrie and coworkers [4] reported the administration by mouth of 48 gm. of the same salt daily to a patient who had acromegaly and hypoglycemia; no untoward effects occurred. Apparently osmotic and other factors acting within the lumen of the small bowel prevent too rapid absorption of potassium salts into the blood stream. The experiments of Winkler, Hoff and Smith [5] in the dog support such a point of view. Therefore, to ascertain the single maximal nontoxic dose of a potassium salt given by mouth to a human subject seemed to be a pharmacologic problem worthy of further study. We shall proceed to outline such an attempt in which normal volunteers were subjects and endeavor to follow the lead so ably given by Ringer and Murrell [6] in 1878 in their well-known experiments in the frog. Ringer interpreted their results as revealing that potassium salts in large doses had a toxic effect on the nervous and muscular tissues, but emphasized that they had a widespread toxic action on all "nitrogenous tissue."

Effect of Potassium on the Kidney

Our first objective was to determine the effect of a large single dose of a potassium salt on the renal clearance of potassium and inulin. Seven normal persons ingested 12.5 to 17.5 gm. of potassium chloride or bicarbonate and

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after absorption had occurred the clearances were estimated.* The routine procedure in these studies consisted in obtaining a control specimen of urine in the early morning, the ingestion of a given potassium salt in 6 to 25 per cent solutions, and then collection of hourly or half hourly specimens of urine. No food was taken in four studies, but to prevent epigastric burning in three a small breakfast was eaten just before ingestion of the potassium salt. This consisted of either two soft boiled eggs or a small portion of oat porridge and 50 c.c. milk. In order to have a satisfactory volume of urine for clearance studies 100 to 200 c.c. of water were ingested every half to one hour throughout the observations. The inulin clearance was determined after the intravenous injection of 100 c.c. of a 10 per cent solution of inulin containing 0.9 per cent sodium chloride, and the chemical determinations in the blood and urine were made according to Alving's [7, 8] method.

In five subjects the potassium clearance rose from the normal fasting level of 6 to 14 c.c.[9, 10] to 41 to 105 c.c., but that of inulin was within the normal range, 93 to 146 c.c. (table 1). During the clearance periods the concentration of potassium in the serum varied from 22 to 28 mg. per 100 c.c. Similar

TABLE I

Renal Clearances: Large Single Dose of Potassium Salt without Toxic Action

Subject (normal person) and surface area, sq. m.	Dose, gm.		Experimental period, minutes	Urine minute volume, c.c.	Plasma and serum clearance*			
	Potassium salt	Potassium†			Inulin	Potassium	Urea	Ratio of potassium to inulin
Ki. 1.97	12.5 $KHCO_3$	4.9	30	3.5	115	52		0.45
	9.5 KCl‡	5.0	67	2.6	143	58	67	0.41
Bu. 1.75	12.5 $KHCO_3$	4.9	31	14.8	137	105		0.77
Ke. 1.95	17.5 $KHCO_3$	6.8	30	2.8	134	41		0.31
Gr. 1.92	17.5 $KHCO_3$	6.8	30 30	3.2 2.9	93 115	49 44		0.53 0.38
Ro. 1.74	12.5 KCl	6.5	30	4.1	146	49	77	0.34
Mean					126	57		0.46

* Calculated as c.c. per 1.73 sq. m. per minute.
† 0.06–0.1 gm. per kilogram of body weight.
‡ Injected intravenously in ninety minutes.

* The potassium in the serum was determined by the method of Kramer, Benjamin and Tisdall, F. F.: A clinical method for the quantitative determination of potassium in small amounts of serum, Jr. Biol. Chem., 1921, xlvi, 339–349. This method in our hands has checked with gravimetric procedures. The precipitation by sodium cobalti-nitrite was allowed to proceed for 45 minutes at icebox temperature in order to obtain a good granular precipitate. If this and other steps are adhered to rigorously, the method is a satisfactory procedure. Potassium in urine was estimated by this method after ashing.

doses of the same salts, however, administered to two additional normal persons, caused the potassium clearance to rise to 37 and 64 c.c., and the concentration of potassium in the serum to 19 to 33 mg. per 100 c.c., but at the same time caused a fall in both the inulin and the urea clearance [11] to less than the normal levels. The minimal values for inulin were 71 and 77 c.c. and for urea 50 and 47 c.c. in these two subjects (table 2). Therefore, in

TABLE II

Renal Clearances: Large Single Dose of Potassium Salt with Toxic (?) Action

Subject (normal person) and surface area, sq. m.	Dose, gm.		Experimental period, minutes	Urine minute volume, c.c.	Plasma and serum clearances*			
	Potassium salt	Potassium†			Inulin	Potassium	Urea	Ratio of potassium to inulin
Fl. 1.80	Control		30	13.8	107	7	99	0.07
			30	10.3	119		76	
	17.5 $KHCO_3$	6.8	30	3.6	71	64	50	0.90
			30	3.8	72	50		0.69
Wi. 1.98	Control		30	10.7	143	10	70	0.07
			30	8.7	145	9	65	0.06
	12.5 KCl	6.5	30	9.1	77	37	64	0.48
			35	2.7	80	36	47	0.45

* Calculated as c.c. per 1.73 sq. m. per minute.
† 0.08–0.1 gm. per kilogram of body weight.

two subjects the large dose of potassium disturbed normal renal excretion. The decreased inulin clearance indicates reduced glomerular filtration. The results of all seven studies reveal that a single dose of a potassium salt containing 80 to 100 mg. of potassium per kilogram of body weight may or may not have a toxic action on the kidney. Obviously such a dose is close to the toxic level for the normal kidney.

Considerable evidence has accumulated that the diseased kidney often can excrete potassium salts satisfactorily.[12, 5] This has been particularly true in cases of chronic glomerulonephritis with edema in which these salts have produced a diuretic action.[12] On the other hand, we now know that it is dangerous to give potassium salts in cases of nephritis in which severe renal insufficiency is present. Caution in their use is especially indicated in cases of severe acute renal failure, of severe passive congestion of the kidney [13, 14, 15] and during the terminal phase of uremia.[15]

EFFECT OF POTASSIUM ON SENSORY NERVE ENDINGS

Because of a certain amount of discomfort in the epigastrium after the ingestion of these large doses of potassium salts it seemed expedient to study

the effect of a potassium salt on renal function after intravenous injection. Such an attempt was made on two healthy volunteers. At the onset of the intravenous injection of 1 per cent solution of potassium chloride both subjects complained of severe burning pain extending from the site of injection in the elbow vein up and along the arm vein to the shoulder. The site and type of pain were apparently similar to those experienced after the rapid intravenous injection of 10 per cent solutions of calcium and magnesium chloride. Further attempts in several other veins also were attended by pain in one volunteer, but the second volunteer managed to take slowly, over a period of 91 minutes, 950 c.c. of the 1 per cent solution of potassium chloride without pain. The concentration of potassium in the serum rose from the control value of 18.3 to 22 mg. per 100 c.c. and the clearance to 58 c.c., but the clearances of inulin and urea were normal, 143 and 67 c.c. respectively (subject Ki. [normal person], table 1). Therefore, this injection did not have any demonstrable toxic effects on the function of the kidney but confirmed the findings of others that a too rapid intravenous injection of a solution of potassium salts gives rise to severe pain along the vein into which the injection is made. Pudenz and his co-workers [16] reported that their patient suffering from periodic familial paralysis, during an attack of paralysis, when the concentration of potassium in the serum was decidedly low, was given intravenous injections of a 0.5 to 2.0 per cent solution of potassium chloride, a total of 1.0 gm. in 10 minutes; the patient complained of such severe burning pain along the vein that on one occasion he stated that he would rather remain paralyzed than submit to such an injection.

The localization of this pain along the veins into which the injection was made naturally suggested that sensory nerve endings for pain in these vessels had been stimulated by the potassium solution. Moore and his co-workers [17, 18] showed experimentally in the cat that intra-arterial injections of potassium salts stimulate the nerve endings for pain in these arteries. Moore also observed, however, that intravenous injections in these animals had no such effect. The investigations of Häbler and Hummel [19] in 1928 revealed that in human subjects intracutaneous injection of isotonic solutions of potassium salts always caused pain within 20 seconds whereas isotonic solutions of similar sodium salts did not cause pain. These facts with regard to the possible stimulation of sensory nerve endings for pain in different tissues by potassium salts offered us a possible explanation of a certain untoward symptom described by Arden [20] after the ingestion by mouth of 15.0 gm. of potassium chloride or potassium bicarbonate. Arden described the development of paresthesia in the hands and feet some 40 minutes after the salt had been taken and its subsequent persistence for three to four hours. Could this paresthesia in the hands and feet be related to an increase in the concentration of potassium in the serum, which in turn stimulated peripheral nerve endings?

In one of our observations the volunteer ingested 12.5 gm. of potassium chloride and while we were studying his potassium and inulin clearances he

complained of paresthesia in his hands and feet. This subject was one of the two volunteers, mentioned already, in whom low inulin and urea clearances developed (subject Wi. [normal person], tables 2 and 3, figure 1). This lat-

TABLE III

Serum Potassium and Paresthesia of Hands and Feet:* Large Single Dose of Potassium Salt; Subject Wi. (Normal Person)

Dose, gm.		Serum potassium, mg. in 100 c.c.	Inulin clearance‡	Paresthesia of hands and feet, grade
Potassium salt	Potassium†			
12.5 KCl	6.5	33.2	77	3

* Two to three hours after ingestion of salt.
† 0.08 gm. per kilogram of body weight.
‡ Calculated as c.c. per 1.73 sq. m. per minute.

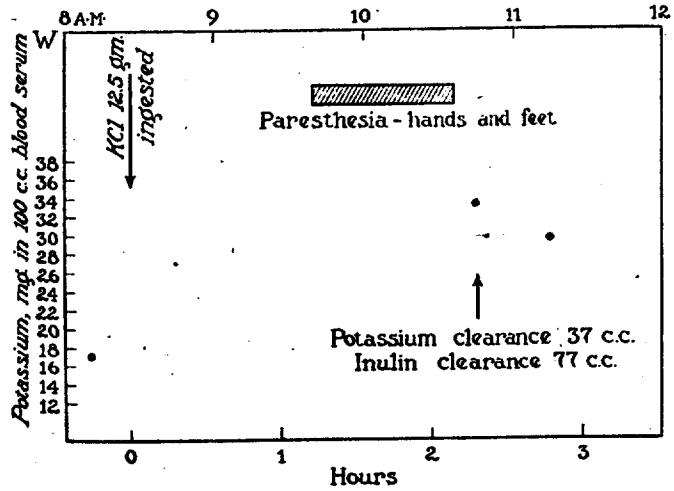

FIG. 1. Subject Wi. (normal person; tables 2 and 3). Concomitant development of paresthesia in hands and feet with a rise in concentration of potassium to 33.2 mg. per 100 c.c. of serum and a reduction of inulin clearance to 77 c.c.

ter finding suggested a possible toxic effect on the kidney while the presence of paresthesia suggested a toxic effect on the sensory nerve endings in the peripheral tissues of the hands and feet. It was noted also that at the time the clearances were estimated and the paresthesia was felt, the concentration of potassium in the serum had increased to 33.2 mg. per 100 c.c. In a similar study of another volunteer we have since observed the development of paresthesia in the hands and feet accompanied by a concentration of potassium in the serum of 32.8 mg. per 100 c.c. (subject Ke. [normal person], figure 2). In a third study, a patient suffering from diffuse cardiovascular disease and hypertension consented to take the same dose of potassium

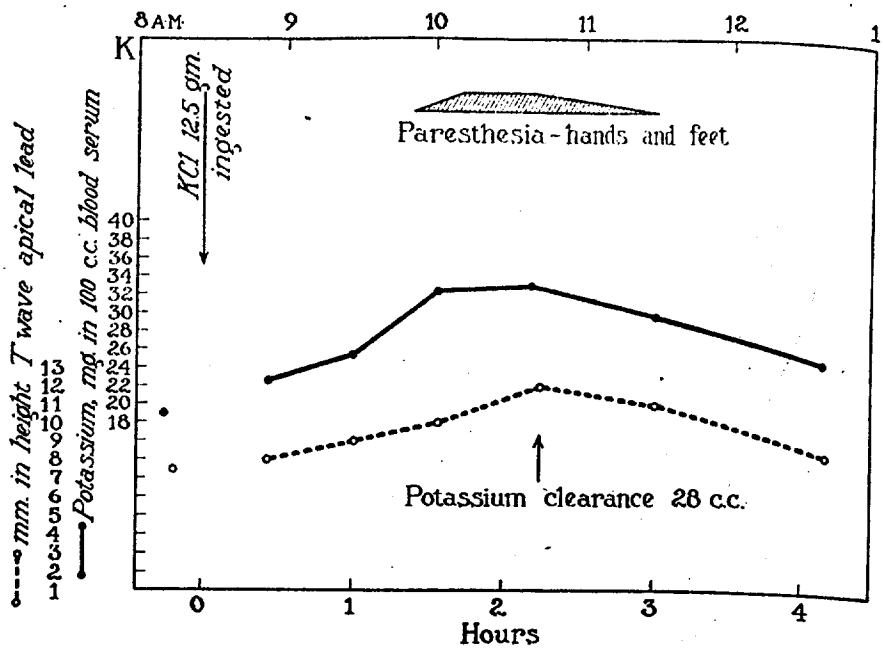

FIG. 2. Subject Ke. (normal person; tables 1 and 4). Concomitant development of paresthesia in hands and feet with a rise in concentration of potassium to 32.8 mg. per 100 c.c. of serum and a rise of 4.5 mm. in the T-wave of the electrocardiogram.

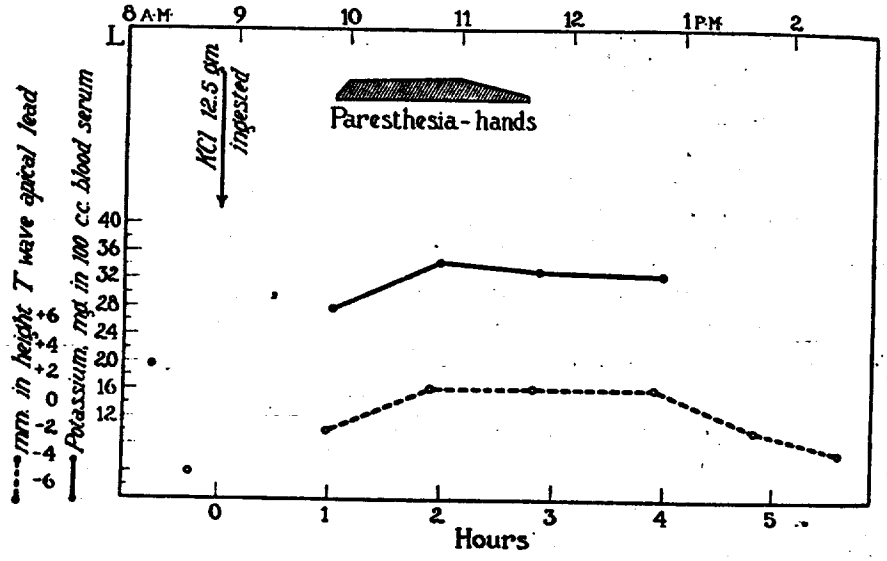

FIG. 3. Subject La. (patient; table 4). Simultaneous occurrence of paresthesia in hands with a rise in concentration of potassium to 34.2 mg. per 100 c.c. of serum and a rise of 6.0 mm. in the T-wave of the electrocardiogram. Note also slow fall of both serum potassium and T-wave.

chloride. Paresthesia developed in his hands but not in his feet when the concentration of potassium rose to 34.2 mg. per 100 c.c. of serum (subject La. [patient], figure 3). On the other hand, a normal volunteer ingested the same amount of potassium chloride but paresthesia did not develop nor did the concentration of potassium rise to more than 26.3 mg. per 100 c.c. of serum. However, he excreted potassium rapidly in the urine, the clearance amounting to 95 c.c. (subject Bu. [normal person], figure 4).* Norn [21] in 1929 re-

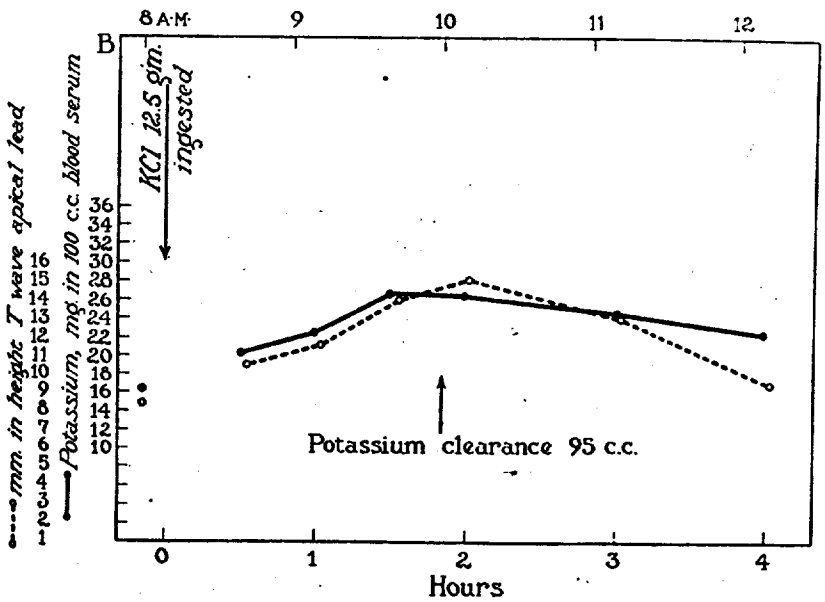

FIG. 4. Subject Bu. (normal person; tables 1 and 4). Simultaneous occurrence of a rise in concentration of potassium to 26.3 mg. per 100 c.c. of serum, a rise of 6.5 mm. in the T-wave of the electrocardiogram and a potassium clearance of 95 c.c.

ported a similar experience to subject Bu. in which paresthesia did not occur. This type of study suggests that there is a close relation between a rapid rise in the concentration of potassium from normal values to 30 mg. per 100 c.c. of serum, and development of peripheral paresthesia.

EFFECT OF POTASSIUM ON THE HEART

Blake, in 1839, and subsequent investigators have suggested that death which followed a rapid intravenous injection of a potassium salt into an animal was due to the sudden cessation of the movements of the heart. Potassium appears to have a dual toxic action on the heart, affecting both the conduction system and the muscle of the heart. Winkler, Hoff and Smith [22]

* This subject's intake of water and output of urine were high in both this and a previous study (table 1). The large intake of water may have played an important rôle in the resulting high potassium clearances; however, in other persons a large volume of urine was accompanied by a much lower potassium clearance.

have demonstrated in their recent experiments the progressive changes that develop in a series of electrocardiograms taken as the toxic action on the animal's heart increases. Similar changes have also been demonstrated in the cat by Chamberlain, Scudder and Zwemer.[22a] Their results confirm the earlier observations of Wiggers [23] in dogs that potassium salts cause a rise in the T-wave of the electrocardiogram early and before auricular or ventricular fibrillation sets in. McLean, Bay and Hastings [24] perfused isolated hearts of rabbits with a fluid containing various concentrations of potassium chloride and demonstrated that increasing the concentration of potassium to 35.3 mg. per 100 c.c. caused a marked rise in the T-wave whereas decreasing it to 11.8 mg. per 100 c.c. had the reverse effect.

There is increasing evidence that similar changes in the T-wave of the electrocardiogram can occur in man. For example, after a normal person has ingested a large dose of a potassium salt a rise in the T-wave can be demonstrated. There are also patients who have certain clinical conditions in which the concentration of potassium in the serum may be increased or decreased abnormally and who show a corresponding rise or fall in the T-wave. Thomson [25] in 1939 demonstrated in examination of a patient who had Addison's disease that a high concentration of potassium in the serum was associated with an increased T-wave in the electrocardiogram. He subsequently showed a similar relation in patients who had cardiac disease and who were ingesting considerable amounts of potassium salts.[26]

In order to follow Thomson's studies further, two normal subjects and a patient who had diffuse cardiovascular disease and hypertension [27] consented to take 12.5 gm. potassium chloride, in a single dose. It is of interest that an electrocardiographic tracing of this patient taken during a control period showed inversion of the T-waves in Leads I and IV. Before and at periodic intervals after the ingestion of the salt, electrocardiographic tracings of the three volunteers were taken and estimations of the concentration of

TABLE IV

Serum Potassium and T-Wave in Electrocardiogram:* Large Single Dose of Potassium Salt

Subject (normal person or patient)	Dose, gm.		Serum potassium, mg. in 100 c.c.	Electrocardiogram, rise in T-wave, mm.‡
	Potassium salt	Potassium†		
Ke.	12.5 KCl	6.5	32.8	4.5
La.§	12.5 KCl	6.5	34.2	6.0
Bu.	12.5 KCl	6.5	26.3	6.5

* Two to three hours after ingestion of potassium salt.
† 0.069 to 0.104 gm. per kilogram of body weight.
‡ T-wave in apical lead.
§ Patient had diffuse cardiovascular disease with hypertension.

potassium in the serum were made.* Both the concentration of potassium in the serum and the T-waves in the electrocardiograms showed in all three studies a steady rise until they reached a maximum in approximately two hours (table 4, figures 2, 3, 4, 5 and 6). In the two normal volunteers

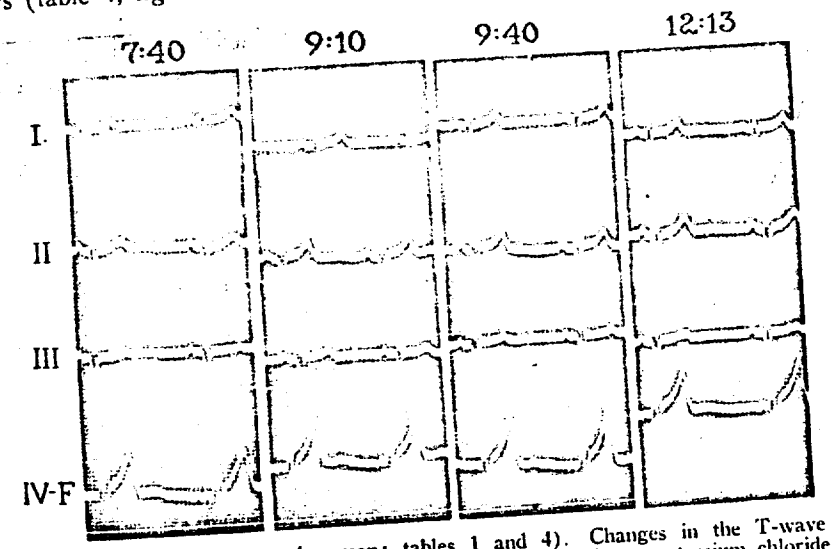

FIG. 5. Subject Bu. (normal person; tables 1 and 4). Changes in the T-wave of the electrocardiogram of a normal person after ingestion of 12.5 gm. potassium chloride at 8:08 a.m.

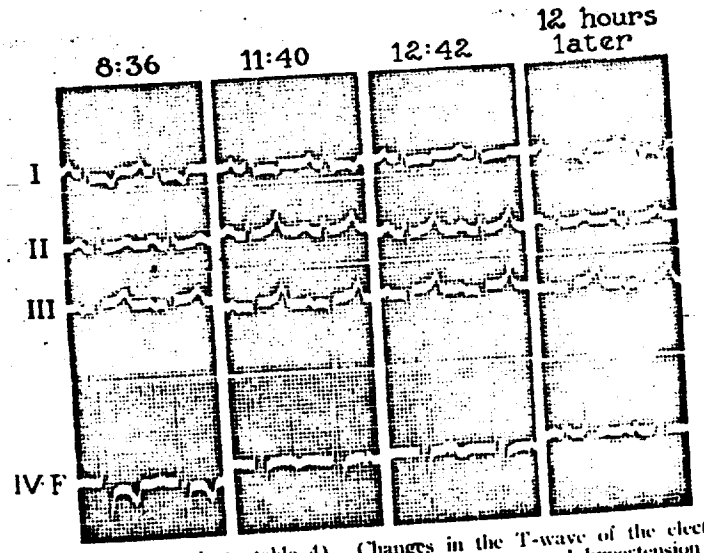

FIG. 6. Subject La. (patient; table 4). Changes in the T-wave of the electrocardiogram of a patient suffering from diffuse cardiovascular disease and hypertension after ingestion of 12.5 gm. potassium chloride at 8:49 a.m.

*The pulse rate was estimated periodically and no marked changes from the normal control rate were observed. Blood pressure readings were not taken during these studies.

there was a gradual decline in the serum potassium and in the T-waves until control levels were approached in four to six hours. However, in the patient who had vascular disease (subject La.) the decline in concentration of potassium in the serum was much slower and even after 12 hours the T-waves had not receded to the control level (figures 3 and 6). It also should be pointed out that in the normal subject Bu. the T-waves rose the highest but the rise in serum potassium was the least (table 4, figure 4). This observation is in agreement with Thomson's findings in patients, that usually the increase in the T-wave was associated with a rise in the concentration of potassium in the serum, but this relationship varied and was not a constant one.

Janota and Weber [28] in 1928 obtained an electrocardiogram in examination of a patient suffering from familial periodic paralysis during a paralytic episode and observed a distinct lowering of the T-wave. With recovery from the paralysis the electrocardiogram became normal. A similar finding was reported later by Zabriskie and Frantz.[29] Several investigators beginning with Aitken, Allott, Castleden and Walker [30] in 1937 have made the interesting observation that during the paralytic attack in such a patient the concentration of potassium in the serum is markedly reduced and that with recovery it rises to normal. Stoll and Nisnewitz [31] early in 1941 made both an electrocardiographic tracing and a serum potassium estimation during an attack of paralysis in one of these patients. As was to be expected, both the serum potassium and the T-waves were reduced definitely.

Simultaneous Diffuse Effects of Potassium

It is quite clear from the foregoing studies that potassium salts after absorption into the blood stream may exert widespread effects throughout the body. Several simultaneous actions in different organs of subjects Wi., Ke., Bu. (normal persons) and La. (patient) are depicted graphically in figures 1, 2, 3 and 4. In subject Wi. (normal person, figure 1) paresthesia in the hands and feet and reduced renal clearances of inulin and urea occurred at a time when the concentration of potassium in the blood stream had risen to 33.2 mg. per 100 c.c. of serum. Apparently at this high concentration of potassium in the serum, the ability of the kidney to excrete inulin and urea was reduced, and the nerve endings in the skin of the hands and feet were stimulated abnormally. In subject Ke. (normal person, figure 2) in addition to the high concentration of potassium of 32.8 mg. per 100 c.c. of serum and the presence of paresthesia in the hands and feet, there is a distinct rise of 4.5 mm. in the T-wave of the electrocardiogram. In this subject we learn that with a similar high concentration of potassium in the serum, as observed in the previous study, abnormal peripheral sensory disturbances occur at the same time as the T-waves in the heart are altered. In subject La. (patient, figure 3) the results are similar to those in the previous subject Ke. (normal person). It is of interest, however, that the T-waves were abnormally low in the electrocardiogram taken previous to the ingestion of potassium and

that the low T-waves were increased distinctly when the concentration of potassium in the serum became distinctly high.

The application of certain technical procedures in these four studies has shown that scattered abnormal functional disturbances sometimes can be correlated with an abnormal symptom, and further that if paresthesia in the hands or feet, or in both, develops after the ingestion of a potassium salt, it is fairly safe to conclude that the concentration of potassium has increased to 30 mg. per 100 c.c. of serum and that T-wave changes can be demonstrated in the electrocardiogram.

Comment

Certain practical considerations emerge from the facts presented in this study. First of all we have learned that protective mechanisms are called into play when too large doses of potassium salts are injected intravenously or ingested by mouth. The stimulation of pain along the vein into which the solution is injected protects the organism from too rapid an entry of potassium into the vascular system and tissue spaces. Likewise after the ingestion and absorption of a considerable dose of potassium the onset of paresthesia in the hands and feet should warn us that the concentration of potassium in the serum has risen quickly to a level which may cause diffuse toxic effects. In a similar manner T-wave changes in the electrocardiogram may be a danger signal, although from present knowledge it is quite clear that their presence alone does not indicate significant dysfunction of the heart. Another practical point is that there are definite indications that in both severe adrenal and renal insufficiency the tissues have a reduced tolerance for potassium salts. This may also apply to patients who have diabetes mellitus.[4] This fact seems true both after experimental adrenalectomy [32, 33] and in patients who have Addison's disease.[34] The problem is not so clear with regard to renal insufficiency. In some abnormal renal states there is greater intolerance than in others to potassium salts. The closer the renal insufficiency is to total loss of renal function in either experimental or clinical conditions,[35, 36, 37, 38] the more likely it seems that there will be intolerance to potassium. However, in moderate renal insufficiency patients often tolerate it well. From these practical considerations one finally concludes that there is considerable individual variation as to what constitutes a toxic dose of a potassium salt, that potassium salts can be given safely in considerable doses in a variety of disease conditions, and that the method of administering moderate repeated doses is safer than giving a large single one.

The fundamental action of potassium salts is related intimately to cellular function * in various tissues. This applies to muscular activity whether in voluntary muscle or the myocardium. Numerous investigators so far have failed to explain adequately the mechanism of potassium in muscular physiology. Some have thought that it was associated intimately with reactions in

* The term "cellular function" refers to both the membrane enclosing the cell and the intracellular protoplasm.

the myoneural junctions, others that its rôle was linked closely with action of hormones such as epinephrine, insulin and cortin within the muscle cell.[39] The beneficial action of potassium salts in familial periodic paralysis and in myasthenia gravis suggests a neuromuscular effect.

A discussion of the diuretic action of potassium salts is of some theoretical interest. Normally after 12 hours of fasting the renal clearance of potassium is remarkably small when compared with that of inulin;[3, 10, 5] the potassium-inulin clearance ratio averages 0.10. This ratio would seem to indicate that a large percentage of the potassium filtered by the glomeruli is reabsorbed by the tubules. With large doses of potassium salts the potassium-inulin clearance ratio rises; in six of our studies on normal persons, the ratio rose to from 0.31 to 0.77. This variable increase in potassium clearance occurred when there was only a moderate increase in the concentration of potassium in the serum, actually from 22 to 28 mg. per 100 c.c. This rise in concentration of potassium in the serum presumably also occurred in the glomerular ultrafiltrate. If from these data one computes the possible reabsorption and excretion of potassium by the tubules, it will be seen that when the potassium-inulin clearance ratio is between 0.31 and 0.45 the absolute amount of potassium reabsorbed is always greater than that excreted. In the one case in which the potassium-inulin ratio reached 0.77, the amount excreted exceeded greatly the amount reabsorbed. The implication of these calculations would seem to be that increased excretion of potassium by the kidney is not always due simply to a rejection of potassium by the tubular cells but is the result of a variable alteration in the balance between reabsorption and excretion in these cells. The diuretic action of potassium salts would appear, therefore, to be the result of a temporary change in the cellular function of the cells of the renal tubules.

The results reported in this paper reveal that the effects of potassium are widespread throughout the body and are in all probability dependent on altered cellular activity. Further knowledge regarding the potassium problem is dependent on the continuance of similar studies as well as on an increased understanding of fundamental cellular physiology.

Summary

A considerable amount of several potassium salts may be ingested by the normal person without demonstrable toxic effects. Similar doses may be given with safety to patients suffering from various diseases, but their use is specifically contraindicated in cases of severe renal and adrenal insufficiency. An important symptom, indicative of toxic action, is the development of paresthesia in the hands and feet. It is accompanied by a rapid rise in the concentration of potassium in blood serum to approximately 30 mg. per 100 c.c. The diffuse action of potassium is revealed by the simultaneous production of effects on the functions of the heart, kidney and peripheral nerve endings.

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Management of Severe Hypertension

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In our hypertension clinic currently attended by 321 patients who have presented with severe hypertension over the past 15 years we now commonly use a combination of a beta adrenergic blocking agent and a peripheral vasodilator. In addition, a drug of the benzothiadiazine group is always combined with other treatment. A recent review of results shows that in every patient with severe hypertension blood pressure can be satisfactorily controlled using a combination of these different agents. In the patients with the most severe and refractory forms of hypertension the use of orally administered diazoxide has had a dramatic effect in several patients, and this drug is sometimes combined with a beta adrenergic blocking agent.

Our aim has been to control the blood pressure at a level of 150/90 mm Hg or below with a minimum of side effects. Controlled trials have shown that this can often be achieved with a combination of a beta adrenergic blocking agent and a drug of the benzothiadiazine group. When this combination fails to achieve adequate control, the addition of a peripheral vasodilator will often achieve a satisfactory response.

Our experience in patients with impaired renal function and severe hypertension suggests that sodium status and control of hypertension should be regarded separately. Not only does diazoxide, the most potent agent presently available, cause marked sodium retention but also, in a few patients, malignant hypertension will disappear or become much more easily controlled during correction of sodium depletion. This apparent paradox may in part represent suppression of very high renin levels.

Severe hypertension implies much more than a high reading on a sphygmomanometer. No matter what precautions are taken to determine that the method of measurement is accurate and reflects not only the intraarterial pressure but also the usual level in the patient under consideration, there are many other factors to be considered. Some of these are well recognized; for example, the relatively benign course of hypertension in a woman over age 50 has often been contrasted with the severe consequences of hypertension in a young man. Hypertension in children and adolescents is rare and usually of renal origin[1] and thus often carries a serious prognosis. Blood pressure increases with age in most European populations.[2-4] This finding has probably had a major influence on the conservative management of hypertension in older persons and raises many questions: Does it reflect the fact that hypertension is a very common disease? Are the rising blood pressure levels merely part of the process of aging? What attitude should one adopt in the individual patient over the age of 70 years?

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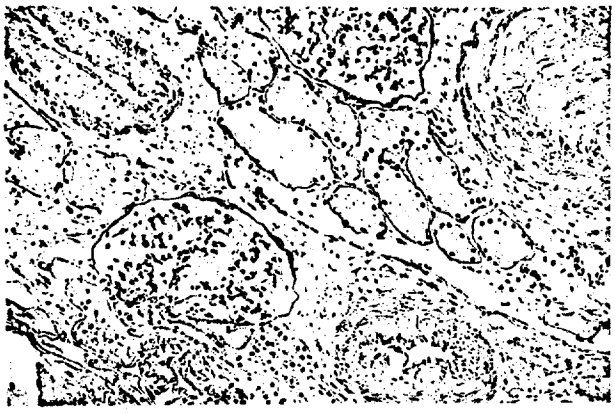

FIGURE 1. Vascular lesions that developed during pregnancy in a young woman whose blood pressure never rose above 140/90 mm Hg.

Apart from age and sex, race is undoubtedly important. Blood pressure does not increase with age in some races;[5] furthermore, even mild hypertension seems to carry serious implications in some races, in black Americans[6] for example.

Pregnancy and the "hypertensive vascular" lesions associated with pregnancy (Fig. 1) not only develop in association with lower levels of blood pressure[7] but also may actually precede and cause hypertension.[8] Vascular lesions are also much more likely to be found in black American women than in white American women with pregnancy-induced toxemia.[6]

How does the internist or family physician reconcile his previous teaching about the benign course of hypertension in older patients with the scientifically correct epidemiologic evidence that his 55 year old patient with a diastolic blood pressure of 105 mm Hg is more likely to have a stroke if he is not treated[9,10] and with the apparent paradox that a young man with a diastolic blood pressure of 110 mm Hg may be admitted to a controlled trial in which it is planned to give him a placebo for 5 years?

It is perhaps unfortunate that blood pressure readings are so easily obtained in large numbers. Multiple readings of figures have a great appeal to the statistician and epidemiologist, but a patient cannot and should not be looked upon as a systolic and diastolic blood pressure reading.[11] We would not have to rely so heavily on long-term large scale controlled trials if we could differentiate between a patient with true "severe hypertension" and one with high blood pressure readings but a good prognosis.[11] To make this distinction we usually have to wait for complications to develop in the retina, heart, brain or kidney. Although no one would doubt the life insurance statistics showing that a high blood pressure carries a diminished life expectancy for the group, let us also recall that the individual patient known to have a very high blood pressure for as long as 30 years may live to a ripe old age and show no signs of hypertensive vascular disease.[11,12]

Mechanisms Underlying Severe Hypertension That May Influence Management

Although the renin-angiotensin story has provoked considerable scientific interest it has until now produced little of value to the individual patient or of interest to the practicing physician. The exciting observations of Laragh's group[13] provide some hope that it may be possible to predict which patients are likely to experience vascular complications of hypertension. Although not all investigators have been able to relate vascular complications to renin levels, this is a field in which we have learned to expect different results from different groups. Perhaps differences in methods of renin estimation and their relation to sodium status will provide the explanation for conflicting findings.

The suggestion that one can detect the difference between "severe hypertension" in terms of vascular complications and high blood pressure readings in patients who will not experience vascular complications is so important that it is mandatory that groups able to investigate this relation should do so. If the findings of Laragh's group[13] are confirmed, then "low renin hypertension" could well represent what has been called "hypertonia"[11] and clinical trials could be considerably shortened by selective entry of the high risk, "high renin" patients.

Because most patients with "severe" renal hypertension also have high renin activity, perhaps we can also find an explanation for "albuminuric retinopathy" or the fact that patients with renal disease are more likely to show retinal hemorrhages and exudates than patients with essential hypertension and the same blood pressure level.

The theory that high renin levels predispose to vascular damage is appealing because angiotensin in acute experiments[14,15] produces profound vascular changes that may be dissociated from the increase in blood pressure.[15] The effects of angiotensin are more striking in renal vessels.[14] The alternating constrictions and dilatations that appear with the onset of malignant hypertension[16] resemble those produced by angiotensin[14] and could represent not only the result of high renin levels but also the morphologic change that initiates and causes the vascular manifestations of the most severe form of hypertension, namely, malignant hypertension.[14]

It is conceivable that flow phenomena operating within the renal circulation after the appearance of constricted and dilated areas in renal arteries and arterioles lead to thrombus formation,[14] as has been shown to occur with flow disturbances in in vitro models[17] and in the microcirculation.[18] Even in the absence of thrombus formation, individual platelets may damage the endothelium.[19] These factors could initiate the intravascular coagulation and microangiopathic hemolytic anemia that accompany malignant hypertension[14,20] and are responsible for some if not all of the resulting vessel damage.[14]

Microangiopathic hemolytic anemia and intravascular coagulation could account for the striking clini-

cal differences between malignant and benign hypertension. The dramatic alteration in the course and clinical features that accompanies the onset of the malignant phase of hypertension could be the result of vascular manifestations of intravascular coagulation. Identical vascular lesions develop in association with intravascular coagulation in other states at a time when the blood pressure is normal although it may increase rapidly after the lesions develop.[8]

Management of Severe Hypertension

Salt and Hypertension

Although it is widely held by the lay public and by the medical profession that "salt is bad for the blood pressure," it is difficult to find evidence that salt restriction short of the strict rice diet introduced by Kempner[21] in 1944 is beneficial in the management of hypertension. Although salt intake is of no real consequence in the management of most patients with hypertension, sodium restriction may be deleterious in some because it produces a fall in the glomerular filtration rate and an increase in blood urea nitrogen and creatinine. Because Laragh's group[13] placed such emphasis on sodium excretion (which reflects sodium intake) in defining groups with low, normal and high renin levels, it is interesting to speculate whether giving additional salt may, by suppressing renin secretion, be capable of converting a patient from a "high renin" to a "low renin" status. This is of particular interest in relation to the vascular lesions seen in the group with high renin levels[13] and to the experimental lesions produced by angiotensin.[14]

Sodium in patients with severe hypertension and impaired renal function: We have repeatedly noted that in certain patients with analgesic nephropathy severe and even malignant hypertension may fluctuate or disappear, usually during improvement in renal function.[22,23] We have documented the course of several patients whose severe or malignant hypertension disappeared,[23,24] at times permanently, during administration of saline solution aimed at correcting the severe sodium depletion that may accompany renal failure in these patients (Fig. 2). This alleviation of hypertension during weight gain and sodium administration has been most clearly demonstrable in analgesic nephropathy in which the medullary lesion predisposes to severe sodium depletion. Although considered a paradox by some, this reduction in blood pressure during sodium administration is not peculiar to patients with analgesic nephropathy (Fig. 3). All forms of impaired renal function are associated with an inability to conserve sodium and, even in advanced glomerulonephritis, particularly in patients whose disease has been arrested at a late stage, large salt supplements may be necessary to prevent sodium depletion. Salt supplements could play a part in the improvement of function that has been observed in such patients.[24,25]

Chronic sodium loading will increase the single nephron glomerular filtration rate[26] and may be a factor in promoting the remarkable nephron hypertrophy commonly seen in analgesic nephropathy.[22-24] In occasional patients renal size may increase during recovery.[23] Sodium loading appears to be helpful

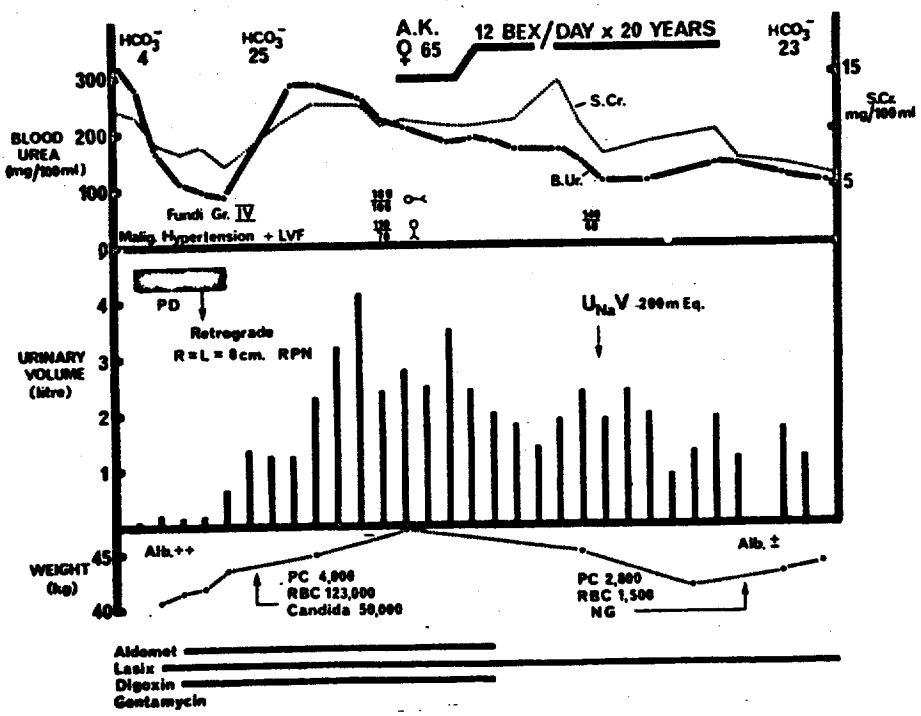

FIGURE 2. Course in a patient who presented with severe renal failure (urea 300 mg/100 ml, creatinine 15 mg/100 ml) and malignant hypertension. In spite of replacement of salt and water and weight gain of over 5 kg, the hypertension, which initially required diazoxide for control, was subsequently easily controlled. Two weeks after admission when her weight was at its peak she required only small doses of methyldopa (Aldomet®), and her hypertension subsequently disappeared, the blood pressure remaining normal without treatment for years. Alb. = albuminuria; BEX = aspirin-phenacetin-caffeine proprietary mixture; B.Ur. = blood urea; Gr. = grade; L = left; L.V.F. = left ventricular failure; N.G. = no growth; P.C. = leukocytes; P.D. = peritoneal dialysis; R = right; R.B.C. = red blood cells; R.P.N. = renal papillary necrosis; S.Cr = serum creatinine; $U_{Na}V$ = urinary excretion of sodium. Arrows indicate urinary findings.

and not harmful in relation to control of the blood pressure in such patients (Fig. 2).

Sodium in patients with preeclamptic toxemia: Although patients with preeclamptic toxemia may not have very high levels of blood pressure, they may nonetheless have "severe hypertension" in relation to vascular lesions, particularly those in the kidney and retina.[7,8] This is another form of hypertension in which there is some clinical and experimental evidence[27,28] that sodium restriction may be harmful and sodium administration beneficial. It is also one of the forms of hypertension in which diazoxide, a sodium-retaining drug, has been most helpful in the control of severe hypertension.[29-31]

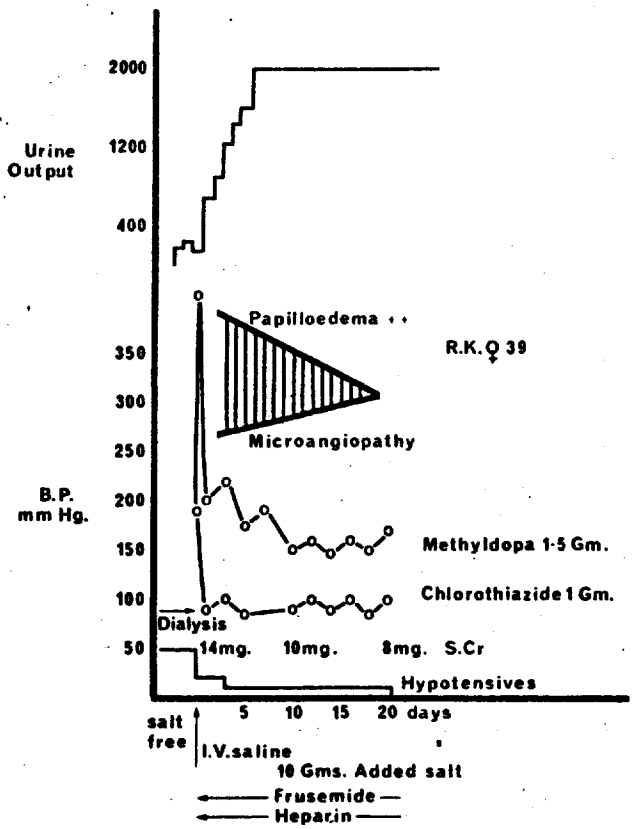

FIGURE 3. Course of a 39 year old woman with essential hypertension and normal sized kidneys who presented with "uncontrollable" hypertension after 3 months of severe salt restriction and peritoneal dialysis. She had been receiving large doses of guanethidine, methyldopa, bethanidine and reserpine, but had very severe papilledema and a blood pressure of 360/180 mm Hg. In spite of energetic replacement of salt and water, which were severely depleted, the blood pressure became more easily controlled and she required steadily diminishing amounts of hypotensive agents during continued sodium administration. The malignant hypertension resolved rapidly, renal function improved and further dialysis was not necessary. Heparin and frusemide were also used in her management and it is difficult to evaluate the separate effects of the different therapeutic measures but salt administration appeared to be beneficial and did not increase the blood pressure. I. V. Saline = intravenously administered saline solution; S.Cr = serum creatinine.

Sodium-Retaining Vasodilator Drugs

It may be coincidental that the two most dramatically effective hypotensive agents currently available for the treatment of severe hypertension, diazoxide[29,31,32] and minoxidil,[33] are both sodium-retaining vasodilators. Furthermore, the diuretic agents that produce the greatest natriuesis, frusemide and ethacrynic acid, are less effective than the thiazide diuretic drugs in reducing blood pressure.[34]

Our own experience of the dramatic effects of diazoxide (400 to 800 mg/day orally)[35,36] reflects that of Pohl et al.[32] although we have studied many fewer patients because this agent has been available in limited amounts. From preliminary data, minoxidil seems to be very similar to diazoxide in its sodium-retaining and peripheral vasodilator actions and, like diazoxide, it seems to be highly effective in severe hypertension resistant to treatment with other hypotensive agents.[33]

If these two agents were generally available the need for bilateral nephrectomy for the control of severe hypertension[37] would probably not arise. It would be difficult to defend bilateral nephrectomy as a method of controlling hypertension when the oral administration of diazoxide would control blood pressure and preserve renal function.[32] The side effects of diazoxide are minor compared with the considerable morbidity and mortality of bilateral nephrectomy, dialysis and transplantation.

Beta Adrenergic Blocking Agents

Following early reports that blood pressure decreased during administration of pronethalol for angina pectoris, numerous publications have confirmed the value of beta adrenergic blocking agents in hypertension. Propranolol has been studied more extensively than any other agent in this group and in our experience has been very effective in severe hypertension.[35,36]

Although many other beta adrenergic blocking agents such as practolol, oxyprenolol, alprenolol and prindolol are effective in reducing blood pressure, their value in severe hypertension has not yet been established. Theoretically they have a potential disadvantage in the treatment of severe hypertension because they possess an intrinsic sympathomimetic activity that may detract from their antihypertensive effects when large doses are used. With two agents, namely, prindilol and practolol, that we have used in uncontrolled studies this sympathomimetic effect has probably been responsible for diminishing effect with large doses. However, in a double-blind crossover controlled comparison of prindilol and propranolol in 35 patients there was no significant difference between the effects of a mean dose of 57.5 mg/day of prindilol and 289.3 mg/day of propranolol.[38] Patients whose severe hypertension was difficult to control were excluded from this study, and it was therefore not designed to test the effects of large doses of beta adrenergic blocking agents. The trial

showed better control of supine systolic and diastolic blood pressures and fewer side effects with both prindilol and propranolol than with the previous treatment.

The great advantage of propranolol in the treatment of severe hypertension is that it shows a continuing dose response curve up to very large doses of 2 g and over.[35,39] When used in doses of 1 to 3 g daily it is very effective in the control of severe hypertension and unlikely to show serious side effects or reduction of the blood pressure to levels below normal.[39] Nightmares and some drowsiness are the major troublesome side effects we have observed. Patients with a history of asthma should not be treated with beta adrenergic blocking agents since the drugs may cause severe bronchospasm.

Mode of action of beta adrenergic blocking agents: Various possible mechanisms have been suggested to explain the hypotensive effect of beta adrenergic blocking agents. It is likely to be in some way related to their beta adrenergic blocking activity because all beta blocking agents tested thus far, including MK950 which is still undergoing preliminary trials,[35] show an antihypertensive effect. With propranolol in particular the dose required to produce beta blockage is often considerably less than the dose required for control of blood pressure, thus suggesting that some effect other than the beta adrenergic blocking action is at least partly responsible for the reduction of blood pressure.

Various mechanisms have been proposed, namely, a baroreceptor effect,[39] an effect of metabolites,[40] an effect on the central nervous system,[41] a gradual fall in peripheral resistance in response to the low cardiac output,[42] and an effect on renin.[43] Of these, the one of greatest interest in relation to earlier discussion is the suggestion by Bühler et al.[43] that propranolol exerts its major effect by reducing plasma renin activity. Many of the patients we treat have severe malignant or premalignant renal hypertension. Although we have not estimated plasma renin activity routinely in such patients, one can predict that many would be classified in the "high renin" group. Such patients have shown impressive responses to propranolol.[35] In a double-blind study we did not encounter any patients resistant to propranolol, and subsequent review suggests that many of these patients may correspond to Laragh's "high renin" group.[44]

Combination of beta adrenergic blocking agents and peripheral vasodilators: Several studies have shown that the combination of propranolol and hydralazine is effective and associated with few side effects.[46-48] Propranolol produces a slight increase in peripheral resistance which is counteracted by hydralazine. On the other hand, the troublesome side effects of hydralazine resulting from reflex sympathetic stimulation, tachycardia, nausea, headache and angina pectoris—are effectively counteracted by propranolol.

Although we have not used hydralazine extensively we have studied prazosin in 53 patients. This drug is effective when used alone and seems to have fewer side effects than hydralazine.[35] In the treatment of severe hypertension the addition of prazosin in a dose of 7.5 to 20 mg daily often brings about a dramatic reduction in the blood pressure in a patient already receiving a moderate or large dose of propranolol.[35] We are presently comparing prazosin and hydralazine in a double-blind study in which the vasodilator is added to propranolol at a stage when the blood pressure has not yet been adequately controlled.

The other peripheral vasodilator we have used in combination with beta adrenergic blocking drugs is diazoxide. This combination permits reduction in the daily dose of diazoxide and may result in excellent control of severe hypertension resistant to other treatment.[36]

Other Drugs in the Control of Severe Hypertension

Benzothiadiazines. When chlorothiazide became available in 1957 it revolutionized the treatment of severe hypertension. The addition of chlorothiazide to other available hypotensive agents, such as reserpine and ganglion blocking agents, permitted much easier control of blood pressure.[35] The same benefit from addition of thiazide diuretic drugs has since been demonstrated with all hypotensive agents in current use.

It is standard practice to introduce the thiazide initially and add other treatment as required. It is particularly important to combine thiazides with beta adrenergic blocking agents, which may occasionally precipitate heart failure, or with drugs that may produce edema such as reserpine, methyldopa or peripheral vasodilators. The effect of the benzothiadiazines is not related to their diuretic activity.[35] A complete separation of diuretic and hypotensive action is seen in the case of diazoxide, which causes sodium and water retention but which is nonetheless the most powerful hypotensive agent currently available.[29-32]

Controlled trials have demonstrated that the potent natriuretic agent frusemide is a less effective agent in controlling blood pressure than hydrochlorothiazide.[34] This serves to emphasize that diuresis and natriuresis are not major factors in relation to the hypotensive effect of this group of drugs.

Spironolactone: In a double-blind controlled study in patients with severe refractory hypertension we demonstrated that addition of spironolactone in a dose of 400 mg daily may have a dramatic effect.[35,36] This effect of spironolactone may be very important in some patients, particularly those in whom orally administered diazoxide is contraindicated.

Methyldopa: For the past 10 years methyldopa has played an important part in the control of severe hypertension. Large doses have often been needed to achieve control. The reduction in side effects and

better control of the supine blood pressure that can be achieved with beta adrenergic blocking agents[38] will probably mean that these agents will gradually replace methyldopa as the most commonly used hypertensive agent. This trend is already apparent in our clinic.[36]

Clonidine: Clonidine, which acts predominantly on the central nervous system, can play a key role in the treatment of severe hypertension.[47,48] Like methyldopa, it is being replaced in our clinic by the beta adrenergic blocking agents, particularly in view of the success achieved by a combination of these agents and peripheral vasodilators. The major disadvantage of clonidine is that large doses may produce drowsiness and, at times, coma.[35] We have observed these effects mainly in patients with poor renal function receiving a combination of clonidine and beta adrenergic blocking agents.[35,36] Other side effects that may limit the usefulness of clonidine in severe hypertension are the sudden increase in blood pressure, agitation and insomnia that may accompany withdrawal.[49]

Sympatholytic agents: Sympatholytic agents such as guanethidine, bethanidine and debrisoquine have been replaced by other agents in our clinic over the past decade.[36] In an occasional patient, one of the sympatholytic drugs still provides good control, but the fall in the blood pressure that occurs on standing and such disadvantages as alterations in blood pressure with physical activity and temperature make these agents far less acceptable than other forms of treatment such as beta adrenergic blocking agents.[38] When the blood pressure cannot be adequately controlled with a beta adrenergic blocking agent and a thiazide, the addition of a peripheral vasodilator will permit adequate control with few side effects in most patients.[36] The large doses of sympatholytic drugs required for adequate control of severe hypertension make the side effects particularly troublesome.

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The Effect of Dietary Sodium on the Blood Pressure of Normotensive Man*

(Preliminary Report)

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Introduction

Epidemiologic studies in the United States and abroad have indicated a strong relationship between the level of habitual salt intake and the prevalence of hypertension [1–6]. Hypertension is infrequent in population groups whose habitual diet is low in sodium and high in potassium, the customary and natural diet in man through his evolutionary history. In the New Guinea Highlands, for example, the intakes of electrolytes are potassium 200 mEq/day and sodium

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10 mEq/day with a potassium-sodium ratio of 20. There hypertension is rare [5, 6].

On the other hand, hypertension is highly prevalent in populations which ingest a high sodium but low potassium diet, one in which salt is added to the food. In the United States, for example, a typical diet contains potassium, 100 mEq/day, sodium, 200 mEq/day, with a potassium-sodium ratio of 0.5 [8].

Animal studies relating sodium intake to hypertension usually have been carried out in rats [9-11]. These rodents are particuarly susceptible to high sodium intakes in regard to the development of chronic systolic blood pressure elevation. The blood pressure response in rats to salt intake is affectd by many factors, including age, sex, and heredity [12]. This effect has not been noted in the cat, rabbit, and dog [13]. There have been a few studies which include the manipulation of both sodium and potassium intake and data from these show that rats eating excess sodium chloride plus extra potassium chloride have an increased survival rate and moderation of the hypertension compared to lower potassium controls [7, 14].

Metabolic studies in man relating salt intake to blood pressure elevation are few in number [15–18]. Most have followed the protocol of adding salt to a low sodium control diet and following blood pressure with certain metabolic and electrolyte measurements. These studies have not usually controlled dietary potassium levels, and where sodium of over 300 mEq/day was added to the diet, observations were not prolonged beyond two weeks except in one patient [19]. Studies generally have been done in hypertensive patients.

In the course of a study unrelated to the effects of dietary salt, two normal subjects were inadvertently given a total of 350 mEq sodium/day from a baseline intake of 260 mEq. Subsequently, in two weeks they developed blood pressure elevation. When the sodium content of their diet was reduced to 80 mEq/day, their blood pressure returned to the baseline of normal values. These findings suggested that the increased blood pressure might have resulted from the large amount of dietary sodium. For this reason, a prospective study of the relationship of salt and blood pressure was developed. We studied the blood pressure and certain physiologic functions in normotensive man after prolonged (1 month) exposure to low (10 mEq/day), moderate (250 mEq/day), and high (410 mEq/day) sodium intake. Potassium content was maintained constant so that the potassium-sodium ratios varied from 10 to 0.24.

In the study we explored the questions:

1. Will large intakes of dietary sodium (compatible with intakes of some Americans) cause increased blood pressure in man?
2. Will these diets have an effect on vascular reactivity, body fluids or electrolytes with or without changes in blood pressure?

This study is unique because previous workers have not monitored sodium intake for as long as one month in normotensive subjects, have generally not controlled potassium intake in human studies, and have not measured vascular reactivity on a regular schedule in patients with low and high salt intakes. Since the studies have been completed and analyzed on only four of the six volunteers, these results are necessarily preliminary.

Materials and Methods

The subjects were white male prisoner volunteers between the ages of 27 and 47 years. All were within 20% of normal weight, as indicated by height and weight tables. The men were physically healthy, as determined by history, a routine physical examination, urinalysis, postprandial blood sugar, blood count, serum electrolytes, creatinine clearance, and a chest X-ray. All subjects had blood pressures below 150/90 mm Hg when measured on several occasions on at least three different days.

Each volunteer was studied for a minimum of twelve weeks, subdivided into three dietary periods of at least four weeks each. Sodium in the diet varied from 10 mEq (low sodium), 210 mEq (moderate), to 410 mEq (high). The potassium in the diet was kept constant at 100 mEq/day and the potassium-sodium ratio varied from 10 to 1 with the low sodium diet to 1 to 4.1 with the high sodium intake. Variation in dietary sodium from one period to another was random and singleblind (Table I), so that physicians and nurses measuring the responses

Table I. Profile of subjects and diets

Subject	Age	Diet sequence		
		10 mEq Na	210 mEq Na	410 mEq Na
M.B.	44	1	2	3
R.H.	27	3	1	2
D.L.	40	3	2	1
J.O.	47	2	1	3

did not know the sodium intake. Early in the study, each of three patients had one dietary period which was extended from one to four weeks because of logistic problems.

The basic diet was a formula containing 40% fat from cocoa butter and peanut oil; 15% protein from powdered skim milk, Casec and Cellu; 40% carbohydrate from sucrose, corn starch and dextrimaltose; 1000 mg of cholesterol from egg yolk; 2 g of calicum, 2 g of phosphorus, 350 mg of magnesium, and sufficient calories to maintain the weight constant for each individual. To this basic low sodium, low potassium formula diet were added solutions of KCl and NaCl to increase the potassium content to 100 mEq in all diets and the sodium to 210 and 410 mEq during the moderate and high salt intakes. Calcium, phosphorus, and magnesium intake were constant for each individual, but varied slightly among different subjects depending on caloric intake. Distilled water was used in the formula, and for all other fluids ingested. The formula diet contained vitamins and mineral supplements and provided adequate nutrients as specified by the National Research Council [8].

Each volunteer was weighed daily in the morning after he had evacuated his bladder. Blood pressure was checked daily at 8:00 a.m., 12:00 noon, 4:00 p.m. and 8:00 p.m., both supine and standing by trained nurses. Systolic and fourth and fifth phase diastolic blood pressures were recorded. Mean blood pressure

was calculated using fifth phase diastolic plus 40% of pulse pressure. A 24-h urine specimen was collected daily and the volume recorded. The daily excretion of sodium, potassium, and creatinine was measured in a 24-h urine aliquot initially, and subsequently, after the first three months of this study, from pooled weekly urine samples. Measurements of urinary excretion of calcium, magnesium, and phosphorus were done weekly. Serum sodium, potassium, chloride, blood urea nitrogen, serum creatinine, cholesterol, triglycerides and creatinine clearance were measured weekly. Methods employed to analyze these substances were as follows: creatinine [20], blood urea nitrogen [21], cholesterol [22], triglycerides [23], calcium [24], magnesium [25], and phosphorus [25]. Sodium and potassium in the urine were measured by atomic absorption spectrophotometry [26].

During the final week of each dietary period, total body water [27], exchangeable sodium [27], total body potassium [28], inulin clearance [29], and inulin space [30] were measured. On another day of the last week changes in forearm blood flow (plethysmograph), intra-arterial blood pressure and heart rate [31] were studied in response to 1) pooling of blood in the lower extremities by exposure of the lower half of the body to subatmospheric pressure, a maneuver known to trigger sympathetic adrenergic activity and reflex vasoconstriction [32], and 2) to intra-arterial norepinephrine and tyramine [33].

Blood for peripheral vein renin was drawn in all subjects at 11:00 a.m. on the final week of the study after the subject had been up for 4 h. Plasma renin activity was analyzed by the method of Pickens et al. [34]. Twenty-four hour urine was collected for aldosterone excretion in the 24-h period after the blood for the plasma renin activity was drawn. Aldosterone was analyzed by Bioscience Laboratories, Los Angeles, California, who used the method of Kliman and Peterson [35].

Results

a) Blood Pressure (Tables II, III, and IV). There was no change in either systolic, diastolic (recorded as muffling or disappearance of sound) or mean blood pressure during any of the three periods when the subjects were supine or upright. Also, there was no consistent change in any one subject during the study. Despite this, the intra-arterial blood pressure measured in two patients at the time of the vascular reactivity studies were higher with increased dietary sodium. Also, when one compared the blood pressure values from all of the control periods during the vascular reactivity studies, the same trends were maintained (Table IV). Thus, not only did the average blood pressure rise in subject J.G. from 76–84 mm Hg and in M.B. from 90–97 mm Hg as the salt intake was increased from 10–410 mEq/day but the intra-arterial blood pressure values obtained when the subjects were on the 210 mEq of sodium daily were higher than during the 10 mEq diet in 4 of the 6 observations when there was a change.

b) Sodium Excretion (Tables II and III). There was a progressive increase of sodium in the urine from a mean of 16 mEq/day when the subjects were on the 10 mEq diet to 166 mEq/day while on the 210 mEq sodium diet, 291 mEq/24 h

Table II. Separate values for each subject on the three diets

Dietary sodium	Subj.	Mean B.P. mm Hg		Wt. kg	24 h Urinary excretion							ml/min CrCl	Blood chemistry				
					Total	Na	K	Ca	Mg	P	Creat.		mEq/l		mg-%		
		L	S		Vol. ml	mEq	mEq	mg	mg	mg	mg		Na	K	Bun.	Chol.	Trig.
10 mEq	MB	90	89	79.3	2761	18	a	b	b	b	1408	101	141	4.3	17	199	153
	RH	86	86	77.2	3261	19	65	349	231	559	2032	139	139	4.5	14	175	141
	DL	94	95	72.2	2213	2	26	183	127	615	1060	66	140	4.7	13	209	163
	JG	93	94	74.3	4144	26	52	295	221	1145	1760	107	140	4.6	12	218	147
210 mEq	MB	93	89	80.5	2733	191	71	340	123	855	1231	85	139	4.4	17	204	145
	RH	88	80	78.1	3316	209	75	401	182	1048	1518	146	137	4.4	13	182	131
	DL	98	96	71.7	2769	121	50	161	112	603	836	54	141	4.2	12	201	159
	JG	93	98	74.1	4367	141	58	267	165	883	1308	91	144	4.8	12	204	137
410 mEq	MB	92	89	79.3	2620	270	72	504	139	890	1531	106	142	4.3	18	191	160
	RH	84	80	78.4	3327	383	85	412	170	933	1642	138	141	4.6	10	171	140
	DL	97	95	72.3	2380	196	57	133	108	664	884	66	142	4.7	12	200	169
	JG	94	94	74.7	4304	315	62	330	212	1158	1598	99	142	4.6	11	231	141

a Intake varied.
b Not obtained.
Blood pressure and body weight represent values obtained in the 4th week of the study. The urine excretion of Na and K and total volume are values from the 2nd and 3rd weeks of the study because some urine samples were lost during the 4th week of the study when many other tests were done. The Ca, Mg, P and creatinine excretion and blood chemistry values are the mean of weeks 2, 3, and 4.

Table III. Averages for four subjects on each diet

Dietary sodium	Wt. kg	Mean B.P. Lying	Mean B.P. Stand.	TBW L	In space L	C In ml/min	C Cr ml/min
10 mEq	75.8	91	91	45.2	11.2	106	101
210 mEq	76.1	93	91	45.8	12.6	112	91
410 mEq	76.2	92	90	45.5	13.1	127	102

Dietary sodium	PVR Ng angio/ml	U aldo µg/day	Exch. Na mEq	TBK mEq
10 mEq	6.0	40	3120	3826
210 mEq	3.1	15	3252	3854
410 mEq	2.7	17	3317	3538

TBW – Total body water; In space – Inulin space; C In – Inulin clearance; C Cr – Creatinine clearance; PVR – Peripheral vein renin activity; U aldo. – Urinary aldosterone; Exch. Na – Exchangeable sodium; TBK – Total body potassium.

while on the 410 mEq sodium diet. There was no suggestion that any of these patients had diarrhea during the study and spot checks on stool sodium indicated that less than 5 mEq of sodium was lost in the stool per day. The subjects were observed while they took the formulae and there was general acceptance. The volunteers were observed in the Clinical Research Center and the urine volume and other electrolyte excretion indicated that the urine was not lost consistently.

Aliquoting of the formula diets for determination of sodium and potassium content was done routinely. The 10 mEq Na diet was consistently found to contain 15–20 mEq of Na. Analysis of the higher salt formulas varied from 80–120% of the calculated intake. The salt solution which was added to the higher sodium formula was always found to be accurate ±0.01% on analysis. Therefore, since >90% of the sodium was provided by this solution in the 210 and 410 mEq Na diet, the 40% variation in analysis is thought to be from difficulties in the aliquoting procedure and not from variations in the dietary intake. The potassium content of the formulas was found to be 60–80% on analysis. Since more of the dietary potassium was provided by food sources, it

Table IV. Control observations of mean intra-arterial blood pressure (mm Hg) during vascular reactivity studies

	Diet	I	II	III	IV	Ave.
JO	10	70	77	80	76	76
	210	84	72	83	71	78
	410	86	81	87	83	84
MB	10	87	90	90	92	90
	210	90	90	90	95	91
	410	97	92	98	100	97

is reasonable that the actual potassium intake was less than 100 mEq, but consistently so. On the 10 mEq Na diet, the mean sodium excretion of 16 mEq/day mirrors the analyzed dietary intake (15–20 mEq/day). There was great individual variation in the amount excreted, ranging from 2–26 mEq. None of the subjects were thought to be in negative sodium balance. It is well known that adults can achieve sodium balance with intakes below 10 mEq/day [15]. There was no evidence that our subjects had any disease, particularly renal disorders, and there was no evidence of progressive signs of sodium depletion over the one month of low salt intake.

Mean sodium excretion on the higher salt diets was 70–80% of intake, and there was considerable day-to-day and individual variation. It is possible that these variations were due to sweat sodium losses which were unmeasured in this study and to potent antinatriuretic stimuli [36].

c) Peripheral Plasma Renin Activity and Aldosterone Excretion (Tables III and VI). Peripheral plasma renin activity decreased in all four patients as the sodium content of the diet was increased from 10–410 mEq. Values in the fourth week of the 210 mEq sodium diet were lower in two of the three subjects than were the values during the 10 mEq sodium diet period. Also, urinary aldosterone excretion decreased in all four individuals as they moved from a diet of 10–410 mEq of sodium. The aldosterone values obtained after four weeks of the 210 mEq diet were also lower than during the 10 mEq sodium session. However, two of these values were lower and two were higher than the aldosterone values obtained while the subjects were on the 410 mEq sodium diet. These responses generally are consistent with the known effect of dietary sodium on renin activity and aldosterone excretion [37].

d) Body Weight (Tables II and III). Body weight measured in the fourth week of each session increased a mean of 0.4 kg as the diet was changed from 10–410 mEq of sodium in random order. Three of the four subjects had an increase in their body weight during the higher sodium intake. The values for body weight during the 210 mEq sodium period were intermediate between those obtained for the other two intervals.

e) Inulin Space (Tables III and VII). Inulin space was used as a measure of extracellular fluid. In each subject as the salt content of the diet rose to 410 mEq of sodium, inulin space increased as compared to 10 mEq sodium intake period. Values for the inulin space while the subjects were on the 210 mEq diet were higher in one instance than on the 10 mEq diet and lower in three subjects than values obtained on the 410 mEq diet. Thus, in general, there was a rise in extracellular fluid which paralleled the increase in salt intake. This was mirrored by the average figures for the four subjects shown in Table III.

f) Glomerular Filtration Rate (Tables II, III, and VI). Glomerular filtration rate measured by the clearance of inulin increased with increasing sodium intake in 7 of 8 diet alterations. The mean inulin clearance rose from 106 ml/min while the subjects were on the 10 mEq sodium diet to 112 on the 210 mEq and 127 on the 410 mEq sodium diet. However, clearances of creatinine (Table III) were much more variable than inulin clearance and the increase in creatinine clearance with increasing sodium intake was not seen. There was enormous variation in creatinine excretion from day to day which may have accounted for our inability

to recognize a trend in this measurement. It is of interest that in three of the four subjects, the blood urea nitrogen and serum creatinine were lower in the 410 mEq sodium intake period than during the period of 10 mEq sodium intake.

g) Excretion of Calcium, Phosphorus, Potassium, and Magnesium (Tables II and V). There was a progressive increase in potassium excretion as sodium intake increased. Since there was no increase in dietary potassium as salt intake went up, the increase must have been associated with either an increased

Table V. Averages for four subjects in each diet

Dietary sodium	24 h Urinary excretion						
	(ml) Total Vol.	Na mEq	K mEq	Ca mg	Mg mg	P mg	(mg) Creat.
10 mEq	3095	16	48	276	193	773	1565
210 mEq	3296	166	64	292	146	847	1236
410 mEq	3158	291	69	345	157	911	1414

potassium load presented to the kidney or a change in the renal handling of potassium. It is of interest that in the two subjects in whom we have total body potassium measurements on all three diets, there was a loss in total body potassium of 281 and 434 mEq as they moved from the 10 mEq sodium diet to the 410 mEq sodium one (Table VI). There was no significant change in the urinary excretion of calcium, magnesium or phosphorus during the three experimental intervals although there was a tendency for magnesium excretion to decrease and phosphate excretion to be greater as sodium intake rose (Table II).

h) Serum Sodium, Potassium, Cholesterol, and Triglycerides (Table II). Serum sodium increased in all four subjects as the dietary intake of salt increased from 10–410 mEq. Although consistent, these changes were small. There were no directional changes noted in the values for the other serum components.

i) Total Body Water, Exchangeable Sodium, and Total Body Potassium (Tables III and VI). There was an average increase in total body water in the two subjects in which this was measured in each of the three dietary periods, but the actual figures indicate that total body water went down in one instance at the 410 mEq diet, and went up in the other. On the average, total body potassium fell as sodium intake increased, but there was a great variation from one session to the next. Exchangeable sodium rose in three of the four patients as they went from 10–410 mEq of sodium with an average increase of about 200 mEq of sodium during the session on the higher salt intake.

j) Vascular Reactivity (Tables IV and VII). As noted previously, during the baseline observations intra-arterial blood pressure was higher with the higher salt intakes in the two subjects studied. But subjects had less vasoconstrictor response to −40 mm Hg lower body negative pressure when on the 10 mEq sodium diet than when on higher salt intakes. Also, although the response was

Table VI. Separate values for each subject on the three diets

Dietary sodium	Subj.	C In ml/min	In space L	TBW L	Exch. Na mEq	TBK mEq	PVR ng/ml	Urine aldo. pg/day
10 mEq	MB	101	14.7	43.8	3220	5488	8.3	31
	RH	107	13.6	49.8	3710	*	7.3	44
	DL	106	8.4	*	2774	3784	2.4	35
	JG	109	8.3	42.2	2777	4007	6.1	51
210 mEq	MB	103	13.8	46.1	3211	*	*	16
	RH	130	13.6	49.1	3613	5379	2.2	15
	DL	110	*	46.5	2891	4019	3.7	7
	JG	103	10.6	41.7	3254	3688	3.3	21
410 mEq	MB	110	15.2	43.1	3859	*	3.8	9
	RH	148	15.0	51.5	3541	*	1.4	22
	DL	127	9.7	47.8	3021	3503	2.2	26
	JG	122	12.6	*	2847	3573	3.3	9

* Not done.
See Table III for abbreviation explanation.

not a graded one, the forearm blood flow and blood pressure responses to norepinephrine in both men indicated a greater vasoconstrictor response while on the higher salt intakes compared to responses during the 10 mEq sodium diet intake. The responses to tyramine did not indicate any particular pattern.

Discussion

The question of importance to which we addressed ourselves was whether salt in amounts consumed by man in the Western World causes or unmasks high blood pressure. Despite epidemiologic and animal studies which suggest that this might be possible in humans with good kidney function, there is little or no direct evidence to support the hypothesis that hypertension can be produced in this manner.

McQuarrie and his colleagues [38] administered diabetic children the adult equivalent by body weight of 70–140 g of NaCl. With this large amount of salt, they observed an increase in body weight of about 5% and an increase in both systolic and diastolic blood pressure of 30–50% above control levels. McDonough and Wilhelmj [39] gave 37 g of NaCl daily above the usual salt intake to a normotensive person and caused facial puffiness and progressive increase in blood pressure. Perera and Blood [40] reported that 4 and 15 g of NaCl added to a salt poor diet increased blood pressure in 5 of 6 hypertensive patients. Gros, Weller, and Hoobler [41] found no increase in blood pressure in 3 normotensive volunteers when their daily sodium intake was increased from 191–279 mEq. They followed the patients from 2–9 weeks. Brown, Brown, and Kirshan [42] gave diets containing 20 mEq and 200 mEq of sodium to 8 normotensive volunteers and studied them after 6 days on each of the two diets. They found an increase in exchangeable sodium, an increase in body weight and serum sodium but no change in plasma volume or mean arterial pressure.

Table VII. Effect of lower body negative pressure (LBNP), tyramine and norepinephrine infusions on forearm blood flow (FBF) in 2 subjects with 3 different salt intakes

	Dietary sodium mEq/day	Control		LBNP −20		LBNP −40		Control		Tyramine 5/min		Tyramine 18/min		Tyramine 36/min		Control		Norepinephrine 0.0375/min		Norepinephrine 0.075/min		Norepinephrine 0.15/min		Control	
		FBF	MAP	FBF	MAP	FBF	MAP	FBF	MAP	FBF	MAP	FBF	MAP	FBF	MAP	FBF	MAP	FBF	MAP	FBF	MAP	FBF	MAP	FBF	MAP
G	10	5.5	70	4.3	72	4.1	70	7.0	77	3.7	77	2.3	78	2.6	80	5.7	80	4.2	75	3.8	69	3.7	72	6.0	76
	210	5.6	84	4.2	82	4.2	81	4.6	72	4.1	78	2.1	78	—	—	4.4	83	3.7	79	3.3	72	2.8	77	4.3	71
	410	5.0	86	4.8	81	3.9	81	5.4	81	5.0	75	3.1	89	2.7	94	5.7	87	5.5	94	3.6	73	2.7	75	4.5	83
M	10	4.8	87	2.6	83	2.5	80	5.4	90	3.9	94	2.5	91	2.4	86	4.4	90	2.9	87	2.3	86	2.6	92	4.3	92
	110	4.6	90	2.4	88	1.9	90	5.5	90	4.2	88	2.7	88	3.0	88	4.8	90	2.7	88	2.6	87	2.5	87	7.1	95
	410	10.0	97	6.1	93	6.6	90	6.7	92	5.9	90	3.9	97	4.9	92	4.5	98	4.2	98	3.8	97	3.7	98	4.2	100

FBF = Forearm blood flow (ml/min); MAP = Mean arterial blood pressure (mm Hg); LBNP = Lower body negative pressure (mm Hg); Tyramine dose in mcg/min; Norepinephrine dose in mcg/min.

We gave our volunteers three different sodium intakes for at least four weeks since it was suspected that salt, except in the very susceptible individual, would not rapidly induce hypertension. Dahl has shown that in adult rats, sensitized to salt ingestion, one must continue a high salt diet for at least two to three weeks before blood pressure elevation develops and many animals do not have a rise for another one to three weeks [43]. We selected the upper level of salt intake as 410 mEq since many patients coming to our Hypertension Clinic excrete over 300 mEq of sodium in their urine per day. In order to avoid variables which might be the result of age, sex or race, we limited our observations to middle-age, white, adult males. To insure patient compliance, we studied these patients in a well-controlled environment of a clinical research unit.

Even with relatively small numbers, there was a consistent increase in weight, extracellular volume and serum sodium. There were also less well-defined trends toward an increase in exchangeable sodium and in potassium and phosphate excretion, a decrease in magnesium excretion or in total body potassium with higher salt intakes. There was no consistent change in total body water. Importantly, blood pressure measured indirectly showed no increase either in the supine or upright position. Nevertheless, mean intra-arterial blood pressure measurements on two patients studied showed a distinct increase as dietary sodium rose from 10–410 mEq/day. Since the intra-arterial measurements were made at the time of vascular reactivity studies, one might assume that anxiety associated with the examinations might have provoked rises in pressure. Even if this were so, the excellent correlation of the higher pressures with the higher sodium intake is not explained. It is most likely that the blood pressure rises were associated with either an increase in cardiac output or peripheral resistance. Resting forearm blood flow was not significantly different between the various dietary sessions and it seemed on the basis of this, that an increase in peripheral resistance is the most logical explanation for the blood pressure rise.

The increase in inulin clearance and presumably glomerular filtration rate was impressive. This effect of a sodium load has not previously been reported in man although it is known to occur in dogs [45]. In general, acute sodium loads are usually excreted from the kidney by a decrease in proximal and distal sodium reabsorption [44]. The regular increase in inulin clearance suggests that there was either a greater perfusion pressure to the kidney or an increase in efferent arteriolar tone of glomerular vessels. Creatinine clearance did not parallel inulin clearance since it stayed roughly the same with increasing salt intake. We are inclined to believe that the inulin clearance data is more representative of the hemodynamic differences after salt ingestion since there was enormous day-to-day variation in creatinine excretion which has been noted by others [46, 47]. In this regard, it is of interest that the inulin clearance increase paralleled the increase in intra-arterial blood pressure observed in the two subjects so studied.

The studies of vascular reactivity are of great interest, even though these preliminary results show only trends. Obviously, one could have a change in vascular smooth muscle either locally or in autonomic nervous system function with various salt intakes which might cause an altered response to standard

stimuli. Indeed, it is conceivable that such alterations in vascular reactivity or tone might appear before blood pressure elevation could be recognized. Although there were suggestions that such changes had occurred in the two patients studied, there was not adequate consistency to place great emphasis on these results and we must await completion of the remainder of the studies to recognize significant differences.

There was no change in serum cholesterol or triglyceride during the series of diets. This is in contrast to the observation in rats and dogs [48] that chronic salt ingestion is associated with a rise in serum lipids. However, since the dogs were fed for 1–4 years, and the rats from 2–6 months, it is possible that, in comparison, our studies were not prolonged enough to duplicate those findings.

The decrease in total body potassium and the increase in urine potassium with increasing doses of salt was not expected. Baldwin, Alexander, and Warner [36] studied 13 patients with varying levels of salt intake and found that urine potassium excretion was generally constant despite changes in NaCl loading. The reduction in total body potassium and the increase in urinary potassium excretion occurred at a time when both plasma renin activity and urinary aldosterone excretion were relatively low. It seems unlikely therefore that the renin-angiotension-aldosterone system was responsible for the change in potassium excretion. It is known that with acute sodium loading, potassium excretion will rise [49]. The duration of the kaluresis is not known since careful long-term studies of potassium balance after chronic salt administration have not been reported. On the basis of our observations, one can not be certain whether the increase in potassium excretion is the result of physical displacement of potassium from stores by sodium, from the increased potassium load presented to the kidneys or from some other mechanism, such as an unmeasured change in acid-base balance.

What do these observations mean? It appears evident that normotensive white males respond to low sodium diets much like hypertensives [15]. They respond to a high salt diet in a manner which indicates that after one month, a new steady state exists which is conducive to the development of higher blood pressure. Whether arterial hypertension actually develops with high salt diets may depend on genetic predilection, time of exposure and other factors such as neurogenic and hormonal stimuli which apparently cause exaggerated vascular responses when excess salt is present.

During this study, the potassium-sodium ratio changed from 10 to 0.24. It is not possible with our experimental design to separate the effect of the change in this ratio from any effect which might have been caused by dietary salt changes. Nevertheless, it is recognized that the relationship of these two major cations may be more important in producing cardiovascular changes than the level of sodium alone.

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EFFECTS OF CHRONIC EXCESS SALT INGESTION

INHERITANCE OF HYPERTENSION IN THE RAT*

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In man, hereditary factors are recognized as essential in the etiology of hypertension. The genetic expression is, however, difficult to assess because it is not known how many genes are involved, how they interact with one another and with environmental factors, or by what mechanisms they exert their influence.

In rats, the genetic influence on blood pressure regulation is definitely established. If similar factors operate in humans, rat colonies may provide prototypes of heredity and blood pressure regulation for the study of human health and disease. Work at this laboratory demonstrated in 1962 that the development of salt-induced hypertension strongly depended upon the genetic background of the rats used (1, 2). By selective breeding, two colonies were derived from a single Sprague-Dawley strain. In one colony the rats rapidly developed hypertension on the same salt (NaCl) intake to which members of the other colony were resistant. Later we confirmed these differences with other techniques generally used to induce experimental hypertension. These techniques included: DOCA plus NaCl, unilateral renal artery compression without NaCl, cortisone, adrenal regeneration, and uninephrectomy without NaCl (3–5). If these experimental models have relevance to man, then several forms of human hypertension would also require for their emergence an appropriate genetic substratum which depends upon environmental determinants for its expression (4).

In the present work, which extended over a six year period, we have explored the mode of inheritance of hypertension in these two strains of rats. Approximately 2000 animals were studied in an effort to determine the number of genes involved. The data indicated that regulation of blood pressure is multigenic; the probable number of genes was calculated to be from two to four. The simplest model compatible with our data consists of two nonlinked, autosomal, diallelic loci with a sex-modified expression of the genes at one locus.

Materials and Methods

Rats were housed three to a cage in air-conditioned rooms, lighted for 10 hr each day. The food pellets were made to order and contained a total of 8% NaCl (w/w). Pellets and

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tap water were always available. Systolic blood pressures (BP)[1] were measured by our modification of the tail microphonic method of Friedman and Freed (6).

All rats were weaned at 21–23 days of age and started on the 8% NaCl diet; they were observed continuously thereafter for 52 wk unless they died from natural causes before that time. BP and weight were recorded once a week from wk 4–12 after weaning, every other week from wk 14–24, and every 4 wk thereafter. If there was a sustained weight loss of more than 10 g the values obtained after the weight-loss started were not used in the analyses, thus avoiding the inclusion of secondary effects. In many cases this precaution may be unnecessary, but it has been consistently applied in all our recent work (7).

The generations were designated as indicated in Fig. 1: R and S stand for parent generations, F_1 and F_2 for the first and second filial generations, BR and BS for the backcrosses between F_1 and the R or S parent population, respectively.

Parent Populations.—R and S rats from our own colonies were used. The development of these strains, and their reaction to dietary salt has been reported in several papers (1–4, 7–9). On a low sodium diet both strains thrive and develop normally; S rats will slowly reach a BP of about 140 mm Hg after a year, whereas R rats will average about 120 mm Hg. A diet containing 8% NaCl is remarkably well tolerated by R rats, but it devastates S rats; after 3–4 wk their BP starts to climb and between 6 and 12 wk after start of this diet most die with a BP in excess of 200 mm Hg. In the R colonies, on the other hand, females have been uniformly resistant; even after 1 yr there is no significant difference in their weight and only a moderate increase in BP when compared with control groups on low salt diets. R males are less predictable as they grow older and we are still collecting data on their long-term reaction to 8% NaCl. After 1 yr on this diet, they have shown individual variations in weight, morbidity, and blood pressure; many animals have presented systolic BP in the range 140–160 mm Hg.

We discuss our R and S colonies as if they were genetically homogeneous, but variations occur in both colonies. We therefore test our breeders by the performance of their first litters: weanling S rats are given 8% NaCl in their food, are expected to develop blood pressures above 180 mm Hg after 8 wk, and to die within 3 months. R rats are given 8% NaCl in their food and triiodothyronine (Cytomel(R)) 0.05 mg/liter in their drinking water; on this regimen their blood pressures should remain below 140 mm Hg after 8 wk. Only if the first litter passes the test will the parents participate in the breeder pool. The original breeders for both strains, furthermore, came from the same line of Sprague-Dawley rats (1, 2). The early generations were obtained by brother–sister matings, but when polydactyly, syndactyly, runting, and infertility began to occur we developed breeding pools of 40 males and females from each strain by testing each pair on the basis of their progeny.

Crossbred Populations.—From 13 R and 13 S litters by tested breeders, one male and one female rat were selected and mated as shown in Fig. 1. The matings were planned to insure equal representation of the same genetic lines in both reciprocal mating types. Nonproductive matings forced us, however, to compromise both in the F_1 and other hybrid populations.

We followed each individual rat for a year or until it died. The rats that participated in the study could therefore not be mated and we had to duplicate the F_1 generation to get breeders for the F_2 and backcrosses. The F_1 rats used for further breeding were kept on a low sodium diet and the same was true for the R and S parents. The information about parental response to high salt applies, therefore, to the population from which the breeders were drawn, and is not based on direct observations of the actual parents. To analyze the results we used the methods developed for population genetics (10). For each of the permutations shown in Fig. 1 we observed approximately 100 rats.

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[1] Abbreviations used in this paper: BP, systolic blood pressure; SHR, spontaneously hypertensive rats; V_g, genetic variance; V_r, random variance; V_t, total variance.

OBSERVATIONS

F_1 Generation.—From 26 matings we obtained 19 litters of F_1 rats for observation. A group of 7 litters had no reciprocal matching of same lineage, while 12 litters could be arranged in 6 pairs of double first cousins. The average blood pressure of the whole group was intermediate to the R and S values. When the sexes were analyzed separately, the male average was close to the midparental average while the female mean was closer to the R parents. Sex, therefore, appeared in F_1 rats to have a profound influence on the phenotypic expression of the genetic and environmental determinants. This was also reflected in the

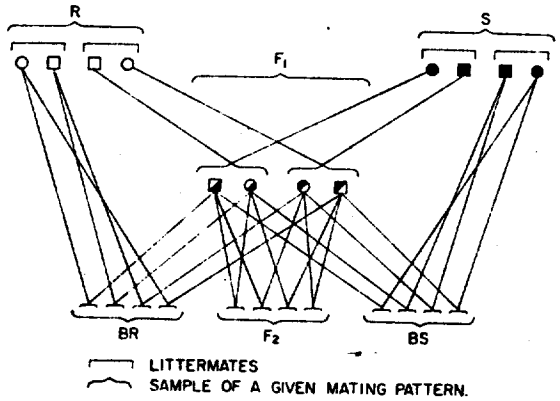

FIG. 1. Diagram of matings for genetic study. From 13 R and 13 S litters, a male and a female were selected and crossmatched to produce the F_1 generation. For the F_2 and backcrosses (BR and BS) a sufficient number of littermates were selected to have each line represented in all the permutations of mating patterns.

fact that within every litter but one the males had the higher blood pressures. Table I presents the cumulative average values after 24 wk of observation.

On the other hand there was no evidence for a sex-linked inheritance. Factorial analysis of the matching litters supported the conclusion that reciprocal matings produced equivalent offspring. A hierarchical analysis of variance involving the entire F_1 generation in the progression (sex, mating type, litter, individuals) further showed that in comparing rats of the same sex, littermates were likely to be more uniform than rats chosen at random from different litters. This is interpreted as evidence that there was a genetic variance in the parent populations.

For Tables I and II, the last reliable blood pressures of dead animals (cumulative values) were included in the results. To show the influence of mortality on the results, we entered in Table III "actual" and "cumulative" averages for each sex at selected intervals. The actual values are those obtained on the

TABLE 1

Blood Pressure Values, Standard Error, and Number of Rats of Each Sex in F_1 Litters

Mating (type dam × sire)	Sex		Mean blood pressure *, SEM, and No.‡ of rats															
			Nonmatching litters							Matching litters						Totals		
																Sex	Cross	All
(S × R)	Female	Mean	125	135						156	136	129[d]	123	136[d]	149	135	(Mean) 147	
		SEM	4.0	5.1						11.2	3.9	5.1	4.7	6.4	8.1	2.4	(SEM) 2.6	
		No.	8	8						5	5	10	6	7	7	56	(No.) 93	
	Male	Mean	147	182						180	158	143[d]	172	148[d]	185	164		(Mean) 148
		SEM	5.8	10.1						10.8	9.6	7.0	5.9	9.8	9.3	4.0		(SEM) 1.6
		No.	3	3						5	4	7	5	4	6	37		(No.) 212
(R × S)	Female	Mean			124	135	142[d]	126	165[d]	128	133	126	128	124	126	132	149	
		SEM			5.0	14.2	4.5	2.3	11.1	4.0	3.5	5.9	5.0	2.0	4.1	2.2	2.0	
		No.			3	4	9	5	3	7	3	6	8	2	4	54		
	Male	Mean			150	160	141[d]	160	178[d]	158	172	159	173	170	156	162	119	
		SEM			4.4	5.3	4.0	6.0	4.4	4.5	2.2	2.5	11.6	3.2	3.5	1.9		
		No.			8	6	3	5	8	6	2	8	5	8	6	65		

Except in one litter, males had higher average pressure than female littermates. The difference was significant ($P < 0.05$) in all litters except for the four marked *d*.

Each column represents one set of reciprocal matings of littermates. No entry indicates that only one of the reciprocal matings succeeded. Values are calculated from readings taken 24 wk after weaning, or from the last reliable pressure previous to that date.

* Mean, mean blood pressure in millimeters of Hg.

‡ No., number of rats.

survivors at a given time. The table also gives the number and fraction of survivors. In the F_1 generation males had both a higher blood pressure and a higher rate of mortality than females.

F_2 Generation.—Similar observations for the F_2 generation (also given in Table III) revealed that male F_2 rats had average blood pressures equivalent to

TABLE II

Analysis of Variance of F_1 Generation

SOURCE	DF	MS		F	
		Main	Partitioned		
Among sex	1	44773		189.24	0.[illegible]
Among mating type, within sex	2	166		0.70	0.[illegible]2
Female	1		203	0.86	0.3[illegible]51
Male	1		129	0.55	0.4[illegible]01
Among litter, within mating type and sex	34	808		3.41	0.0000
Female, S × R	7		789	3.33	0.0024
Female, R × S	10		527	2.23	0.0183
Male, S × R	7		1488	6.29	0.0000
Male, R × S	10		625	2.64	0.0051
Among individuals, within litter, mating type, and sex	174	237		1.00	
Total	211				

The sex difference was highly significant ($F = 189.24$, $P < 0.01$). There was no difference between the reciprocal mating types ($F < 1$; $P > 0.2$) indicating no sex-linked inheritance. The differences between litters were significant for both sexes and both mating types ($F = 2.23$–6.29; $P < 0.02$) indicating a genetic heterogeneity in one or both parental strains. DF, degrees of freedom; MS, variance; F, Snedecor's F ratio; P, probability. The column headed "partitioned" refers to subdivisions within each main source of variance.

male F_1 rats, but a different mortality curve; the F_2 had a higher mortality at the beginning of the year, and a higher survival ratio at the end. The F_2 females had higher average blood pressures than F_1 females. The sex influence on blood pressure phenotype was thus less obvious in the F_2 than in the F_1 generation. Although the average for males was significantly higher than for females ($P < 0.01$), the absolute difference between sexes was less, and there were litters in which females had higher pressures than males.

Backcrosses.—In the backcrosses the average pressures were intermediate to

TABLE III

[illegible] Standard Error, and No. of Rats of Each Sex in the Crossbred Populations after Different Periods on High Salt Diet

Cross		4 wk				12 wk				24 wk				36 wk				48 wk			
		Ave.*	Cum.‡	No.§	f‖	Ave.	Cum.	No.	f	Ave.	Cum.	No.	f	Ave.	Cum.	No.	f	Ave.	Cum.	No.	f
		mm	mm			mm	mm			mm	mm			mm	mm			mm	mm		
F_1	F	141 (1.35)	141 (1.35)	110	1.00	133 (1.50)	133 (1.50)	110	1.00	134 (1.69)	134 (1.63)	104	0.95	141 (2.21)	141 (2.09)	97	0.88	138 (2.30)	142 (2.03)	69	0.63
	M	147 (1.45)	147 (1.45)	102	1.00	152 (1.86)	155 (2.03)	96	0.94	159 (1.70)	163 (1.89)	90	0.88	179 (3.91)	175 (1.97)	31	0.30	171 (4.73)	173 (1.75)	8	0.08
F_2	F	137 (1.44)	137 (1.44)	202	1.00	135 (1.87)	137 (1.96)	184	0.91	143 (2.20)	148 (2.29)	172	0.85	147 (2.51)	154 (2.48)	164	0.81	153 (2.09)	161 (2.21)	136	0.67
	M	142 (1.34)	142 (1.34)	216	1.00	148 (1.89)	154 (1.93)	178	0.82	160 (1.66)	166 (1.68)	156	0.72	166 (1.55)	171 (1.48)	113	0.52	162 (1.76)	170 (1.47)	75	0.35
BR	F	113 (1.01)	113 (1.01)	209	1.00	119 (1.11)	119 (1.17)	205	0.98	122 (1.06)	123 (1.12)	202	0.97	128 (1.27)	128 (1.32)	199	0.95	126 (1.07)	127 (1.17)	188	0.90
	M	121 (0.96)	121 (0.96)	209	1.00	130 (1.25)	129 (1.25)	203	0.97	139 (1.28)	139 (1.30)	198	0.94	145 (1.45)	146 (1.45)	185	0.89	145 (1.40)	148 (1.38)	143	0.68
BS	F	155 (1.55)	155 (1.55)	185	1.00	168 (3.11)	192 (2.62)	89	0.48	170 (3.16)	196 (2.43)	70	0.38	177 (3.04)	201 (2.15)	58	0.31	175 (3.70)	202 (2.09)	38	0.20
	M	159 (1.63)	159 (1.63)	196	1.00	176 (4.15)	197 (2.16)	53	0.27	176 (2.97)	200 (1.95)	37	0.19	183 (2.88)	202 (1.84)	21	0.11	171 (6.14)	201 (1.88)	7	0.04

All reciprocal mating types in each cross are combined; sexes are presented separately. Males have a significantly higher blood pressure than females in F_1, F_2 and BR crosses, but not in BS. In all crosses, males have a higher mortality than females.

* Ave., average blood pressure (± standard error) for surviving rats.

‡ Cum., cumulative values (last reliable pressures of dead rats included in results).

§ No., Number of surviving rats.

‖ f, fraction of survivors relative to the number present at 4 wk.

mm, millimeters of Hg.

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F_1 and the respective parent strains. BR rats (R × F_1) evinced a demonstrabl sex influence on the phenotype while no sex difference in blood pressure coul

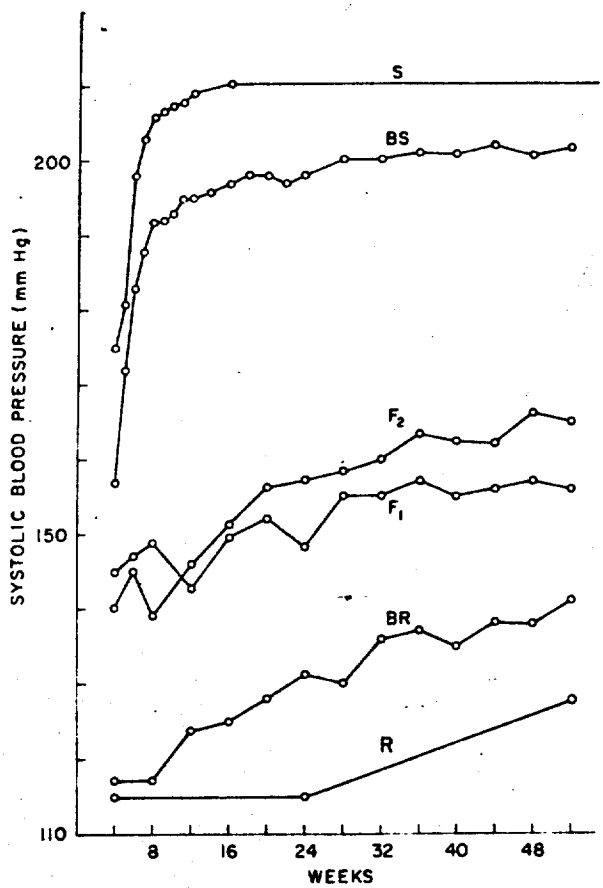

FIG. 2. Average blood pressures of different rat populations on 8% NaCl. The curves give the average cumulative systolic blood pressure for each cross during a year of observation. Although R, BR, F_1, and F_2 showed significant differences in BP based on sex (males higher than females), the values have been pooled for the purpose of this graph which illustrates the definite genetic influence on the reaction to salt intake.

be shown for the BS (S × F_1) rats. In all crosses represented in Table III the males had higher mortality rates than females.

The information tabulated in Table III is presented graphically at closer time intervals in Figs. 2 and 3, which also includes data from representative R and S populations.

The data show conclusively that mendelian inheritance provides etiological factors for hypertensive disease developing as a result of high salt intake. To

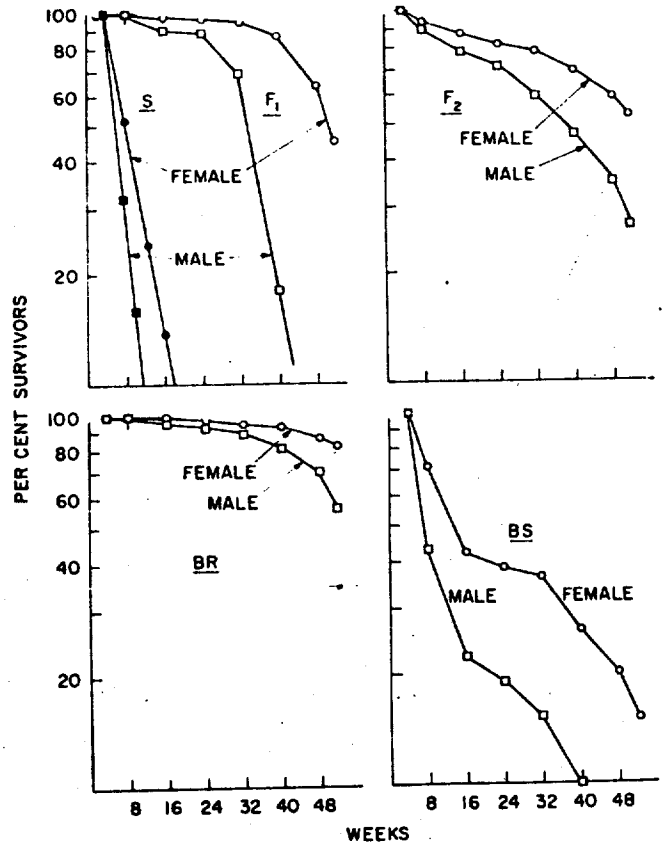

FIG. 4. Survivors in different rat populations on 8% NaCl. The graphs demonstrate how mortality on 8% NaCl parallels blood pressure development (see Fig. 2) and has a significant element of genetic determination. R rats are not shown. Mortality among R females during the first year cannot be correlated to salt or blood pressure; data on R males indicate practically no mortality in the first 36 wk and better than 80% survival after 48 wk. In all these populations exposed to high salt diet we have thus observed a higher mortality rate among males than among females. Note that coordinate is logarithmic.

investigate the mode of inheritance the cumulative values at 24 wk (the last reliable pressures of dead rats are included in the averages) are presented with sample statistics in Table IV. The choice of 24 wk was a compromise. The time was long enough to separate "pure" S rats from hybrids and provide some spread in the blood pressures of the latter, but short enough to avoid the high

mortality figures caused by chronic hypertensive disease. However, the sex influence on BP of R rats was not fully demonstrated; the observed sex difference was not statistically significant, but increased in the following months and was probably real. The statistics show that S rats had the highest averages and

TABLE IV

Observations after 21 Wk on 8% NaCl

Cross	Sex	No. of rats observed	Mean blood pressure	Variance: Total V_t (measured)	Variance: Random V_r (estimated)	Variance: Genetic $V_g = V_t - V_r$	SD due to genetic variance ($=\sqrt{V_g}$)	Heritability $V_g/V_t \times 100$
			mm Hg				*mm Hg*	%
R	Female	50	114	94	94	0	0	
	Male	28	123	151	150	0	0	
S	Female	70	212	660	660	0	0	
	Male	48	210	1146	650	396	20	
F_1	Female	108	134	294	220	74	8.6	
	Male	102	163	366	390	<0		
F_2	Female	202	148	1038	300	758	27.5	74
	Male	216	166	613	400	213	14.6	35
BR	Female	209	123	262	150	112	10.6	43
	Male	209	139	353	240	113	10.6	32
BS	Female	185	196	1090	570	520	22.8	48
	Male	196	200	743	390	153	12.4	20

Last reliable pressures of dead rats are included in the results. Compare SD to estimates from model in Table VI.

V_t, total variance. Value calculated from data. V_r, variance caused by random factors. For the R populations this variance is determined by independent tests. For the other populations it has been estimated by assuming the total S female variance to be random, and interpolate for average blood pressures. V_g, genetic variance. Calculated by subtracting V_r from V_t. The considerable genetic variance in S male and F_1 female is probably an indication of the uncertainty of the V_g estimates. $V_g/V_t \times 100$, heritability, or degree of genetic determination. Part of V_g in females is due to dominance. Strictly, the expression should include additive genetic variance, only. The male data may therefore be more relevant.

the largest variances. Repeated weekly readings on R rats have shown that most of the variance found in R populations is due to uncontrollable factors such as physiologic fluctuations, metabolic state, and anesthesia. The rapid blood pressure increases in the terminal phase of the S rats prevented a similar test for that strain and must have worked to increase the observed variance in the present study. Since blood pressures were read at weekly intervals the last reliable pressure could have been recorded in some animals days before the peak

value was reached. Hence, in the S parent population, the observed average values are presumably lower, and the variance larger, than we might have found by a continuous, or even daily, record of the pressures.

Genetic variances in the F_2, BR, and BS populations are only partly responsible for the observed phenotypic variance (V_t) entered in Table IV, as these populations must be subject to the same random fluctuations as the more homogeneous groups. If we assume that the data on R and S females represent the random variances at average readings of 114 and 212 mm Hg respectively, and further assume a linear relationship between random variance and average BP, we may estimate the expected random variance of the other population samples by interpolation. These estimates are given in the column V_r in Table IV. The environment was kept as uniform as possible: there was no change in rooms, personnel, food supply, or working routine during the years of observation. The difference between total variance (V_t) and random variance (V_r) is therefore the best available estimate of genetic variance (V_g). Table IV lists the estimated genetic variances and the corresponding standard deviations (SD).

INTERPRETATION

The high variance of the S males, and the high average BP of the BS females do not fit a simple, noncontroversial genetic model. Except for these data the findings are consistent with the hypothesis that blood pressure regulation and the constitutional tendency to hypertensive disease depend on several autosomal genes, that in males the effects of these genes are additive while in females the allele for lower blood pressure is dominant at one or more of the loci. Fig. 4 displays mean blood pressures from Table IV. The male averages fall close to a straight line, while the female averages show dominance. To fit a straightforward genetic pattern the BS females should have averaged 175 mm Hg.

We can arrive at estimates of the degree of genetic determination (heritability) and the number of genes involved by using the equations derived by Wright (10). From the male data we calculated two to four genes and a heritability of 20–35% (Table IV). Due to dominance these equations are not as applicable to the female data. The strains were fairly well established by the third generations (1, 2), indicating that the number of genes involved must be small; this is consistent with the present estimates.

The observations that blood pressures increase with age at different rates in different rats, that mortality acts as a selection against high pressures, that the parent populations probably are not uniform, that only the males seem to express an additive effect of the genes, and that environmental factors have different effects on the different genotypes all warn against placing too much emphasis on any given number. We have tested models with two, three, and

four nonlinked autosomal diallelic loci. There is no unique solution; in general the more complex models can be manipulated to correspond better with the observations. We feel, however, that any complex model should be justified by independent evidence. Random fluctuations so cloud the results that any purely genetic model which describes these particular data with reasonable success is

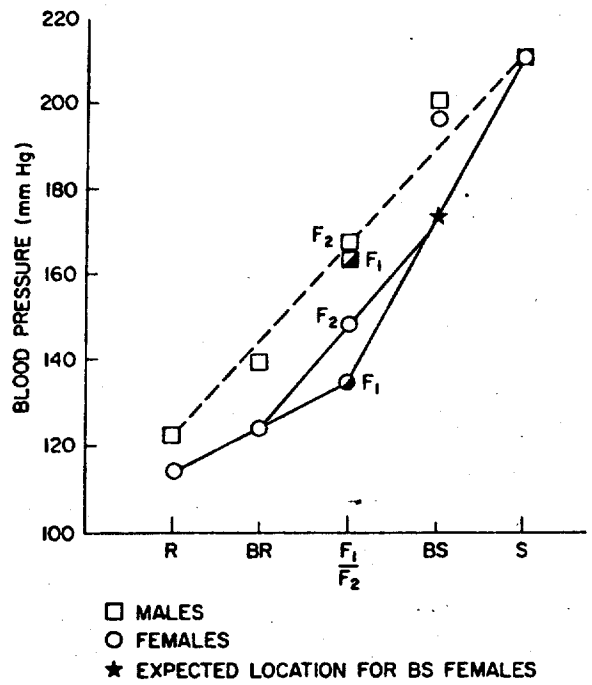

FIG. 4. Mean blood pressure of each sex in R, S, and cross bred populations at 24 wk. The values are consistent with an additive genetic effect in the males, and dominance in the females. However, the BS females broke the pattern; the asterisk indicates the location which would have fitted with the other data.

likely to be as correct as one which reproduces them in great detail based on speculative assumptions.

We also tested the consequences of logarithmic transforms of the observations. They improved the form of the distribution curves and made the variances of the different populations more equal. They did not, however, remove the difficulties with the S male, and BS female data. As in the case of complicated models, we feel that transforms should be justified by physiological reasoning, not introduced solely to improve sets of data.

If we tentatively ascribe the deviations as due to sampling variance in the

selection of breeders, the simplest model that gives a fair prediction of the findings consists of two nonlinked diallelic loci. The allelic effects are additive in the male but the R allele dominates at one locus in the female. Ascribing the gene values 0 to the R alleles and 1 to the S alleles, the locus where R is dominant can only contribute 2 (for genotype 11), or 0 (for genotype 01 and 00) to the total genotypic values of the females.

If we make the simplifying assumption that R and S strains are uniform (00)/(00) and (11)/(11) respectively, we may calculate probabilities of combina-

TABLE V

Calculation of Genotype Distribution in F_2 Generation

				Locus A				
				Male			Female	
	Genotype			11	01	00	11	01 00
		Value		2	1	0	2	0
			f	0.25	0.50	0.25	0.25	0.75
Locus B (Both sexes)	11	2	0.25	0.0625	0.125	0.0625	0.0625	0.1875
	01	1	0.50	0.125	0.250	0.125	0.125	0.375
	00	0	0.25	0.0625	0.125	0.0625	0.0625	0.1875

	Genotype value				
	0	1	2	3	4
Female, f	0.1875	0.375	0.250	0.125	0.0625
Male, f	0.0625	0.250	0.375	0.250	0.0625

Assumptions: 2 autosomal, nonlinked, diallelic loci. R and S parental strains homozygous at both loci. Values additive over loci. At one locus, in the female only, the 0 allele dominates. Otherwise the allelic effects are additive.

Genotype distributions for the other crossbred populations were calculated in the same manner.

f, frequency.

ions as demonstrated for the F_2 generation in Table V. The predicted distribu-
ions for all crosses are given in Table VI. Plotting average genotypic values
rom this table against the observed average blood pressures in Table IV gives
a good fit for all but the female BS rats (Fig. 5). The rank correlation between
he predicted genotypic and the observed phenotypic variances is also sig-
nificant. ($p < 0.05$).

DISCUSSION

Colonies with genetically determined elevations of BP have been studied by others. In 1958, Smirk and Hall first reported on one such colony isolated by

Their rats differ from ours in at least one respect; their's develop hypertension on low salt intake (17). In a study of the inheritance in SHR rats, Louis, Tabei, Sjoerdsma, and Spector (18) found several autosomal loci and concluded that the SHR were not completely homogeneous as regards their hypertensive genetic complement. They also found higher average values in males than in females.

Our findings are thus in general agreement with those on similar rat populations and raise the possibility that all these strains have become selected for the same mutations. If not, the completed model must be more complex than the one under discussion here.

Alexander, Hinshaw, and Drury (19, 20) studied a strain of spontaneously hypertensive rabbits and concluded that the trait was inherited. They found higher values in males than in females and the range of BP was similar to that of rat and man. Schlager and Weibust (21–23) have analyzed the genetic control of BP in mice. Among several strains, they found that variations within the normal range were genetically determined. By crossing the strains, they concluded that several genes were involved. The effects were additive over loci in the males, whereas the female mouse represses the elevation of BP (23). They calculated the heritability as 20% in males and 77% in females (21, 22). These conclusions agree with ours. They furthermore reported in their early studies a possibly greater influence of the genetic contribution of the sire (22), an observation compatible only with some of our preliminary results (24). We later found, however, that spurious evidence for sex-linked inheritance appeared because a high-risk line of the R rats were disproportionately represented in one of the reciprocal matings. (In subsequent mouse studies, no evidence for sex-linked inheritance has been observed. [Schlager, G., personal communication]).

We have also tested our model by trying to predict segregation into parental phenotypes and by estimating frequency distributions of blood pressures in each cross. We further tested the consequences of extrapolating the model to man. There is a peril in manipulation of numbers on the basis of assumptions. Nonetheless, the results suggest that model and data are compatible. The additional assumptions appear reasonable, were consistently applied, and may constitute clues to as yet unexplored relations.

Segregation of Hybrid Colonies into Parental Phenotypes.—We define the parental phenotypes as follows: R animals live 48 wk on 8% NaCl in the diet and their blood pressures remain below 140 mm Hg; S animals die within 16 wk on the same diet. These definitions are too strict as nearly half of the R males fail to live up to this ideal, but they permit a comparison of crosses by counting, rather than by biometric methods. In the F_1 population, 30% of the females and none of the males were phenotype R. We therefore assigned all of genotype O and 30% of genotype 1 to phenotype R; we further assumed that geno-

types 3 and 4 gave rise to phenotype S and that genotype 2 was in between. On this basis we predicted the number of phenotypes in each cross and compared this with the actual counts. Table VII gives the results. The model with these assumptions predicts a higher segregation into R phenotypes in the male F_2 and BR rats than we observed. To remove this discrepancy, the model and assumptions must be modified to allow for a sex difference in the R rats.

Frequency Distributions of Blood Pressures in Hybrid Colonies.—We may use the model to predict frequency distributions in each cross if we assign a blood

TABLE VII

Predicted and Observed Segregations into Parental Phenotypes

Cross	Sex	R		S	
		Predicted	Observed	Predicted	Observed
		%	%	%	%
R	Female	100	100		
	Male	100	50		
S	Female			100	85
	Male			100	100
F_1	Female	30	30	0	2
	Male	0	0	0	9
F_2	Female	32	20	18	15
	Male	14	2	31	24
BR	Female	66	61	0	2
	Male	41	18	0	3
BS	Female	8	0	50	58
	Male	0	0	75	78

pressure range to each genotype. Fig. 5 indicates that each unit increase in genotype adds 20–25 mm Hg to the average pressure. This and the increase in variance from R to S phenotypes formed the basis for assuming the following correlations:

Genotype	Systolic BP (mean ± SD)	
	Female	*Male*
0	115 ± 10	120 ± 10
1	140 ± 14	140 ± 14
2	165 ± 18	165 ± 18
3	190 ± 22	190 ± 22
4	215 ± 26	215 ± 26

Then we applied these distribution curves to the genotype combinations presented in Table VI, and compared them with the observed distributions. The results and the goodness of fit are presented in Table VIII and in Figs. 6 and 7.

Frequency Distributions of Blood Pressures in Human Populations.—In applying the model to data from human populations we assumed, in analogy to the rat, that genotypes 3 and 4 give rise to malignant hypertension, that 1 and 2 provide candidates for essential benign hypertension, and that genotype 0 is normotensive. (Further, to make estimates of gene frequencies possible, we stipulated that malignant hypertension occurs in less than 1% of adults, twice as

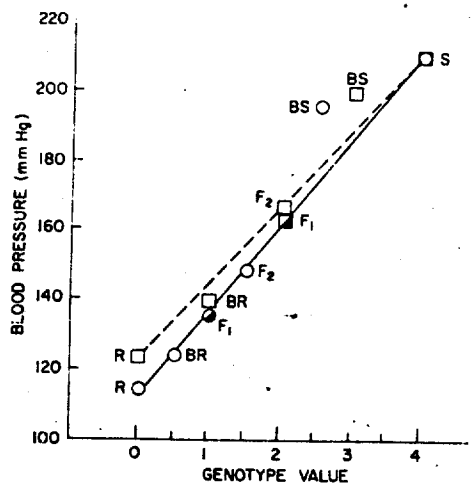

FIG. 5. Mean blood pressure of each cross and sex vs. mean genotype value. □, male; ○ female.

frequently in males as in females, while 70% or more of the population is normotensive.) The gene frequency $P = 0.13$ for the hypertensive allele at both loci led to a satisfactory development. In a randomly mating population, these gene frequencies give the genotype distributions calculated in Table IX. The same correlations between blood pressure ranges and genotypes that we used for rats (see above) lead to the frequency distributions of blood pressures which are presented in Table X and Fig. 8. They compare favorably with observations on adults in the age group 35–44 yr (25). The critical issue is not so much the "goodness of fit" in this example. Other age groups in the same study (25) showed different distributions, and other studies have presented other distributions for this particular age group. But all studies of young adult human populations show a pattern similar to panel *d* in Fig. 8, and this is the type of distribution pattern the model generates. (Fig. 8, panel *c*). The example there-

TABLE VIII

Expected and Observed Distribution of Blood Pressures in Different Rat Populations

(mm Hg)	F_1				F_2				BR				BS			
	Female		Male		Female		Male		Female		Male		Female		Male	
	Exp.* No.	Obs.* No.	Exp. No.	Obs. No.	Exp. No.	Obs. No.	Exp. No.	Obs. No.	Exp. No.	Obs. No.	Exp. No.	Obs. No.	Exp. No.	Obs. No.	Exp. No.	Obs. No.
<-99					3.0	4	0.5		7.9	2	1.0	1				
100-109	1.7	3			10.8	16	2.9	2	28.0	34	9.2	6	0.8			
110-119	7.1	15	0.6	1	19.7	17	8.7	2	46.7	58	25.2	17	3.2	1	0.3	
120-129	18.4	27	2.1	1	22.5	26	15.4	6	41.5	56	35.9	48	8.8	6	1.3	1
130-139	29.2	34	6.0	8	25.4	23	21.3	17	33.5	32	37.5	42	15.4	3	3.8	1
140-149	28.5	17	12.6	14	26.7	27	25.6	26	27.6	8	34.5	36	18.9	7	8.6	7
150-159	17.1	1	19.5	22	22.9	22	26.9	40	16.2	8	26.2	28	18.9	9	15.2	3
160-169	6.3	7	22.3	22	18.1	20	26.4	32	5.9	4	17.4	13	18.3	9	21.5	10
170-179	1.4	3	18.9	13	14.6	8	21.2	28	1.3	2	11.0	11	18.0	14	25.7	21
180-189	0.2	3	11.9	10	11.5	12	20.1	23	0.2		6.3	6	17.2	22	26.9	22
190-199			5.5	8	8.6	9	15.4	20			2.8	0	15.8	26	25.4	29
200-209			1.9	1	6.3	9	11.0	10			1.0	1	13.9	15	21.7	26
210-219			0.5	0	4.5	2	7.5	5			2.0		11.5	18	16.8	20
220-229			0.1	2	3.1	4	4.7	3					8.8	23	11.8	21
230-239					2.0	1	2.7	1					6.2	14	7.7	17
240-249					1.2	2	1.5	1					3.9	5	4.6	14
250-259					1.0		1.2						2.2		2.5	2
260-269													1.1		1.2	2
270-279													0.7		0.8	
X^2		36.9		3.9		9.1		25.5		31.9		11.0		75.6		50.3
DF		5		6		11		11		7		8		12		11
P		<0.001		>0.50		>0.50		<0.01		<0.001		>0.20		<0.001		<0.001
Total no. obs.		110		102		202		216		209		209		184		196

The calculations of predicted distributions are based on the assumptions outlined in the text. The observed (Obs.) data are the cumulative observations after 24 wk. (Last reliable values of dead rats are included). The distributions are graphically presented in Figs. 6 and 7.

* No., number of rats.

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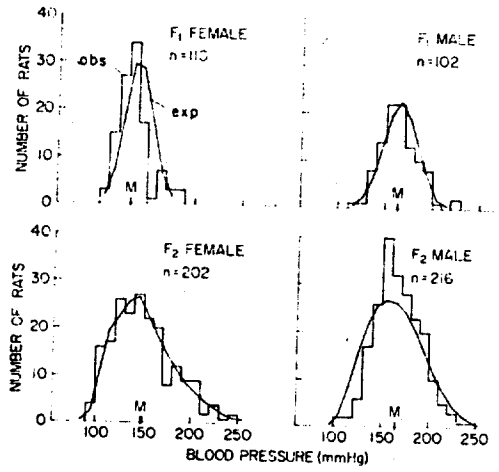

FIG. 6. Comparisons of expected and observed blood pressure distributions for each cross and sex. Histograms present observations, frequency polygons present expectations. *M* stands for mean.

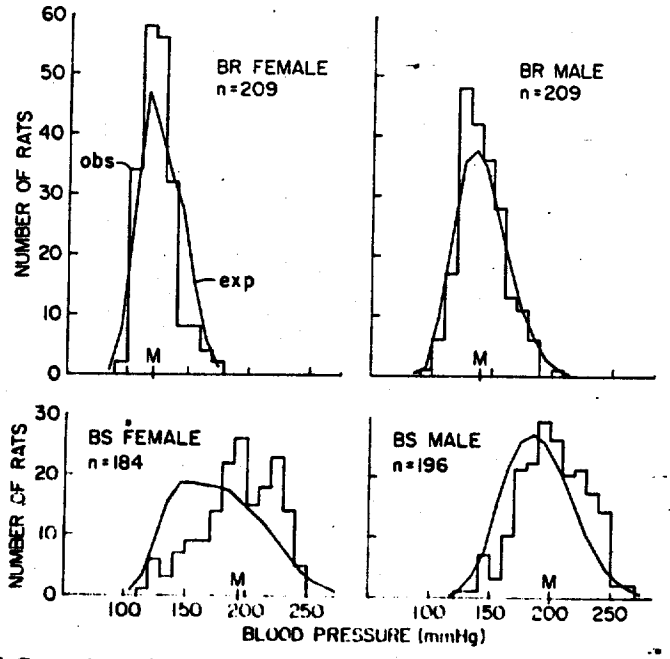

FIG. 7. Comparisons of expected and observed blood pressure distributions for each cross and sex. Histograms present observations, frequency polygons present expectations. *M* stands for mean.

fore strengthens our hope that it will be possible to design a genetic model based on rat experiments and apply that model to a set of human data.

The administration of a number of drugs and hormones as well as the development of endocrine and renal diseases affect the blood pressure of rat and man in a similar manner. Whether the analogy extends to epidemiological and genetic studies still has to be proven, but circumstantial evidence holds forth promise in this respect. Doyle and Fraser studied the correlation between vascular reactivity and hypertensive disease and showed that a hereditary pattern is com-

TABLE IX

Genotype Distributions for a Randomly Mating Population Where the Gene Frequency is 0.13 at Each Locus for the Hypertension-Producing Allele

				Locus A				
				Male			Female	
	Genotype			11	01	00	11	01 00
		Value		2	1	0	2	0
			f	0.0169	0.2262	0.7569	0.0169	0.9831
Locus B (Both sexes)	11	2	0.0169	0.0003	0.0038	0.0128	0.0003	0.0166
	01	1	0.2262	0.0038	0.0512	0.1712	0.0038	0.2224
	00	0	0.7569	0.0128	0.1712	0.5729	0.0128	0.7441

	Genotype value				
	0	1	2	3	4
Female, f	0.7442	0.2224	0.0294	0.0038	0.0003
Male, f	0.5729	0.3424	0.0768	0.0076	0.0003

Explanation of assumptions in text. The calculations are based on the proposed model and an assumed frequency of the allele for high pressure of 0.13 at each locus.

f, frequency.

mon to both these aspects of circulatory function (26). The analogy to the findings that hypertension-prone rats have increased vascular reactivity is obvious (12, 13). The epidemiological studies of Dahl and Love (27) and of Isaacson, Modlin, and Jackson (28) on the correlation between average salt intake and prevalence of hypertension in different populations, parallel closely the findings of Meneely et al. on unselected rat populations (29). Our S and R colonies indicate that salt sensitivity is dependent on the presence of certain genes in the rat. It is reasonable to expect that the same may be found to be true for man and would be compatible with the conclusions of Thomas and Cohen that genetic factors are modified by environmental agents (30).

Many studies on selected human populations have been published with a number of contradictory observations and often conflicting interpretations of

TABLE X

Expected and Observed Blood Pressure Distributions in Human Populations, Based on the Model and Observations of the U.S. Health Survey

		<90	90–99	100–09	110–19	120–29	130–39	140–49	150–59	160–69	170–79	180–89	190–200	>200	Total No. observed
Expectation	Female, %	0.5	5.0	19.1	29.9	20.9	10.2	6.5	4.1	2.0	0.9	0.5	0.2	0.1	
	Male, %		1.5	8.9	22.2	25.0	16.8	10.9	6.9	3.7	2.0	1.1	0.6	0.5	
Female 25–34 yr	Exp., No.	60	563	2163	3378	2363	1150	736	457	230	100	54	26	24	11291
	Obs., No.	102	928	2440	4174	2255	935	222	116	73	34	14			
Female 35–44 yr	Exp., No.	66	614	2361	3687	2580	1255	804	499	240	109	55	29	25	12325
	Obs., No.	16	391	2134	3571	2794	1761	747	456	245	87	102	21	20	
	Obs., %	0.13	3.17	17.31	28.97	22.67	14.29	6.06	3.54	1.99	0.71	0.83	0.17	0.16	
Male 25–34 yr	Exp., No.		158	911	2286	2567	1728	1124	708	381	203	112	57	44	10281
	Obs., No.		203	1129	2390	3187	2025	927	311	88	8	13			
Male 35–44 yr	Exp., No.		175	1007	2528	2839	1912	1243	783	421	224	124	63	50	11373
	Obs., No.		95	1012	2755	2894	2153	1171	703	267	240	21	12	50	
	Obs., %		0.83	8.89	24.22	25.44	18.93	10.30	6.18	2.35	2.11	0.18	0.11	0.44	

Predicted values are calculated from the frequency distributions in Table IX, transformed into blood pressure values in the same manner as explained for Table VIII. Observed data are from U.S. Health Survey, 1960–1962. **23:** 7, 22, 25, 29, 30.

the same data. A polarization of opinions is personified by Platt and Pickering. Their views have the virtue of representing extremes and providing a convenient framework for discussions.

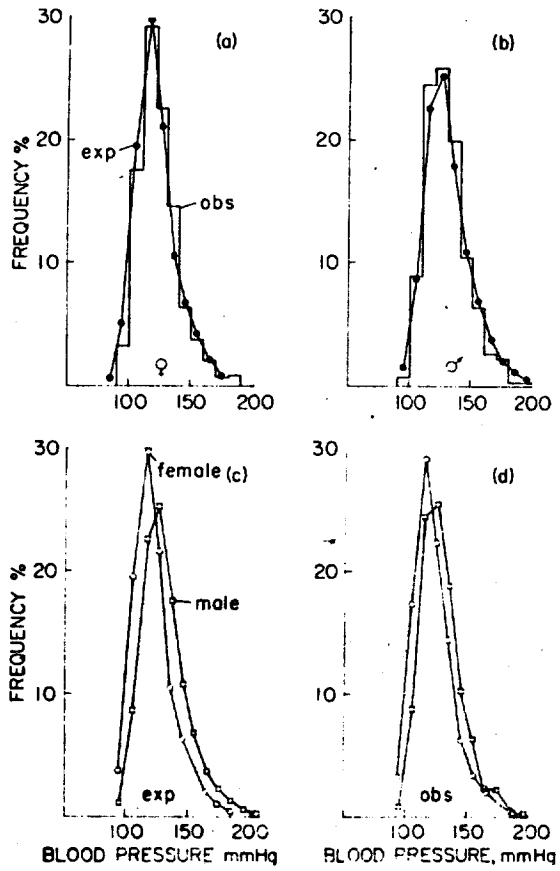

FIG. 8. Comparisons of expected and observed frequency distributions in human populations. Data for the graphs are taken from Table X. (*a*) Histogram of the male age group 35–44 yr superimposed on the predicted frequency polygon for males. (*b*) Histogram of the female age group 35–44 yr superimposed on the predicted frequency polygon for females. (*c*) Comparison of male and female distributions generated by model. □, male; ○, female. (*d*) Comparison of male and female curves observed in age group 35–44 yr.

Platt views essential hypertension as a clinical entity, qualitatively different from the normotensive state, and characterized by an inherited tendency to develop high blood pressure in middle life. He does not rule out multigenic inheritance but considers a single gene with partial dominance as a sufficient hy-

pothesis. Environmental variance does not occupy a major place in his arguments. Pickering claims that a number of genes are involved, that blood pressure resembles stature in that it has a continuous and unimodal frequency distribution, that no natural dividing line can be drawn between normotension and hypertension, and consequently that the concept "essential hypertension" has no useful function in the clinic. Both schools base their analyses on distribution curves in the general population, comparison of relatives of hypertensives and of controls, and studies of blood pressures in twins.

At the root of these controversial interpretations is the fact that a number of nongenetic influences confound the results. While most studies permit the conclusion that heredity is important for the development of blood pressure diseases, the data do not yield to an impartial analysis of the quantitative aspects of this inheritance. One has to adopt a point of view and look for inconsistencies; if none occur, the data are compatible with the postulated model. Whenever discrepancies disturb, imponderable features may be invoked to explain them. Pickering and Platt do not agree on the ground rules. Pickering's school uses sex- and age-adjusted scores developed by Roberts (31) rather than the raw data as a basis for analysis. Platt's thesis denies the validity of such scores; some persons will show an increase in blood pressure with age, others will not. In Platt's words: "If we are going to study essential hypertension it is necessary to study people at the age of 50 to 60 or at the widest, 45 to 65." (32)

We followed our rats for one year. The natural life span of the rat is 2-4 yr and the reproductive period is about 15 months (33). By analogy it would correspond to epidemiological studies in man restricted to the age group below 30-50 yr, the age when Platt wants studies to start. Since we have not observed the rats into senium, we have no way of knowing whether the scores developed by Pickering's school have any relevance to our rat populations; conversely, we can test our model only against data obtained on young adults.

For this reason we have been unable to compare specific predictions of our model with the data presented by Miall and Oldham from two populations in Wales (34). They calculated regression coefficients for the scores of first degree relatives and found average values of 0.3. Our model would predict regression coefficients for the raw data from 0.3 to 0.5, but we cannot match their values or make allowance for the effects of scoring except in general terms.

Our observations suggest that modifying factors, genetic and environmental, are multiple but that the main tendency to hypertensive disease may be explained by as little as two determining genetic components. The difference between malignant hypertension and the more common varieties of essential hypertension may be one of degree; it takes a combination of strong genetic and environmental agents to produce the malignant forms. In one respect the data favor Platt's view; there is an inherited difference among rats in the rise in blood pressure we observe with age. Miall and Lovell found that the increase in man observed over a 4 yr interval depended on the level of the initial pressure rather

than on the age of the individual (35). The same pattern is evident in our rats. Individuals with high pressures have a tendency to rise further, while those in the low blood pressure ranges tend to remain low. Age adjusted scores may therefore be of value in comparing populations of mixed genetic composition, but be quite misleading when applied to individuals or to members of a single family.

SUMMARY

Two strains of rat have been developed by selective breeding: one strain (R rats) is resistant to salt hypertension, the other strain (S rats) is highly susceptible. The inheritance of these traits has been explored in the first (F_1) and second (F_2) generation of crossbred rats and in backcrosses between parent and first filial ($F_1 \times R$, $F_1 \times S$) generations. Male F_1 rats had an average blood pressure close to the mid-parental (R and S) values, and the average of F_2 males was equivalent to that of F_1. Male offspring of F_1 with R, or F_1 with S also showed averages close to the respective mid-parental values. Female offspring showed deviations from this linear relationship, indicating a significant dominance in the female for the genes of normal blood pressure. A model of two autosomal, nonlinked diallelic loci, with a dominance deviation at one locus in the female, gave predictions with a reasonable agreement to the observed values. The same model also appeared compatible with human data if we assume a gene frequency of 0.13 for the hypertensinogenic allele on both loci. Random fluctuations in blood pressure, and incomplete homogeneity of parental strains permit several alternative models. The major conclusions are: that more than one locus is needed to explain the findings though as few as two loci may possibly suffice; the allelic effect seems additive in males, but there is a sex-determined influence on the expression in females; there is no consistent evidence for sex-linked inheritance. Furthermore, this model developed from the study of rats may provide a framework for analysis of human data.

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Hypertensive Vascular Disease Produced by Salt

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EXPERIMENTAL EVIDENCE has been accumulating that salt may play a significant role in the production of hypertension. Several investigators have reported that increase in salt consumption alone results in elevated blood pressure.[6, 7, 14, 15, 17, 23] Most of these studies were of relative short duration. The present work describes the injurious effects of continuous ingestion of excess salt over a long period of time. The results show clearly that such a regimen results in the production of hypertension and vascular and renal disease.

METHOD

Young, adult, white male rats weighing about 180 Gm. were divided randomly into experimental and control groups. The former was placed on a diet of pellets* and 1% sodium chloride for drinking and the latter on pellets and tap water. The diets were offered ad libitum. No other procedures of any type were carried out. The two groups differed only in respect to their drinking fluid. All animals were housed and maintained in similar manner.

The animals were weighed periodically. Blood pressure was measured by the plethysmographic technic. Readings were taken during the week prior to experiment and about once weekly thereafter. Pressures over 150 mm. Hg were considered hypertensive. On fairly numerous occasions, at intervals of

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* Supplied by Rockland Farms, New City, N.Y. Sodium content is 0.88%.

1 to 16 months, fluid intake, urinary output, and quantitative albuminuria (Esbach method) were measured over a 24-hour period. Blood urea nitrogen was determined prior to onset of the experiment, at occasional intervals thereafter, and routinely at the time of death or sacrifice. Terminally many animals also had analyses for total serum protein and albumin-globulin ratio. Autopsies were performed on all animals and microscopic sections were made routinely of practically every organ except brain. Heart and kidneys were weighed, the former after excising the atria and expressing the blood content. Cardiac indices were derived from the formula $\frac{\text{heart wt.}}{\text{body wt.}} \times 1000$.

There was a total of 25 experimental and 28 controls rats. The latter were sacrificed at time intervals corresponding to the death of the experimental animals.

RESULTS

Control Rats

These were in good condition, gained weight as expected, and remained normotensive during the course of the experiment. Only one animal, sacrificed 15 months after start of the experiment, showed necrotizing vascular disease. This consisted of polyarteritis nodosa and was limited to a segment of mesentery.

The majority of kidneys revealed no abnormality. However, some animals did show glomerular lesions such as exudation of plasma into the capsular space, adhesions, hyalinization, and accumulation of fat. The corresponding tubules contained colloid casts. Tubular atrophy with interstitial fibrosis and lymphocytic exudate was present occasionally. As a rule all these changes were focal and relatively insignificant, although in a few animals they were more diffuse.

Fluid intake ranged from 22 to 75 cc. daily, and urinary output was generally about half the intake. Albumin output in 24-hour urine specimens varied from 0.11 to 0.64 Gm. Total serum protein ranged from 4.7 to 6.1 Gm. per 100 cc. and A-G ratios from 1.1 to 1.8. Blood urea nitrogen was within normal limits.

Salt Rats

The animals died or were sacrificed from 7 to 16½ months after being placed on experiment. Hence their approximate age terminally ranged from 9 to 18½ months. As a rule, sacrifice was limited to rats in poor condition or moribund. The principal cause of death was intercurrent infection, especially pneumonia. It was apparent that the salt regimen reduced life span materially,

since the corresponding control animals were generally in good condition when sacrificed at the same time interval.

For the most part the weight curves were similar to those of the control rats. However, some animals, after about the sixth month, gained weight less rapidly than the controls or showed a more or less stationary weight. Daily intake of 1% saline varied from 15 to 70 cc. and the corresponding urinary output often equaled or exceeded intake. There were a few instances of distinct polyuria in the latter part of life among animals with well-developed renal disease.

Of the 25 salt rats, 15 (60 per cent) developed hypertensive vascular disease; 3 were hypertensive but did not show vascular lesions; 2 others had vascular lesions but exhibited no elevation of blood pressure. Thus the over-all incidence of hypertension was 72 per cent and of vascular disease 68 per cent. Five animals showed neither hypertension nor vascular disease.

Hypertension

The animals with hypertension revealed intermittent elevation of blood pressure rather than a sustained high level. The highest values—170 to 190 mm. of mercury—were usually observed in the later months of life and at this time the elevation tended to be sustained.

Vascular Disease

Lesions were often observed grossly, especially in the mesentery, as nodules

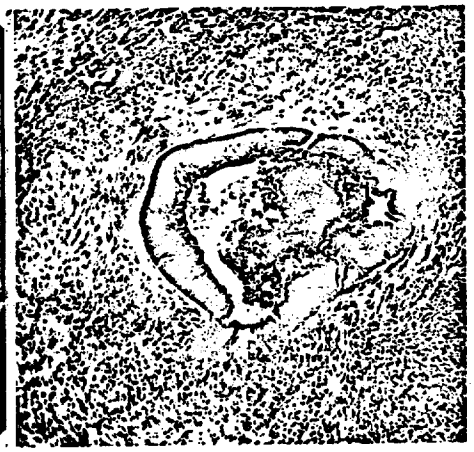

Fig. 1 *(left)*. Polyarteritis nodosa of mesenteric arteries in rat drinking 1% salt for 14 months. **Fig. 2** *(right)*. Polyarteritis nodosa of mesenteric artery in animal drinking 1% salt for 15½ months. (H&E; × 77)

near the attachment of small bowel or along the mesenteric arteries (Fig. 1). Microscopically, the lesions involved arteries and arterioles, varied in extent, and were often generalized. They were found in mesentery, pancreas, omentum, gastrointestinal tract, testis, liver, spleen, adrenal, kidney, heart, lymph nodes, and periaortic tissue.

The vascular disease was usually of the polyarteritis nodosa type (Fig. 2). However, atypical lesions such as polyarteritis without necrosis were also found, and in addition there were instances of pure fibrinoid necrosis, especially in the kidneys and heart. Variability of lesions was sometimes evident in adjacent segments of the same artery. Other vascular changes consisted of thickening of the media, intimal hyalinization and periadventitial fibrosis. These probably represented, at least in part, a chronic or healed stage of the necrotizing disease.

Renal Damage

Fig. 3. Granular nephrosclerotic kidneys in animal drinking 1% salt for 16½ months.

This was present in 16 of the 25 salt rats. The injury was graded as equivocal or slight in 4 animals, moderate in 5, and as severe in 7 animals.

The kidneys with severe damage were enlarged, showed granular outer surfaces, an indistinct cortical pattern, and resembled human arteriolar nephrosclerosis (Fig. 3). Microscopically, the main features were diffuse glomerular lesions, massive colloid cast formation, and necrotizing vascular disease (Figs. 4-6). The glomeruli showed fibrinoid necrosis, accumulation of fat, adhesions between tufts, and various degrees of hyalinization. In places there was tubular atrophy, usually associated with interstitial fibrosis and lymphocytic exudate. However, other tubules were enlarged and commonly the seat of fatty or hyaline droplet degeneration. The vascular disease involved the arterial tree from interlobar vessels to vasa efferentia and consisted mainly of fibrinoid necrosis, occasionally accompanied by proliferative and exudative components. In some animals polymorphs were found within an occasional tubule and one animal had pyelitis. Over-all, however, there was no satisfactory evidence that a pyelonephritic process was concerned in the development of the renal disease.

Slight to moderate kidney damage was observed mainly in those animals on a salt regimen which died after a relatively short time—7 to 13 months. Study of these aided in constructing the natural history of the renal lesion. The earliest changes were clearly glomerular and consisted of exudation of plasma-like fluid into the capsular space. The corresponding tubules were filled with col-

4 5 6

Fig. 4. Dilated renal tubules and colloid casts in animal drinking 1% salt for 14 months (H&E; × 77). **Fig. 5.** Adhesions between tufts, hyalinization, and fat in glomeruli of animal drinking 1% salt for 16 months (H&E; × 135). **Fig. 6.** Fibrinoid necrosis of afferent arteriole and glomerular tufts in animal drinking 1% salt for 14 months (H&E; × 180).

loid casts. Subsequently there was thickening of the basement membrane, swelling of epithelial cells, adhesions between tufts, fibrinoid necrosis of capillary loops, and accumulation of fat. Progressive hyalinization led to obliteration of many glomeruli.

The glomerular lesions were focal at first and gradually became widespread. Tubular alterations such at atrophy, hypertrophy, and fatty degeneration appeared to be secondary to glomerular injury, while interstitial fibrosis and lymphocytic exudate followed damage to the tubules. Vascular necrosis usually paralleled glomerular disease in severity, although there were instances of vascular lesions in kidneys with minimal glomerular change.

Rats with progressive glomerular disease showed albuminuria. The latter gradually became more severe and in some instances the amount of protein excreted in the urine exceeded 3 Gm. a day. Terminally, total serum protein varied from 5.1 to 7.0 Gm. per 100 cc., but albumin was reduced and A-G ratios ranged from 0.4 to 0.8. However, this was not accompanied by edema. Correlation between albuminuria and renal damage was excellent. For example, daily quantitative albumins in animals with insignificant or slight kidney damage were less than 0.5 Gm.

Renal function was not seriously compromised in the salt rats, and no deaths could be attributed to uremia. However, elevation of blood urea nitrogen was present in several animals, usually in the latter part of life, with values up to 96 mg. per 100 cc. In general the highest levels occurred in animals with the most severe renal disease.

The mean cardiac index for the hypertensive salt animals was 4.3 as compared to 4.1 for the nonhypertensive salt rats and 2.9 for the animals on normal diet. This probably indicates that the hypertensive state was accompanied by cardiac hypertrophy. However, the high index for nonhypertensive experimental animals suggests that other factors associated with salt ingestion may have contributed to enlargement of the heart.

Of the 12 animals with hypertensive vascular disease, 4 showed myocardial lesions. These consisted of focal necrosis of fibers, fibrinoid necrosis of vessels, and fibroblastic proliferation.

DISCUSSION

None of the control animals in this study developed hypertension, and this is in keeping with our experience that spontaneous hypertension in the rat is rare. Only 1 control animal showed polyarteritis nodosa. The latter, too, has been observed only rarely as a spontaneous lesion in this laboratory, probably in part because our studies have been concerned mainly with young adult rather than senile rats. Wilens[24] studied 487 rats which died of natural causes and reported polyarteritis in about 10 per cent, most of which were females. Among males the incidence was only 3.5 per cent. The disease was limited essentially to old animals averaging 700 days at the time of death, and was not observed before 500 days. The cause of the spontaneous disease is obscure. No connection with renal disease, hypertension, or defect in salt metabolism is known, although probably these items have not been adequately studied.

The only spontaneous lesions observed with some frequency in the control rats involved the kidneys and mainly the glomeruli. The changes were similar to those present in the salt animals except that in the latter they occurred earlier, tended to be progressive, became diffuse, and were accompanied by fibrinoid necrosis of glomerular loops as well as necrotizing vascular disease of small arteries and arterioles. Possibly the lesions in the control rats were related to low tolerance for the salt present in the standard diet.

It is fairly well established that excessive intake of sodium chloride over a period of time may cause hypertension. Sapirstein, Brandt, and Drury[17] found that substitution of 2 per cent sodium chloride for drinking water caused elevation of blood pressure in the rat. Gepts[7] observed lesions of the heart and pancreas, including vascular necrosis, in rats on this regimen. Toussaint and associates[23] were unable to obtain hypertension in rats given 2 per cent salt for drinking but did produce hypertensive vascular disease when a supplement of 5 per cent sodium chloride was incorporated into the diet. Meneely *et al.*[15] reported that sustained hypertension occurred in rats chronically fed diets rich

in sodium chloride (as high as 9.8 per cent). Some animals also developed severe renal disease of nephrotic type. Hypertension was obtained in rabbits ingesting excess salt[6] and also in chickens given isotonic or slightly hypertonic saline to drink in place of water.[14] Selye[19] described renal lesions in chicks which drank 0.9 per cent saline instead of water.

Sodium chloride is also an important factor in steroid hypertension, such as that produced by administration of desoxycorticosterone.[20] Here the hypertension presumably depends on retention of sodium and this activity of the steroid is aggravated by salt-loading. Both steroid and salt hypertension may well have the same basic pathogenesis, although the combination of steroid and exogenous sodium commonly shortens the time required for development of the disease. Morphologically the vascular, renal, and cardiac lesions of salt and steroid hypertension are virtually indistinguishable.

In the rat, renal injury results in hypertensive vascular disease usually without need of exogenous salt-loading. However, there is evidence that a normal or high level of sodium chloride in the diet promotes the development of vascular lesions in renal hypertension.[10, 16] It was shown in this laboratory[11] that the amount of exogenous salt available to an animal with kidney damage was a critical factor in determining the presence or absence of both hypertension and vascular disease. Moreover, the NaCl seemed to complement renal injury in the production of hypertensive vascular disease. Mild injury plus high salt intake yielded the disease, whereas it was absent with normal or low salt intake.

In the present study the control and experimental animals differed only in that the latter drank 1 per cent saline instead of tap water. Hence there could be little doubt that increased intake of salt was responsible for the development of hypertensive vascular disease. The amount of extra NaCl consumed by the experimental animals was approximately 0.3 Gm. daily. The animals showed wide individual variation in their tolerance to salt. Some were normotensive and had no vascular or renal lesions even after 14 months on this regimen, while others, for reasons not known, revealed hypertension after only a few months and/or showed renal disease by 7 months and vascular disease by 9 months. Over-all, however, the changes evolved slowly, and a fairly long period of time—at least 10 months—on 1 per cent salt was required to produce well-developed hypertension and diffuse vascular lesions.

Information on sequence of development of the various lesions was incomplete, since regular interval sacrifice of animals was not performed. However, the data permit certain observations along this line. In the later stage of disease the association between hypertension, vascular disease, and renal damage was fairly close, although not obligatory. At an earlier stage hypertension

clearly preceded the development of both vascular and renal lesions in some instances. This suggests that renal disease was not a requisite for the development of hypertension. Whether elevated blood pressure per se caused the vascular disease is not known, but the latter bore no significant relation either to severity or duration of the hypertension. A few animals showed disparity between the degree of vascular disease and renal damage. The latter was primarily glomerular and perhaps due to injury of the basement membrane of capillary tufts by excess sodium.

The notion has been entertained that imbalance of electrolytes in the tissues, especially sodium and potassium, may be a factor in causing hypertension. In salt hypertension the imbalance is presumably initiated by retention and intracellular accumulation of sodium, and perhaps replacement of potassium by sodium. Such a process might well entail altered distribution of other cations and anions, as well as of water, and perhaps change in local *p*H. Evidence has been cited[22] that change in electrolyte composition can increase vascular tonus and hence serve to initiate hypertension.

That a cellular-electrolyte upset may cause tissue necrosis is illustrated by the potassium deficiency state.[3, 5, 18, 21] Here necrosis of myocardial fibers, similar to that observed in hypertension, is associated with reduction in potassium content of the heart and increase in sodium content. The necrotizing process is diminished by low salt intake[1, 4] and accelerated by high salt intake.[1] This suggested to Cannon, Frazier, and Hughes[1] that replacement of potassium by sodium produced a toxic effect on intracellular metabolic processes. Another possibility is that electrolyte imbalance causes cellular necrosis through alteration in the physical state of the actomyosin complex or by interference with the energy-producing mechanism, both of which are influenced by relative concentration of the alkaline ions.[5] Perhaps a similar mechanism is operative for the vascular necrosis in long-term salt feeding and for myocardial necrosis in the potassium-deficient state. However, in this regard it should be noted that apart from myocardial damage, neither vascular lesions nor hypertension have been described in potassium-deficient rats.

So far there have been several studies on electrolyte changes in the tissues of hypertensive animals. These were mainly concerned with alterations in sodium, potassium, and water in extracellular and intracellular phases. Changes in sodium and potassium[2, 12] or in total sodium[8] were reported, but the results apparently may vary both according to the technic employed to induce hypertension and with the type of tissue analyzed.[22] Thus no constant pattern of electrolyte disturbance has been established and in general the results have been inconclusive.[9] Doubt has been expressed as to whether alteration in sodium and potassium actually reflected intracellular change rather

than expansion of the extracellular space.[13] Also, there is the possibility that the electrolyte changes were secondary to some other basic tissue alteration associated with the hypertension. In general the available data indicate some kind of association between hypertensive vascular disease and electrolyte changes in tissues, but they have so far failed to establish a causal relationship.

SUMMARY

Hypertensive vascular disease frequently develops in rats which drink 1 per cent saline instead of tap water over a long period of time. The vascular lesions are diffusely distributed, often severe, and mainly of the polyarteritis nodosa type.

The salt regimen also produces renal damage, principally a glomerulitis, which is progressive and leads to albuminuria and mild or moderate azotemia. However, the changes responsible for elevated blood pressure can occur before there is a significant morphologic renal lesion.

The mechanism of salt injury is not known. Possibly it is related to retention and intracellular accumulation of sodium, which lead to critical imbalance of electrolyte concentration and distribution in the tissues, especially the vascular bed.

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The inhibition of chorda formation in the frog embryo by chemical action

by

F.E. Lehmann

The suppression of chorda formation by chemical means can readily be achieved in the embryo of the salamander Triton alpestris. If the embryos are placed in LiCl solution at the beginning of gastrulation and returned into the incubating solution after several hours of treatment, the presumptive chorda material does not develop into the chorda, but into somitic substance: it becomes "mesodermized" [Lit. see Lehmann, Naturwiss. 1926, 403, and Lehmann, Revue Suisse Zool. 44:1, 1937]. Repeated experiments on anura, particularly Rana fusca, to induce an absence of chorda by LiCl treatment alone did not lead to satisfactory results. The treatment did give rise to the long known anomalies in the head region (see Leplat, Arch. de Biol. 30, 1919), but the mesodermal axillary organs in the trunk were less strongly affected than in the triton. Stronger concentrations caused cellular decomposition in various regions of the embryo, but without giving rise to appreciable morphologic alterations.

Studies on triton embryos to determine the effects of combined solutions of KCl and LiCl led to a surprising observation.

While according to Runnström [Acta Zool. (Stockholm) 9, 1928] and Lindahl [Acta Zool. (Stockholm) 17, 1936] the addition of KCl inhibits the effect of LiCl on see urchins, LiCl solution has a stronger effect on the triton if KCl is added to it than when it is used alone. This observation has been confirmed on frogs. If embryos of Rana fusca were treated as blastulae with a mixture of KCl (conc. 1:160) and LiCl (conc. 1:200) for 17 hours at 18°, embryos developed in which the trunk region of the neural plate was severely altered. At later stages these embryos revealed a very defectively developed front end, in which no eyes were found and only very abnormally developed brain portions. In the anterior portion of the trunk there was a neural tube with a sagital cleft situated inside a partially bilateral band of somitic substance (Fig. 1b). The chorda was completely absent from the anterior end down to this region. The chorda appeared in a further caudal direction. Aside from a somewhat stronger necrotization of all tissues, conditions resembled those found in the triton after Li treatment. Frog embryos raised in 1: 80 KCl (Fig. 1a) and those raised in 1:200 Li developed normally.

It is important for an evaluation of the physiology of the organizer that in urodeles and anura chorda formation can be inhibited by analogous agents. It must nevertheless be stressed that in addition to this concordance with regard to the effect upon the chorda mesoderm of urodeles and anura there are also considerable variations in the response to the agents used (for example to their concentration). The findgins referred to are

being further analyzed.

Figures

1. 40x magn. a) Cross section through the pronephros region of a frog embryo treated in the blastula stage for 17 hours with 1:80 KCl. The chorda, provertebra and neural tube are present and of normal structure. b) Cross section through the anterior trunk region of an embryo treated at the blastula stage with a salt mixture (LiCl 1:200, KCl 1:160). The neural tube and somitic substance are present, the chorda is completely absent. The embryo, of the same age as the embryo in Fig. 1a, is somewhat retarted in differentiation, the tissues contain some necrotic cells.

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Kurze Originalmitteilungen.

Für die kurzen Originalmitteilungen ist ausschließlich der Verfasser verantwortlich.

Hemmung der Chordabildung beim Froschembryo durch chemische Mittel.

Die Unterdrückung der Chordabildung durch chemische Mittel gelingt bei Keimen des Molches Triton alpestris ohne Schwierigkeiten. Werden die Keime zu Beginn der Gastrulation in Lösungen von LiCl gebracht und nach mehrstündiger Behandlung wieder in Zuchtlösung zurückgebracht, so entwickelt sich das präsumptive Chordamaterial nicht zu Chorda, sondern zu Somitenmaterial: es wird „*mesodermisiert*" [Lit. s. LEHMANN, Naturwiss. 1926, 403 und LEHMANN, Rev. Suisse Zool. 44, 1 (1937)]. Mehrfach wiederholte Versuche auch bei Anuren, vor allem bei Rana fusca, durch Behandlung mit LiCl allein, Chordalosigkeit zu erzeugen, gaben keine befriedigenden Resultate. Die Behandlung rief zwar die schon lange bekannten Störungen im Kopfbereich [vgl. LEPLAT, Arch. de Biol. 30 (1910)] hervor, während die mesodermalen Achsenorgane im Rumpf weniger beeinflußt wurden als bei Triton. Stärkere Konzentrationen bewirkten in verschiedenen Keimbereichen Zellzerfall, ohne aber wesentliche morphologische Änderungen hervorzurufen.

Beobachtungen an Tritonkeimen über die Wirkungen von kombinierten Lösungen von KCl und LiCl führten zu einer überraschenden Feststellung. Während nach RUNNSTRÖM [Acta zool. (Stockh.) 9 (1928)] und LINDAHL [Acta zool. (Stockh.) 17 (1936)] der Zusatz von KCl die Wirkung von LiCl auf Seeigelkeime hemmt, wirken Li-Lösungen bei Triton stärker, wenn ihnen KCl zugesetzt wird, als wenn sie ohne Zusatz angewandt werden. Diese Erfahrung hat sich auch beim Anurenkeim bestätigt. Wurden Keime von Rana fusca als Blastulae während 17 Stunden bei 18° mit einem Gemisch von KCl (Konz. 1:160) und LiCl (Konz. 1:200) behandelt, so entwickelten sich Embryonen, bei denen auch der Rumpfbereich der Neuralplatte stark verändert war. Auf späteren Stadien zeigten diese Keime ein sehr mangelhaft entwickeltes Vorderende, bei dem keine Augen und nur

sehr abnorm entwickelte Gehirnteile gefunden werden konnten. Im vorderen Rumpfbereich fand sich ein Neuralrohr mit Sagittalspalt. Es ist einem teilweise bilateralen Band von Somitenmaterial eingelagert (Fig. 1b). *Die Chorda fehlt vom Vorderende bis in diese Region vollständig.* Weiter caudal erscheint sie. Abgesehen von einer etwas stärkeren Nekrotisierung aller Gewebe gleichen die Verhältnisse denjenigen, die bei Triton nach Li-Behandlung auftreten. Froschkeime, die in KCl 1:80 (Fig. 1a), und solche, die in Li 1:200 gezüchtet wurden, entwickelten sich normal.

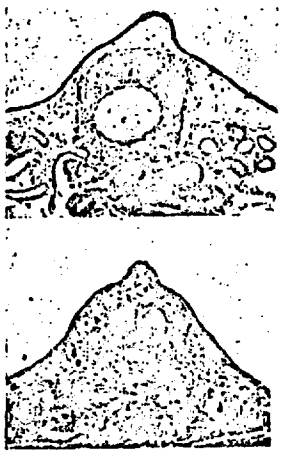


Fig. 1. Vergr. 40mal.
a) Querschnitt durch die Vornierenregion eines Froschkeims, der als Blastula 17 Stunden mit KCl 1:80 behandelt wurde. Chorda, Urwirbel und Neuralrohr vorhanden und normal gebaut.
b) Querschnitt durch die vordere Rumpfregion eines Keims, der als Blastula mit einem Salzgemisch (LiCl 1:200, KCl 1:160) behandelt wurde. Neuralrohr und Somitenmaterial vorhanden, Chorda fehlt vollständig. Keim, gleichalt wie Keim der Fig. 1a, in der Differenzierung etwas zurückgeblieben, Gewebe enthalten zum Teil nekrotische Zellen.

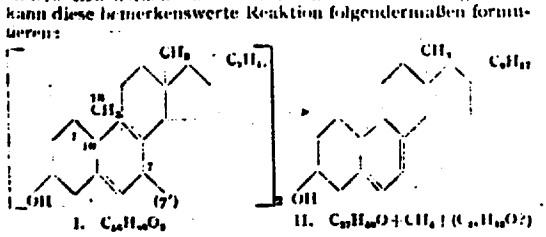
Es ist für die Beurteilung der Physiologie des Organisators wesentlich, daß nun *bei Urodelen und Anuren die Chordabildung durch analoge Mittel gehemmt* werden kann. Immerhin muß hervorgehoben werden, daß neben dieser Übereinstimmung in der Beeinflußbarkeit des Chordamesoderms von Urodelen und Anuren auch wesentliche Verschiedenheiten in der Ansprechbarkeit auf die angewandten Mittel (z. B. auf ihre Konzentration) bestehen. Die erwähnten Befunde werden weiter analysiert.

Bern, Zoologisches Institut der Universität,
den 22. Januar 1937. F. E. Lehmann.

Übergang von Sterinen in aromatische Verbindungen. Umwandlung von Cholesterin in Iso-Equilin.

Das Problem der Umwandlung von Sterinverbindungen in Oestron bzw. in Stoffe, die der Follikelhormongruppe angehören, ist im wesentlichen das Problem der Eliminierung der quartären Methylgruppe vom Kohlenstoffatom 10 des Vierringsystems.

Diese Methylgruppe am C_{10}, die normalerweise sehr fest haftet, kann, wie das Beispiel des „Ergopinakons"[1] lehrt, unter „gewissen" Voraussetzungen außerordentlich beweglich sein. Das „Ergopinakon" (I) zerfällt bekanntlich beim Erhitzen unter Abspaltung von Methan[2] in das im Ring B aromatisierte Neo-ergosterin (II) und ein oder mehrere andere Bruchstücke unbekannter Zusammensetzung. Man kann diese bemerkenswerte Reaktion folgendermaßen formulieren:



I. $C_{28}H_{46}O_2$ II. $C_{28}H_{44}O + CH_4 + (C_{27}H_{46}O?)$

Die hier für das „Ergopinakon" aufgestellte, noch nicht sicher bewiesene Konstitution läßt den vorstehend formulierten, so überraschend leicht verlaufenden Zerfall etwas besser verstehen. Bei Anwendung der Schmidtschen Doppelbindungsregel erkennt man nämlich, daß sich der auflockernde Einfluß der beiden Kerndoppelbindungen, wie sie im „Ergopinakon" angenommen werden, sowohl auf die Kohlenstoffbindungen C_{10}—C_{18} (bzw. C_{10}—C_1) als auch C_7—$C_{7'}$ auswirkt, und zwar jeweils additiv. Hierdurch wäre das Auseinanderbrechen des Di-ergosterin-moleküls an den C-Atomen 7 und 10 zu erklären. Sicherlich hat ferner auch die im Ring B durch die Molekülverknüpfung hervorgerufene Spannung eine Bedeutung für diesen Zerfall, der schon bei 135° vor sich geht.

Die Tatsache, daß das Ergosterin selbst erst bei 250° Methan abspaltet, wurde also dahingehend zu deuten sein, daß 1. die C_7—C_8-Doppelbindung in die C_8—C_9-Lage verschoben werden muß, und daß 2. die sog. „Pinakonbindung" fehlt.

Bei meinen Versuchen zur Darstellung ähnlich konstituierter mehrfach ungesättigter Oxo- und Oxy-Verbindungen von Steroiden[1] ist es mir nun gelungen, aus Cholesterin über *Dibrom-androstan-ion* durch Abspaltung von Bromwasserstoff Produkte darzustellen, die beim Erhitzen ebenfalls glatt Methan abspalten und hierbei *aromatische Stoffe mit phenolischen Eigenschaften* liefern. Aus den Reaktionsgemischen ließ sich ein Stoff mit folgenden Eigenschaften abtrennen:

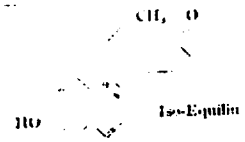
Er ist fast unlöslich in Äther und kristallisiert aus Alkohol-Wasser in feinen Nadeln. Der Schmelzpunkt lag nach einmaligem Umkristallisieren bei 248—250° unter Rotfärbung, restloses Zusammenfallen bei 252° (unkorr.): Rotfärbung und schwaches Sintern setzten etwa 10° unterhalb des Schmelzpunktes ein. Dieses Präparat zeigte in Dioxanlösung eine optische Drehung von $[\alpha]_D^{20} = +170°$. Nach einer weiteren Umkristallisation war der Schmelzpunkt unverändert. Die Elementaranalysen zweier Präparate ergaben folgende Werte: gef. C 80,75, 80,18; H 7,31, 6,99; $C_{18}H_{20}O_2$ ber. C 80,55, H 7,52.

Das Absorptionsspektrum des 2mal umkristallisierten Produktes weist Maxima bei 265, 275 und 334 mμ auf. Die physiologische Auswertung ergab folgendes Resultat: 60 γ waren, bei 6maliger Unterteilung in wässeriger Lösung innerhalb von 2 Tagen, im Allen-Doisy-Test an der Ratte voll wirksam, während 30 γ keine Wirkung mehr zeigten.

Auf Grund dieser Befunde läßt sich folgendes sagen[2]. Die Analysen weisen mit Ausnahme eines H-Wertes auf die Summenformel $C_{18}H_{20}O_2$ hin, wonach es sich um eine Verbindung von der Zusammensetzung des Equilins handelt. Eine Identität mit Equilin (F. 238—240°, $[\alpha]_D$ + 308°) ist jedoch schon durch Schmelzpunkt und Drehung ausgeschlossen. Ferner zeigt das Spektrum im Gegensatz zu dem des Equilins sowie auch des Oestrons ein Maximum bei 334 mμ. Im ganzen ist das Spektrum mehr dem des Equilenins ähnlich. Eine Identität mit Equilenin kommt aber, abgesehen von der Analyse, ebenfalls wegen der Drehung nicht in Betracht. Der Mischschmp. mit einem Equilenin-Präparat vom Schmp. 245—247° lag bei 243—244°, zeigte also eine geringe Depression.

Bemerkenswert ist die relativ hohe physiologische Grenzdosis, die bei etwa 50 γ angenommen werden darf, während das Equilin unter den gleichen Bedingungen fast ebenso wirksam ist wie das Oestron selbst.

Ich glaube daher annehmen zu können, einen neuen Vertreter der Follikelhormongruppe von der Formel $C_{18}H_{20}O_2$ in Händen zu haben und möchte diesem Stoff die nebenstehende Konstitution eines Iso-Equilins zuerteilen, wobei vorausgesetzt wird, daß bei der Aromatisierung keine unerwarteten Umlagerungen stattgefunden haben.



Iso-Equilin

Die vierte Doppelbindung wird auf Grund des Absorptionsmaximums bei 334 mμ in Konjugation zum Benzolkern

[1] Die Bezeichnung „Ergopinakon" ist unzutreffend und könnte durch „Di-ergosterin" ersetzt werden; vgl. Lettré u. Inhoffen, Über Sterine, Gallensäuren usw. S. 135. Stuttgart: Ferdinand Enke 1936.

[2] H. H. Inhoffen, Liebigs Ann. 497, 130 (1932).

[1] Ber. dtsch. chem. Ges. 69, 1134, 1702, 2141 (1936).

[2] Es erscheint möglich, daß der Stoff noch nicht restlos einheitlich ist. Das Vorhandensein wesentlicher Mengen von Begleitsubstanzen ist jedoch unwahrscheinlich, da schon die Rohprodukte relativ scharf und hoch schmolzen, (um 240 bis 245°).

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ARTERIAL HYPERTENSION IN THE CHICKEN[1]

1000

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For some time we have been interested in the production of hypertension in the chicken in order to study the interrelationship between high blood pressure and atherosclerosis. In our preliminary studies on the chicken we attempted to produce hypertension by the Goldblatt principle by ligation or partial constriction of the main renal arteries (1). In some of these animals after partial constriction of the renal arteries on one side, the ureter of the opposite kidney was ligated in order to increase the ratio of ischemic to normal kidney tissue (2). Unilateral and bilateral obstruction of the ureters was also tried. However, none of these measures consistently increased the blood pressure for any significant period. Even when both ureters were occluded and acute bilateral hydronephrosis was produced, a condition which consistently causes a rise in pressure in mammals (3), no increase in pressure was seen in the chickens and the animals died in 12 to 15 hours in acute hyperuricemia (4).

Seyle (5) observed that cardiovascular and renal changes could be produced in chickens by adding NaCl to the drinking water of the animals. The initial changes observed were generalized anasarca, cardiac dilatation and renal alterations resembling subacute glomerulonephritis. Later, dehydration, cardiac hypertrophy and glomerular sclerosis were the outstanding features of this salt intoxication syndrome. These changes suggested the possibility that the animals had developed hypertension, but blood pressures were not reported. The possibility that hypertension had been produced seemed worthy of further investigation. After the present studies were begun, Krakower and Heino (6) published data on the blood pressure of chickens on a salt diet which also suggested that a rise in blood pressure occurred. Our results with methods differing from those of Krakower, but somewhat similar to those of Selye, are presented in this report.

METHODS

Eight white leghorn chicks, six weeks old, were placed on a starting diet of mash and tap water for 16 days. During this period three control blood pressures on each chicken were measured with the Hamilton manometer by means of a small needle inserted into the sciatic artery. The drinking water was then replaced by a 0.9 per cent NaCl solution and the chickens permitted to drink this salt solution ad libitum. Blood pressures were taken every five to eight days. After 33 days, when the rise in pressure was seen to be moderate, the NaCl con-

[1] Aided by the Sam Ehrlich Memorial Club Fund for Cardiovascular Research.
[2] The Department is supported in part by the Michael Reese Research Foundation.

centration was increased to 1.2 per cent. The salt solution was replaced 26 days later by tap water for 13 days. A second period of salt administration (1.2 per cent) was then instituted for 22 days, during which period blood volume, body weight and hematocrit values were determined in addition to the blood pressures. After a final period of 10 days on tap water the chickens were killed.

RESULTS

The systolic and diastolic blood pressures recorded during the experiment in the eight birds are given in table 1, and the average for the entire group is shown in

TABLE 1. EFFECT OF SALT INGESTION ON THE BLOOD PRESSURE OF THE CHICKEN

DAY	FLUIDS GIVEN	CHICKEN NUMBER AND BLOOD PRESSURE IN MM.Hg							
		50	51	52	53	54	55	56	57
0	tap water	122/112	120/105	140/125	130/120	133/116	142/120	140/125	125/115
6	" "	130/119	125/115	150/140	145/135	143/127	142/128	150/135	162/145
12	" "	122/112	120/110	140/133	140/130	134/129	135/125	133/123	140/130
18	salt 0.9%								
22	" "	165/155	125/115	145/133	143/133	130/120	125/115	125/115	136/125
29	" "	158/138	135/125	173/163	155/135	162/142	165/145	160/145	150/135
34	" "			180/165	145/125	140/133		155/136	155/137
42	" "	168/150	143/127	175/155	130/115	155/135		152/138	145/128
50	" "	168/150	150/130	175/160	155/145	145/135		150/140	162/148
51	" 1.2%								
56	" "	143/128	170/127	175/153	173/148	135/120		165/148	162/148
62	" "	183/158	158/134	190/160	180/150			155/138	162/145
69	" "	178/163	168/138	195/167	165/145			145/130	185/163
76	" "	195/170	190/150	190/160	158/138			165/145	205/160
77	tap water								
83	" "	133/120	135/123	135/120				140/125	155/140
89	" "	137/120	128/115	167/147				133/118	165/145
90	salt 1.2%								
99	" "	160/145	148/123	170/155				175/157	168/148
109	" "	165/150	165/150	190/170				175/153	180/160
112	tap water								
121	" "	136/120	155/145	177/163				180/155	160/138

figure 1. The control blood pressure at the beginning of the experiment averaged 132/117 mm. Hg. The values in the several birds did not vary significantly from this value, which is in the range obtained in our laboratory on a large number of controls (7). The high level of the normal diastolic pressure in the chicken appears to be related to its high normal body temperature of 42°C. (7).

At the onset of the 0.9 per cent salt administration the blood pressure did not rise significantly, but at the end of 17 days it had risen 22/17 mm. Hg. It then remained unchanged until the salt concentration was increased to 1.2 per cent. This produced a further increase of 25/9 mm. with the average pressure reaching 183/154 mm. Hg. The total rise over the control values was 51/37 mm. Hg. When the salt was discontinued a prompt fall in pressure occurred, averaging

43/28 mm. Hg and bringing the pressure nearly to the control levels. With the readministration of salt (1.2 per cent) in the drinking water, a rise in pressure was again observed, with a fall after the animals were again returned to ordinary tap water. The weight of five birds during the second period of salt intake showed an average decrease of 330 grams, a 20 per cent reduction over a period of 15 days. Within nine days after the salt intake was ended there was an average gain in weight of 40 grams.

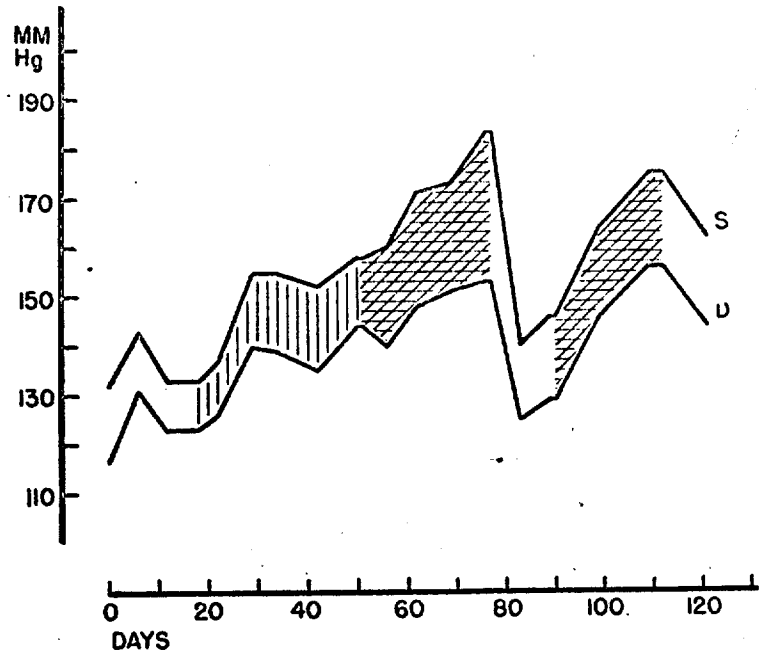

FIG. 1. EFFECT OF INCREASED SALT INGESTION on the average blood pressures of eight chickens. S and D systolic and diastolic blood pressure, respectively. The various periods are indicated by markings between systolic and diastolic pressure lines; periods of tap water ingestion are indicated by blank spaces; 0.9 per cent saline, by vertical-lined period; 1.2 per cent saline, by cross-hatched periods. Ordinates indicate mm. Hg.; abscissae, time in days.

The average hematocrit in these five birds, determined at the same time, showed only a slight increase, from 36 to 39 per cent, during the period of salt administration. Blood volumes measured during the salt intake period in four of these birds showed no consistent deviation from the normal.

The amount of salt solution consumed daily averaged approximately 700 cc. per chicken for the 0.9 per cent salt solution and one liter for the 1.2 per cent concentration. Diarrhea was profuse throughout the period of salt administration. At times the feces were streaked with blood. Obvious anasarca was not observed at any time. No dyspnea was noted. The comb, however, became very pale

soon after the salt administration was discontinued. A certain lethargy was also noted while salt was being given.

One bird was found dead on the 16th day of the first salt intake period. It showed no evidence of edema or gross abnormalities except for a small pericardial effusion and slight pulmonary edema. A second bird died 10 days after the salt concentration had been increased to 1.2 per cent. It was distinctly dehydrated; moderate pulmonary edema and a pericarditis with a large effusion and thin pericardial adhesions were present; all organs were markedly hyperemic. A third bird was killed for comparison at the end of the first salt intake period. It was dehydrated, had no pericardial effusion and showed no other gross changes.

Gross autopsy findings in the five birds surviving the four-month period were not remarkable. Two birds had slight pulmonary edema. None had any marked effusions. The pericardium appeared normal in all. One bird had small subepicardial hemorrhages in the region of the left ventricle. No other microscopic changes were found in the heart. Only one bird had a possible cardiac enlargement in comparison to the other birds. The ratio of wet heart weight to body weight ranged between 0.45 and 0.60 per cent. This compares with control values ranging from 0.40 to 0.53 per cent (8). Both Selye (5) and Krakower and Heino (6) found cardiac hypertrophy to be a consistent finding in the salt intoxication syndrome. Krakower's figures are distinctly higher than ours for which differing experiment conditions may be responsible. However, he hesitated to attribute the consistent cardiac hypertrophy to a blood pressure elevation, because the hypertrophy occurred regardless of the presence or absence of hypertension.

None of the birds had contracted kidneys. Histological changes were confined to the kidneys. Variations were apparently related to the time of death. Thus, the bird that died during the early part of the study showed no renal changes of note. The kidneys of the bird dying soon after the salt concentration had been increased to 1.2 per cent showed a moderate proliferation of the glomerular tufts and degenerative changes in the tubules. The most pronounced alterations were found in the kidneys of the chicken killed at the end of the first salt intake period. In this bird, there was a marked proliferation of the glomerular tufts with conspicuous hyperplasia of the individual cells and a moderate proliferation of Bowman's capsule. The proliferated glomerular tufts completely filled the capsular space and the capillaries were compressed and ischemic. The kidneys of the five birds killed at the end of the study showed similar changes but to a lesser degree. Proliferation of glomerular tufts and hyperplasia of Bowman's capsule was still evident, but the capsular spaces were not completely obliterated and the capillaries appeared less compressed. Krakower and Heino (6) found glomerular enlargement to be variable in their salt-treated birds, dependent mainly on the normal rate of growth. Inflammatory or sclerotic changes were not encountered in our series. The tubules were for the most part intact or showed a mild cloudy swelling. Arterial and arteriolar changes were likewise absent. It should be noted that in the normal chicken these vessels present a very well-developed muscular coat.

Gastrointestinal hemorrhages were not found at autopsy in our birds, although they may have been present earlier in the course of the experiment, since blood-streaked excreta were observed in several birds.

DISCUSSION

The intake of 0.9 per cent saline by mammals is generally followed by an immediate transfer of this fluid and salt across the circulatory bed into the interstitial fluid. If a large amount of saline is ingested, it may be greater than can be excreted by the unadapted kidney and thus will lead to the development of edema. This is apparently what occurred in the experiments of Selye (5) and of Krakower and Heino (6). In our experiments, a transient questionable edema developed in some animals but all our animals soon lost weight and actually became dehydrated. This may have been due to an adaptation of the excretory function of the animal to the increased load.

Normally the chicken reabsorbs much of the urinary water in the cloaca and excretes only a paste composed of uric acid and other nitrogenous substances. Under the conditions of our experiments, however, the increased fluid intake occasioned by the high salt content of the water results in an apparent overwhelming of the dehydrating mechanism and in a consequent production of a fluid excreta.

The increase in both systolic and diastolic blood pressures during the course of high salt intake is considered significant because the upward trend was present in all of the birds. By comparison, a control series of blood pressures in five normal young chickens taken over a period of 80 days was found to be remarkably constant; the average variation in systolic and diastolic pressures in this group was 8 mm. Hg, with no tendency for the blood pressure to change significantly during the period.

No less striking was the effect of the return to tap water, which produced an average drop in pressure of 43/28 mm. Hg. Since this phenomenon was observed twice, we feel justified in assuming a direct relationship between high salt intake and the rise in blood pressure in the chicken. The mechanism of this phenomenon is not entirely clear. An increased blood volume is probably not involved as our direct measurements show, and as the marked weight loss and dehydration would seem to indicate.

The production of hypertension in the chicken by a high salt intake in the drinking water appears pertinent to the general problem of the genesis of hypertension. It has been known for some time that desoxycorticosterone, which affects the excretion of Na and K, also produces changes in the blood pressure in man and other animals (cf. 9). Particularly is this true in animals on a high salt diet. The low blood pressures seen in Addisonian patients is also associated with a low plasma sodium concentration, and this hypotension can be treated by the oral administration of sodium chloride (10). Further, the recent emphasis on the use of salt-free diets in the treatment of arterial hypertension (cf. 11), which originated primarily on an empirical basis, may be considered to have some support from these and other findings.

The mode of production of the hypertension in the chicken is not clear at the present time. The proliferative renal changes which are observed may begin as an adaptive response to high salt intake, but finally may act to increase the intrarenal pressure and thus decrease the blood flow through the kidney. The relationship of the sodium chloride to the blood pressure changes would, however, emphasize the possibility that the adrenal cortex has an important rôle in the hypertensive process.

SUMMARY

1. The blood pressures of chickens were measured at frequent intervals before, during and after periods of substitution of 0.9 and 1.2 per cent of saline solution for the drinking water. Some determinations of body weight, hematocrit and blood volume were also obtained.

2. The average systolic and diastolic blood pressures increased progressively during the period of salt administration from an average of 132/117 mm. Hg at the beginning of the increased salt intake to a maximum average value of 182/154 mm. Hg. The blood pressure fell promptly to control levels after the withdrawal of NaCl. Similar observations were made during two periods of salt administration and two periods of return to the drinking of tap water. The degree of hypertension appeared to depend upon the concentration of salt in the ingested water.

3. Hyperplasia and proliferation of glomerular tufts and Bowman's capsule with compression of the capillaries were found after prolonged periods of the substitution of saline for drinking water. This hyperplasia is reversible.

4. The relation between the kidney and factors affecting salt metabolism in the production of this hypertension is discussed.

We are indebted to Dr. O. Saphir of the Pathology Department for his critical review of the microscopic sections.

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A STUDY OF THE TOXICITY OF STRONTIUM AND COMPARISON WITH OTHER CATIONS EMPLOYED IN THERAPEUTICS*

9010

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VULPIAN (1885) is credited with having introduced strontium as a remedy. J. V. Laborde (1890) proved that it was nontoxic and harmless when pure. Since then, strontium has been employed in medicine by oral administration in the place of sodium and potassium cations as a carrier for a number of anions such as bromide, iodide, salicylate, lactate and others. As with potassium salts, the anions are evidently the active therapeutic agents for the reason that both potassium and strontium are only slightly and irregularly absorbed from the digestive tract. This probably accounts for the failure to establish more convincing evidence in the literature as to the therapeutic value of strontium.

Strontium is a divalent in the same chemical group as the alkali earths magnesium, calcium and barium. A number of pharmacologists have studied the effect of strontium on cells, isolated tissues and blood and compared the effects with those exerted by other elements including members of the same group. The impression gained from a research of these reports is that it resembles more nearly the effect of calcium, but that it is weaker. Sollmann[1] states: "This cation resembles calcium and may take its place in some of its characteristic pharmacologic relations; but it is much weaker and also less toxic."

Like calcium, strontium hastens the coagulation of the blood. This is not true of barium or magnesium. It is eliminated by the intestines. The urine contains only traces of strontium when given subcutaneously. It is said to increase the elimination of uric acid and have an effect on bony tissues which are imperfectly calcified. Like calcium, it exerts a digitalis-like action on the heart, but weaker. Paralysis of the heart by quinine and arsenic is said to be neutralized by strontium and calcium.

There have appeared in recent literature, reports of the intravenous injection of strontium salts, particularly of the bromide. These reports indicate its application in the treatment of urticaria and other skin affections, parathyroid tetany, spasmophilia, etc., and other conditions indicating the relationship to the effect of calcium.

A review of the literature on strontium reveals a lack of information regarding its action and toxicity when administered intravenously. We have deemed it desirable that experiments be carried on to determine the definite toxicity of strontium and to compare, if possible, the toxicity and tolerance with the elements more commonly employed in therapeutics such as sodium, potassium, magnesium, and calcium. We submit herewith the tabulated results. We employed white rats and the same technic of intravenous injection we described in 1924.[2]

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Table I represents the results of the intravenous injection of strontium bromide in the form of a five per cent solution indicating the doses, grams per kilo weight, equivalent dose for human of 70 kilos and the amount of elemental strontium and bromine grams per kilo weight.

TABLE I

TOXICITY TESTS: STRONTIUM BROMIDE 5 PER CENT—$SrBr_2.6H_2O$

RAT NO.	DOSE GRAM/K	EQUIV. HUMAN DOSE (70K)	WEIGHT OF RAT	C.C. INJECTED	STRONTIUM G/K	BROMINE G/K	COMMENT
1084	0.250 Gm.	17.5 Gm.	150 Gm.	0.75 c.c.	0.061 Gm.	0.112 Gm.	
1085	0.375	26.25	132	1.00	0.092	0.169	
1100	0.450	31.50	135	1.22	0.110	0.202	
1086	0.500	35.00	260	2.60	0.123	0.225	
1096	0.500	35.00	140	1.40	0.123	0.225	Died 45 min.
1099	0.500	35.00	115	1.15	0.123	0.225	
1097	0.550	38.50	130	1.43	0.135	0.247	Died 2 min.
1098	0.615	43.05	120	1.48	0.151	0.276	Died on table
1087	0.750	52.5	200	3.00	0.185	0.337	Died on table

With the smaller doses, the animals reacted with the same symptoms following the intravenous injection of sodium bromide, becoming stupefied and remaining lethargic for a long time after recovery from the shock of the injection. It will be observed, however, that the toxicity of strontium bromide is greater than that of sodium bromide. White rats tolerate up to 2.50 gm. per kilo of sodium bromide, whereas our maximum tolerated dose is barely 0.5 gm. per kilo of the strontium salt. With the larger doses, the rats struggled violently toward the end of the injection, the hind leg becoming paralyzed before death ensued.

In view of the evident sedative effect of the bromide anion, we believed it desirable to ascertain the toxicity of strontium chloride and strontium iodide and arrive, if possible, at results which would clearly indicate the toxicity of strontium cation with due consideration for the effect of the accompanying anions.

Table II represents the results of the intravenous injection of strontium chloride in the form of a 5 per cent solution.

TABLE II

TOXICITY TESTS: STRONTIUM CHLORIDE 5 PER CENT—$SrCl_2.6H_2O$

RAT NO.	DOSE GRAM/K	EQUIV. HUMAN DOSE (70K)	WEIGHT OF RAT	C.C. INJECTED	STRONTIUM G/K	CHLORINE G/K	COMMENT
1088	0.250 Gm.	17.5 Gm.	123 Gm.	0.60 c.c.	0.082 Gm.	0.066 Gm.	
1089	0.300	21.0	150	0.90	0.098	0.079	
1104	0.300	21.0	135	0.81	0.098	0.079	
1101	0.350	24.5	140	0.98	0.115	0.093	
1103	0.375	26.25	200	1.50	0.123	0.100	Collapsed but recovered
1102	0.425	29.75	145	1.24	0.139	0.113	Died in 2 min.
1090	0.500	35.00	205	2.05	0.164	0.132	Died in 15 min.
1091	0.625	43.75	138	1.73	0.205	0.165	Died in 3 min.

These rats exhibited much more toxic symptoms. Their struggles were much more violent; great difficulty in breathing and violent twisting of tail before final effect of lethal dose. Greatest tolerance is approximately 0.350 gm. per kilo.

Table III represents the results of the intravenous injection of strontium iodide in the form of a 5 per cent solution.

TABLE III

TOXICITY TESTS: STRONTIUM IODIDE 5 PER CENT—$SrI_2.6H_2O$

RAT NO.	DOSE GRAM/K	EQUIV. HUMAN DOSE (70K)	WEIGHT OF RAT	C.C. INJECTED	STRONTIUM G/K	IODINE G/K	COMMENT
1092	0.250 gm.	17.5 gm.	125 gm.	0.62 c.c.	0.049 gm.	0.141 gm.	No visible effect
1093	0.375	26.25	130	0.97	0.074	0.211	
1105	0.425	29.75	110	0.94	0.084	0.240	
1094	0.500	35.00	160	1.60	0.098	0.282	Died 60 hr.
1106	0.500	35.00	135	1.35	0.098	0.282	
1108	0.550	38.50	130	1.43	0.108	0.310	
1107	0.625	43.75	110	1.38	0.123	0.352	Died 10 min.
1095	0.625	43.75	110	1.38	0.123	0.352	Very toxic

The rats did not struggle during the injection, and as a whole the solution appeared to be least immediately toxic. Rats which survived regained their previous weight within one week and retained their weight for another week while under observation. The minimum lethal dose of strontium iodide is apparently 0.625 gm. per kilo.

Table IV presents for comparison the approximate minimum lethal dose of strontium bromide, strontium chloride and strontium iodide, the equivalent strontium per kilo and the equivalent haloids per kilo, their atomic weight and the ratio of atomic weights to lethal dose.

TABLE IV

COMPARISON OF MINIMUM LETHAL DOSE

	$SrBr_2.6H_2O$	$SrCl_2.6H_2O$	$SrI_2.6H_2O$
Dose per kilo	0.5 Gm.	0.375 Gm.	0.625 Gm.
Strontium per kilo	0.123	0.123	0.123
Haloid per kilo	0.225	0.100	0.352
Atomic weight of halogen in even figures	80	35.5	127
Ratio of atomic weights halogens	2.25	1	3.55
Ratio of lethal dose haloid	2.25	1	3.52

A comparison of the approximate minimum lethal dose established very definitely that the toxicity of strontium bromide, chloride and iodide is essentially dependent upon the strontium content. In all three instances, the strontium per kilo weight is 0.123 gm. This is further confirmed by the fact that the haloid constituents at the minimum lethal dose are in the ratio of their atomic weights.

Table V indicates the minimum lethal dose of sodium bromide, chloride and iodide.

TABLE V

MINIMUM LETHAL DOSES OF SODIUM SALTS (10 PER CENT SOLUTIONS USED)

	NaBr	NaCl	NaI
Dose per kilo	2.5 gm.	1.8 gm.	1.3 gm.
Dose haloid per kilo	1.94 gm.	1.08 gm.	1.10 gm.

Comparing the minimum lethal dose of the respective salts of sodium and strontium, we find:

On a $6H_2O$ basis:

Strontium Bromide is 5 times more toxic than sodium bromide.
Strontium Chloride is 4.8 times more toxic than sodium chloride.
Strontium Iodide is 2.08 times more toxic than sodium iodide.

On an anhydrous basis:

Strontium Bromide is 7.2 times more toxic than sodium bromide.
Strontium Chloride is 8.1 times more toxic than sodium chloride.
Strontium Iodide is 3.0 times more toxic than sodium iodide.

In a previous paper,[2] we drew attention to the relative toxicity of potassium as compared to sodium as deciding our choice of sodium iodide for the intravenous administration of massive doses of iodides. In this instance, we employed a 10 per cent weight to volume solution as the solution employed clinically was of that concentration. For a comparison of the toxicity of strontium to that of potassium, we chose the chlorides and continued the choice of the chloride for comparison with the elements in the same group. We maintained a concentration of 5 per cent except in the case of magnesium and of barium which exception will be noted later.

We submit Tables VI, VII, VIII, and IX as indicating the toxicity of potassium, magnesium, calcium, and barium.

TABLE VI

TOXICITY TESTS: POTASSIUM CHLORIDE 5 PER CENT—KCl (0.67 N)

NO. RATS INJECTED	C.C. PER 100 GM.	GM. KCL PER KILO.	POTASSIUM G/K	CHLORINE G/K	COMMENT
4	0.120	0.060	0.031	0.029	Fairly well tolerated
1	0.150	0.075	0.039	0.036	Very sick
3	0.200	0.100	0.052	0.048	All died within 5 minutes

The minimum lethal dose is approximately 0.090 gm. KCl per kilo.

Lower doses produced symptoms of extreme agitation or uneasiness in rats after release from board. Toxic doses produced convulsive contortions, then coma, noted slight twitching of paws, heartbeat irregular for three to five minutes before death ensued.

TABLE VII

TOXICITY TESTS: MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$ 2.06 PER CENT) (0.2025 N)

RAT NO.	C.C. INJECTED PER 100 GM.	GM. $MgCl_2.6H_2O$ KILO	MAGNESIUM G/K	CHLORINE G/K	COMMENT
1110	0.80	0.164	0.019	0.058	Survived
1122	0.80	0.164	0.019	0.058	Survived
1109	0.85	0.176	0.021	0.061	Died within 5 min.
1111	0.85	0.176	0.021	0.061	Died within 5 min.
1120	0.85	0.176	0.021	0.061	Died within 4 min.
1121	0.85	0.176	0.021	0.061	Died within 3 min.
1123	0.85	0.176	0.021	0.061	Died within 3 min.

This clearly indicated that $MgCl_2.6H_2O$ is lethal to white rats in doses of 0.176 gm. per kilo. In all fatal cases, the animals passed through the following stages: violent convulsions, twisting of tail, coma, no visible breathing, irregular heartbeat which continued for some time after respiration ceased.

TABLE VIII

TOXICITY TESTS: CALCIUM CHLORIDE—5 PER CENT—$CaCl_2$ (0.901 N)

NO. RATS INJECTED	C.C. PER 100 GM.	GM. $CaCl_2$ PER KILO	CALCIUM G/K	CHLORINE G/K	COMMENT
2	0.142	0.071	0.025	0.046	No immediate reaction
4	0.250	0.125	0.045	0.080	Increased respiration. Tendency to minor convulsions
5	0.284	0.142	0.051	0.091	Increased respiration. Convulsions and toxic symptoms
6	0.322	0.161	0.058	0.103	Very toxic—three immediate deaths
5	0.356	0.178	0.064	0.114	Three immediate deaths. Two barely survived

The minimum lethal dose is approximately 0.170 gm. $CaCl_2$ per kilo.

At lower doses noted increased respiration. Toxic doses produced convulsive contortions, gasping for breath and then coma for short period.

It will be observed from Table IX that when employing a 5 per cent solution of barium chloride, death ensues rapidly after injecting as small a volume as 0.05 c.c. per 100 gm., making it practically impossible to secure accurate measurement. We were of necessity compelled to employ a dilution of 0.5 per cent. The results are indicated in Table X.

TABLE IX

TOXICITY TESTS: BARIUM CHLORIDE—5 PER CENT—$BaCl_2$ (0.48 N)

NO. RATS INJECTED	C.C. PER 100 GM.	GM. $BaCl_2$ PER KILO	BARIUM G/K	CHLORINE G/K	COMMENT
1	0.20	0.100	0.066	0.034	Died immediately
1	0.15	0.075	0.049	0.026	Died immediately
1	0.10	0.050	0.033	0.017	Died immediately
1	0.05	0.025	0.016	0.009	Died within 30 sec.

TABLE X

TOXICITY TESTS: BARIUM CHLORIDE—0.5 PER CENT $BaCl_2$ (0.048 N)

NO. RATS INJECTED	C.C. PER 100 GM.	GM. $BaCl_2$ PER KILO	BARIUM G/K	CHLORINE G/K	COMMENT
1	0.40	0.020	0.013	0.007	Died immediately
1	0.30	0.015	0.010	0.005	Survived
1	0.20	0.010	0.007	0.003	Survived

The minimum lethal dose is about 0.020 Gm. $BaCl_2$ per kilo. Since death occurred quickly it was difficult to ascertain immediate cause. The dying animals ran the gamut of violent contortions and twisting of tail and then ceasing of respiration and heartbeat. For a period of one minute after death, there persisted a twitching or tremor of injected leg or lower jaw, other signs of life having disappeared.

We had at the outset determined to confine our comparisons with the cations employed in therapeutics. We were prompted to extend our studies to barium which is rarely used in therapeutics, for the reason that the toxicity of magnesium, calcium, and strontium was in an order inversely to the increase

in atomic weights. Barium is generally recognized as the most toxic of the elements of this group and we believe it to be of interest to include it in these comparisons. It will be observed that the lowering of toxicity with the increase of atomic weight does not hold as to barium.

We submit herewith a summary of the toxicity of the chlorides of sodium, strontium, calcium, potassium, magnesium, and barium, placing them in the order of their increasing toxicity. It will be observed that at the concentration we employed, strontium occupies a position next to sodium, being less toxic than the other cations employed in therapeutics.

SUMMARY OF TOXICITY TESTS OF CHLORIDES

	APPROX. MINIMUM LETHAL DOSE PER KILO ANHYDROUS SALT	CATION	ANION (Cl)
Sodium Chloride ($NaCl$)	1.08	0.425	0.655
Strontium Chloride ($SrCl_2$)	0.223	0.123	0.100
Calcium Chloride ($CaCl_2$)	0.170	0.061	0.109
Potassium Chloride (KCl)	0.090	0.046	0.044
Magnesium Chloride ($MgCl_2$)	0.082	0.021	0.061
Barium Chloride ($BaCl_2$)	0.02	0.013	0.007

THE ANTAGONISTIC EFFECT OF STRONTIUM TO MAGNESIUM

In view of the affirmed similarity of strontium action to that of calcium and of the interest recently displayed in the antagonistic effect of calcium on the respiratory depressive action of magnesium, we carried on the following experiments. In order to avoid complications by the evident sedative effect of the bromide anion, we selected magnesium chloride and strontium chloride for this purpose.

In Table VII, we demonstrated that magnesium chloride ($6H_2O$) is lethal in doses of 0.176 gm. per kilo. In order to test out the effect of strontium, we prepared a similar solution containing in addition strontium chloride on a 1/5 equivalent basis.

Table XI indicates the results of the injection of a solution containing magnesium and strontium.

TABLE XI

TOXICITY TESTS: 2.06 PER CENT MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$ 2025 N) 0.55 PER CENT STRONTIUM CHLORIDE ($SrCl_2.6H_2O$ 0.0405 N)

RAT NO.	C.C. INJECTED	WEIGHT OF RAT	GM. $MgCl_2.6H_2O$ KILO	GM. $SrCl_2.6H_2O$ KILO	COMMENT
1112	1.14	133	0.176	0.047	Survived
1113	1.03	120	0.176	0.047	"
1124	.98	115	0.176	0.047	"
1125	1.28	150	0.176	0.047	"
1126	.98	115	0.176	0.047	"
1114	1.05	110	0.195	0.052	Died 3 min. symptoms of magnesium alone

While these rats struggled toward the end of the injection, they recovered quickly from the shock and did not go into coma.

It will be observed in Table VII that all five rats died when injected with 0.176 gm. of magnesium chloride per kilo. When 1/5 equivalent of

strontium chloride was injected with the same dose of magnesium chloride, all the rats survived whereas the same equivalent of strontium chloride was incapable of antagonizing the effect of 0.195 magnesium chloride.

Meltzer and Auer[3] failed to demonstrate that strontium neutralizes the inhibitory effect of magnesium. These authors, however, employed the subcutaneous method of injection into the rabbit, an animal evidently less sensitive to magnesium. They state, however, that strontium causes a slight improvement of the respiration, but seems rather to aggravate and hasten other inhibitory symptoms due to magnesium, especially the paralysis.

To ascertain the effect of a smaller quantity of strontium, we prepared a solution containing 1/10 of equivalent strontium; 2.06 per cent magnesium chloride (0.2025 N), 0.275 per cent strontium chloride (0.02025 N).

At a dose of 0.176 gm. magnesium chloride ($MgCl_2$) one rat died within three minutes after going into coma. Another rat struggled violently, passed into coma with irregular breathing and heartbeat, finally recovering. It was evident that it requires a larger amount of strontium to neutralize the effect of the lethal dose of magnesium. We have shown, however, that strontium like calcium, in relatively small quantities, does exhibit an antagonistic effect to magnesium.

SUMMARY

We have ascertained by intravenous injection into animals that the minimum lethal dose of strontium is approximately 123 milligrams per kilo when the chloride, bromide, and iodide salts are employed.

We have compared the toxicity of strontium with that of sodium, the least toxic of the cations employed in therapeutics. By continuing the comparison with potassium and elements in its own periodic group, we have established that strontium is among the least toxic of the therapeutic cations, occupying the second position in the following order of increasing toxicities: sodium, strontium, calcium, potassium, magnesium, and barium.

In view of the extended clinical administration of strontium bromide and the comparatively low toxicity of strontium demonstrated by these experiments, it is to be concluded that strontium is adaptable for intravenous administration, providing properly controlled solutions and doses are employed.

We have demonstrated also that strontium antagonizes the toxic effect of magnesium by counteracting the respiratory depressive action thus corroborating the impression that the action of strontium resembles that of calcium.

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Editorial

Hypertension in children

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The notion that hypertension of unknown etiology is uncommon in children has been commented upon in the pediatric literature for some time.[1-5] However, in 1966, Tackeuchi,[6] after mass determinations of blood pressure in Japan, reported that essential hypertension may start at an earlier age than was previously thought. More recently, in February, 1971, Zinner and his co-workers[7] concluded from their studies that a familial influence on blood pressure can be detected in childhood, and they postulated that the factors probably responsible for essential hypertension are acquired in childhood. The results of our recently published investigation[8] of 74 hypertensive children add further evidence that this is probably the case.

At the beginning of our studies the definition of hypertension in childhood presented a problem. The published values for normal children varied widely and reliable standards for office practice were unavailable. It was therefore first necessary to establish normal values. The mean values, standard deviations, and the 80 and 90 per cent ranges for each sex and year of age were calculated for systolic and diastolic pressures based on normal children examined in office practice. The population sample consisted of 795 boys and 798 girls, 3 to 15 years of age. Appropriate size, wraparound cuffs, and the mercury sphygmomanometer were used and the pressures were obtained in a standard manner.[9-10]

Our investigations on the hypertensive children were an outgrowth of the above studies and were conducted on 74 children, 4 to 18 years of age, who were asymptomatic and who either came for routine physical examinations to the private office of one of the authors (S. L.) or to his office in the Pediatrics Department of the St. Louis Labor Health Institute. The elevated pressures noted in these patients were incidental findings.

As suggested by Master and his coworkers[11] in their studies of adult patients, children whose systolic and/or diastolic blood pressures were repeatedly recorded above the ninetieth percentile and occasionally above the ninety-fifth percentile for age and sex were classified as hypertensive. All but 2 were under 15 years of age when their hypertension was first detected and they were followed for an average of 3½ years and in some cases for as long as 8 years. The youngest age at which elevated pressure was observed was 3 years. Only patients who were hypertensive for a year or longer were included in the study. Laboratory studies for possi-

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ble causes of hypertension were performed on 33 individuals as outpatients. Because of the paucity of abnormal findings in this group, 41 patients were more extensively investigated as inpatients in the Clinical Research Unit of St. Louis Children's Hospital.

Possible causes for hypertension were found in only 5 patients, who had abnormalities of the urinary tract. The presence of obesity in 53 per cent of the children and a family history of parental hypertension in 44 per cent, however, were significantly higher than in normotensive subjects. The systolic and/or diastolic pressures were still elevated in 65 per cent of 46 patients who were followed for 3 to 8 years.

The absence of an apparent cause for the hypertension in all but 5 of the patients, the high prevalence of obesity and of parental hypertension, and the persistence of elevated pressure in two thirds of the children followed for over 3 years, raises the question whether this type of juvenile hypertension is a prelude to essential hypertension in later life. Additional studies by other investigators and continued study of our patients into adulthood may help clarify this perplexing question.

Why has it been considered that hypertension in children is usually secondary? The reason may be the lack of a logical frame of reference for the practitioner taking blood pressures of children. Because of this, authors writing about juvenile hypertension have arbitrarily considered 135 to 140/85 to 90 mm. Hg as the upper limit of normal. However, since these values are higher than the ninetieth percentile for children under the age of 10 years in our studies,[9-10] younger children with moderately elevated blood pressures would have been excluded. Furthermore, whereas the patients in our study were asymptomatic children whose elevated pressures were incidental findings, the cases usually reported in the literature were hospitalized, symptomatic, and severely hypertensive children.

As previously reported for both adults and children,[12-15] the blood pressures of our patients fell after they were in the hospital. Can it be that reliance on hospital blood pressure determinations alone may overlook children whose pressures may be moderately elevated in day-to-day life? Perhaps normal ranges for hospitalized children should also be established.

Since intensive investigation revealed conditions possibly related to hypertension in only 7 per cent of our patients, extensive and expensive hospital studies are not indicated in this type of child. The following kind of outpatient study would seem reasonable: inquiry for family history of hypertension; complete physical examination, including lower extremity blood pressure determination; blood and urine examination; creatinine clearance determination, checks on serum potassium and urea nitrogen levels; and a rapid-sequence intravenous pyelogram.

Recent evidence indicates that the treatment of adults with long-standing essential hypertension reduces the risks of complications.[16] Should treatment, therefore, be started in asymptomatic children with hypertension of unexplained origin? Probably not, because the pathogenesis of essential hypertension is as yet unknown, and there is no evidence at this time that these hypertensive children will develop fixed hypertension in later life. Also, the antihypertensive drugs have well-known undesirable side effects, and we question the wisdom of their use in growing children. Finally, there is no information available as to whether the institution of antihypertensive therapy in childhood hypertensives will prevent the progression of the disease. It is known that obesity is a contributing factor and there is suggestive evidence that excessive sodium intake[17] may play a role. There is therefore justification for recommending weight reduction in the hypertensive child and probably some reduction in his dietary salt intake. These children should have annual physical examinations, including funduscopic examinations and urinalysis. From the standpoint of prophylaxis, hypertensive parents should be apprised of the influence of heredity, obesity, and possibly of salt intake in essential hypertension. It should be stressed that all advice be offered without creating needless anxiety.

The youngest age at which we have been able to obtain reliable auscultatory blood pressure readings under office conditions

was 3 years. Since we have documented elevated pressure as early as 3 years, it is highly probable that hypertension may begin at an even earlier age. The necessity for establishing reliable blood pressure standards from early infancy to 3 years of age is obvious. There are now instruments available for the measurement of blood pressure in infancy,[18] and it is hoped that pediatricians will make use of these new techniques. Once reliable standards are established for this age group, the question of how early hypertension appears may be answered.

The much higher incidence of hypertension and cardiovascular disease among black adults than among white leads to the question of how early in life this difference appears. Unfortunately, there were not enough black children in the population studied by us to answer this question. Rose,[19] who compared black and white tenth-grade children, found no difference. Whether this is true for younger children awaits further investigation.

Our observations point to broader horizons for preventive pediatrics. The importance of spotting an early tendency to hypertension needs no emphasis to the readers of this Journal. However, the importance of careful routine blood pressure determinations does need emphasizing to the practitioner treating children. The implications from the public health standpoint are clear. Furthermore, just as the routine tuberculin test in the young sometimes leads to uncovering adult tuberculosis, so may the finding of hypertension in children lead to the uncovering of hypertension in parents who may not have had blood pressure determination for a long time.

The studies herein discussed also indicate the necessity of continuing extramural research on healthy populations, as well as continuing research in medical centers, which concern themselves mainly with sick people. Only by continued observations of healthy infants and children will we be able to spot minor but significant deviations from the normal which may affect longevity.

The educational campaign on the concept of risk factors in atherosclerosis and coronary artery disease which has been mounted by the American Heart Association has been aimed exclusively at the adult population. It may well be that both these diseases, as well as essential hypertension, have their origin in infancy or childhood. When more information becomes available and the pathogenesis of these diseases is better understood, the risk factor campaign can be broadened to include the entire population. Also, it is of vital importance to attempt to develop, through research, the profile in infancy and childhood of the individual who is prone to develop atherosclerosis, coronary artery disease, or essential hypertension. Hopefully this knowledge may eventually lead to effective prophylactic programs against these dreaded diseases.

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Research in infant nutrition: the untapped well[1]

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Intromed

The Chairman of this Symposium has asked me to fulfill two separate functions: to comment on the communications presented; and to review some of the research challenges facing those individuals investigating the nutrition of infants and children. He has, in addition, offered me an opportunity to speculate on the relationship of vascular disease and nutrition.

Both speakers were kind enough to permit me to view a synopsis of their comments so as to fulfill the first function assigned.

Let me begin by commenting on the broad issue of the relationship between the salt content of infants' diets and the development of hypertension in adult life. Several questions come to mind: *1*) Is there a correlation between salt intake of adults (i.e., their customary or chronic salt intake) and the development of hypertension? *2*) Are today's infants consuming excess salt and is there any evidence that a high salt intake in infancy predisposes to high salt intake among adults? *3*) Is there any evidence of secular increases in salt intake by American infants? *4*) Is there any evidence that current levels of salt intake are injurious to infants?

The issue in adults rests in a large part on epidemiologic information. A recent summary of this evidence concluded as follows: "The evidence associating salt intake and hypertension in man is inconclusive" (1). The statement then summarizes the pros and the cons concluding that, on balance, the issue remains questionable.

There is no question that the practice of adding salt to prepared infant food during manufacture increases the salt intake of American infants. According to a recent survey, averages of the intake range from 2.2 to 5.7 mEq/100 kcal or 9 to 60 mEq/day. The lower numbers are for younger infants and the larger value for infants approaching 1 year of age. A study reported 5 years ago gave rather similar data (2) with intakes at 1 month of age averaging 0.3 mEq/100 kcal rising to 6.4 mEq/100 kcal at 1 year.

Attempts to relate these intakes to those of adults have frequently been made by comparing the intake on a per kilogram basis. When such comparisons are made, the 70-kg adult consumes 25 g NaCl/day if given salt equal to that taken by infants with the highest intakes. In contrast, the average adult on his customary diet consumes between 2 to 4 mEq/kg or 8 to 16 g/day (3). When the comparison is made on the basis of sodium intake per 100 kcal of diet, the picture is rather different. An average man, eating 2,800 kcal/day, would ingest a total of 10.5 g NaCl if he consumed salt at the same rate as the infant having the highest intake quoted above, 6.4 mEq/100 kcal. This is a value significantly below the average of 12 g NaCl/day usually taken by adults consuming a mixed average diet.

Although it is easily shown that the salt intake of American infants is substantially higher than it would be if they were breast fed, there remains no evidence that the present levels of intake have produced morbid changes in structure or function. The ability of the infant kidney to excrete a sodium load is probably not compromised beyond the 1st few weeks of life, although the period is longer in the premature infant. On the other hand, solids on the average, are introduced at approximately 6 weeks of age, and under these circumstances, there is

[1] This paper was presented as part of the Symposium on Factors in Childhood That Influence the Development of Atherosclerosis and Hypertension at a joint meeting of the American Federation for Clinical Research and The Society for Pediatric Research on May 1, 1971 in Atlantic City, New Jersey.

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little fear of a physiologic inability to handle the levels of salt intake prevailing when proprietary foods provide a significant source of nutrients. Milk continues to supply well over one-half the calories and protein in the diet until well beyond the 2nd year of life.

Whether the increased Na content of certain prepared infant food conditions infants to a higher Na intake in later life remains debatable, although Guthrie (4) claims to have demonstrated a long-term effect suggesting that high intakes early in life program the infant to continue this practice in adult life. Certainly the added Na does not increase acceptability of the food, for studies have shown the willingness of infants to accept prepared food without salt added by manufacturers, but otherwise similar.

It has been stated that the Na is added in manufacturing as a means of satisfying the palate of the parent rather than that of the infant. In part the producer hopes that the mother, when tasting the baby's food as she almost invariably does, will find his product attractive. In fact, the claim has been made that if the product is unsalted, the mother will add salt.

This brings us to the interesting question—why does the mother add salt? Undoubtedly, she does so because she associates a specific degree of saltiness with specific food. But, why does she salt her food? Here habit may be at work, but it is also possible that she is responding to a specific biological need when she salts her own food. We are all familiar with the anecdote of the child with undiagnosed Addison's disease who amazed his parents with the amount of table salt added at each meal. Accurate diagnosis and appropriate treatment were followed by reduction of his intakes to normal. No one questions that the healthy individual has an uncanny ability to maintain sodium homeostasis. We meet our needs from a variable diet and give evidence of intuitive knowledge of need, just as we do for calories and water. No one speaking of water, as opposed to alcoholic beverages, says a persons drinks too much. Day in and day out, with rare exceptions, he consumes his required amount. So too with salt.

What determines the mother's estimate of need? Quite possibly, in part, her hormonal status may be under genetic control. But also, in part, her need for salt may reflect environmental temperatures as well as intake of potassium. Wild animals, living on forage, consume a high K diet and travel large distances to find a source of sodium chloride; modern knowledge of renal physiology has provided an explanation for this characteristic behavior of all foraging animals. The rate of excretion of hydrogen and potassium ions by the distal renal tubule is a function of the availability of sodium; on a high potassium diet, the amount of sodium available may be rate limiting and, in theory, if the disproportion between sodium and potassium in the diet is sufficiently great, hyperkalemia and acidosis may ensue.

A diet of meat, vegetables, and cereal without added salt becomes a high K, low salt diet (Table 1). The solid foods we first give to infants, vegetables and fruits, have virtually no sodium, whereas they contain relatively large amounts of potassium. Although the issue may remain unresolved until better evidence becomes available, I propose that the salting of food represents the need for Na and what the mother has learned of her own salt needs during her lifetime of eating, she now translates into the need

TABLE 1

Salt, protein, and caloric content of selected infant foods[a]

	Na, mEq	Kcal/100 g	Protein, g	Na, mEq [illegible]	[illegible]
Cow's milk	2.5	70	3.5	[illegible]	0.7
Human milk	0.7	75	1.1	[illegible]	0.6
Strained beef	10	100	14.7	10	0.6
Junior beef	12	118	19	[illegible]	0.6
Chicken dinner	11	100	7.4	11	1.5
Beef dinner	11	87	7.4	[illegible]	1.5
Green beans	9	37	1.4	24	6.5
Oatmeal	19	375	16.5	[illegible]	1.1
High protein cereal	28	357	35.2	8	0.8

[a] Taken from Agriculture Handbook [illegible] adapted by Fomon, S. J. *Infant Nutrition*. Philadelphia: Saunders, 1967, p. 280. (Numbers [illegible] rounded to nearest decimal.)

TABLE 2
Secular trends in infant food composition*

Years	Na, mg/100 g, product	Protein, g/100 g product	Protein, g/100 kcal
1957	223	5.05	5.1
1960	219	5.06	5.0
1965	205	4.48	4.7
1966	202	4.42	4.7

*Prepared from data made available by Dr. Robert Stewart.

of the infant as he begins to consume foods with which she has had experience.

A recent statement by the Food and Nutrition Board on the issue of salt in infant food took the following position: "In essence, the committee has found no evidence that, insofar as healthy infants are concerned, addition of sodium chloride at current levels to infant foods is either harmful or beneficial to the infant. There is no valid scientific evidence available in support of the contention that addition of salt to infant foods contributes to development of hypertension or other disease states in adult life, nor is there valid evidence that the practice is not harmful, or that salt levels now consumed by infants in the United States overburden excretory mechanisms" (1).

It seems difficult to sustain the recommendation contained in the statement that salt content of prepared infant foods be reduced to a fixed level of 0.25%. There is evidence neither for nor against this specific level, but logic would argue that at least some cognizance be taken of the K content of the specific food so that the Na:K ratio be maintained in the range of 0.5 and 1. In any case, no evidence can be found that the content of Na in manufacturing infant foods is steadily increasing (Table 2) and, hence, an argument for maintaining status quo has as much justification as any proposal for initiating regulations limiting manufacturing practice.

The second issue before us concerns the broad question of the relationship at all ages between diet and the development of vascular degenerative disease. Specifically, we are asking whether the diet of the infant affects his propensity for, or resistance to, development of vascular disease in old age. The report given by Dr. Glueck today indicates that very real progress can be made in the early specific identification and prompt beneficial treatment through diet of one form of hyperlipidemia associated with atherosclerosis. We now must examine what remains of this question.

Interest in this problem arose, at least in part, from the observations made during World War II and the Korean War that young men were dying of coronary artery disease, and necropsies on soldiers dying of other causes showed a significant and unexpected prevalence of coronary artery disease. Heretofore, atherosclerosis had been considered predominantly a disease of the aging or the aged. As the lesions were seen in relatively young individuals, it appeared logical to seek the pathogenesis in some factor of life characteristic of the individual in childhood or even infancy.

At the same time, epidemiologic studies in adults of various ages were beginning to develop evidence that plasma and dietary lipid levels were related to the appearance of vascular disease. The tangled story need not be reviewed here in detail, but implicated as causative have been, in turn, total dietary lipids, cholesterol, saturated lipids, sucrose, and perhaps even total calories to the extent that a correlation has been made between obesity and the frequency of various forms of vascular disease (5).

The interplay between genetic factors, exercise, and dietary variables complicates the problem. As dietary lipids have been indicted in the pathogenesis of atherosclerosis, present emphasis is placed upon a reduction of saturated fats in the diet and an isocaloric replacement by polyenoic fats.

Though lacking conclusive or really convincing evidence on which to implicate a dietary factor or factors in adults, it has been proposed that one might prevent coronary vascular disease among the relatively young or even among the elderly by manipulating the diet of infants and children.

One must question whether the fatty streaks found in the aorta of the young are the antecedents of atherosclerosis. To the extent that no convincing sequence of development of vascular lesions from minimal to

maximal from infancy to adult life has appeared, the issue must remain debatable. Furthermore, until we have a convincing case for indicting a specific dietary lipid in adult vascular disease, it seems difficult to advise, with any conviction, that specific changes should be made in the dietary fats of infants and children.

Dr. Fredrickson has demonstrated convincingly that there are at least five genetically determined causes of hyperlipidemia, more than one of which may be associated with premature atherosclerosis and each of which requires type-specific dietary modifications for treatment. Although a causal relationship between hyperlipidemia and vascular disease now seems beyond doubt, it must be apparent to all that one cannot make the most effective therapeutic recommendations, let alone initiate prophylaxis for the general population, without knowledge of the heterogeneity, genetic and otherwise, among different subjects. National recommendations will only be valid insofar as the regimen modulates specific types of hyperlipidemia and then only to the extent that the particular forms of hyperlipidemia are represented in the population as a whole.

Caution, therefore, seems indicated and one would be loathe to initiate any nationwide change in our feeding practices for infants and children. This is not to claim that current practice is ideal, as it may not be. But we do not have the evidence with which to recommend rational change.

I would now like to move to the second part of my assignment, that of commenting on some of the unknowns in nutrition of the fetus, the infant, and the child and indicate where the well of knowledge can be found by the dowsing of science.

Three major advances in medical knowledge are responsible for the remarkable drop in infant mortality during the past generation, not only in this country, but in all economically advanced nations. The advances include the development of chemotherapy for infectious diseases, perfection of treatment for diarrheal disease and, finally, mastery of infant feeding techniques. At the turn of the century and well into the 2nd decade of this century, a principle art of the pediatrician was the ability to design a milk formula f[illegible] artificial feeding. Today over 70% of all i[illegible]fants receive commercial proprietary form[illegible]las concocted according to recipes deriv[illegible] by these pediatricians and pro[illegible]d by fi[illegible] years of experience. Many yo[illegible] pedia[illegible]cians, although far more knowi[illegible] about m[illegible]lecular genetics than their f[illegible]ars, [illegible] considerably less learned a[illegible] form[illegible] preparation. Faced with the ne[illegible] conc[illegible] a formula, they might flounder [illegible] sudde[illegible] realize that the preparation of [illegible]milk m[illegible]ture adequate to sustain health [illegible] grow[illegible] is an art as well as a science.

The very success of commerc[illegible] prepar[illegible]tions has lulled us into believing t[illegible]t we ne[illegible] to know little about nutrition, a[illegible] all infa[illegible] thrive on any of a number of form[illegible]las read[illegible] available at the pharmacy and, in some [illegible]stances, the supermarket. Is our sense of co[illegible]fidence well founded? I am not certain. L[illegible] me at least point to areas in need of seri[illegible] research.

The lipid composition of modern in[illegible] formulas is quite different from that of h[illegible]man milk or, the substitute with which [illegible] have had most experience, cow's milk. T[illegible] contemporary products use mixtures of veg[illegible]table oils which, when consumed, produce [illegible] least two effects not seen when breast milk [illegible] consumed. The cholesterol level in plasma [illegible] very low and stool calcium disturbingly hi[illegible] at least during the 1st weeks of life. Wheth[illegible] either of these differences is significant, [illegible] do not know; but I would propose that un[illegible] we can simulate human milk in every w[illegible] there is much need for active research.

Modern cow's milk formula products [illegible]tempt to simulate human milk by adjusti[illegible] the ratio between casein and lactalbumin. [illegible] produce this, it is necessary to fraction[illegible] cow's milk, in some cases, electrophor[illegible]cally. Whereas the final product simul[illegible] the protein ratio in human milk, we do [illegible] know what has been lost in the product[illegible] process. Serious consideration must be gi[illegible] to the possibility that trace metals of un[illegible]parent importance have been removed. W[illegible] this to be true, a strong argument could [illegible] made for the early introduction of solid fo[illegible] as they might logically become the source[illegible] trace metals.

Although we have developed guidelines for fortifications of milk with vitamins and even minerals, it is unnerving to remember that our evidence is not better than empirical judgment. We know with precision neither the minimal daily requirement nor the optimal intake of any single nutrient for the infant. It is true that any pediatric text provides a table of requirements, but the reader is never told that shaky evidence buttresses these numbers. We either infer them by extrapolation from adult requirements or make estimates derived from knowledge of the composition of human milk. This guide might be satisfactory if all children were well at all times and if all infants received breast milk. Neither is true.

The nutrient content of human milk is proper for that particular mixture of fats, proteins, minerals, and vitamins, but probably no other. Several obvious examples will make the point. The amount of phosphorus present determines whether or not the calcium content is adequate. The folate content will be adequate only if the carbon content is specified. The vitamin E content will suffice only as long as the ratio of saturated to unsaturated lipids is fixed. One could continue the list, but the examples cited should suffice. We simply do not know a priori whether a given mixture will provide nutrients for optimal growth. Too often, in the past 2 decades, we have been reminded how little we know about the interaction and interdependence of nutrients only when infants exhibited specific deficiency syndromes. Our knowledge of the interrelation between folate and ascorbic acid developed only after infants receiving several proprietary formulas developed megaloblastosis. The effects of B_6 deficiency on the central nervous system came to the attention of pediatricians when a change in manufacturing process reduced the B_6 content of a formula below a critical level. The lack of iodine in soy formulations was only discovered after a number of infants developed goiters.

The problem becomes more troublesome when we realize that drugs can influence the nutritional requirements of infants as well as of adults. This influence is usually, but not always, in the direction of increasing the need. The examples that follow should illustrate the problem and indicate how important it is that we develop greater knowledge through research of the nutrient needs of infants.

Vitamin D has been recognized as an essential nutrient for almost 50 years and we probably have better knowledge of the requirement and toxicity of this vitamin than of any other. Most nutritionists feel confident that consumption of 400 IU/day is not only safe but probably ideal. Suddenly a new facet of this vitamin has appeared. In the past 3 years, three reports have identified the fact that 15% of young epileptics (6) and 22% of a group of epileptics of all ages (7) had roentgenographic and chemical evidence of osteomalacia at a time when all subjects were receiving an intake of vitamin D that is usually considered normal. Subjects in both groups had been receiving therapy with a variety of anticonvulsants; when daily intake of vitamin D was increased, the osteomalacia disappeared despite continuation of anticonvulsant therapy.

This same group of anticonvulsive agents has been implicated in the production of folate deficiency in adults (8, 9) and in children (10). They appear to cause a significant lowering of the folate level in serum.

Neither in the case of vitamin D nor folate is it clear how the anticonvulsant drugs changed the requirement for a vitamin, although it has been suggested that stimulation of the microsomal, drug metabolizing enzyme system by barbital-like substances may have increased catabolism of the vitamin.

The effect of antimicrobial agents upon the microflora of the gut and intestinal absorption has been appreciated for many years. Recently the rather specific effects of one agent, neomycin (11), have been studied in detail and it is now clear that following therapy with this agent, there is a loss in the gastrointestinal tract of essential nutrients such as vitamin B_{12} and carotene as well as of iron; in addition, lactase activity (12–14) falls and specific morphologic changes appear in intestinal biopsy specimens.

Antimicrobial agents also appear to effect vitamin K requirement, but marginal dietary intake is probably necessary before antibiotic therapy can precipitate hypoprothrombinemia.

Isoniazid administration has been shown

to lower the blood level of vitamin B_6 but functional studies, using tryptophan loading, suggest that frank deficiency does not appear even after prolonged administration (15).

During early life, the infant's formula is his sole source of nutrition; he cannot rely upon other dietary constituents to supplement his diet as can the adult. Errors in formulation may have morbid or mortal consequences. More importantly, nutrient quality is dependent upon the nutrient environment, the relationship of the one nutrient to the other, and of nutrients to nonnutrient products consumed by the infant. It becomes increasingly important to learn more about nutrient requirements and interaction not only to carry an infant safely through an illness but, more importantly, because during the next decade we will witness increasing experimentation with novel formula preparations. As milk protein becomes increasingly expensive, we will seek out vegetable sources. In this country the issue may be less acute than in developing nations. For the latter it may be a matter of milk substitutes or starvation. Until we have a more complete knowledge of true requirement, as well as the interaction among nutrients, such undertakings may be hazardous if not lethal.

My final comments will relate to an outstanding opportunity for utilizing nutrition knowledge in the interest of public health. I am addressing particularly the distressing issue of our continuing high level of infant mortality. In analyzing the cause, it has become apparent to all that the greatest contribution to infant mortality comes from low birth weight infants. Our infant mortality rate is now about 20/1,000 live births, which places us about 14th among advanced nations. Our low position does not mean we have made no progress. Indeed we have, for in 1940 the infant mortality rate was 50/1,000 live births, and in 1950 it was 30/1,000. Other countries have made more progress. At the same time that our infant mortality rate has continued a slow fall, the prematurity rate, births under 2,500 g, has continued to rise and is now over 8.2% (1968). An analysis of infant mortality data indicates that three-fourths of the deaths in the 1st year of life occur during the 1st month and, therefore, are in large measure due to prematurity. Hence the greatest improvement in infant mortality could be expected from any procedure that reduced the number of low birth weight infants.

Dr. Mervin Susser of Columbia University (Table 3) has been kind enough to make available data on the birth weight of infants and weight gain of their mothers [illegible] deliveries at the Harlem Hospital [illegible] New York. All mothers were patients of the hospital at the time of delivery, but no information is provided on the type or duration of antepartum care.

What emerges from analysis of these data are a series of conclusions, some of which have been previously observed, but several of which are unique. All are of interest and bear on the prevention of low birth weights.

The best predictor of birth weight is the weight gain of the mother during gestation (Table 4). The overall correlation coefficient of weight gain of mother on birth weight of infant is 0.94 with a confidence limit of 0.91 to 0.97. If her prepregnancy weight is removed from the regression, we still account for 51% of the variation; the mother's prepregnancy weight accounts for 37% of the variation. Similar conclusions have been drawn from the data accumulated by participants in the collaborative study of the National Institute of Neurological Diseases and Stroke.

The second observation is also of interest (Table 4). Although the weight gain of the mother is highly correlated with the birth weight of the infant, the rise in infant weight is not continuous, reaching, on the average, a maximum at approximately 3,500 g regardless of the prepregnancy weight of the mother or the absolute weight gain during pregnancy. This suggests that there is a biological norm and, above this level, neither the mother's weight nor her weight gain has a positive effect upon the birth weight.

The third observation of interest relates to the proportion of weight gain during pregnancy that is reflected in birth weight of the infant. The greater the weight gain of the mother, the smaller is the percent of the weight gain that is converted into fetal tissue. The remainder must be maternal tissue. (For equal weight gains during pregnancy, the heavier the woman, the smaller is the per

TABLE 3

Mean birth weights in grams, by weight gain and prepregnancy weight, for all registered singleton liveborn infants, between 37 and 44 weeks gestation, and numbers of individuals in each category, Harlem Hospital, 1964 to 1967

Weight gain, kg	Prepregnancy weight mean							
	46	51	55	60	61	64	73	77
1.36	(28)	(43)	(55)	(48)	(48)	(36)	(27)	(15)
	2,657	2,704	2,790	2,837	3,006	3,011	3,055	3,013
3.64	(65)	(139)	(159)	(131)	(92)	(61)	(34)	(24)
	2,661	2,800	2,907	2,915	3,034	3,136	3,147	3,170
5.91	(139)	(260)	(241)	(208)	(117)	(88)	(63)	(33)
	2,874	2,879	2,923	2,922	3,051	3,113	3,169	3,200
8.18	(176)	(292)	(299)	(279)	(126)	(103)	(60)	(30)
	2,877	2,917	3,026	3,053	3,146	3,193	3,196	3,189
10.44	(177)	(260)	(312)	(196)	(148)	(79)	(61)	(28)
	3,000	3,004	3,073	3,050	3,147	3,289	3,252	3,392
12.73	(96)	(205)	(210)	(217)	(122)	(73)	(44)	(16)
	3,051	3,102	3,158	3,165	3,192	3,154	3,275	3,281
15.00	(50)	(111)	(111)	(117)	(81)	(38)	(25)	(13)
	3,090	3,112	3,186	3,197	3,203	3,313	3,324	3,176
17.27	(21)	(41)	(56)	(63)	(59)	(35)	(20)	(13)
	3,128	3,290	3,264	3,249	3,237	3,369	3,380	3,446
19.54	(10)	(13)	(28)	(24)	(22)	(12)	(10)	(4)
	3,070	3,184	3,300	3,087	3,350	3,316	3,480	3,375

TABLE 4

Average birth weight as a percent of weight gain

Weight gain, lb	Prepregnancy weight, lb							
	100–109, %	110–119, %	120–129, %	130–139, %	140–149, %	150–159, %	160–169, %	170–179, %
3	195	198	205	208	220	220	224	221
8	73	77	80	80	83	86	87	87
13	49	49	49	49	52	53	54	54
18	35	36	37	37	38	39	39	39
23	29	29	29	29	30	32	31	32
28	24	24	25	25	25	25	26	26
33	21	21	21	21	21	22	22	21
38	18	19	19	19	19	20	20	20
43	16	16	17	16	17	17	18	17

centage of this weight gain converted to fetal tissue.)

For each level of weight gain in women of similar weight, a different percent of weight goes to increase fetal weight and for each prepregnancy weight, a specific weight gain during pregnancy has a different effect upon infant weight. This must raise a question concerning the general practice of fixing the weight gain of women by some arbitrary standard (Fig. 1).

These observations can be illustrated by exploring interaction using three levels of prepregnancy weight. For the woman with a prepregnancy weight of 115 lb, a 10-lb increase in weight gain adds an additional 122 g to the birth weight of her infant; for the woman starting pregnancy at 140 lb, a similar increase in weight gain adds 99 g; and for the woman weighing 165 lb, the same weight gain confers 77 g on the fetus. It is of special interest that for any weight gain during pregnancy, the birth weight of the infant is a fixed percentage of the actual weight gain re-

gardless of the mother's prepregnant weight. For very small weight gains, there is a slight increase in the benefits of starting pregnancy with a higher body weight.

These observations on weight may have other importance. Recently, Thompson, Hytten and Billewicz (16) examined the efficiency of lactation. They questioned the general recommendation that the maternal diet be increased by 1,000 kcal/day during lactation. They base this challenge on the conclusion that the weight gain of the mother during pregnancy, after accounting for the weight of fetus and decidua, is largely fat and that this fat provides the energy for lactation. The orderly weight loss following pregnancy reflects the utilization of depot fat for the energy demanded by lactation.

They calculate that 1 kg of body weight loss postpartum will provide 6,500 kcal. If a mother produces 500 ml of milk daily, and assuming that the process is close to 100% efficient, the calories required for milk production amount to 350. The 1 kg of body weight will supply energy for 500 ml of milk for approximately 23 days. Thus, if a mother gains 10 kg in excess of fetal and placental weight, she will have a storage of 65,000 kcal for lactation of 3.25 months' duration; that is, assuming that her milk production keeps pace with the infant's need and consumption proceeds from a volume of 750 ml at 1 month to 900 ml at 3 months and 1 liter thereafter. All this appears very reasonable, as breast feeding up to 3 months or longer is certainly common practice in societies in areas where formulas are unavailable. Thus, the weight gain of the mother may play a useful role other than merely providing a bigger baby; it prepares her for lactation.

The correlation between weight gain of mothers and birth weight takes on special significance if encouraging a mother to eat ad libitum and gain weight can prevent low birth weight infants. The data presented for correlation examination lack information on low birth weight infants. We can infer, however, from the published data of Brewer (17) that a nutritious diet producing a generous weight gain during pregnancy can, in fact, remarkably reduce the incidence of low birth weight. In 1,500 pregnancies among a lower

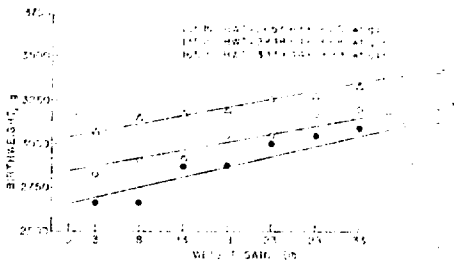

FIG. 1. The relationship between bir[illegible] weig[illegible] and weight gain during pregnancy.

economic group of women, the pren[illegible]turi[illegible] rate (birth weight below 2,500 g) was [illegible].2% and even among 381 primipara from 14 t[illegible] 21 years of age, the rate was only [illegible].7% These numbers should be compared with th[illegible] national average of 8.2% (1968) and 15% in lower income black women.

These conclusions challenge the conve[illegible]tional wisdom, which demands constraint o[illegible] weight gain by caloric restriction, a limita[illegible]tion of salt intake, and use of saline diuretics. None of these were used in the Brewer serie[illegible]

If these observations are confirmed i[illegible] other and larger series, we will be in a pos[illegible]tion to explain what to date has confounde[illegible] those epidemiologists studying infant morta[illegible]ity rates and prematurity rates. Why have w[illegible] failed to reduce infant mortality as effective[illegible] as other modern nations and why is our pr[illegible]maturity rate rising, a factor of life in [illegible] other advanced nation?

The answer may well lie in our prenat[illegible] regimens. It looks as if we can make re[illegible] progress on both questions merely by feedi[illegible] pregnant women.

It would be unfortunate to close this sem[illegible]nar without providing some possible corre[illegible]tion between these nutritional observatio[illegible] and the pathogenesis of atherosclerosis. [illegible] recent review states as follows: "Among t[illegible] factors associated with the accelerated a[illegible]pearance of atherosclerotic vascular dise[illegible] the level of blood pressure has been repe[illegible]edly shown to be a potent variable in all [illegible] the large epidemiological studies" (18).

Therefore, one might, on being challeng[illegible] attempt to seek a correlation between so[illegible] element of nutrition and the developmen[illegible] hypertension, recognizing full well that [illegible]

all subjects with atherosclerosis have hypertension and that hypertension may appear in a predisposed individual only if other contingent factors coexist.

My hypothesis is as follows: the predisposing condition to hypertension is intrauterine malnutrition. We know that the incidence of toxemia of pregnancy is from three to five times as common among women in the lowest socioeconomic groups as it is among the population at large. There is direct evidence that the fetus participates causally in the hypertension of toxemia for, following delivery, there is dramatic abatement of hypertension. It has been suggested that the juxtaglomerular apparatus of infants born dead to mothers with toxemia shows hyperplasia similar to that seen in sodium-restricted animals and some humans with hypertension. Perhaps it is the living infants, born either to toxemic mothers or mothers with subclinical toxemia, who will be programmed to develop hypertension in later life. Whereas the hypothesis is at best a surmise, it is at least susceptible to testing by retrospective studies. Furthermore, it is consistent with at least several epidemiologic coincidences of interest, the relatively high incidence among blacks of hypertension, low birth weight infants, and toxemia of pregnancy. It is attractive in that if proven, we can, at one and the same time by providing sound nutrition during pregnancy and by encouraging weight gain, moderate not only the infant mortality rate but perhaps eliminate one cause of hypertension and the ensuing vascular disease.

We have wandered far over the terrain of nutrition, reviewing several critical aspects, all in the hope of developing nutrition programs for the infant and child that might prevent the development of degenerative cardiovascular disease in later life. My conclusions, though tentative, are as follows:

1) We can find no compelling evidence that the present level of salt in baby food or the total salt content of infant diets in this country can be implicated in the development of hypertension in adult life.

2) There are no convincing data to argue that a change in current feeding practice for infants with a modification of fat intake or change in lipid types will act beneficially in preventing atherosclerosis. In the absence of this evidence, prudence would suggest we maintain status quo.

3) There are many intriguing and important areas of nutrition research demanding to be explored by the curious and ingenious. There is even a sense of urgency and necessity that they be explored.

4) It is possible and even probable that improvement of nutrition during pregnancy by encouraging weight gain can improve infant mortality by reducing frequency of low birth weight infants.

5) A speculation has been advanced that the substrate upon which hypertension in adult life appears is intrauterine malnutrition.

We are indebted to Dr. Samuel Greenhouse for the statistical analysis provided.

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strength lactated Ringer's injection U. S. P. On the following day, fever, diarrhea, and vomiting persisted. He had become irritable and had not voided for six hours. He was therefore admitted to the hospital.

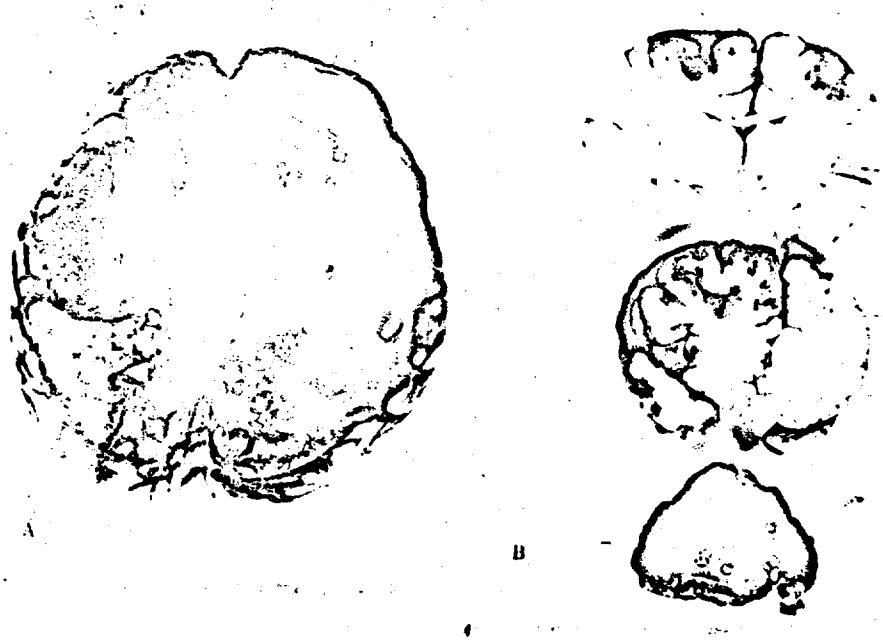
On admission, he was in no distress, but was mildly dehydrated. The vital signs were as follows: temperature 37.5 (99.5 F); pulse 126 and respirations 32, per minute, blood pressure 115/90; weight 8,300 gm. Except for mild dehydration, a depressed fontanel, and a liver edge palpable 2 cm. below the right costal margin, there were no abnormal physical findings. Laboratory studies showed hemoglobin 9.0 gm/ml.; white blood cell count 10,500; polymorphonuclear leukocytes 55%, and lymphocytes 43%. The urine was cloudy; specific gravity, 1.035; pH, 6.0; protein, 3+-4+; sugar, trace. Microscopic study (centrifuged specimen) showed 5-6 rbc per high-power field, and many hyaline and granular casts. Serum analyses showed NPN 70 mg/100 ml., sodium 159 mEq/L., CO_2 13.2 mEq/L., chloride 132 mEq/L., and potassium 5.5 mEq/L.

The infant took a milk formula well during the first 48 hours of hospitalization. He voided four times within the first 24 hours and five times on the second day. The temperature, however, remained around 38-39 C (100.4-102.2 F). Throat, blood, and urine cultures were negative. A nasopharyngeal culture showed a few colonies of hemolytic Staphylococcus aureus. Chest x-rays and an electrocardiogram were normal. The urine continued to show 3+-4+ protein, numerous white blood cells and red blood cells, but fewer casts. On the third hospital day he had a generalized convulsion. Despite intramuscular phenobarbital, he had another convulsion four hours later. Serum analyses at this time were as follows: NPN 80 mg/100 ml.; sodium 174 mEq/L.; chloride 127 mEq/L.; potassium 4.6 mEq/L.; CO_2 11.5 mEq/L.; glucose 240 mg/100 ml.; calcium 11.3 mg/100 ml., and phosphorus 5.7 mg/100 ml. Several hours later he began to breathe more rapidly, with a rate varying from 30 to 50 per minute, and had a pulse rate of 120-160 per minute. Rales were heard in the lungs. He was given one-fourth of the estimated dose of a digitalis glycosides preparation (Digalen) required for full effect. He voided only once that day, and the blood pressure stabilized at 120/60. A few hours later, however, he suddenly became hypotensive and died.

Autopsy.—The body was that of a well-nourished and developed boy, weighing 8,500 gm. There were numerous venipuncture sites in both femoral triangles. The organs were pale. No

Fig. 1 (Case 1).—*A*, dorsal view of the brain, showing bilateral subarachnoid hemorrhage of venous drainage areas. The hemorrhage is more marked on the right and is associated with swelling of the right cerebral hemisphere.

B, coronal sections of the brain, showing bilateral hemorrhagic softening, greater on one side than the other. The lowest section is the frontal poles.



Hemorrhagic Encephalopathy Induced by Hypernatremia

9095.

I. Clinical, Laboratory, and Pathological Observations

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Hyperosmolarity is characterized by an increase in the solute concentration of body fluids brought about by loss of water, inadequate water intake, or administration of large amounts of hypertonic solutions. When the osmolar concentration of the extracellular fluid is increased, transfer of water from cells to extracellular fluid exceeds that in the opposite direction. Net water movement is thus out of the cells, with resultant dilution of extracellular fluid and concentration of the fluid within cells themselves. If the solute which increases the extracellular fluid osmolarity is one which is in the main excluded from intracellular fluid, such as sodium and chloride ions, this disturbance in water distribution cannot be mitigated by movement of solute into cells. Hence, the ultimate effect of hypernatremia is dehydration of cells. This physicochemical disorder is most frequently encountered in infants and children with severe diarrhea and vomiting, but it also occurs in adults.

Schmidt,[1] in 1850, reported high chloride concentration in the serum of some infants with diarrhea, and the first description of this state is generally attributed to him. In the United States, clinical interest in hyperosmolarity was aroused by Rapoport[2] in the late 1940's. Several authors[3-8] have emphasized central nervous system disturbances in patients with severe dehydration and hypernatremia. The question is raised, therefore, whether central nervous system lesions cause hyperosmolarity, or whether hypernatremia produces central nervous system lesions. In these patients, the neurological symptoms were particularly evident when the biochemical disorder developed rapidly. Subarachnoid and subdural effusions certainly occurred in several infants,[5,7] suggesting that hemorrhagic lesions may be of primary importance in the encephalopathy associated with hypernatremia. Our purpose is to describe the clinical, laboratory, and pathological findings in three infants with neurological disorders due to severe dehydration and hypernatremia. This provides certain basic information for experimental studies on hyperosmolarity in cats, to be described in a subsequent paper.

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From the Division of Neurological Medicine, The Johns Hopkins Hospital; the Pediatric Division, Baltimore City Hospital, and the Department of Pediatrics, The Johns Hopkins University School of Medicine.

Report of Cases

CASE 1.—Patient admitted Nov. 7, 1957; died Nov. 11, 1957.

An 8-month-old Negro boy was seen in the outpatient department with a history of vomiting and diarrhea of three days' duration. He appeared only mildly dehydrated, with signs of an upper respiratory infection and a temperature of 38.9 C (101.1 F). He was given penicillin and streptomycin intramuscularly. Administration of clear fluids by mouth was advised. During the following day, he vomited twice and had two loose stools, but urinary output seemed adequate. He did, however, look sick and dehydrated, and his fever was unchanged. There were no other abnormal physical signs of note. Spinal fluid was normal, and a blood culture subsequently proved sterile. He was given a trisulfapyrimidenes suspension (triple sulfonamides), more penicillin and streptomycin, and a hypodermoclysis of 250 cc. of one-fourth-

Fig. 2 (Case 1).—*A*, section showing organizing thrombi of the sagittal sinus and bridging veins of the dura mater. H and E stain; reduced to 61% of mag. × 35.
B, section through the parietal cortex showing hemorrhagic necrosis and thromboses of dural vessels. H and E stain; reduced to 61% of mag. × 10.

lesions were seen in the heart. The pleural surfaces of the lungs were smooth. There were several clots in the pulmonary arteries to the left lung, which, in the lower lobe, appeared to distend the vessels and may have represented emboli. The liver was pale and its architecture obscure. The gallbladder, bile ducts, duodenum, pancreas, ureters, bladder, esophagus, stomach, rectum, spleen, and adrenals were normal.

There was an adherent clot in the superior sagittal sinus. Dural veins were markedly distended. There was extensive subarachnoid bleeding over the cerebral hemispheres. This hemorrhage was more prominent on the right, with softening of the underlying cortex (Fig. 1*A* and *B*). The gyri were flattened and the convolutions narrowed. There was bilateral uncal herniation, and the peduncles appeared flattened. The hypophysis was normal, as were the arteries of the circle of Willis. The cranial nerves were intact. On section, bilateral hemorrhagic necrosis, involving the gray and white matter to a depth of 1.5 cm., was most prominent in the region of the motor strip on the right (Fig. 1*B*). The ventricular system was not dilated, and the choroid plexuses were intact. The cerebellum and brain stem were unremarkable.

Microscopically, the heart was normal. Acute and chronic submucosal inflammatory foci were present in the trachea. The lungs were congested with multiple pulmonary thrombi. Numerous hemosiderin-pigmented macrophages were found in patchy areas of infarction. The spleen showed sinusoidal prominence with lymphoid hyperplasia, and moderate numbers of acute splenic tumor cells. Severe hepatic cytoplasmic vacuolation was present, with central venous congestion. The gastric mucosa was denuded. The kidneys were completely normal, as were also pancreas, gallbladder, testes, prostate, thymus, skin, muscle, hypophysis, aorta, and bone marrow. There was an organizing thrombus in the sagittal sinus with early fibroblastic ingrowth (Fig. 2*A*). Massive areas of hemorrhagic necrosis were present in the brain along with widespread subarachnoid hemorrhage (Fig. 2*B*).

An 8-month-old boy was admitted to the hospital with a four-day history of vomiting and diarrhea. Serum electrolyte determinations disclosed hypernatremia. Despite fluid replacement therapy, oliguria developed. Serum sodium concentration continued to rise. Convulsions and death soon followed. At necropsy, thrombosis of the superior sagittal sinus was found, with bilateral hemorrhagic softening of the involved venous drainage areas.

CASE 2.—Patient admitted Oct. 8, 1954; died Oct. 13, 1954.

This was the first hospital admission of the patient, a 2-month-old Negro girl, with diarrhea and vomiting of four days' duration. The family, past, and developmental histories were all noncontributory. The infant was apparently entirely well until four days before admission, when she was exposed to two siblings with acute respiratory infections. Late in the evening that day, she developed diarrhea and vomiting. Three days before admission, she was seen at another hospital and was treated with Coca-Cola syrup. In the 48-hour interval before admission, she had five to eight loose, watery stools per day with an indefinite number at night. For 12 to 18 hours before coming to the hospital she was tremulous and irritable and refused feedings. In the accident room she was started on treatment with an oral electrolyte solution. Overnight, however, she had 10 further

large, loose watery stools and was, therefore, admitted to the hospital.

Examination on admission revealed an extremely dehydrated, moribund infant. The vital signs were as follows: temperature 99.8 F, pulse 160 and respirations 55, per minute; weight 3,090 gm. The eyeballs were soft and sunken, and the anterior fontanel was depressed. Respirations were shallow, though the lungs were clear. The heart was normal. The liver edge was just palpable. The skin and mucous membranes were dry. The left eardrum was inflamed. The neck was supple. Superficial and deep tendon reflexes were depressed throughout. No pathological reflexes were elicited. There were no obvious cranial nerve signs, and the fundi were normal.

Initial laboratory studies showed a white cell count of 12,000 per cubic millimeter, with a normal differential count, and hemoglobin 10 gm/100 ml. Urinalysis showed specific gravity 1.018, pH 5.0, 2+ proteinuria, no sugar, a few epithelial cells, and 2-3 red blood cells per cubic millimeter. Serum analyses were as follows: sodium 179 mEq/L.; chloride 143 mEq/L.; CO_2 8.2 mEq/L.; potassium 5.2 mEq/L.; BUN 62.8 mg/100 ml., and calcium 5.2 mg/100 ml. The electrocardiogram was normal. The spinal fluid was grossly bloody, though the pressure was not recorded. Centrifuged immediately, it gave a deeply xanthochromic supernatant fluid. Blood, nose, throat, and urine cultures revealed no abnormalities.

The infant was immediately given intravenous and subcutaneous fluids, antibiotics, and oxygen. In the first 12 hours she seemed to improve clinically, although she failed to void. Twenty-two hours after admission, diuresis followed intravenous administration of additional 10% dextrose and water. Thereafter, however, she became tremulous, and an electrocardiogram showed changes indicative of hypocalcemia and hypokalemia. Temporary improvement followed transfusion and replacement of fluids, with additional calcium. An attempt was then made to start the patient on oral feedings, but little was taken. She became progressively more lethargic, though tremulous, and had two convulsions. She died on the morning of the fourth hospital day, 15 minutes after the second convulsion. Serum analyses on the morning of death showed sodium 165 mEq/L., chloride 140 mEq/L., CO_2 10.1 mEq/L., potassium 10.8 mEq/L., calcium [illegible] mg/100 ml., phosphorus 13.2 mg/100 ml., and BUN 41.6 mg/100 ml.

Autopsy.—The body was that of a poorly nourished female infant, weighing 3,435 gm. The contents of the abdominal cavity appeared grossly normal. The heart was normal. The lungs weighed 60 gm. Trachea and bronchi were normal. On cut section, the basal lobes were poorly aerated and meaty in consistency. Foamy fluid was expressed from the small bronchi. The liver, gallbladder, spleen, gastrointestinal tract, and pancreas were normal. The combined weight of the kidneys was 33 gm. They were pale but normal in size, shape, and anatomical position. On cut section, the cortices and pelves were normal. The adrenals weighed 5 gm. They were normal in appearance.

The leptomeninges were markedly congested with blood in the vicinity of many vessels. Small red blood clots were found in the subarachnoid spaces of the posterior fossa and in the third and lateral ventricles. The hypophysis was reddish in color.

Microscopically, there was focal heme pigment in the convoluted tubules of the kidneys. The collecting tubules were filled with acidophilic debris and urate spheroids, with dilatation of the proximal tubular apparatus (Fig. 3*A*). There were multiple microscopic pulmonary emboli with widespread edema of the lungs. The brain showed dilatation and thrombi of subependymal vessels of the lateral and third ventricles (Fig. 3*B*), in addition to intracerebral (Fig. 3*C*), subarachnoid (Fig. 3*D*), and intraventricular bleeding. There was a massive hemorrhage of the pars posterior of the hypophysis (Fig. 3*E* and *F*). The meningeal vessels were dilated and congested.

A 2-month-old infant with diarrhea and vomiting of four days' duration was admitted to hospital in a moribund state. Oliguria, tremulousness, convulsions, and death occurred, despite fluid replacement, antibiotics, and symptomatic treatment. Pathological examination disclosed intracranial bleeding and massive hemorrhage of the pars posterior of the hypophysis. There were thrombi in subependymal vessels and intraventricular hemorrhage. The complicating posterior pituitary lobe hemorrhage may have contributed further to terminal irreversible water and electrolyte disturbances. The renal changes may well have resulted from inspissation of urine, and precipitation of protein urates and blood pigment in the collecting tubules.

CASE 3.—Patient admitted April 21, 1957; died April 21, 1957.

This one-month-old, premature Negro girl was admitted to the hospital with a seven-day history of regurgitation of feedings and diarrhea. In the week before admission, she became increasingly lethargic and had to be awakened for feedings. Her respirations became more shallow and rapid. One day before admission, she had an increasing, but indefinite, number of green, watery stools. In the accident room, she passed four loose stools in

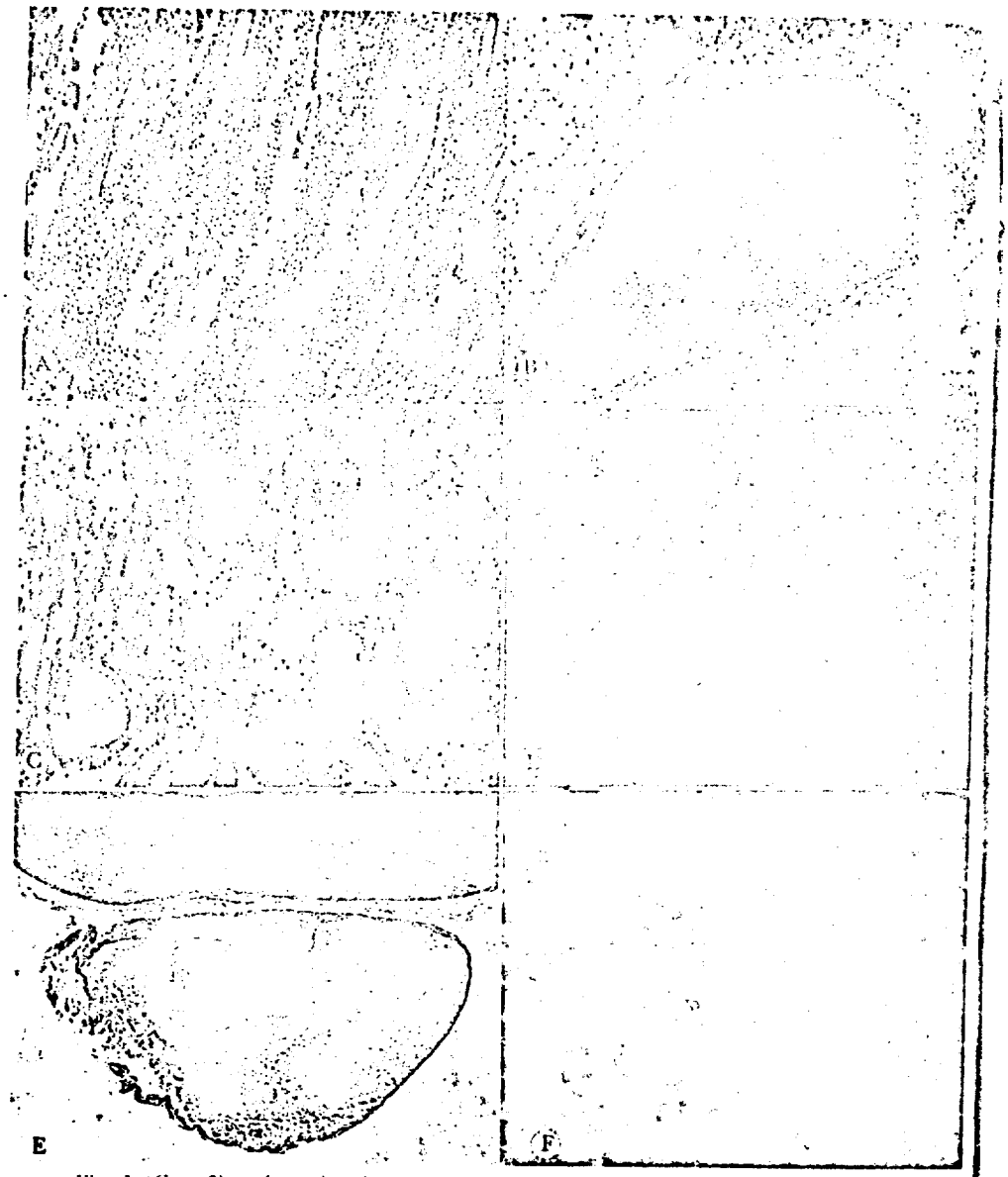


Fig. 3 (Case 2).—*A*, section from the renal medulla, showing collecting tubules filled with acidiphilic debris and urate spheroids. H and E stain; reduced to 61% of mag. × 125.

B, section through the hypothalamus, showing an organized thrombus of a small vein. H and E stain; reduced to 61% of mag. × 125.

C, section through the hypothalamus, showing hemorrhagic necrosis and edema and an organized thrombus in a small vein. H and E stain; reduced to 61% of mag. × 125.

D, section through the cerebral cortex, showing edema, and intense vascular congestion and subarachnoid hemorrhage in the depth of a sulcus. H and E stain; reduced to 61% of mag. × 100.

E, section through the hypophysis, showing massive hemorrhage into the pars posterior. H and E stain; reduced to 61% of mag. × 50.

F, high-power magnification of the pars posterior of the hypophysis, showing hemorrhage and loss of nerve cells. H and E stain; reduced to 61% of mag. × 750.

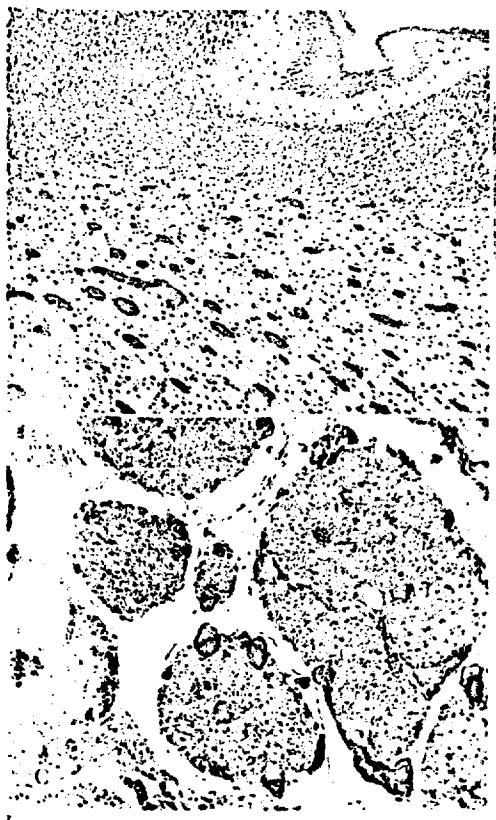
30 minutes. Throughout the illness there had been progressive weight loss.

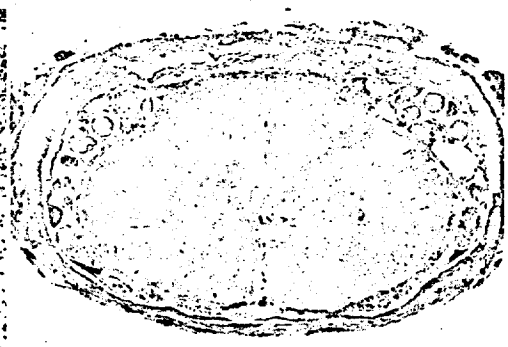
On admission, the infant was acutely ill and tachycardic. The vital signs were as follows: temperature 97.4 F; pulse 150 and respirations 72, per minute; weight 2,079 gm. She seemed alert. The cry, however, was piercing and shrill. The anterior fontanel was depressed and the eyes sunken and soft. Skin turgor and elasticity were poor and the mucous membranes dry. The chest was clear to auscultation and percussion. Heart sounds were difficult to hear. The abdomen was somewhat distended, with the liver felt one fingerbreadth below the costal margin. The spleen was not palpable. The neurological examination was unremarkable.

While in the treatment room, the infant stopped breathing during an attempt to start fluid therapy. Repeated suction and intracardiac epinephrine 0.2 cc. of 1:1,000 solution, were ineffectual. She died 15 minutes after hospital admission. Immediate postmortem specimens of blood and spinal fluid were obtained for chemical analysis and culture. The white blood cell count was 30,000, with a normal differential count. Hemoglobin was 18 gm/100 ml. The spinal fluid was grossly bloody and xanthochromic. Postmortem serum analyses showed sodium 180 mEq/L., potassium 6.3 mEq/L., chloride 151 mEq/L., and CO_2 7.7 mEq/L. Blood and spinal fluid cultures were negative. X-ray examination of the whole body and chest were normal.

Autopsy. The body was that of a premature, dehydrated female infant, weighing 2,079 gm. The skin was wrinkled. The contents of the abdominal cavity were normal. The lungs were of normal appearance, and on cut section appeared crepitant and pinkish red in color. The neck organs were normal. Moderate congestion of superficial meningeal vessels was noted. Intradural hemorrhages were seen near the midline of the hemispheres and over the inner aspects of the inferior surfaces of the frontal lobes. Scattered small subarachnoid hemorrhages were present, especially in the depths of the sulci. The hypophysis was reddish in color. Spinal fluid in the subarachnoid space about the spinal cord was also bloody.

Microscopic examination revealed moderate edema of the lungs, with desquamation of epithelial cells. There were moderate congestion of the splenic sinusoids and mild hyperemia of adrenal medullary capillaries. Extensive hepatic cytoplasmic vacuolation was present. The remaining thoracic and abdominal organs were normal. There





B

Fig. 4 (Case 3).—*A*, section through the cerebellum, showing vascular congestion of small veins and capillaries in the white matter of the cerebellum. H and E stain; reduced to 61% of mag. × 125.

***B*, section through cervical spinal cord, showing subarachnoid hemorrhage and vascular congestion. H and E stain; reduced to 61% of mag. × 20.**

***C*, section through the cauda equina, showing intense congestion of capillaries within the nerve roots. H and E stain; reduced to 61% of mag. × 125.**

was extensive vascular congestion of capillaries and small veins of the cerebral hemispheres and cerebellum (Fig. 4*A*), midbrain, pons, and medulla. Occasional small, petechial hemorrhages were found in the cerebral cortex and centrum semiovale, the white matter of the cerebellum, and the gray and white matter of the spinal cord. There was moderate hyperemia of the anterior lobe of the hypophysis. Representative sections from different levels of the spinal cord all showed subarachnoid hemorrhage (Fig. 4*B*) and marked congestion of capillaries and small veins. This was particularly striking in the capillaries of the spinal nerve roots (Fig. 4*C*).

A neglected one-month-old female infant, with poor fluid intake, diarrhea, and vomiting of one week's duration, died 15 minutes after admission to the hospital. Immediate postmortem serum analyses confirmed the diagnosis of hypernatremia. Pathological examination of the nervous system showed intradural and subarachnoid hemorrhage. There was intense vascular congestion of small veins and capillaries throughout the nervous system with occasional small, petechial hemorrhages of the brain and spinal cord. The kidneys appeared normal.

Comment

The threat of hypernatremia is greater in children than in adults. One obvious reason for this is the higher incidence of diarrhea and vomiting in children, coupled with greater water requirements per kilogram. Another is the increased chance of accidental salt ingestion with its attendant complications. In young infants, an additional factor of significance in the pathogenesis of this syndrome is the relative inadequacy of maximal renal osmotic concentration.[9,10]

The problem of hypernatremia, however, is not limited to infants with diarrhea and vomiting. Hyperosmolarity in adults has appeared in several seemingly unrelated clinical settings. Of these, head injuries or intracranial lesions have received considerable emphasis.[4,11,12] Indeed, it has been suggested by some that hyperosmolarity is secondary to some disturbance of water-regulating mechanisms of neurogenic origin. However, Zierler[13] analyzed the published protocols of patients with hyperosmolarity attributed to such causes and showed that, when sufficient clinical and laboratory data were given, the hyperosmolarity could be explained as a consequence of insufficient water intake in the face of excessive water loss. He concluded, therefore, that there is little evidence that the syndrome is in general the result of an intracranial lesion.

Hypernatremia may readily be produced in the hospital by administration of large amounts of sodium chloride, even as isotonic saline, especially to patients with selective renal tubular damage. This process may well be accentuated by the concomitant use of agents for the reduction of increased intracranial pressure, such as urea, which in itself produces a solute diuresis removing appreciable amounts of water in excess of sodium. Not uncommonly, hypernatremia may occur and be unrecognized in comatose and stuporous patients maintained for prolonged periods on concentrated tube feedings. An awareness of these possibilities and daily serum sodium determinations can circumvent this complication in the unconscious patient. In this sense, the comatose, stuporous adult is much like an infant, whose fluid requirements must be maintained by careful balance studies.

Clinically, hypernatremia or hypertonic dehydration has itself been noted to be frequently accompanied by central nervous system symptoms. Finberg and Harrison[5] studied such a group of dehydrated infants, in whom the management of the physiological disturbance was difficult, despite the adequate replacement of water with solutions that were ordinarily effective. Cerebrospinal fluids characteristically showed increased concentration of protein or subarachnoid hemorrhage. Subdural effusions have been demonstrated in some of the acutely ill infants, whereas others have shown significant neurological residua ranging from moderate to severe disability.[5,7]

In the present cases, as in others,[1,5,7] the predominant neurological manifestations of hypernatremia were alterations of con-

sciousness, varying from lethargy to coma. Tremulousness, muscle twitching, and frank convulsions were also common. The pathological causes for these clinical derangements have not been completely clear or uniform. In the cases reported here, however, hemorrhagic encephalopathy was the prominent pathological lesion. Gross subarachnoid hemorrhage, as well as intradural, intracerebral, and intraventricular bleeding, was also observed. In one case, there was a sagittal sinus thrombosis with hemorrhagic necrosis of the venous drainage areas from both cerebral hemispheres. Another showed subependymal thromboses of small veins and a massive hemorrhage into the posterior lobe of the hypophysis.

It seems clear, therefore, that hypernatremia may be associated with extensive intracerebral, subdural, and subarachnoid bleeding. The present data afford, however, no direct information on mechanisms underlying this vascular damage. It is tempting to consider a purely mechanical explanation. Thus, the stresses created by shrinkage, from cellular water loss, of brain tissue within the rigid cranium might be reflected by stretching, and even tearing, of unsupported vessels. The relatively soft consistency of the immature brain, and its higher normal water content, could theoretically provide more favorable conditions for disproportionate shrinkage than exist in the adult, and thus explain the higher incidence in infancy of such vascular damage. Perhaps, too, the liability of small blood vessels to mechanical injury by such factors as these is heightened in the presence of hypernatremia. In this connection, it may be relevant that Haddy, Emmanuel, and Scott have recently demonstrated vasodilation and decreased responses of small vessels to pharmacological agents when sodium concentration is elevated locally.

This is not by any means to suggest that the devastating effects of hypernatremia in infancy are due solely to vascular damage. Indeed, there is good evidence that other factors are involved as well. For example, Luttrell and Finberg[15] have produced comparable intracranial hemorrhagic lesions in kittens with equiosmolar intraperitoneal infusions both of sodium salts and of urea. Yet the urea-treated animals survived, while those receiving sodium were usually moribund or dead within 24 hours. Apparently, then, there is some deleterious factor associated with hypernatremia beyond the hyperosmolarity and resultant vascular damage. Finberg, Luttrell, and Redd[8] have shown that brain adapts in a different way to excessive sodium than muscle. Both tissues show transfer of water from cells. In muscle, however, there is considerable entry of sodium into cells, whereas in brain there is little or none. It seems probable that alterations of this nature in the chemical anatomy produce within cells "idiogenic osmols,"[16] presumably derived from the dissociation of complex intracellular ions or proteins. In brain, this process appears to go on to a significantly greater degree than in muscle because of the relative exclusion of sodium. This metabolic defect, if sufficiently intense, may itself result in permanent neurological damage, and even death.

Adequate treatment of hypernatremia depends on its early recognition. If proper fluid replacement therapy is instituted early,[17] some of these devastating neurological complications may be avoided. An awareness of this problem should result in more careful follow-up and judicious management of those conditions leading to the development of hypernatremia.

Summary

The clinical, laboratory, and pathological findings in three infants with neurological disorders due to dehydration and hypernatremia are described.

Important neurological manifestations of hypernatremia are alterations of consciousness, tremulousness, muscle twitching, and frank convulsions. Possible causative mechanisms of these brain hemorrhages are discussed.

The relative significance of these structural vascular lesions is compared with that

of chemical alterations associated with hypernatremia.

It is emphasized that adequate treatment of hypernatremia depends on its early recognition.

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MINERAL SALTS AS TOXIC FACTORS IN URINARY PROLAN CONCENTRATES[1]

9002

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During a recent investigation of the urinary prolan excretion of a non-pregnant human female (1), we experienced great difficulty in that when using the alcohol precipitation method and the original Aschheim-Zondek technic, the urinary concentrate proved toxic to many of the animals. Believing that the source of toxicity might be due to the mineral salts it contained, 3 specimens of the solutions of concentrates which had proven toxic were analyzed for various cations.[2]

The results were as follows; the metals being reported as the chlorides:

Soln. 1. ¼ of precipitate from 24-hr. specimen of urine, dissolved in 6 cc. H_2O.
KCl = 58.1 mg./cc.
Soln. 2. ¼ of 24-hr. specimen in 6 cc. H_2O.
$CaCl_2$ = 1.22 mg./cc.
Soln. 3. ¼ of 24-hr. specimen in 9 cc. H_2O.
NaCl = 35.10 mg./cc.
KCl = 15.83 mg./cc.
$CaCl_2$ = 0.20 mg./cc.
$MgCl_2$ = 0.07 mg./cc.

In order to determine the relation of these concentrations to the M.L.D., 21-day-old female rats were injected subcutaneously with solutions of pure salts. I believed it unwise to use the data in the literature on the toxicity of these salts for adult rats. Solutions were made up in the convenient strengths of 10% NaCl and 5% KCl, $CaCl_2$, and $MgCl_2$, and the dose was always diluted to 1 cc. for injection. The results and approximate time of death following the injections are as follows:

	M.S.D./kg.[1]	M.L.D./kg.[2]	M.L.D./30 gm.[3]	Time[4]
	gm.	gm.	mg.	hr.
NaCl	3.0	3.5	105	5
KCl	1.9	1.2	36	1
$CaCl_2$	0.7	1.0	30	15
$MgCl_2$	0.7	0.9	27	1

[1] Maximal safe dose per kg.: the largest dose given 5 rats where none died.
[2] Minimal lethal dose per kg.: the smallest dose given 5 rats where 3 or more died.
[3] Minimal lethal dose for a 30 gm. rat. The weight of 30 gm. was selected as the average weight of the 21-day rats we were using.
[4] Time: approximate time after injection of M.L.D. when animals died.

[1] Since completion of this paper, analysis of a composite of 5 urinary concentrates (first morning specimen in 9 cc. H_2O), which had proven toxic in doses of 1.0 cc. twice daily, gave the following results: Na (as NaCl) = 40.1 mg./cc.; K (as KCl) = 16.6 mg./cc. One specimen which was toxic in doses of 0.5 cc. twice daily, had the following surprising composition: Na (as NaCl) = 37.6 mg./cc.; K (as KCl) = 39.1 mg./cc.

[2] I am indebted to Dr. R. C. Neale for the analyses.

The amounts of Ca and Mg in solutions of the prolan precipitate are negligible so far as their M.L.D.'s are concerned. However, the amount of K per cc. in *soln.* 1 (28 mg.) is 28/36 of the M.L.D. for a 30-gm. rat, and the Na in *soln.* 3 (35 mg./cc.) is 35/105 of the M.L.D. Although both are below their M.L.D's, if given 3 times daily, as with the Aschheim-Zondek technic, it may well be understood why many animals die. Especially in the case of Na, where death occurs in about 5 hours, the effects can easily become cumulative. However, it seems most probable that it is the K which is responsible, since I have never noticed any hyperirritability typical of Na poisoning in rats dying during the injections of prolan concentrates. This agrees with the statement of Sollman (2) that 85% of the toxicity of the urine is due simply to its K salts. A point which may be of importance in explaining the death of the animals following repeated administration of sub-lethal doses is that they are often so depressed that they do not drink or eat.

Consequently, it appeared necessary to determine if the amount of K found in *soln.* 1 would prove toxic when administered 3 times daily for 2 days, and if a solution similar to *soln.* 3 would also prove toxic. Five female rats, 21 days old, weighing from 26 to 34 gm., were injected with synthetic *soln.* 1 (K) at 3-hour intervals, and 4 died, 3 of these after the third injection. Five more rats, weighing from 25 to 30 gm., were injected with synthetic *soln.* 3 (Na-K-Mg-Ca), at 3-hour intervals, 3 times daily for 2 days. Two of these rats died on the second day. In practically all cases, the smallest rats were the ones which died. This proves that the Na or K in the prolan concentrate is adequate to explain the toxicity, at least in part.

The possibility remained that in such a mixture of salts as in *soln.* 3, ion antagonism might occur, so that even if the M.L.D. of Na were administered, the Ca present might antagonize it. However, an attempt to demonstrate ion antagonism or synergism using adult mice, between certain ion pairs, as Na-K and Na-Ca, failed. The difficulty may lie in the fact that *a*), these salts may produce death by affecting different organs, *b*), their rates of absorption may differ, and *c*), that their time of death differs. For instance, administration of ½ the M.L.D. of Na occasionally prevents death by the simultaneous administration of an M.L.D. of K. This may be due merely to the fact that since both salts were administered in one solution, the hypertonic solution will so slow the absorption of the K that it can be excreted as rapidly as it is absorbed. Consequently, a fatal concentration will not accumulate. Because of these difficulties, the problem was abandoned.

The injection of NaCl alone, when determining the M.L.D., produced an amazing hyperirritability in the rats, probably due to what is mistermed Ca tetany. K, Mg, and Ca all produced depression, but rats dying of Ca showed strongly contracted hearts at autopsy.

Following a single injection of the approximate M.L.D. of 2 salts, it is usually easy to determine from which the animal dies, by the effects and the time of death. For instance, an animal injected with K and Ca—if it apparently dies of K poisoning—does so in an hour. If, not, it dies overnight with the strongly contracted heart typical of Ca poisoning.

The question as to how to diminish this toxicity of urinary concentrates may be solved in several ways, *a*), by using rats of a large strain weighing over 30 gm. at 21 days of age, *b*), by strictly spacing the injections at least 4 hours apart, to prevent cumulation, or better *c*), by taking up the precipitate in larger amounts of water in order to dilute the salts (as in 9 cc. instead of 6 cc.) and *d*), by giving injections twice daily instead of 3 times, or *e*), by previous removal of part of the salts, as by dialysis through Dupont cellophane tubing.[3] Application of principles *c*), and *d*) has proven very successful, in that many specimens of urinary concentrate solutions which were toxic with the Aschheim-Zondek technic, were injected without fatalities by using 9 cc. and 9 injections over 5 days, with but one exception out of 30 (1).

However, there are probably other toxic factors besides Na and K present in these urinary concentrates, such as the H^+ concentration.

SUMMARY

Urinary prolan concentrates made by the alcohol precipitation method contain sufficient amounts of Na and K to explain their toxicity. Suggestions for circumventing this toxicity are made. The M.L.D.'s of Na, K, Ca, and Mg chlorides for immature rats are given.

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ORAL SODIUM LOADING IN NORMAL INDIVIDUALS

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10001

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At the commencement of the Peru Burn Project (1) in 1951, great apprehension was aroused in many by the use of large quantities of isotonic saline solutions[5] in the treatment of burned patients, for it was believed that such therapy in individuals retaining sodium due to the stress reaction would overload the circulation and lead to death in pulmonary edema. The reason for this fear was based upon the knowledge that man, in contrast to the dog, retained a great part of the sodium administered as isotonic sodium chloride over a considerable period of time (2, 3). Yet, during the course of the study of 110 burned children and 83 burned adults to whom had been given orally a quantity of an isotonic saline solution equivalent to 10 per cent of body weight during the first 24 hours after thermal injury and half that amount during the second 24-hour period, no untoward effects due to sodium, such as vomiting, generalized edema or massive pulmonary edema, were encountered (1), in spite of the fact that these patients demonstrated marked water and sodium retention (largely the result of accumulations in the burned area as edema fluid). The experimental conditions of sodium loading in previous studies of normal subjects did not duplicate those utilized in the treatment of burned patients Hence, a quantity of isotonic saline solution similar to that given to burned patients was administered to normal persons over the same time period in order to clarify the effects of such sodium loading upon the kidney and circulation.

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[5] The term 'saline solution' throughout this paper refers to a solution of a mixture of sodium chloride and sodium bicarbonate. For exact composition, see Materials and Methods.

MATERIALS AND METHODS

Renal function. Twelve healthy Peruvian males between the ages of 20 and 28 years were studied. None had a medical history of previous renal or cardiovascular disease. The subject was not permitted to take any liquid or food after 8 P.M. of the day previous to the performance of the study. After voiding on arrival at the hospital at 8 A.M., he was weighed nude and placed in bed in a recumbent position. The bladder was catheterized, venous pressure was measured carefully in the antecubital vein, and three twenty-minute clearance periods using inulin and sodium para-aminohippurate (PAH) by the technique of Goldring and Chasis (4) were accomplished. Five hundred cc. of water had been given approximately one and one-half hours previous to the measurement of the first clearance period, and suprapubic pressure and injection of air were used to empty the bladder. The catheter was then removed. At 11 A.M., after the subject had voided, a gastric tube was introduced into the stomach via the nose, and a constant drip of an isotonic saline solution containing 137 mEq. per L. of Na, 93 mEq. per L. of Cl and 44 mEq. per L. of HCO_3 was administered by the gastric tube at a uniform rate of 4 to 5.5 cc. per min. Each subject was given a total quantity of saline solution equivalent to 10 per cent of body weight over a period of 21 hours, during which time the subject remained recumbent and received no food or water. At 2-hour intervals the subject voided spontaneously, and the urine volume was measured and the urine saved. At 8 A.M. the next morning, when the total quantity of saline solution had been given, he was again weighed after voiding and then catheterized. Venous pressure was measured, and three clearance periods of 15 to 20 minutes each were repeated with inulin and PAH, this time without giving water previously. At the termination of the clearance studies the subject was weighed once more.

In the urine and plasma collected during the clearance periods PAH was analyzed by the method of Smith, Finkelstein, Aliminosa, Crawford, and Graber (5), inulin by the method of Schreiner (6), sodium and potassium by the flame photometer using lithium as internal standard (7), and chloride by the method of Van Slyke and Hiller (8). In the first blood sample drawn at the control period and the period after completion of the sodium loading test, hematocrit was determined by the Wintrobe method, total plasma protein by the copper sulfate method (9), alkaline reserve by the manometric

method of Van Slyke (10), and concentration of sodium and potassium in red blood cells separated from plasma after one-half hour centrifuging at 3,000 rpm by the flame photometer method. Sodium and potassium concentrations were determined also in the 2-hour urine samples collected. Afterwards, the separate 2-hour samples of urine were mixed and the total quantity of urine excreted over the period of sodium administration was analyzed for sodium and potassium. Glomerular filtration and renal plasma flow were calculated during the control period and at the termination of the oral sodium loading. Total quantity of sodium and potassium excreted (UV) during the same periods, as well as during the 2-hour collection periods, was calculated. In addition, percentage of filtered sodium excreted was calculated during all clearance periods.

Plasma volume study. Eleven healthy Peruvian males between the ages of 20 and 28 years (including seven of the former subjects who volunteered for this test one month after completion of the previous one) were studied. All of the same conditions were maintained as before, except for the fact that no renal clearances were performed. Instead, at 9 A.M. on the day of the beginning of the test, plasma volume was measured by the four sample techniques using T-1824 (11). Again the subjects received a quantity of the saline solution equivalent to 10 per cent of body weight via gastric tube uniformly by constant drip over a 21-hour period. After completion of administration of the saline solution (8 A.M.), plasma volume determination was repeated as on the day before.

RESULTS

Hemodynamics

In Table I are listed glomerular filtration rate, renal plasma flow and plasma volume during the control period and during the period just after the administration of a quantity of the isotonic saline solution equivalent to 10 per cent of body weight. In eleven of the twelve subjects the glomerular filtration rate increased significantly over the control value and in only one individual did it stay the same. The average value shows a significant increase (+ 32 per cent) over the control value after the sodium load. On the other hand, renal plasma flow increased over the control value in six of the twelve subjects, decreased in five and stayed the same in one. The average value of all the subjects does not show a significant increase over the control value. The plasma volume increased over the control value in four of twelve individuals, decreased in six and remained the same in one after the administration of the saline solution. The average value of all the subjects shows no significant change over the control value. Venous pressure was measured during the control period (average, 104 mm.) and after sodium load-

TABLE I

Hemodynamic studies in normal subjects with oral sodium loading

Subject	No. of clearances	Glomerular filtration cc./min./1.73 m²			Renal plasma flow cc./min./1.73 m²			Plasma volume cc.		
		Control*	After*	Diff.	Control*	After*	Diff.	Control	After	Diff.
J. C.	3	105	161	55	614	725	111	3,055	3,358	303
M. C.	2	160	253	93	599	925	326			
L. M.	2	129	195	66	752	730	−22	2,110	2,400	290
P. L.	2	162	198	36	1,099	1,375	276			
E. R.	3	130	126	−4	648	577	−71	2,610	2,483	−127
A. R.	3	108	139	31	759	587	−172	3,354	3,600	246
A. V.	3	138	167	29	668	668	0			
J. G.	3	103	146	43	635	605	−30			
V. F.	3	118	163	45	631	750	119			
L. Q.	3	145	186	41	825	863	38	3,027	2,731	−296
R. C.	3	102	133	31	685	649	−36	3,035	3,048	13
C. G.	3	114	137	23	728	768	40	2,800	2,650	−150
A. L.								3,510	3,097	−413
J. R.								2,256	2,320	64
J. E.								2,720	2,455	−265
J. M.								2,941	2,463	−478
Average values		126	167	41†	720	768	48	2,856	2,782	− 74
Standard error of average				6.9			40.7			84

* Each number represents the average value of the clearance periods.
† Significant at the 1 per cent level.

TABLE II

Water and electrolyte excretion in normal subjects with oral sodium loading

Subject	No. of clearance periods	Urine volume* cc./min.		$U_{Na}V$* mEq./min.			U_{Na}* mEq./L.		U_KV* mEq./min.		U_K* mEq./L.	
		Control	After	Control	After	Sum	Control	After	Control	After	Control	After
J. C.	3	2.65	4.22	0.345	0.660	1.005	135.3	158.8	0.0689	0.0390	26.4	9.3
L. M.	2	3.79	3.03	0.310	0.520	0.830	85.7	172.0	0.0425	0.0449	11.7	14.8
P. L.	2	4.88	4.40	0.560	0.934	1.494	118.8	212.5	0.0943	0.0892	19.8	20.3
M. C.	2	9.75	3.85	0.526	0.718	1.244	54.1	187.0	0.0911	0.0294	9.4	7.6
E. R.	3	3.89	6.53	0.485	0.701	1.186	130.6	113.2	0.1168	0.0684	31.2	10.8
A. R.	3	4.02	3.65	0.528	0.572	1.100	148.4	155.5	0.0473	0.0277	13.1	7.6
A. V.	3	3.96	4.15	0.403	0.794	1.197	103.0	192.9	0.1021	0.0543	26.2	13.2
J. G.	3	4.69	1.63	0.286	0.396	0.682	63.4	242.0	0.0642	0.0437	14.1	26.7
V. F.	3	4.14	4.43	0.457	0.758	1.215	125.6	176.3	0.0586	0.0701	16.4	16.5
L. Q.	3	1.66	4.01	0.422	0.751	1.173	265.0	91.6	0.0367	0.0260	23.0	4.9
R. C.	3	1.36	5.04	0.345	0.841	1.186	49.4	167.1	0.0396	0.0400	4.9	8.0
C. G.	3	5.29	9.33	0.613	0.961	1.574	124.7	103.2				
Average value		4.16	4.52	0.440	0.717		117.1	164.3	0.0690	0.0484	17.8	12.7

Analysis of variance of $U_{Na}V$ †

Source	df	Sum of squares	Mean square	F
Mean	1	8.0342		
Total	23	0.8804		
Control vs After	1	0.4611	0.4611	68.82 ‡
Subjects	11	0.3455		
Interaction	11	0.0738	0.0067	
				F = 9.65

* Each number represents the average value of the clearance periods.
† $U_{Na}V$ = Total Na excretion; U_{Na} = Na concentration in urine excreted.
U_KV = Total K excretion; U_K = K concentration in urine excreted.
df = Degrees of freedom.
‡ Significant at the 1 per cent level.

ing (average, 133 mm.) in nine individuals, but no significant change was noted.

Excretion of water and electrolytes

In Table II are shown the concentrations and total quantity of the ions, Na and K, excreted during the clearance periods performed before and after the administration of the saline solution. The concentrations of sodium increased significantly after sodium loading, while the concentration of potassium decreased. The total quantity of sodium excreted augmented significantly after sodium loading (+ 63 per cent), while potassium diminished (− 30 per cent). The sodium excreted during the control period represented 2.58 per cent of the filtered load, while the sodium excreted after the administration of the sodium load represented 3.33 per cent of the filtered load. Although the volume of urine increased in seven of twelve individuals, the average urine volume after sodium loading showed no significant increase over the

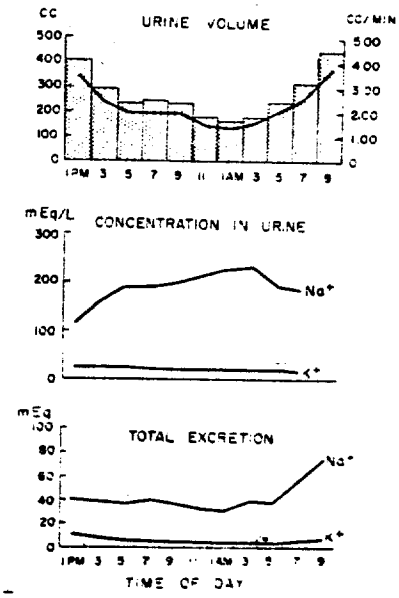

FIG. 1. DIURNAL VARIATIONS WITH ORAL SODIUM LOADING

TABLE III

Fluid and electrolyte balance in normal subjects with oral sodium loading *

Subject	Fluid intake cc.	Electrolyte intake mEq. Na	K	Cl	Urinary output cc.	Urinary excretion mEq. Na	K	Weight change Kg. After Na loss	[illegible]
M. C.	6,650	822	0	558	2,620	432	40	+2.1	+1.6
L. M.	5,300	685	0	465	3,340	455	43	+1.5	[illegible]
	5,300	726	0	493	4,000	480	46	+0.5	
P. L.	6,000	822	0	558	4,860	690	53	+0.5	−1.1
E. R.	5,000	685	0	465	3,020	382	38	+1.7	+0.9
	5,400	740	0	502	2,540	315	62	+1.7	
A. R.	6,000	810	0	534	2,925	375	22	+2.4	+1.4
	6,000	810	0	534	960	232	38	+4.4	
A. V.	6,600	904	0	614	4,960	660	66	0.0	−1.1
J. G.	7,000	959	0	651	2,000	340	68	+3.3	+2.9
V. F.	6,000	822	0	558	3,600	490	59	+0.7	−0.5
L. Q.	6,000	822	0	558	3,500	517	39	+1.5	
	6,000	822	0	558	2,680	493	66	+2.2	
R. C.	7,000	959	0	651	3,300	609	64	+3.3	+2.5
	7,000	959	0	651	3,060	536	59	+3.3	+2.1
C. G.	6,300	863	0	586	3,900	554	78	+1.1	−0.4
	6,000	822	0	558	2,780	378	98	+2.0	
J. R.	5,000	685	0	465	1,540	408	55	+1.8	
J. E.	5,300	726	0	493	4,540	654	91	+0.3	
J. M.	6,300	863	0	586	1,540	442	31	+2.9	
Average	6,000	815	0	552	3,083	472	56	+1.85	+0.82

* Table does not include one subject in which the sodium load produced diarrhea and another who had large saliva loss.

control value due to the fact that the control value itself represented a slight water diuresis.

In Figure 1 are demonstrated the average urine volume, concentration of sodium and potassium and total excretion of sodium and potassium of the 2-hour urine collections during the administration of the saline solution. The first 2-hour sample showed an increase of water and sodium excretion as compared to the values prior to the administration of the saline solution. Water, sodium and potassium excretion all decreased during the night hours with sodium excretion rising notably during the early morning hours above the values encountered at the beginning of the test on the previous day. Sodium concentration in the urine rose during the administration of the sodium load with only a moderate effect noticeable due to changes in water excretion, while potassium concentrations remained relatively constant regardless of water excretion.

In Table III are listed the total intake and total output of water and electrolytes during the administration of the sodium load, together with the changes of weight affected by the sodium administration. It is noted that 51 per cent of the water and 58 per cent of the sodium administered were excreted via the kidney during the period of oral administration of sodium. From the weight data, it can be observed that at the completion of the sodium loading only 31 per cent of the water administered (1.85 Kg.) was retained, which rapidly diminished to 14 per cent (0.82 Kg.) three hours after the termination of the sodium administration. The different responses of a single subject receiving the same sodium load on two separate occasions are also given.

Blood analyses

Table IV demonstrates the changes in hematocrit, total plasma proteins, plasma concentration of sodium, potassium, chloride and bicarbonate and erythrocyte concentration of sodium and potassium before and immediately after the administration of the sodium load. Although some of

TABLE IV

Blood analyses in normal subjects with oral sodium loading

Analyses	Control Average ± S.D.	After Na loading Average ± S.D.
Hematocrit, %	46.2 ± 2.48	44.0 ± 7.2
Tot. Proteins, *gm.* %	7.66 ± 0.34	7.17 ± 0.47*
Plasma Na, *mEq./L.*	139.1 ± 3.51	137.2 ± 3.39
Plasma K, *mEq./L.*	4.13 ± 0.37	3.86 ± 0.30*
Plasma Cl, *mEq./L.*	101.3 ± 0.95	100.7 ± 0.55
Plasma HCO_3, *mEq./L.*	24.6 ± 0.41	23.0 ± 0.51*
RBC K, *mEq./L.*	90.8 ± 3.5	87.9 ± 4.2*
RBC Na, *mEq./L.*	14.5 ± 0.35	14.7 ± 1.84

* The difference between the control value and the value after the sodium load has statistical significance.

these values changed significantly due to the administration of the large quantities of saline solution, none of the changes appear to be of any real importance.

DISCUSSION

The excretion of 58 per cent of a large oral sodium load during the period of its administration challenges the widely held belief that man, unlike the dog, is sluggish in excreting sodium (2). Since previous studies in the literature concerned with sodium loading in man have all been performed giving smaller total amounts of physiologic sodium chloride solution (1 to 3 liters), but by more rapid intravenous infusion (13 to 65 cc. per min.), it is possible that the previously unreported experimental conditions used in this study are responsible for the observed difference.

Earlier investigators have held that man excreted sodium sluggishly compared to the dog due to the fact that glomerular filtration did not increase in man with the intravenous administration of sodium chloride. In the present study, however, there was usually a consistent and significant increase of glomerular filtration at the termination of oral sodium loading. Since the percentage of filtered sodium excreted increased significantly from 2.58 per cent to 3.33 per cent, it is evident that decreased tubular reabsorption over and above the increased amount filtered by the glomerulus accounted for a part of the augmented sodium excretion. Even though it is difficult to compare the results of this study with prior ones, it can be stated, nevertheless, that water and sodium excretion after oral sodium loading fell to values between those reported in non-prehydrated (2) and prehydrated (12) subjects maintained in the recumbent position.

The magnitude of the sodium and water diuresis after oral sodium loading is masked somewhat by the control values, since these latter values themselves demonstrated a slight water and sodium diuresis compared to data in the literature. The slight water diuresis during the control period was due to the administration of a small quantity of water before the commencement of the test. The slight sodium diuresis is more difficult to explain, but is most likely due to an augmented ingestion of sodium in the daily Peruvian diet prior to the test.

Although no attempt was made to study renal hemodynamics during the course of the administration of the sodium load, the gross data of sodium and water excretion on the 2-hour samples collected during that time demonstrated the well-known diurnal variations of water and sodium excretion without sodium loading, but at higher levels than reported under normal circumstances (13).

SUMMARY

1. Sixteen healthy young Peruvian males were given a quantity of an isotopic saline solution equivalent to 10 per cent of body weight in the recumbent position by constant drip via a gastric tube over a period of 21 hours. Renal hemodynamics and electrolyte excretion were studied in twelve of the subjects and plasma volumes in eleven of them before and after the administration of the sodium load.

2. Glomerular filtration rate increased an average of 32 per cent over the control value after administration of the sodium load, while effective renal plasma flow did not change significantly.

3. While body weight was increased significantly at the termination of the sodium load, plasma volume did not change significantly. The increase in body weight largely disappeared within 3 hours after termination of the load.

4. During the period of administration of the sodium load, 58 per cent of the administered sodium and 51 per cent of the administered water were excreted via the kidneys. The concentration and total quantity of sodium excreted in the urine increased significantly at the termination of the sodium load. An increased filtration of sodium by the glomeruli as well as a decreased reabsorp-

tion of sodium by the tubules are considered to be responsible for this phenomenon. Water excretion was augmented also, but its significance was masked by the slight water diuresis present in the control values. The diurnal variations of sodium and water excretion during the course of administration of the sodium load were evident, but at higher levels than under normal circumstances.

5. Total excretion of potassium as well as potassium concentration in the urine decreased after sodium loading.

6. There were no important changes in plasma concentrations of red blood cells, total proteins, or electrolytes.

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SALT TOLERANCE OF TURKEYS*

Scrivner (1946) has suggested that edema and ascites as observed in turkey poults may be caused by sodium chloride, or more specifically, by the sodium in the diet. He based his conclusions on results of experiments in which day-old poults were fed a commercial mash containing 0.5% sodium chloride to which he added up to 3.0% sodium chloride and in another series, various percentages of other sodium salts. His data indicated that

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2.5% sodium chloride was detrimental. More recently James (1946) supplemented a basal diet with 0.25%, 0.5%, 1.0% and 2.0% sodium chloride. He did not state whether or not his basal diet contained sodium chloride *per se*. He concluded that sodium chloride up to 1.0% in the diet was not detrimental to growth or the cause of high mortality. Batchelder (1946) reported on observations made on turkey poults submitted to the Poultry Diagnostic Laboratory of the Rhode Island Agricultural Experiment Station. His conclusion was that salt poisoning may be more wide spread in turkey poults than generally suspected.

We wish to report the results of an exploratory experiment to determine the sodium chloride tolerance of turkey poults. The day-old Bronze turkey poults used were from the Experiment Station flock and were confined to electrically heated brooding compartments. The semi-purified basal mixture, which was essentially "salt" free, consisted of the ingredients listed in table 1. The dietary supplements and experimental results are given in table 2. Potassium chloride was included in lots 5 to 8 because it had been used therapeutically in edema of humans.

TABLE 1.—*Basal Diet*

White Table Corn Meal	49.48	
Casein (commercial)	30.00	
Bagasse	2.00	
Primary Grown Yeast	5.00	
B 43	5.00	
Wheat Germ Oil	2.00	
Limestone	2.00	
Tri-calcium Phosphate	4.00	
Fortified Feeding Oil 4000A-800D	0 50	
Manganese Sulphate	0.02	
	100.00	
Vitamins/100 pounds		
Choline	90.00	gm.
Nicotinic Acid	2.27	gm.
Thiamine	0.095	gm.
Pyridoxine	0.227	gm.
Ca pantothenate	0.635	gm.
Vitamin K	0.0454	gm.

TABLE 2.—*Dietary supplements and experimental results to 23 days*

Lot No.	% NaCl	% KCl	No. Birds	Deaths	Number showing edema	Av. wt. survivors
1	0.5		12	0	0	241.2
2	1.0		12	0	0	285.0
3	2.0		12	0	0	279.2
4	4.0		12	3	3	295.6
5	0.5	1	13	1	0	288.0
6	1.0	1	13	1	0	275.1
7	2.0	1	13	1	1	272.0
8	4.0	1	13	4	4	306.0

The data indicate that turkey poults readily tolerate concentrations of sodium chloride, *per se*, up to 2.0% if the diet is otherwise relatively free from "salt." Potassium chloride at the level fed did not appear to have any preventive action. No omphalitis was observed.

Dam (1944) has shown that the quantity of sodium chloride as well as its ratio to other salts in his experimental diets affected the occurrence of exudative diathesis. He has also demonstrated that the protein-carbohydrate ratio and the quality of fat have an effect on inducing exudative diathesis.

It is possible, therefore, that the lack of complete agreement on the sodium chloride tolerance of young poults may be due to the differences in the composition of the experimental diets used, especially in the amounts of salts other than sodium chloride that may be present.

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Clinical Nutrition

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HYPERTENSION, SALT INTAKE, AND THE INFANT

A growing number of pediatricians, nutritionists and internists are becoming worried over the high salt intake of infants—and with good reason, I believe. Breast milk has a very low sodium content (7 mEq per liter, a concentration lower than that of tap water in many cities). The total sodium intake of the infant who is entirely breast-fed is thus rarely above 10 mEq per day. Thousands of generations have shown that this small amount of sodium is entirely adequate to support healthy growth.

Cow's milk is much higher in sodium—about 30 mEq per liter. An infant reared on cow's milk is thus on a diet very high in sodium, compared with a breast-fed baby. The sodium content of the diet is further increased when solid baby foods are introduced in the diet. When the infant is six months old, milk usually contributes, at most, half the total daily sodium intake; at one year milk contributes normally less than a quarter of the total sodium, with 60 percent of the total coming from processed meats and vegetables. As prepared by U.S. manufacturers, these foods are copiously salted, apparently to make them palatable *to the mothers*! Whereas the breast-fed infant receives about 1 mEq sodium per kilogram, the infant fed cow's milk exclusively receives about 3.5 mEq per kilogram.

A recent study on a large group of healthy American term babies fed cow's milk and ranging in age from 1 to 14 months showed that at one month of age the average daily intake of sodium was 4.6 mEq per kilogram, with the highest value 8 mEq per kilogram. By one year of age, the average had risen to 6.3 mEq per kilogram. The highest value recorded was 10.9 mEq per kilogram in a three month old infant—10 times the intake of a breast-fed infant of the same age! (The intake of 1 mEq per kilogram, incidentally, agrees well with calculated requirements for infants from birth to one year, with the requirements for growth decreasing and the insensible and fecal losses increasing during that period, leaving the overall requirement per kilogram essentially constant.)

Some manufacturers appear to have been stung by the criticism that they were selling "baby foods" on the basis of their being palatable to the mothers (who are used to a high-salt diet), rather than conforming to the infant's requirements. One of their advocates has pointed out that while it is true that the salt intake of infants fed processed baby food is enormous if related to a body weight basis, so are water, calorie and protein intake. He pointed out that "the year old infant takes in 120 to 135 ml of water per kilogram per day, which is equivalent to 8.40 to 9.45 L of water per day for the 70 kg adult." He went on to show that for a 70 kg man the calorie and protein intake equivalent to that of a baby would be 7700 calories and 140 to 245 gm of protein, and suggested that "the restriction of salt to levels of impalatability might do more harm than good."

This, of course, is a meaningless argument. The requirements for the infant have been calculated and agree well with the historically adequate intake of the breast-fed infant. There is no indication that highly salted food is particularly palatable *to the infant*, or that bland food —like essentially tasteless milk—is not the preferred food at that age. Furthermore, there are a great deal of experimental evidence, quite a

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lot of clinical material, and some epidemiologic data pointing to the dangers of high-salt diets.

Epidemiologic studies have shown that primitive peoples, by and large, have low intakes of salt and, generally speaking, no hypertension. By contrast, West Indian Negroes, who have a high intake of salt (salt pork and salt fish), have a much higher prevalence of hypertension than do white inhabitants and Indians in the same area. Lewis K. Dahl, the well-known chief of the medical service in the Medical Center at Brookhaven National Laboratory, divided a large number of adults into three groups, according to low, average and high sodium intakes. He found that over 10 percent of the group with high intakes had hypertension, compared with 7 percent of the average group and less than 1 percent in the low group. Conversely, in another study hypertensive subjects were found to have a higher salt intake than those who were normotensive.

A number of populations do live on very low salt intake with no evidence of harmful effect. Among such peoples who have been investigated are the Eskimo, certain Chinese groups, American Indians of the Northwest, Lapps and the Masai of Africa (a warring people of particularly splendid physique who live essentially on meat, milk and blood and have an average intake of 1.8 gm of salt per day).

Experimental work shows that a high dietary concentration of salt induces permanent hypertension in rats. Some strains of rats are much more sensitive than others, but basically there is a dietary concentration, characteristic of each strain, which will induce this condition. In an experiment involving 30 different varieties of baby foods (all of which showed a sodium content greatly in excess of the unprocessed meats and vegetables from which they were made), Dahl and co-workers found that hypertension developed within four months in most rats predisposed to hypertension that were fed these foods. Dahl also found that very young rats are more immediately sensitive to the effect of high-salt diets than are older animals.

Clinically, it is well known that the tendency for edema to develop in prematurely born infants is a function of the sodium content of the diet. It has also been demonstrated that a high salt content of the diet increases the likelihood of renal cast formation in these infants. We do not need to be reminded of the therapeutic effects of low-salt diets in hypertension.

What can we conclude from this evidence? Certainly that there is a very definite risk that salted baby foods may be harmful. Cigarette smoking, radiation and some poisons are also harmful, but their cumulative effect is slow enough to hide the toxicity for many years. Similarly, we may be just becoming aware of the role of excessive dietary intake of sodium chloride in the etiology of hypertension. It seems particularly dangerous to expose infants to intakes that are proportionally higher than those of adults and enormously higher than those resulting from breast feeding. By contrast, there appears to be no risk whatsoever in low sodium intake.

A reasonable policy would seem to be to instruct mothers not to add any salt to eggs, cereals or other dishes they prepare for their infants. Pediatricians and their professional societies should try to influence manufacturers of baby foods—who in other respects have tried to conform with sound nutritional concepts—to stop adding so much salt (or for that matter, to stop adding any salt) to the foods they prepare. Manufacturers should be invited to participate in educational campaigns directed at the mothers to teach them not to use the same criterion in judging the "salt taste" of food for their infants as for adults.

We should remember that basically prepared baby foods add very little to the diet of milk-fed infants given vitamins. The very young infant spits out most of what is fed to him. What is ingested and absorbed does make a small contribution of iron to the baby's nutrition, at some cost (10 percent of allergic reactions in very young infants). It is perhaps too much to say, that solid foods fed to small infants are really a status symbol for the mother, but it is legitimate to question whether for infants of less than three months, at any rate, they serve any useful purpose.

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NaCl preference in spontaneously hypertensive rats: age and blood pressure effects

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McCONNELL, S. D., AND ROBERT I. HENKIN. *NaCl preference in spontaneously hypertensive rats: age and blood pressure effects.* Am. J. Physiol. 225(3): 624–627. 1973.—Preference for 0.30 M NaCl was determined in spontaneously hypertensive (SH) male rats and in normotensive Wistar/Kyoto (WI/KY) controls of the same strain with a two-bottle preference test for 16 weeks postweaning. Preference for the salt solution in the SH rats increased as blood pressure increased to the hypertensive range as the animals increased in age. Increased preference and hypertension reached maximal levels at 14 weeks of age and remained at these levels thereafter. No significant difference in preference or blood pressure was observed in the normotensive controls during the same period. SH rats in which preference for 0.30 M NaCl was measured for the first time at 14 weeks of age had hypertension similar to that of the SH rats exposed to the salt solution from weaning; elevated preference for 0.30 M NaCl in these two groups of SH rats was also similar. WI/KY normotensive controls first given NaCl at 14 weeks of age had low levels of preference similar to that observed in control rats given NaCl from weaning.

hypertension; taste

IN PREVIOUS INVESTIGATIONS we have shown that adult spontaneously hypertensive (SH) rats exhibit an increased preference for salt solutions containing sodium (2) or potassium (6), but not for the sour or bitter solutions of HCl or quinine sulfate (6). Other workers have reported that rats subjected to latex encapsulation of the kidneys became hypertensive and developed a decreased preference for NaCl 2–3 weeks after the operation (1). These latter data suggested that some temporal relationship existed between the onset of hypertension and changes in salt preference. Since hypertension in SH rats increases with age (4) and since adult SH rats demonstrated an abnormal preference for various salt solutions (6), we wondered whether or not changes in salt preference were age dependent.

In the present study blood pressure and preference for NaCl and other salts were measured in SH rats from weaning through 20 weeks of age when they were considered to be adults.

MATERIALS AND METHODS

Rats were caged individually under controlled conditions of temperature, humidity, and light as previously described (2). Pulverized Purina Laboratory Chow was available ad libitum; deionized, distilled water was used for drinking purposes and to prepare the salt solutions. Testing was initiated after a 5-day period during which rats were presented with H_2O in two graduated drinking tubes (Kontes Glass Co., Vineland, N.J.). Testing was conducted on a 7-day schedule. During the first 4 days a salt solution was offered in one bottle and water was offered in the other. Fluids were switched in bottles on the 3rd day after repeated rinsing of each bottle with distilled water. The bottles were returned to their original positions, reducing any effect of different drinking lips on preference behavior. During the last 3 days rats were offered H_2O in both bottles. Testing for the next 7-day period was carried out in a similar manner and for each 7-day period thereafter. Recordings of fluid intake were made every 24 hr from 9:00 to 9:00 AM.

Body weight and systolic blood pressure were measured weekly. An indirect plethysmographic technique (9) was used to measure blood pressure with an E & M physiograph and a small animal unit (E & M Instrument Co., Houston, Texas). Each animal was placed in a specially constructed box[1] (with an ambient temperature of 37 C) prior to measurement of blood pressure in order to obtain reproducible, tail artery pulse recordings. Rats less than 9 weeks of age were placed in the box for 4–5 min, the older rats, for 10–12 min, prior to measurement of blood pressure.

Preference for the test solution was expressed in percent as:

$$\frac{\text{ml salt solution consumed/24 hr}}{\text{ml total fluid consumed/24 hr}} \times 100$$

Values for the first 4 days of each period were averaged for all animals under each condition, and the mean daily preference was calculated. Total fluid intake (water plus test solution)/100 g body wt and volume of test solution consumed/100 g body wt were also recorded.

Male rats were used in all studies. For convenience the experiments were divided into three parts.

Experiment I. Twelve SH weanling rats, 4 weeks of age (body wt, 70 ± 2 g), were used as experimental animals and six Wistar/Kyoto (WI/KY) rats, 6 weeks of age (87 ± 4 g, mean ± 1 SE), were used as controls. The WI/KY rats are the strain from which the SH rats were obtained, but they are normotensive (5). Both groups were tested simultaneously for preference for 0.30 M NaCl for 11 consecutive weeks.

[1] Produced by the Biomedical Engineering Instrumentation Branch, National Institutes of Health.

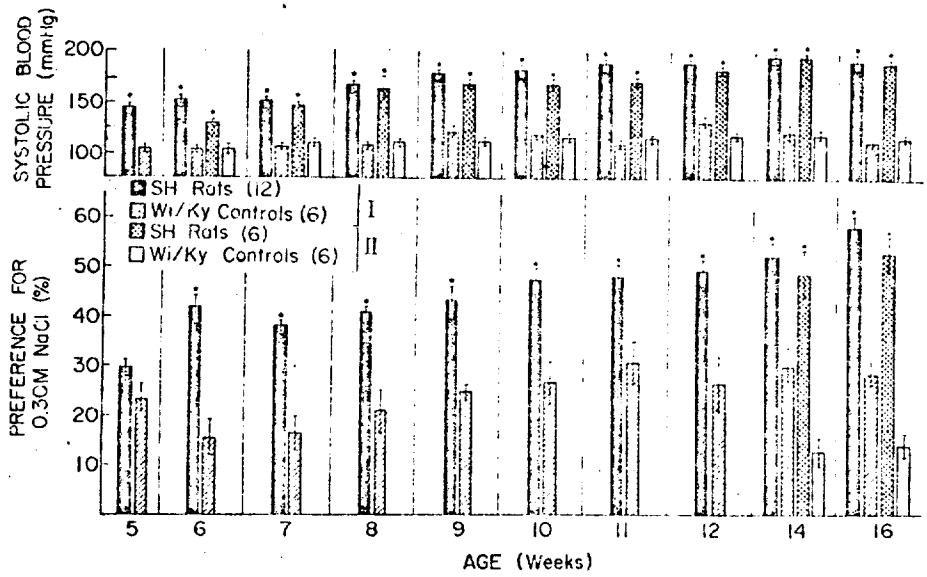

FIG. 1. Preference for 0.30 M NaCl and systolic blood pressure in SH rats and WI/KY controls measured from 5 to 16 weeks of age. In *experiment I* measurements of blood pressure and preference for NaCl were compared in 12 SH rats (black bars) and in 6 WI/KY controls (single-hatched bars) each week for 11 consecutive weeks, 9 of which are shown. In *experiment II* measurements of blood pressure were compared in 6 additional SH rats (double-hatched bars) and in 6 additional WI/KY controls (dotted bars) each week for 10 consecutive weeks, 8 of which are shown; preference for NaCl was compared in these rats for the first time at 14 weeks of age and continued for next 2 weeks, only 1 of which is shown. * $P < 0.001$, t test. In *experiment I*, when choice between NaCl and water was offered continually from 5 weeks of age, preference of SH rats was significantly greater than WI/KY controls at 6 weeks of age and older. However, blood pressure of SH rats was significantly higher than controls from 5 weeks of age to end of study. In *experiment II* choice between NaCl and water was first offered at 14 weeks of age. At that time preference of SH rats was significantly greater than controls. Blood pressure measurements in both SH and WI/KY rats were similar to those of *experiment I*, although they were measured from 6 weeks of age.

Experiment II. Six SH weanling rats, 4 weeks of age (78 ± 2 g), and six WI/KY weanling controls, 4 weeks of age (70 ± 5 g), were given tap water to drink, and preference for NaCl was determined at 14 weeks of age (SH rats, 319 ± 7 g WI/KY rats, 320 ± 5 g), 15 weeks of age (SH rats, 330 ± 9 g, WI/KY rats, 326 ± 15 g) and 16 weeks of age (SH rats, 338 ± 11 g, WI/KY rats, 339 ± 14 g).

Experiment III. Six SH rats were tested for preference for three different salt solutions at different ages: 0.45 M NaCl at 8 and 13 weeks of age; 0.15 M KCl at 9 and 16 weeks of age, and 0.15 M $NaHCO_3$ at 10 and 17 weeks of age.

RESULTS

Experiment I. Changes in preference for 0.30 M NaCl and in blood pressure for SH and WI/KY controls from 5 to 16 weeks of age are compared in Fig. 1. Blood pressure increased in SH rats from 145 ± 5 mm Hg at 5 weeks to 205 ± 6 mm Hg at 14 weeks. Preference for 0.30 M NaCl also increased significantly ($P < .001$, t test) during the same period from 30 ± 2% at 5 weeks to 52 ± 3% at 14 weeks. After 14 weeks there was no further significant increase in either blood pressure or NaCl preference. The controls demonstrated a small, but variable, increase in blood pressure from 109 ± 5 mm Hg at 7 weeks to 123 ± 5 mm Hg at 14 weeks. This level was never exceeded thereafter. During this same period, preference for 0.30 M NaCl increased from 5 ± 4% at 7 weeks to 30 ± 3% at 14 weeks.

Total fluid intake for SH and WI/KY rats during four test periods and four succeeding 3-day intervals when only H_2O was available to drink are shown in Fig. 2. At 5 weeks of age SH rats exhibited significantly greater total fluid intake than controls (38 ± 2 ml/100 g body wt vs. 30 ± 2 ml/100 g body wt, $P < .005$). As NaCl preference increased with age in SH rats, total fluid intake declined. This decline in fluid intake with age was also observed in WI/KY control

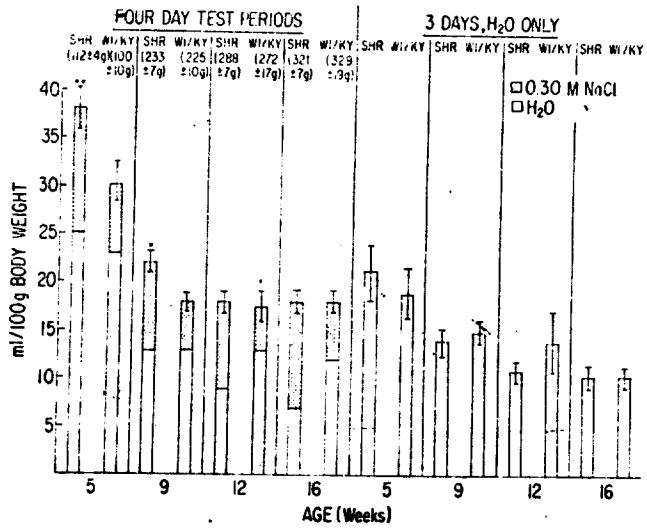

FIG. 2. Mean total fluid intake (0.30 M NaCl—dotted portion of bar. Water—clear portion of bar) ± 1 SE in SH rats and WI/KY controls compared at 5, 9, 12, and 16 weeks of age. ** $P < 0.005$. * $P < 0.05$. Total fluid intake of SH rats was higher than controls at all ages but statistically significant only at 5 and 9 weeks of age. NaCl intake of SH rats was significantly greater than in controls at 9 weeks of age and older ($P < 0.001$). Mean body weight ± 1 SE is shown in parentheses for SH and WI/KY rats at each age studied.

rats. At 9 weeks of age total fluid intake in SH rats was still significantly greater than that of controls, but intake for both groups decreased (22 ± 1 ml/100 g body wt in SH rats, 18 ± 1 ml/100 g body wt in controls, $P < .05$). By 12 weeks of age total fluid intake was similar in both groups of rats (18 ± 1 ml/100 g body wt) at a time when intake of 0.30 M NaCl was significantly greater in SH rats than in controls (preference levels: SH, 49 ± 3%, controls, 26 ± 4%). Intake of total fluid during test periods was maintained at these levels thereafter. Fluid intake during periods when only water was offered to drink was always the same in both SH and control rats at any age.

Experiment II. SH rats given only tap water to drink and then presented with a choice between 0.30 M NaCl and water for the first time at 14 weeks of age showed a preference for 0.30 M NaCl of 49 ± 5% in comparison to 52 ± 3% for the SH rats that had been given a choice between NaCl and water beginning at 5 weeks of age. Preference for NaCl in these SH rats was significantly ($P < .001$) greater than those in WI/KY control rats also offered NaCl for the first time at 14 weeks of age (Fig. 1). These SH rats developed hypertension at the same rate as those offered a choice of salt and water at an early age, and hypertension in both groups stabilized at the same level at the same age, i.e., 14–16 weeks of age.

WI/KY controls given a choice between 0.30 M NaCl and water for the first time at 14 weeks of age exhibited a marked aversion for the salt solution (13 ± 3%); blood pressure at this time was 121 ± 4 mm Hg. Blood pressure in the controls given a choice between salt and water at an early age (123 ± 5 mm Hg) was the same as rats first given a choice at 14 weeks of age; however, preference for NaCl was significantly higher (29 ± 3%, $P < .005$) in rats given a choice at an early age than in rats first given a choice at 14 weeks of age.

Experiment III. Preference for 0.45 M NaCl and 0.15 M KCl and blood pressure increased significantly as SH rats increased in age from 8 to 16 weeks. For 0.45 M NaCl, preference increased from 14 ± 1% at 8 weeks of age to 32 ± 3% at 13 weeks of age ($P < 0.01$), and blood pressure increased from 160 ± 5 mm Hg at 8 weeks to 195 ± 5 mm Hg at 13 weeks ($P < 0.001$). For 0.15 M KCl preference increased from 52 ± 1% at 9 weeks of age to 84 ± 4% at 16 weeks ($P < 0.001$) and blood pressure increased from 170 ± 5 mm Hg at 9 weeks to 200 ± 5 mm Hg at 16 weeks ($P < 0.01$).

DISCUSSION

These studies show that SH rats exhibit an increase in preference for 0.30 and 0.45 M NaCl and for 0.15 M KCl as they increase in age from weanlings to adults. Preferences exhibited at 16 weeks of age were maintained throughout the remainder of their adult lives.

The onset of hypertension in the SH rats preceded the onset of their increased preference behavior. Although blood pressure was significantly greater in SH rats compared to WI/KY controls at the first measurement at 5 weeks of age, mean daily preference was not significantly increased until 1 week later.

The increase in preference for 0.30 M NaCl demonstrated over 12 weeks of testing postweaning paralleled the increase in blood pressure that SH rats develop under normal laboratory conditions. Furthermore, the greatest perference for 0.30 M NaCl occurred at the age when maximum blood pressure levels were attained. Both preference and hypertension remained elevated thereafter.

The data presented for preference and fluid intake are independent of growth rates in the two groups of rats studied for body weights of the SH and WI/KY rats were similar during the time that significant differences in salt preference, fluid intake, and blood pressure were observed. Although other workers have suggested that SH rats are always smaller than controls at any age, the SH rats studied in these experiments did not follow that pattern. However, after 17 weeks of age the WI/KY rats continued to increase in weight, whereas the body weight gain of the SH rats slowed. After 20 weeks of age the SH rats weighed significantly less than did the WI/KY controls (SH rats, 353 ± 10 g, WI/KY rats, 406 ± 7 g, 21 weeks of age, $P < 0.01$). Since the amount of food eaten by the SH and WI/KY rats was similar at all ages studied, the slower growth rate of SH rats after 17 weeks of age cannot be attributed simply to decreased food intake.

These experiments also show that the development of hypertension and elevated preference for 0.30 M NaCl in SH rats are unaffected by prolonged exposure to 0.30 M NaCl under the conditions of choice described. SH rats presented with 0.30 M NaCl for the first time when hypertension had reached maximum levels did not exhibit significantly different preference for NaCl than did SH rats given a choice of 0.30 M NaCl from an early age. However, control WI/KY rats given a choice between NaCl and water at an early age exhibited a significantly greater preference for NaCl than did the control rats given a choice first at 14 weeks, although blood pressure measurements were the same in the two control groups of rats. These results suggest that early exposure to NaCl may influence later intake of NaCl in some rats but may not influence blood pressure in rats, which are not particularly sensitive to the development of hypertension.

It is of considerable interest that SH rats demonstrate a significantly greater preference and intake of NaCl over that of controls without demonstrating an increase in water intake. Although hypertensive rats studied by some investigators increased their water intake when their salt intake increased (1), other workers using rats genetically predisposed to hypertension noted no polydipsia with increased salt intake (10) as did we. It is important to point out that the rats genetically predisposed to hypertension previously studied may not have been hypertensive at the time their preference was measured (10). At best their blood pressure was 10–20 mm Hg above that noted in nonpredisposed rats (115–125 mm Hg, predisposed rats vs. 105 mm Hg, nonpredisposed rats, mean blood pressure) (10). In our studies, when blood pressure in SH rats averaged 145 mm Hg at 5 weeks of age, no significant differences in NaCl preference with the control WI/KY rats were observed. It was only after blood pressure of the SH rats increased to levels that averaged 152 mm Hg at 6 weeks of age that significantly increased preference was observed. The differential blood pressure at 5 weeks of age between SH and WI/KY rats was 36 mm Hg, and at 6 weeks of age it was 48 mm Hg. Whether

the initiation of the preference differences was related to differences in the absolute level of hypertension, to relatively small differential levels of hypertension, or to age differences is not known. Nevertheless, once these differences in NaCl preference appeared, they remained for as long as the SH and WI/KY rats were studied.

All other investigators who have studied NaCl preference in rats made hypertensive by hormonal, surgical, or other artificial manipulation have observed that they exhibit a decrease in NaCl preference (1, 3, 8). This same decrease in NaCl preference was also observed in rats predisposed to hypertension by genetic selection and made hypertensive by feeding diets high in NaCl (10). The mechanism for the decreased NaCl preference in any of these rats has never been clearly described. Contrary to these previous observations the SH rat exhibits an increase in NaCl preference when it is hypertensive, as does man (7). The hypertension of the SH rat occurs whether or not presented with large quantities of NaCl, a phenomenon quite different from that observed by workers studying rats genetically predisposed to develop hypertension (10).

The interrelationships between salt preference and hypertension in SH rats may offer an important clue to our understanding of some significant aspects of hypertension. At the very least it suggests that the SH rat is a useful model in which to study hypertension.

S. D. McConnell is a Fellow of the Campbell Institute for Food Research.

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THE EFFECT OF EXCESS SALT INTAKE ON HUMAN BLOOD PRESSURE*

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IT HAS long been felt that sufficient restriction of sodium chloride intake results in a fall in the blood pressure of both normotensive and hypertensive humans. The following experiment was performed to see the effect of excess salt intake on human blood pressure.

PROCEDURE

One subject, a healthy normotensive young adult male, was used. Ten blood pressure recordings and four radial pulse determinations were made daily, and the means of these values were taken as the daily blood pressure and heart rate according to the method of Wilhelmj *et al* (1). Body weight was also recorded daily. Since such things as smoking, excitement, exercise, and diet can profoundly affect blood pressure, an effort was made to stabilize these factors. Determinations were always done in the same room at the same time (about one hour before lunch), and following a rest period of at least one-half hour. A mercury sphygmomanometer and auscultation with a stethoscope were used to record blood pressure, and a stopwatch was used in recording the pulse. Smoking was discontinued about one hour before readings were made. The diet was simple and did not vary much from day to day.

Control readings were taken for 23 days, then table salt[1] was taken in the dose range of 25-60 gm/day. Four divided doses mixed in water were taken daily. Salt was taken for 23 days then abruptly discontinued. During the post-salt period, readings were carried out for 25 days.

Plasma volume determinations[2] using the Evan's Blue (T-1824) dye method were done during the salt period and again after the blood pressure and body weight had stabilized in the post-salt period.

RESULTS

During the 23 days of salt ingestion, 865 gm of salt were taken over and above the salt taken at meals. During the first few days there was a prompt increase in body weight associated with slight but definite swelling of tissues of the face, arms and legs. Pitting edema never occurred. There was a gradual, progressive, highly significant increase in systolic and diastolic pressures. Changes in heart rate were not significant. When salt was discontinued there was a precipitous drop in systolic and diastolic pressures and body weight. These curves then leveled off and assumed

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1. Morton's White Crystal Salt. NaCl content 99.53%.
2. Through the generosity of R. E. Lemire, M.D.

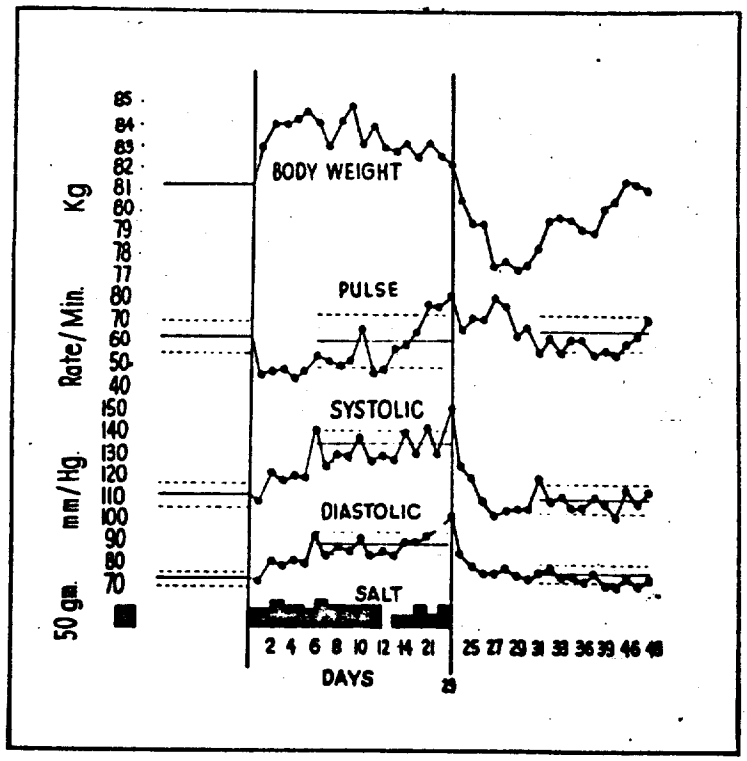

Fig. 1: Broken Lines Represent Standard Deviation from the Mean.

values very close to the values obtained during the control (pre-salt) period (Fig. 1). Plasma volume was slightly but not significantly increased during the salt ingestion (Table 1).

TABLE I

PLASMA VOLUME DETERMINATIONS USING T-1824

	Salt	Post-Salt	Diff
Hematocrit	40.5 mm	44.0 mm	3.5 mm
Total Plasma Volume	4047 cc	3683 cc	364 cc
Total Blood Volume.	7085 cc	6851 cc	234 cc

DISCUSSION

The effect of massive daily doses of salt in this case was a highly significant elevation in systolic and diastolic blood pressure together with an increase in body weight. When salt was discontinued, blood pressure and body weight promptly fell to low levels and then returned to normal. Since the plasma volume was not appreciably affected by the high salt intake, increased vascular volume was probably not the only factor responsible for the elevation of blood pressure. Cellular edema may have been a factor; and edema of the arterioles resulting in increased total peripheral resistance is certainly a possibility.

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The Injection of Chemicals into the Yolk Sac of Fertile Eggs prior to Incubation as a Toxicity Test

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The increasingly large number of food additive chemicals introduced into the market each year has necessitated the development of rapid and reliable methods for the evaluation of their toxicity. Toxicologic studies of all these chemicals by the usual methods using animals are very difficult, and such studies sometimes give inconclusive results.

The toxicity of some chemicals, and especially of food additives, may be determined by injection of the chemical into the yolk sac of fertile eggs prior to incubation and subsequent observation of the effects of the chemical on the embryonic development of the chick. This appears to be a promising method in that it may be carried out much more economically in terms of money and space than would be possible with larger animals. Hundreds of chicken embryos may be observed in a minimum of space, and over a comparatively short period of time. The feasibility of using such large numbers is valuable also in the statistical evaluation of toxicity data.

A review of the literature shows how little work has been done in this field except in a fragmentary way on isolated cases. Most of the reports refer to injections of chemicals made after the fourth or eighth day of incubation and examination of the embryos killed before they hatch.

The earliest work that we have found in the literature was by Féré (1893). During ten years after this date he published about sixty-seven papers: a review article (Féré, 1899) contains a summary of many of his studies. His work consisted mainly of injection before incubation, but the eggs were usually opened on the third day of incubation. His interest was mainly in the teratogenic effect of chemicals.

Since 1900 other articles have appeared in the literature, but most of them concern the effects of one or two chemicals on a small number of embryos. One of the most informative papers is that of Ridgway and Karnofsky (1952) in which they report experiments on compounds containing fifty-five of the elements, mainly the metallic ones, generally injected at either the fourth or eighth day of embryonic development to study toxicity and teratogenic effects. Hamburger and Hamilton (1951) have described an elegant method for determining the stages of development of the chick embryo. More recent investigations employing the chick embryo to study the toxicity of various chemicals have been reported by Kemper (1962), Platt *et al.* (1962), and Goerttler (1962). Finally, the classic books of Romanoff and Romanoff (1949) and Romanoff (1960) contain a wealth of information on the avian embryo.

Preliminary reports of this investigation have been presented by Marliac (1962) and McLaughlin and Mutchler (1962).

EXPERIMENTAL

The fertility and hatchability of eggs and the livability of chicks are dependent on a complex interrelationship of ecological factors, among which are the genetic background and the age of the mated birds, the nutritional status and general management of the flock, and seasonal variations. In view of this the initial phase of our work, which started in 1959, was devoted to a study of the hatchability of our supply of White Leghorn eggs[1] under conditions existing in our laboratories. The data accumulated during two years for control eggs showed that the hatchability of these eggs was, in fact, consistent, reproducible, and very high. The possibility of a seasonal variation occurring in responses to compounds introduced into the eggs was also examined by repeated testing of several chemicals at all seasons of the year. No important variation was detected.

Selection of eggs. Eggs to be injected are first candled in order to discard those that are defective and to outline with a pencil the exact location of the air cell. In our laboratory 9% of 5000 eggs had to be discarded: 2% cracked, 4% with improperly calcified shells, 1.5% with a tremulous air cell, 1% with the air cell in the wrong place, and 0.5% with blood clots. After the elimination of such defective eggs, the hatch of control eggs averages 95%. A further restriction is based on the weight of the eggs: all those weighing less than 52 g or more than 63 g are rejected. After

[1] Truslow Eggs, Chestertown, Maryland.

candling, the eggs are randomized in order to avoid series of infertile eggs in any one experiment.

The initial experiment with a given chemical is for range-finding and is performed at two or more concentrations of the chemical with 10 eggs per level. On the basis of this information, 20 or more eggs are injected with the appropriate amount of the chemical.

If the chemical proves to be nontoxic, the experiment is repeated with the minimum number of eggs that will give a reliable and reproducible value for the hatchability. In the case of a toxic chemical, additional eggs are injected to determine the specific effects of the chemical. The total number of eggs used for a chemical depends upon the data obtained initially and upon the kind of information desired. Hence, data for some chemicals are based on less than one hundred eggs, whereas data for others are based on several hundred eggs.

Technique of injection. The injections of pure chemicals, chemical solutions, or suspensions are made at volumes up to 0.10 ml. When necessary, dilutions are made with solvents such as water, propylene glycol, corn oil, peanut oil, or other nontoxic solvents.

In order to avoid contamination, the injections are carried out in an Isolator Box[2] with a sterile atmosphere created by using formaldehyde vapors (produced by mixing 2 g of potassium permanganate and 50 ml of 37% formalin). During the period of a year, more than fifteen hundred noninjected eggs were exposed to formaldehyde vapors; no toxic effect was noticed. After exposure to these vapors for 30 minutes, the eggs are ready for injection.

The large end of the egg is wiped with a sterile gauze pad moistened with a 70% alcohol solution, and a hole is drilled in the shell in the center of the surface over the air cell (Fig. 1). Care must be taken not to damage the shell membrane with the point of the drill[3]; this is to avoid, if possible, contact of the air with the egg membrane. Fine particles of shell are removed with an aspirator to prevent the needle from carrying them into the yolk.

Immediately before the injection, each egg is shaken with a quick twist of the wrist. Since the germinal disc occasionally sticks to the air cell and it is possible to damage it with the needle, this movement will allow the disc to float free in the egg.

2 Kewaunee Scientific Company, Adrian, Michigan.
3 Burgess Vibrocrafters Inc., Grayslake, Illinois.

The needle (hypodermic, 1 inch long, either no. 22 or no. 27, depending on the viscosity of the liquid to be injected) is inserted horizontally through the air cell into the yolk (Fig. 1). Care must be taken in withdrawing the needle to avoid damaging the vitelline membrane since such damage could cause the yolk to spread out in the albumen. If the end of the needle has some yolk on it, the injection is not satisfactory. The needle should be wiped with a sterile gauze pad between each injection. As soon as the egg has been injected, the hole in the shell is covered with a small piece of Scotch tape, care being taken not to cover the entire air cell.

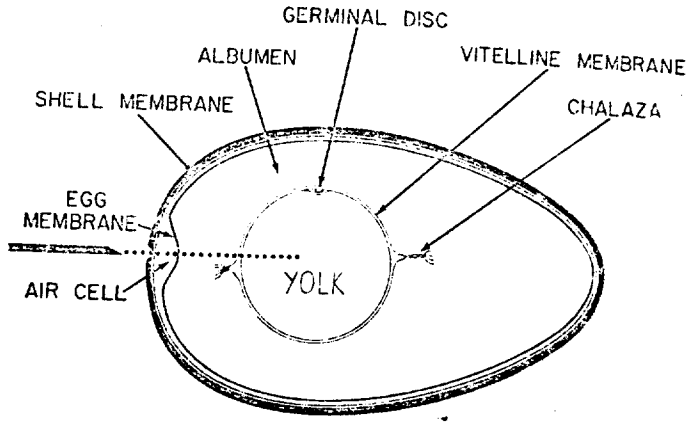

FIG. 1. Diagram of egg and position for injection.

Incubation and hatching. The injected eggs are put into the incubator trays with the large end up; the trays are placed in the incubator,[4] which automatically rotates hourly and is maintained at an optimum temperature of 38°C and a relative humidity of 60%. The eggs are candled on the fifth day of incubation and every day thereafter. Clear eggs and dead embryos are removed for examination. On the seventeenth day of incubation the fertile eggs are transferred to the hatcher[5] and kept at a temperature of 37°C until they hatch.

EVALUATION OF DATA

The injection of the chemical into the egg may produce one of four possible results: (1) the chemical is highly toxic at the level injected, and

[4] Humidaire Incubator Co., New Madison, Ohio; model no. 50, capacity 450 eggs.
[5] Brower Manufacturing Co., Quincy, Illinois.

all the embryos are killed during the first 20 hours of incubation (before the two-somite stage); (2) the chemical is toxic but allows a number of embryos to develop only up to a certain point, and some possibly even to hatch; (3) the chemical has little effect on the hatch; and (4) the chemical has no effect on the hatch or on the posthatch development of the chick ("no effect" level).

If the chemical appears to be highly toxic and all the embryos are killed, the experiment is repeated with smaller doses of the chemical until some hatch is obtained. If the chemical is toxic, but allows the embryos to develop for a longer period of time, dead embryos are examined pathologically and the chicks that do hatch are examined for eye damage, color of the feathers, weight, length of the legs, form of the beak and of the rump, hematologic changes, and condition of the internal organs (liver, kidneys, heart, gall bladder, and spleen).

It is advisable in all cases to observe the chicks for a period of a few weeks in order to detect any delayed effects. Since as much as 30% of the yolk remains at the time of hatching and is absorbed during the first 7 days thereafter, effects of a toxic chemical may first be observed at this time. There may be weight retardation, death during the first week, or the appearance of nerve damage occurring as late as 2–6 weeks after hatching.

The toxicity of a chemical is evaluated mainly from the percentage of hatch at varying dosages of the chemical as compared to noninjected (control) eggs, from a study of the embryonic development of the eggs that fail to hatch, and from a study of the appearance and development of the chicks that do hatch. However, several other factors must also be considered in this evaluation: these are specific gravity, solubility, coagulating effect, pH, and the ionic concentration of the chemical tested.

If the chemical has a high specific gravity, there is the possibility of its settling out in the bottom of the egg and thereby giving a value of apparently low toxicity.

The solubility is quite important since the availability of the chemical for utilization in the chick embryo is partially dependent on its solubility in the egg. However, since egg yolk is an emulsion, solubility problems are somewhat minimized. In the case of insoluble chemicals that are injected as suspensions, it is also necessary to consider particle size in the evaluation of toxicity data.

In order to have a toxic effect, the chemical must come in contact with the embryo either directly or indirectly through the bloodstream. Chemicals such as the lower aliphatic alcohols have a coagulating effect on the pro-

tein, and this coagulation may decrease the availability of the chemical as well as some yolk nutrients, and thereby alter the response of the embryo.

If the pH is highly acidic or basic, a pH effect differing from the true toxic effect of the chemical may be obtained due to interference with the normal acid-base equilibrium in the egg.

The ionic concentration is also important for a similar reason. The observation of increasing toxicity with increasing concentration of a chemical should be interpreted cautiously, since highly concentrated solutions may upset the physical equilibrium of the yolk by causing osmotic effects.

Finally, the introduction of a chemical into the yolk may cause a special type of toxicity because it destroys, alters, or combines with essential nutrients such as vitamins and minerals.

EXPERIMENTAL DATA

Twenty-five thousand eggs have been used in our laboratory during the past three years to test more than 100 chemicals with the following

TABLE 1
NONTOXIC CHEMICALS

Chemical	Solution injected		Per cent hatch
	Concentration	Quantity (ml)	
Water (boiled)	—	0.05	95
Propylene glycol	Undiluted	0.05	95
Corn oil	Undiluted	0.05	90
Peanut oil	Undiluted	0.05	90
Sodium chloride	0.9% in water	0.05	90
	5.0% in water	0.05	70
	5.0% in water	0.10	30
	10.0% in water	0.05	20
Dextrose	5.0% in water	0.05	90

results: (1) nontoxic chemicals injected at an appropriate level allowed the embryo to develop and to hatch as did the controls: (2) toxic chemicals produced effects at dose levels which may be compared to those producing effects in feeding experiments using animals: and (3) this technique often provided toxicologic information which had not been shown by conventional methods.

Table 1 lists the results obtained with some chemicals in common use in food and shows the dosages used and the percentages of hatched chicks.

TABLE 2
CHEMICALS WITH A HIGH ORDER OF TOXICITY

Chemical	Solution injected: Concentration	Solution injected: Quantity (ml)	Per cent hatch	Remarks
Lead acetate	10% in water	0.05	0	—
	2% in water	0.05	0	Hydrocephalus in embryos that failed to hatch
	0.5% in water	0.05	20	—
Mercuric chloride	1% in water	0.05	0	All embryos died at the beginning of incubation
Thiourea	10% in water	0.05	0	Some embryos lived up to 34 days
	5% in water	0.05	20	Hatched on the 28th day of incubation; growth retardation
Triorthocresyl phosphate	Undiluted	0.02	0	Most of the developing embryos died on the 15th day of incubation
	Undiluted	0.01	40	Hatched chicks showed growth retardation and developed paralysis 2–6 weeks after hatching
p,p'-Diaminodiphenylmethane	10% in ethanol	0.05	30	Leg damage; beak deformity (short mandible)
Sodium selenite	0.2% in water	0.05	0	—
	0.1% in water	0.05	20	—
	0.1% in water	0.02	80	—
Dibutyl-tin-dilaurate	Undiluted	0.01	0	Beak deformity
Aroclor 1242 (chlorinated polyphenyls)	Undiluted	0.025	0	—
	Undiluted	0.01	5	Beak deformity (short upper beak); edema; growth retardation

These data, supplemented by an examination of the nonviable eggs and an autopsy of the chicks that hatched, have not indicated any hazard from their use in food. However, it must be pointed out that any chemical, added at a sufficiently high concentration, may have some toxic effect. Since our data and those reported in the literature indicated safety, this phase of the study was not carried beyond a preliminary examination to ascertain that nontoxicity also was shown for these chemicals by the chick embryo technique. This is of theoretical as well as practical importance for the evaluation of any new technique to be used in toxicologic studies.

Table 2 lists our results with several chemicals which have been shown to be highly toxic to animals. In each case there is not only the low percentage of hatch at a low level of the chemical tested, but there are also congenital abnormalities and other responses that raised extremely serious questions as to the safety to the consumers of any food contaminated with these chemicals.

Lead acetate resulted in no hatch at a level of 1 mg per egg. Autopsy of the dead embryos showed extensive brain damage, as has been reported by de Franciscis and Bocalatte (1962) and by Karnofsky and Ridgway (1952).

Mercuric chloride showed no hatch even at a level of 0.5 mg per egg.

Thiourea is a known carcinogen with a basic effect on the thyroid gland. The hatch time was delayed with increasing amounts of this chemical. At a level of 5 mg per egg no chicks hatched and the embryo required 35 days to develop to the stage normally attained at 20 days. At 2.5 mg per egg some chicks hatched, but most of them had to be helped out of the shell. This effect has been reported also by Yushok (1950).

Cavanagh (1954) reported that triorthocresyl phosphate (TOCP), a well-known plasticizer for nonfood use, causes paralysis when fed to adult chickens. We observed this paralysis in some chicks which hatched from eggs injected with 10 mg of the undiluted chemical.

The compound *p,p'*-diaminodiphenylmethane has been reported by Zylberszac (1951) to cause cirrhosis of the liver in rats. We found this chemical to be extremely teratogenic at a level of 5 mg per egg: more than 90% of the chicks had a short mandible and leg damage consisting of a severe bending of the tibia and a general shortening of the bones of the leg.

Sodium selenite proved to be highly toxic. At a level of 0.1 mg per egg, no embryos developed to more than the 5-day stage.

Dibutyl-tin-dilaurate showed no hatch at a level of 10 mg per egg. The majority of the embryos, which did not live more than 15 days at this

TABLE 3

CHEMICALS WITH AN INTERMEDIATE ORDER OF TOXICITY

Chemical	Solution injected: Concentration	Quantity (ml)	Per cent hatch	Remarks
Acetone	Undiluted	0.05	70	
n-Butanol	Undiluted	0.01	95	
		0.02*	70	* At these levels: corneal opacity, cataract, cleft palate, nerve and kidney damage
		0.03*	20	
		0.04	0	
Carbon tetrachloride	Undiluted	0.05	40	
		0.10	0	
Diethylene glycol	Undiluted	0.05	80	
		0.10	55	
Ethyl acetate	Undiluted	0.05	35	
		0.10	15	
Ethyl alcohol	Undiluted	0.05	95	
		0.10	60	
		0.30	0	
Ethylene glycol	Undiluted	0.05	80	
		0.10	65	
Di-2-ethylhexyl phthalate	Undiluted	0.05	95	
Heptachlor	1% in propylene glycol	0.05	75	
		0.10	65	
Hydrochloric acid	4% in water	0.05	80	
Isopropanol	Undiluted	0.05	35	
		0.10	15	
Malathion	Undiluted	0.05	60	Short legs; bleaching effect on chick down
		0.10	0	
Methanol	Undiluted	0.05	90	
		0.10	65	
Styrene	5% in 80% ethanol	0.05	95	

level, had a short and/or flexed mandible; in addition, some embryos showed subcutaneous edema.

Aroclor 1242 gave no hatch at a level of 25 mg per egg. At a level of 10 mg per egg, one chick hatched out of 20 injected eggs, but died 2 days later. Some embryos, which were examined after they died, showed beak deformities (often a short upper beak), edema, and growth retardation.

Table 3 lists preliminary data on the toxicity of some chemicals which have been shown to be toxic in some degree to animals, and which may be found in some processed foods. Included in this group are various solvents, plasticizers, and insecticides. Some of the chemicals on this list require further study, including observation of the chicks until they reach maturity, before they may be classified as to low or high toxicity.

DISCUSSION

The injection of chemicals into the yolk of fertile eggs prior to incubation is a method which can be advantageously used as an element in the evaluation of the safety of food additive chemicals and drugs, and which could be used to screen new products and eventually to correlate their toxicity with that of similar products already tested.

If one considers that a chemical injected into the yolk may be compared to a substance which has the power to cross the placental barrier, this technique, in addition to being an embryonic feeding study, assumes further importance in that it is also a reproduction study. The unfortunate experiences recently suffered with chemicals that have teratogenic effects in humans, and the failure of conventional testing methods to produce this effect in animals, emphasize the urgent necessity for new methods of analysis. Preliminary work that we have done in this area has given satisfactory results (Verrett and McLaughlin, 1963).

Since this represents a system in which the chemical is in direct contact with the embryo throughout development, it is more than likely that any toxic or teratogenic effects would be readily observed. However, there is always the possibility that the chicken will not be a species susceptible to a particular compound, just as it has been shown that the other commonly used species of animals do not respond to all chemicals in a similar manner. It is also possible for the chicken to be more sensitive to a chemical than other species.

Finally, this technique may be applied also to the study of the synergistic effects of chemicals. Results of experiments in our laboratory on

the potentiation of a few pesticides have been very encouraging (Marliac and Mutchler, 1963).

SUMMARY

An evaluation of toxicity by injection of the chemical into the yolk sac of fertile eggs prior to incubation gave the following results:

Water, propylene glycol, corn oil, peanut oil, isotonic saline solution, and isotonic glucose solution showed no toxicity or a very low order of toxicity.

Mercuric chloride, lead acetate, selenium, triorthocresyl phosphate, *p,p'*-diaminodiphenylmethane, thiourea, Aroclor 1242, and dibutyl-tin-dilaurate showed a high order of toxicity and or teratogenic effects at certain levels.

Acetone, methanol, ethanol, *n*-butanol, diethylene glycol, ethylene glycol, isopropanol, di-2-ethylhexyl phthalate, hydrochloric acid, carbon tetrachloride, ethyl acetate, malathion, heptachlor, and styrene showed an intermediate order of toxicity.

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THE INFLUENCE OF RIGID SALT RESTRICTION IN THE DIET OF CHRONIC NEPHRITIS.[1]

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A year ago Dr. F. M. Allen reported his observations upon the influence of salt restriction in the diet of patients with chronic nephritis and so-called essential vascular hypertension. He stated that the measures usually attempted were ineffective because inadequate, and that rigid chloride restriction, as nearly absolute as possible, would achieve genuine benefits. He reported that following such withdrawal of salt from the dietary he has regularly seen a material fall in the blood-pressure. Among other beneficial effects he also saw improvement in the retinitis and other eye changes of nephritis. I recall his interesting speculation that we may in time come to conclude that sodium chloride is the long-sought-for poison of uremia.

It is generally recognized that the excessive use of salt is harmful, and that its restriction in nephritis is to be recommended; but I have not been able by chloride restriction as ordinarily practised to produce results comparable with those of Allen. Following, then, his suggestion that our restriction of the past has not been sufficiently rigid, I have studied in a small number of hypertension patients over a limited period of time the effect of the almost absolute elimination from the diet of all chlorides. Because the patient frequently complained that this diet produced an intense weakness, it was necessary to limit the length of such observations;

[1] Read before the American Society for Clinical Investigation, Atlantic City, May 9, 1921.

Case No.	Diet.	Systolic blood-pressure. Mm.		Blood chemistry.								Urine (24-hr. specimen).						Remarks.
				Urea N. Mgm.		Creatinin N. Mgm.		Sugar. Per cent.		Chlorides. Mgm.		Chlorides. Gm.		Albu min.		Casts.		
		Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	
1612	15 days, salt-poor 64 days, salt-free	220	205	18.7	8.5	1.00	2.20	0.11	0.17	.692	.493	12.60	1.27	+	++	+	+	
2654	62 days, salt-poor 16 days, salt-free	190	165	15.0	10.8[1]	1.35[2]	1.87	0.12	0.12[1]	.499	.504[1]	16.05	0.89[1]	+	+	0	+	[1]On 63d day. [2]On 9th day.
2693	9 days, salt-poor 18 days, salt-free	250	220	8.9	19.2[1]	1.10	2.60	0.11	0.10	.491	.453	15.50[2]	1.30[1]	+	+++[1]	0	+[1]	[1]On 21st day. [2]On 9th day.
2891	51 days, salt-free	200	240	5.6[1]	14.8	1.50	1.80[1]	0.11	0.12[1]	.492	.492	..	..	+	++	+	0	[1]On 14th day.
3033	11 days, salt-free	200	240	12.7	13.4	1.40	1.55	0.13	0.17	.549	.511	19.80	?	+	+	+	0	
3048	19 days, salt-poor 38 days, salt-free	218	190	12.4	23.0	0.90	1.64	0.10	0.10	.421	.473	2.79	0.60	++++	++++	+	++	
3059	15 days, salt-free	180	200	5.3	14.5	1.40	1.55	0.11	0.15	.531	.481	?	0.70[1]	++	++	0	+	[1]On 7th day.
3093	13 days, no restriction 10 days, salt-free	198	155[1]	16.6	18.7[1]	2.08	1.90	0.11	..	.550	.450[1]	7.87	1.30	++	++[1]	0	0	[1]At end of s.-free period.
4006	9 days, salt-free	240	270	20.0	30.0	2.70	2.67	0.07	0.09	.481	.506	?	1.47	++++	++++	+++	+	
H.H.	57 days, salt-poor	215[1]	172	13.5[1]	12.8	2.14[1]	1.42	..	0.13	..	.547	4.48[1]	4.38	++++[1]	++++	+[1]	+	[1]On 49th day.

The above table gives data obtained at the beginning and at the end of these observations. The data obtained at various intermediate periods are omitted. "Salt-poor" and "salt-free" periods of diet alternate. Note is made above, however, merely of the total number of "salt-free" and "salt-poor" days.

and because of the many pitfalls which surround attempts at accurate clinical control of intake and output, the final number of patients upon whom I can report was reduced to ten.

Note was made of the influence of this diet upon the patient's general condition, his blood-pressure, any possible eye changes and certain chemical bodies of his blood. The chloride content of the urine was used as a check upon the accuracy with which the dietary restriction was observed.

Distinction is made in these charts between the salt-poor and the salt-free diets, the latter, of course, being a relative term. With the salt-poor form the patient was given the usual low-calorie nephritis diet with no addition of salt to the food after it reached the table. It is estimated that thus the patient received daily the equivalent of about 2 gms. of sodium chloride. For the salt-free diet the bread and all other foods were cooked without salt and the patient received only such an amount of chlorides as was present in the native foodstuffs. It is estimated that upon this diet the patient received daily the equivalent of less than 1 gm. of sodium chloride. One or two weeks upon the salt-free diet with a variable interim of salt-poor food, and then a second salt-free period, was the rule, although one patient was able to maintain the more rigid diet for a continuous period of sixty-four days.

To summarize: The following results of the salt-free diet were noted.

1. The patient found the food unappetizing and usually ate but little. This factor cannot be disregarded.
2. The blood urea instead of decreasing showed a tendency to increase.
3. The blood chlorides, irrespective of diet, varied but little; while the urine chlorides, reflecting the degree of the patient's adherence to the diet, fell to a very low figure.
4. The systolic pressure, as a rule, showed a moderate fall. This fall was never marked.
5. In two of the patients there developed weakness and prostration to a distressing degree.
6. One of the patients suddenly experienced retinal hemorrhages and other fundus changes at the end of two weeks of this diet.

Conclusion. From these limited observations I would conclude that the almost complete elimination of chlorides from the diet of patients with nephritis and vascular hypertension accomplishes little if anything more than does the salt-poor diet ordinarily prescribed.

EFFECTS OF EXCESSIVE INGESTION OF SODIUM AND POTASSIUM SALTS ON CARBOHYDRATE METABOLISM AND BLOOD PRESSURE IN DIABETIC CHILDREN [1]

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NINE FIGURES

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In a preliminary communication (Thompson and McQuarrie, '34), the finding of an apparently significant influence of excessive salt ingestion on arterial blood pressure and carbohydrate metabolism was reported. The present paper records the results from a more extensive investigation of the subject.

Our interest in the question of a possible interrelationship between salt ingestion and carbohydrate metabolism was first aroused in 1930, when one of our younger diabetic patients, suffering from an abnormal craving for salt, appeared to show some freakish variations in her ability to utilize carbohydrate according to variations in the amounts of sodium chloride which she was allowed to consume. Lack of cooperation on the part of the patient and her premature discharge from the hospital frustrated our first attempts to investigate the problem. A second opportunity presented itself, however, in the autumn of 1933, when one of our clinic patients, a 15-year-old boy with moderately severe diabetes manifested a similar but even more marked craving for salt.

Obviously the first question presenting itself was that regarding the factors responsible for this patient's requiring

[1] Aided by a grant from the Medical Graduate Research Fund of the University of Minnesota.

between 80 and 90 gm. of table salt daily to satisfy his abnormal craving. Since he had been on a relatively high vegetable diet (high in K) for a long time, had partially depleted his reserve of base as a result of ketosis and had vomited an undetermined number of times just before being admitted to the hospital (thereby losing a considerable amount of chloride), this symptom was first tentatively explained on the simple basis of temporarily physiological need for both Na and Cl. That this explanation was entirely inadequate soon became apparent, however, when the craving was observed to continue only slightly abated long after all evidence of ketosis had disappeared and after a simplified diet low in potassium had been substituted for the ordinary diabetic diet. The unexpected discovery, that this patient's blood pressure was elevated temporarily and his glycosuria was decreased as a result of the extra salt ingestion, impressed us as being of sufficient interest to warrant immediate investigation. It appeared probable too that the factors involved in these reactions might incidentally provide an answer to our query concerning the symptom of salt craving.

Following confirmation of our original observation regarding the effects of high NaCl intake, a number of pertinent new questions presented themselves. Among these were the following: 1) Are the observed reactions peculiar to diabetic subjects suffering from an abnormal craving for salt or do other diabetics and even normal persons behave similarly under the same conditions? 2) Are the observed effects due specifically to the Na or the Cl ion alone or to the two combined? 3) Are the two effects (i.e., decrease in glycosuria and increase in blood pressure) mutually dependent or is it possible to obtain one without the other? 4) May the effect on blood pressure be due to increased tonus in the peripheral arterioles, to effects on the kidneys or merely to the mechanical effect of increased blood volume? 5) Is the diminution in glycosuria due merely to retention of sugar with retained water or is it due to improved ability to store or oxidize glucose? 6) If either of the latter, what is the nature of the

mechanism involved? 7) What nervous or endocrine functions, if any, are involved? 8) Has a high salt intake any influence on the metabolism of protein and fat?

METHODS AND MATERIALS

Repeated studies were carried out on each of four diabetic patients and on one normal subject, all in the age group between 13 and 15 years. Not more than two patients were under observation at one time. At such time they were kept in a small metabolism ward constantly under the supervision of special nurses, who weighed them every 12 hours, collected the urine and feces in special containers and made certain that diets and medications were taken precisely as ordered. Urine was collected quantitatively in 6- or 12-hour periods. The feces for different periods were marked by carmine according to standard technic. Systolic and diastolic blood pressure measurements were made by means of the same mercury sphygmomanometer before meals and after the patient had been in the recumbent position for at least ½ hour. Measurements were frequently checked independently by two observers. The averages of these readings were charted each day. The room temperature was held between 68° and 74°F. and the relative humidity between 40 and 60 per cent to prevent sweating or chilling. The body weight was determined every 12 or 24 hours immediately after the bladder was emptied and before meals or water were given.

The daily diet, which was constant for each patient throughout the entire experimental period, was divided into four identical meals given at 6-hour intervals. For the sake of uniformity and accuracy, it was made up from the following foods: powdered whole milk, egg yolk, egg white, cane sugar, water, unsalted butter, clear lemon juice and a small amount of white bread. The mineral constituents of a typical basic diet, containing protein 64, fat 96, and carbohydrate 132 gm., were as follows; Na, 1.03; K, 1.34; Ca, 0.68; Mg, 0.16; Cl, 1.83; P, 0.93 and S, 0.76 gm. It is seen that this is relatively low in sodium, potassium and choride. The amounts of protein,

values very close to the values obtained during the control (pre-salt) period (Fig. 1). Plasma volume was slightly but not significantly increased during the salt ingestion (Table 1).

TABLE I

PLASMA VOLUME DETERMINATIONS USING T-1824

	Salt	Post-Salt	Diff
Hematocrit	40.5 mm	44.0 mm	3.5 mm
Total Plasma Volume	4047 cc	3683 cc	364 cc
Total Blood Volume.	7085 cc	6851 cc	234 cc

DISCUSSION

The effect of massive daily doses of salt in this case was a highly significant elevation in systolic and diastolic blood pressure together with an increase in body weight. When salt was discontinued, blood pressure and body weight promptly fell to low levels and then returned to normal. Since the plasma volume was not appreciably affected by the high salt intake, increased vascular volume was probably not the only factor responsible for the elevation of blood pressure. Cellular edema may have been a factor; and edema of the arterioles resulting in increased total peripheral resistance is certainly a possibility.

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The Injection of Chemicals into the Yolk Sac of Fertile Eggs prior to Incubation as a Toxicity Test

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The increasingly large number of food additive chemicals introduced into the market each year has necessitated the development of rapid and reliable methods for the evaluation of their toxicity. Toxicologic studies of all these chemicals by the usual methods using animals are very difficult, and such studies sometimes give inconclusive results.

The toxicity of some chemicals, and especially of food additives, may be determined by injection of the chemical into the yolk sac of fertile eggs prior to incubation and subsequent observation of the effects of the chemical on the embryonic development of the chick. This appears to be a promising method in that it may be carried out much more economically in terms of money and space than would be possible with larger animals. Hundreds of chicken embryos may be observed in a minimum of space, and over a comparatively short period of time. The feasibility of using such large numbers is valuable also in the statistical evaluation of toxicity data.

A review of the literature shows how little work has been done in this field except in a fragmentary way on isolated cases. Most of the reports refer to injections of chemicals made after the fourth or eighth day of incubation and examination of the embryos killed before they hatch.

The earliest work that we have found in the literature was by Féré (1893). During ten years after this date he published about sixty-seven papers; a review article (Féré, 1899) contains a summary of many of his studies. His work consisted mainly of injection before incubation, but the eggs were usually opened on the third day of incubation. His interest was mainly in the teratogenic effect of chemicals.

Since 1900 other articles have appeared in the literature, but most of them concern the effects of one or two chemicals on a small number of embryos. One of the most informative papers is that of Ridgway and Karnofsky (1952) in which they report experiments on compounds containing fifty-five of the elements, mainly the metallic ones, generally injected at either the fourth or eighth day of embryonic development to study toxicity and teratogenic effects. Hamburger and Hamilton (1951) have described an elegant method for determining the stages of development of the chick embryo. More recent investigations employing the chick embryo to study the toxicity of various chemicals have been reported by Kemper (1962), Platt *et al.* (1962), and Goerttler (1962). Finally, the classic books of Romanoff and Romanoff (1949) and Romanoff (1960) contain a wealth of information on the avian embryo.

Preliminary reports of this investigation have been presented by Marliac (1962) and McLaughlin and Mutchler (1962).

EXPERIMENTAL

The fertility and hatchability of eggs and the livability of chicks are dependent on a complex interrelationship of ecological factors, among which are the genetic background and the age of the mated birds, the nutritional status and general management of the flock, and seasonal variations. In view of this the initial phase of our work, which started in 1959, was devoted to a study of the hatchability of our supply of White Leghorn eggs[1] under conditions existing in our laboratories. The data accumulated during two years for control eggs showed that the hatchability of these eggs was, in fact, consistent, reproducible, and very high. The possibility of a seasonal variation occurring in responses to compounds introduced into the eggs was also examined by repeated testing of several chemicals at all seasons of the year. No important variation was detected.

Selection of eggs. Eggs to be injected are first candled in order to discard those that are defective and to outline with a pencil the exact location of the air cell. In our laboratory 9% of 5000 eggs had to be discarded: 2% cracked, 4% with improperly calcified shells, 1.5% with a tremulous air cell, 1% with the air cell in the wrong place, and 0.5% with blood clots. After the elimination of such defective eggs, the hatch of control eggs averages 95%. A further restriction is based on the weight of the eggs: all those weighing less than 52 g or more than 63 g are rejected. After

[1] Truslow Eggs, Chestertown, Maryland.

candling, the eggs are randomized in order to avoid series of infertile eggs in any one experiment.

The initial experiment with a given chemical is for range-finding and is performed at two or more concentrations of the chemical with 10 eggs per level. On the basis of this information, 20 or more eggs are injected with the appropriate amount of the chemical.

If the chemical proves to be nontoxic, the experiment is repeated with the minimum number of eggs that will give a reliable and reproducible value for the hatchability. In the case of a toxic chemical, additional eggs are injected to determine the specific effects of the chemical. The total number of eggs used for a chemical depends upon the data obtained initially and upon the kind of information desired. Hence, data for some chemicals are based on less than one hundred eggs, whereas data for others are based on several hundred eggs.

Technique of injection. The injections of pure chemicals, chemical solutions, or suspensions are made at volumes up to 0.10 ml. When necessary, dilutions are made with solvents such as water, propylene glycol, corn oil, peanut oil, or other nontoxic solvents.

In order to avoid contamination, the injections are carried out in an Isolator Box[2] with a sterile atmosphere created by using formaldehyde vapors (produced by mixing 2 g of potassium permanganate and 50 ml of 37% formalin). During the period of a year, more than fifteen hundred noninjected eggs were exposed to formaldehyde vapors; no toxic effect was noticed. After exposure to these vapors for 30 minutes, the eggs are ready for injection.

The large end of the egg is wiped with a sterile gauze pad moistened with a 70% alcohol solution, and a hole is drilled in the shell in the center of the surface over the air cell (Fig. 1). Care must be taken not to damage the shell membrane with the point of the drill[3]; this is to avoid, if possible, contact of the air with the egg membrane. Fine particles of shell are removed with an aspirator to prevent the needle from carrying them into the yolk.

Immediately before the injection, each egg is shaken with a quick twist of the wrist. Since the germinal disc occasionally sticks to the air cell and it is possible to damage it with the needle, this movement will allow the disc to float free in the egg.

[2] Kewaunee Scientific Company, Adrian, Michigan.
[3] Burgess Vibrocrafters Inc., Grayslake, Illinois.

The needle (hypodermic, 1 inch long, either no. 22 or no. 27, depending on the viscosity of the liquid to be injected) is inserted horizontally through the air cell into the yolk (Fig. 1). Care must be taken in withdrawing the needle to avoid damaging the vitelline membrane since such damage could cause the yolk to spread out in the albumen. If the end of the needle has some yolk on it, the injection is not satisfactory. The needle should be wiped with a sterile gauze pad between each injection. As soon as the egg has been injected, the hole in the shell is covered with a small piece of Scotch tape, care being taken not to cover the entire air cell.

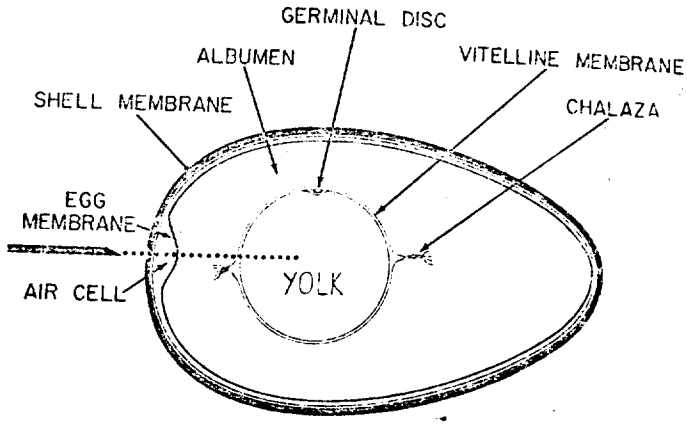

FIG. 1. Diagram of egg and position for injection.

Incubation and hatching. The injected eggs are put into the incubator trays with the large end up; the trays are placed in the incubator,[4] which automatically rotates hourly and is maintained at an optimum temperature of 38°C and a relative humidity of 60%. The eggs are candled on the fifth day of incubation and every day thereafter. Clear eggs and dead embryos are removed for examination. On the seventeenth day of incubation the fertile eggs are transferred to the hatcher[5] and kept at a temperature of 37°C until they hatch.

EVALUATION OF DATA

The injection of the chemical into the egg may produce one of four possible results: (1) the chemical is highly toxic at the level injected, and

[4] Humidaire Incubator Co., New Madison, Ohio; model no. 50, capacity 450 eggs.
[5] Brower Manufacturing Co., Quincy, Illinois.

all the embryos are killed during the first 20 hours of incubation (before the two-somite stage); (2) the chemical is toxic but allows a number of embryos to develop only up to a certain point, and some possibly even to hatch; (3) the chemical has little effect on the hatch; and (4) the chemical has no effect on the hatch or on the posthatch development of the chick ("no effect" level).

If the chemical appears to be highly toxic and all the embryos are killed, the experiment is repeated with smaller doses of the chemical until some hatch is obtained. If the chemical is toxic, but allows the embryos to develop for a longer period of time, dead embryos are examined pathologically and the chicks that do hatch are examined for eye damage, color of the feathers, weight, length of the legs, form of the beak and of the rump, hematologic changes, and condition of the internal organs (liver, kidneys, heart, gall bladder, and spleen).

It is advisable in all cases to observe the chicks for a period of a few weeks in order to detect any delayed effects. Since as much as 30% of the yolk remains at the time of hatching and is absorbed during the first 7 days thereafter, effects of a toxic chemical may first be observed at this time. There may be weight retardation, death during the first week, or the appearance of nerve damage occurring as late as 2–6 weeks after hatching.

The toxicity of a chemical is evaluated mainly from the percentage of hatch at varying dosages of the chemical as compared to noninjected (control) eggs, from a study of the embryonic development of the eggs that fail to hatch, and from a study of the appearance and development of the chicks that do hatch. However, several other factors must also be considered in this evaluation: these are specific gravity, solubility, coagulating effect, pH, and the ionic concentration of the chemical tested.

If the chemical has a high specific gravity, there is the possibility of its settling out in the bottom of the egg and thereby giving a value of apparently low toxicity.

The solubility is quite important since the availability of the chemical for utilization in the chick embryo is partially dependent on its solubility in the egg. However, since egg yolk is an emulsion, solubility problems are somewhat minimized. In the case of insoluble chemicals that are injected as suspensions, it is also necessary to consider particle size in the evaluation of toxicity data.

In order to have a toxic effect, the chemical must come in contact with the embryo either directly or indirectly through the bloodstream. Chemicals such as the lower aliphatic alcohols have a coagulating effect on the pro-

tein, and this coagulation may decrease the availability of the chemical as well as some yolk nutrients, and thereby alter the response of the embryo.

If the pH is highly acidic or basic, a pH effect differing from the true toxic effect of the chemical may be obtained due to interference with the normal acid-base equilibrium in the egg.

The ionic concentration is also important for a similar reason. The observation of increasing toxicity with increasing concentration of a chemical should be interpreted cautiously, since highly concentrated solutions may upset the physical equilibrium of the yolk by causing osmotic effects.

Finally, the introduction of a chemical into the yolk may cause a special type of toxicity because it destroys, alters, or combines with essential nutrients such as vitamins and minerals.

EXPERIMENTAL DATA

Twenty-five thousand eggs have been used in our laboratory during the past three years to test more than 100 chemicals with the following

TABLE 1
NONTOXIC CHEMICALS

Chemical	Solution injected		Per cent hatch
	Concentration	Quantity (ml)	
Water (boiled)	—	0.05	95
Propylene glycol	Undiluted	0.05	95
Corn oil	Undiluted	0.05	90
Peanut oil	Undiluted	0.05	90
Sodium chloride	0.9% in water	0.05	90
	5.0% in water	0.05	70
	5.0% in water	0.10	30
	10.0% in water	0.05	20
Dextrose	5.0% in water	0.05	90

results: (1) nontoxic chemicals injected at an appropriate level allowed the embryo to develop and to hatch as did the controls; (2) toxic chemicals produced effects at dose levels which may be compared to those producing effects in feeding experiments using animals; and (3) this technique often provided toxicologic information which had not been shown by conventional methods.

Table 1 lists the results obtained with some chemicals in common use in food and shows the dosages used and the percentages of hatched chicks.

TABLE 2
CHEMICALS WITH A HIGH ORDER OF TOXICITY

Chemical	Solution injected: Concentration	Solution injected: Quantity (ml)	Per cent hatch	Remarks
Lead acetate	10% in water	0.05	0	—
	2% in water	0.05	0	Hydrocephalus in embryos that failed to hatch
	0.5% in water	0.05	20	—
Mercuric chloride	1% in water	0.05	0	All embryos died at the beginning of incubation
Thiourea	10% in water	0.05	0	Some embryos lived up to 34 days
	5% in water	0.05	20	Hatched on the 28th day of incubation; growth retardation
Triorthocresyl phosphate	Undiluted	0.02	0	Most of the developing embryos died on the 15th day of incubation
	Undiluted	0.01	40	Hatched chicks showed growth retardation and developed paralysis 2–6 weeks after hatching
p,p'-Diaminodiphenylmethane	10% in ethanol	0.05	30	Leg damage; beak deformity (short mandible)
Sodium selenite	0.2% in water	0.05	0	—
	0.1% in water	0.05	20	—
	0.1% in water	0.02	80	—
Dibutyl-tin-dilaurate	Undiluted	0.01	0	Beak deformity
Aroclor 1242 (chlorinated polyphenyls)	Undiluted	0.025	0	—
	Undiluted	0.01	5	Beak deformity (short upper beak); edema; growth retardation

These data, supplemented by an examination of the nonviable eggs and an autopsy of the chicks that hatched, have not indicated any hazard from their use in food. However, it must be pointed out that any chemical, added at a sufficiently high concentration, may have some toxic effect. Since our data and those reported in the literature indicated safety, this phase of the study was not carried beyond a preliminary examination to ascertain that nontoxicity also was shown for these chemicals by the chick embryo technique. This is of theoretical as well as practical importance for the evaluation of any new technique to be used in toxicologic studies.

Table 2 lists our results with several chemicals which have been shown to be highly toxic to animals. In each case there is not only the low percentage of hatch at a low level of the chemical tested, but there are also congenital abnormalities and other responses that raised extremely serious questions as to the safety to the consumers of any food contaminated with these chemicals.

Lead acetate resulted in no hatch at a level of 1 mg per egg. Autopsy of the dead embryos showed extensive brain damage, as has been reported by de Franciscis and Bocalatte (1962) and by Karnofsky and Ridgway (1952).

Mercuric chloride showed no hatch even at a level of 0.5 mg per egg.

Thiourea is a known carcinogen with a basic effect on the thyroid gland. The hatch time was delayed with increasing amounts of this chemical. At a level of 5 mg per egg no chicks hatched and the embryo required 35 days to develop to the stage normally attained at 20 days. At 2.5 mg per egg some chicks hatched, but most of them had to be helped out of the shell. This effect has been reported also by Yushok (1950).

Cavanagh (1954) reported that triorthocresyl phosphate (TOCP), a well-known plasticizer for nonfood use, causes paralysis when fed to adult chickens. We observed this paralysis in some chicks which hatched from eggs injected with 10 mg of the undiluted chemical.

The compound *p,p'*-diaminodiphenylmethane has been reported by Zylberszac (1951) to cause cirrhosis of the liver in rats. We found this chemical to be extremely teratogenic at a level of 5 mg per egg; more than 90% of the chicks had a short mandible and leg damage consisting of a severe bending of the tibia and a general shortening of the bones of the leg.

Sodium selenite proved to be highly toxic. At a level of 0.1 mg per egg, no embryos developed to more than the 5-day stage.

Dibutyl-tin-dilaurate showed no hatch at a level of 10 mg per egg. The majority of the embryos, which did not live more than 15 days at this

TABLE 3

CHEMICALS WITH AN INTERMEDIATE ORDER OF TOXICITY

Chemical	Solution injected: Concentration	Quantity (ml)	Per cent hatch	Remarks
Acetone	Undiluted	0.05	70	
n-Butanol	Undiluted	0.01	95	
		0.02*	70	* At these levels: corneal opacity, cataract, cleft palate, nerve and kidney damage
		0.03*	20	
		0.04	0	
Carbon tetrachloride	Undiluted	0.05	40	
		0.10	0	
Diethylene glycol	Undiluted	0.05	80	
		0.10	55	
Ethyl acetate	Undiluted	0.05	35	
		0.10	15	
Ethyl alcohol	Undiluted	0.05	95	
		0.10	60	
		0.30	0	
Ethylene glycol	Undiluted	0.05	80	
		0.10	65	
Di-2-ethylhexyl phthalate	Undiluted	0.05	95	
Heptachlor	1% in propylene glycol	0.05	75	
		0.10	65	
Hydrochloric acid	4% in water	0.05	80	
Isopropanol	Undiluted	0.05	35	
		0.10	15	
Malathion	Undiluted	0.05	60	Short legs; bleaching effect on chick down
		0.10	0	
Methanol	Undiluted	0.05	90	
		0.10	65	
Styrene	5% in 80% ethanol	0.05	95	

level, had a short and/or flexed mandible; in addition, some embryos showed subcutaneous edema.

Aroclor 1242 gave no hatch at a level of 25 mg per egg. At a level of 10 mg per egg, one chick hatched out of 20 injected eggs, but died 2 days later. Some embryos, which were examined after they died, showed beak deformities (often a short upper beak), edema, and growth retardation.

Table 3 lists preliminary data on the toxicity of some chemicals which have been shown to be toxic in some degree to animals, and which may be found in some processed foods. Included in this group are various solvents, plasticizers, and insecticides. Some of the chemicals on this list require further study, including observation of the chicks until they reach maturity, before they may be classified as to low or high toxicity.

DISCUSSION

The injection of chemicals into the yolk of fertile eggs prior to incubation is a method which can be advantageously used as an element in the evaluation of the safety of food additive chemicals and drugs, and which could be used to screen new products and eventually to correlate their toxicity with that of similar products already tested.

If one considers that a chemical injected into the yolk may be compared to a substance which has the power to cross the placental barrier, this technique, in addition to being an embryonic feeding study, assumes further importance in that it is also a reproduction study. The unfortunate experiences recently suffered with chemicals that have teratogenic effects in humans, and the failure of conventional testing methods to produce this effect in animals, emphasize the urgent necessity for new methods of analysis. Preliminary work that we have done in this area has given satisfactory results (Verrett and McLaughlin, 1963).

Since this represents a system in which the chemical is in direct contact with the embryo throughout development, it is more than likely that any toxic or teratogenic effects would be readily observed. However, there is always the possibility that the chicken will not be a species susceptible to a particular compound, just as it has been shown that the other commonly used species of animals do not respond to all chemicals in a similar manner. It is also possible for the chicken to be more sensitive to a chemical than other species.

Finally, this technique may be applied also to the study of the synergistic effects of chemicals. Results of experiments in our laboratory on

the potentiation of a few pesticides have been very encouraging (Marliac and Mutchler, 1963).

SUMMARY

An evaluation of toxicity by injection of the chemical into the yolk sac of fertile eggs prior to incubation gave the following results:

Water, propylene glycol, corn oil, peanut oil, isotonic saline solution, and isotonic glucose solution showed no toxicity or a very low order of toxicity.

Mercuric chloride, lead acetate, selenium, triorthocresyl phosphate, *p,p'*-diaminodiphenylmethane, thiourea, Aroclor 1242, and dibutyl-tin-dilaurate showed a high order of toxicity and or teratogenic effects at certain levels.

Acetone, methanol, ethanol, *n*-butanol, diethylene glycol, ethylene glycol, isopropanol, di-2-ethylhexyl phthalate, hydrochloric acid, carbon tetrachloride, ethyl acetate, malathion, heptachlor, and styrene showed an intermediate order of toxicity.

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THE INFLUENCE OF RIGID SALT RESTRICTION IN THE DIET OF CHRONIC NEPHRITIS.[1]

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A year ago Dr. F. M. Allen reported his observations upon the influence of salt restriction in the diet of patients with chronic nephritis and so-called essential vascular hypertension. He stated that the measures usually attempted were ineffective because inadequate, and that rigid chloride restriction, as nearly absolute as possible, would achieve genuine benefits. He reported that following such withdrawal of salt from the dietary he has regularly seen a material fall in the blood-pressure. Among other beneficial effects he also saw improvement in the retinitis and other eye changes of nephritis. I recall his interesting speculation that we may in time come to conclude that sodium chloride is the long-sought-for poison of uremia.

It is generally recognized that the excessive use of salt is harmful, and that its restriction in nephritis is to be recommended; but I have not been able by chloride restriction as ordinarily practised to produce results comparable with those of Allen. Following, then, his suggestion that our restriction of the past has not been sufficiently rigid, I have studied in a small number of hypertension patients over a limited period of time the effect of the almost absolute elimination from the diet of all chlorides. Because the patient frequently complained that this diet produced an intense weakness, it was necessary to limit the length of such observations;

[1] Read before the American Society for Clinical Investigation, Atlantic City, May 9, 1921.

Case No.	Diet.	Systolic blood-pressure. Mm.		Blood chemistry.								Urine (24-hr. specimen).						Remarks.
				Urea N. Mgm.		Creatinin N. Mgm.		Sugar. Per cent.		Chlorides. Mgm.		Chlorides. Gm.		Albu min.		Casts.		
		Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	Begin.	End.	
1612	15 days, salt-poor 64 days, salt-free	220	205	18.7	8.5	1.00	2.20	0.11	0.17	.692	.493	12.60	1.27	+	++	+	+	
2654	62 days, salt-poor 16 days, salt-free	190	165	15.0	10.8[1]	1.35[2]	1.87	0.12	0.12[1]	.499	.504[1]	16.05	0.89[1]	+	+	0	+	[1]On 63d day. [2]On 9th day.
2693	9 days, salt-poor 18 days, salt-free	250	220	8.9	19.2[1]	1.10	2.60	0.11	0.10	.491	.453	15.50[2]	1.30[1]	+	+++[1]	0	+[1]	[1]On 21st day. [2]On 9th day.
2891	51 days, salt-free	200	240	5.6[1]	14.8	1.50	1.80[1]	0.11	0.12[1]	.492	.492	..	..	+	++	+	0	[1]On 14th day.
3033	11 days, salt-free	200	240	12.7	13.4	1.40	1.55	0.13	0.17	.549	.511	19.80	?	+	+	+	0	
3048	19 days, salt-poor 38 days, salt-free	218	190	12.4	23.0	0.90	1.64	0.10	0.10	.421	.473	2.79	0.60	++++	++++	+	++	
3059	15 days, salt-free	180	200	5.3	14.5	1.40	1.55	0.11	0.15	.531	.481	?	0.70[1]	++	++	0	+	[1]On 7th day.
3093	13 days, no restriction 10 days, salt-free	198	155[1]	16.6	18.7[1]	2.08	1.90	0.11	..	.550	.450[1]	7.87	1.30	++	++[1]	0	0	[1]At end of s.-free period.
4006	9 days, salt-free	240	270	20.0	30.0	2.70	2.67	0.07	0.09	.481	.506	?	1.47	++++	++++	+++	+	
H.H.	57 days, salt-poor	215[1]	172	13.5[1]	12.8	2.14[1]	1.42	..	0.13	..	.547	4.48[1]	4.38	++++[1]	++++	+[1]	+	[1]On 49th day.

The above table gives data obtained at the beginning and at the end of these observations. The data obtained at various intermediate periods are omitted. "Salt-poor" and "salt-free" periods of diet alternate. Note is made above, however, merely of the total number of "salt-free" and "salt-poor" days.

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and because of the many pitfalls which surround attempts at accurate clinical control of intake and output, the final number of patients upon whom I can report was reduced to ten.

Note was made of the influence of this diet upon the patient's general condition, his blood-pressure, any possible eye changes and certain chemical bodies of his blood. The chloride content of the urine was used as a check upon the accuracy with which the dietary restriction was observed.

Distinction is made in these charts between the salt-poor and the salt-free diets, the latter, of course, being a relative term. With the salt-poor form the patient was given the usual low-calorie nephritis diet with no addition of salt to the food after it reached the table. It is estimated that thus the patient received daily the equivalent of about 2 gms. of sodium chloride. For the salt-free diet the bread and all other foods were cooked without salt and the patient received only such an amount of chlorides as was present in the native foodstuffs. It is estimated that upon this diet the patient received daily the equivalent of less than 1 gm. of sodium chloride. One or two weeks upon the salt-free diet with a variable interim of salt-poor food, and then a second salt-free period, was the rule, although one patient was able to maintain the more rigid diet for a continuous period of sixty-four days.

To summarize: The following results of the salt-free diet were noted.

1. The patient found the food unappetizing and usually ate but little. This factor cannot be disregarded.

2. The blood urea instead of decreasing showed a tendency to increase.

3. The blood chlorides, irrespective of diet, varied but little; while the urine chlorides, reflecting the degree of the patient's adherence to the diet, fell to a very low figure.

4. The systolic pressure, as a rule, showed a moderate fall. This fall was never marked.

5. In two of the patients there developed weakness and prostration to a distressing degree.

6. One of the patients suddenly experienced retinal hemorrhages and other fundus changes at the end of two weeks of this diet.

Conclusion. From these limited observations I would conclude that the almost complete elimination of chlorides from the diet of patients with nephritis and vascular hypertension accomplishes little if anything more than does the salt-poor diet ordinarily prescribed.

EFFECTS OF EXCESSIVE INGESTION OF SODIUM AND POTASSIUM SALTS ON CARBOHYDRATE METABOLISM AND BLOOD PRESSURE IN DIABETIC CHILDREN [1]

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NINE FIGURES

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In a preliminary communication (Thompson and McQuarrie, '34), the finding of an apparently significant influence of excessive salt ingestion on arterial blood pressure and carbohydrate metabolism was reported. The present paper records the results from a more extensive investigation of the subject.

Our interest in the question of a possible interrelationship between salt ingestion and carbohydrate metabolism was first aroused in 1930, when one of our younger diabetic patients, suffering from an abnormal craving for salt, appeared to show some freakish variations in her ability to utilize carbohydrate according to variations in the amounts of sodium chloride which she was allowed to consume. Lack of cooperation on the part of the patient and her premature discharge from the hospital frustrated our first attempts to investigate the problem. A second opportunity presented itself, however, in the autumn of 1933, when one of our clinic patients, a 15-year-old boy with moderately severe diabetes manifested a similar but even more marked craving for salt.

Obviously the first question presenting itself was that regarding the factors responsible for this patient's requiring

[1] Aided by a grant from the Medical Graduate Research Fund of the University of Minnesota.

between 80 and 90 gm. of table salt daily to satisfy his abnormal craving. Since he had been on a relatively high vegetable diet (high in K) for a long time, had partially depleted his reserve of base as a result of ketosis and had vomited an undetermined number of times just before being admitted to the hospital (thereby losing a considerable amount of chloride), this symptom was first tentatively explained on the simple basis of temporarily physiological need for both Na and Cl. That this explanation was entirely inadequate soon became apparent, however, when the craving was observed to continue only slightly abated long after all evidence of ketosis had disappeared and after a simplified diet low in potassium had been substituted for the ordinary diabetic diet. The unexpected discovery, that this patient's blood pressure was elevated temporarily and his glycosuria was decreased as a result of the extra salt ingestion, impressed us as being of sufficient interest to warrant immediate investigation. It appeared probable too that the factors involved in these reactions might incidentally provide an answer to our query concerning the symptom of salt craving.

Following confirmation of our original observation regarding the effects of high NaCl intake, a number of pertinent new questions presented themselves. Among these were the following: 1) Are the observed reactions peculiar to diabetic subjects suffering from an abnormal craving for salt or do other diabetics and even normal persons behave similarly under the same conditions? 2) Are the observed effects due specifically to the Na or the Cl ion alone or to the two combined? 3) Are the two effects (i.e., decrease in glycosuria and increase in blood pressure) mutually dependent or is it possible to obtain one without the other? 4) May the effect on blood pressure be due to increased tonus in the peripheral arterioles, to effects on the kidneys or merely to the mechanical effect of increased blood volume? 5) Is the diminution in glycosuria due merely to retention of sugar with retained water or is it due to improved ability to store or oxidize glucose? 6) If either of the latter, what is the nature of the

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mechanism involved? 7) What nervous or endocrine functions, if any, are involved? 8) Has a high salt intake any influence on the metabolism of protein and fat?

METHODS AND MATERIALS

Repeated studies were carried out on each of four diabetic patients and on one normal subject, all in the age group between 13 and 15 years. Not more than two patients were under observation at one time. At such time they were kept in a small metabolism ward constantly under the supervision of special nurses, who weighed them every 12 hours, collected the urine and feces in special containers and made certain that diets and medications were taken precisely as ordered. Urine was collected quantitatively in 6- or 12-hour periods. The feces for different periods were marked by carmine according to standard technic. Systolic and diastolic blood pressure measurements were made by means of the same mercury sphygmomanometer before meals and after the patient had been in the recumbent position for at least ½ hour. Measurements were frequently checked independently by two observers. The averages of these readings were charted each day. The room temperature was held between 68° and 74°F. and the relative humidity between 40 and 60 per cent to prevent sweating or chilling. The body weight was determined every 12 or 24 hours immediately after the bladder was emptied and before meals or water were given.

The daily diet, which was constant for each patient throughout the entire experimental period, was divided into four identical meals given at 6-hour intervals. For the sake of uniformity and accuracy, it was made up from the following foods: powdered whole milk, egg yolk, egg white, cane sugar, water, unsalted butter, clear lemon juice and a small amount of white bread. The mineral constituents of a typical basic diet, containing protein 64, fat 96, and carbohydrate 132 gm., were as follows; Na, 1.03; K, 1.34; Ca, 0.68; Mg, 0.16; Cl, 1.83; P, 0.93 and S, 0.76 gm. It is seen that this is relatively low in sodium, potassium and choride. The amounts of protein,

fat and carbohydrate were adjusted to meet each patient's caloric and nitrogen requirements. The total water intake, based on the maximum amount taken during periods of high NaCl ingestion, was the same throughout individual experiments. The insulin dosages, in those cases on insulin therapy, were given at 6-hour intervals and were adjusted at levels which allowed some degree of glycosuria in every period of the day. When extra salt was added, it was given in the form of weak solutions and in gelatin capsules, the total for the day being administered along with the diet in four equal parts. Only chemically pure salts were used.

The urinary glucose was determined on the fresh samples in 6- or 12-hour periods by the method of Benedict ('11). The total nitrogen and the various mineral constituents were determined on 24-hour samples. The chemical methods used for the analyses of urine and feces were as follows: total nitrogen, Kjeldahl (1883); sodium, Salit ('32); potassium, Shohl and Bennett ('28); chloride, Volhardt, as modified by Harvey ('10). For blood analyses the methods were as follows: glucose, Folin and Wu ('20); sodium, Butler and Tuthill ('31); potassium, Shohl and Bennett ('28); chloride, Wilson and Ball ('28). The blood volume was determined by the dye method of Keith, Rowntree and Geraghty ('15) as modified by Hooper, Smith, Belt and Whipple ('20). Fasting respiratory quotients were kindly determined for us by Mr. A. J. Beber in the laboratory of Dr. George Burr.

EXPERIMENTAL RESULTS

For the sake of brevity, representative data from the various experiments are here presented by means of one short table and a series of graphic charts.

Table 1 contains the data from a single study on the various effects produced by the ingestion of 60 gm. of NaCl daily for a period of 1 week in the case of L.R., the 15-year-old boy who craved salt. It will be seen that the degree of glycosuria fell far below the control level soon after the extra salt was introduced and remained comparatively low throughout the experi-

mental period. That this change was due to the salt is further evidenced by the fact that glucose output again gradually rose to its previous level during the 4-day-post-saline control period. Approximately 27 gm. of NaCl was retained during the first day of the experimental period, but thereafter the output was practically equal to the intake. The gain of 2.5 kg. in body weight, which accompanied the retention of salt, undoubtedly represented chiefly retention of water. At an assumed Na concentration of 152 milli-equivalents per liter, that

TABLE 1

The effects of ingesting 60 gm. of NaCl daily on L.R. age 15 years. Severe diabetic. Diet contained protein 64, fat 132 and carbohydrate 168 gm. Insulin 32 units daily. No ketosis

DAY	INTAKE (GRAMS)				OUTPUT (GRAMS)					WEIGHT (KILOGRAMS)	BLOOD PRESSURE
	Na	Cl	K	N	Na	Cl	K	N	Glucose		
1	1.57	2.43	1.48	10.36	1.86	2.27	0.86	12.40	73	38.3	116/80
2	25.16	38.84	1.48	10.36	14.26	13.38	0.86	11.10	33	38.2	116/76
3	25.16	38.84	1.48	10.36	21.17	42.23	0.86	7.87	13	40.8	140/88
4	25.16	38.84	1.48	10.36	23.55	38.16	1.40	9.23	14	40.3	150/98
5	25.16	38.84	1.48	10.36	26.56	43.06	0.93	9.63	33	40.3	174/110
6	25.16	38.84	1.48	10.36	24.38	33.58	1.56	9.67	31	40.2	166/108
7	25.16	38.84	1.48	10.36	24.83	40.36	1.40	9.78	26	40.4	164/112
8	25.16	38.84	1.48	10.36	27.28	41.85	1.21	10.87	26	40.4	176/118
9	1.57	2.43	1.48	10.36	4.99	7.88	1.44	8.78	30	40.4	164/98
10	1.57	2.43	1.48	10.36	1.86	2.41	0.82	8.91	49	39.2	130/88
11	1.57	2.43	1.48	10.36	2.57	3.80	1.13	9.47	57	39.1	112/80
12	1.57	2.43	1.48	10.36	1.75	2.24	1.05	10.87	72	38.7	106/74

ordinarily assigned to extracellular body water, the retained water would account for but 22 gm. of the NaCl not excreted by way of the urine and feces, leaving 5 gm. unaccounted for. Since visible sweating did not occur, it is obvious that the concentration of NaCl in the body fluids was increased or that the extra salt was stored in some other form than in aqueous solution. Actual determinations of the Na and Cl in the blood serum a few days before and again a few days after the ingestion of the extra 60 gm. of NaCl per day were as follows: before, Na 303 mg. and Cl 281 mg. per 100 cc. of serum; after:

Na 342 mg. and Cl 342 mg. per 100 cc. The fasting blood sugar values for the corresponding days were 290 and 131 mg. per 100 cc. respectively. Increased concentration of salt in the body fluids of this particular patient, therefore, account in all probability for most of the extra NaCl retained. A comparison of the average per diem excretion of potassium for the experimental period with that for the fore-period and after-period shows an increased output of this element during the period of high NaCl intake.

The total nitrogen excretion was definitely decreased during the periods of high salt ingestion. As may be observed from table 1, the nitrogen balance was negative during the low sodium chloride period when glycosuria was most marked, but was positive during the high salt period. This protein-sparing effect has been observed repeatedly in the series of experiments thus far completed. It is an additional proof of improved carbohydrate utilization.

The systolic and diastolic blood pressure levels both rose well above the upper limits of normal after the second day of high salt ingestion and remained elevated until the second day following withdrawal of the salt. The systolic level was increased approximately 40 per cent while the diastolic rose about 30 per cent. All of the changes recorded showed a period of lag at the beginning and at the end of the experiment, accompanying respectively the initial retention and then the gradual excretion of extra salt. The patient complained of headache on several occasions when his systolic pressure rose above 165 mm. of mercury.

Certain of the data from the foregoing experiment are presented graphically in figure 1, which is largely self-explanatory. In addition, the chart shows the ineffectiveness of adrenal cortical extract (Eschatin P.D.) as a substitute for NaCl in this relationship, when given in average-sized doses. Obviously this is not a crucial or altogether satisfactory test of this point because maximum doses were not given.

Since it at first appeared possible that the response to high sodium chloride ingestion just described might be peculiar to

patients suffering from abnormal salt craving, similar studies were made on other experimental subjects both diabetic and non-diabetic, who manifested little or no such craving.

Data are graphically presented in figure 2, which demonstrate clearly that other diabetic subjects than those marked

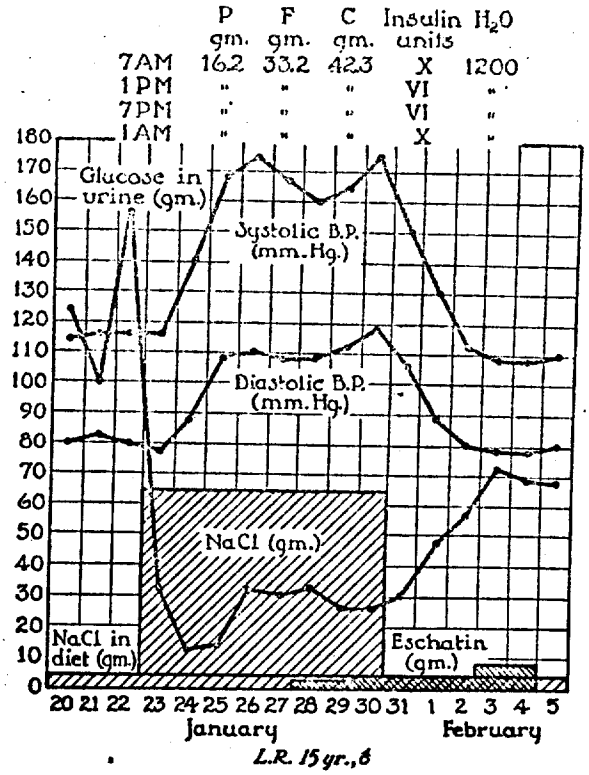

Fig. 1 Effects of sodium chloride on glycosuria and blood pressure. Non-effect of extract of adrenal cortex. L.R. severe diabetes.

by excessive salt craving respond to a sustained high intake of NaCl. It will be seen that the amount of glucose excreted per day by patient H.E. during the fore-period, namely between 40 and 70 gm. fell to between 10 and 20 gm. daily when the NaCl intake was increased from 4 to 48 gm. per day. At

the same time the systolic blood pressure rose from a basic level varying between 108 and 116 mm. of mercury to a new level ranging between 140 and 155 mm., while the diastolic level rose from between 74 and 85 to between 100 and 110 mm. of mercury. Soon after the extra sodium chloride was with-

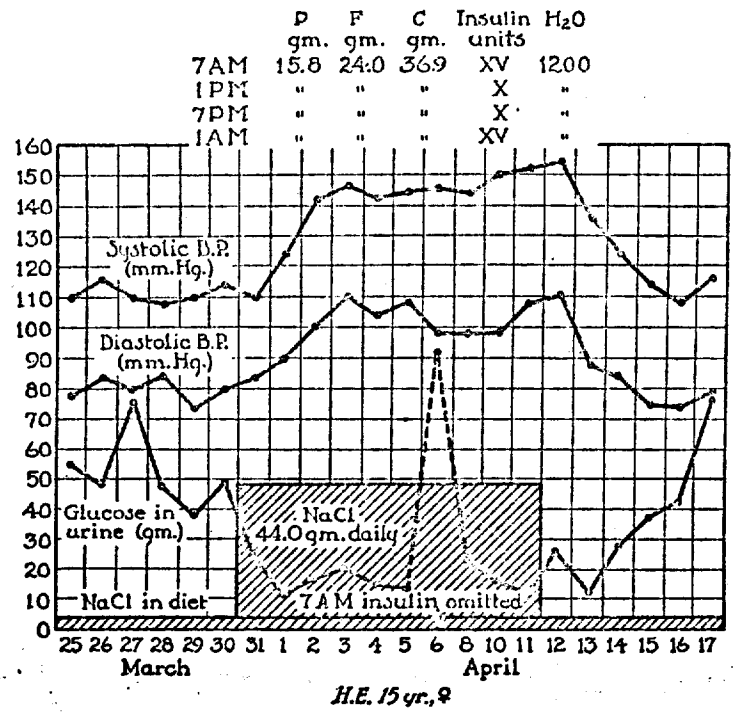

Fig. 2 Effects of sodium chloride ingestion on glycosuria and blood pressure. Dotted line shows effect on glycosuria of omitting insulin for 12 hours. H.E. severe diabetes.

drawn, the previous levels of glycosuria and of blood pressure were resumed.

An additional point of interest is shown in this figure, namely, that withholding of insulin for 12 hours from this moderately severe diabetic subject caused a prompt and marked increase in the degree of glycosuria without a corre-

sponding fall in the blood pressure. The effect of this withdrawal on the glycosuria curve is represented by the interrupted line.

To determine whether or not other than diabetic subjects react to overfeeding of NaCl in the foregoing manner, a single control study was made on a healthy, 14-year-old boy, L.K., who at the time of the experiment was in the late stages

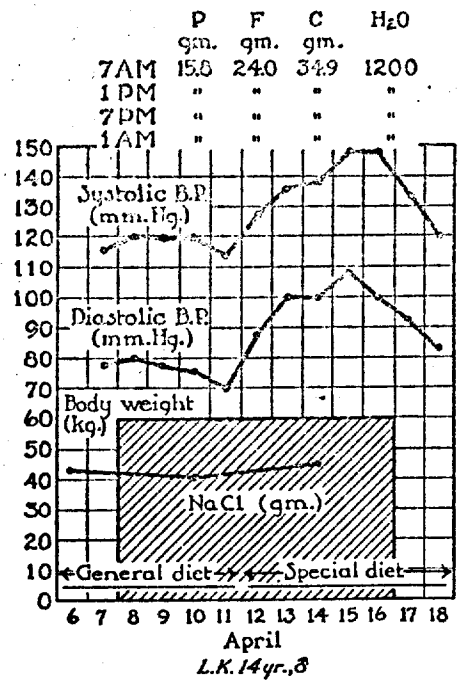

Fig. 3 Response of blood pressure to high-sodium, low-potassium intake in normal subject, L.K.

of convalescence from an operation for club foot. Since he did not have glycosuria or any disturbance of his carbohydrate metabolism, this phase of the problem could not be investigated by means of the technic employed here. It may simply be noted that he did not show any signs of hypoglycemia at the height of the salt effect. The effect on blood pressure, however, was very definitely like that of the diabetic patients.

Reference to figure 3 shows that he had no rise in either his systolic or diastolic pressure from the ingestion of 60 gm. of NaCl daily during the first 5 days while he was on the ordinary hospital diet. However, when he was given the same diet as that taken by patient H.E., namely, the simplified diet, which

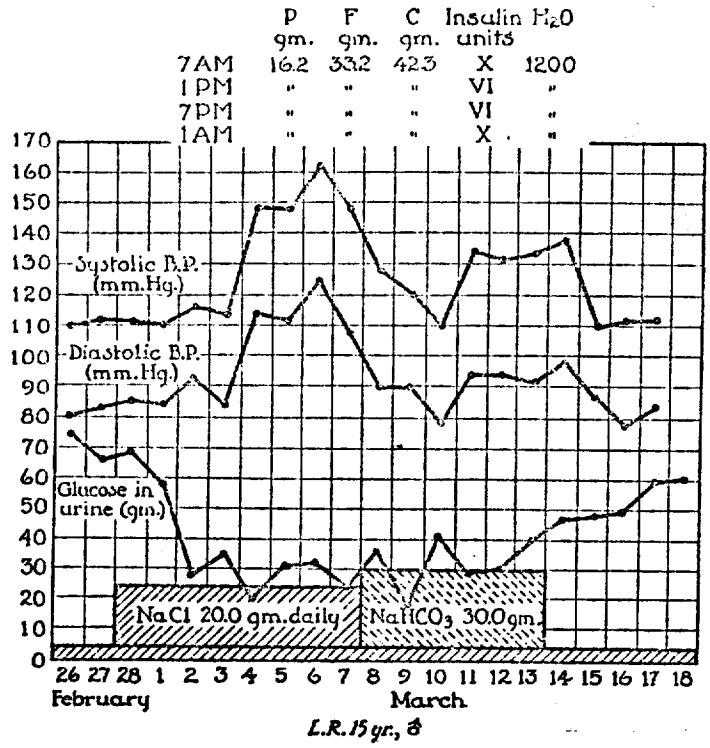

Fig. 4 Effects of sodium bicarbonate on glycosuria and blood pressure. L.R. severe diabetes.

was comparatively very low in K, his systolic pressure rose to between 130 and 150 mm. of mercury, while his diastolic level rose to between 90 and 108 mm. of mercury. These values gradually returned to normal following withdrawal of the extra salt.

The next objective was to determine which of the two ions, Na^+ or Cl^-, is primarily responsible for the reactions observed. The following four charts present data pertaining to this phase of the study.

An attempt to evaluate the specific effect of Na is represented by the data graphically recorded on figure 4. After eliciting the typical reaction to sodium chloride in our most

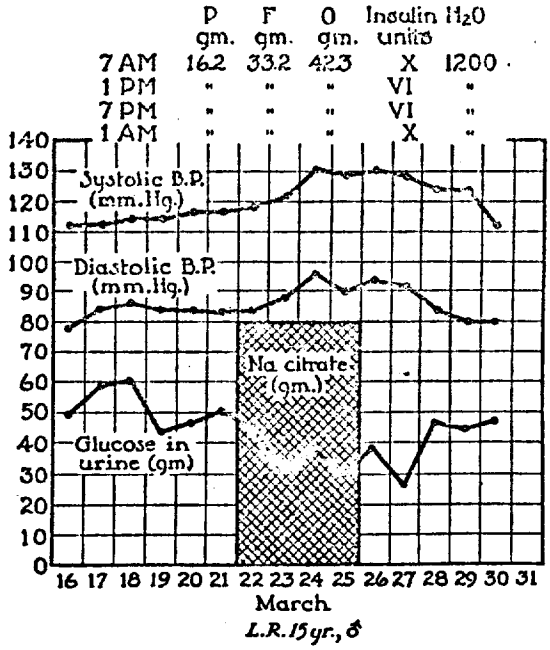

Fig. 5 Effects of sodium citrate on glycosuria and blood pressure.

responsive subject, L.R. (who manifested a craving for salt) with an extra salt intake of but 20 gm. daily, an equivalent amount of Na was substituted in the form of sodium bicarbonate. The object in giving a smaller amount of salt in this experiment than that used in the previous tests was that of avoiding, if possible, a drastic displacement of the acid-base equilibrium of the body fluids toward the alkaline side, which in itself may influence carbohydrate metabolism (Murlin and

Kramer, '16). It is seen that 20 gm. of extra NaCl daily finally resulted in a moderate decrease in the degree of glycosuria and an accompanying elevation of blood pressure after a certain amount of the salt had been retained. When the sodium bicarbonate was substituted, the effects produced by the NaCl were sustained but somewhat less efficiently. Withdrawal of the bicarbonate then resulted in a return of the values for the glycosuria and the blood pressure readings toward their original control levels.

In the next experiment (fig. 5) another sodium salt, the citrate, was administered in daily amounts approximately equivalent to 40 gm. of NaCl. It is evident from the course of the urinary sugar curve and that of the curves representing changes in the blood pressure that the effects of this salt are similar to those of NaCl though less marked. In agreement with the preceding, this experiment appears to indicate that sodium is the element which is responsible in large part for the effects observed.

The next step was to determine the effect of the chloride ion when given with some basic ion other than sodium. The potassium salt was employed because it occurs naturally in the animal body and because, like NaCl, its effects on the acid-base equilibrium are minimal. This salt was found to exert somewhat surprising effects as shown in the two following figures.

Inspection of figure 6 reveals the fact that KCl, when ingested in much smaller amounts, had effects on both the blood pressure and glycosuria of a third diabetic child, J. P., which were diametrically opposite those of NaCl. During the first 4 days shown on the figure, when she was receiving 44 gm. of extra NaCl daily, the urinary glucose varied between 20 and 30 gm. per day and the blood pressure varied between 132/98 and 150/110. Shortly after the extra NaCl was omitted, the glucose excretion rose to between 60 and 70 gm. per day, while the blood pressure fell to a level averaging around 110/78, where it remained throughout the next week. Potassium chloride was then given in gradually increasing doses over the

next 6 days, reaching a maximum of 20 gm. per day for the last 2 days. Apparently as a direct result of this, the glucose excretion increased to new levels varying from 70 to 100 gm. daily, whereas the blood pressure fell slightly below the basic level to between 86/60 and 95/62. Discontinuance of the KCl was followed within 36 hours by a return of all values to the control levels.

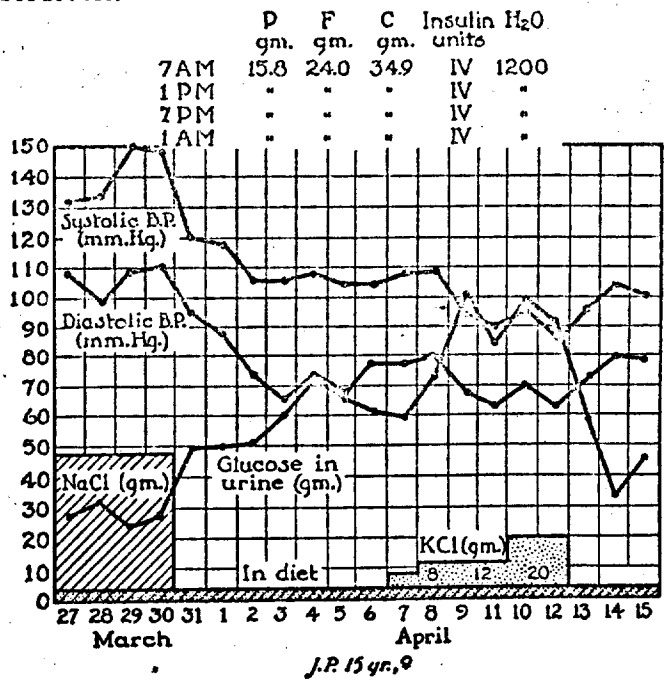

Fig. 6 Comparison of effects of potassium chloride with those of sodium chloride on blood pressure and glycosuria. J. P. moderately severe diabetes.

It was next attempted to ascertain whether or not Na and K exert antagonistic effects when given at the same time and, if so, in what equivalent proportions the one is capable of neutralizing the influence of the other.

Under basic conditions similar to those already described, the patient H.E. was given 40 gm. of NaCl daily over a period of 19 days. Without any other modification of the regimen,

KCl was given as follows: 12 gm. on the eleventh day, 16 gm. on the twelfth and thirteenth days and 4 gm. on the fourteenth day, after which it was withdrawn. The effects of the latter procedure are shown graphically in figure 7. It is seen that 16 gm. of KCl more than neutralized the effects of 40 gm. of NaCl. Since the potassium in 16 gm. of KCl is chemically equivalent to the sodium in but 12.6 gm. of NaCl, it would appear from the present experiment that 1 milli-equivalent of K

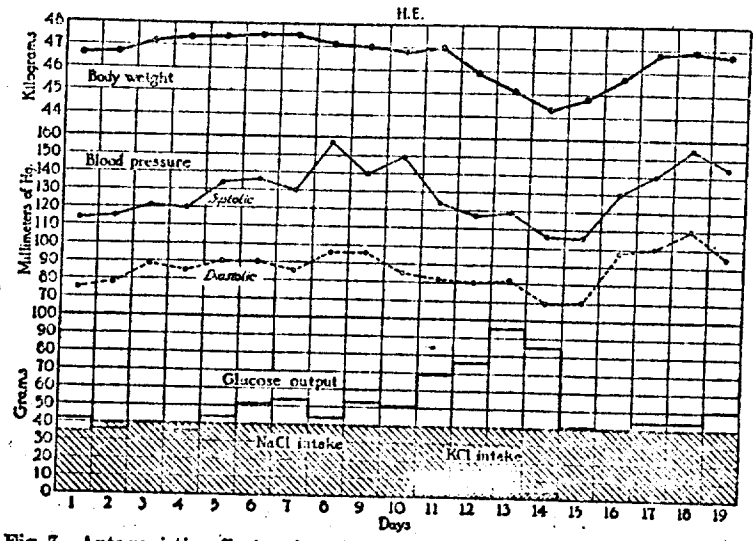

Fig. 7 Antagonistic effects of sodium and potassium on glycosuria and blood pressure. H. E. severe diabetes.

is capable of antagonizing the effect of at least 3 milli-equivalents of Na, as far as the carbohydrate metabolism and blood pressure are concerned. The difference in the effects of the two salts on the water balance of the body should be noted. It is obvious that the sodium ion is chiefly responsible for the retention of water as it is for the decrease in glycosuria and the increase in blood pressure. The decrease in glucose output as a result of NaCl ingestion was far less striking in this than in the previous experiment on H.E. (fig. 2), apparently because she was suffering from a mild upper respiratory infection during the first week of the present experiment.

The studies presented thus far, with the exception of that on one normal subject, were all carried out on severely diabetic patients who required insulin therapy. In order to determine the specific effects of the various salts without this complicating factor, the next experiment was carried out on D.R., a 14-year-old girl, whose diabetic state could be controlled fairly satisfactorily by dietary measures alone. After a pre-

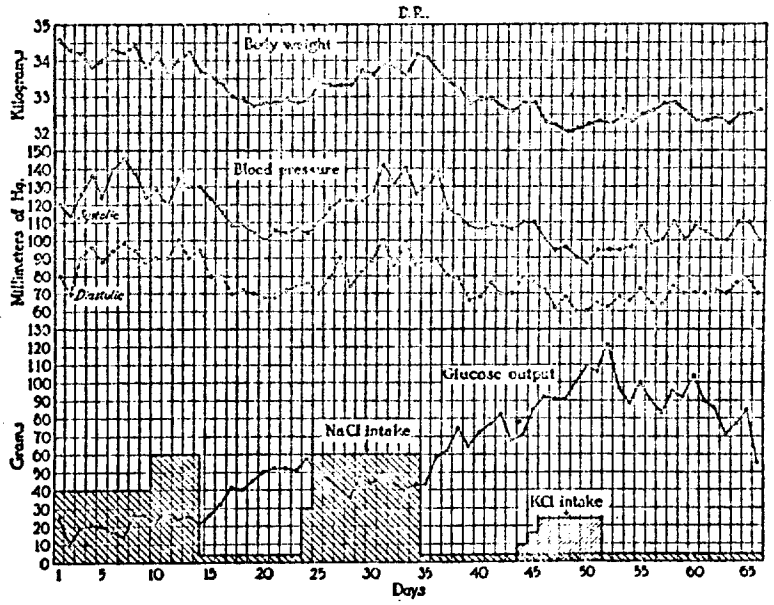

Fig. 8 Effects of sodium chloride and of potassium chloride on glycosuria and blood pressure in diabetic patient not on insulin therapy.

liminary test period, her diet was adjusted at such a level that between 50 and 75 gm. of glucose appeared in the urine daily during control periods. She was then given the salts as indicated in figure 8.

The data recorded on this figure demonstrate that the antagonistic effects of large amounts of NaCl and of KCl were qualitatively the same in this diabetic patient, who was not under the insulin therapy, as they were in the preceding cases.

The fasting serum potassium in the case of this patient was significantly lower during the period of high NaCl intake than during the control periods, the values being 27.2 mg. per 100 cc. before, 12.5 mg. during and 19.6 mg. 1 week after the high-salt period.

The next project undertaken was that of determining the effect of NaCl ingestion on the level of the blood sugar. A

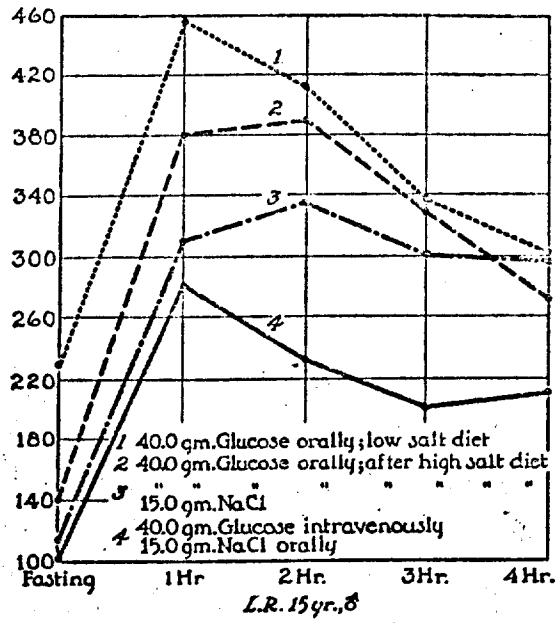

Fig. 9 Effects of sodium chloride on fasting blood sugar and on sugar tolerance curves.

series of sugar tolerance tests were made on patient L.R. under a variety of conditions as indicated on figure 9. It will be observed that all curves obtained were of the diabetic type, the ingestion of salt having no demonstrable beneficial effect under the emergency conditions of such a test. The only difference in the blood sugar findings during the control period and during the periods of high salt feeding was a tendency for the fasting values to be distinctly lower

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under the latter circumstances. Nevertheless, this latter finding indicates a definite increase in the ability to utilize carbohydrates.

Additional evidence that the metabolism of carbohydrate is significantly improved by the excessive ingestion of NaCl is found in the fact that the fasting respiratory quotient was found to be slightly higher during the period of high intake than during the period of low intake in the few instances in which this factor was determined. For example, patient H.E. showed an R.Q. of 0.763 on the high salt regimen and 0.714 on the low salt, while corresponding values for L.R. were 0.747 and 0.712. Ketosis was found to develop earlier in the severely diabetic subjects after withdrawal of insulin during the period of low NaCl intake than during the periods of high intake. On several occasions patients, whose insulin dosages were sufficiently large to prevent marked hyperglycemia but not sufficient to cause hypoglycemic reactions during periods of very low NaCl intake, were found to experience typical insulin reactions when from 40 to 60 gm. of this salt were given daily.

Whether or not simultaneous administration of the potassium will prevent the latter reactions has not as yet been determined. In one extremely severe adult case of spontaneous hypoglycemia associated with acromegaly, however, we observed a steady but temporary rise in the blood sugar with partial relief of symptoms following the administration of between 24 and 48 gm. of KCl daily. Much to our surprise, no toxic symptoms were observed in the case of this man, although he finally refused to take the larger doses because of gastric discomfort. In less severe cases of spontaneous hypoglycemia, a low-sodium, high-potassium regimen might prove to be beneficial.

DISCUSSION

The foregoing data afford ample proof that the daily ingestion of sodium chloride in large amounts causes an elevation in the blood pressure and at the same time increases the ability of the diabetic subject to utilize carbohydrate. These effects are not observed if the diet is high in potassium. The effect of potassium chloride was to lower blood pressure and increase glycosuria.

A satisfactory explanation for these effects is not apparent at the present time. The effects referred to are not obtained with the small amounts of salt and water required to prevent depletion of the body's reserves. The reports of Atchley and others ('33) and Peters, Kydd, Eisenman and Hald ('33) and their co-workers have adequately described the disturbances in water and electrolyte balances which develop in uncontrolled diabetes mellitus. Kydd ('33) has emphasized the depletion of chloride in excess of other electrolytes, as if the base lost with the ketone acids might be derived from NaCl rather than from bicarbonate. He stresses the need for replacement of chloride during recovery from diabetic coma. However, in the present studies the dietary and other control factors were purposely adjusted at levels such that ketosis was extremely slight if present at all.

Since the fluid balance of the body is modified to a considerable degree and in opposite direction by the ingestion of large amounts of sodium and potassium salts, this factor must be taken into account. The decrease in glycosuria due to NaCl ingestion followed the retention of water, whereas the increase in glycosuria from potassium administration was accompanied by a mild diuresis. This observation is in keeping with the finding of Schiff and Choremis ('26) that a normal or increased hydration in the tissues favors carbohydrate utilization. It is contrary to the view of Andrews ('26) that dehydration enhances insulin action. That the glucose retained is not merely held in solution in the retained water is easily shown by comparing the small quantity so held with the total amount seen to be retained over the period of a week

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(table 1). Assuming the concentration of glucose in the retained water to equal that of the blood plasma (e.g., 150 mg. per 100 cc.), the 2½ kg. of retained water would contain less than 4 gm., whereas in the course of 1 week over 300 gm. were actually retained. In those cases in which insulin therapy was employed, it might be assumed that the beneficial effect of water and salt retention in the extracellular spaces is due to the slower release of subcutaneously injected insulin. This explanation could not hold, however, in diabetics not on insulin therapy. Various data are presented which indicate that glucose is more efficiently oxidized and probably more readily stored as glycogen when large amounts of NaCl are ingested. The protein-sparing effect, fasting respiratory quotient measurements and lessened tendency to development of ketosis, as well as the decrease in glycosuria and hyperglycemia, all point in this direction.

Reference to the literature of the pre-insulin era reveals the fact that other observers obtained similar results from feeding large amounts of sodium salts. For example, Murlin and Kramer ('16) found that sodium hydroxide and carbonate increased in utilization of glucose in diabetic dogs while Murlin and Craver ('16) and Underhill ('17) observed marked decreases in glycosuria in diabetic patients following the administration of alkali. These workers attributed the effects observed to changes in the acid-base equilibrium. Beard ('18) found little or no effect from the giving of sodium bicarbonate, but observed that two of his diabetic patients, who voluntarily took increased amounts of sodium chloride and water, experienced increases in their carbohydrate tolerance. No reference is made in these papers to potassium or to the effect on blood pressure.

More recent studies on the effect of acid-base balance on carbohydrate metabolism are somewhat contradictory. Thompson, Mitchell and Kolb ('33) found in normal subjects that acid administration was followed by diminution of carbohydrate tolerance as indicated by blood sugar curves, while alkalosis had little effect. In a series of short-time experiments on normal children, Johnston and Maroney ('35) more

recently found the oxidation of dextrose to be accelerated on the acid side of neutrality and inhibited on the alkaline side. They also found the oxidation of dextrose to be depressed by the ingestion of sodium chloride and water during the short time of their retention in the body, but found it to be accelerated when the salt and water were released. Of course, these acute experiments on normal subjects cannot be compared directly with our prolonged experiments on diabetic patients. It is unlikely that the neutral NaCl or KCl, as given in our longer experiments, caused any important shift in the acid-base equilibrium.

The somewhat surprising discovery, that potassium exerts effects directly antagonistic to those of sodium, suggests the possibility that the beneficial influence of high sodium intake on carbohydrate metabolism in some way is dependent upon this interrelationship. It has been reported that serum potassium is characteristically elevated in diabetes mellitus. Rathery and Bertoliatti ('34) for instance, found values averaging 35 per cent above normal in seven cases of diabetes not on insulin therapy and 22 per cent above normal in seven insulin-treated cases. Harrop and Benedict ('24) found potassium as well as inorganic phophorus and glucose of blood to be reduced by insulin in normal and diabetic subjects. Briggs and co-workers ('23) found a decrease of 24 per cent in the serum potassium in fasting dogs following the administration of insulin in large doses. Harrop and Benedict suggest that the potassium may enter into the intermediary metabolism of carbohydrate in association with the phosphorus-carbohydrate combination. The few potassium determinations made thus far in our studies indicate that high NaCl ingestion results in a significant decrease in serum K, similar to that occurring after institution of insulin therapy. This phase of the problem is still under investigation. One case is cited above in which severe spontaneous hypoglycemia appears to have been temporarily controlled by administration of KCl in large doses.

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The mechanism by which sodium salts result in an increase and potassium salts in a decrease in arterial pressure is unknown. Purely mechanical factors, such as a possible change in plasma volume and change in resistance to blood flow due to accumulation or depletion of fluid in the interstitial fluid reservoir of the body after excessive ingestion of the various salts, may play some role. Measurements of blood volume were attempted in a few instances but the results were inconclusive. Some specific effects either direct or indirect on the arterioles or the kidneys are more likely the important factors. While the observations of McLester ('22), Mosenthal and Short ('23) and others failed to confirm the claims of Allen ('22) that a 'salt free' diet results in a lowering of blood pressure in arterial hypertension, the potassium content of the diets used by the different workers may have differed sufficiently to account for their obtaining conflicting results. We are attempting at the present time to determine the effect of low-sodium, high-potassium diets on young subjects with hypertension.[2]

The possible involvement of certain nervous and endocrine factors are also being investigated by means of animal experiments. Because of the relationships of pituitary and adrenal functions to blood pressure and to the water, mineral and carbohydrate metabolism these glands are receiving first attention.

The cause of marked salt craving in two of our diabetic patients remains obscure. An underlying disturbance in the potassium metabolism was suggested by a violent, anaphylactic-like reaction experienced by L.R. when given potassium chloride in much smaller dosage than that taken without any ill effect whatsoever by other subjects studied.

Studies on the blood changes and on the mineral balances will be presented in subsequent reports.

[2] After the present paper was set up for printing, the authors discovered a paper by W. L. T. Addison (Can. Med. Assoc. J., 1928, vol. 18, p. 281) describing effects of Na and K salts on the blood pressure of adult patients with arterial hypertension, which were similar to those recorded here for non-hypertensive children.

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SUMMARY

1. The effects of ingesting excessive quantities of sodium and potassium salts on the blood pressure and carbohydrate metabolism have been studied in one non-diabetic and four diabetic children. Repeated tests were made in the case of each diabetic subject. While the most striking effects were observed in the case of one particular diabetic, who consistently required between 60 and 90 gm. of NaCl daily to satisfy his craving for salt, all patients submitted to the tests responded in like manner.

2. The daily ingestion of between 1 and 2 gm. of NaCl per kilogram of body weight resulted within a period of from 2 to 4 days in a gain of from 4 to 5 per cent in body weight and an increase in both systolic and diastolic blood pressure to new plateaus between 30 and 50 per cent above the control levels. These higher pressures were maintained so long as the salt was taken.

3. In addition to this effect on blood pressure, the excessive ingestion of NaCl was found to cause a marked reduction in the degree of glycosuria in the diabetic subjects studied. The effects were qualitatively the same in one mildly diabetic patient not given insulin as in those patients who received insulin at regular 6-hour intervals.

4. The fasting blood sugar was found to range at lower levels after a few days of the high NaCl ingestion than during the foreperiod, the patient's food intake, insulin dosage and activity remaining unchanged. The fasting R.Q. was slightly higher during the period of high NaCl intake than previously. The shape of the sugar tolerance curve, however, was not measurably influenced by salt ingestion.

5. In severe diabetes, ketonuria appeared earlier after withdrawal of insulin when the NaCl intake was low than when it was very high.

6. With a constant protein intake estimated to maintain nitrogen balance under ordinary conditions the nitrogen output exceeded the intake during periods of low NaCl intake. During periods of high NaCl intake, however, the nitrogen balance became positive.

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7. Typical insulin reactions were found to occur at times during the period of high NaCl intake in patients receiving insulin in dosages found during the low-salt periods to be sufficient to completely prevent glycosuria.

8. Sodium bicarbonate and sodium citrate when given in amounts with equivalent sodium values had similar though less marked effects than those of NaCl.

9. The maximum effects of NaCl on both the B.P. and the carbohydrate metabolism were obtained only when the patients were given simplified diets which were low in potassium. Ordinary diets, high in potassium, either prevented or greatly lessened the effects of the NaCl.

10. Potassium chloride, when given in doses of 10 to 20 gm. daily along with a simplified diet low in sodium, resulted in a slight fall in both systolic and diastolic blood pressure and at the same time a significant increase in the degree of glycosuria, thus exerting effects diametrically opposite those of the sodium salt.

11. When the two salts were given simultaneously one part of potassium was found to completely abolish the effects of at least three chemically equivalent parts of sodium.

12. A small number of serum potassium determinations have been made. The high NaCl intake depresses the serum potassium.

CONCLUSIONS

1. When ingested in amounts varying between 1 and 2 gm. per kilogram of body weight daily, sodium chloride exerts a favorable influence on the carbohydrate metabolism of diabetic children taking simplified diets low in potassium. This effect is observable usually on the second or third day.

2. At the same time both the systolic and diastolic blood pressure levels are elevated significantly.

3. Sodium appears to be chiefly responsible for these effects since other salts of this element as well as the chloride exert similar, though less marked, effects.

4. Potassium chloride has diametrically opposite effects on both glycosuria and blood pressure.

5. In terms of chemical equivalents, potassium completely antagonizes the effects of sodium when given simultaneously in amounts as little as one-third that of sodium.

6. The physiological mechanisms involved in these reactions are at present obscure but are being further investigated.

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GEORGE R. MENEELY

Toxic Effects of Dietary Sodium Chloride and the Protective Effect of Potassium

Sodium chloride is naturally present in nearly all living things and, therefore, has always been a component of food. By the beginning of recorded history, the human use of extra sodium chloride added to the diet as a condiment was well established. How it came to be so is entirely speculative. There is some evidence that use of salt is habit forming in the same sense that tobacco is, but there certainly is no doubt that people accustomed to its use have fought wars to possess a source of it, and numberless men have risked death to smuggle it. There are parts of the ancient world where superficial excrescences of it occur naturally, and the process of obtaining it by evaporation of saline waters is only a procedure copied by man from nature in areas where it occurs spontaneously on the shores of dead seas. The antiseptic properties of brine must have been noticed early by man. The use of salt to preserve foods that otherwise would decay seems a reasonable pathway whereby early man may have developed his taste for it.

The elemental composition of sodium chloride was learned at the turn of the nineteenth century. Scheele discovered chlorine in 1774 but thought it contained oxygen. Davy named it in 1810, recognizing that it was an element. He had isolated sodium by electrolysis in 1807. By the middle of the nineteenth century, there were reports of disturbances of sodium balance in disease states, especially in cholera in which depletion of sodium is a striking feature. Physiologists were fascinated by sodium chloride in the latter half of the nineteenth century, and extensive speculations on the relation of the salt content of the herbivorous diet compared with the diet of the carnivores led to the

theory, attributed to von Bunge, that herbivorous animals are compelled to go to salt licks to obtain extra sodium chloride to balance the high intake of potassium characteristic of herbivorous diet. It had been established by 1840 that the sodium chloride content of the natural herbivorous and carnivorous diets is the same and that the difference lies in the much higher potassium of the former. This established the error of the earlier theory that herbivores got their salt from salt licks and the carnivores got theirs from the herbivores. The concept that sodium chloride is required to protect against high dietary potassium seems quite fallacious now. Indeed, the reverse is probably the case. As will be seen in the discussion of potassium in the diet, there is good animal and human experimental evidence that extra potassium exerts a protective effect on the toxic effects of excess sodium chloride ingestion.

Sodium is widespread in nature. It is the most abundant of the alkali elements and the seventh element in amount in the crust of the earth. It is highly reactive and never found free. In living organisms, it occurs in manifold combinations, and it is erroneous to think of it as occurring always as the chloride, although frequently it is so expressed in chemical analyses. Sodium enters the body in five ways: first, there is sodium in nearly all drinking water, quite a lot in certain areas, and, where water softeners are used, two atoms of sodium are added for every atom of calcium or magnesium exchanged. If the water supply contains as much as 25 mg of sodium per 100 ml, and if an intake of water in food and drink is taken to be 2 liters, the sodium intake will be as high as 500 mg per day. In Galveston, Texas, the municipal water supply contains 34 mg per 100 ml, and in Crandal, Texas, it contains 170 mg per 100 ml. The latter is rarely drunk but it is used for cooking. The high sodium content of Galveston drinking water is well known to the medical profession as a problem in patients with congestive heart failure.

Second, sodium occurs naturally in all foods, although in many the content is very low; for example, fresh peas contain only 0.9 mg of sodium per 100 g.

The third pathway is via food processing, and a great deal of sodium, usually in the form of sodium chloride, may be added. For example, canned peas, even with the liquor poured off, contain 230 mg of sodium per 100 g. Expressed as sodium chloride this is 580 mg. Even the conventional freezing process uses sodium chloride so that frozen peas contain 100 mg per 100 g. A point worth noting (see also the discussion of potassium) is that, at the same time the sodium is added, the potassium is depleted. Fresh peas contain 380 mg of potassium per 100 g,

while in canned peas drained of the liquor the potassium is reduced to 180 mg, and in frozen peas it is reduced to 160 mg.

The fourth pathway is addition of salt in the cooking process within the home. It is highly variable from household to household, as is the matter of the disposition of the cooking water. It appears probable that the old custom of saving all or almost all cooking waters as *pot-au-feu* or pot liquor is vanishing in the United States. Generally, in the preparation of vegetables, salted water is brought to a boil and raw or frozen vegetables added. At the end of the cooking period, the vegetables are drained, the cooking water being discarded. The net effect is a further addition of sodium and a further depletion of potassium.

The fifth pathway is addition of common salt to food at the table. Again there is great individual variation, dependent, apparently, upon individual taste for salt. Dahl observed that patients on diets containing up to 250 mg of salt per day immediately noticed the addition of 1 g per day, whereas among subject eating 10 to 20 g per day the addition of 5 or even 10 g would go unnoticed. On the other hand, Dahl found it easy to alter individual taste for salt, much easier than for some other well-recognized tastes.

There are only a limited number of studies on the human requirement for sodium. It has long been recognized as essential to life. Estimates of daily requirement have ranged as high as 15 g (as sodium chloride) per day, but clearly 5 g is ample and 1 g per day is more probably correct. Many studies have shown that humans with normal kidney function maintain salt balance easily on daily intakes below 1 g.

That sodium chloride might have harmful effects if eaten in excess was suspected long before any objective evidence on the subject existed. Widespread use and familiarity with salt led most, however, to the assumption that it was a harmless substance. The earliest association of salt with human hypertension is thought by Chapman, in the Bell Symposium, to be the work of Ambard and Beaujard in 1904. Benefit in a disease state by restriction of an element in the diet does not, of course, establish it as having a causal relation to the disease. Therapeutic restriction of salt fell in and out of favor throughout the first four decades of this century, but the merit of salt restriction in controlling edema in congestive heart failure was certainly firmly established by the fourth decade. The association with hypertension proved more subtle. The effect of salt restriction in human hypertension, however, is now very well documented. In general, patients so treated fall into one of three categories: some experience a return of the blood pressure to normal levels, some experience a reduction in blood pressure but not to normal,

and some show no fall in blood pressure at all. Again, speaking generally, the longer the duration of the hypertension and the more severe its manifestations, the less likely is a completely favorable response. The success of the natriuretic agents in controlling hypertension further documents the association of human hypertension and dietary sodium chloride.

It has not proved easy to demonstrate a causal relation between sodium chloride intake and human hypertension. Suspicion of such a relationship was strong by 1951 when Braun-Menendez commented, at the Bell Symposium in Minneapolis, that, whatever the experimental method for producing hypertension, increased salt intake facilitated its obtainment and salt restriction prevented it. Selye, Hall, and Rowley and Knowlton, Loeb, Stoerk, and Seegal found hypertension produced by desoxycorticosterone acetate with the addition of extra sodium chloride. With hypertonic saline as the sole source of liquid, Lenel, Katz, and Rodbard produced hypertension in the chicken; Sapirstein, Brandt, and Drury in the rat; and Fukuda in the rabbit.

In 1951, the author and his colleagues began experiments that showed that chronic ingestion of excess sodium chloride as the sole variable in an otherwise wholesome diet reliably produced hypertension in the rat that appeared to duplicate human hypertension clinically and morphologically.

Various levels of sodium chloride were investigated, but attention focused finally on three ranges as most revealing and most realistically related to the human problem. The first range, the "control" level, is from 0.15 to 2.0 percent. Over this range only minor differences in growth rate were found, and animals eating such diets never became hypertensive. The second range of interest is 2.8 to 5.6 percent, which represents a moderate excess of sodium chloride. Finally, 7.0 to 9.8 percent sodium chloride in the diet represented a high level of excess salt. It may seem that these levels are out of the range of human consumption, but this is not the case. These levels may be compared on a percentage of nutriment basis: 2.8 percent sodium chloride in the purified rat diet corresponds to 14 g of sodium chloride daily in an ordinary diet for man. The equivalent human intake in grams per day may be obtained by multiplying the rat diet percent composition by 5. Thus the control (0.15 to 2.0 percent) level corresponded roughly to 0.75 to 10 g of NaCl daily for man. The "moderate" excess range (2.8 to 5.6 percent) corresponded to 14 to 28 g per day and was frankly hypertensigenic and life-shortening. Finally, the "high" level of excess salt (7.0 to 9.8 percent), equivalent to 35 to 49 g daily, has its counter-

part in man in only a few parts of the world, most notably northern Japan. It was drastically hypertensigenic in the rat and it is drastically hypertensigenic in the Japanese.

At all levels of salt feeding the rats evidenced growth and good development, but at levels above the control there were successive decrements in growth. These persisted throughout the life of the animal. The water consumption (and urine production) was proportional to the dietary sodium chloride content. This association of water drinking, urine formation, and salt intake is well known, the earliest observation being recorded in Pliny's *Natural History*. It was, for example, commented on by Thoreau who wrote in Walden: "Finally, as for salt, that grossest of groceries . . . if I did without it altogether, I should probably drink the less water."

Consistent and reproducible blood pressure observations were obtained in early experiments about the ninth month, at which time hypertension was well established in animals eating above the control levels of salt. It is of considerable interest to note that, although the mean blood pressure of each group was successively higher and essentially proportional to the sodium chloride content of the diet, there was a very considerable scatter of individual values. The mean systolic blood pressure at control levels of salt was approximately 122 mm Hg. At the lower level of excess salt feeding (2.8 percent) the mean of the systolic blood pressures had risen to about 130. Yet there were individuals in this group who had pressures as high as 160 mm Hg and others as low as 114. By the same token, at a high level of salt feeding (9.8 percent), the mean systolic blood pressure for the whole group of animals had risen to 152 mm Hg, but there were rats with pressures as high as 205 and one rat with a pressure of only 125. It is further of considerable interest that those animals lying significantly above or significantly below the means of their group tended to maintain this relative position throughout life. Thus, it is evident that even in fairly homogenous experimental material such as the male Sprague-Dawley rat there are substantial differences in susceptibility to hypertension induced by this means. One could reliably predict that those animals that had the higher levels of hypertension for their group were the more likely to show severe electrocardiographic changes later in life and also to show serum cholesterol elevation. Conversely, those that had the lower pressures were the less likely to evidence severe electrocardiographic changes and less likely to manifest abnormally high cholesterol. The time relations of the blood pressure change was such that at high levels of excess salt feeding the blood pressure rose rapidly to high

levels, while at lower levels of excess salt feeding the blood pressure rose less rapidly to intermediate hypertensive levels.

Among the hypertensive animals, specific abnormalities of the electrocardiagram were observed which were closely similar to those seen in humans with hypertension, namely, a high incidence of T wave abnormalities, S-T segment abnormalities, left axis deviation, left ventricular strain pattern, and prolonged duration of the QRS complex. On the other hand, arrhythmia and P-R interval abnormalities occurred only in low incidence.

Serum cholesterol levels tended to increase with increasing dietary sodium chloride. There was a strong positive correlation between serum cholesterol and the observed level of the blood pressure.

Perhaps the most sensitive index of the adverse effects of sodium chloride upon these rats was the survival data. No significant difference in survival was seen between control animals and those eating the lower levels of high salt (2.8 to 5.6 percent) until the seventeenth month of the experiment. For human beings, this would correspond roughly to age 51 if 10 days for a rat is equivalent to a year for man. Thereafter the difference increased. The median duration of life for rats eating 5.6 percent sodium chloride was many months less than that of controls. In the case of the higher levels of high salt feeding, for example, 8.4 percent, there was an 8-month difference between the median duration of survival in the high-salt rats and the controls. If one were to translate this figure to man, this would be equivalent to a difference in the median duration of life of about 32 years. Repeated experiments have shown that the slopes of the survival curves were associated with the level of dietary salt and were identical in comparable experiments.

One experiment of especial interest was performed on a small number of elderly rats made available through the courtesy of Dr. Kenneth Kohlstaadt of Eli Lilly and Company. This colony of 26 male rats, nearly 2 years in age, had been maintained previously on a chow diet. When placed on test diets containing 5.6 and 9.8 percent sodium chloride, they gradually become hypertensive, but it is important to note that the levels of elevated blood pressure attained were always less than was the case with young rats who ate the same ration for the same length of time. While sodium chloride did indeed have a hypertensigenic effect when started late in life, the effect was not nearly so great as when started early in life.

In young female rates fed corresponding levels of increased sodium chloride, the level of blood pressure obtained was never as high as that for the male rats.

Each animal that died was autopsied, the organs were weighed and tissue sections obtained for microscopic examination. The weight of the kidneys and the weight of the heart increased in proportion to the sodium chloride in the diet and, of course, to the level of blood pressure which was also proportional to the salt in the diet. The findings in the adrenals were less consistent: at high levels of salt feeding there was a significant increase in adrenal weight but the variability was considerable and there was only a general trend for the adrenals to increase in weight as sodium chloride increased. The characteristic alteration noted in the microscopic examination of the tissues among animals with severe hypertension was a diffuse disease involving especially the arterioles and small arteries. In the kidney, this was manifest by relatively bloodless enlarged glomeruli with swelling and vacuolation of both endothelium and epithelium and large amounts of sudanophilic lipid. The tubules were dilated and the epithelium was hyperplastic and swollen with lipid and hyaline droplets. The small arteries and arterioles showed fatty degeneration of muscle cells and often underwent smudgy eosinophilic necrosis. Similar arteriolar lesions were found in other viscera, most particularly the pancreas and the testes. The late Dr. Ernest Goodpasture examined the tissues of all of the rats from Experiment I and drew particular attention to the apparent similarity between the morphological lesion seen at the high levels of high salt feeding and that seen in human malignant hypertension. He further found that when arteriolar lesions were prominent in the kidneys they were also always present in the testes. Since the testes of rats are readily available for biopsy, this suggested to him the possibility that one might follow serially the development of the morphological lesion. In rats that exhibited the clinical course of benign essential hypertension, there was a lack of striking anatomical alteration in the microscopic sections, as is true with humans. In fact, with ordinary hematoxylin and eosin stains, the several pathologists who examined these tissues were unable to find any consistent differences between these animals and the controls.

It is worthwhile to examine why the picture of chronic sodium chloride toxicity is so clear in animal experimental work and so blurry in human studies. Dahl has assembled an impressive body of evidence by examining the salt intake in *different* populations. The association of hypertension and salt intake is overwhelming. In contrast, studies *within* populations have largely been unconvincing. There are several factors that probably account for this latter finding. In any given region there tends to be an average level of salt intake with considerable

variation about this mean but, in most instances, not a sufficient spread among a sufficient number of individuals to reveal the hypertensigenic salt effect in the presence of other variables. Of these, the most notable is the probable role of heredity, which we will discuss more below, but also, there are a number of ways of acquiring hypertension, especially in association with diseases of the kidney. In one investigation in Nashville, Tennessee (unpublished), some 3,000 individuals were queried concerning salt intake and blood pressure was measured. Three findings germane to the thesis of this report emerged. First, the distribution of observed blood pressures was not wide, due, it was thought, to the youth of the population studied (potential military draftees). Further, when hypertension was found in this group, it was almost always possible to elicit a history of kidney disease from the individual manifesting it. Finally, from a random sample of 24-hour urine collections it was abundantly apparent that there was no correlation between the amount of salt these individuals thought they were eating and the amount actually consumed. Another field study of a local population by this author and his colleagues gave equally indeterminate results. It is evident that a local population with a relatively low mobility comes to consume about the same amount of salt by reason of common local custom and identical sources of food. There will always be a few individuals distributed well away from the mean but apparently not enough to bring out the hypertensigenic salt effect from among the other variables. On the other hand, as Dahl has so clearly shown, when different populations with widely differing salt-eating habits are investigated, the effect is abundantly evident.

It has long been known that there was a familial factor in hypertension, although it has not been clear whether the higher incidence of hypertension among the offspring of hypertensives was purely hereditary or in some way acquired in childhood. The reports of Brest and Moyer, of Schroeder, of Thomas, of Platt, of Pickering, and of many others in this connection are well known. It now appears that Dahl has resolved the problem. He interbred rats that developed high hypertension on increased salt intake and also interbred those that developed little or no elevation of blood pressure on high salt diets. By repeating this procedure, it proved possible to develop two distinct strains of rats, one extremely sensitive to increased salt intake and the other extremely resistant to it. It thus appears reasonable to believe that the toxic effect of chronic excess sodium chloride eating in man is conditional and depends upon his hereditary material. Given the appropriate hereditary proclivity and given a salt intake in excess of normal requirement, the result will be hypertension.

Throughout nature, potassium is the principal intracellular cation. It therefore is present in all foods. There have been parts of the world where potassium salts have been used as a condiment. Lapicque found natives of Africa extracting the ash of certain plants (lixiviation) to make a condiment used somewhat like common salt. Many potassium salts, however, have a bitter disagreeable taste (salt of wormwood = potassium carbonate) and its use as a condiment has never been widespread. It is extremely difficult to make an animal (or a normal human being) potassium deficient since potassium is so widespread in nature, and there is no evidence that normal man or animals ever eat an excessive amount of it. In disease states in man, however, excess and deficiency conditions of potassium exist. The former are usually associated with impaired renal formation. One cannot increase total body potassium in normal subjects by feeding extra potassium, as the author and his colleagues found some years ago. On the other hand, in kidney disease, serious states of potassium intoxication occur frequently as a clinical problem. Hyperkalemia, excessive potassium in the blood, may occur, but the electrocardiogram is a more sensitive indicator, both of excess and of deficit. There is about 20 times as much potassium in the cellular fluid as in the extracellular, and the electrocardiogram reflects changed intracellular energetics due to potassium deficit or excess before changes in plasma potassium level are detectable. Potassium deficiency occurs as a clinical problem most usually as an iatrogenic disorder following administration of diuretics or the mineralacorticoid steroids. Alimentary tract loss in mucus colitis and certain rare tumors, and renal loss in certain uncommon renal disorders also can induce potassium deficit. While exogenous hyperkalemic states are hard to induce, and probably impossible by mouth in the presence of normal kidneys, endogenous hyperkalemia readily occurs among individuals with borderline kidney function when disease or crushing injury destroys large numbers of cells, releasing the high concentration of intracellular potassium into the extracellular compartment from which a moderately impaired kidney may be unable to drain it rapidly enough to avoid potassium intoxication.

In animal experiments, it is easy to demonstrate a protective action of extra potassium against the toxic effects of chronic excessive dietary sodium chloride. The addition of extra potassium chloride to rat diets high in sodium chloride increased the median duration of life by 7 or 8 months. The concurrent blood pressure observations were of special interest. As previously stated, at moderate levels of excess salt feeding a moderate hypertension developed. When extra potassium chloride was added to a diet containing 5.6 percent sodium chloride there was no

change in the hypertensigenic action of the extra sodium chloride, but there was a tremendous improvement in survival. The median duration of life was increased by 7 months, and yet throughout life the blood pressure persisted at intermediate levels, just as in animals who received no extra potassium. In contrast to this, at levels of excess salt feeding, which ordinarily produced high levels of hypertension, the extra potassium chloride had the effect of ameliorating the hypertensigenic action of sodium chloride so that only moderate hypertension developed. Again, a dramatic increase in the median duration of life of approximately 8 months was observed.

It was evident from these data that there were at least two kinds of hypertension in the animals eating excessive amounts of salt. The first, a moderate hypertension from moderate levels of excess salt feeding, which was not significantly altered by the addition of extra potassium chloride; and the second, a high hypertension associated with high levels of extra salt feeding, which was held to intermediate levels by the addition of extra potassium chloride. It was probably significant that the total body sodium of the moderate salt-eating animals was essentially the same as that of controls, whereas in animals eating high levels of excess sodium chloride there was a significant increase in total body sodium. Further, upon the addition of potassium chloride to each of these diets, no significant effect upon total body sodium was observed at moderate levels of sodium chloride increase, whereas at high levels of sodium chloride feeding with extra potassium the total body sodium stayed within normal limits.

The above observations shed light upon the puzzling situation with regard to human beings and dietary sodium and potassium. It is evident that there were five quite different categories of rats, and there should probably be at least five corresponding categories of man. First, there were those with optimal sodium and potassium intake who lived a long life and did not develop hypertension. Second, there were those with moderately increased sodium intake and normal potassium intake. These animals developed a moderate hypertension and exhibited a shortened life span, but did not have any significant alteration in total body sodium content. Third, there were those with moderately excessive sodium intake and extra potassium intake. These animals were distinguishable from the second category only in that they survived significantly longer. Fourth, there were those with a highly excessive sodium chloride intake and a normal potassium intake. These animals developed a severe hypertension, their life span was greatly shortened, and their total body sodium was significantly elevated. In the fifth

category were those rats with a highly excessive sodium chloride intake and an increased potassium intake. This group was characterized by the development of a moderate hypertension, a substantial prolongation of life when compared with animals eating the same amount of sodium chloride but without the supplemental potassium chloride, and a total body sodium that remained within normal limits.

If these data were transferable directly to man, it would account for the fact that some hypertensive patients exhibit a fall in blood pressure when extra potassium chloride is provided and others do not. One could hardly expect potassium to produce a fall in blood pressure unless the individual (a) had a hypertension corresponding to the high salt intake hypertension of the rat and (b) was not already receiving a sufficient amount of potassium. Further, the benefit of extra potassium fed to an individual eating an intermediate level of high salt could be detected, so far as one is able to determine, only by observing a prolongation of life. This is obviously impossible in the individual.

The protective effect of extra potassium in diets high in sodium chloride has been confirmed in animals by Dahl. Some most interesting human studies by Sasaki confirm the effect in man.

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J. Nutrition

CHRONIC SODIUM CHLORIDE TOXICITY IN THE ALBINO RAT

I. GROWTH ON A PURIFIED DIET CONTAINING VARIOUS LEVELS OF SODIUM CHLORIDE

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The importance of dietary salt has been recognized from time immemorial, and dietary sodium has been suspected for over 30 years as a primary cause of arterial hypertension in man (Allen and Sherrill, '22); yet little evidence has accumulated on the chronic toxicity of sodium chloride. The more primitive type of renal glomerulus in fowl as compared to the mammal makes it more susceptible to sodium chloride poisoning; hence most studies have been made on birds (French, '35; Krakower and Goettsch, '45; Lenel, Katz and Rodbard, '48). Campbell ('46) reported on the growth and reproduction of the rat on diets containing 1.32, 2.59 and 5.06% of sodium chloride. No great differences were observed in growth, apparent well-being or ability to reproduce. There were some indications of renal damage at the highest level of sodium chloride intake. Meyer et al. ('50) found that high level feeding of sodium retarded somewhat the growth of rats.

This report covers the growth, ration consumption, early mortality, and general observations on rats fed a purified diet containing sodium chloride at 7 different levels.

EXPERIMENTAL

Ration I contained casein, vitamin-test,[1] 25.0%; cane sugar, 51.9%; shortening, all-vegetable (Crisco), 20.0%; a mineral mixture,[2] 2.9%; and a vitamin mixture,[3] 0.2%. It is a low-sodium diet (no sodium chloride added but containing 0.01% of sodium chloride by analysis). Rations II to VII, inclusive, were made by intimately mixing ration I with the calculated amount of sodium chloride, C. P. powder, in a Hobart mixer. Ration II contained an adequate reasonable amount of sodium chloride equivalent to incorporating the Hubbell, Mendel and Wakeman ('37) salt mixture at about a 2.0% level. Rations III to VII, inclusive, were progressively higher in sodium chloride content, 20, 40, 50, 60 and 70 times the Hubbell, Mendel and Wakeman formula fed at a 2% level. Rations were made fresh at no more than 15-day intervals and were stored at 7°C. until placed in feed cups.

Male, Sprague-Dawley, albino rats, 33 to 40 days old and weighing from 86 to 154 gm, were divided into 7 matched groups and placed on demineralized water and the purified diets ad libitum. The rats were kept in steel wire cages (several rats per cage) in a constant temperature room (27° ± 2°C.). They were fed, watered and observed daily, and individually weighed and carefully examined weekly. The rate of food and water consumption was studied for the first 5 weeks of the experimental regimen. Scatter-proof feed

[1] Manufactured by Nutritional Biochemicals, Inc., Cleveland, Ohio.

[2] The mineral mixture is a modification of the Hubbell, Mendel and Wakeman ('37) formula by weight, as follows: $CaCO_3$, A. R., 543.0; KH_2PO_4, A. R., 212.0; KCl, A. R., 112.0; $FePO_4$, N. F., 38.1; $MgCO_3$, A. R., 25.0; $MgSO_4$, A. R., 16.0; $KF \cdot 2H_2O$, A. R., 2.20; $CuSO_4 \cdot 5H_2O$, A. R., 1.41; $ZnSO_4 \cdot 7H_2O$, U. S. P., 0.90; $MnSO_4 \cdot H_2O$, A. R., 0.39; $AlK(SO_4)_2 \cdot 12H_2O$, U.S.P., 0.31; KI, C. P., 0.08; and $CoSO_4 \cdot 7H_2O$, C. P., 0.01.

[3] The vitamin supplementation in weight per kilogram of ration I was as follows: thiamine hydrochloride, 5.0 mg; riboflavin, 5.0 mg; pyridoxine hydrochloride, 2.5 mg; vitamin B_{12}, 40.0 μg; i-inositol, 1.0 gm; p-aminobenzoic acid, 2.5 mg; biotin 24.0 μg; niacin, 20.0 mg; calcium pantothenate, 30.0 mg; choline chloride, 1.0 gm; menadione bisulfite, 4.8 mg; α-tocopherol, 30.0 mg; folic acid, 1.0 mg; and concentrated oleovitamin A and D, U.S.P., 0.4 gm.

cups[4] and inverted wide mouth bottles with stainless steel water feeder tubes[5] were used.

RESULTS

Growth

Table 1 lists the per cent of sodium chloride in the ration and the group means and standard deviations of the gross body weight fortnightly for the first 20 weeks of the experimental regimen. This tabulation includes only those rats which exhibited good health throughout the test period. Excluded here were some 11% of the rats which developed abnormalities such as intercurrent infection or which sustained injuries and were removed from the colony. This omission was made in order to keep the number of animals in each group constant throughout the 20-week period. However, the growth pattern of the excluded animals did not differ from that of their respective group until edema or other diseased states intervened. A separate report is to be made on the pathologic changes observed in these animals (Meneely et al., '52).

Food consumption

Table 2 lists the rate of food consumption during the first 5 weeks of the experimental regimen. The values are the weekly means expressed as grams of food per rat per day. These values represent an over-estimation of the amount of food eaten by the rats, because some undetermined wastage occurred. The semi-plastic consistency of the food and the scatter-proof feed cups tended to reduce the food wastage to a minimum.

Water consumption

Table 3 lists the rate of water consumption during the first 5 weeks of the experimental regimen. The values are the

[4] Manufactured by C. H. Sommers and Associates, Cincinnati, Ohio.
[5] See footnote 4.

TABLE 1

Growth data (gross body weight in grams at bi-weekly intervals)

TIME IN WEEKS	RATION I 0.01% NaCl		RATION II 0.15% NaCl		RATION III 2.8% NaCl		RATION IV 5.6% NaCl		RATION V 7.0% NaCl		RATION VI 8.4% NaCl		RATION VII 9.8% NaCl	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Initial	116	19	119	17	118	17	117	19	121	17	118	21	121	22
2	170	30	184	26	179	18	176	21	180	21	176	20	173	21
4	207	41	251	28	244	24	229	19	244	22	237	22	228	21
6	226	45	296	29	297	28	276	22	290	28	277	27	269	23
8	256	44	334	31	330	28	315	24	322	30	313	29	301	27
10	281	49	360	32	354	30	339	24	341	31	334	31	324	25
12	315	58	392	34	380	29	365	23	365	32	353	29	341	25
14	340	55	412	36	393	30	374	24	377	33	367	30	349	24
16	359	60	432	38	412	33	394	24	396	35	385	36	368	28
18	388	56	454	38	435	34	414	27	411	35	399	37	383	26
20	416	43	472	39	449	34	432	26	424	36	413	35	395	28
N	10		30		29		28		24		26		25	

weekly means expressed as milliliters of water per rat per day. These values also represent some over-estimation of the water drunk by the rats as an unavoidable amount of spillage occurred.

TABLE 2

Mean ration consumption. All figures are in grams per rat per day

RATION	I	II	III	IV	V	VI	VII
% NaCl	0.01	0.15	2.8	5.6	7.0	8.4	9.8
1st Week	11.1	11.4	11.4	11.5	12.6	12.3	11.7
2nd Week	10.3	12.4	12.8	12.9	14.0	13.7	13.3
3rd Week	10.5	13.4	13.3	13.5	13.8	14.3	13.9
4th Week	10.4	14.2	13.7	12.7	14.7	15.6	15.0
5th Week	10.2	13.9	14.0	14.8	14.6	14.6	13.9
Mean	10.5	13.1	13.0	13.1	13.9	14.1	13.6

TABLE 3

Mean water consumption. All figures are in milliliters per rat per day

RATION	I	II	III	IV	V	VI	VII
% NaCl	0.01	0.15	2.8	5.6	7.0	8.4	9.8
1st Week	24.0	26.4	47.9	55.7	60.0	72.1	73.4
2nd Week	28.6	30.2	40.6	63.4	71.9	77.8	84.9
3rd Week	29.4	38.9	46.1	65.8	76.1	84.0	90.0
4th Week	30.8	45.2	58.0	65.1	82.9	102.7	91.2
5th Week	25.0	49.0	63.1	74.3	87.7	104.1	89.2
Mean	27.6	37.9	51.1	64.9	75.7	88.1	85.7

Mortality and morbidity

The rats grew well on all of the experimental rations. Only 17 rats out of a total of 193 died or were sacrificed during the 20-week period covered in this report. Table 4 summarizes the mortality data. The morbidity included two rats on ration IV with mechanical injury to the snout, one rat on ration V which contracted respiratory infection and one rat on ration VI which developed edema.

DISCUSSION

The diet herein described is of known composition, is reproducible from available, chemically pure nutrients, and is of a desirable consistency for feeding to small animals. It is very low in cholesterol, the only source being the concentrated oleovitamin A and D oil. The diet contains adequate amounts of all vitamins which are known to be required by the rat. In addition a few vitamins are incorporated of which, presumably, the rat does not require a dietary source, the reason being that sufficiently long-term studies have not been

TABLE 4

Mortality experienced during the first 20 weeks on the purified rations

RATION	CAUSE OF DEATH	NUMBER
I	Respiratory infection	1
II	Fractured nose	1
III	Respiratory infection	1
IV		0
V	Renal failure with edema	2
	Respiratory infection	2
	Fractured nose	1
VI	Renal failure with edema	2
	Respiratory infection	1
VII	Renal failure with edema	4
	Renal failure without edema	1
	Accidental hemorrhage	1

reported in every case, and it was deemed desirable not to risk the occurrence of vitamin deficiencies in the present long-term study.

The sodium chloride added to the rations was in a powdered form, freshly screened to render it uniform, and adequately mixed into the diet at high speed in a Hobart mixer. The rat had no chance to reject the added salt from his diet. The dispersion was such that if the rat ate any of the ration, he was forced to ingest the calculated fraction of sodium chloride.

The low-sodium diet (ration I) caused mild anorexia. The small group of rats on this diet ate less, drank less water,

and grew more slowly than did the controls (ration II). These animals exhibited a continuous salt hunger. This was manifest by their habit of licking any object in the cage, particularly wherever fingerprints were left. There was considerable variation in the rate of growth of the animals on ration I as demonstrated by the large standard deviations in gross body weight (table 1).

The high sodium chloride diets (rations III to VII, inclusive) depressed the rate of growth. Table 5 indicates the significance of the differences between the means of the control animals and the other 6 groups. Although the data on

TABLE 5

The probability (P) that the mean body weight of the sample differs from the mean body weight of the controls (ration II). Values in italics indicate that the difference is statistically significant

RATION	START	4 WEEKS	8 WEEKS	12 WEEKS	16 WEEKS	20 WEEKS
I	>.6	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*
III	>.6	>.3	>.4	>.1	*<.05*	*<.02*
IV	>.5	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*
V	>.8	>.3	>.1	*<.01*	*<.02*	*<.01*
VI	>.8	*<.05*	*<.01*	*<.01*	*<.01*	*<.01*
VII	>.6	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*

ration consumption indicate that the rats on high salt diets ate as much or more than the controls, they did not consume as many calories as did the control animals and, consequently, did not gain as rapidly in gross body weight. The fact that most of the rats on the high salt diets grew and thrived demonstrates the remarkable ability of the rat kidney to excrete enormous quantities of salt. As anticipated, these rats exhibited a marked polydipsia and polyuria.

Although food and water spillage by the rat introduces some error into the data on ration consumption (tables 2 and 3), the relative values are reasonably correct. Trends are apparent from a casual inspection of the data. There can be little doubt that the low salt regimen had a depressing

effect on the appetite and that the high salt diets caused thirst but did not depress the appetite to a marked degree.

The general appearance of the rats on the 7 purified diets was good throughout the test period with the following exception. When first placed on the high salt diets, many of the animals developed a condition of extreme dehydration characterized by marked porphyrin staining of the hair and extremities. This occurred during the first week of the experiment. By the end of the second week the condition had almost disappeared and did not recur. The rats had successfully adapted to the high-sodium diet.

Of the 17 deaths occurring during the 20-week period (table 4) only 9 were directly attributable to dietary stress. Less than 5% of the total number of animals involved succumbed to pathologic changes induced by sodium chloride toxicity or 10% of the animals on the three higher levels of sodium intake.

Sporadic renal damage was manifest by the sudden appearance of massive edema. The extracellular fluid measured with radioactive sodium-24 rose from control values of $26.2 \pm 1.88\%$ of body weight to 57.3 and 57.8% of body weight in two rats. During the week of most rapid edema formation, body weight gains averaged 62.8 gm for 9 edematous rats in contrast to an average gain of 10.1 gm a fortnight earlier for the same animals. The edematous rats were characterized by marked anemia, hypoproteinemia, lipemia, and azotemia. Histologic examination of the kidneys revealed extensive glomerular and tubular lesions. The pathology of these rats will be reported when the study is completed.

The surviving animals on all 7 rations were of uniform, healthy appearance indistinguishable with respect to fur, skin, color and behavior when handled from controls fed on Purina Chow. The size of the animals was a notable exception.[6]

[6] The control rats on ration II actually exceeded the Purina Chow-fed rats in rate of growth. However, the test was not legitimate, because the Chow-fed rats were the extremes in weight rejected from the sample at the start of the experiment, and the data are omitted specifically for that reason.

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The rate of growth on ration II (controls) compares favorably with other reports in the literature (Copping et al., '51; Freudenberger, '32; Mendel and Hubbell, '35; Zucker et al., '41). However, the "rapidly growing" rats of Pickens et al. ('40), and the small group of rats raised on a purified diet by Mayer ('48) grew more rapidly than did our control animals.

SUMMARY

Young male rats have been raised on a purified diet, containing 7 different levels of sodium chloride, and water ad libitum with the following results:

Rats fed diets high (2.8 to 9.8%) and low (0.01%) in sodium chloride grew more slowly than did rats on a control ration (0.15% NaCl).

High sodium chloride feeding caused polydipsia and polyuria but did not have a marked effect on appetite. A low-sodium diet caused mild anorexia.

Edema developed during this first 20-week period in 10% of the rats fed diets containing from 7.0 to 9.8% of sodium chloride. The majority of the animals (89%) remained in excellent health throughout the 20 weeks.

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CHRONIC SODIUM CHLORIDE TOXICITY IN THE ALBINO RAT

II. Occurrence of Hypertension and of a Syndrome of Edema and Renal Failure

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Plates 6 and 7

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The need for common salt in the diet is an ancient, inveterate cognition, deeply rooted in human mores, to such an extent that little thought has been given to the chronic toxicity of sodium chloride. A search of the literature reveals a number of papers which describe toxic effects of sodium chloride in fowl (1), but a paucity of information relative to mammals. Campbell (2) reported the possibility of kidney damage in the rat fed a diet containing more than 5 per cent of sodium chloride. Mosier (3) studied the adrenal glands in rats on diets very high in sodium chloride. Sapirstein, Brandt, and Drury (4) and Gross (5) produced hypertension in rats fed diets high in sodium chloride and restricted in the amount of fluid. This was accomplished by substituting saline solution for drinking water. Binet, Dejours, and Lacaisse (6) raised rats on a high sodium chloride regimen and described a resulting renal hypertrophy and functional adaptation. A few experiments of a chronic nature have been made using the mouse (7), the albino and the kangaroo rat (8), the dog (9), and the cow (10).

Recently Meneely, Tucker, and Darby (11) reported on the growth of rats fed for 20 weeks on purified diets containing seven different levels of sodium chloride. The present paper describes certain pathologic manifestations of chronic sodium chloride toxicity which were observed in these animals. Abstracts of these findings have appeared (12).

Materials and Methods

The experimental regimen has been described (11). Male, albino, Sprague-Dawley rats, weighing an average of 119 gm., were placed on purified rations and demineralized water *ad libitum*. The purified rations contained seven different levels of sodium chloride as follow: ration I (low Na diet), about 0.01 per cent NaCl; ration II (control diet), 0.15 per cent NaCl; ration III, 2.8 per cent NaCl; ration IV, 5.6 per cent NaCl; ration V, 7.0 per cent NaCl; ra-

tion VI, 8.4 per cent NaCl; ration VII, 9.8 per cent NaCl. Eleven rats were placed on ration I and thirty or thirty-one rats on each of the other six diets. The experiment was designed to cover the life span of the rat. Systolic blood pressure measurements were made by the cuff method of Kersten *et al.* (13).[1] At the time of sacrifice the mean arterial blood pressure in a number of rats was determined manometrically by direct cannulation of the carotid artery under diallyl barbituric acid with urethane (Ciba Pharmaceuticals Products, Inc., Summit, New Jersey) anesthesia. A few animals developed intercurrent disease, usually respiratory infection, and died or were sacrificed. Other animals for sacrifice were chosen at random from each group. Autopsies were performed as soon after death as possible, immediately in the case of all sacrificed animals. The tissues were fixed in 10 per cent formaldehyde solution and blocked, sectioned and stained by standard histologic techniques except for those prepared by frozen section for fat stains.

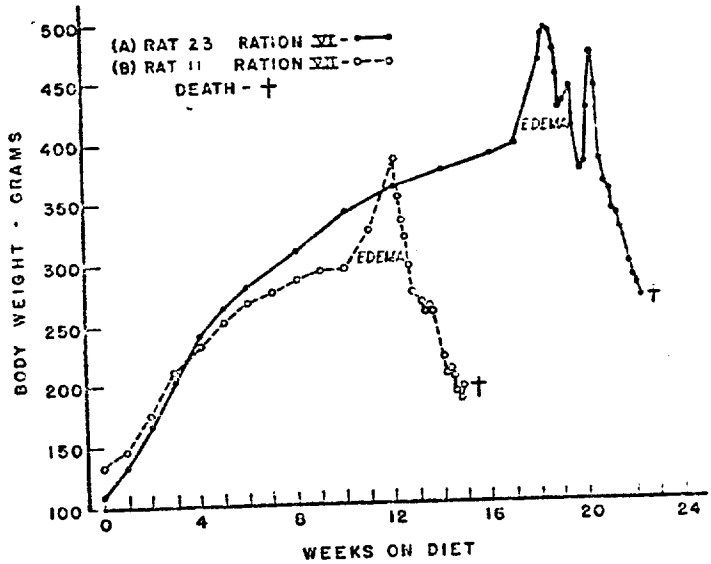
RESULTS

Edema.—As previously reported, all seven groups of rats tolerated the diets and gained weight, although the animals on sodium restriction (ration I) and those on excessive salt gained less rapidly than did the controls (ration II). After the 2nd week the rats all appeared healthy upon inspection. During the ensuing 2 months the rats on the higher levels of salt intake consumed large amounts of water and maintained an impressive polyuria, approximately proportional to the amount of sodium chloride in the diet. Beginning in the 3rd month there appeared sporadic instances of rapidly developing, massive edema among those three groups eating the highest levels of salt (from 7.0 to 9.8 per cent). This occurred with moderate frequency until the 6th month. Since that time edema has been observed but rarely.

Two typical weight curves of edematous rats are shown in Text-fig. 1. During the phase of edema the weight of the animal usually was unstable, fluctuating in the manner seen in curve (A). Most of the rats which developed edema died or were sacrificed, but those that passed through the edematous episode exhibited a sudden and precipitate fall in weight, became emaciated, cachectic, and died. Two rats entered a terminal cachectic phase directly without ever exhibiting detectable edema. The total number of rats that showed these changes was sixteen, which is 18 per cent of the ninety initially eating the three high salt diets (Table I).

Examination of these edematous animals revealed quite uniform findings. Interstitial fluid was greatly increased, as evidenced by the appearance of the animal before death (Fig. 1) and by the obvious edema of all the tissues and organs at autopsy (Fig. 2). Measurements of the sodium space with the radioisotope Na^{24} revealed apparent volumes of extracellular fluid as high as 57.5 per cent of body weight (Table II); this value may be contrasted with an average of 23.8 per cent among the controls at that time. The plasmas from these edematous animals were lipemic in the gross. The total serum proteins were greatly diminished, in some instances to values as low as 3.5 gm. per

[1] The photoelectric tensometer used in this experiment was manufactured by Metro Industries, Long Island City, New York.



TEXT-FIG. 1. Growth curves for two rats which developed massive edema. A. Solid line. Rat 23 on ration VI (8.4 per cent NaCl). B. Broken line. Rat 11 on ration VII (9.8 per cent NaCl).† Death.

TABLE I

Data on Rats Which Became Edematous from the 7th until the 23rd Week of the Experiment

Rat No.	Ration	Time of onset of edema	Weight at onset of edema	Maximum weight	Time of death	Weight at death	Anemia	Cachexia	Died or sacrificed	Lowest total serum proteins	Lowest hematocrit reading	Remarks
		wk.	*gm.*	*gm.*	*wk.*	*gm.*				*per cent*	*per cent*	
54	VII	7	241	346	12	113	+	+	Died	4.62	24.4	
24	VII	7	259	457	10	427	+		Sacrificed	4.13	17.0	See Figs. 1 and 2.
22	V	8	231	315	10	278	+		Died	—	19.1	
51	VI	9	286	350	10	270	+		"	3.52	27.5	
52	V	9	294	440	12	380	+		Sacrificed	—	—	
42	VII	10	320	363	15	178	+	+	Died	5.89	—	
11	VII	11	295	384	15	196	+	+	"	5.10	—	See Text-fig. 1.
17	VI	13	324	406	15	375	+		"	4.15	—	
23	VI	18	395	489	23	267	+	+	Sacrificed	—	30.0	NPN, 77–175 mg. per cent. See Text-fig. 1.
64	VII	20	410	430	29	294	+	+	"	—	—	Slight edema.
6	VII	21	396	427	25	330	+	+	Died	—	38.0	NPN. 82 mg. per cent. Slight edema.
18	VII	23	425	549	27	305	+	+	"	—	—	

cent. A severe anemia was present, the hematocrit reading falling as low as 17.0 per cent in one instance. Azotemia, as indicated by elevation of the plasma non-protein nitrogen, was observed terminally in animals on rations V through VII. Blood pressure measurements, when made, revealed hypertension in most of the edematous rats. At autopsy, the organ weights were obtained, but the edema obscured their interpretation. Therefore they are not presented at this time.

Microscopic examination of the tissues of animals in this stage showed spectacular lesions of the kidneys in every instance. All components of the nephron were affected, but the glomeruli were most uniformly and severely damaged. The tufts were enlarged but appeared almost bloodless, and overall cellularity seemed diminished. Individual cells, both endothelial and epithelial, were swollen, and the cytoplasm was empty or vacuolated. Nuclei

TABLE II

Na^{24} Space Expressed as Per Cent of Gross Body Weight

	Sample	Rations	Per cent NaCl	N	Mean Na^{24} space	S.D.
From 2–4 mos.	Controls	II	0.15	7	23.8	2.00
	High salt (without edema)	V, VI, VII	7.0, 8.4, 9.8	11	26.1	2.03
	High salt (with edema)	V, VII	7.0, 9.8	2	57.5	—
From 10–11 mos.	Controls	II	0.15	8	21.6	0.96
	High salt (without edema)	V, VI, VII	7.0, 8.4, 9.8	20	25.8	2.87

were misshapen and often pyknotic, but some were vesicular. The basement membranes were greatly swollen, frayed, degenerated, and tended to fuse with the cellular elements so that capillary structure was virtually obliterated. Staining with sudan IV demonstrated large quantities of lipid throughout the glomerular tufts. This occurred in both fine and coarse droplets and was largely intracellular. It appeared that the endothelial cells uniformly were more affected, but even the capsular epithelium was not exempt. The convoluted and straight tubules were generally dilated and contained protein precipitate varying in amount and density. The lining epithelium was of pale granular appearance with abundant sudanophilic lipid in the cytoplasm. The renal arterioles were uniformly diseased, and many small arteries were affected. Stainable lipid formed vacuoles in smooth muscle cells, some of which were necrotic. In many arterioles the elastic lamina was greatly swollen, frayed, and intensely eosinophilic; the lumina were narrowed or even oc-

cluded (Figs. 3 and 4). Extrarenal arterial lesions occurred only occasionally in this group of animals and were usually less severe than those in the kidney.

In the rats on rations V, VI, and VII, which survived only 6 to 9 months and which did not become edematous, the kidneys again showed widespread lesions with somewhat more severe tubular degeneration. Among these animals visceral arterial lesions were fairly constant and were almost invariably present in the heart, pancreas, testis, and gastro-intestinal tract. Vascular lesions in the heart were associated with small areas of scarring. In none was the aorta sclerotic. The microscopic structure of the adrenal glands was not different from that of control animals.

Hypertension.—Most of the rats (134 out of 193) forming the initial seven groups in this study are still alive after 14 months, and the experimental regimen continues. With few exceptions the animals appear healthy and alert

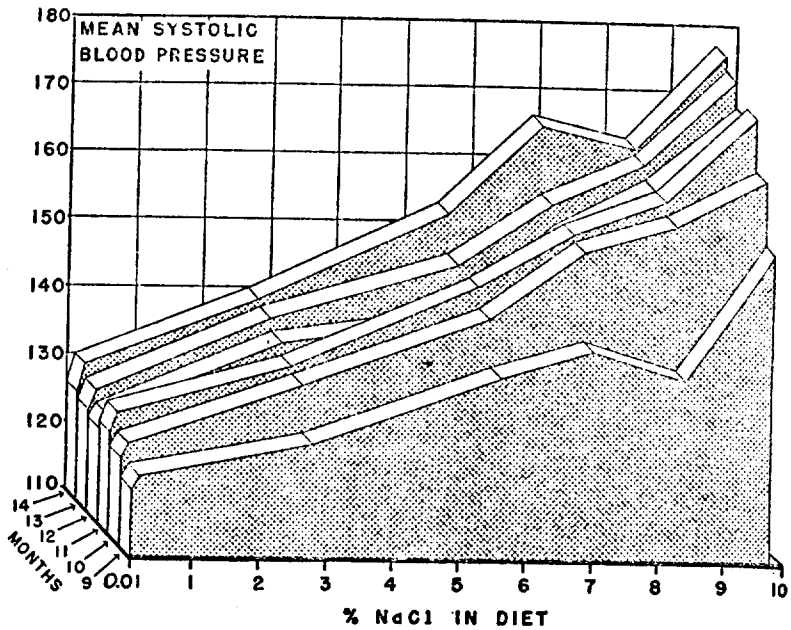
TABLE III

Mean Body Weight

Ration	Per cent NaCl	N	Mean weight		S.D. + 1 yr.
			Start	+ 1 yr.	
I	0.01	8	113	614	44
II	0.15	25	120	670	73
III	2.8	25	121	592	50
IV	5.6	27	118	566	41
V	7.0	21	119	543	40
VI	8.4	18	125	538	50
VII	9.8	18	120	491	41

with sleek coats. Several of the control animals and some of those on rations I, III, and IV are actually obese (Table III). None of the animals on rations I through IV has shown significant visceral lesions. Despite their generally healthy appearance, many of the rats are not normal. Practically all the rats on rations V, VI, and VII have sustained arterial hypertension, and most of the rats on rations III and IV have significant elevations in blood pressure. Reliable determinations of blood pressure on the colony as a whole have been obtained beginning in the 9th month of the experiment. Previous to the 9th month the rats were insufficiently trained. Text-fig. 2 shows that striking changes in blood pressure have appeared among the animals eating rations III through VII. In this figure the means of the systolic blood pressures at monthly intervals are plotted for each group. All the data obtained from the beginning of the 9th month until the end of the 14th month of the experiment are included in this graphic summary. Part of the intragroup fluctuation is due to sacrifice or death of some animals.

The animals on diets V, VI, and VII, sacrificed while in apparent good health, showed renal lesions which resembled those described above but which were somewhat less severe and much less uniform. In fact many nephrons appeared uninjured while neighboring units were effectively destroyed. The arterial lesions, both renal and general visceral, were less impressive and less constant, usually consisting of medial hypertrophy. However, in the continuing study some of the hypertensive rats which were sacrificed at about 14

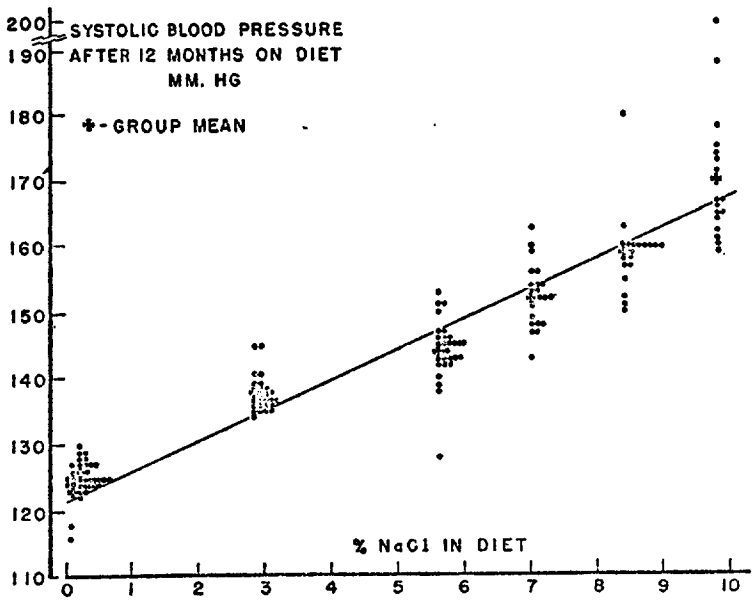


TEXT-FIG. 2. The oblique lines represent the means of the systolic blood pressures of the seven different groups at monthly intervals.

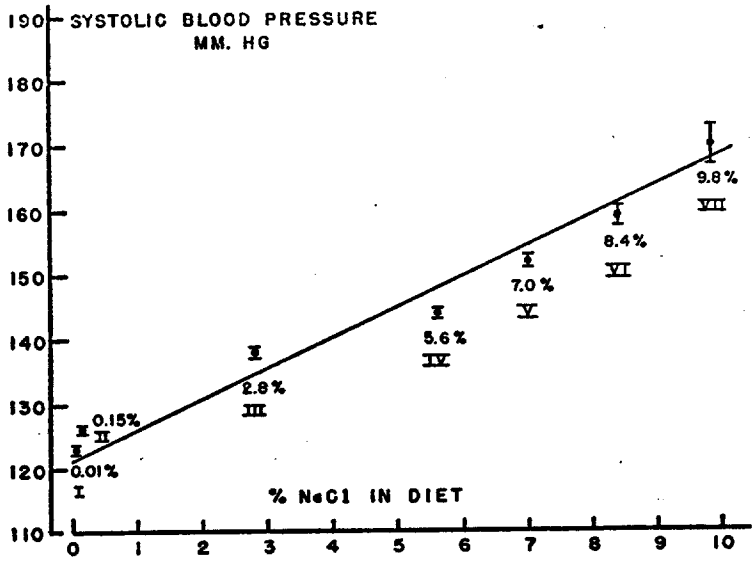
months and were manifestly declining before death, exhibited both renal and arterial disease equal to that observed in the edematous group.

DISCUSSION

Hypertensive disease was provoked in the rat by increase in the ration of sodium chloride. The diet fed was essentially cholesterol-free. The rats were housed in suspended steel wire cages; hence they had only limited access to their feces. After several months of the dietary regimen, a sustained arterial hypertension developed in the rats eating the high salt rations. The average blood pressure was related in a linear fashion to the amount of sodium chloride (Text-figs. 3 and 4). Within a given salt intake group the distribution of



TEXT-FIG. 3. Scatter diagram of systolic blood pressure values after 1 year of the dietary regimen. Identical values are arrayed to the right of the perpendicular.



TEXT-FIG. 4. The mean systolic blood pressure and the standard error of the mean after 1 year of the dietary regimen.

blood pressures showed some scatter; the group means, however, fell close to a straight line. No more than random departure from linear regression was indicated. The product moment coefficient of correlation was $+0.91 \pm 0.08$. These effects occurred without restricting the amount of drinking water, although others have claimed that it is necessary to restrict water intake to produce hypertension with salt (4).

The measurements of blood pressure were made with a standardized photoelectric device (13). In a number of rats which were sacrificed, these observations were confirmed by direct measurement of the blood pressure in the cannulated carotid artery.

Rats which developed marked edema on the three highest rations of salt (7.0 to 9.8 per cent) evinced similar signs and strikingly similar alterations of their organs in the gross and upon microscopic examination. The syndrome of massive edema, lipemia, anemia, hypoproteinemia, and azotemia resembles nephrosis as seen in human beings, but might differ in other respects from the human disease. Neither the pathogenesis of salt injury in rats nor the pathogenesis of human nephrosis is known. The renal lesion observed in these rats is different from that seen in human nephrosis principally in that there is initial and severe involvement of the glomeruli in the rat kidney. The severity of hypertension in these rats may well account for the similarity of the arteriolar lesion in these animals to the arteriolar lesion seen in malignant hypertension in man. Also it may be noted that the experimental syndrome developed mainly among the rats while they were young, that is, during the first one-fifth of the usual life span, an age which corresponds to the time of occurrence of nephrosis in man.

Some animals went into terminal renal failure without exhibiting detectable edema. In these the histologic picture was indistinguishable from that seen in rats which passed through an edematous phase. It seems that the clinical course exhibited by an individual rat depends upon a balance of those factors which favor salt and water retention as opposed to those which lead to salt and water loss. In all cases impairment of renal function inevitably predisposed to an early death.

Owing to the fact that the initial reading of blood pressures dates from the 9th experimental month, by which time segregation of groups with respect to blood pressure had occurred (Text-figs. 2 to 4), the earliest time of origin of the hypertension cannot be stated.

Pathologic changes have not been found as yet by usual staining techniques in the animals of groups III and IV, although mild hypertension has developed in both of these groups. It is possible that recognizable lesions will appear later in the course of the experiment. This suggests that physiologic changes resulting in hypertension occur before there is any readily demonstrable anatomic lesion in the kidney or elsewhere.

Except for edematous rats, the Na^{24} space measured in rats fed high salt diets for 2 to 4 months was not significantly greater than in control rats. However, after approximately 10 months a significant difference was observed (Table II). This apparent increase in volume of extracellular fluid probably is accounted for in part by differences in total body fat; some of the control rats have become obviously obese (Table III).

SUMMARY

Sustained arterial hypertension developed in male, albino rats chronically fed diets rich in sodium chloride with demineralized drinking water available *ad libitum*. After 12 months of the experimental regimen a positive, linear correlation ($r = 0.91$) was found between the systolic blood pressure and the concentration of sodium chloride in the diet.

A syndrome of edema and renal failure was observed in 18 per cent of the group fed at the level of 7.0 to 9.8 per cent of sodium chloride. Significant histologic changes occurred in the kidneys and certain other organs in rats consuming rations containing these levels of NaCl.

The relative volume of the radiosodium space was increased in the rat by high dietary sodium chloride.

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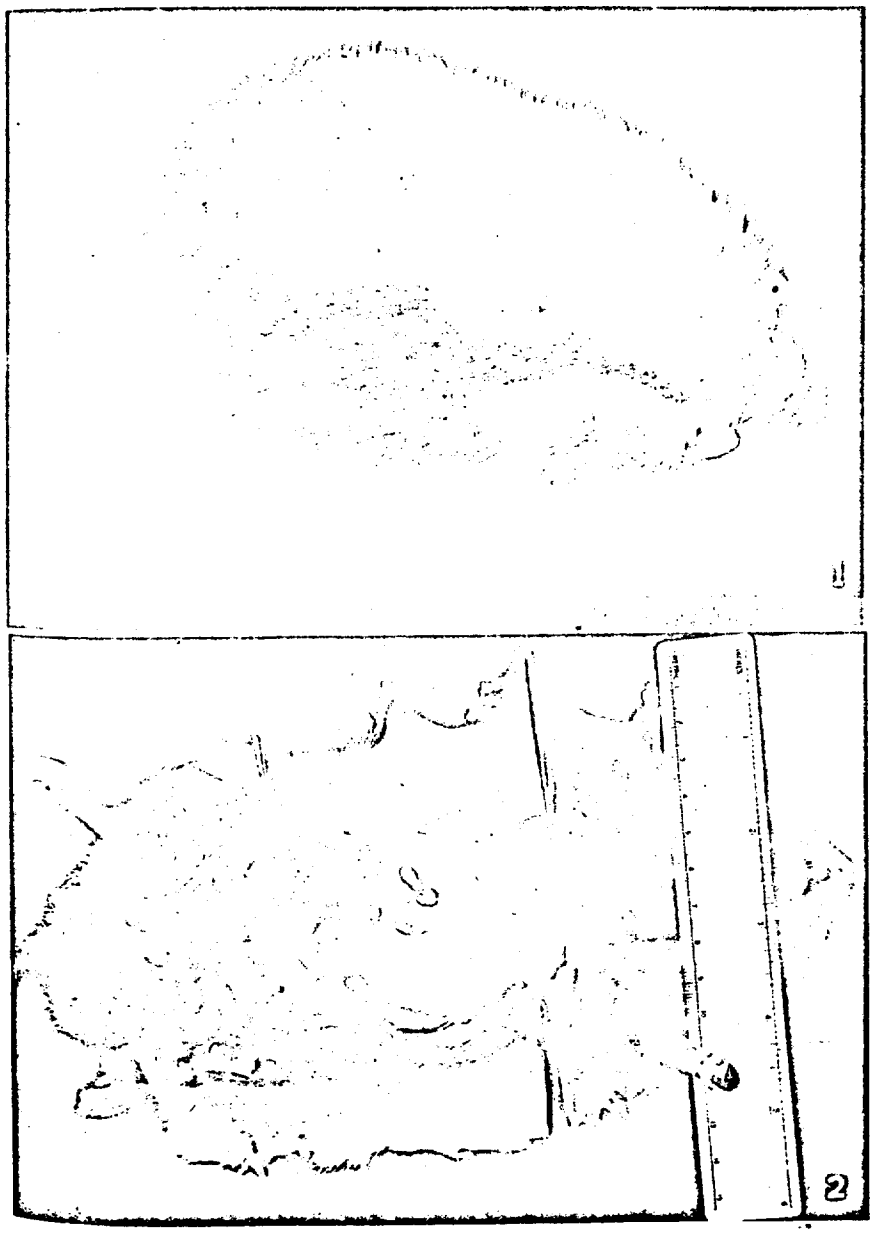

(Meneely *et al.*: Sodium chloride toxicity. II)

PLATE 7

FIG. 3. Rat 24 kidney stained with hematoxylin and eosin. × 100. This rat (on ration VII) was sacrificed in the edematous phase. All elements of the nephron tissue are involved. Extensive arteriolar disease is also present.

FIG. 4. Rat 24 kidney stained with sudan IV. × 75. This photomicrograph shows accumulation of lipoid in the glomeruli, the tubules and the arterioles in the same kidney illustrated in Fig. 3.

EXPLANATION OF PLATES

PLATE 6

FIG. 1. Rat 24. After eating ration VII (9.8 per cent NaCl) for 8 weeks, the rat developed massive edema. This photograph was taken in the 10th week when the body weight was about 440 gm. The pit on the nose was produced by finger pressure.

FIG. 2. Appearance of edematous rat 24 (ration VII) at autopsy. This photograph was taken shortly after Fig. 1.

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CHRONIC SODIUM CHLORIDE TOXICITY: HYPERTENSION, RENAL AND VASCULAR LESIONS *

By GEORGE R. MENEELY, M.D., F.A.C.P., ROBERT G. TUCKER, Ph.D., WILLIAM J. DARBY, M.D., Ph.D., and STEWART H. AUERBACH, M.D., *Nashville, Tennessee*

AN investigation of so common a substance as salt needs an introduction. While used in abundance by nearly all of us every day, we have certainly learned the merit of restricting it in most patients with edema, and there is a growing body of evidence of its rôle in hypertension.[1] A spate of publications on electrolytes in recent years makes one think that nothing before ever was known about salt, and there is some danger of missing the wood for the trees.

Scientific knowledge of the composition of sodium chloride did not exist earlier than 1774, when Scheele first discovered "dephlogisticated marine acid," the greenish yellow gas chlorine. He was confused about it, however, thinking it contained oxygen. In 1807 Davy isolated potassium, the first metal isolated by electricity, and that same year he isolated sodium, also by electrolysis. He named chlorine in 1810. In this, the early part of the nineteenth century, the rise and development of chemical theory was a tumultuous procession. Earlier, Lavoisier had laid the ghost of the phlogiston theory. Besides Davy, Berzelius, Gay-Lussac, Avogadro, Faraday and Liebig were on the stage. The structure and even the electrical nature of salts were understood clearly for the first time by the midpoint of the century. This knowledge replaced the mysteries of the thirteenth century alchemist Geber and the curiously correct speculations of Glauber, he of the salt which is still esteemed as a medicinal in many corners of the globe.[2] By 1850, Carl Schmidt was reporting determinations of sodium and potas-

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sium in the blood in his essay on epidemic cholera,[3] and the era of understanding of the physiologic rôle of electrolytes had begun.

These discoveries in the fundamental science of chemistry stimulated a chain reaction among the biologists of that day. Scholars as they were, they looked backward to history as well as forward by observation and experiment. Sodium chloride claimed much attention during the latter half of the nineteenth century in the sphere we have come to call the cultural anthropological, as well as in the fundamental sciences as such.[4]

It is certainly difficult, and perhaps impossible, to trace the earliest human use of common salt. It has been in use by civilized people since earliest recorded history. Evidence from linguistics suggests that salt was not known as such when the stem language developed. There is thought to be no common Indo-European root for the word. Evidence has been assembled [5, 6] which suggests that salt came into use at the time man made the transition from a nomadic hunter-fisher, on a roasted meat and milk diet to an agriculturist on a cereal grain and vegetable diet. The fact that herbivorous animals manifest a craving for salt by travel for long distances to salt licks, while carnivorous animals do not, intrigued the physiologic chemists of the latter half of the nineteenth century. Bunge [7] may have been the first to advance the concept that the high potassium content of a grain and vegetable diet required in the herbivora an increased sodium intake. "Herbivorous animals take at least three or four times as much of the salts of potassium as the carnivorous. This fact leads me to imagine that the abundance of potassium in vegetable food is the cause of the need of salt in the herbivora." [8] Whether this is the true reason agricultural man began adding salt to his diet, certainly once he had he took a great fancy to it. The preservative effect of salt must have been noted early, and food so preserved became vital through the non-harvest seasons and during voyages and migrations. There are many references to salt in the Bible, and there is a rich and entertaining literature concerned with the history, symbolism and financial problems of salt.[9, 10, 11] Lack of salt has been propounded as a major factor in the fall of the Confederacy.[12]

In spite of the popularity of salt, some have surmised that the popular consumption of it was excessive. Jonathan Swift felt it was "an effect of luxury," Crusoe's man Friday despised it, the explorer Stefansson found he did not need it, and Bunge, who from his experimental work had reason to know, said, "We are accustomed to take far too much salt with our viands. Salt is not only an aliment, it is also a condiment and easily lends itself, as all such things do, to abuse." [13]

Two years ago the thought occurred to us that sodium chloride consumption in excess of the amount required for normal nutrition might be a factor in degenerative disease. A search of the literature reveals surprisingly little information about the chronic effects of excess sodium chloride ingestion. Chapman [1] has traced the history of our understanding

TABLE I

Composition of Diets

Basic Ration		Sodium Chloride to Make	
		I (Low NaCl)	0.01%
Cane Sugar	51.9%	II (Control)	0.15%
Casein, Vitamin-test	25.0%	III	2.8 %
Shortening, All-vegetable	20.0%	IV	5.6 %
Mineral Mixture	2.9%	V	7.0 %
Vitamins	0.2%	VI	8.4 %
		VII	9.8 %

of the rôle of sodium chloride in disease. Carrion and Hallion in 1899 produced pulmonary and peripheral edema with salt solutions. Widal and Lemierre in 1903 showed that the edema of Bright's disease was increased by giving salt. Ambard and Beaujard in 1904 were the first to observe a decline in blood pressure following salt restriction in hypertensive patients, but, like others later, they thought that the chloride rather than the sodium was the important factor. Sixteen years later, Allen found a low salt diet effective in the treatment of some patients with hypertension. Kempner [14] in 1944 reported his now well known low-sodium rice-fruit diet. Others have reported on injury due to increased dietary sodium chloride in lower animals, especially fowl, which have a primitive glomerulus, more readily susceptible to injury than that of the mammalia.[15]

Most of the reports on experimental animals included sodium chloride variations and something else, such as steroid medication, parabiosis, fluid restriction, et cetera. The few investigations on salt alone were usually short in duration, with relatively small numbers of animals. Therefore, a long-term experiment was initiated using the male albino rat. The growth and development of these rats have been reported elsewhere,[15] and a detailed

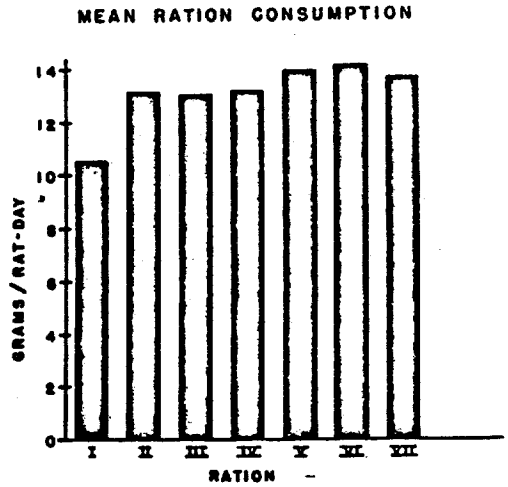

FIG. 1. Mean ration consumption for first five weeks of experiment.

report of other observations is in preparation for publication.[16] It is the purpose here to review in a general way the findings to date.

A basic purified diet was prepared containing all known nutrients in the amounts considered optimal for rats but with as little sodium chloride as possible without going to extremes. This diet by analysis contained 0.01 per cent sodium chloride by weight, perhaps twice an amount so low as to cause failure of growth, decline and death. To this basic ration finely powdered sodium chloride was added to produce six additional rations (table 1). Ration number II, the control diet, contained 0.15 per cent sodium chloride, the amount commonly used in nutritional experiments. The remaining five diets contained 20, 40, 50, 60 and 70 times this amount (2.8 per cent, 5.6 per cent, 7.0 per cent, 8.4 per cent and 9.8 per cent of

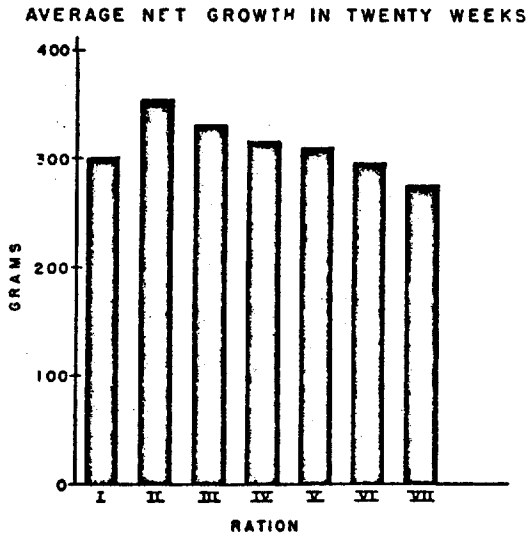

FIG. 2. Average net growth in first 20 weeks of experiment.

NaCl). Thirty male Sprague-Dawley rats were placed on each of the diets except the low-sodium diet, which group was smaller. They were housed in suspended steel wire cages at 27° C. Somewhat surprisingly, after a few days the rats were eating these rations with apparent relish almost without exception, and by the end of the second week they seemed well adjusted, healthy and happy. Those on restricted salt (Ration I) had less appetite (figure 1). The animals all gained weight, but none so well as the control group (figure 2).

Demineralized or distilled water was provided ad libitum. The rats on the higher rations of salt developed and maintained an impressive polyuria and a consequent polydipsia (figure 3). This is not a new observation. Lord Somerville[17] remarked upon increased thirst in cattle given salted

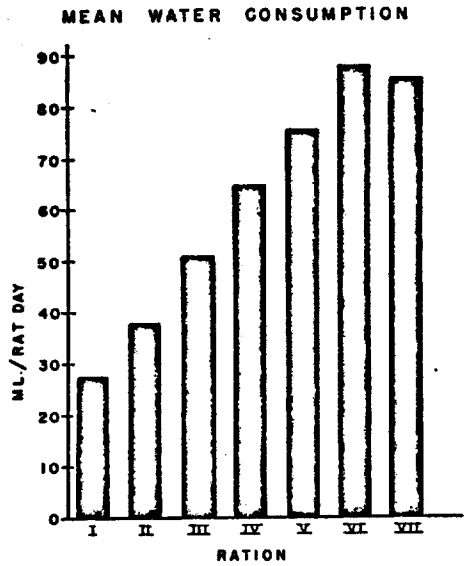

FIG. 3. Mean water consumption for first five weeks of experiment.

hay in an address to the Board of Agriculture in 1814, and it was doubtless well known long before that.

Beginning after two months and continuing thereafter for several months, episodes of massive edema began to occur among the animals eating the three highest rations of salt, that is, from 7.0 to 9.8 per cent. A total of 18 per cent of the 90 rats on these rations were so affected. In such animals, after a period of apparently normal growth an abrupt increase in weight occurred. In some animals the volume of extracellular water estimated with the radioactive isotope of sodium increased to more than twice the normal. Most of the animals who became edematous died or were sacrificed for study. A few passed through the edema phase to a dry cachectic state terminating in emaciation and death.

The clinical syndrome (table 2) exhibited by these edematous animals bore a striking resemblance to the syndrome of nephrosis seen in humans. (We particularly wish to emphasize that this is merely a resemblance. At this time the data are not nearly complete enough to reach a firm conclusion.) Besides the edema, the blood pressure was found to be elevated in most animals in which it was measured. There was a profound anemia.

TABLE II

Clinical Features of a Syndrome Occurring in 18 Per Cent of Rats Eating from 7.0 Per Cent to 9.8 Per Cent NaCl in the Diet at 27°C.

Edema	Lipemia
Hypertension	Hypoproteinemia
Anemia	Azotemia

The plasma was grossly lipemic. The blood proteins were low, and the animals were in a state of nitrogen retention, presumably due to renal failure.

Aside from the relatively small group of rats which developed edema and renal failure, the majority of the remainder of the colony continued in apparent good health, with only the attrition to be expected from intercurrent infection. Reliable blood pressure measurements on the entire colony were obtained beginning in the ninth month (figure 4). As early as this there were frankly hypertensive animals, even in the group on the lowest increased salt diet (2.8 per cent), and most of the animals on the highest ration of salt had striking elevations of blood pressure. Statistical analysis of these data shows that all groups above the control group, that is, groups III, IV, V, VI and VII, had highly significant increases in arterial blood pressures.

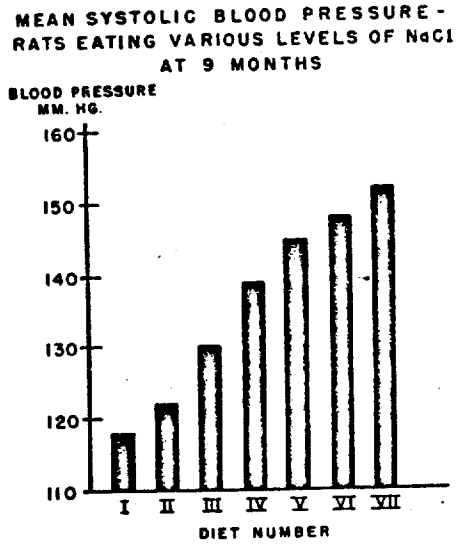

FIG. 4. Mean systolic blood pressure at nine months.

Moreover, the relationship of the blood pressure to the amount of salt in the diet was linear.

Most of these rats are still alive. Hypertension persists among the high salt groups and is increasing. Now, from time to time, instances of renal failure of a somewhat more chronic and slowly developing nature than that previously described are being encountered. In addition, a few animals have developed a syndrome characterized by dyspnea, moderate edema, cardiac enlargement and elevated venous pressure. Seemingly, this represents congestive heart failure. A few of the rats with hypertension have been subject to epistaxis and convulsions.

Some animals from groups III and IV have been sacrificed, although to date there have been no clinical manifestations attributable to hyperten-

sion among them despite the significant elevations of blood pressure. Examination by ordinary methods of the tissues of these rats, including the kidneys and adrenals, does not reveal any microscopic anatomic alterations not seen among control animals similarly sacrificed.

There are important pathologic changes among the animals eating the three highest levels of salt (V, VI and VII). The ground plan of these changes seems similar, whatever the clinical picture the animal may have exhibited. The important differences are in degree and extent of the changes, dependent presumably upon the time of onset of the change, its severity and its distribution. The earliest lesion is seen in the glomerulus, which is relatively bloodless, increased in size, degenerated and containing foam cells which on Sudan IV staining are seen to contain lipid. A more severe stage involves further glomerular deterioration and involvement of the tubules in a devastating degenerative process. The arterioles are involved early, and in advanced stages of the lesion may be seen to have undergone changes resembling those in human malignant hypertension. In such cases, arteriolosclerosis is widespread through the other organs and tissues examined. The adrenals have not revealed structural changes not seen in the control rats. In animals on high salt diets, sacrificed while in apparent good health despite hypertension, the nature of the lesion is the same but is less in degree, and the renal lesion is spotty in distribution, some nephrons apparently escaping entirely while others are severely involved.

We must be cautious in our conclusions from these data. We have demonstrated that increases in salt in the diet of the male rat produce physiologic and pathologic changes which are proportional to the amount of the increase. It is probable that as time goes on rats in groups III and IV which do not reveal microscopic anatomic changes as yet will begin to show such changes, because most of these animals have sustained hypertension. The parallel to human hypertension is of course obvious, but nothing we have shown here can be directly applied to the human without extensive further study to evaluate the sensitivity of humans to salt. Nevertheless, we can hardly fail to mention that diet III (2.8 per cent NaCl) compares roughly on a weight-for-nutrient basis with a human intake of about 12 gm. of salt per day, a value which is often regarded as the national average.

Summary

In view of the paucity of information relative to the chronic effects of sodium chloride in the diet of mammalia, a long-term study has been undertaken in the male albino rat. Purified rations, containing from 0.01 per cent to 9.8 per cent of NaCl, and water were fed at will from the age of five weeks. Diets both high and low in sodium chloride reduced the rate of growth. Water consumption was related directly to the amount of salt in the diet. A low-sodium diet depressed the appetite, but diets high in sodium chloride did not. Edema developed in 18 per cent of the rats eating

the diets which contained from 7.0 per cent to 9.8 per cent of NaCl. Pathologic lesions were observed in the kidneys and to a lesser extent in various other tissues of rats consuming these high levels of salt. Sustained arterial hypertension was observed in the rats eating high levels of sodium chloride after nine months of the dietary regimen. A linear relationship was found to exist between the NaCl concentration in the diet and the systolic blood pressure.

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ON THE TOXICITY OF POTASSIUM IONS AT DIFFERENT PERIODS OF THE ANIMAL LIFE

III. EXPERIMENTS ON MICE

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(Received for publication 23. VI. 1939)

In the present publication we communicate additional evidence concerning the age variations in the resistance to potassium ions of white mice (cf. Communication 1 in the Bulletin de Biologie et de Médecine Expérimentale de l'URSS, vol. VIII, No. 2-3, 1939).

These data concern the earliest period of animal's life (from birth up to 14 days), the period from 3-4 weeks to sexual maturity, the pregnancy and the post-parturition period.

Three hundred sixty-four experiments were carried out in all, not less than 10 experiments for each point of the curve.

The mortality of the mice subcutaneously injected at different life periods with potassium chloride in a dose of 1 mg per 1 g body weight is illustrated by three figures.

Fig. 1 (curve *1*) shows that for newborn mice and mice of the first 3 days of life the above potassium dose is not lethal. During the period comprizing the 3rd to 7th day of life 20 to 25% of mice succumb. On the 8th day mortality greatly increases amounting to 80%. On the 9th day it attains 100% and is maintained on this level very long (5-6 weeks). The days of marked decrease of resistance to potassium (8-9th) are characterized by the fact that in the mice there appears fine down and in some of them the visual function is established.

The age of more adult mice was determined indirectly by the weight.

Fig. 2 (curve 2) demonstrates the mortality of the mice weighing 5 to 16 g. The mice weighing 5-7 g (3 to 6-weeks'-old) show also 100% mortality caused by the same potassium dose. In more adult groups the death rate gradually decreases attaining a minimum in mice weighing 13 to 16 g (onset of sexual maturity), viz. 21%. In adult mice the mortality is somewhat higher, viz. 22% in females and 26% in males. Pregnancy from its very outset raises the death rate to 100% (Fig. 3, curve *3*) while as mentioned in communication 1 the embryos (dissection of the succumbed mother) prove alive; this resistance brings them close to newborn animals. During the first day after parturition the mortality is still high (60%), but already on the second day it drops to 14% being maintained on this level during about 7 days.

It was pointed out in communication 1 that at the stage characterized by a lesser resistance of the mice to potassium ions the prophylactic administration of atropine may greatly raise the resistance. In the present study we have succeeded in following up the period of life when the positive effect of atropine makes its appearance and vanishes. It was found indeed that from the third to the seventh day of life, *i.e.*, when mortality caused by 1mg potassium amounts to 20%, preliminary administration of atropine (for 10 minutes)

in a dose of 5 mg per 1 g weight does not reduce the death rate (cf. curve 4, Fig. 1). Beginning with the eighth day when the death rate caused by potassium attains 80% the atropine effect becomes quite distinct: mortality decreases from 80 to 20%, *i.e.*, by four times. In mice from 5 to 7 g weight mortality is reduced by atropine to one half, *i.e.*, to 47%, and this is also the case with mice weighing 9-11 g, viz. from 78 to 40%. In mice weighing 11-13 g there is noted almost no decrease of mortality (40 *versus* 32%), in 13-16 g mice it is not reduced at all (21-19%) (cf. curve 5, fig. 2). In both curves (4 and 5) every point comprizes 15 to 25 experiments. The relatively

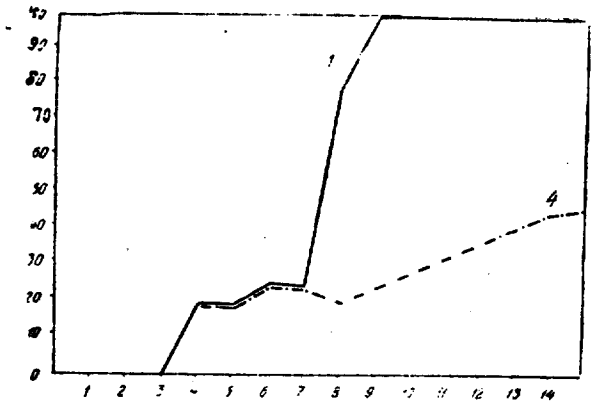

Fig. 1. Curve *1* — death rate of mice during the first days of life (KCl — 1 mg per 1 g weight), Curve *4* — same upon atropine treatment (KCl + atropine—5 γ per 1 g weight). Ordinate — death rate in per cent, abscissa — days after birth.

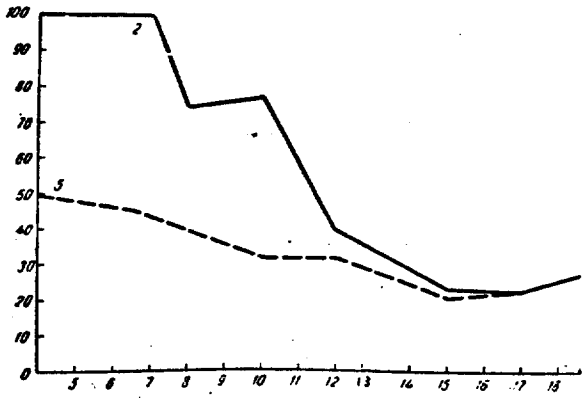

Fig. 2. Curve — death rate of mice 5 weeks prior to the end of growth (KCl — 1 mg per 1 g weight). Curve 5 — same upon atropine treatment (KCl + atropine—5 γ). Ordinate — death rate in per cent, abscissa — weight in g. After 16 g — adult mice.

lesser effect of atropine in mice aged 11 days to 5 weeks may be due to the fact that for this age 1 mg potassium per 1 g weight surpasses the lethal dose. This assumption is confirmed by the following findings. With a dose of 0.9 mg per 1 g weight which likewise gives 100% death rate atropine decreases mortality to 28%, *i.e.*, more than by 3 times, and with the dose of 0.8 mg per 1 g weight which gives 47% mortality—to 7%, *i.e.*, by more than 6 times.

Hence at the age when the mice are particularly susceptible to excess of potassium atropine treatment is most effective.

During pregnancy preliminary atropine treatment decreases the death rate by 3 times (curve *6*, fig. 3), *i.e.*, during this period the efficiency of atropine is very high (cf. Communication I).

During the post-parturition period preliminary atropine treatment caused a decrease of mortality to zero even in those cases when it was very high (60% during the first day after parturition).

treatment while suppressing one of the components of the potassium effect brings the death rate to about the same level in all the ages, viz. 20, 25, 28, 40, 32, 19, 22, 26 and 30%. (An exception is offered by the newborn animals and by the post-parturition period.)

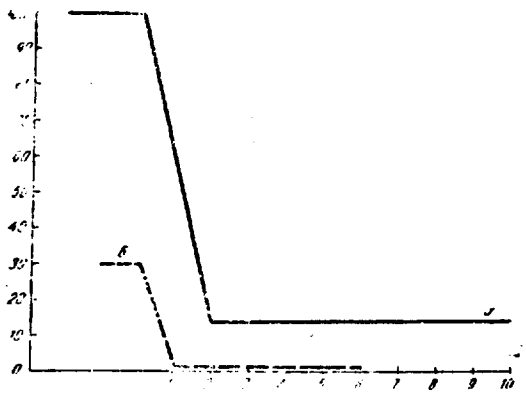

Fig. 3. Curve 3 — death rate of mice during pregnancy and after parturition (KCl—1 mg per 1 g weight). Curve 6 — same upon atropine treatment (KCl + atropine — 5γ per 1 g weight). Ordinate — death rate in per cent, abscissa — days after parturition.

Further experimental evidence concerning the factors increasing the resistivity of the organism to excess of potassium ions will be communicated in the next publication.

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Pathomorphology of Sodium Chloride Poisoning. III. Histopathological and Histoenzymatic Studies of Changes in the Kidneys

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ABSTRACT

The kidneys of swine and rabbits poisoned with sodium chloride (NaCl) were examined. The experiment was carried out in two groups, that of acute poisoning and that of chronic poisoning. In addition to histopathological examination, alkaline and acid phosphatases were detected in the kidneys.

The occurrence of marked damage, especially to further segments of the nephrons (collecting tubules), with the breakdown and atrophy of the epithelia and cystic dilatation of the lumen, was found in NaCl poisoning.

A great increase in the reaction for the enzyme which takes part in active transport i.e. for alkaline phosphatase, indicates an increased permeability of the cell membranes, and the simultaneous increase in reaction for acid phosphatase in the renal tubules suggests their impairment.

A greatly increased reaction for acid phosphatase was also found in the walls of afferent arterioles, which provides evidence of damage to the juxtaglomerular apparatus, or disturbances in the formation of renin.

З. Михальска: *Патоморфология отравления хлоридом натрия.* III. *Гистопатологические и гистоэнзиматические исследования изменений в почках.*

Исследовали почки свиней и кроликов, отравленных хлоридом натрия (NaCl). Опыты проводили в двух группах, вызывая в одной острое отравление, в другой—хроническое. Кроме гистопатологических исследований в почках находили кислую и щелочную фосфатазы. Доказано, что при отравлениях NaCl значительно повреждаются нефроны, особенно дальние отрезки (сборные канальцы), в которых наблюдается разложение и пропадание эпителия и кистовидное расширение света. Усиление реакции на фермент, участвующий в активном транспорте, т. е. на щелочную фосфатазу, свидетельствует об увеличении проницаемости клеточных оболочек, а выступающее одновременно усиление реакции на кислую фосфатазу в канальцах почки — об их повреждении.

Особенно усиливается реакция на кислую фосфатазу в стенках приносящих артериол, что указывает на повреждение приклубочного аппарата, т. е. на нарушение реакции в образовании ренина.

Sodium chloride poisoning induces disturbances in the general homeostasis of the organism by unsettling its normal fluid balance. The renin-angiotensin-aldosterone system and so-called factor III or natriuretic hormone take part in electrolyte-water regula-

tion [28**, 36**, 42**, 3, 14]. Changes in the adrenals, which produce aldosterone, following an excessive supply of NaCl were discussed in the second part of the studies on the pathomorphology of sodium chloride poisoning [9].

The purpose of the present work is to demonstrate the histopathological and histoenzymatic changes in the kidneys, which produce renin, in acute and chronic poisoning with common salt.

Investigation of enzymatic reactions in the kidneys is the objective of many of the latest experimental studies, for it reflects all the functional processes that occur in this organ. These studies are concerned, among other things, with changes in the enzymatic activities of the kidneys during transplantation [1], sudden ischaemia [15], and in experimental poisoning with carbon tetrachloride CCl_4 [7] and vapour of benzene [4].

MATERIAL AND METHODS

Kidneys removed from 25 swine and 48 rabbits subjected to acute or chronic sodium chloride poisoning were used. In addition, kidneys of 6 swine and 6 rabbits were used as controls. The data concerning the experimental animals and the scheme of experiments (dosage of NaCl, made of its administration, survival time of animals) are given in part I of this study [8].

Fragments of the kidneys were fixed in 5—10 per cent neutral formalin. Paraffin sections were stained with haematoxylin and eosin (HE). Alkaline phosphatase (AlPase) and acid phosphatase (AcPase) were detected using Gomori's precipitation method [20*]. Incubation time was 10 minutes for AlPase and 40 minutes for AcPase. Incubation without substrate was applied as control reaction.

RESULTS

HISTOPATHOLOGY OF KIDNEYS

Acute poisoning. Venous congestion occurs throughout the kidneys or only in their cortical layer. The epithelia of the convoluted tubules, Henle's loops, and collecting tubules undergo parenchymatous degeneration and at times also hyaline or vacuolar degeneration. Casts, mostly granular ones, are encountered in the lumen of damaged tubules. Further segments of the nephron (collecting tubules) are subject to plasmo- and karyolysis. However, neither in swine nor in rabbits do the degenerative changes occur uniformly, there being areas of normal structure in the kidneys. The renal glomeruli are usually unchanged or bloodless and compressed by the swollen tubules. In exceptional cases protein fluid accumulates in Bowman's capsule.

Chronic poisoning. Marked passive congestion is observed, especially in deeper layers of the kidneys. Large portions of the parenchyma show protein and hyaline degeneration accompanied by the simultaneous formation of appropriate casts. The dilatation of renal tubules and pronounced flattening of the epithelia which line them occur in the cortical and superficial medullary layers, especially in the swine. Complexes of small cysts are observed in such sections (Fig. 1). In further segments of the nephron the cells are often subject to damage and breakdown. Malpighian bodies are small and bloodless. In rabbits there are, in addition, hyaline changes in the vascular network of glomeruli and a slight swelling of Bowman's capsules.

** References marked with two asterisks are quoted in the second part of this study (Folia Histochem. Cytochem. 9, 51—76, 1971).

* References marked with an asterisk are quoted in part I of this study (Folia Histochem. Cytochem. 9, 31—50, 1971).

HISTOENZYMATIC REACTIONS

Alkaline phosphatase (AlPase)

Control animals. Cell membranes in the convoluted tubules show a weak reaction. The brush border reacts most intensely. No reaction is detectable in renal corpuscles and collecting tubules.

Acute poisoning. Reaction is distinctly intensified but non-uniform and occasionally diffuse throughout the wall of the convoluted tubule. An intense reaction occurs also in large complexes of Henle's loops and even in the collecting tubules, which are arranged in dark bands in the deep layers of the kidney. In rabbits the strongest reaction can be seen between the 12th and 24th hour of poisoning.

Chronic poisoning. In swine a fairly intense and most often diffuse reaction occurs in large portions of the deep layers of the cortex. It is, however, non-uniform and varies in intensity even within the same kidney. In rabbits, from the 10th day of poisoning onwards, the epithelia of large groups of convoluted tubules, Henle's loops, and collecting tubules show a strongly phosphatase-positive reaction. It is more intense than the reaction found in acute poisoning, but very non-uniform (Figs. 2—4).

Acid phosphatase (AcPase)

Control animals. The cytoplasm of cells of the convoluted tubules contains the largest amounts of lysosomal granulations, whereas Henle's loops, the collecting tubules, and the endothelia of glomeruli react weakly.

Acute poisoning. A greatly intensified reaction occurs in the convoluted tubules, being somewhat weaker in the loops and collecting tubules. Granulations corresponding to lysosomes are densely scattered throughout the cytoplasm of epithelial cells. An increase in the reaction is visible as early as the 6th hour of poisoning (rabbits). The vascular network of glomeruli and the first segments of afferent arterioles show, especially in swine, an intense reaction.

Chronic poisoning. The strongest reaction is observed in the deep cortex and in the superficial medullary layer. Here the reaction is often diffuse, non-uniform, and concerns the epithelia of collecting tubules. The vascular network of glomeruli reacts very intensely, this being expecially true of the walls of afferent arterioles (Figs. 5—8).

DISCUSSION

The histopathological changes in the kidneys in acute sodium chloride poisoning are not very specific. A more characteristic picture of the toxic action of NaCl will only be found in chronic poisoning, where further segments of the nephron, which are actively engaged in the exchange of electrolytes, are seriously damaged.

The microscopic changes are closely reflected by the histoenzymatic reactions of the kidneys. The reaction for alkaline phosphatase is greatly intensified and concerns both the proximal tubules (mainly in acute poisoning) and the distal ones (mostly in chronic poisoning). However, the zonal arrangement of the reacting collecting tubules indicates that not the whole population of nephrons takes part in active filtration. The reaction for

acid phosphatase presents a characteristic picture. It is very intense, includes both the proximal and distal tubules, and is arranged, like that for alkaline phosphatase, in bands or radially. In chronic poisoning the deeper layers of the kidneys react more strongly. An increased reaction is also observed in the network of vessels of the renal glomeruli, stronger in the swine than in the rabbit. A very great increase in the intensity of the reaction for acid phosphatase in the epithelial and other cells of the walls of afferent arterioles deserves special emphasis. This increase is of essential significance, since the afferent arterioles belong to the so-called juxtaglomerular apparatus (JGA), which, by the medium of the humoral renin-angiotensin system, takes part in the mechanism of auto-regulation of the blood flow in the kidney. According to PYTASZ [13], the renin-angiotensin system is activated particularly in cases of long-lasting changes in the renal blood flow, most often pathological ones.

The JGA forms an aggregation of cooperating cells. There are several types (three have been described hitherto) of these cells, which contain granules probably taking part in the formation of renin. The number of granules is larger in animals than in man and undergoes fluctuation in various morbid conditions [11]. The fluorescence method has lately been used to determine the differences in the number of JGA granules [6]. According to OLIVER and BRODY [10], prolonged anoxia induced by a decrease in the blood flow in the kidneys is the factor which stimulates the epitheloid cells of the walls of afferent arterioles to increase the production of secretory grains. None the less, the factors that stimulate the JGA to form renin are not as yet sufficiently well known. Studies carried out by WICHERT *et al.* [16] showed that no fluctuations in the number of granules of the JGA occur in the Icenko-Cushing syndrome.

The greatly increased reaction for acid phosphatase in the walls of afferent arterioles found in the present study indicates impairment to the cells of the JGA, which may well be connected with the disturbances in the production of renin and, in consequence, in the whole renin-angiotensin-aldosterone system. It is known that a diet of high salt content itself reduces or inactivates auto-regulation and lowers the renin level in the kidneys [5]. However, sodium chloride tolerance is also dependent on the specific membership of animals used for experiments, since only slight deviations from the normal state of renal function were detected in sheep and dogs during similar studies [12, 17]. This may partly have been associated with the fact that these animals were not deprived of drinking water during the experiment. It has also been observed that in animals kept for some time on a salt-free diet a stronger reaction occurs after the administration of NaCl and the simultaneous applications of substances which exert an influence on the regulation of sodium level (angiotensin II) than in control animals [2].

The degree of alkaline phosphatase activity in the epithelia of the renal tubules indicates a great intensity of active transport through the cell membranes. A marked rise in this reaction in sodium chloride poisoning gives evidence of a raised level of renal metabolism in connection with the intense exchange of electrolytes. The distal tubules are particularly greatly involved in this exchange. A simultaneous lack of reaction in large portions of the renal parenchyma may suggest that some of the nephrons do not take part in active filtration and that the high sodium chloride concentration damages the cell membrane. This damage, affecting especially the distal tubules, can well be seen in the

microscopic images. The great increase in the reaction for acid phosphatase also points to the intensification of katabolic processes in the renal tubules. The injury to the lysosomal membranes is observed mainly in the distal tubules and the walls of afferent arterioles, as has been mentioned above.

The occurrence of diffuse reactions for alkaline and acid phosphatases and also the intensification of these reactions in chronic poisoning indicate that the prolonged administration of sodium chloride leads to severe disturbances in the homeostasis of the organism, which disturbances find expression in morphological changes within the kidneys.

CONCLUSIONS

1. Chronic poisoning with sodium chloride brings about marked impairment to the kidneys, especially to their further tubules. The breakdown and atrophy of the cells of the epithelium and the cystic dilatation of the lumen of changed tubules occur in the microscopic images.

2. In sodium chloride poisoning the intensity of reactions for alkaline and acid phosphatases increases in the deeper layers of the kidneys.

3. A great increase in reaction for acid phosphatase in the walls of afferent arterioles suggests damage to the cells of the juxtaglomerular apparatus and, consequently, disturbances in the formation of renin.

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EXPLANATION OF FIGURES

Fig. 1. Swine kidney. Chronic poisoning (34th day). Groups of small cysts arising from dilated renal tubules (HE). × 100.

Fig. 2. Control swine kidney. Reaction for alkaline phosphatase visible in brush border of convoluted tubules. × 100.

Fig. 3. Swine kidney. Chronic poisoning (28th day). Remarkably increased and diffuse reaction for alkaline phosphatase throughout the walls of convoluted tubules. × 100.

Fig. 4. Rabbit kidney. Chronic poisoning (21st day). Increased and non-uniform reaction for alkaline phosphatase in collecting tubules of superficial medullary layer. × 60.

Fig. 5. Control swine kidney. Weak reaction for acid phosphatase in convoluted tubules and vascular network of renal glomeruli. × 160.

Fig. 6. Swine kidney. Acute poisoning (36 hours). Increased reaction for acid phosphatase in the cytoplasm of cells of convoluted tubules. × 160.

Fig. 7. Swine kidney. Acute poisoning (101 hours). Strongly increased reaction for acid phosphatase in the vascular network of the glomerulus and the wall of the afferent arteriole. × 160.

Fig. 8. Swine kidney. Chronic poisoning (34th day). Very intense reaction for acid phosphatase in the wall of the afferent arteriole. × 250.

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PATHOLOGIC FEATURES OF EXPERIMENTAL SODIUM CHLORIDE POISONING IN CHICKS

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SUMMARY

Pathologic changes in young chicks poisoned by sodium chloride were studied. Water consumption increased gradually and was accompanied by marked deterioration in condition and anorexia. Most chicks exhibited nervous disorders. By the 3rd week, the mean average weight of the surviving experimental chicks was only about half that of the control group. All chicks had poor musculature, edematous subcutaneous tissue, and variable amounts of fluid in the pericardial sac, thoracic cavity, and peritoneal cavity. Microscopic changes in kidneys, liver, spleen, pancreas, adrenals, eosophagus, crop, proventriculus, small intestine, lungs, heart, skeletal muscles, and central nervous system included degeneration with very little inflammatory reaction. Encephalomyopathy consisted of neuronal vacuolation, neuronal degeneration and necrosis, perineuronal and periglial edema and proliferation of oligodendroglial cells. Eosinophilic meningitis, primarily involving the lumbar segment of the spinal cord, was observed in only 4 cases. The cellular reaction was principally eosinophilic, with some lymphocytes, large mononuclear cells, and plasma cells. Vacuolar and necrotic changes were also seen in the ganglionic cells of the adrenal medulla.

Chickens, especially baby chicks, among other domestic fowls, are susceptible to salt (sodium chloride) poisoning (1,3,5,6,8,9,11, 12). Clinical signs include muscular weakness, diarrhea, nervous disorders and respiratory distress (3,6,11). Cardiac hypertrophy

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and enlarged, congested kidneys with marked glomerular hypertrophy have been seen in chicks as a result of sodium chloride toxicity (3,5,8).

In the many studies of sodium chloride poisoning in poultry, little attention has been given to the occurrence of histopathologic changes in different tissues, especially in the central nervous system (CNS) and ganglionic cells associated with visceral organs. Reported here are pathologic alterations, including neuropathologic changes induced by saline-water intoxication of young chicks.

MATERIALS AND METHODS

One-day-old chicks of the Babcock strain were maintained on an unmedicated commercial pellet-type feed containing 0.25% sodium chloride throughout the experiment. Thirty-two of them were divided at two weeks old into an experimental group of 26 and 6 controls. After they were weighed, the experimental group was given 1% sodium chloride (Carey Ev'r-Fl. Salt, refined, 95% purity, Carey Salt Co., Hutchinson, Kansas) in distilled drinking water for 10 days, and then 1.5% sodium chloride for the remainder of the 6-week experimental period. Control birds were given distilled water throughout the experiment. Otherwise, both groups were maintained under identical conditions. The birds were observed at least twice daily. Surviving birds were weighed two weeks after the experiment began, and packed cell volume was determined with heparinized microhematocrit capillary tubes. Tissues of birds found dead, or decapitated when moribund, were collected and fixed in 10% neutral buffered formalin as follows: esophagus, crop, gizzard, proventriculus, small intestine, liver, pancreas, spleen, kidneys, adrenals, heart, muscles from thigh, especially musculus iliotibialis, and breast, trachea, lungs, brain, and spinal cord. The brains were sectioned transversely at 3 different levels: a) through the mid-portion of the forebrain; b) tangentially through the mid-portion of the cerebellum, passing through the optic lobes; c) caudal to the cerebellum and posterior to the origin of the 12th cranial nerve. Transverse sections were made of the spinal cord at 3 levels: a) center of the cervical region; b) middle of the thoracic enlargement; and c) lumbar region just anterior to the rhomboidal sinus. Two control birds were killed at 2 weeks and the remainder at 6 weeks, and identical tissues were collected. Tissues preserved in buffered formalin were dehydrated, embedded in paraffin, sectioned at a thickness of 5-6 μ and stained with hematoxylin and eosin (H & E) for histopathologic studies.

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RESULTS

Clinical signs. The experimental group was dull and depressed at 48 hours; water intake was markedly increased, and feed consumption was slightly reduced. After 48 hours, water intake increased, body condition deteriorated markedly, and anorexia was apparent. Most of the treated chicks huddled together under the heat source and chirped continuously; some had such nervous signs as convulsions, leg-weakness, and incoordination. The feces of all were watery. There was no marked difference in the mean hematocrit value between the experimental and control groups except that one treated bird had a level of 50% (Table 1). In the 3rd week, the mean average weight of surviving experimental chicks was only about half that of the control group (Table 1). Four chicks died during the 1st week, 8 in the 2nd week, and 2 in the 4th week. Five

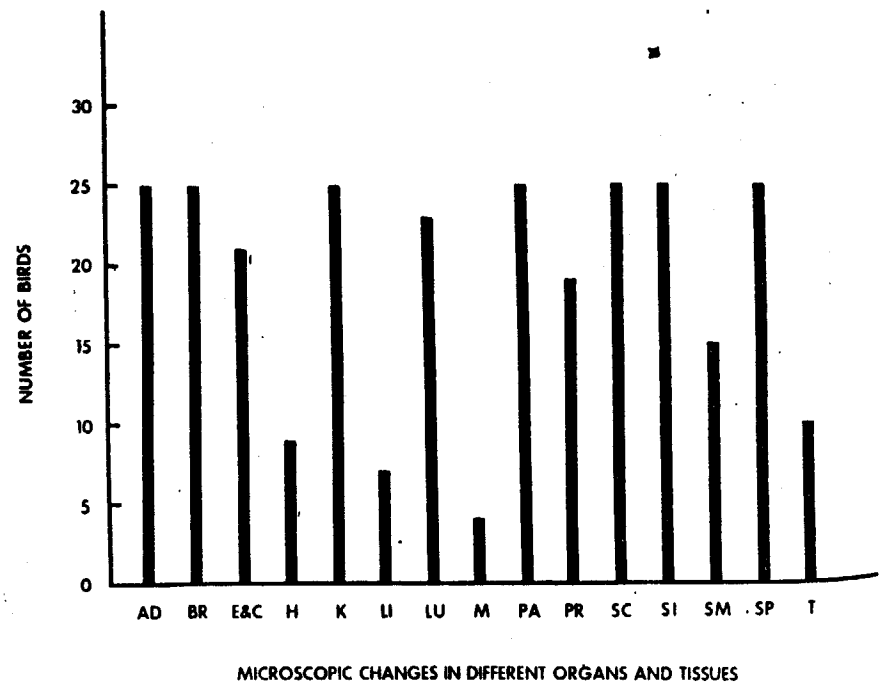

Fig. 1. Graphic representation of birds with microscopic changes in indicated organs and tissues.
Key: AD, adrenals; BR, brain; E & C, esophagus and crop; H, heart; K, kidneys; LI, liver; LU, lungs; M, meninges; PA, pancreas; PR, proventriculus; SC, spinal cord; SI, small intestine; SM, skeletal muscles; SP, spleen; T, trachea.

Table 1. Weight and hematocrit values of experimental and control chicks.

Sl. no.	Weight (g) 2 weeks old	Weight (g) 4 weeks old (2 weeks PE[A])	Hematocrit value 4 weeks old (2 weeks PE)
Experimental group			
1	122	115	30%
2[B]	130	122	50%
3	127	115	38%
4	125	126	36%
5	113	135	36%
6	124	131	41%
7	123	112	32%
8	119	133	40%
9	115	136	35%
Mean av.	125	122	37.55%
Control group			
1	120	262	36%
2	121	220	37%
3	132	210	39%
4	117	268	39%
5	128	274	37%
6	118	257	35%
Mean av.	122.66	242.5	37%

[A]PE (postexperimental period).
[B]Comatosed state.

moribund chicks were destroyed in the 2nd, 1 in the 3rd, 4 in the 5th, and 2 in the 6th week.

Macroscopic changes. Gross changes were almost identical in most experimental chicks except for minor individual variations. All the carcasses were emaciated and the musculature was congested or slightly edematous and the subcutaneous tissue was edematous. The pericardial sac, thoracic cavity and peritoneal cavity contained variable amounts of clear fluid. Chicks destroyed in the 5th and 6th weeks had little generalized edema. Gross alterations, as previously reported (3,5,8,11,12), were observed in heart, lungs, kidneys, crop, proventriculus, and intestines and consisted of pale and dilated heart, edematous lungs, pale and enlarged kidneys with red mottling and mucosal thickening of the crop. The mucous membranes of the proventriculus and intestine were pale with occasional petechiae and there was mucus in their lumina. The pancreas was slightly congested in some chicks and pale in others, with scattered chalky-white foci. The adrenals were enlarged in most chicks, and the cerebral hemispheres and cerebellum were slightly swollen in all. No gross abnormalities were noted in the control chicks.

Histopathologic changes. Figure 1 is a graphic representation of the birds with microscopic changes in different organs and tissues. One chick with postmortem autolytic changes was not included.

Esophagus and crop. The mucosa of the crop was slightly to

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moderately thickened and, in a few cases, mild hyperkeratosis and parakeratosis were seen. Early evidence was observed of mucous degeneration of the esophageal glands.

Proventriculus. Focal areas of desquamation of lining cells, with evidence of regeneration, were observed in most cases, and granular degeneration with hydropic changes in others. In a few cases, the mucosal lining of the isthmus and proventriculus had mucous degeneration. The lumina of some of the proventricular glands were distended with eosinophilic granular material. Isolated lining cells were hydropic. Mucous degeneration was noticed in some proventricular glands, and the lumina of some were partly filled with mucus. The lamina propria and submucosa were edematous in a few cases, but infiltration of a few heterophils into lamina propria and submucosa was observed in only 4 chicks.

Small intestine. Mild to moderate changes were noticed only in the duodenum, and catarrhal duodenitis was seen in a few cases. Cloudy swelling and hydropic degeneration was evident in some, while a few others had mucous degeneration of lining cells and cells of the duodenal glands. Seen in some others were coagulative necrosis of isolated cells to focal areas of coagulative necrosis, focal desquamation, and regeneration of lining cells with heterophilic infiltration into the lamina propria and submucosa. Lymphocytic infiltration admixed with large mononuclear cells and a few plasma cells in the lamina propria and submucosa, was observed in a few cases; the mucosal lining was intact, with evidence of regeneration.

Liver. Changes were focal. Scattered isolated cells had albuminous degeneration and vacuolation. In a few focal areas, the sinusoids were distended with blood, and the interstitial tisues were edematous. In a few cases, there were scattered, isolated necrotic cells, with a few heterophils accumulated in the sinusoids. The lining cells of a few bile ducts had focal areas of desquamation, with vacuolar changes in isolated cells.

Pancreas. In comparison with controls, there was a paucity of islets of Langerhans in a few sections, and vacuolar degeneration was noticed in a few cells of the remaining islets. Coagulative necrosis of isolated cells of some pancreatic acini was found in many chicks. A few acinar cells had vacuolar changes and some vacuoles contained eosinophilic granular debris.

Kidneys. Glomeruli were hypertrophic and were associated with hypertrophy of endothelial cells and epithelial cells of the visceral layer of Bowman's capsule. Lining cells of the convoluted tubules had albuminous degeneration which, in many cases, was

accompanied by hydropic changes. The changes were prominent in the proximal convoluted tubules. Coagulative necrosis was evident, affecting isolated lining cells in a few cases, to extensive areas with desquamation in others. Lumina of some tubules contained eosinophilic granular debris. Hydropic changes and cloudy swelling were seen in the epithelium of collecting tubules. Intratubular mineralized foci were observed in a few sections.

Adrenals. Hydropic changes were evident in the cytoplasm of isolated cells in the cortex and medulla, with coagulative necrosis of a few scattered cells. Intracytoplasmic vacuoles of variable sizes, which almost destroyed the cytoplasm in many cells, were noticed in the ganglionic cells of the adrenal medulla (Fig. 2). The cytoplasm of some cells had a cribriform appearance from many vacuoles (Fig. 3). The changes were associated with edema of the interstitial tissue, especially around the cells. Many cells were apparently destroyed, which resulted in vacuolated spaces occasionally filled with necrotic debris. Many ganglionic cells were in various stages of necrosis.

Spleen. Prominent changes in the spleen included lymphoid depletion, disorganization of lymphoreticular tissue and proliferation of reticulo-endothelial cells. Many blood vessels, primarily capillaries and muscular arteries, were seen in many birds. The blood

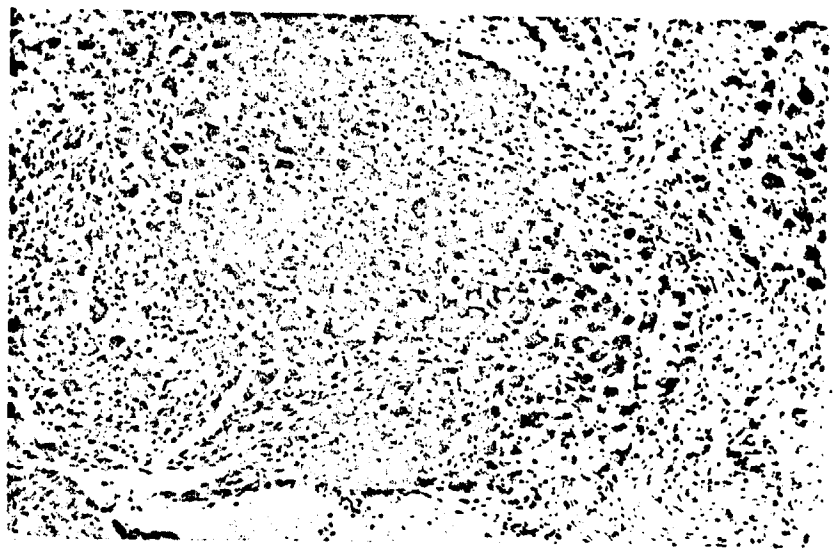

Fig. 2. Intracytoplasmic vacuoles of variable sizes in the ganglionic cells of the adrenal medulla. H & E stain; ×125.

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vessels were often surrounded by more reticuloendothelial-type cells than in control birds.

Heart. No significant changes were noticed in most chicks; however, one had a few foci of Zenker's degeneration associated with focal areas of sarcolemmal cell proliferation, and two had interstitial myocarditis, manifest by infiltration of a few heterophils. Mild interstitial edema was seen in a few chicks.

Skeletal muscles. Cloudy swelling and Zenker's degeneration were evident in focal areas involving isolated muscle fibers. In a few cases, isolated muscle fibers were fragmented, often associated with focal areas of sarcolemmal proliferation. In most cases, the interstitial tissues were edematous, with some focal separation of muscle fibers. Focal areas of mineralization were observed in muscles of one bird.

Trachea. Increased mucous secretion was observed in some chicks. Tracheal lining cells in some had vacuolation with mucinous material adhering to the lining cells in focal areas. Scattered foci of heterophilic infiltration were seen in the lamina propria and submucosa of a few chicks.

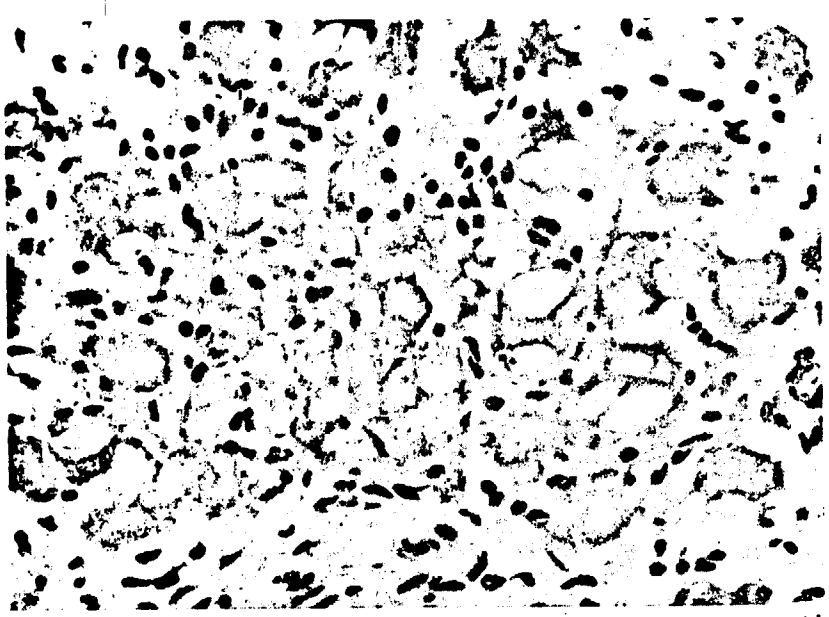

Fig. 3. Cytoplasm of many ganglionic cells of the adrenal medulla with cribriform appearance due to multivacuolation. H & E stain; ×500.

Lungs. Mild edema was evident in the interstitial tissue associated with proteinaceous material and some threads of fibrin. Alveolar ducts and alveolar spaces in many chicks contained proteinaceous material admixed with a few threads of fibrin. Mucous degeneration was seen in the lining cells of the secondary bronchi in a few chicks.

Forebrain. Changes were mainly limited to neurons in the region of hyperstriatum accessorium and included intracytoplasmic vacuoles of various sizes (Fig. 4). In a few cells, the nuclei were eccentric and hyperchromatic. A few neurons had a cribriform appearance due to multivacuolation in which the cytoplasm was reduced to narrow bands forming outer margins of the intracytoplasmic vacuoles (Fig. 5). A few neurons were apparently destroyed, leaving empty vacuolar spaces (microcysts). Some of the microcysts might have resulted from oligodendroglial mucocytes. Perineuronal edema was evident in many sections. The intersititial tissue of the forebrain appeared edematous and was accompanied by proliferation of glial cells which were primarily oligodendroglia. Edema was evident around most of the oligodendroglial cells. Edema of the Virchow-Robin's spaces was seen in many birds.

Cerebellum. Edema was evident in Purkinje's cell layer and white matter of the cerebellum. The Purkinje's cells contained

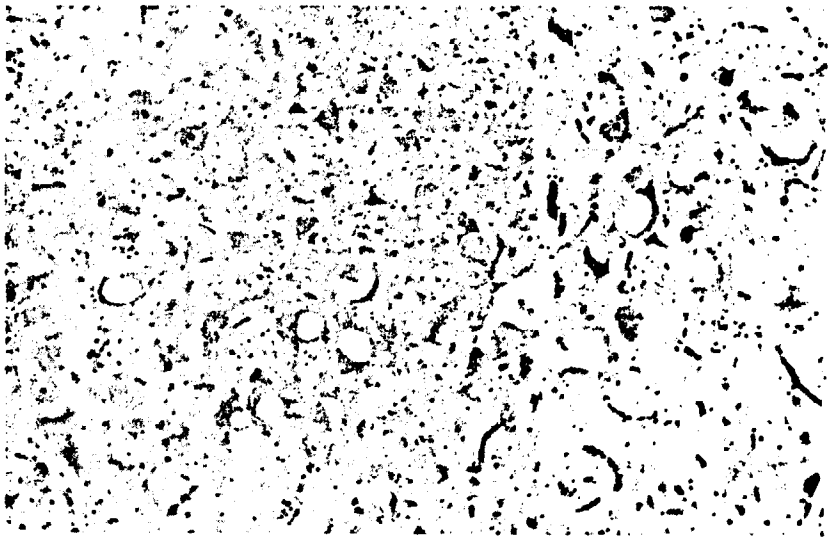

Fig. 4. Cytoplasmic neuronal vacuolation and presence of microcysts in the forebrain. H & E stain; ×125.

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intracytoplasmic vacuoles. A few Purkinje's cells were necrotic and few in number in focal areas. Some neurons in the region of the cerebellar nuclei had perineuronal edema and intracytoplasmic vacuoles. Pyknosis was noticed occasionally in isolated Purkinje's cells of the cerebellum of the control birds.

Changes in the brain stem, pons, and medulla oblongata were identical to those described for the forebrain.

Spinal cord. Changes were noticed in the three regions of the spinal cord, i.e., the cervical, thoracic, and lumbar. Edema was evident in both gray and white matter. Many neurons contained vacuoles in their perikaryons. The cytoplasm in a few neurons was destroyed by multivacuolation, and a few neurons were necrotic. A few microcysts containing granular eosinophilic debris were observed. Edema around the neurons and glial cells, especially the oligodendroglia, was evident. Proliferation of oligodendroglia was marked in the edematous areas. Perivascular edema and edema of Virchow-Robin's spaces were seen in many areas.

Meninges. The meninges were edematous. Eosinophilic meningitis was seen in only 4 chicks and involved the lumbar region. The thoracic segment of the spinal cord was also involved but in only one chick with eosinophilic meningitis. The inflammatory reaction of the meninges was characterized by numerous eosinophils

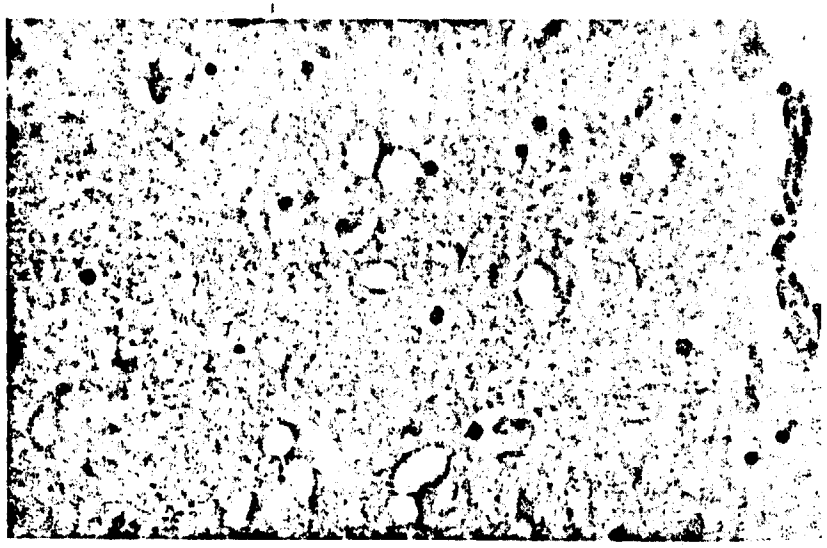

Fig. 5. Neurons with multivacuolation and narrow bands of cytoplasm between the vacuoles in the forebrain. H & E stain; ×500.

admixed with a few mononuclear cells, i.e., lymphocytes, histiocytes, and plasma cells (Fig. 6). The relative proportion of the various mononuclear cells varied in different areas, with lymphocytic cells being more numerous. The cellular reaction extended to the Virchow-Robin's spaces and, in a few areas, the perivascular spaces were distended by the infiltration of the inflammatory cells (Fig. 7).

DISCUSSION

Our results indicate that clinical manifestations and gross pathologic changes in chicks affected with salt intoxication are almost identical to those reported previously (1,3,5,6,8,9,11). Degenerative changes were present in kidneys, liver, spleen, pancreas, adrenals, esophagus, crop, proventriculus, small intestine, lungs, heart, striated muscles, and central nervous system. Similar histopathologic changes have been reported in kidneys, liver, heart, and lungs by other workers (3,5,8). Degenerative changes, characterized by edema, albuminous degeneration, focal areas of coagulative necrosis, and vacuolar degeneration were also observed in various organs. Inflammatory reaction was minimal and appeared to be

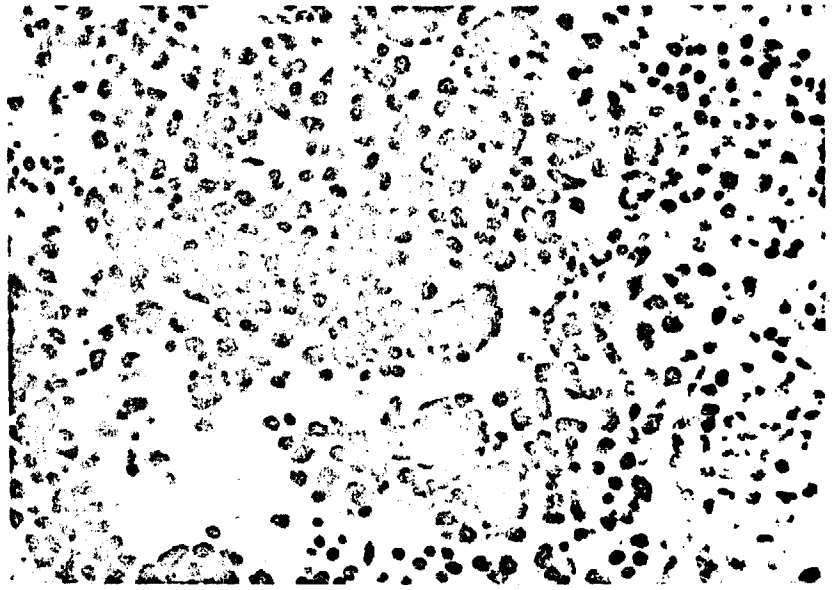

Fig. 6. Eosinophilic meningitis in the lumbar segment of the spinal cord with many eosinophils admixed with a few lymphocytes, histiocytes, and plasma cells. H & E stain; ×500.

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secondary to cellular degenerative changes. The many capillaries seen in the spleen apparently resulted from lymphoid depletion, which made the vessels more prominent than proliferating capillaries. The encephalomyelopathic changes in the brain and spinal cord were characterized by perineuronal, periglial, and perivascular edema. Intracytoplasmic vacuoles were detected in neurons of brain, spinal cord, and ganglionic cells of the adrenal medulla. Some neurons had a cribriform appearance due to multivacuolation, and a few were degenerated and necrotic. Regressive lesions in nerve cells of the brain and ganglions due to salt toxicity in pigs and rats have been reported (2,4,7,13, 14).

Our findings indicate encephalomyelopathic changes in the central nervous system and regressive changes in the ganglionic cells of affected chicks. The changes seemed to be rather constant compared with changes in other tissues. Eosinophilic meningitis occurred in only 4 chicks (15.4%). The reaction was limited to the lumbar region of the spinal cord except that it also involved thoracic segment in one chick. Eosinophilic meningoencephalitis is reported as a frequent finding in pigs affected with sodium-chloride intoxication (4,13,14). Observations by Trainer and Karstad (15)

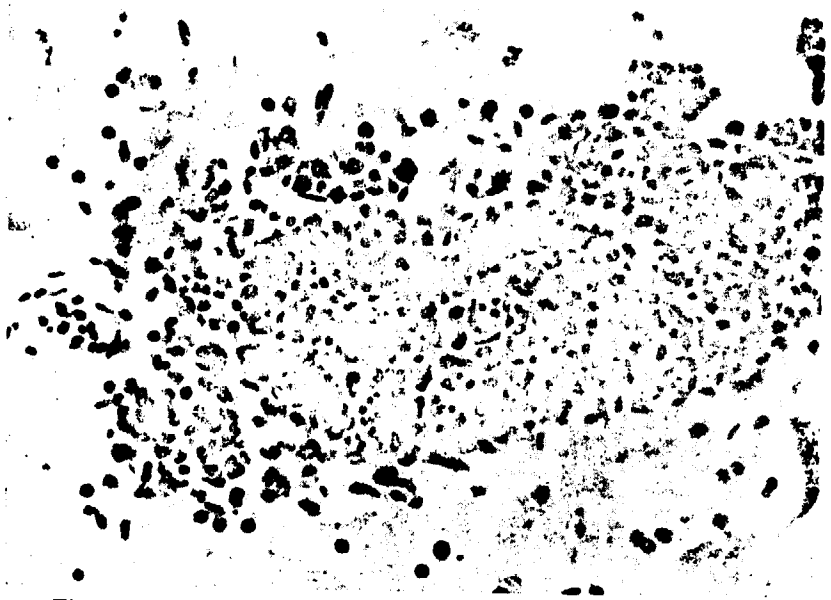

Fig. 7. Eosinophilic spinal meningitis with cellular reaction extending into the Virchow-Robin's space. H & E stain; ×500.

indicated the irregular presence of eosinophils in the meninges of sodium-chloride-intoxicated pheasants and rabbits. We found that eosinophilic meningitis also occurs in salt-intoxicated chicks.

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