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ARTERIAL HYPERTENSION *

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The great importance of arterial hypertension or the conditions associated with it, as a widespread and apparently increasing cause of disability and death, is a self-evident incentive to investigation. At the same time it must be recognized that our knowledge of the subject is in a very confused state, as respects its phases of etiology, mechanism and treatment. Just as with diabetes, the literature contains almost every conceivable variety of opinions and suggestions. The first task of an investigator to choose is what seems significant and valuable among this mass of conflicting material, and the second is to build concrete facts with accurate methods in a clinical field which is still largely a morass of tradition and imagination.

THEORIES REGARDING THE CAUSES OF HYPERTENSION

More or less restriction of salt has been employed for many years by some observant clinicians in the treatment of the various forms of nephritis. The first scientific foundation of both theoretical and practical importance is afforded by the work of the French school, beginning with Widal's demonstration of the rôle of the chlorids, particularly in the causation and treatment of edema, and with the application of this principle to hypertension by Ambard,[1] Combe,[2] Laufer[3] and others. Similar results were obtained in arteriosclerosis by Bayer;[4] but with only a few exceptions the tests with nephritis in Krehl's clinic were negative or doubtful, and the general German experience was opposed to a strict relationship between retention of chlorids and elevation of blood pressure.[5]

In American literature, salt and fluid restriction in hypertension is mentioned casually and incompletely if at all, and the protein intoxication theory is undoubtedly the dominant one. Treatment consists chiefly in low protein diets, the elimination of supposed toxins, or the artificial reduction of pressure by drugs, bleeding, electricity, and the like. Mental and bodily rest is advised to a degree which largely terminates usefulness in life, and many conservative practitioners refrain from any serious attempt to reduce the pressure, and devote themselves to keeping the patient as comfortable as possible, with resignation as to the results.

There are various reasons for this confusion and pessimism. In the German work the cases were not always well chosen and the restriction of salt was generally inadequate, though this work does furnish evidence against any invariable association of hypertension and gross chlorid retention. On the other hand, whether or not protein has any influence on blood pressure, it is impossible to accept Ambard's assertion that it is harmless in nephritis. Ambard considers fluid restriction unnecessary, and in adopting milk diet he comes back to the identical therapeutic program used by the followers of the intoxication hypothesis. The fluid restriction of Laufer, who accepts von Noorden's limit of a liter daily without reckoning the important additional quantities contained in the food, is too lax for the worst cases. Likewise a much stricter exclusion of salt than represented by milk diet is essential for results in really severe cases. These inaccuracies are responsible for an unnecessarily high percentage of cases of absolutely or relatively irreducible hypertension in French, German, American and all other records, and also for the unnecessarily frequent and early deaths from apoplexy, cardiac failure, pulmonary edema and convulsions (misnamed uremic). For the same reason there have been no differences in therapeutic results sufficiently marked to establish any one theory to the satisfaction of all parties.

RESTRICTION OF WATER AND SALT

It would be wrong to ignore the unknown and uncontrollable factors in hypertension and the unsolved problems which they present. But any practitioner who will conform his treatment to one reasonable and definite condition, namely, the necessity of the organism to force a filtrate of water and dissolved substances through a damaged and partially blocked glomerular filter, can readily demonstrate for himself the compensatory element in the hypertension, by observing the fall in pressure and the relief of some attendant symptoms when proper diet reduces the quantity of this filtrate to a minimum. The opposite treatment under the intoxication hypothesis, through unsuitable diets and attempts at "flushing out," entails much harm. In some hypertension cases the mere reduction of the overload of salt and water brings relief which is comparable to the benefits of diet in diabetes.

Fuller laboratory study, particularly by blood analyses, contributes new information of theoretical, prognostic and therapeutic importance. Among the nephritic cases treated in our clinic to date, there have been twenty examples of marked hypertension. Most of these were treated in private hospital status, but four were outpatients. The observations are necessarily empiric, and are subject to modification from wider clinical experience, and particularly to more fundamental and decisive interpretations when animal experiments become available. Numerous data of these cases, function tests according to Ambard's principles, and details of individual histories and results, must be reserved for a more complete publication at a later date.

It may suffice here to state that the patients' ages ranged from 25 to 69 years, with the usual predominance of those past 40. All the cases were of chronic type. Their severity was mostly too great to yield to mere rest and hospital care, and some were known as highly severe cases which had proved intractable to a variety of customary treatments for several years. Bleeding was used temporarily in three critical cases, and brief experiments have been tried with some other measures; but in general the patients were not kept in bed or confined to the house but were treated entirely by diet.

To establish the general principle, the effects of sudden dosage with salt or water in suitable cases are illustrated by two experiments.

In the first experiment (Table 1), attention may be called to the absence of real retention of either salt or water (contrary to the French reports), and to the

* Read before the American Society for Experimental Pathology, Cincinnati, Dec. 30, 1919.

* Preliminary communication, based on a clinical study in collaboration with Drs. J. W. Mitchell and J. W. Sherrill.

1. Ambard, L.: Thèse de Paris, 1905; Semaine méd. 26: 361, 1906.
2. Combe, A.: Monatsschr. f. Kinderh. 4: 13, 82, 1905.
3. Laufer, R. J.: Compt. rend. Soc. de biol. 56: 249, 1904.
4. Bayer, R.: Arch. f. exper. Path. u. Pharmakol. 57: 162, 1907.
5. Loeb, A.: Deutsch. Arch. f. klin. Med. 85: 348, 1905-1906. Brodski, J.: Berl. klin. Wchnschr. 43: 906, 1906; Deutsch. Arch. f. klin. Med. 93: 310, 1908. Lowenstein, C.: Arch. f. exper. Path. u. Pharmakol. 57: 137, 1907.

remarkably high plasma chlorid figures [6] and the elevation of blood pressure accompanying these.

The second experiment (Table 2) was performed on a different patient. The plasma chlorid percentage was not high, and fell lower with the water drinking, while the blood pressure rose markedly.

The first patient received salt, but showed no effect from a corresponding test with water alone. Because of the close relations between salt and water, it is

TABLE 1.—SALT TOLERANCE TEST, SEPT. 25, 1919

Time	Water Given, C.c.	Urine Vol., C.c.	Urine Sp. Gr.	Urine NaCl %	Urine NaCl,* Gm.	Plasma Chlorids Mg. per 100 C.c.	Blood Urea Mg. per 100 C.c.	Blood Pressure Systolic	Blood Pressure Diastolic
8 a.m.	200	...	.....	....	....	...	....	202	104
9 a.m.	200	65	1.011	0.72	0.47	...	....	...	...
10 a.m.	200	410	1.006	0.30	1.21	...	....	...	...
11 a.m.*	200	505	1.007	0.34	1.63	672	19.6	200	100
12 m.	200	120	1.008	0.44	0.53	672	19.6	198	91
1 p.m.	200	160	1.015	1.15	1.84	684	19.6	194	98
2 p.m.	200	105	1.016	1.21	1.27	676	....	224	100
3 p.m.	200	120	1.016	1.30	1.55	704	....	242	110
4 p.m.	200	210	1.007	0.90	1.83	...	....	266	110
5 p.m.	200	500	1.007	0.41	2.20	...	....	238	100
6 p.m.	200	250	.....	0.60	1.49	648	....	210	100
6 p.m. to 8 a.m.	550	830	1.009	0.41	3.40	644	19.6	182	80
Total	2,750	3,275			16.87				

* Ten grams of sodium chlorid were given at 11 a. m.

difficult to distinguish accurately between their effects, and space requirements necessitate leaving other tests to a later publication. The rise in pressure in both cases was accompanied as usual by the subjective symptoms of headache, dizziness, weakness and general malaise. Precautions were taken so that no patients suffered harm from such tests, but in severe cases there is danger that the reckless administration of either salt or water may cause alarming symptoms and even death. The accuracy of the tests is often interfered with by a protective diarrhea which also prevents dangerous disturbances.

The results of a short test may be negative in some patients or at some stages, and bad effects may become

TABLE 2.—WATER TEST, NOV. 19, 1919

Time	Water Given, C.c.	Plasma Chlorids, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c.	Blood Pressure Systolic	Blood Pressure Diastolic
8:30 a.m.	.....	571	30.2	215	130
8:40 to 9:20 a.m.	1,000	...	....	...	...
9:25 a.m.	.....	...	....	226	128
9:20 to 9:50 a.m.	600	...	....	...	...
9:55 a.m.	.....	...	....	245	130
10:00 to 11:10 a.m.	1,400	...	....	...	...
11:15 a.m.	.....	...	....	238	140
11:20 a.m. to 12:20 p.m.	800	...	....	...	...
12:30 p.m.	.....	543	23.7	254	120
2:25 p.m.	.....	...	....	220	125
4:00 p.m.	.....	...	....	188	100
5:00 p.m.	.....	...	....	190	100
9:30 a.m.	.....	...	....	200	120

evident only after some days. The effects of salt are in general more powerful and obvious, as demonstrated by the success of Ambard and others in numerous cases. A very close restriction of water may be impracticable at the beginning of treatment, because of the salt stored in the patient's body and the inadvisability of imposing any real suffering from thirst. Also in many cases no rigid fluid restriction is necessary, and moderate quantities (from 1 to 3 liters daily) may be actually beneficial for flushing out salt or nitrogen. But the prolonged combination of excessive fluid and little salt possibly involves osmotic and eliminative difficulties which are responsible for the anasarca, pulmonary edema and cardiac failure sometimes described in connection with long continued salt-poor diet.

No rigid classification, either anatomic or functional, of the cases listed in Table 3 was attempted, partly because the experience is insufficient, and partly because every case of nephritis is in some respects an individual problem, in which tests of the efficiency of different functions should be made and individual treatment prescribed accordingly. Different tests will give different classifications, and there are presumably types of cases not shown in this series. The phenolsulphonephthalein excretion is given here as one indication of renal action, but the complete publication will include other function tests. The data shown are otherwise limited to those which seemed most essential for treatment and prognosis. The order of cases is not merely consecutive or accidental, but forms a series based on these data.

TABLE 3.—EFFECTS OF DIETETIC TREATMENT IN TWENTY CASES OF HYPERTENSION

Case No.	Phenolsulphonephthalein Excretion in 2 Hrs., %	Before Treatment: Plasma Chlorids, Mg. per 100 C.c.	Before Treatment: Blood Urea, Mg. per 100 C.c.	Before Treatment: Blood Pressure Systolic	Before Treatment: Blood Pressure Diastolic	After Treatment: Plasma Chlorids, Mg. per 100 C.c.	After Treatment: Blood Urea, Mg. per 100 C.c.	After Treatment: Blood Pressure Systolic	After Treatment: Blood Pressure Diastolic
1	....	624	29.2	208	...	569	28.0	166	98
2	34.5	610	27.1	(300) 215	170	588	20.0	134	70
3	51.0	696	21.8	240	110	541	24.5	140	80
4	42.8	616	19.8	210	120	586	21.2	140	95
5	36.0	620	20.5	240	110	509	10.4	166	75
6	....	690	23.5	220	140	556	23.5	178	98
7	46.0	698	32.2	195	92	501	26.9	162	80
8	18.0	613	30.7	230	98	550	28.5	110	80
9	....	611	32.6	220	70	576	16.2	130	66
10	41.3	612	47.0	220	110	555	31.4	154	80
11	4.0	556 (625)	60.4	212	104	556	52.6	156	80
12	30.2	664	94.4	210	120	598	26.4	172	100
13	....	659	104.5	198	100	545	62.5	90	60
14	5.0	648	131.0	214	170	537	42.2	140	100
15	....	597	27.1	190	92	597	....	108	70
16	38.2	569	36.4	190	90	595	14.6	165	100
17	....	534	16.7	210	100	553	56.2	180	95
18	27.4	576	55.0	235	115	543	23.7	170	98
19	12.1	548	58.2	(205) 215	170	505	18.0	180	120
20	64.8	565	21.0	254	190	559	19.4	180	120

* Parentheses in the systolic pressure column indicate that the pressure in these cases was repeatedly near 300 m.m., but the actual pressure on beginning dietetic treatment was 215. In Case 19 this reduction had been accomplished only by heavy bleeding in another hospital.

CASES OF PURE HYPERTENSION

The first cases are more or less typical examples of so-called pure hypertension. One of them (Case 2) had been observed for over a year, and occasional urinalyses, even when the blood pressure was as high as 300 m.m., never showed albumin. In recent months, with more frequent urinalyses, traces of albumin have sometimes been found. The others of this group have traces of albuminuria. Omitting individual peculiarities, it may be said that this group is characterized by activity of excretion and apparent absence of retention in tests with nitrogen, water and even salt. The kidney retains power to concentrate, and both the blood urea and the urea function according to the Ambard principle may be fully normal. But the chlorid threshold, whether determined mathematically or by salt privation, is high, and these patients purchase their apparent efficiency of salt excretion at the price of abnormally high blood salt and blood pressure. Extraordinary strictness of salt-free diet may be necessary to control these, and the therapeutic success corresponds to the restriction in salt intake. This single feature some-

6. For chlorid method, see Donleavy, J. J.: J. Biol. Chem. 37: 551 (April) 1919.

times makes the difference between health and invalidism. While protein and water create no symptoms, on general principles they should be limited to some extent. Without assuming that all possible kinds of cases are represented in this list, and without overlooking other abnormalities revealed by sufficiently thorough study, it can be stated that pure hypertension, so far as it has occurred in this series, has been essentially a salt nephritis.

HYPERTENSION WITH KIDNEY IMPAIRMENT

As all standards of kidney function are to some extent arbitrary, this group passes by gradual transition into the second and larger group, in which there is obvious impairment of several kidney functions, as indicated by retention of urea and other products, impaired excretion of urea and chlorids by Ambard's laws, more or less fixation in the chlorids and specific gravity of the urine, and especially chlorid retention with or without evident edema.

The degree of impairment in the nitrogen economy calls for corresponding restriction of protein, and influences the prognosis. But the hypertension is affected only through the salt and water functions. Careful fluid restriction is necessary as a rule, but attention should be given to the nitrogen retention thus entailed in certain toxic cases. This is an additional reason for limiting the protein intake, though it is well known that the percentage of blood urea is not an infallible index of danger. Even though chlorid retention be far greater than in the first group and the other signs more evident as stated, the actual chlorid threshold may be lower and the actual salt restriction required to control the pressure may be less stringent in the second group.

It is well known that retained salt may be diffused through edema fluid without raising the percentage in the blood. Patient 11 thus had much edema, and as this diminished, the chlorid percentage in the blood rose as shown in parentheses. With this proviso, the great majority of these cases had high chlorids in the blood plasma, and it is probable that the same will hold for the majority of all hypertension cases. But in appoaching Group 3, we find a small group of hypertension patients with continuously low blood chlorids and low chlorid threshold, and often with normal or actually low blood urea.

HYPERTENSION WITH DIABETES OR SEVERE NEPHRITIS

The six closing cases of this table may be divided into two groups of three each. The first group includes three cases (15, 16 and 17) of diabetes. In them the prognosis is good with thorough treatment of the diabetes, sometimes with the aid of salt and water restriction and sometimes without it. Though there are presumably rare cases of intractable hypertensive nephritis associated with diabetes, there is generally the strong probability that proper treatment, associated with reduction of the blood sugar, will reduce the hypertension.

The last three cases of the table are nondiabetic, and have proved the most difficult and intractable of the entire series. Limitation of salt, protein and fluid, reducing the twenty-four hour urine volume below 300 c.c., has given symptomatic relief and more or less complete checking of retinitis, but the influence on the pressure has on the whole been slight and the reduction shown in the table has not been permanent. Notwithstanding the innocent looking blood analyses, according to function tests these are cases of severe nephritis, and the worst element in their prognosis from the standpoint of hypertension seems to be the absence of a high blood chlorid before treatment.

LIMITATIONS IN TREATMENT

Certain precautions or limitations should be mentioned with regard to the practical application of these principles in treatment.

First, a diet which is reasonably satisfying and at the same time sufficiently poor in salt is not so easy to arrange as it may appear. Both physician and patient may sometimes believe conscientiously that strict abstinence from salt has been maintained, when analyses will show the blood chlorids undiminished and an output of from 5 to 10 gm. in the twenty-four hour urine. Such analyses are important for controlling the treatment, and no pressure is rightly called irreducible unless the daily chlorid excretion has been reduced to practically zero.

Second, some quantity of salt is considered indispensable in the permanent ration. This is stated as about 2 gm. for normal persons, but the limits for nephritics have never been determined. On the one hand, it is not certain that the intake need be as great when the output is obstructed. On the other hand, there may be danger in withdrawing salt from certain nephritics, especially with nitrogen retention, who seem to need much more than 2 gm. of salt for its diuretic action to ward off uremia. As a rule, the outstanding effect of undue salt privation has been weakness. It is necessary, therefore, to work out salt rations for individual nephritics which may range from almost zero to a considerable quantity.

Third, restriction of salt or fluid, like every dietetic treatment of organic disease, is only palliative. Success is not as uniform as in the treatment of diabetes, and complete or partial failures are more numerous. When the first results are more or less satisfactory, there is still reason to fear that the same patients may pass into a hopeless condition in the course of months or years. Futhermore, the most effectual relief of the hypertension demonstrates strongly that the essential trouble is more than mechanical. Reduction of the blood pressure nearly or completely to normal may still leave the patient an invalid, either from weakness due to the strict salt privation which is necessary to hold down the pressure, or still worse, if salt is given so as to allow the pressure to go up. They may also be subject to attacks of hypertension and accompanying symptoms from little or no evident cause. The greatest defect of the French work is the disposition of those authors to treat the entire question as a mere mechanical matter of salt retention. Obviously, even the mechanics of hypertension remains largely unsolved, and the fundamental condition thus far baffles both research and treatment.

Notwithstanding these limitations, the benefit of suitable chlorid restriction for hypertension is still great, in making the patients more comfortable, diminishing the danger of apoplexy, and possibly checking the progressiveness of the disorder. So many of these patients give a history of copious use of salt that this may be a possible contributory factor. Granting a primary infectious or toxic etiology of the renal or vascular damage, the prolonged irritation by the excess of salt and the wear and tear of the hypertension itself

may well be regarded as a cause of progressive aggravation. By far the best and easiest results are obtainable in the earliest cases, when the hypertension is intermittent rather than continous. Early diagnosis is therefore important, and in advance of the sphygmomanometer it may be hoped that chemical tests, such as for the blood chlorids and chlorid threshold (the normal values for which are still undecided), may give advance warnings to guide prophylaxis.

Such studies may also throw some light on the origin and the control of arteriosclerosis and some other disorders. One of the most interesting branches of the investigation has concerned ophthalmic disorders, and with the cooperation of several oculists, evidence has been gathered indicating that many cases of retinitis are due chiefly or solely to chlorids and are benefited by chlorid restriction. More cases of retinitis and of glaucoma are needed for this study. The promising study of salt metabolism will probably soon be extended more widely by those who are in position to undertake parallel determinations of various salts and to develop the more fundamental problems through animal experiments. The present investigation, which has had to be self-supporting to date, has been limited and superficial for lack of these things. The clinical facts collected, however, warrant urging that unfounded theories be discarded, the widespread abuse of drugs, particularly nitrites and iodids, stopped, and the possibilities of modern laboratory methods utilized for the guidance of treatment.

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ANON

POTASSIUM AND GASTROINTESTINAL LESIONS I

Ulcers and stenosis in the small intestine have occurred in a small percentage of patients receiving enteric-coated tablets of potassium chloride. In some cases, surgical intervention was necessary. If the symptoms were recognized early enough, discontinuance of the tablets cleared the condition. Potassium chloride other than that in these tablets had no such effect.

Within the past few years, a number of patients have been observed who had gastrointestinal abnormalities referable to potassium chloride therapy. In most cases, this compound was incorporated with one of the thiazide drugs in an enteric-coated tablet. The thiazide drugs presumably were prescribed for control of hypertension, since a large percentage of the patients were over 50 years of age and had some cardiovascular difficulty (F. D. Lawrason, E. Alpert, F. L. Mohr, and F. G. McMahon, *J. Am. Med. Assn.* **191**, *641 (1965)*).

These drugs enhance urinary excretion of sodium and at the same time, but to a lesser degree, increase excretion of potassium. The loss of potassium produces a low serum level, with the consequent danger of cardiac abnormalities if the low potassium level is permitted to persist. Since addition of potassium chloride to the thiazide drug does not interfere with reduction of blood pressure (*Nutrition Reviews* **20**, *195 (1962)*), the two compounds are frequently given together. The level of potassium chloride in each tablet is usually either 500 or 1,000 mg

(*Physicians Desk Reference to Pharmaceuticals, Medical Economics Inc., Oradell, New Jersey* (*1963*)).

Reports of complications associated with the use of the tablets containing both a thiazide drug and potassium chloride have appeared only within the past few years. One of these by B. Lindholmer, E. Nyman, and L. Raf (*Acta Chir. Scandinav.* **128,** *310* (*1964*)) described four patients who were operated for ileus (obstruction of the bowel attended with severe colicky pain, vomiting, and often fever and dehydration). These patients were over 50 years of age, and had been treated with the tablets containing both compounds for over a year prior to development of symptoms.

The patients first noticed loose stools some weeks prior to operation. Colicky pain developed about the same time. The pain increased until it became so severe that medical attention was sought. Laparotomy revealed a stenotic region, about ½ to 1 cm. long, midway down the small intestine. The lumen was practically closed by the stricture. The serosa in the area of the stenosis was reddened, except for an elongated purple ulcer.

Examination of records of patients operated for obstruction of the small bowel at the same hospital, and at one nearby, turned up an additional 20 patients, practically all of whom had received thiazide therapy. Most or all of the patients had also received potassium therapy. No specific compound or mode of therapy was implicated by Lindholmer, Nyman, and Raf.

Another report (D. R. Baker, W. H. Schrader, and C. R. Hitchcock, *J. Am. Med. Assn.* **190,** *586* (*1964*)) indicated that there were only 170 cases of small bowel ulceration reported in the literature prior to 1963. In a 15-month period thereafter, 12 patients with ulcerating, predominantly stenosing, and obstructive lesions of the small intestine were treated in the surgical service of one hospital. One patient at the time of operation had an undigested enteric-coated potassium chloride tablet at the site of perforation. "The solitary, focal character of the ulcer, the lack of serosal, muscular, lymphatic, or mesenteric involvement, and the absence of any significant lymph node or granulomatous reaction in these nonspecific ulcerations appears to provide adequate separation" from ulcers due to bacterial infection.

All patients operated on by Baker, Schrader, and Hitchcock showed stenosis of the small intestine. Actually, a recent report by F. Shuster and E. H. Berg (*J. Am. Med. Assn.* **194,** *570* (*1965*)) suggests that partial obstruction of the small intestine precedes development of the ulcer. The obstruction results in dissolution of the enteric-coated tablets, and release of potassium chloride in high concentrations.

These and other reports prompted the Food and Drug Administration to call a conference in November, 1964, at which 89 case reports were reviewed (Lawrason *et al., loc. cit.*). When the conference was held, a survey of 488 hospitals in this country and abroad was underway to determine (1) the frequency of ulcerative-obstructive lesions of the small intestine, (2) the possible relationship between therapeutic administration of diuretics or potassium and development of the lesions, and (3) any unusual geographic distribution of the cases (Lawrason *et al., loc. cit.*).

This survey indicated that one-half of 395 patients with small intestinal lesions had been given diuretics or potassium, while the rest did not receive either. These figures were based on questionnaires answered by 377 hospitals in the United States and 63 from abroad. Again, the majority of the patients were over 50 years of age, and 70 per cent had an obvious cardiovascular disease. There was no difference in sex distribution among the patients. Practically all who received a diuretic also received potassium, usually in combination with the diuretic.

The survey suggested that there were

geographic areas where the incidence of the lesion was especially high. Less than 15 per cent of the hospitals had most of the patients. A check was made to determine whether questionable batches of medication were sent to the areas reporting most of the cases. This possibility was ruled out when it was shown that there were no chemical or physical differences in lots shipped by the manufacturers. The authors (Lawrason *et al., loc. cit.*) give no indication as to whether most of the cases were seen in large metropolitan hospitals, or in hospitals located in certain areas of the United States. They conclude that "at present, it is not known why there are apparent concentrations of lesions in certain geographic areas."

Surgical intervention is usually not essential if the condition is recognized early enough. Recovery occurred in a 64 year old man after potassium therapy was terminated (A. A. Abbruzzese and C. A. Gooding, *J. Am. Med. Assn.* **192,** *781 (1965)*). This patient developed "creeping abdominal pain" 22 days after being started on a regimen of enteric-coated tablets containing 50 mg. hydrochlorothiazide and 1,000 mg. potassium chloride. Besides the pain, frequent bowel movements, nausea, and vomiting had bothered the man.

Intravenous fluids were given for the first few days of his hospital stay. When he was discharged at the end of nine days, he was apparently free of symptoms. On his own, he resumed the thiazide-potassium chloride therapy, only to develop the same symptoms over the next four weeks. Cessation of the tablet therapy cleared up his complaints with no further recurrences.

These reports leave a number of questions unanswered. One of them is the time elapsing from the date when the tablets first appeared on the market to the first report of small intestinal stenosis. Most of the reports indicate that the first patients with this difficulty were seen about 1960 (L. Morgenstern, M. Freilich, and J. F. Panish, *J. Am. Med. Assn.* **191,** *637 (1965)*). Lawrason and co-workers, making their survey for the principal manufacturers, should have been in a position to make such a comparison.

Another question is why one tablet containing 1 g. of potassium chloride should be ulcerogenic in some patients? Related to this is the great variability in individual susceptibility. Some patients have developed ulcers after ingesting only 1 or 2 g. of potassium chloride, where others showed no symptoms until after five years of daily therapy (S. J. Boley *et al., J. Am. Med. Assn.* **192,** *763 (1965)*).

If ulcer development depended primarily on total intake of potassium, the daily ingestion of 1 g. potassium chloride should not cause any difficulty under normal circumstances. The variation in daily intake of potassium is equivalent to from 2 to 5 g. of the chloride (S. Davidson, A. P. Meiklejohn, and R. Passmore, *Human Nutrition and Dietetics, p. 147. The Williams and Wilkins Co., Baltimore, Maryland, 1959*). One additional gram should not make much difference under such circumstances. Furthermore, there appears to be no correlation between the dose of potassium chloride, as the enteric-coated tablet, and the development of lesions in man (Lawrason *et al., loc. cit.*).

Finally, why should some individuals develop ulcers when the great majority of patients who are given this therapy show no detectable disturbances? Although a large number of patients apparently have shown signs of intestinal lesions, it is still a small fraction of the total number treated (Baker, Schrader, and Hitchcock, *loc. cit.;* Lawrason *et al., loc. cit.*). One estimate is that only one in 35,000 patients receiving the enteric-coated potassium chloride tablets develops any detectable disturbance (R. M. Diener, D. H. Shoffstall, and A. E. Fa[illegible] *Toxicol. Appl. Pharmacol.* **7,** *746 (1965)*).

These reports emphasize the fact that what may appear to be an innocuous for[illegible]

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of therapy may become harmful for some individuals. Since practically all clinical reports implicate the enteric-coated tablets containing potassium chloride, it would appear that the problem may relate to local concentrations of the salt in the small intestine. When the potassium chloride is given in some form other than the enteric-coated tablet, no difficulties have been seen (Baker, Schrader and, Hitchcock, *loc. cit.*). This would suggest that any means whereby the potassium chloride could be incorporated into a routinely used dietary ingredient should help to eliminate this problem.

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SODIUM INTAKE AND BLOOD PRESSURE

Both genetic and environmental factors regulate levels of blood pressure. There is increasing evidence that sodium intake may account for some of the variability in the level of blood pressure observed in human populations.

Hypertensive cardiovascular disease is still a major cause of death and disability in many contemporary societies, although there is accumulating evidence of an improvement in mortality experience from this cause within the past 15 or 20 years (E. D. Freis, in *Second National Conference on Cardiovascular Disease, Vol. I, p. 336, E. C. Andrus and C. H. Maxwell, Editors. Washington, D.C., 1965*). Presumably this is the result of more effective medical management.

Among the factors associated with accelerated clinical appearance of atherosclerotic vascular disease, the level of blood pressure has been repeatedly shown to be a potent variable in all of the large epidemiologic studies. The known potentiating role of increased blood pressure in experimental atherosclerosis dates back to the early studies of N. Anitschkow (*Beitr. Z. Path. Anat. V. Z. Allg. Path.* **59**, *306 (1914)*). As with another major risk factor, blood lipids, there is evidence that blood pressure is regulated by both constitution and environment. Among the environmental factors, dietary sodium may be an important element in setting the mean blood pressure level of population groups. The type of association reported by S. Kaneta, K. Ishiguro, S. Kobayashi, and E. Takahashi (*Tohoku J. Exp. Med.* **83**, *398 (1965)*) has lent support to this hypothesis. On the other hand, whether the salt accounts for the variability of blood pressure within a population group has been much harder to document. Quantitating the "usual" consumption of such a widespread mineral as sodium, one whose intake can vary manyfold in man, presents a formidable problem.

At least the beginnings of experimental animal models which may illuminate the interplay of host and environmental regulators of blood pressure already exist.

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Genetic factors have been shown to be important determinants of blood pressure; strains of rats which manifest spontaneous hypertension have been bred out. However, hybridization of these rats with normotensive animals has shown how very complicated are the genetic mechanisms involved (W. J. Louis, R. Tabei, A. Sjoerdsma, and S. Spector, *Lancet* 1, *1035 (1969)*). Their findings suggest a non-sex-linked, polygenic mode of inheritance. In contrast, L. K. Dahl, M. Heine, and L. Tassinari (*J. Exp. Med.* 115, *1173 (1963)*) have bred out strains of rats responding quite differently to high levels of dietary sodium—some developing hypertension, others not. Unlike the spontaneously hypertensive rats, the latter animals did not manifest a difference in blood pressure on a diet of usual sodium content.

Epidemiologic studies on man have recently been extended by the report of I.A.M. Prior *et al.* (*New Engl. J. Med.* 279, *515 (1968)*). These investigators have examined the interrelationships among blood pressure, sodium intake, and the urinary excretion of sodium in two populations of Polynesians of similar ethnic and climatic background, but geographically separate. Their samples consisted of the entire adult population of the atoll, Pukapuka (365 adults, age 20 and over) and 455 adults (98 per cent) of the population of a city on Rarotonga. The former population lives on a subsistence level economy, the latter on a cash economy with a diet that includes imported canned foods.

Blood pressure was carefully measured under standardized conditions. Among the Pukapukans, males showed no age related increase and females a moderate increase in systolic pressure of 108 mm Hg in the 20 to 29 year old group to 129 in those over 70 years. Rarotongans, on the other hand, had higher systolic and diastolic pressures even in the 20 to 29 year old age groups of both sexes and showed striking age related increases, more marked in females than males. Among subjects 40 years of age and older, 28 per cent of men and 47 per cent of women had pressures above 159 systolic or 94 diastolic. Pressures of this order are quite comparable to these reported in the United States (*National Center for Health Statistics, Series 11, Number 4, 1964*). Among Pukapukans, the prevalence of pressures of this magnitude was 3 per cent in males and 7 per cent in females.

Though Rarotongans tended to be slightly taller and heavier than Pukapukans, the authors concluded that only very little of the observed difference in mean blood pressures between the two populations could be thus accounted for.

Twenty-four hour dietary intakes established estimates of mean sodium intake in the two areas at 50 to 70 mEq per day in Pukapuka and 120 to 140 mEq per day in Rarotonga. The higher intake in the latter was mainly from canned fish, corned beef, and salted coconut sauce. Further validation of these dietary estimates was provided by analysis of casual urine samples for sodium. Samples were collected from almost the entire study series in both areas. Sodium concentration was approximately twice as high (126 versus 65 mEq per liter) in Rarotongans. A subsample of 51 Rarotongans and 70 Pukapukans furnished two 24 hour urine collections. Here too, 24 hour urinary excretion of sodium was approximately twice as high in Rarotongans, both male and female.

Variability in blood pressure *within* members of either population group could not be accounted for by any of the various estimates of sodium intake. Small sample size, methodologic problems in accurate estimates, day to day variability—

all make this very difficult. Genetically determined variability in blood pressure regulation and the response to sodium must also be assumed to be at play here.

In balance, the authors have presented strong evidence for sodium intake's accounting for at least some of the observed differences in blood pressure found in human populations, and further, shown that these differences may be manifest by age 20.

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COMMENTS

SALT, PALATABILITY AND FUTURE HEALTH

RECENTLY there have been increasing numbers of warnings in the medical Press about the probable positive correlation between the prolonged ingestion of food containing a high sodium chloride concentration and the later development of hypertension. As a result, attention has been drawn to the potential dangers of feeding infants with products, especially strained foods, which contain unnecessary amounts of salt.

Dr Derek Denton, Principal Research Fellow of the Howard Florey Laboratories, University of Melbourne, speaking on the A.B.C.'s radio programme "Insight",[1] warned parents that the salt content of many infant

[1] *The Sydney Morning Herald*, April 4, 1970.

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proprietary food mixtures was equivalent to concentrations ingested by adults in northern Japan, who have probably the highest incidence of hypertension in the world. It is thus not inconceivable that in their first two to three years of life modern infants could eat the same amount of salt as did their parents over a period of 20 to 30 years.

A further reason for caution has been raised by workers studying possible aetiological factors associated with the occurrence of gastric carcinoma, a disease most common in Japan.[2] The role played there by a high, regular salt intake is rather suspect.

It would seem that a major reason for the added salt in baby foods has been an economic one. Processors have catered more for the mother's palate, realizing that she will tend to buy "tasty" food for her offspring, having presumed that her preference approximates that of her child. However, the latter conclusion is not valid.

Infants' taste preferences are uncertain, but the sodium content of human milk, for example, is only approximately 24 mg/100 kcal, suggesting that food palatability for them should not depend heavily on salt content. S. J. Fomon *et alii*,[3] to test this theory, recently conducted a survey in the U.S.A. on the consumption of salted and unsalted baby foods by normal four- and seven-month-old infants. Most of the children were drinking commercially available milk formulas or whole cow's milk, often supplemented by vitamins and minerals. These provided a baseline sodium intake of approximately 52-86 mg/100 kcal.

Twenty four-month-old infants were fed salted and unsalted foods on alternate weeks, eleven of them having salted preparations during weeks one and three, and nine during week two only. Ten seven-month-old infants ate salted foods during the first week only, as they had been used to a wider variety of these preparations over a longer time than their younger counterparts. The mothers did not know that the salt content of the samples varied.

The mean intakes of sodium from strained foods were then calculated, and it was found to be four times greater when salted foods were given, compared with unsalted. The mean intake of sodium was 200 mg/100 kcal by the four-month-old babies, and 243 mg/kcal by those seven months old.

However, as Fomon *et alii* comment, there was a wide variation in the sodium intake, this varying with the proportion of the child's diet that was derived from strained foods. One girl, four months of age and weighing only 6 kg, had a total daily sodium intake of 830 mg/day; one boy, seven months of age, was given 1,329 mg of sodium per day. Both these children ingested only one-third of this amount if given unsalted food, but neither they nor the other babies showed a definite preference for the saltier products.

Modern research has raised doubts about the advisability of ingesting high concentrations of salt over a long period, and workers such as Fomon *et alii* have shown that a high concentration is unnecessary for palatability. Doctors and child health personnel, aware of the possible dangers, should carefully supervise the diet régimes suggested for children under their surveillance. Mothers should be encouraged to avoid using an excess proportion of proprietary strained foods, while manufacturers, reassured that their product will be no less acceptable to prospective customers, should lower the salt content in them.

[2] WHO Press Release, April 6, 1970.

[3] *J. Pediat.*, 1970, 76: 242 (February).

EVOLUTION AND HUMAN BEHAVIOUR

Most medical people regard an anthropologist as someone preoccupied with the physical classification of mankind or the unravelling of primitive cultures. But these stereotypes no longer hold, because nowadays many anthropologists cut across disciplinary boundaries and attempt an objective synthesis of issues beyond the scope of other social or medical scientists. Such an approach is taken by Alexander Alland,[1] who looks at the way human behaviour has been shaped during the immense time scale of evolution. He preserves a commendable detachment, and unlike some of his colleagues, does not dwell on behaviour in the value-judgement terms of western civilization. Alland sees behavioural evolution as a self-regulating system subject to all the checks and pressures of natural selection. He takes neo-Darwinian theory as his cornerstone — that is, Darwin's concept of evolution allied to modern genetics. On this firm basis, which occupies the first half of the book, Alland then relates the contribution of learning and culture to human behaviour and brings in the lessons from work on animals. The latter is contentious stuff, and extrapolation to man is often suspect. But at present there is little alternative, and when comparisons are made with honesty and skill, they can be both stimulating and fascinating. Certain patterns of behaviour appear to be strongly inherited; others have such complex dynamics that no useful analysis is possible.

By keeping close to biological principles, Alland has written a book that has much value for any doctor interested in the intricacies of human behaviour. There are no easy answers, but it is helpful to have a scholarly framework on which to base the hypotheses that must be tested to cover vast gaps in our existing knowledge. Although written in a fluent and pleasant style, the book contains an extensive core of instruction in genetic and evolutionary principles whose assimilation requires some intellectual perseverance. There are a few minor gaffes with reference to man—for example, a simplistic view of diabetes and a too-ready acceptance of Huxley's genetic model for schizophrenia—but these are of little consequence. This book should prove useful to those who appreciate that the understanding of health and disease in human populations is improved if thought is given to the way man has evolved as a functioning unit in a particular culture.

[1] "Evolution and Human Behaviour", by Alexander Alland; 1969. London: Tavistock Publications; Sydney: Hicks Smith & Sons Pty Ltd. 8½" x 5½", pp. 254, with diagrams. Price: $2.60.

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ANON.

Detection of Hypertension in Childhood

There is already some evidence that treating mild hypertension in young adults may help to reduce complications in later life, so that perhaps treatment in childhood might be even more effective. This is a long way off as a practical therapeutic possibility, and the groundwork could be laid for it only if it were true that a relatively high blood pressure in childhood predisposed to a relatively high blood pressure in early adult life. The possibility that it might do so follows from observations that blood pressure in early adult life is statistically correlated (though only very roughly) with that observed in the fourth decade and after. Moreover, when the blood pressure is raised in early adult life, even though it is within the normal range, it may rise more rapidly with the passage of time. Thus, from early adult life onwards the higher the blood pressure, the faster and further it rises with the passage of time.

Recently a study from Canada by Carol Buck[1] has provided suggestive evidence that, if raised blood pressure is recorded at the age of 5, it is likely to be still above the average at the age of 11-12. However, the increased blood pressure Buck observed was not very remarkable. For those children whose blood pressure at the age of 5 was over one standard deviation above the mean for their group Buck observed that the blood pressure at age 12 was approximately 9 mm systolic and 6 mm Hg diastolic above the average for the age. Though statistically it seems probable that a relatively high blood pressure at the age of 5 predisposes to higher blood pressures throughout childhood and probably into early adult life, it would be unwise in the present state of knowledge to embark on a clinical trial of the treatment of marginal hypertension in childhood with drugs.

The argument could be put forward that, if children who might later be at risk for hypertension could be identified early, simpler prophylactic measures should be taken. For instance, the amount of salt in the diet might be kept low, and at a later stage girls could be advised not to start taking the contraceptive pill. But for any screening procedure to be worthwhile its expense, inconvenience, and perhaps also its potential for causing hypochondriasis have to be weighed against the possible benefits in life expectancy. On this score alone it is difficult to recommend wholesale investigation or screening of the blood pressure of young children. The most that might be recommended is that it should be taken annually in children when the family history is extremely strong—for example, with both parents under treatment for hypertension. Whether it would be reasonable to recommend treatment for a 12-year-old with a blood pressure of, say, 120/80 mm Hg is debatable. At present treatment would not be reasonable except for higher blood pressures. The number of children likely to be found below the age of 10, or indeed during the second decade, in need of treatment by active drugs is likely to be exceedingly small. In view of the expense and inconvenience alone screening would be difficult to justify. It would seem that for hypertension in childhood much the same applies as for hyperlipidaemia in childhood, a subject recently discussed in these columns.[2] A raised serum cholesterol early in childhood can statistically predict a raised cholesterol later in childhood; but attempts to lower the serum cholesterol by active means are scarcely justifiable.

The situation may change in the next decade, because if the treatment of hypertension in young adults comes to be accepted as beneficial and an established part of medical practice, the screening of 20-year-olds will be easily justified, for it is easy to control blood pressure elevations of a mild degree in young adults. For example, guanethidine 10 mg daily, or perhaps propranolol 20 mg twice daily, or bendrofluazide 5 mg daily will in many cases restore normal levels of blood pressure without any detectable side effects. If the blood pressure is left untreated until it is perhaps 160/110 mm Hg at the age of 35, it may no longer be easy to restore it to a normal value for the age without at least some unpleasant side-effects.

The possible long-term ill-effects of drugs in common use need to be borne in mind. At present we do not know whether, for example, starting small doses of guanethidine in childhood might interfere severely with fully adequate sexual function in later life. Nor do we know whether the use of beta-blockers over several decades has long-term ill-effects, perhaps on the brain. And we do not know whether the possible risk of potassium depletion and impairment of glucose tolerance by thiazide diuretics over many decades may not in itself reduce life expectancy. The earlier the physician starts to treat hypertension, and the milder the degree of hypertension, the greater is his responsibility to

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ensure that the treatment is justifiable. At present the routine screening of blood pressure in normal children or teenagers has no prophylactic value.

[1] Buck, C. W., *Journal of Chronic Diseases*, 1973, 26, 101.
[2] *British Medical Journal*, 1973, 1, 690

Arena, J.M. 1970

Poisoning, 2nd edition

Chas. C. Thomas, Springfield, Ill. 1970
pp. 516-517

Decrease of Cardiac Mass Following Excess Dietary Potassium Chloride in the Rat

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Am. J. Physiol.

THE INFLUENCE of electrolyte balance on the heart and kidney has been observed in several studies (1). An excess of sodium in the body, due to sodium flooding or to DCA-induced sodium retention, may be responsible for the development of hypertension and cardiac hypertrophy associated with nephrosclerosis and renal tubular dilatation (1). The importance of the adrenal cortex in relation to certain cardiovascular conditions has been established (2, 3). Investigations on the action of DCA and of electrolytes on the blood pressure of rats have shown that the hypertensive action of the hormone is dependent on the presence of the sodium ion (4, 5).

Selye *et al.* (6, 7) observed that certain acidifying salts are capable of opposing the cardiovascular toxicity of DCA; the action of these salts was referred to their sodium depleting effect. Darrow and Miller (8) observed that the cardiac lesions produced by excess DCA or by potassium deficiency are apparently due to the deficit of potassium and are prevented by potassium chloride treatment. Other workers (5, 6) observed, however, that potassium chloride treatment sufficient to maintain the normal blood potassium level did not depress the signs of hypertension induced by DCA overdosage in the rat.

This paper reports findings which indicate that potassium chloride flooding in the rat induces a depression of cardiac mass, and a hyperactivity of the adrenal glomerular zone.

MATERIALS AND METHODS

Female rats of the Wistar strain were used. Fourteen rats were placed, 2 in a cage, and fed, *ad libitum*, a diet of 'Purina laboratory chow' and a 2.5 per cent aqueous solution of potassium chloride as the sole source of fluid. At the end of 105 days 10 of these animals were killed; the remaining 4 animals were supplied with tap water in place of the KCl for the rest of the experiment. These animals were killed 1 month after the KCl was discontinued. Six control rats received food and tap water *ad libitum* for 105 days at the end of which time they were killed.

Intake of food and of fluid by these animals was observed at 2- to 3-day intervals throughout the duration of the experiment. After death the heart and the paired kidneys were weighed. The adrenals and the kidneys were prepared for

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histological studies. One adrenal of each animal was used for the detection of ascorbic acid, and the other for the detection of cholesterol and of other lipids by methods used in a previous study (9). The heart and left gastrocnemius muscle of some animals of each group were analyzed for water content after a modification of the oven-drying method used by Laramore and Grollman (10).

Results

Organ weight data are presented in table 1; also presented are the percentage deviations of the observed weights from organ weights calculated on the basis of equations proposed by Addis and Gray (11). The observed heart weights in the normal control animals were very similar to the weights predicted by the equation

Table 1. Body, heart and kidney weights of rats on fluid intake of water, 2.5% KCl solution, and 2.5% KCl for 105 days followed by water for 30 days

	Controls	KCl flooding	KCl followed by water
Final body wt., gm. ± S.D.[1]	221±12	226±16	229±13
Heart wt., mg/100 gm. ± S.D.[1]	324±19	270±34[3]	304±28
% deviation from prediction[2]	−0.7	−18.5	−5.6
Kidney wt., mg/100 gm. ± S.D.[1]	731±31	845±112[3]	643±38
% deviation from prediction[2]	+20.0	+40.0	+8.0

[1] $S.D. = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$

[2] Absolute organ weights predicted by equations: log (heart wt. in mg.) = 1.1286 + 0.7375 log (body wt. in gm.); log (2 kidney wt. in mg.) = 1.7313 + 0.5951 log (body wt. in gm.). Equations apply to female albino rats (11).

[3] Significantly different from controls.

of Addis and Gray. The kidney weights predicted from the equation were much lower than the weights observed in normal control animals.

A significant correlation was observed between the heart and kidney weights of the normal animals and persisted in the other 2 groups of animals (r for treatments = 0.796, $P < 0.05$; the difference between treatment correlations is insignificant, $F_{2, 14} = 1.40$, $P > 0.05$). The mean heart weight of the animals flooded with KCl for 3½ months was significantly less than that of control animals ($t_{17} = 4.148$, $P < 0.01$); the mean kidney weight in the KCl-flooded animals was significantly greater than that of control animals ($t_{17} = 3.751$, P 0.01). The organ weights returned to normal levels in the animals which were taken off KCl flooding. These findings are verified on comparison of the predicted organ weights with the observed organ weights.

The animals under KCl flooding consumed relatively more fluid than did the animals of the other groups (table 2). An apparent decrease was observed in the water content of the gastrocnemius muscle of the KCl-flooded animal. No appreciable change was observed in the water content of the hearts of animals under

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treatments. Histological study of the adrenals indicated that the glomerular zone of the KCl-flooded animal showed signs of hypertrophy, confirming a previous report (9). The adrenal of the animal reversed from KCl was essentially normal in appearance.

Discussion

The data indicate that dietary excess of KCl in rats induces adrenal glomerular zone hyperactivity, renal hypertrophy and a decreased cardiac mass. It has been found that changes in cardiac mass are highly correlated with changes in blood pressure, and heart weight has been suggested (12, 13) as an index of blood pressure. On the basis of this relationship, it is possible that the decreased heart weight observed in the KCl-flooded animals reflected a decrease in blood pressure, but this is not certain since blood pressure determinations were not made. It is possible that the increase of renal mass observed in the KCl-flooded animals was the result of a

Table 2. Effects of KCl consumption on body weight, fluid and food consumption, tissue water content and adrenal histology

	controls	KCl flooding	KCl to H_2O
No. of rats	6	10	4
Initial body wt., gm	204	206	206
Final body wt., gm	221	226	229
Fluid intake, cc/100 gm/day	13	21	11
Food intake, gm/100 gm/day	7	7	5
Tissue water content, mg/gm			
Heart[1]	744±13	755±13	
Gastrocnemius[1]	746±5	734±3	
Adrenal histology	Normal	Hyperactive	Normal

[1] In each case, 4 animals were used for determination.

compensatory work hypertrophy intended to aid in ridding the body of the excess electrolyte.

The hypertrophic changes observed in the adrenal glomerular zone were similar to changes observed in a previous study. It is probable that the temporary relative mineralocorticoid insufficiency occasioned by the increased supply of KCl served as the stimulus to hypertrophy and hyperactivity. It is noteworthy that a similar temporary mineralocorticoid insufficiency may be induced by sodium deficiency. The mineralocorticoid insufficiency induced by sodium deficiency has been prevented by simultaneous DCA treatment (14, 15).

Potassium chloride excess may exert its effect on the heart either by depleting the sodium reserve, by the increased 'utilization' of DCA occasioned by the depression of the Na/K ratio in the body, or by depressing the production of pressor substances by the kidneys. It is noteworthy that Friedman (16) proposed a relationship between the active renal mass and the level of blood pressure and heart weight; according to that hypothesis an increased renal mass may diminish the amount of circulating pressor substances secreted by the adrenal cortex. The observations in this study are in consonance with that hypothesis.

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Summary

Data are presented to indicate that potassium chloride flooding to rats for $3\frac{1}{2}$ months induces an increase in the weight of the kidneys, a decrease in the weight of the heart and adrenal glomerular zone hypertrophy. These changes are reversible on cessation of KCl flooding.

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REFERENCES

1. Selye, H. *Stress.* Montreal: Acta, 1950.
2. Anderson, E., E. W. Page, C. H. Li and E. Ogden. *Am. J. Physiol.* 141: 393, 1944.
3. Page, E. W., E. Ogden and E. Anderson. *Am. J. Physiol.* 147: 471, 1946.
4. Friedman, S. M., C. L. Friedman and J. R. Polley. *Am. J. Physiol.* 153: 226, 1948.
5. Knowlton, A. I., E. N. Loeb, H. C. Stoerk and B. C. Seegal. *J. Exper. Med.* 85: 187, 1945.
6. Selye, H., J. Mintzberg and H. Stone. *J. Pharmacol. & Exper. Therap.* 85: 42, 1945.
7. Selye, H. and H. Stone. *J. Pharmacol. & Exper. Therap.* 83: 56, 1945.
8. Darkow, D. C. and H. C. Miller. *J. Clin. Investigation* 21: 601, 1942.
9. Bacchus, H. *Am. J. Physiol.* 163: 326, 1950.
10. Laramore, D. C. and A. Grollman. *Am. J. Physiol.* 161: 278, 1950.
11. Addis, T. and H. Gray. *Growth* 14: 49, 1950.
12. Rytand, D. *J. Clin. Investigation* 17: 391, 1938.
13. Schroeder, H. A. *J. Exper. Med.* 75: 513, 1942.
14. Deane, H. W., J. H. Shaw and R. O. Greep. *Endocrinology* 43: 133, 1948.
15. Knowlton, A. I., E. N. Loeb, B. C. Seegal and H. C. Stoerk. *Endocrinology* 45: 4, 1949.
16. Friedman, S. M. and C. L. Friedman. *Canad. M. A. J.* 61: 596, 1949.

Fatal Poisoning From Salt Used as an Emetic

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A 3-year-old boy was given salt as an emetic and later received gastric lavage with saline solution following accidental ingestion of aspirin. He became comatose and laboratory values revealed severe hypernatremia and hyperchloremia. He died despite therapy with fluids, peritoneal dialysis, and mechanical ventilation.

The use of salt to induce vomiting in cases of intoxication is widespread. Recommendations for its use as an emetic are widely publicized in newspaper articles, labels of household products, various manuals of medical emergencies, a textbook of pediatrics,[1] a pediatric journal,[2] and a government publication.[3] However, fatal poisoning due to gastric lavage with hypertonic saline in children[4] and adult deaths due to the use of salt as an emetic agent[5,6] have been reported. Severe salt intoxication in infants and children has also been described under other circumstances.[7,8]

DeGenaro and Nyhan[9] have recently called attention to the dangers of salt as an antidote and reported a child fatality after its use as an emetic. We here report an additional child fatality resulting from salt administered as an emetic.

Report of a Case

The patient was a normally developed 3-year-old white boy with a one-day history of fever and anorexia. There was no diarrhea or vomiting. He seemed to improve early in the morning of the admission day. At approximately 9:00 AM he took 36 baby aspirin tablets (2.9 gm) and shortly thereafter his mother gave him two glasses of water with an unmeasured amount of table salt and mustard added in an unsuccessful attempt to induce vomiting. The patient was taken to a hospital where at noon a gastric lavage with a saline solution was carried out.

Shortly after the lavage, the patient became unresponsive and was transferred to another hospital where he arrived at 3:15 PM in an unconscious state, at which time his temperature was 39.4 C; heart rate, 200 beats per minute; and respiration rate, 44 breaths per minute. A few minutes after arriving, the patient had a tonic clonic convulsion. An intravenous infusion of 5% dextrose in 0.22% saline with 50 mEq of sodium bicarbonate added to a 500 ml bottle (total sodium content, 138 mEq/liter) was begun. Two hours later this was changed to 5% dextrose in distilled water with no additions. The child received approximately 120 ml of each fluid.

Laboratory studies revealed the following values: hematocrit, 35%; hemoglobin, 12.9 gm/100 ml; white blood count, 19,000/cu mm; serum salicylate level, 27 mg/100 ml; sodium, 188 mEq/liter; potassium, 3.6 mEq/liter; CO_2, 20 millimols/liter; and chloride, 160 mEq/liter. The patient was then transferred to the Texas Children's Hospital intensive care unit where he arrived 11 hours after the aspirin ingestion and eight hours after the gastric lavage. On admission, respiration rate was 50 breaths per minute; heart rate, 220 beats per minute; and blood pressure, 90/40 mm Hg. Funduscopic examination results were normal and his pupils were dilated, responding sluggishly to light. The child was not weighed on admission. The lips were mildly cyanotic, the neck was supple, and the lungs were clear to percussion and auscultation.

Cardiovascular examination results indicated tachycardia, but there were no murmurs and no signs of cardiac enlargement. There were no signs of dehydration or edema. Abdominal examination findings were normal. The patient was comatose, responding slightly to deep pain. Deep tendon reflexes were 1+ and symmetrical; there were no abnormal reflexes. At the time of admission, urinalysis results revealed a specific gravity of 1.013, pH of 5.0, and moderate acetonuria, but findings were normal otherwise. Other values on admission were: serum sodium, 186 mEq/liter; chloride, 150 mEq/liter; potassium, 2.9 mEq/liter; total CO_2, 13 millimols/liter; salicylate, 50.9 mg/100 ml; glucose, 160 mg/100 ml; and blood urea nitrogen, 20 mg/100 ml. Chest roentgenogram results were normal and an electrocardiogram showed evidence of sinus tachycardia, but was normal otherwise. Spinal fluid examination results, including culture, were negative except for the presence of fresh red blood cells. Blood culture was also normal.

Fluid therapy consisted of 5% dextrose in 0.22% saline with 30 mEq of sodium bicarbonate and 20 mEq of potassium chloride added to each liter. Ten hours after admission the patient experienced a respiratory arrest and was given respirator assistance. In view of the persistent hypernatremia (sodium, 181 mEq/liter) and the lack of neurological improvement, peritoneal dialysis was begun; at that time the salicylate level was 9 mg/100 ml. The dialysis solution was prepared by combining equal quantities of a commercial dialysis preparation (Inpersol, 1.5% dextrose) with 5% dextrose in distilled water. The sodium concentration of this solution was 80 mEq/liter and potassium was added to a concentration of 4.0 mEq/liter. Six hours after dialysis was started, the sodium level was 154 mEq/liter and chloride, 124 mEq/liter.

The patient continued to be unresponsive and required mechanical ventilation until his death. Two electroencephalograms (24 hours apart) showed no electrical activity. Hyperglycemia (550 mg/100 ml) resulting from the peritoneal dialysis was treated with insulin. Eight hours following completion of dialysis, the serum electrolyte concentrations were as follows: sodium, 129 mEq/liter; potassium, 4.8 mEq/liter; chloride, 95 mEq/liter; and total CO_2, 18.5 millimols/liter. The mild hyponatremia was considered to be due to persisting hyperglycemia. Ten hours after cessation of dialysis, the patient had a cardiac arrest and was pronounced dead. Permission for autopsy was not granted.

Comment

In acute salt intoxication the rise in

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sodium and chloride concentrations account for an abrupt increase in the tonicity of extracellular fluid space. This causes an acute shift of water from the cells to the extracellular space resulting in an expansion of the extracellular and blood volumes that may be manifest clinically as acute circulatory congestion. Extracellular dilutional acidosis may also occur.[10] The shift of water from the cells diminishes the volume and raises the tonicity of the cells. The resulting interference with intracellular metabolism may further amplify the development of a metabolic acidosis.[11]

The most serious consequences of salt intoxication are seen in the central nervous system. The shrinkage of the brain in acute hypernatremic states depends upon loss of water from brain cells and cerebrospinal fluid (CSF) due to the differential transport rates of water and ions across the vascular CSF "barrier." The combination of brain shrinkage and distension of intracranial blood vessels associated with the increase in blood volume may result in subarachnoid and intraventricular hemorrhage as well as capillary thrombosis and hemorrhage throughout the brain substance. The change in volume and tonicity of the brain cells also undoubtedly interferes with the metabolism of these cells. These pathophysiological changes may be manifest clinically as coma, convulsions, reflex changes, and interference with the vital centers in the brain stem. These clinical and pathological findings have been described in detail elsewhere.[12] In addition, severe central nervous system sequelae to experimental salt intoxication have been reported in animals.[13] Elton et al[12] have also described renal and pulmonary damage in acute salt intoxication.

We cannot be certain of the relative contribution of the use of salt as an emetic and its use in the gastric lavage solution to the salt intoxication experienced by this child. However, beginning signs of central nervous system involvement were present before the lavage and these signs became very severe too soon after the lavage to allow time for significant transport of sodium from the lavage solution to the blood vascular space to occur. In addition, one would assume that most of the lavage solution was recovered. We therefore feel that the salt given as an emetic three hours before the marked worsening of his central nervous system status was the dominant factor.

At any rate, the hypernatremia was very severe and was believed to be the principal cause of the catastrophic course of this patient. Using the Done normogram,[14] the salicylate level six hours after ingestion falls into the asymptomatic range and the 11-hour concentration plotted on the same normogram falls into the mild intoxication range. Although there is a discrepancy between the values obtained at the two hospitals, the results of both determinations seem to indicate that salicylate intoxication was not a major factor in the child's course. Ironically, the use of saline to produce emesis in this case was unsuccessful. This patient did not have any signs or symptoms to suggest congestive heart failure. The central nervous system abnormalities, however, were profound and resulted in the child's death. Peritoneal dialysis was effective in lowering the concentration of sodium and chloride, but was ineffective in this instance in reversing the central nervous system symptoms.

The toxic potential of sodium chloride has been well known for years. It is our view, based on the experiences reported here and elsewhere,[5,6,9] that the use of salt as an emetic should be condemned. Most of the publications that advocate its use to promote emesis do not prescribe an exact amount of salt, but rather suggest that a "strong salt solution" be administered. In this case the mother dumped an unknown amount of salt into two glasses of water; in the case reported by DeGenaro and Nyhan,[9] four tablespoons of salt were added to each of four glasses of water. One level tablespoon of salt would contain at least 250 mEq of sodium and, if retained and absorbed, would be expected to raise the serum sodium level by 25 mEq/liter (based on a total body water of about ten liters in a 3-year-old child). Two tablespoons, if absorbed, would raise the serum sodium concentration by 50 mEq/liter. The salt given as an emetic might cause emesis, thereby reducing the load; however, it might not—as in our case. Cashman and Shirkey report that salt is only one fourth as effective as ipecac in promoting emesis. This unreliability makes its use all the more dangerous.

While the use of isotonic saline solutions are acceptable for lavage, we would recommend that hypertonic saline solutions for lavage should not be used.

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References

1. Nelson WE, Vaughan VC, McKay JR: *Textbook of Pediatrics*, ed 9. Philadelphia, WB Saunders Co, 1969, p 1491.
2. Adams WC: Emetics in accidental poisoning. *Pediatr Clin North Am* 8:351-352, 1961.
3. *Your Child From One to Six*. Department of Health, Education, and Welfare, Children's Bureau, No. 30, 1966, p 92.
4. Carter RF, Fotheringham BJ: Fatal salt poisoning due to gastric lavage with hypertonic saline. *Med J Aust* 1:539-541, 1971.
5. Laurence BH, Hopkins BE: Hypernatremia following saline emetic. *Med J Aust* 1:1301-1303, 1969.
6. Ward DJ: Fatal hypernatremia after a saline emetic. *Br Med J* 2:432, 1963.
7. Finberg L, Kiley J, Luttrell CN: Mass accidental salt poisoning in infancy. *JAMA* 184:187-190, 1963.
8. Calvin ME, Kneeper R, Robertson WO: Hazards to health: Salt poisoning. *N Engl J Med* 270:625-626, 1964.
9. DeGenaro F, Nyhan WL: Salt: A dangerous antidote. *J Pediatr* 78:1048-1049, 1971.
10. Shires GT, Holman J: Dilution acidosis. *Ann Intern Med* 28:557-559, 1948.
11. Sotos JF, Dodge PR, Talbot NB: Studies in experimental hypertonicity: II. Hypertonicity of body fluids as a cause of acidosis. *Pediatrics* 30:180-193, 1962.
12. Elton NW, Elton WJ, Nazareno JP: Pathology of acute salt poisoning in infants. *Am J Clin Pathol* 39:252-264, 1963.
13. Finberg L, Luttrell CN, Redd E: Pathogenesis of lesions in the nervous systems in hypernatremic states. *Pediatrics* 23:46-53, 1959.
14. Done AK: Salicylate intoxication. Significance of measurements of salicylate in blood in case of acute ingestion. *Pediatrics* 26:800-807, 1960.
15. Cashman TM, Shirkey HC: Emergency management of poisoning. *Pediatr Clin North Am* 17:525-534, 1970.

lymphocytic leukocytosis then occurred. At first large young lymphocytes appeared that were difficult to differentiate from monocytes, but, as time went on, these cells matured and were more easily identified. This reaction was accompanied by fever, adenopathy and a rising serum agglutination titer. The blood picture gradually returned to normal.

Nonspecific immunotransfusion should be performed primarily in septicemia. Specific immunotransfusion should be made possible by early isolation of the causative organism, preparation of a vaccine and active immunization of a suitable donor.

Cooperation between bacteriologist and attending physician is most important.

EDEMA AS INFLUENCED BY A LOW RATIO OF SODIUM TO POTASSIUM INTAKE

CLINICAL OBSERVATIONS

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The purpose of this paper is to present a few of many observations made on cases of edema which have been influenced chiefly by the dietary mineral control. This material is offered only as evidence of the probable importance of the daily intake of sodium and potassium in the control of chronic cases of disturbed water balance of cardiac and renal origin.

It is not necessary to enter into a long discussion of sodium, potassium, the chlorides and acid-base equilibrium, which have been so ably discussed by Blum, Widal, Bunge, Peters, Van Slyke, Keith and many others included in the editorials of THE JOURNAL. It is sufficient to say that there have been repeated statements of the importance of sodium rather than the chlorides in fluid storage and that there is abundant evidence of the antagonistic or the displacing influence of sodium by potassium with a resultant fluid loss. However, no definite program has come out of this great amount of work which has been of real practical clinical use.

Important advances have been made by Haldane, Keith, Gamble, Linder, their co-workers and others who have shown the great influence that acid-forming salts (calcium chloride, ammonium chloride, ammonium nitrate) and the mineral acids (hydrochloric acid) have on base excretion and water balance. The important relation of serum osmotic pressure to edema as influenced by the levels of the serum proteins has been emphasized by Starling, Epstein, Van Slyke, Leiter, Barker and Kirk, Shelburne and Egloff, and others. The rôle played by the motor power of the heart, the quality of the blood and renal function need no emphasis. Realizing that there are many influences concerned in controlling body fluid equilibrium, any one or all of which may be at fault at the same time in an edematous patient, I wish to suggest that a dietary regimen in which the sodium and potassium intake are carefully controlled can be instituted which may bring about an occasional clinical improvement after all other measures seem to have failed.

Read in part before the Chicago Society of Internal Medicine, Dec. 15, 1930.

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EXPERIMENTAL EVIDENCE

In the course of study of experimental edema, the possibility of throwing further light on some of the clinical observations immediately suggested itself.[1] As a result, a series of experiments was planned whereby dogs were made edematous or potentially edematous by reducing their blood serum proteins. Various salts were added to their diets and the most striking of these experiments were those in which sodium and potassium chloride were given.[2] When sodium chloride was added to the diet of such potentially or slightly edematous animals, fluid storage was prompt and often marked (as much as 40 per cent of the animal's weight in five days) and continued as long as sodium chloride was given. If sodium chloride was omitted, the edema would very gradually wear away. If, however, potassium chloride was substituted for the sodium chloride in equal amounts at any period during fluid storage, a prompt and marked diuresis occurred. Similar observations have been made independently by Shelburne and Egloff.[3] Not only would the animals lose all their edema but their weight would run below that of the control periods even though the serum proteins remained at the same levels. When these observations were made and repeatedly confirmed, it became obvious that a clinical application should be attempted.

CLINICAL SODIUM-POTASSIUM STUDIES

Patients with gross edema were selected for this study. They were chiefly of two classes; first, cases in which treatment had not been given so that they might be considered untreated cases; and, second, those in which all the present-day accepted forms of treatment had been given either without improvement or, if improvement had occurred in the past, with present failure.

The first step taken was to restrict the sodium intake in all its forms, the fluid intake being unregulated. Occasionally, a rather remarkable and prolonged improvement would occur following this one change alone. Going into the diet of these cases, it was found all too frequently that a patient with severe congestive heart failure, although he was on a salt poor diet and taking full doses of digitalis, together with ammonium chloride or ammonium nitrate and diuretics, was allowed at the same time large quantities of citrus fruits, or baking soda for gas and distention, or was given alkaline waters by his physician. It was immediately obvious why the salt free diet, digitalis and the diuretic regimen had failed.

The second step consisted in giving the patient a full maintenance diet of foods which were thought to be adequate in every respect except that the foods containing sodium were restricted when possible, while foods high in potassium were given. It is difficult to get a patient to take a diet for a long period unless it meets the physiologic requirements, and he will find great difficulty in taking any diet without salt. Both objections have been quite overcome by making the diet very liberal and by allowing the patient to shake potassium chloride on his food for taste. To make diets successful in the ambulatory clinic, they must be simple in outline. The plan now in use in the renal clinic at

1. Barker, M. H., and Kirk, E. J.: Experimental Edema in Dogs in Relation to Edema of Renal Origin in Patients, Arch. Int. Med. 45: 319 (March) 1930.
2. Barker, M. H.: The Effect of Low Proteinemia as Related to Specific Ions, read in a symposium on the kidney in health and disease at Minneapolis in July, 1930.
3. Shelburne, S. A., and Egloff, W. C.: Experimental Edema, Arch. Int. Med. 48: 51-69 (July) 1931.

Northwestern University Medical School is scheduled in tables 1 and 2.

When the diet outlined in the tables is started, 5 Gm. (one teaspoonful) of potassium chloride crystals is placed in a salt shaker in the morning. The patient uses from the shaker all he wishes on his food during the day and at the end of the day places the remainder in water and drinks it. It may be necessary to give more potassium chloride to promote diuresis in obstinate cases of the nephrotic type, but 5 Gm. is ordinarily sufficient in the edemas of cardiac or cardiovascular origin. In a few cases, a rather severe dehydration has resulted, so that the potassium chloride had to be reduced greatly or omitted for a time. Such cases, however, appear to be rare.

Table 1.—*Diet: Low Sodium-High Potassium with Acid Excess*

Fruits
3 servings daily, fresh or stewed, but should include either prunes, plums, cranberries or currants once daily.

Vegetables
2 large servings daily, especially beets, carrots, brussels sprouts, yellow corn, kohlrabi, lettuce, mushrooms, peas, spinach, kidney beans, parsnips.

Meat
2 servings daily.

Eggs
2.

Milk
1 glass.

Cream
½ glass.

Butter
Salt-free, 6 squares.

Cereal
Oatmeal or wheatena; farina, puffed wheat or rice occasionally.

Bread
Graham bread, 3 large slices or 6 small slices daily.

Rice, macaroni or spaghetti
1 serving daily.

Potato
1 serving.

Jelly, preserves or honey
2 level tablespoons.

Sugar
ad libitum—at least 1 tablespoonful.

NOTES

All foods are to be prepared without salt and no salt is to be served with meals. Potassium chloride (from 2 to 5 Gm. in shaker) may be given as salt substitute. Spices—cinnamon, sage, paprika, pepper, cloves, nutmeg, allspice—may be used. Small servings of citrus fruits may be added after fluid volume is established. Additional vitamin D to be supplied.

Coffee or tea, 1 cup daily.

A neutral diet may be obtained in one of the following three ways:
1. Reduction of meat, rice, macaroni, bread, cereal.
2. Increase of fruit and vegetables.
3. Addition of figs, molasses, raisins.

The latter method is recommended.

This diet is designed preferably for the treatment of convalescent and ambulatory patients. Very ill patients will have difficulty in eating all the diet, in which event, the diet may be reduced, the same food substances to be given, but in smaller amounts.

CHEMICAL STUDIES

Frequent determinations of the sodium, potassium, calcium, carbon dioxide combining power, p_H and cholesterol of the blood were done. No attempt will be made to discuss the results of these observations except to state that in general nothing striking appeared.[4] No significant changes in blood sodium or potassium were noted. Certainly there was no potassium storage as measured by increased levels in the blood stream. Tissue sodium and potassium determinations were not made. Occasionally a slight elevation of potassium above the control level of 17 to 19 mg. to a level of 21 to 25 mg. was noted after a few days, but this always fell back to the original level in one or two weeks. Blood serum proteins occasionally were reduced in the edemas of cardiac origin and they would return to normal as the edema reduced. Brown and Rowntree[5] have indicated that hydremic plethora does not exist in nephrotic and cardiac edemas in which there is no anemia, but the frequent apparent concentration of the proteins following diuresis in this group of cases speaks for some dilution. No shift in acid-base equilibrium was noted even after months of such management, which is in contrast to the acid-base balance following the administration of full doses of calcium chloride and hydrochloric acid.

REPORT OF CASES

The following case reports will serve to illustrate the types of patients used in the study and some of the results obtained.

Case 1.—E. S., a man, aged 56, a manufacturer, came with a history of weakness, dyspnea, orthopnea, irregular heart action, edema and ascites of five years' duration. A past history of chorea, rheumatic fever or syphilis could not be obtained. His symptoms had been slight at the beginning and he had been relieved by digitalis. Later, his symptoms became more severe and he was again quite relieved by taking ammonium chloride. In 1929, he became severely edematous and was forced to give up work. When I first saw him, in July, 1930, he was taking 0.1 Gm. of digitalis leaves three times daily, from 6 to 8 Gm. of ammonium chloride daily, and salyrgan once or twice a week.

The patient was a large man with marked dyspnea, orthopnea, and cyanosis of the lips, hands and lower extremities. He was coughing up large quantities of liquid sputum. The pupils reacted to light and the fundi were not remarkable except for

Table 2.—*Sample Menus*

1	2	3
Breakfast	*Breakfast*	*Breakfast*
Stewed currants	Plums	Stewed prunes
Oatmeal	Wheatena	Farina
Two poached eggs	Scrambled eggs	Soft cooked eggs
Graham toast	Graham toast	Graham toast
Sweet butter	Sweet butter	Sweet butter
Honey	Jelly	Honey
Cream	Cream	Cream
Sugar	Sugar	Sugar
Dinner	*Dinner*	*Dinner*
Roast beef	Broiled steak	Broiled lamb chop
Mashed potatoes	Buttered macaroni	Buttered spaghetti
Buttered beets	Stewed tomatoes	Buttered spinach
Sliced peaches	Banana salad	Grapefruit cup
Graham bread	Graham bread	Graham bread
Sweet butter	Sweet butter	Sweet butter
½ glass milk	½ glass milk	½ glass milk
Supper	*Supper*	*Supper*
Broiled chicken	Broiled liver or sweetbreads	Broiled whitefish
Buttered rice	Baked potato	Mashed potatoes
Jelly	Celery salad	Buttered brussels sprouts
Lettuce salad	Cherries	Pineapple and nut salad
Orange cup	Graham bread	Graham bread
Graham bread	Butter	Sweet butter
Sweet butter	Jelly	Jelly
½ glass milk	½ glass milk	½ glass milk

Menu	Protein	Fat	Carbohydrate	Cal.	Vitamin A	Vitamin B	Vitamin C	Vitamin D	Vitamin E
1....	89.3	140.4	235.9	2565	++++	+++	+++	+	++
2....	77.2	131.4	215.7	2355	++++	+++	+++	+	++
3....	84.2	130.1	227.6	2417	++++	+++	+++	+	++

Mineral Content

Ca	Mg	K	Na	P	Cl	S	Fe	Acid	Base
0.660	0.278	3.362	1.126	1.514	1.485	1.139	0.0177	46.4	34.0
0.627	0.268	3.263	1.038	1.332	1.556	0.975	0.0156	42.6	34.6
0.638	0.299	3.483	1.061	1.471	1.47	1.177	0.0168	45.4	40.5

4. The mass of clinical observations and the chemical data accumulated in the study will be published elsewhere.

5. Brown, G. E., and Rowntree, L. G.: The Volume and Composition of the Blood and Changes Incident to Diuresis in Cases of Edema, Arch. Int. Med. 35: 129 (Jan.) 1925.

some caliber changes and narrowing of the arteries with nicking at the arteriovenous crossings. The chest was barrel-shaped. There were no signs of fluid, but there was a large amount of moisture all through the bases. The heart was enlarged to percussion, the right border dulness being 4 cm., the left border 13 cm. and the base seemed not to be increased. No thrills were felt, but the apex was forceful. The rhythm was absolutely irregular, with a pulse of 56 and an apex rate of 88. A rough blowing systolic murmur was heard at the apex and transmitted to the base. There were no diastolic murmurs. Abdominal examination revealed a large amount of ascites and a tender liver 8 cm. below the costal margin. The lower extremities showed marked cyanosis and deep pitting edema.

The blood pressure was 156 systolic and 90 diastolic. The blood showed: red blood cells, 4,990,000; white blood cells, 9,600; hemoglobin 77 per cent (Newcomer). The Wassermann and Kahn tests were both negative. The urine showed a specific gravity of 1.018 and a slight trace of albumin, and was acid. The sediment showed an occasional hyaline and granular cast and an occasional white blood cell, but there were no red blood cells. Electrocardiography showed auricular fibrillation with a rather low electromotive force and considerable spread and slurring of the QRS complex. The T waves were inverted in lead 3 and they were regarded as a digitalis effect in that they became upright when digitalis was omitted.

A diagnosis of chronic myocarditis with auricular fibrillation, probably of hypertensive origin, was made, and after five days the patient was placed on the diet low in sodium with normally high potassium. All other medication was stopped, but he was given 5 Gm. of potassium chloride daily to shake on his food. As he had been ambulatory at home, he was discharged from the hospital so that he would be in his most natural state. Two days after starting this regimen, he noted a slight increase in the volume of urine and a decrease in body weight (chart 1). His weight fell from 92 to 75 Kg. in thirteen days and at that time he was entirely free from edema, and his skin hung in folds. Under this marked edema, which he had carried for years, there was rather severe wasting of the muscles. The liver gradually decreased in size and six months later his cough had cleared up and he was back at his work. Now, after a year and a half, he is very active; he feels well and has no cardiac symptoms. About one month after starting the diet he became extremely dehydrated, so the potassium was reduced to 2 Gm. daily and he was allowed small feedings of citrus fruits. He has been more careless of his diet in the past six months but, on the whole, he adheres closely to the plan outlined and seems remarkably well. He has taken 0.2 Gm. of digitalis folia daily during the past six months because the heart rhythm is more regular, but no symptomatic benefit has been observed.

This case seemed to be one of congestive heart failure which had finally failed to respond to management that was above criticism. The patient was apparently taking sufficient digitalis (0.3 Gm. daily) and he was on a low salt diet. He was taking ammonium chloride and finally he was forced to take salyrgan to hold the edema down. The response to the low sodium-high potassium management after a discontinuance of all other forms of therapy was striking. The events are best shown in chart 1. The inset shows a rather sharp concentration of the serum proteins coincident with the loss of the edema.

Case 2.—M. R., a married woman, aged 27, developed acute nephritis in August, 1929, which subsided, leaving her with an albuminuria of 16 Gm. a day. Four months later (December, 1929) the serum proteins had became gradually depleted, so that she presented the classic symptoms and indications of the so-called nephrosis group. She felt well except that she was tired. Physical examination was negative except for marked pitting edema of the ankles and thighs, and a moderate ascites. The blood pressure ranged about 90 systolic and 55 diastolic. The urea nitrogen of the blood was characteristically low at 8 mg. per hundred cubic centimeters. There was no anemia, and the Wassermann test of the blood was negative. Renal function tests were entirely normal. The urine contained from 12 to 18 Gm. of albumin daily and the sediment was entirely negative except for numerous hyaline casts, a rare hyaline cast with granules and numerous doubly refractile lipoid bodies in the casts and free in the urine. With physical strain or exposure and in the presence of infections of the upper respiratory tract, a shower of red blood cells would be seen in the urine for a few days.

Management consisted of a low sodium-high potassium diet and the protein was increased to 150 Gm. This diet was tolerated very well and she seemed to feel much stronger, but the proteinuria and edema persisted. Eight grams of ammonium chloride daily reduced the edema materially but it failed to keep her free from edema. The same was true of ammonium nitrate in 10 Gm. daily feeding. The sharpest diuresis and the most prolonged effect was caused by giving

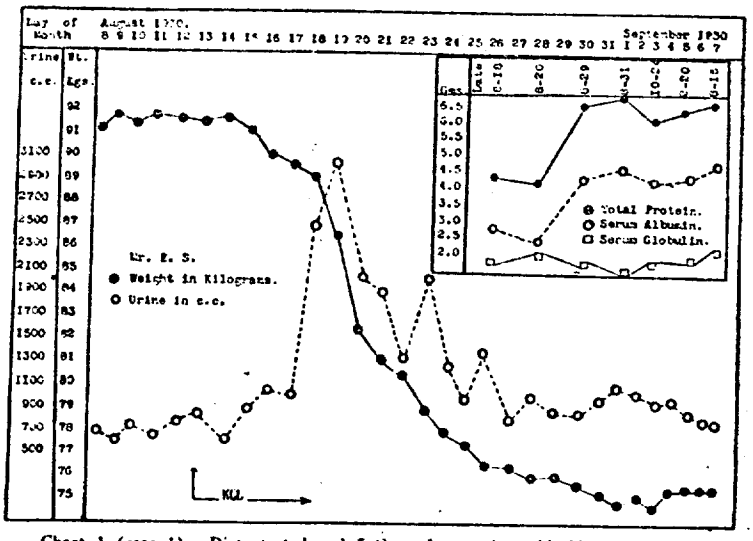


Chart 1 (case 1).—Diet started and 5 Gm. of potassium chloride started Aug. 13, 1930, and continued. The urinary output and the weight loss are graphed. The inset shows a rather reduced total serum protein, chiefly at the expense of the albumin fraction. A quite normal level of 6.5 per cent was found on the 29th after the edema had disappeared. These normal levels have obtained since that time. The last protein levels graphed were determined after one year.

4 cc. of dilute hydrochloric acid in water with each meal. However, in all these periods of therapy with the acid-forming salts and the hydrochloric acid, there was an associated decrease in the carbon dioxide, an increase in the chlorides and proteins, and a shift of the p_H from 7.22 to as low as 7.14. When this shift occurred, water was released from the body but the patient complained of not feeling well. She complained of dull headaches, weakness and loss of appetite. No aggravation of the renal picture was noted. Finally, potassium chloride was given in daily amounts of 5 Gm. A sharp diuresis occurred with a loss of all the ascites and the edema. When the potassium salt was discontinued, the fluid gradually returned, and with the resumption of potassium chloride feeding the edema again disappeared (chart 2). The patient was maintained free from edema for weeks at a time by this method.

This case is of interest in that the patient presented a nephrotic syndrome which not only responded to the giving of acid and acid-forming salts but relieved her of edema by the neutral salt, potassium chloride. The diuretic influence of large amounts of alkali as given by Osman[6] in this type of case is of interest in that he gives potassium salts in large amounts. Again, an

6. Osman, A. A.: The Use of Alkalis in the Treatment of Bright's Disease, Guy's Hosp. Rep. 77: 386 (July-Aug.) 1927.

apparent concentration of the serum proteins was associated with diuresis whether it was produced by ammonium chloride, hydrochloric acid or potassium chloride (inset, chart 2). The patient was much more willing to eat the low salt-high protein diet as soon as she was given potassium chloride, which served as a salt substitute as well as a diuretic. It was especially gratifying that the patient had no depressing symptoms while taking potassium chloride, and there were no measurable changes in the acid-base equilibrium, which was in contrast to the acidosis and symptoms produced by hydrochloric acid and the ammonium salts.

Case 3.—L. L., a laborer, aged 36, began to suffer weakness, dyspnea and nocturia from five to eight times in July, 1931, and later edema of the ankles appeared. The

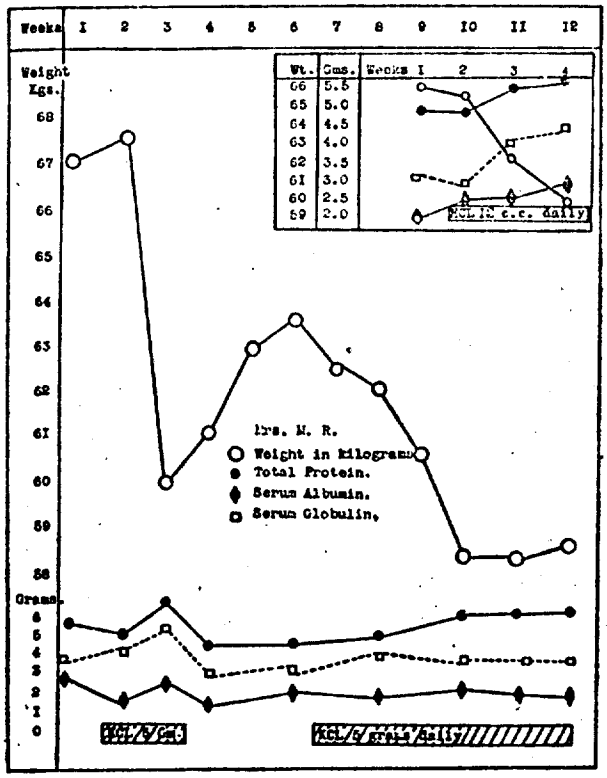


Chart 2 (case 2).—In addition to the diet, 5 Gm. of potassium chloride was given daily beginning with the second week. A great reduction in edema followed as shown by the fall in body weight. The potassium chloride was discontinued and a gradual increase in weight was noted, which again fell to normal when the potassium chloride feedings were resumed. The lines at the bottom of the chart show an increase in the level of the total serum proteins as the edema disappeared. Both serum albumin and serum globulin seemed somewhat increased. The inset shows a similar drop in weight and an increase in serum proteins in one patient associated with the giving of 4 cc. of dilute hydrochloric acid in water with each meal.

patient had had left hemiplegia in 1925, for which he had been observed in the neurologic clinic. Albumin and hyaline casts were found in the urine and the blood pressure was 225 systolic and 150 diastolic, so he was referred to the renal clinic. Physical examination showed only a slight residual of the old hemiplegia. The pupils reacted sharply to light and in accommodation. The fundi showed edema of the disk margins. The retinal vessels were small and tortuous, and there was nicking at the arteriovenous crossings. No hemorrhages were seen, but there were two small areas of exudation in the inferior temporal quadrant of the right eye. The throat and neck were normal and the chest was clear. The heart was moderately enlarged, the right border dulness being 3 cm. and the left border dulness 13 cm. The rate was regular at 90, and no murmurs were heard except for a slight systolic blow at the apex. The liver was about 5 cm. below the costal margin and it was very tender. There was marked edema of the legs and thighs. The blood pressure was now 196 systolic and 138 diastolic. There was no anemia; the Wassermann reaction was negative and the renal function tests were essentially negative. The day volume was 250 cc. with a specific gravity of 1.022, and the night volume was 1,440 cc. with a specific gravity of 1.012. The phenolsulphonphthalein excretion was reduced to 15 per cent, as might be expected in congestion of the liver. The urea nitrogen of the blood was only 14.5 mg. The electrocardiogram showed a rate of 88, a sinus mechanism, low P waves in all leads and a slurring of the RS complex in all leads. The T wave was inverted in lead 1 and diphasic in lead 2.

The patient was regarded as one having hypertensive vascular disease in which congestive heart failure had developed. He remained ambulatory and continued on the low salt diet of the preceding five years. He was given 5 Gm. of potassium chloride to sprinkle on his food; within a week he noted an increase in the urinary output and a decrease in the dyspnea, and his friends told him that his color was improved. The edema began to disappear rapidly and after two months' time he was quite dehydrated, having lost 30 pounds (13.6 Kg.) (chart 3). He was no longer cyanotic or dyspneic, and he boasted that he was walking two miles daily. He contracted an influenzal type of infection in January, 1932, followed by pneumonia and death.

This patient is of especial interest in that he was known to be hypertensive for six years during which time he had been on a low salt-low protein diet. He had taken no medication except potassium iodide from time to time. He finally developed a typical congestive heart failure from which he seemed to be entirely relieved by taking 5 Gm. of potassium chloride daily (chart 3). No cardiac therapy, bed rest or other circulatory supportive measures were instituted. It was striking to watch the circulatory picture improve as fluid balance was established. The table inserted in chart 3 indicates a rather concentrated state of the serum proteins. A slight return toward normal levels seems to follow clinical improvement.

COMMENT

To date, sixteen suitable cases have been studied in detail; four of them failed to respond. Clinical differences or laboratory observations have not been determined on which to predict which cases will respond and which cases will not. The cases that have been helped most have been those of congestive heart failure of the nonvalvular type. Two cases of chronic cardiac valvular disease with massive edema have shown good responses. One patient with high-grade mitral stenosis remained edema free for four months and he was up and around, when he suddenly died of pulmonary embolism. The second was a case of stenosis of the mitral and pulmonary valves. This patient was kept edema free for six months and then she went on to congestive failure similar to that present when she was first seen. One severe nephrotic syndrome has been maintained edema free only by the giving of 10 Gm. of potassium chloride daily. The standard care of numerous cases of congestive failure not included in this paper has apparently been greatly augmented by the general plan described.

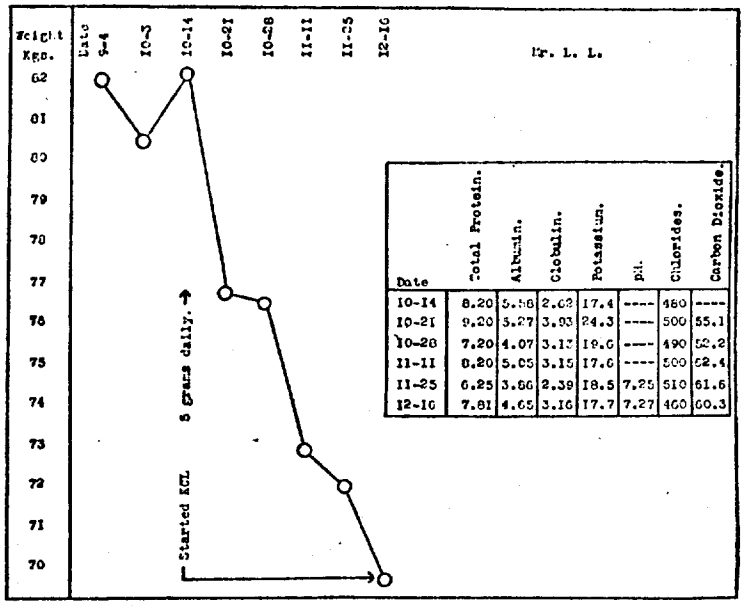
The diet employed is general and it is felt that it is well adapted to the maintenance of a person with chronic disease. Great care must be given in prescribing diets that are deficient in any way if they are to be taken for a long period of time. This point is not

sufficiently emphasized in the clinical literature. Too many patients are placed on unbalanced or deficient diets which render them less able to maintain their body weight during the long strain of chronic disease. In many prescribed diets, it seems that the normal sodium to potassium ratio is greatly disturbed owing to the restricting of meats and the feeding of more fruit and vegetables. In the same fashion a great excess of alkaline ash is introduced into the body, which probably greatly facilitates fluid storage. Lashmet [7] has recently emphasized the addition of acid substances to the neutral diet. It will be noted that the diet outlined in table 2 leaves a definitely acid ash. That may explain why it has rarely been necessary to prescribe acid-forming salts.

The total amounts of specific mineral given in the diet agree quite closely with the averages given by Sherman [8] in 150 American dietaries. His figures are 1.94 Gm. of sodium and 3.39 Gm. of potassium a day, as compared to 1.07 and 3.33 Gm., respectively, given to our patients. The chief difference is the increased ratio of potassium to sodium, but often no diuretic influence was noted until further potassium was added as potassium chloride. The helpful diet of Keith, Smith and Whelan [9] differs from the plan used in these cases in that they attempt to keep all minerals low. Their first diet contained 3.74 Gm. of potassium, but this was reduced to 1.62 so that all ions were low in amount. The possible dangers of restricting important minerals such as sodium, calcium, phosphorus and potassium is not to be discussed here. The other chief difference is that the Keith low mineral diet leaves an excess alkaline ash.

Although no deleterious effects have been noted in this group of cases in which potassium chloride was given, the extreme toxicity of the potassium ion should be kept in mind. Several observers, Osman,[6] Addison [10] and others, have used large doses of potassium substances over long periods without trouble even in severe cases of vascular and renal disease. However, overdosage and, especially, poor excretion of potassium should be closely watched in that either may result in storage sufficient to produce severe symptoms and death.

In connection with the occasional marked diuretic response to potassium chloride feeding, the work of Harrison and Pilcher and their co-workers [11] should be borne in mind. These workers have shown that tissue potassium is often at a low level in cases of congestive heart failure. Whether or not a relation exists between their observations and the clinical studies presented here will be worked out in the future. The influence of the potassium ion alone or its action in the presence or absence of sodium and in special circumstances or combinations with the chlorides will be answered only by much careful study. Certainly more thought should be given to the minerals ingested by the patient chronically ill with edema.



Date	Total Protein.	Albumin.	Globulin.	Potassium.	pH.	Chlorides.	Carbon Dioxide.
10-14	8.20	5.18	2.02	17.4	----	480	----
10-21	9.20	5.27	3.93	24.3	----	500	55.1
10-28	7.20	4.07	3.13	19.0	----	490	52.2
11-11	8.20	5.05	3.15	17.0	----	500	52.4
11-25	8.25	3.66	2.39	18.5	7.25	510	61.6
12-16	7.81	4.65	3.16	17.7	7.27	460	60.3

Chart 3 (case 3).—This graph shows the dramatic loss of body weight in congestive heart failure, associated with the giving of 5 Gm. of potassium chloride daily when the sodium intake was reduced. A complete symptomatic restoration of circulatory efficiency was effected without specific cardiac therapy. The small table inset shows a rather high percentage of serum proteins. The chlorides, carbon dioxide and *pH* remained essentially unchanged.

SUMMARY

Experimental observations on the relation of sodium and potassium to body fluid balance have been applied to cases of cardiac and nephrotic edema. It has been found that twelve of sixteen patients lost their edema when given potassium chloride, provided they were placed on a full general diet with an acid ash excess in which a low ratio of sodium to potassium was maintained. Some cases responded dramatically and others less. No cause for the success or failure of this regimen has been found. No ill effects from the procedure have been noted in cases followed as long as a year and a half. No conclusions can be drawn from these observations, but it is hoped that this material might be of help to an occasional patient who has failed to respond to the usual measures.

303 East Superior Street.

7. Lashmet, F. H.: The Treatment of Nephritic Edema by Acid, J. A. M. A. 97: 918 (Sept. 26) 1931.

8. Sherman, H. C.: Chemistry of Food and Nutrition, ed. 3, New York, Macmillan Company, 1930, p. 271.

9. Keith, N. M.; Smith, Florence F., and Whelan, Mary: The Therapeutic Use of Diets Low in Water and in Mineral Content, Arch. Int. Med. 37: 550 (April) 1926.

10. Addison, W. L. T.: The Use of Sodium Chloride, Potassium Chloride, Sodium Bromide and Potassium Bromide in Cases of Arterial Hypertension Which Were Amenable to Potassium Chloride, Canad. M. A. J. 18: 281 (March) 1928.

11. Pilcher, Cobb; Calhoun, J. A.; Cullen, G. E., and Harrison, T. R.: Studies in Congestive Heart Failure: Potassium Content of Skeletal Muscle Obtained by Biopsy, J. Clin. Investigation 9: 191 (Oct.) 1930.

Spermatogenesis in Advanced Age.—Recently I appeared as an expert witness in the law courts in a suit which turned on whether a man of 83 could be the father of a child. We won our case, not only because the collateral evidence was strong, but because I could quote many instances of spermatogenesis in the testes of men of 80 and even of 90. Metchnikoff actually cites the case of a man of 103 in whose testes spermatozoa were found. And if examples are required of the retention of sexual passion we have only to think of Goethe, who at the age of 74 fell so desperately in love with a girl of 17 that when his suit went badly he was prostrated with grief and no longer desired to live. Indeed, it was this episode in his life, and a previous one in which he had been more successful, that suggested to him the theme of Faust.—Walker, Kenneth: The Accidents of the Male Climacteric, *Brit. M. J.* **1:** 50 (Jan. 9) 1932.

Proc. Soc. Exp. Biol. Med. 136: 565-568, 1971

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Enhanced Salt Toxicity in the Spontaneously Hypertensive Rat (35312)

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In 1950, Sapirstein *et al.* (1) demonstrated that rats developed hypertension when given 2% NaCl solution to drink for 6 weeks. Dahl *et al.* (2, 3) developed two strains of Sprague-Dawley rats, one of which became hypertensive rapidly when fed an excess salt diet while the other was resistant to salt. These two strains remained consistent as hypertension-sensitive and hypertension-resistant with other methods of creating experimental hypertension, such as clipping one renal artery (4). In 1963, Okamoto and Aoki (5) produced by selective inbreeding a spontaneously hypertensive strain of Wistar rats (SHR) whose members developed elevated blood pressures as they matured. Louis *et al.* (6) tested the effect of salt consumption on this SHR strain and concluded that their blood pressures were relatively unresponsive to excessive sodium intake. Such a result is at variance with the experience of Dahl and also is inconsistent with Okamoto's observations that SHR responded with a greater rise in blood pressure than control rats when given DOCA plus 1% NaCl for 10 weeks or following unilateral nephrectomy (7). The present study was undertaken to examine the question as to whether SHR developed more severe hypertension than normal (non-SH) Wistar rats when subjected to a prolonged period of excess salt ingestion.

Methods. Six male and 8 female SHR derived from the strain developed by Okamoto and Aoki were divided into two groups each consisting of 4 females and 3 males. The membership of the groups was determined by matching average systolic pressures and body weights. One group received 1% NaCl in tap water *ad libitum* while the control group was given plain tap water. All rats ate Wayne chow. They were housed in plastic cages, three males or four females to a cage. Two control groups of 3-month-old normal (non-SH) Wistar rats were also formed. Three males and three females received the 1% NaCl solution while one female and two males received tap water without excess salt.

All rats were kept in a room controlled at a constant temperature of 78°F. Systolic blood pressures were recorded approximately every 2 weeks in this room by means of a tail cuff and mercury-in-rubber resistance gauge. The inflatable cuff was connected to a Statham transducer attached to a Hewlett-Packard carrier preamplifier and 2-channel recorder. The other channel of the recorder was connected through another preamplifier and a small Wheatstone bridge to the resistance gauge which detected the change in tail circumference occurring at the time of the first pulse appearing distal to the tail cuff. The rats were lightly anesthetized with ether during pressure measurements. The animals were weighed every 2 weeks.

Results. Water consumption. SH rats receiving 1% salt solution drank 2 to 3 times more than those on tap water from the third week and 1 to 3 times more than the normal rats on the same salt regimen (Table I). Water consumption in the SH rats receiving salt increased with time until the majority had died. Normal rats receiving excess salt drank less than double the amount consumed by their litter mates who were on tap water.

Mortality. Three of the 4 female SHR, drinking 1% NaCl, died between the weeks 18 and 21 of the study. One male SHR, receiving 1% salt water, died in week 14 and another of the same group died in week 19. One SHR male and one SHR female are still alive after 28 weeks on the high salt regimen. All SHR who received tap water without

TABLE I. Average Water Consumption (ml/rat/day) in Various Groups.

Time in study (weeks)	SHR				Normal rats			
	Controls (tap water)		1% NaCl		Controls (tap water)		1% NaCl	
	Male	Female	Male	Female	Male	Female	Male	Female
3	44	27	92	64	32	21	46	38
4	59	30	118	66	31	12	50	45
5	44	25	101	61	34	12	49	58
9	42	25	148	68	—	25	44	42
14	34	21	176	128	—	—	—	—
19	40	—	110	139	—	—	—	—
20	—	—	—	—	25	17	28	25
21	39	25	111	84	—	—	—	—

excess salt are alive with the exception of one female who was accidentally over-etherized in week 26. One normal Wistar female receiving 1% NaCl died in week 19. All of the other normal rats remained alive and in good health through 20 weeks.

Weights. Comparing the same sexes, both SHR groups showed the same growth rates except when an individual in the salt group was approaching death. Such rats would usually show a slight weight gain followed by a rapid and substantial weight loss (Fig. 1). Both groups of normal rats grew at the same rate until week 7 when the males and females receiving salt began to grow more slowly. With the exception of the females receiving salt, the normal rats grew faster than the SHR on similar regimens.

Blood pressures. By the week 3 after beginning the study the average blood pressure of all SHR on the higher salt regimen was greater than that of their tap water counterparts (Table II). By week 9 this difference was significant ($p<.01$) and remained significant through week 20 ($p<.005$) after which time most of the rats receiving excess salt died and the levels of the blood pressures of the remaining control rats began to reach that of the two remaining rats on excess salt (Table II). No significant differences were found between the normal control rats receiving 1% salt and those receiving tap water.

Pathology. Prior to death the SH rats receiving excess salt had a scruffy, ragged appearance. Later they developed abdominal bloating, edema of the front and the back legs, and decreased motor activity. Terminally, these rats showed rhythmic twitching movements and labored breathing.

The SH rats receiving excess salt also had periarteritis of the mesenteric vessels. The kidneys were small and presented granular surfaces. Two of these rats, one male and one female, had left ventricular scarring. All five rats that died showed left ventricular hypertrophy. The control SHR (sacrificed at 27

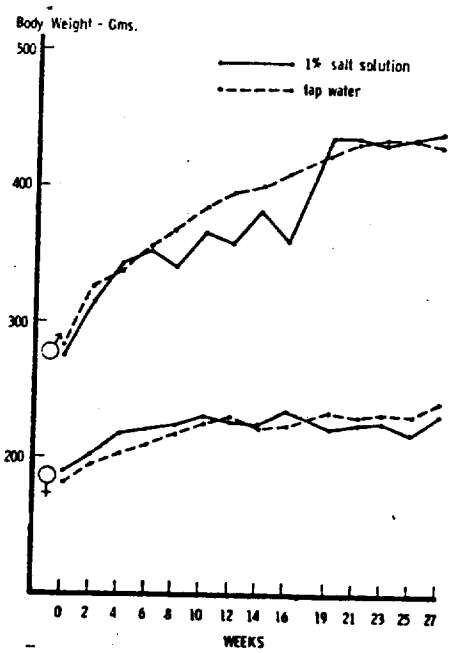

FIG. 1. Changes in body weight of SH rats on tap water (- -); or 1% salt (—) during study period. Males shown above and females below.

TABLE II. Blood Pressure Changes in Various Groups of Rats.

Time in study (weeks)	Systolic blood pressure (mm Hg)			
	SHR		Normal	
	Controls (tap water)	1% NaCl	Controls (tap water)	1% NaCl
0	121 ± 17	120 ± 23	107 ± 8	106 ± 15
1	136 ± 14	129 ± 14		
3	142 ± 23	144 ± 25	111 ± 12	104 ± 18
5	152 ± 28	169 ± 26		
7	152 ± 20	167 ± 23		
9	148 ± 14	184 ± 31	91 ± 5	105 ± 16
15	155 ± 20	196 ± 14	106 ± 11	103 ± 19
20	153 ± 19	186 ± 2	104 ± 8	103 ± 10
24	146 ± 19	176 ± 12		
26	172 ± 18	179 ± 6		
28	160 ± 26	185 ± 33		

weeks) displayed none of the above changes. The female normal rat which died had no hypertension or edema and apparently died of a respiratory infection.

Histological examination of the kidneys of the SH rats, given 1% salt solution who died, disclosed the typical changes of malignant nephrosclerosis with fibrinoid necrosis of the media and intimal hyperplasia of the renal arterioles. The control SHR given tap water showed a normal renal architecture on histological examination. All of the normal Wistar rats were sacrificed between weeks 20 and 21. No abnormalities were found in the heart, mesentery, or kidneys of these rats.

Discussion. Many investigators have described the pathology of the accelerated phase of hypertension in rats to be rapid with loss of weight, edema, apathy, twitching or convulsions, and death (9–13). Renal damage shown by granular outer surfaces and a thin and indistinct cortical layer on cut section is common as is cardiac hypertrophy and periarteritis. The rats also may have fibrotic areas in the myocardium. Histological examination shows fibrinoid necrosis of the renal arterioles. These pathological changes developed only in the SHR on 1% salt and were not seen in the normal rat or SHR on tap water during the period of the experiment.

In the study of Meneely and associates (9), 18% of their rats fed 7, 8.4, or 9.8% NaCl died between months 3 and 6. In Koletsky's study (11) 40 weeks were required on 1% NaCl to produce well-developed hypertension in normal rats compared with 9 weeks for a significant elevation of blood pressure in our SH rats.

The present results are similar to those of Dahl (4) in which his "salt-sensitive" rats, while developing hypertension more slowly when initiation of a high salt diet was delayed until the rats were 3 months old, still showed an increased blood pressure and death within 8 months or less. Louis and his associates (6) concluded that the SH rat shows a relative insensitivity of its hypertension to changes in dietary sodium. However, their observations on salt-loaded rats did not extend beyond 10 weeks; and after 7 weeks the blood pressure was significantly higher in the SH rats given excess salt. Therefore, their results do not differ greatly from the present observations.

Summary. Chronic ingestion of 1% salt solution causes accelerated hypertension leading to death in the SH rat at a more rapid rate than in the normal rat given 1% salt or in the SH rat given tap water.

1. Sapirstein, L. A., Brandt, W. L., and Drury, D. R., Proc. Soc. Exp. Biol. Med. 73, 82 (1950).

2. Dahl, L. K., Heine, M., and Tassinari, L., Nature (London) 194, 480 (1962).

3. Dahl, L. K., Heine, M., and Tassinari, L., J. Exp. Med. 115, 1173 (1962).

4. Dahl, L. K., Heine, M., and Tassinari, L., J. Exp. Med. 118, 605 (1963).

5. Okamoto, K., and Aoki, K., Jap. Circ. J. 27, 28 (1963).

6. Louis, W. J., Tabei, R., Spector, S., and Sjoerdsma, A., Circ. Res. 14, 15 (1969).

7. Okamoto, K., Int. Rev. Exp. Pathol. 7, 227 (1969).

8. Dahl, L. K., Knudsen, K. D., Heine, M., and Leitl, G. J., Circ. Res. 22, 11 (1968).

9. Meneely, G. R., Tucker, R. G., Darby, W. J., and Auerbach, S. H., J. Exp. Med. 98, 71 (1953).

10. Okamoto, K., Aoki, K., Nosaka, S., and Lukushima, M., Jap. Circ. J. 30, 703 (1966).

11. Koletsky, S., Lab. Invest. 7, 377 (1958).

12. Smirk, F. H., and Phelan, E. L., J. Pathol. Bacteriol. 89, 57 (1965).

13. Byrom, F. B. (F. Gross, ed.), p. 125. Springer-Verlag, New York (1966).

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THE EFFECT OF SODIUM CHLORIDE ON HYPERTENSION *

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In 1904, Ambard and Beaujard,[1] in France, put forth the claim that there was a direct relationship between the retention of sodium chloride and the level of arterial tension. This was followed by attempts on the part of French clinicians to treat patients with an elevated blood pressure by reducing the sodium chloride intake. In this country, Allen and his pupils have been the chief advocates of this method of treating hypertensive patients, while Mosenthal and his co-workers have led the opposition to this form of therapy.

In 1922, Allen and Sherrill[2] reported on 180 cases of hypertension in which the patients were treated by close restriction of the sodium chloride intake. Fully normal blood pressure was restored in 18.9 per cent of these patients, while in 41.9 per cent there was sufficient improvement to regard the outcome as "a distinct therapeutic success." Transitory benefit followed by relapse or death resulted in 8.9 per cent of the patients, while complete failure occurred in 30.5 per cent of the cases. The failures were believed to be due to organic changes, chiefly in the kidneys. In this report Allen set up plasma chlorides of 580 mg. as a dividing line, and stated that patients with plasma chlorides above this figure had a much better prognosis than those with chlorides below this level.

Isolated case reports favorable to this line of therapy were also published by Musser,[3] by Konikow and Smith,[4] by Houghton[5] and by others at about the same time.

In 1923, Selman[6] (one of Allen's co-workers) reported ten more cases of hypertension in which the patients were treated by salt restric-

* Submitted for publication, May 22, 1929.
* From the Medical Service of Mt. Sinai Hospital.

1. Ambard, L., and Beaujard: Arch. gén. de méd. **81**:520, 1904.
2. Allen, F. M., and Sherrill, J. W.: J. Metab. Research **2**:429, 1922.
3. Musser, J. H.: New York M. J. **112**:570, 1920.
4. Konikow, M. J., and Smith, M.: Boston M. & S. J. **185**:281, 1921.
5. Houghton, H. A.: M. Rec. **101**:441, 1922.
6. Selman, J. J.: Ohio State M. J. **19**:852, 1923.

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tion with reduction to normal pressure in 60 per cent of the cases. In 1925, Allen[7] again reiterated his statements previously made and claimed striking success in 60 per cent of his cases. He said that he also believed the salt restriction to be of benefit in mitigating the symptoms and prolonging life even in the majority of cases classed as failures. Furthermore, he stated that others who had obtained no benefits from salt-free diets in hypertension had failed because their diets were not accurately salt free.

Carrying on Allen's work, Vogel[8] recently reported the results in 212 cases of hypertension in which the patients had been treated at Allen's institute since the report of 1922. Seventy-four per cent of these patients were improved, while 26 per cent were classed as unimproved. In the majority of the unimproved cases, the patients were thought to have had an associated nephritis.

In opposition to the claims of Allen and his co-workers, O'Hare,[9] in 1921, arranged a series of forty-five cases of hypertension according to the height of the maximum blood chlorides and showed that those with the normal chloride values averaged the higher blood pressures. In 1922, Mosenthal[10] showed that there was no close relationship between the blood pressure and the concentration of salt in the blood, and, furthermore, that types of nephritis characterized by salt retention have as their outstanding features albuminuria, edema and no increase in blood pressure. Moreover, he had not succeeded in affecting the arterial tension by the administration of 10 Gm. of sodium chloride per day. In 1923, Mosenthal and Short[11] once more reiterated their claims of the inefficacy of sodium chloride restriction as a therapeutic measure in cases of hypertension. They emphasized the marked spontaneous variations which occur in the blood pressure of all hypertensive persons and showed that there was no evidence that sodium chloride raises the blood pressure. They concluded that sodium chloride may be safely administered in large doses to patients with hypertension, provided that there are no complications or sequelae present.

As an interesting sidelight on this question, mention may here be made of the work of Addison,[12] who claimed that it was the sodium and not the chloride ion which was harmful to patients with hypertension, and he treated these patients with potassium chloride.

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7. Allen, F. M.: New York State J. Med. **25**:726, 1925.
8. Vogel, S. A.: New York State J. Med. **28**:318, 1928.
9. O'Hare, I. P.: Proc. Am. Soc. for Clin. Invest., 1921, p. 6.
10. Mosenthal, H. O.: M. Clin. N. Amer. **5**:1139, 1922.
11. Mosenthal, H. O., and Short, J. J.: Am. J. M. Sc. **165**:531, 1923.
12. Addison, W. L. T., and Clark, H. G.: Canad. M. A. J. **15**:913, 1925. Addison, W. L. T.: Canad. M. A. J. **18**:281, 1928

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METHOD

The purpose in this work was to determine the effects of various amounts of sodium chloride in the dietary of patients with hypertension. This has been attempted in a series of thoroughly controlled and carefully studied cases. We have also checked the results in two of these cases by repeating the sodium chloride studies after waiting several months. The difficulties encountered in such a study are numerous. In the first place, we have found it necessary to hospitalize our patients, as otherwise no accurate check could be kept on the diets. In the second place, daily determinations of sodium chloride had to be run on the specimens of urine to act as controls on the daily salt intake. Moreover, we have found that symptoms of salt privation prevented prolonged administration of diets containing less than 1 Gm. of sodium chloride per day. Also we must not omit the fact that patients usually find diets with a low salt content rather unpalatable and object to keeping up this regimen for a long period; after a while they tend to begin to "cheat" on the diet.

We have purposely refrained from using the term "salt-free" in connection with diets, as there is, accurately speaking, no such a thing as a strictly salt-free diet. Instead, we have designated our diets as salt-poor when they contained less than 2 Gm. of sodium chloride per day and as very poor in salt when they contained less than 1 Gm. of sodium chloride per day. In order to go to the opposite extreme in our tests we have also given these patients 10, 20 and even 30 Gm. of sodium chloride per day in addition to the amount present in the diet, and in some cases have also tried the effect of large doses of sodium chloride intravenously. It must be emphasized also that all our diets have been checked by daily determinations of the chloride in the urine.

In these patients we endeavored to vary the daily salt intake while keeping all the other factors as constant as possible. We also first observed the patients on an unrestricted diet for a period of time until they had become accustomed to hospital routine and their pressures had reached as stable a level as is possible with this type of patient (whose blood pressure, as we all know, is subject to frequent spontaneous variations).

We here report the results of varying the sodium chloride intake of eleven patients with hypertension. In addition, we made two distinct studies on two of these patients at different times. The average stay in the hospital was thirty-nine days; the shortest stay was nineteen and the longest seventy-five days. These patients ranged in ages from 31 to 57 years. Three were men and eight were women. Nine were white and two were colored. All of these cases belong to those commonly classified under the heading "essential hypertension"; that is, the patients had elevated blood pressure without evidence of real kidney

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damage and without any other readily ascertainable cause for the increased tension. The heart rate was regular in all cases. The pressures at the time of admissions varied from 150 systolic and 100 diastolic to 264 systolic and 134 diastolic. Diabetes (of a mild type) was present in one case; otherwise there were no complications.

In addition to these patients, we have also made observations on the effect of changes in the sodium chloride intake on the blood pressure of two other patients—one with nephrosis (for fifty-three days) and the other with acute glomerular nephritis (for eighty-one days).

With all the other factors constant as far as possible, the patients were kept on salt-poor diets and on diets containing varying amounts of salt for a sufficient length of time to observe whether there was any relationship between the pressure and the diet.

TABLE 1.—*Summary of Blood Pressures on Varying Salt Intakes*

Case	Unrestricted Diet	Low Salt (Less Than 2 Gm./Day)	Very Low Salt (Less Than 1 Gm./Day)	5 Gm. Salt/Day	10 Gm. Salt/Day	15 Gm. Salt/Day	20 Gm. Salt/Day	25 Gm. Salt/Day	30 Gm. Salt/Day
1 A. S.	184/110	198/123	.....	.....	201/119	214/127	.....	.....	.....
2 J. F.	228/109	.....	230/104	.....	242/112	236/112	.....	237/117	.....
3 F. R.	201/110	194/111	195/112	.....	.....	.....	.....	.....	.....
3 F. R.	219/117	216/117	.....	.....	215/111	225/113	.....	.....	.....
4 W. G.	195/124	.....	190/118	.....	.....	.....	.....	.....	.....
5 N. W.	226/125	217/132	.....	.....	201/121	204/122	203/119	.....	.....
6 L. K.	174/196	.....	168/98	.....	142/95	.....	.....	.....	.....
6 L. K.	164/90	183/110	189/116	.....	.....	.....	.....	.....	.....
7 C. W.	152/94	138/98	155/95	.....	.....	.....	.....	.....	.....
8 G. S.	200/112	.....	158/109	.....	.....	.....	.....	.....	.....
9 S. B.	166/98	.....	152/102	.....	148/90	.....	.....	.....	.....
10 E. L.	186/102	.....	156/90	.....	182/118	.....	.....	.....	.....
11 E. K.	221/100	.....	206/95	.....	194/91	.....	.....	.....	.....
12 L. R.	136/72	109/77	.....	.....	115/77	109/71	.....	.....	119/82
(Nephrosis)	.....	121/83	.....	.....	.....	.....	.....	.....	.....
13 J. R. (age 11). Acute Nephritis	164/92	.....	.....	133/75	.....	.....	.....	.....	.....

In order to conserve space, we reproduce only four of the charts, showing the daily (or twice daily) blood pressure readings, the daily chloride output and the salt intake. In reporting our cases, we think it would be too confusing for the reader and would take up too much of his time to give all the pressure readings, and we are therefore giving only the average blood pressure level on each type of diet in each case. Table 1 gives a summary of the average blood pressure levels in each patient under the varying amounts of salt intake.

Before going on to the case reports, it is worth while to emphasize that patients with hypertension commonly show spontaneous variation from day to day and even during the course of the same day. Deductions from the pressure curves must therefore be made with great caution, and only appreciable changes in pressure which are maintained over a period of time should be considered as having any significance. In order that the personal equation might be eliminated as far as pos-

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When the patient was on an unrestricted diet, the blood pressure averaged 219 systolic and 117 diastolic; on 10 Gm. of sodium chloride per day, it averaged 215 systolic and 111 diastolic; on 20 Gm. per day, 225 systolic and 113 diastolic, and on a low salt diet (less than 1.5 Gm. per day), 216 systolic and 117 diastolic.

Case 4.—W. G., a colored man, aged 49, was admitted to the hospital on May 19, 1923, and was discharged on June 12, 1923. The chief complaints were shortness of breath of six months' duration and swelling of the ankles of three weeks' duration. The blood pressure was 182 systolic and 130 diastolic. The heart was moderately enlarged. The liver was palpable three fingerbreadths below the costal margin, and there was a moderate degree of pretibial edema. The urine showed considerable albumin but no casts. The blood chemistry was normal, and the plasma chlorides 597 mg. per hundred cubic centimeters.

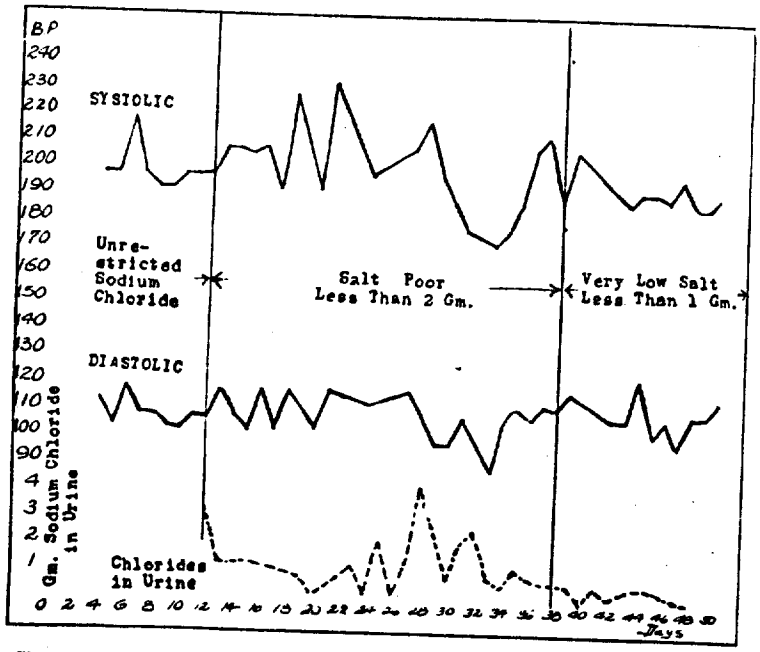

Chart 1.—Blood pressure readings, daily chloride output and salt intake in case 3 during the patient's first admission.

With the patient on an unrestricted diet, the blood pressure average was 195 systolic and 124 diastolic, and on a low salt intake (less than 0.5 Gm. per day) it averaged 190 systolic and 118 diastolic.

Case 5.—N. W., a woman, aged 43, was admitted to the hospital on March 19, 1923, and was discharged on June 2, 1923. The chief complaints were headaches, backaches, spots before the eyes and frequency of urination. She had known of her hypertension for about two years. The blood pressure was 252 systolic and 130 diastolic. There was slight cardiac enlargement to the left and no signs of decompensation. The blood chemistry was normal, and the plasma chlorides were 582 mg. per hundred cubic centimeters. The urine at times showed traces of albumin and occasional casts.

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When the patient was on an unrestricted diet, the blood pressure averaged 220 systolic and 115 diastolic; on a low salt intake, 217 systolic and 132 diastolic; on 10 Gm. of sodium chloride per day, 201 systolic and 121 diastolic; on 15 Gm. of sodium chloride, 204 systolic and 122 diastolic, and on 20 Gm. of sodium chloride per day, 203 systolic and 119 diastolic.

CASE 6.—L. K., a woman, aged 40, was admitted to the hospital on May 1, 1922, and was discharged on May 21, 1922. The complaints were chiefly of headaches, and of aches and pains in different parts of the body for about seven years. The blood pressure was 175 systolic and 95 diastolic. There was slight cardiac enlargement, but no signs of decompensation. The blood chemistry was normal. The urine showed slight amounts of albumin at times and occasional hyaline and granular casts.

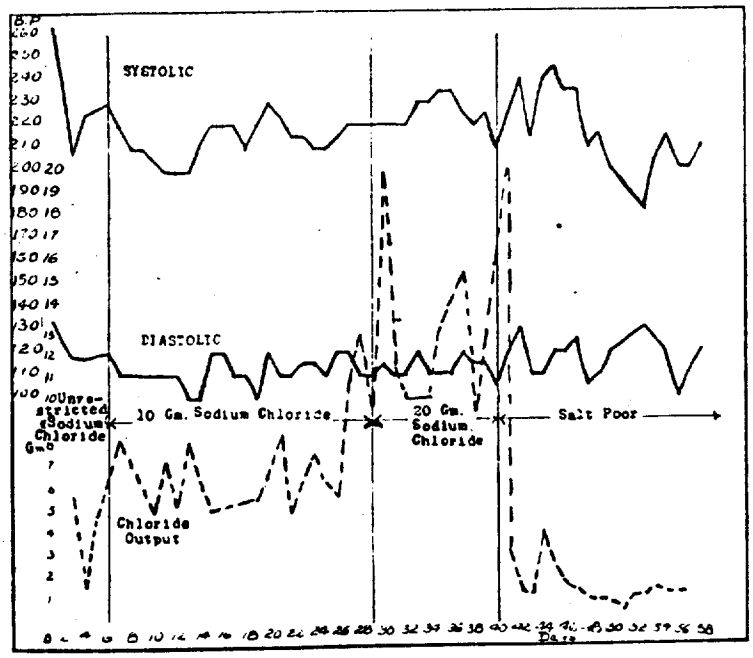

Chart 2.—Blood pressure readings, daily chloride output and salt intake in case 3 during the patient's second admission.

The blood pressure with the patient on an unrestricted diet averaged 174 systolic and 96 diastolic. On a low salt intake (less than 1 Gm. per day) for nine days, the pressure averaged 168 systolic and 98 diastolic. At the end of this period, the patient became so weak (due to salt privation) that she was unable to get out of bed. At this time her urinary chloride excretion was 0.2 Gm. per day. Coincidentally with this marked anorexia, the blood pressure fell to 135 systolic and 90 diastolic. The patient was then placed on an intake of 10 Gm. of salt per day for nine days, and soon began to show marked improvement. The pressure remained down for about a week, but at the time of discharge it had risen to 175 systolic and 110 diastolic.

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The patient was readmitted on Feb. 13, 1923, and discharged on March 4, 1923. This time the patient complained chiefly of headaches. The blood pressure at the time of admission was 164 systolic and 90 diastolic. The blood chemistry was normal and the plasma chlorides were 622 mg. per hundred cubic centimeters. The urine was normal, except for traces of albumin.

With the patient on an unrestricted diet, the blood pressure averaged 164 systolic and 90 diastolic. On a low salt intake, it averaged 183 systolic and 110 diastolic, and on a very low salt intake, 189 systolic and 110 diastolic.

Case 7.—C. W., a man, aged 30, was admitted to the hospital on June 12, 1924, and was discharged on July 23, 1924. The chief complaints were swelling of the legs and generalized weakness of two months' duration. The blood pressure was 150 systolic and 100 diastolic. There was slight edema of the ankles. The urine showed large amounts of albumin and on microscopic examination granular casts. The blood chemistry was normal and the plasma chlorides 597 mg. per hundred cubic centimeters.

When the patient was on an unrestricted diet, the blood pressure averaged 152 systolic and 94 diastolic; on a low salt intake (less than 2 Gm. per day), it averaged 138 systolic and 98 diastolic, while on a very low salt intake, it averaged 155 systolic and 95 diastolic.

Case 8.—G. S., a woman, aged 45, was admitted to the hospital on June 24, 1924, and was discharged on July 12, 1924. The chief complaints were headaches and throbbing sensations of one year's duration. The blood pressure was 200 systolic and 112 diastolic. The orthodiagram showed slight left ventricular enlargement and diffuse aortic widening. There were no signs of decompensation. The blood chemistry was normal, and the plasma chlorides 626 mg. per hundred cubic centimeters. The urine contained no albumin, but occasional granular casts were found on microscopic examination.

With the patient on an unrestricted diet, the blood pressure averaged 200 systolic and 112 diastolic; on a very low salt intake, it averaged 158 systolic and 109 diastolic.

Case 9.—S. B., a woman, aged 31, was admitted to the hospital on April 7, 1924, and was discharged on May 13, 1924. The chief complaints were shortness of breath, fainting spells and pain in the left side of the chest of three and one-half years' duration. The blood pressure was 190 systolic and 110 diastolic. There were no signs of decompensation. The orthodiagram showed the aorta to be slightly widened and increased in density. The blood chemistry was normal, and the plasma chlorides 586 mg. per hundred cubic centimeters. The urine showed small amounts of albumin at times but no casts.

When the patient was on an unrestricted diet, the blood pressure averaged 166 systolic and 98 diastolic; on a very low salt intake (less than 0.5 Gm. per day), 155 systolic and 102 diastolic, and on a daily intake of 10 Gm. of salt, 148 systolic and 99 diastolic.

Case 10.—E. L., a colored woman, was admitted to the hospital on May 6, 1924, and was discharged on June 12, 1924. The chief complaints were frequent severe headaches and occasional fainting spells. The blood pressure was 240 systolic and 120 diastolic. The orthodiagram showed moderate left ventricular enlargement. There were no signs of decompensation. The blood chemistry was normal and the plasma chlorides 577 mg. per hundred cubic centimeters. The results of urinalysis were negative.

The blood pressure with the patient on an unrestricted diet averaged 186 systolic and 102 diastolic; on a very low salt intake, 156 systolic and 99 diastolic, and on 10 Gm. of salt per day, 182 systolic and 118 diastolic.

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CASE 11.—E. K., a woman, aged 57, was admitted to the hospital on March 9, 1923, and was discharged on June 2, 1923. The chief complaints were shortness of breath, palpitation and precordial pain; as well as generalized aches and pains intermittently for about twelve years. The blood pressure was 240 systolic and 90 diastolic. The orthodiagram showed moderate left ventricular enlargement. The urea nitrogen of the blood was 20 mg. per hundred cubic centimeters, and the plasma chlorides 626 mg. per hundred cubic centimeters. The urine showed a trace of albumin but no casts. The blood sugar at the time of admission was 184 mg. per hundred cubic centimeters, and the first specimen of urine showed a moderate amount of sugar. The diabetes was easily controlled by regulating the diet, and the patient remained "sugar-free" during her stay.

With the patient on an unrestricted salt intake, the blood pressure dropped from 240 systolic and 90 diastolic to 200 systolic and 90 diastolic, but the high

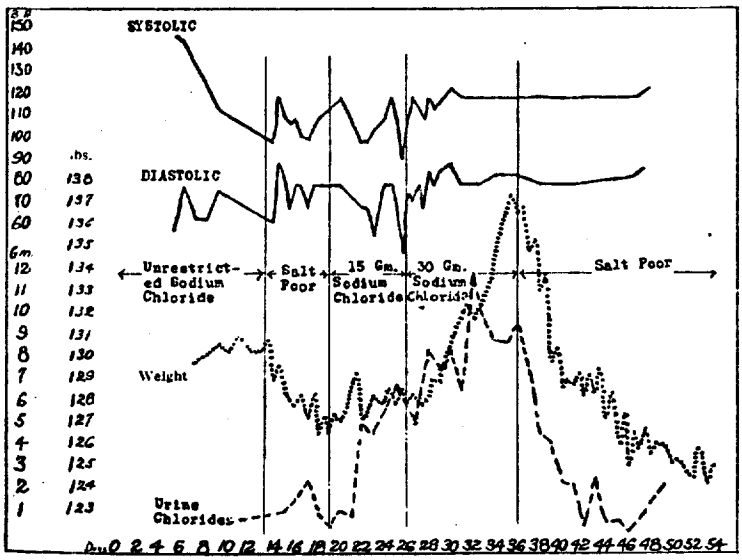

Chart 3.—Blood pressure readings, daily chloride output and salt intake in case 12.

figures obtained at first served to bring the average pressure for this period up to 221 systolic and 100 diastolic. On a salt intake of less than 1 Gm. per day, the pressure averaged 206 systolic and 95 diastolic, and on a high salt intake (reaching an intake at times of 30 Gm. per day), it averaged 194 systolic and 91 diastolic.

CASE 12.—*Nephrosis.*—L. R., a man, aged 26, was admitted to the hospital on May 15, 1923, and was discharged on July 7, 1923. The chief complaints were recurrent swelling of the left submaxillary region and swelling of the ankles and legs of about four months' duration. He felt well otherwise. Examination showed slight puffiness of the eyelids and edema of the legs. The remainder of the examination gave negative results. The blood pressure at the time of admission was 146 systolic and 86 diastolic. The blood chemistry was normal. The plasma chlorides were 626 mg. per hundred cubic centimeters. The urine

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showed large amounts of albumin but no casts, and the specific gravity varied from 1.005 to 1.025. The phenolsulphonphthalein output was 65 per cent in two hours. The eyegrounds were normal.

When the patient was on an unrestricted diet, the blood pressure fell from 146 systolic and 86 diastolic to 115 systolic and 78 diastolic with an average of 136 systolic and 72 diastolic. On a salt-poor diet the pressure then averaged 109 systolic and 77 diastolic. With 15 Gm. of salt per day added to the diet, the pressure averaged 109 systolic and 71 diastolic, and with 30 Gm. of salt per day added to the diet, 119 systolic and 82 diastolic. Following this the patient was again placed on a salt-poor diet, and the blood pressure averaged 121 systolic and 83 diastolic.

Although the blood pressure was not influenced by the salt intake, careful check on the patient's weight (twice daily weighing) showed that the edema increased with the salt intake.

Case 13.—*Acute Glomerular Nephritis.*—J. R., a boy, aged 11, was admitted to the hospital on Nov. 20, 1924, and was discharged on Feb. 9, 1925. About two weeks before admission the patient had a sore throat. While the patient was

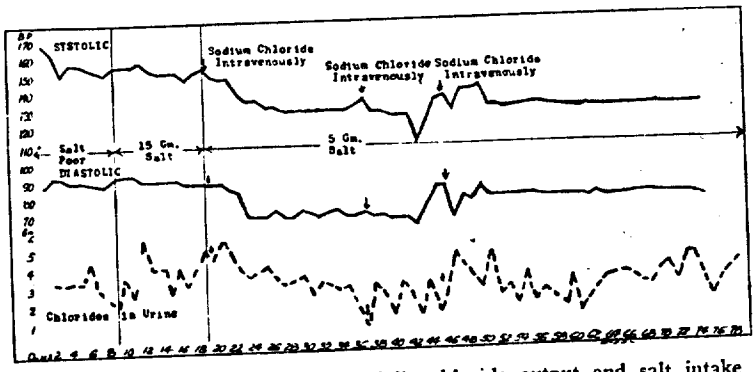

Chart 4.—Blood pressure readings, daily chloride output and salt intake in case 13.

recovering from this, the mother noticed that the child's face was becoming rather puffy, especially around the eyes. Soon after this the legs also began to swell, and in addition the child began to complain of pain in the back just below the ribs on both sides. Since the onset of his illness, the child had had increased frequency of urination.

Examination showed generalized subcutaneous edema, most marked in the face which was puffy and about the eyes where the swelling was prominent. The tonsils were hypertrophied, and there was some redness of the pharynx. The heart was slightly enlarged. The blood pressure was 170 systolic and 90 diastolic. There was tenderness over both kidneys posteriorly. The urine at the time of admission showed gross blood and a large amount of albumin, and microscopically large numbers of red blood cells, white blood cells and casts. The blood chemistry showed nonprotein nitrogen, 50 mg.; urea nitrogen, 23 mg.; creatinine, 1.6 mg., and plasma chlorides, 530 mg. per hundred cubic centimeters.

At the time of admission, the patient was put on a salt-poor diet; following this, 15 Gm. of salt per day was added to his diet and later, 5 Gm. of salt was added. In addition he was given 5 Gm. of sodium chloride (in 5 per cent solu-

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tion) intravenously on three occasions. Following the intravenous administration of sodium chloride, the blood pressure readings were taken at hourly intervals, with the results shown in table 2.

The patient improved slowly, and his blood pressure dropped gradually, without regard to the salt intake. At the time of discharge there was no blood in the urine, and the microscopic examination failed to show any red blood cells or casts. Also the edema had entirely disappeared.

SUMMARY

1. The effect of sodium chloride on the blood pressure has been studied on eleven patients with so-called essential hypertension, on one patient with nephrosis, and on another with hypertension associated with acute glomerular nephritis.

2. These patients have been carefully observed while on diets containing varying amounts of sodium chloride, ranging from intakes of less than 1 Gm. per day to 30 Gm. per day.

3. In only one of these cases (case 10) was there an appreciable drop in blood pressure, which seemed to be due to the salt-poor diet.

TABLE 2.—*Blood Pressure Readings Before and After the Intravenous Administration of Sodium Chloride*

	Dec. 8th	Dec. 26th	Jan. 4th
Before	164/90	136/72	122/72
1 hour after	150/80	138/70	122/72
2 hours after	162/92	120/66	120/70
3 hours after	160/90	128/70	124/68
4 hours after	160/90	136/72	.....

4. In another case (case 8) there was a well marked drop in the systolic pressure, but this case was not observed for a sufficient length of time.

5. In a third case (case 6) the blood pressure fell when the patient showed marked signs of salt privation with accompanying anorexia, weakness, etc., but rose again when her condition improved.

6. Reduction of the sodium chloride intake in all of the other cases did not cause a drop in the blood pressure level.

7. Raising of the sodium chloride intake above the normal level also did not have any appreciable effect on the blood pressure.

8. In the patient with nephrosis the edema (but not the blood pressure) varied with the amount of sodium chloride in the diet.

9. Administration of sodium chloride orally and intravenously even to patients with acute hemorrhagic nephritis had no effect on the blood pressure level.

10. The initial level of the plasma chlorides bore no relation to the subsequent course of the case.

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CONCLUSION

Thirteen patients were carefully observed in the hospital for a period of time ranging from twenty to eighty-one days (average, forty-four days). After an initial period of stabilization, rest in bed, diet, etc., ranging from ten to fourteen days, salt was withdrawn from their diet, their output dropping in most instances to below 1 Gm. per day and in some as low as 0.5 Gm. per day. After they had been kept at this level for periods of from two to five weeks, sodium chloride was added to their diet in quantities of from 10 to 30 Gm. per day, supplemented in several instances by the intravenous administration of from 5 to 15 Gm. We have failed to see any unquestioned modification of the blood pressure curve which could be definitely attributed to variations in the sodium chloride intake.

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Which Factor is Third?

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The term 'Third Factor' was introduced to designate the variable, other than glomerular filtration rate and aldosterone-like activity, that is responsible for the increase in sodium excretion that occurs when saline solutions are infused in normal animals. Although the existence of one or more additional variables had been suggested by many earlier studies and inferred by many earlier investigators, it was the experiments of DE WARDENER and MILLS and their associates [6, 15] that finally convinced everyone, at least after these findings had been confirmed in half a dozen other laboratories, that something other than glomerular filtration and adrenal steroids must be involved in the regulation of sodium excretion [1, 12, 19, 7]. That this is true, is now generally accepted. Beyond that there is almost nothing about which there is general agreement.

Attempts to explain the changes in reabsorption of sodium can be divided into two broad groups of factors – physical and hormonal:

Among the physical factors are changes in the pressures and flows in the peritubular capillaries as proposed, for example, by EARLEY and his associates [14]. These have generally been thought to exert their influence by their effects on the so-called Starling forces that affect the distribution of fluid across the capillary wall between blood and interstitial fluid. The complementary forces determined by the plasma protein concentration are also included in this hypothesis. It is not clear how these forces might exert their influence on the active movement of sodium and water across the tubule wall. It is generally agreed that the rather trivial osmotic forces available from changes in plasma protein concentration are not sufficient to produce much fluid movement across a structure such as the tubule wall which, compared to a capillary, has a very low permeability to solute. There have been rather

vague allusions to the volume of intercellular spaces and the basal infoldings of proximal tubule cells. A satisfactory explanation of how the effect might be produced has not been explicitly stated.

Nevertheless, the fact that there is no adequate explanation of the mechanism cannot be interpreted as evidence that the postulated forces are not important. On the contrary there is quite good evidence that they are. EARLEY and his associates have shown that measures which might be expected to raise peritubular capillary pressure yield a marked increase in sodium excretion – for example, the unilateral production of renal vasodilation especially when arterial pressure is increased. They have also shown that elevation of plasma albumin concentration acutely reduces salt excretion [14].

We have also shown recently that reabsorption in the proximal tubule varies rapidly and directly with changes in the oncotic pressure of the blood

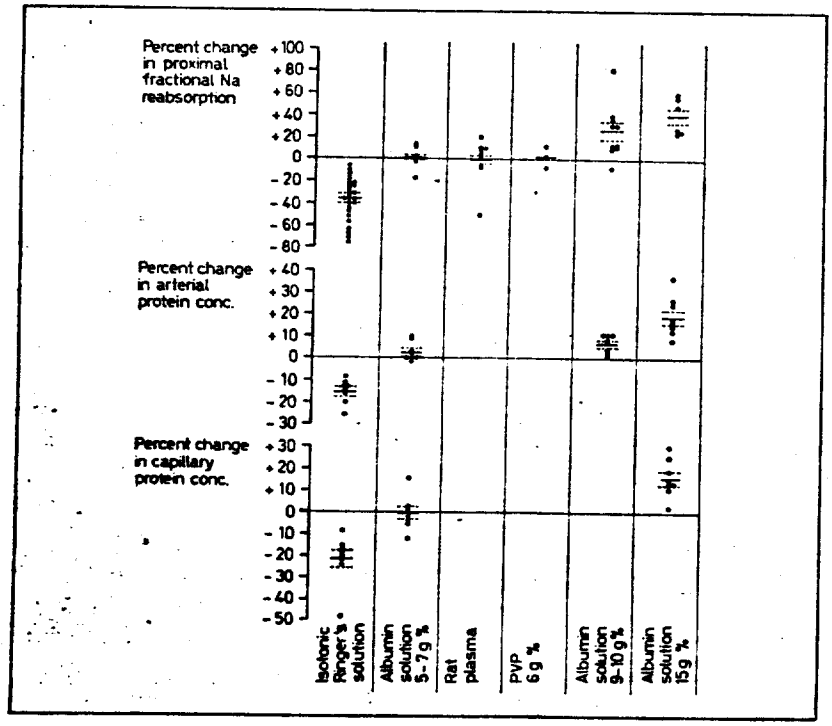

Fig. 1. Changes in proximal reabsorption during intra-aortic injection of fluids of varying oncotic pressure. From BRENNER *et al.* [2].

in peritubular capillaries [2]. In these experiments in rats 2 ml of solutions of varying oncotic pressure were injected into the aorta above the renal arteries and fluid collected from proximal tubules beginning some 10 sec after the start and during the period of the injection. The data, which are summarized in figure 1, indicate that reabsorption is immediately depressed by the injection of Ringer's solution; unaffected by rat plasma, 5% albumin or isoncotic dextran; and increased by 10–15% serum albumin. We have, in addition, measured the protein concentration of blood from peritubular capillaries and found the expected changes. The immediate onset of the changes in reabsorption indicates that the effects observed cannot be attributed to an extrarenal humoral factor. The minimum time for the onset of such an effect would be twice the circulation time; the observed effect is fully developed within that period.

On the other hand, the time course of the changes in reabsorption has an additional implication. Figure 2 shows that, although the immediate effect

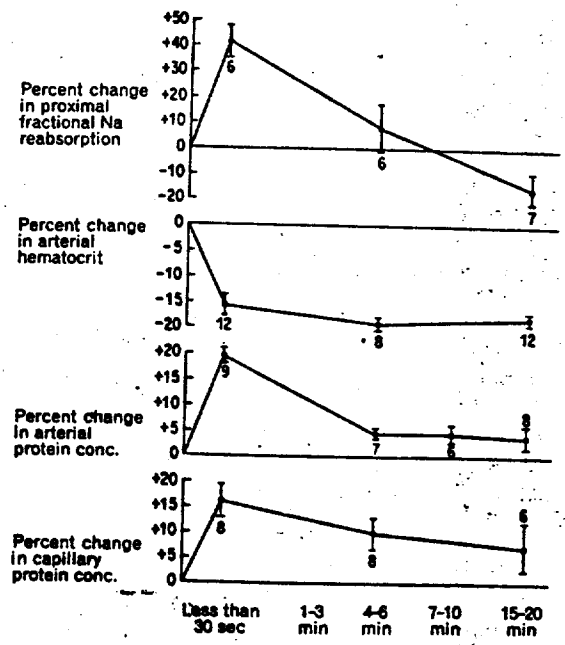

Fig. 2. Time-course of changes in reabsorption in proximal tubule during and following intra-aortic injection of 15% albumin solution. From BRENNER *et al.* [2].

of the injection of hyperoncotic fluid is an increase of reabsorption. 20 min later, at which time peritubular capillary protein concentration has returned nearly to normal, reabsorption has not only returned to normal but is depressed. This depression, which corresponds to that previously observed following the administration of hyperoncotic albumin in dogs [8], is compatible with the operation of a humoral agent. However, missing from all of these experiments is the measurement of capillary hydrostatic pressure. As a consequence of the rather crude methods of measurement that have been used, we do not believe that the measurements that have been made to date have sufficient accuracy and reliability to yield useful information about changes of peritubular capillary pressure of the magnitude likely to be relevant to this problem. Till such measurements are made the possibility that even the late effects of injection may be explained by changes in the balance of hydrostatic and oncotic forces cannot be excluded.

One additional physical explanation for the increased salt excretion of salt loading involves a hypothetical shift of filtration from nephrons that excrete little salt to others that excrete more. Although we have not studied the problem exhaustively, we have not observed any systematic deviation of the filtration rate in superficial nephrons from that of the kidney as a whole. If there is, in fact, redistribution of glomerular filtration during salt loading, an explanation of some other sort is still required for the depression of reabsorption that occurs in surface proximal convoluted tubules [7]. The depression of reabsorption has been demonstrated in many laboratories and with many techniques; this, in fact, is one of very few observations in current micropuncture studies that seem to be reproduced in any laboratory. There are observations in direct contradiction of each other on almost every other point!

There seems to be an adequate basis for concluding that physical factors are important, but there remains considerable question as to whether they can be responsible for the entire phenomenon. There is no lack of proposed hormonal factors that might fill any gap. These proposed humoral factors are conveniently reviewed with reference to the techniques that have been used in attempts to detect and measure them.

In the experiments of DE WARDENER and his associates referred to earlier [6, 15], an attempt was made to demonstrate a humoral effector substance by cross-circulating blood from a saline-infused donor to a hydropenic recipient maintained at constant weight. Because the sodium excreted by the recipient did not exceed about a third that put out by the saline-loaded donor the results were interpreted as negative. The idea of a humoral effector was not

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abandoned but it was suggested that the hypothetical substance might have too short a half-life to attain an adequate concentration in the recipient. Very similar experiments were done by JOHNSTON and DAVIS, differing mainly in that the donors were dogs that had escaped from chronic administration of DOCA [9]. The results, including the marked discrepancy between donor and recipient, were entirely similar to those of DE WARDENER's group, but were interpreted as positive. It is clear, however, that they cannot be considered conclusive.

Perfusion of the blood through an isolated kidney avoids the dilution inherent in the cross-circulation experiment. This technique was also used by DE WARDENER and his associates [6, 15] as well as by LICHARDUS and PEARCE [13]. Both observed rather small increases in sodium excretion when the blood perfusing the kidney was from a salt-loaded animal. Such experiments have the defect that isolated kidneys tend to show an increase in salt excretion with time – a phenomenon observed, for example, in the studies of NIZET and his associates whose success with isolated kidneys is as great as any that has been reported [16]. NIZET has attempted to circumvent this difficulty by retaining one kidney as a control for the other. It is noteworthy that in his experiments he finds the changes in blood composition adequate to explain the increased salt excretion with, in fact, what appears to be remarkable precision [16]. The isolated kidney experiments seem to me to be inconclusive as far as the demonstration of a hormonal agent is concerned.

Attempts have been made, with reported success, to demonstrate the presence of inhibitors of sodium transport in the blood of salt-loaded animals. Such putative inhibitors have been tested for ability to depress sodium transport in frog skin and toad bladder. CORT has reported positive results with samples obtained by precipitation of plasma proteins with trichloroacetic acid, extraction of the trichloroacetic acid with ether and concentration to one tenth the original volume [5]. Since these solutions are markedly hypertonic with sodium concentrations of about 1,500 mEq/l [10], this may account for some of the effect although it is not clear why similar preparations from animals not given salt do not. BUCKALEW, using dialysates of plasma which avoid the problem of hypertonicity, has given a preliminary report of similar results [4]. It cannot be assumed that an agent that depresses transport in isolated amphibian membranes necessarily has similar effects in the kidney. Nevertheless, if these results can be confirmed, the conditions under which the reported factor is present and absent would be strongly suggestive of involvement in regulating sodium balance. The same would perhaps be true of the inhibitor of PAH uptake reported by BRICKER *et al.* [3].

RECTOR and SELDIN and their associates have reported that plasma from saline-infused animals depresses reabsorption in the proximal tubule of rats in which it is injected and that, when dialysates of such plasma are used to establish split-drops in rat proximal tubules, these drops are more slowly absorbed than similar drops made with dialysates of plasma from hydropenic animals [18]. Neither our laboratory nor SCHRIER, DE WARDENER and HOLZGREVE have been able to obtain similar results [20]. We have carried out studies in collaboration with the group in Dallas and they have extended these double-blind studies. All of the results have been negative [17]. This particular reported humoral agent should be eliminated from further consideration.

Humoral agents have also been assayed by the injection of concentrated preparations from plasma or urine into intact assay animals. The material reported by CORT and his associates has been assayed in this way [5]. The terms in which his results are reported are not amenable to evaluation. The percent change in tubular rejection fraction is not, in itself, a meaningful index. For example, a 100 % change in tubular rejection fraction from 0.1 % to 0.2 % of the filtered sodium is a trivial effect. Without additional detail about the condition of the assay animals, the order of injection of various materials, the number of assays in single animals and the number of animals in each assay, an adequate evaluation of the significance of the assay cannot be reached. We have attempted to repeat the results obtained with TCA concentrates of plasma from hydropenic and saline-loaded dogs [10]. The results, shown in table I, reflect the very high salt concentration of the injected material. The second injection elicits a greater increase in salt excretion than the first whether the second extract injected is from a hydropenic or salt-loaded animal.

LARAGH and his associates have reported that the plasma and urine of salt-loaded men and sheep contains a material that increases sodium excretion when injected in water-loaded rats heterozygous for diabetes insipidus [11]. The material is isolated by fractionation on sephadex columns and behaves like a substance with a molecular weight between 5,000 and 70,000 and it is non-dialyzable and destroyed by trichloroacetic acid. Striking increases in sodium excretion lasting several hours were produced. The material is reported to be only minimally active, if at all, in hydropenic animals and in the well-hydrated animals in which it is assayed produces increased salt excretion with little increase in free-water clearance. These facts suggest that the material might depress reabsorption in a distal segment of the nephron and have relatively little effect in the proximal tubule. This

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Table I. Comparison of trichloroacetic acid preparations of 'natriuretic' and 'hydropenic' dog plasmas on renal Na excretion. Results of assays in rats of plasma samples from hydropenic and saline-infused dogs. Samples prepared as described by CORT and associates [5]

Rat No.	Material injected	Urine volume (μl/min)		$U_{Na}V$ (μEq/min)		GFR (ml/min)		Na excretion (% of filtered)		Infusion conc. (meq/l)	
		C	E	C	E	C	E	C	E	Na^+	K^+
1	Natr.	6	20	0.06	0.82					1325	60
	Hydro.	6	28	0.41	2.83					1570	75
2	Natr.	9	21	0.16	0.94	1.95	2.36	0.05	0.27	1238	44
	Hydro.	7	23	0.15	2.21	2.00	1.80	0.05	0.82	1390	65
3	Hydro.	5	22	0.12	3.30	2.54	1.30	0.03	1.71	1390	65
	Natr.	6	35	0.69	8.48	2.13	2.61	0.20	2.10	1238	44
4	Hydro.	5	34	0.17	4.66	2.05	3.52	0.08	0.92	1078	51

together with the rather erratic onset of its effect from immediate to as much as an hour suggests that it cannot, by itself, explain some of the facts of saline diuresis nor can it be the same as the material reported by CORT and his associates.

In summary, a wide variety of mechanisms have been advanced to explain the increased sodium excretion that follows the administration of salt. The evidence concerning no one of them is entirely convincing although it seems fairly certain that physical factors at least play a role. Although the existence of additional hormonal factors is likely, none of the proposed agents has yet been demonstrated unequivocally. It is highly probable that so-called 'Third Factor' is, in fact, multiple.

References

1. BLYTHE, W. B. and WELT, L. G.: Dissociation between filtered load of sodium and its rate of excretion in the urine. J. clin. Invest. *42:* 1491–1496 (1963).
2. BRENNER, B.; FALCHUK, K. H.; KEIMOWITZ, R. I., and BERLINER, R. W.: The relationship between peritubular capillary protein concentration and fluid reabsorption by the renal proximal tubule. J. clin. Invest. *48:* 1519–1531 (1969).
3. BRICKER, N. S.; KLAHR, S.; PURKERSON, M.; SCHULTZE, R. G.; AVIOLI, L. V., and BIRGE, S. J.: *In vitro* assay for a humoral substance present during volume expansion and uraemia. Nature *219:* 1058–1059 (1968).
4. BUCKALEW, V. M.; MARTINEZ, F. J., and GREEN, W. E.: Dialyzable inhibitor of toad bladder sodium transport in plasma of volume expanded dogs. Clin. Res. *17:* 426 (1969).

5. CORT, J. H.; PLIŠKA, V., and DOUŠA, T.: The chemical nature and tissue source of natriuretic hormone. Lancet *1:* 230–231 (1968).
6. DE WARDENER, H. E.; MILLS, I. H.; CLAPHAM, W. F., and HAYTER, C. J.: Studies on the efferent mechanism of the sodium diuresis which follows the administration of intravenous saline in the dog. Clin. Sci. *21:* 249–258 (1961).
7. DIRKS, J. H.; CIRKSENA, W. J., and BERLINER, R. W.: The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. J. clin. Invest. *44:* 1160–1170 (1965).
8. HOWARDS, S. S.; DAVIS, B. B.; KNOX, F. G.; WRIGHT, F. S., and BERLINER, R. W.: Depression of fractional sodium reabsorption by the proximal tubule of the dog without sodium diuresis. J. clin. Invest. *47:* 1561–1572 (1968).
9. JOHNSTON, C. I. and DAVIS, J. O.: Evidence from cross circulation studies for a humoral mechanism in the natriuresis of saline loading. Proc. Soc. exp. Biol., NY *121:* 1058–1063 (1966).
10. KEIMOWITZ, R.; BRENNER, B., and FALCHUK, K.: Unpublished data.
11. LARAGH, J.; SEALY, J., and KIRSHMAN, J. D.: Natriuretic activity in plasma and urine of salt-loaded man and sheep. J. clin. Invest. *48:* 49A (1969).
12. LEVINSKY, N. G. and LALONE, R. C.: The mechanism of sodium diuresis after saline infusion in the dog. J. clin. Invest. *42:* 1261–1276 (1963).
13. LICHARDUS, B. and PEARCE, J. W.: Evidence for a humoral natriuretic factor released by blood volume expansion. Nature *209:* 407–409 (1966).
14. MARTINO, J. A. and EARLEY, L. E.: Demonstration of a role of physical factors as determinants of the natriuretic response to volume expansion. J. clin. Invest. *46:* 1963–1978 (1967).
15. MILLS, I. H.; DE WARDENER, H. E.; HAYTER, C. J., and CLAPHAM, W. F.: Studies on the afferent mechanism of the sodium chloride diuresis which follows intravenous saline in the dog. Clin. Sci. *21:* 259–264 (1961).
16. NIZET, A.; GODON, J. P., and MAHIEU, P.: Quantitative excretion of water and sodium load by isolated dog kidney: autonomous renal response to blood dilution factors. Pflügers Arch. ges. Physiol. *304:* 30–45 (1968).
17. RECTOR, F. C.: Personal communication.
18. RECTOR, F. C. Jr.; MARTINEZ-MALDONADO, M.; KURTZMAN, N. A.; SELLMAN, J. C.; OERTHER, F., and SELDIN, D. W.: Demonstration of a hormonal inhibitor of proximal tubular reabsorption during expansion of extracellular volume with isotonic saline. J. clin. Invest. *47:* 761–773 (1968).
19. RECTOR, F. C. Jr.; VAN GIESEN, G.; KIIL, F., and SELDIN, D. W.: Influence of expansion of extracellular volume on tubular reabsorption of sodium independent of changes in glomerular filtration rate and aldosterone activity. J. clin. Invest. *43:* 341–348 (1964).
20. WRIGHT, F. S.; BRENNER, B. M.; BENNETT, C, M.; KEIMOWITZ, R. I.; BERLINER, R. W.; SCHRIER, R. W.; VERROUST, P. J.; DE WARDENER, H. E., and HOLZGREVE, H.: Failure to demonstrate a hormonal inhibitor of proximal sodium reabsorption. J. clin. Invest. *48:* 1107 (1969).

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Sodium and Potassium in Foods and Waters

Determination by the Flame Photometer[1]

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REASSESSMENT of the occurrence in food of sodium and also of potassium is in order because of the increased use of low-sodium diets for therapeutic purposes. On reviewing the literature one is impressed by the paucity of data for these elements in comparison with others, such as calcium, magnesium, and iron. Until recently there was relatively little interest in the subject, and besides the available analytical methods were discouragingly tedious.

In the classical method, sodium and potassium are separated from all other elements as chlorides; potassium is precipitated and weighed as chloroplatinate, and sodium is estimated by difference. This procedure, described in detail by Fresenius (1) in 1853, remains today the official method of the Association of Official Agricultural Chemists (2) for plant materials. The analytical data given in compilations of the world literature, such as those of Sherman (3), are traceable largely to this method.

Fresenius observed that in the separation of potassium from sodium, the potassium ran about 1 per cent low, because of a solubility loss to which potassium chloroplatinate is subject. Obviously, the loss in potassium appears as a corresponding gain in sodium when the latter is estimated by difference. When the amount of sodium is substantial, this error is trivial, but as the amount decreases, the error grows, becoming enormous percentagewise as the quantity of sodium approaches zero. It is not unusual to find in the textbooks sodium values which are many times greater than they should be. Since many plant products contain much potassium but only traces of sodium, it appears that the chloroplatinate method for sodium determinations is not suitable in the very application for which it is official.

In connection with the present work, Fresenius' observation on solubility loss was confirmed. Samples of spectroscopically pure potassium chloride were found to give a false showing of considerable sodium, which makes it plain that the chloroplatinate method is unsuitable for the determination of sodium in the presence of much potassium.

An exception to the general use of the chloroplatinate method is found in the analytical tables of McCance and Widdowson (4). In this work, sodium was separated as sodium uranyl zinc acetate and the uranium estimated colorimetrically. Potassium was separated as sodium potassium cobaltinitrite and the nitrite estimated by titration. To overcome a tendency to high sodium values when much potassium was present, some of the potassium was removed by precipitating it as perchlorate before the sodium determinations were made (5–7).

EXPERIMENTAL PROCEDURE

The present survey was made with the flame photometer, the analyses thus being physical rather than chemical. The instrument consists essentially of a gas burner, an atomizer for spraying solutions into the flame, and a photoelectric colorimeter for measuring the characteristic light emitted. By this means, the estimation of sodium and potassium is greatly facilitated, and the attainment of quantitative accuracy resolves itself into a matter of instrument design and technique. A description of the instrument and procedure, together with a study of the errors encountered, is given elsewhere (8).

In determining the foods to be analyzed for sodium and potassium, emphasis was given to unprocessed or lightly processed foods, rather than to culinary crea-

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tions in which the use of salt and other sodium compounds is highly variable. It was one purpose of the work to provide data from which low-sodium diets could be formulated, and this consideration was a factor in the emphasis on natural foodstuffs. Cooked foods, except commercially canned ones, were mostly omitted. Meats and other salty foods are known to lose salt when boiled, the amount depending in part on the amount of water used (4, 6). Low-sodium foods may gain sodium from the tap water in which they are boiled. Dry cooking, of course, increases the concentration of sodium and potassium, along with all other non-volatile substances.

No attempt was made to analyze a large number of samples of each food so as to obtain statistically significant average values. It was felt that more knowledge would be gained by surveying a large number of different foods, employing a single sample or at most a few samples of each. The findings seem to justify this view, for they show that fruits as a class, cereal grains as a class, meats as a class, and so on form definite categories as regards their content of sodium. Even if the number of samples of each food had been large enough to give significant average values, the user of the tables would have no means of knowing whether any given sample was typical or deviant.

The processed foods were selected from leading brands in national distribution. Most of the canned fruits and vegetables were Del Monte brand products contributed by the California Packing Corporation. The fresh fuits and vegetables were market commodities which originated in various parts of the country. Each specimen was weighed, ashed, and analyzed in duplicate, and in each analysis at least two readings of the flame photometer were made for both sodium and potassium. Analytical values above 10 were expressed in two significant figures, and those below 10, where sample variation greatly exceeds analytical error, were limited to one significant figure. The findings for about five hundred foods and food adjuncts are recorded in Table 1.

One of the major dietary sources of sodium is drinking water. If one assumes an average daily consumption of 2.5 liters of water in food and drink, it follows that when the water contains as much as 20 mg. of sodium per 100 cc., the patient will receive 500 mg. of sodium from water alone, so that a diet limited to this amount becomes impossible. Samples of the public water supplies of all sections of the country were therefore obtained for sodium and potassium determinations. Specimen bottles which had been treated to remove soluble alkali were mailed to physicians or company representatives, along with the necessary instructions for sampling. Included in this survey are the principal cities of the United States, the capitals of the states, and many other places. Altogether, 28 per cent of the population of continental United States is represented in the 150 cities for which water analyses are recorded in Table 2.

DISCUSSION

The data in Table 1 confirm the rule that foods of animal origin contain much sodium, while foods of vegetable origin usually contain little sodium. They establish that the sodium content in many unprocessed vegetable foods is literally that of a trace element, being about the same as the iron content. They show, furthermore, that in the processing operations to which foods are subjected, some sodium is generally added.

The fresh meats, fish, and fowl mostly contained from 50 to 100 mg. of sodium per 100 gm. of edible portion. Conspicuous exceptions on the high side were kidney, brain, clam, and lobster. The two lowest specimens were rabbit loin and quail breast. It is interesting that in each of the five fowl studied—chicken, duck, goose, quail, and turkey—the breast meat always contained substantially less sodium than the leg meat. Four species of widely used fish—catfish, cod, halibut and salmon—were analyzed in the fresh state. The catfish was the "fiddler" of the Mississippi Valley rivers, and the others were salt water fish. These species were similar in sodium content, ranging from 48 to 60 mg. of sodium per 100 gm. The fresh water catfish contained no less sodium than the ocean species.

The processed meats and fish contained much larger amounts of sodium than the fresh products. Heavy salting is familiar in such items as ham, bacon, and dried fish, but considerable amounts of salt are also added to products ordinarily regarded as fresh. Much of the national fish supply, especially in the inland states, is sold as frozen fillets and steaks. In some processes brine is applied in the washing and freezing of the fish. Thus figures for frozen cod fillets and halibut steak were 400 and 460 mg. sodium per 100 gm., in comparison with 60 and 56 mg. for the corresponding fresh materials.

Eggs and milk are typical animal foods in regard to sodium content. The tests confirmed that most of the sodium of eggs is in the white. Cow's milk contained 50 mg. of sodium per 100 gm., or nearly 500 mg. per quart. The higher sodium content of butter and cheese is due to added salt. Goat's milk contained less sodium and more potassium than cow's milk, while human milk, especially after several weeks of lactation, is much poorer in both sodium and potassium.

The occurrence of sodium as a trace element is seen in the data for unprocessed fruits, grains, nuts, and legume seeds. Excluding coconut, the average sodium content was found to be about 2 mg. per 100 gm. The two highest sodium values were for cantaloupe and cashew nuts, 12 and 14 mg. per 100 gm. respectively. In some of these products the sodium

TABLE 1

Sodium and potassium content of foods. Analyses made on edible portions of unprocessed foods except as otherwise designated

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm.*
All-Bran cereal	1400	1200
Allspice	62	680
Almond		
Raw	3	690
Roasted in oil, salted	160	710
Anchovy paste	9800	200
Apples		
Juice (sweet cider), bottled	4	100
June, less skin and core	0.1	71
Mackintosh, less skin and core	0.2	90
Red Delicious, less skin and core	0.3	76
Sauce, canned	0.3	55
Apricot		
Canned in sirup	2	65
Dried	11	1700
Raw, with skin	0.6	440
Artichoke, globe	43	430
Asparagus		
Spears, canned	410	130
Tips, fresh	2	240
Tips, frozen	3	320
Avocado	3	340
Bacon		
Fried crisp	2400	390
Raw	680	110
Baking powder		
Alum type	10,000	150
Phosphate type	9000	170
Tartrate type	7300	5000
Banana	0.5	420
Barley, pearled	3	160
Beans		
Baked, Heinz, Navy		
With pork and tomato sauce, canned	480	210
With tomato sauce, canned	400	140
Dry, Navy	1	1300
Green, in pods		
Canned	410	120
Fresh	0.9	300
Frozen	2	110
Lima		
Canned	310	210
Fresh	1	680
Frozen	310	580
Beef		
Corned	1300	60
Dried	4300	200
Lean, koshered, raw	1600	290
Lean, raw	51	360
Beer	8	46
Beets		
Canned	36	120
Greens, fresh	130	570
Raw	110	350

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm.*
Blackberry	0.2	150
Blueberry	0.6	89
Bouillon cube	24,000	100
Brain, pig	150	340
Bran, wheat, crude	15	980
Brandy	3	4
Brazil nuts		
Raw	1	670
Roasted in oil, salted	190	730
Bread		
Boston brown, with raisins	280	360
Low-sodium—4 laboratory samples	3	94
Low-sodium cinnamon roll—laboratory sample	2	120
Low-sodium—14 commercial "salt-free" breads:		
Maximum	76	200
Minimum	4	72
Average	28	120
Passover—See matzoth		
Rye and wheat	590	160
White, enriched	640	180
Whole wheat	930	230
Whole wheat and white	620	250
Breakfast cereals—See individual cereal		
Broccoli		
Fresh	16	400
Frozen	13	250
Brussels sprouts		
Fresh	11	450
Frozen	9	300
Butter		
Theoretical sodium value based on U. S. average salt content of 2.5%	980	
4 Indiana samples	880	23
Unsalted	5	4
Buttermilk, cultured	130	140
Cabbage	5	230
Candy		
Bar, Baby Ruth	170	300
Bar, Milky Way	220	150
Bar, Oh Henry	76	420
Gum drop	41	18
Marshmallow	41	6
Milk chocolate	86	420
Necco Wafers	5	2
Peppermint patty, Schrafft's	10	110
Sweet chocolate	35	230
Cantaloupe	12	230
Caraway seed	17	1400
Carbonated drinks—See individual drinks		
Carrots		
Canned	280	110
Scraped and trimmed	31	410
Casein		
Acid-washed	0.4	2
Low-ash commercial	13	39
Vitamin-free	160	900

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm.*
Cashew nuts		
Raw	14	560
Roasted in oil, salted	200	560
Catchup, tomato	1300	800
Catfish (fiddler), Ohio River	60	330
Cauliflower		
Buds	24	400
Buds, frozen	22	290
Caviar, salmon, canned	2200	180
Celery		
Salt	28,000	380
Seed	140	1400
Stalks, less leaves	110	300
Cereals, dry		
Bran		
All-Bran	1400	1200
Crude, unsalted	15	980
Corn flakes	660	160
Farina		
Cream of Wheat, plain	2	86
Cream of Wheat, quick-cooking, enriched	90	84
Grape-Nuts	660	230
Pabena	640	340
Pablum	620	380
Rolled oats	2	340
Ry-Krisp	1500	600
Wheat		
Flakes	1300	320
Germ, malt-flavored, Zing	9	780
Instant Ralston	1	360
Maltex	4	250
Muffets	4	300
Pettijohn's	2	380
Puffed	4	340
Shredded	2	330
Wheatena	2	380
Certo (pectin solution)	15	110
Chard		
Large leaves	210	720
Small leaves	84	380
Cheese		
American Swiss	710	100
Cheddar	700	92
Cottage	290	72
Cream, Philadelphia	250	74
Process	1500	80
Whey, Velveeta	1600	270
Cherries		
Sour, frozen in sirup	2	78
Sweet		
Dark		
Raw	1	260
Canned in sirup	0.8	77
Frozen in sirup	1	280
Light, canned in sirup	3	55
Chestnut	2	410
Chicken, raw		
Breast meat	78	320
Leg meat	110	250

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm.*
Chocolate—See also individual candy		
Sirup, Hershey	60	130
Unsweetened	4	830
Cider, sweet (apple juice), bottled	4	100
Cinnamon	8	200
Citron, candied	290	120
Clam	180	240
Clove	210	1000
Coca-Cola	1	52
Cocoa		
Dutch process	57	3200
Plain, Hershey	5	1400
Coconut		
Dry, shredded	16	770
Meat	29	320
Milk	53	190
Cod		
Raw	60	360
Frozen fillets	400	400
Liver oil	0.1	0
Salted, dried	8100	160
Coffee		
Instant, Nescafé, dry	84	3100
Roasted		
Decaffeinated, Sanka, dry	6	2000
Regular, dry	2	1600
Cookie, salt-free, Betty Bakerite	12	240
Corn		
Flakes	660	160
Meal, yellow, enriched, degerminated	0.7	120
Oil	0.2	0.1
Popcorn, popped and oiled	3	240
Popcorn, popped, oiled, and salted	2000	240
Starch	4	4
Sweet		
White		
Canned	200	200
Milk stage	0.3	240
Yellow		
Canned	210	200
Frozen	9	190
Milk stage	0.4	370
Yellow field, dry—5 varieties	0.6	290
Cowpeas, fresh, shelled	2	560
Crab, canned	1000	110
Crackers		
Graham	710	330
Rye, Ry-Krisp	1500	600
Soda	1100	120
Unsalted, Jewish—See matzoth		
Cranberry		
Raw	1	65
Sauce, canned	1	17
Cream of tartar—theoretical value for pure $KHC_4H_4O_6$	0	20,776
Cream, whipping, 32% fat	40	56
Crisco (vegetable shortening)	4	0
Cucumber, less parings	0.9	230
Currants		
Red	2	160
Zante, dried (Zante raisins)	22	730

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm.*
Curry powder	45	1300
Dandelion greens	76	430
Date, semi-dry, California	1	790
Dextri-Maltose		
No. 1	840	160
No. 2	46	160
No. 3	46	1300
B	52	360
Dextrin	14	14
Dextrose	1	0.4
Dill, seed	13	1000
Duck, domesticated, raw		
Breast meat	68	360
Leg meat	96	210
Egg		
Whites only	110	100
Whole	81	100
Yolks only	26	100
Eggplant, less skin	0.9	190
Endive greens	18	400
Farina		
Cream of Wheat, plain, dry	2	86
Cream of Wheat, quick-cooking, enriched, dry	90	84
Figs		
Canned in sirup	1	105
Dried	34	780
Raw	2	190
Filberts (hazelnut)	1	560
Flour		
Bleached		
Enriched, Gold Medal	1	86
Enriched, phosphated	13	78
Buckwheat	1	680
Gluten	2	24
Rye, dark	1	860
Self-rising	1500	90
Untreated, high-extraction	1	120
Whole wheat (Graham)	2	290
Fruit cocktail, canned in sirup	9	160
Garlic, less skin	6	510
Gelatin		
Dessert, flavored, Jell-O	330	210
Plain	36	22
Gin	0.7	0.3
Ginger	29	1100
Ginger ale	8	0.6
Gizzard, turkey	58	170
Gluten flour	2	24
Goose, raw		
Breast meat	76	420
Leg meat	96	420
Gooseberry		
Frozen	2	150
Raw	0.7	87
Grapes		
Concord, less seeds and skin	3	84
Emperor, less seeds, with skin	4	180
Jam	7	78

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm.*
Grapes, continued		
Juice, Concord, sweetened, bottled	1	120
Thompson Seedless, with skin	4	180
Tokay, less seeds, with skin	0.7	160
Grapefruit		
Fresh	0.5	200
Juice, sweetened, canned	0.4	150
Sections, sweetened, frozen	5	60
Grape-Nuts cereal	660	230
Gravy flavoring, Kitchen Bouquet	86	280
Gum, chewing, spearmint	22	27
Halibut		
Raw	56	540
Steak, frozen	460	500
Ham, raw	1100	340
Hash, corned beef, canned	540	200
Hazelnuts—See filberts		
Heart		
Beef	90	160
Turkey	69	240
Hominy, canned	250	22
Honey	7	10
Horse-radish, prepared	96	290
Ice cream	100	90
Jam, grape	7	78
Kale, leaves and midribs	110	410
Kidney, beef	210	310
Kumquat, pulp and rind, less seeds	7	230
Lactalbumin	47	69
Lactose, U.S.P.	2	0
Lamb		
Chop, lean, raw	98	340
Leg, lean, raw	78	380
Lard	0.3	0.[illegible]
Lemon-lime soda	7	33
Lemons		
Peel		
Candied	50	12
Fresh	9	360
Pulp and juice	0.7	130
Lentils, dry	3	1200
Lettuce		
Head	12	140
Leaf	7	230
Lime, pulp and juice	1	100
Litchi, dried	3	1100
Liver, raw		
Calf	110	380
Goose	140	230
Pig	77	350
Turkey	51	160
Lobster, boiled in tap water	210	180
Lonalac, dry	13	1300
Macaroni, plain, dry	1	160
Mace	45	180
Maize—See corn		
Maltex cereal, dry	4	250
Maple sirup	14	130
Marmalade, orange	13	19

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	mg./100 gm.
Matzoth		
American style (salted)	470	120
Egg	16	160
Farfel (dough balls)	28	130
Meal	4	130
Passover (Passover bread)	1	140
Plain	1	160
Poppy seed	350	110
Thin tea	2	130
Whole wheat	280	420
Mayonnaise	590	25
Meat extract, flavored	11,000	6000
Milk		
Cow's		
Buttermilk, cultured	130	140
Condensed, sweetened	140	340
Evaporated	100	270
Fat	0.4	0.3
Skim	52	150
Whole		
Dry	410	1100
Liquid	50	140
Goat's	34	180
Human		
From 10 mothers, 3 to 10 days post-partum	37	68
From 4 mothers, 49 to 77 days post-partum	11	51
Low-sodium—See Lonalac		
Malted, dry	440	720
Molasses, cane	80	1500
Muffets cereal	4	300
Mulberry	0.7	200
Mushrooms		
Canned	400	150
Raw	5	520
Mustard		
Greens	48	450
Powder	3	840
Prepared paste	1300	130
Nectarine, less skin	2	320
Nutmeg	14	160
Oats, rolled (oatmeal), dry	2	340
Okra, fresh	1	220
Oleomargarine	1100	58
Olives		
Green, pickled	2400	55
Oil	0.2	0.2
Ripe, pickled	980	23
Stuffed, pickled	2800	55
Onion, less tops and dry skins	1	130
Orange Crush	2	100
Oranges		
Juice, unsweetened, canned	0.5	190
Pulp and juice	0.3	170
Temple, pulp and juice	3	220
Oyster, raw	73	110
Pabena cereal, dry	640	340
Pablum cereal, dry	620	380
Pancreas, pig, raw	57	240
Paprika	82	2300

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	mg./100 gm.
Parsley, fresh	28	880
Parsnip, scraped and trimmed, fresh	7	740
Peaches		
Canned in sirup	5	31
Dried	12	1100
Frozen in sirup	3	120
Raw, less skin	0.5	160
Peanuts		
Butter	120	820
Oil	0.2	0.1
Raw, with skin	2	720
Roasted		
Dry, with skin	2	740
In oil and salted, with skin	460	700
Pears		
Bartlett		
Canned in sirup	8	52
Raw, less skin and core	2	100
Peas		
Canned, less liquor	270	96
Dry, split	42	880
Fresh	1	370
Frozen	100	160
Pecan, raw	0.3	420
Pectin solution, Certo	15	110
Pepper (spice)		
Black	16	880
Red	46	2400
White	5	48
Peppermint extract	0.3	5
Peppers, green, empty pods	0.6	170
Pepsi-Cola	15	3
Persimmon, wild	0.6	310
Pettijohn's cereal, dry	2	380
Pickle, dill	1400	200
Pilchard—See sardine		
Pineapple		
Canned in sirup	1	120
Frozen in sirup	1	38
Juice, unsweetened, canned	0.5	140
Raw	0.3	210
Plums		
Canned in sirup	18	110
Raw	0.6	170
Polyvitamin dispersion, Mead's, dry	6	10
Pomegranate, pulp and juice	0.3	200
Popcorn		
Popped		
Oiled	3	240
Oiled and salted	2000	240
Pork		
Lean, raw	58	260
Salt	1800	27
Postum		
Cereal beverage, dry	36	1300
Instant, dry	71	2200
Potatoes		
Chips	340	880

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm.*
Potatoes, continued		
Sweet		
Canned	48	200
Raw less skin	4	530
White		
Canned	350	240
Raw, less skin	0.8	410
Poultry seasoning	26	840
Pretzel	1700	130
Protenum, dry	360	1100
Prunes		
Canned in sirup	3	220
Dried	6	600
Juice, unsweetened, bottled	2	260
Raw, with skin	0.7	210
Pumpkin		
Canned	2	240
Raw, less rind and seeds	0.6	480
Quail, raw		
Breast meat	35	160
Leg meat	44	190
Quince, less skin and core, raw	0.7	290
Rabbit, domesticated, raw		
Foreleg	47	370
Loin	34	400
Radish, with skin	9	260
Raisin		
Seedless	21	720
Zante	22	730
Ralston cereal, Instant, dry	1	360
Raspberries		
Black	0.3	190
Oriental (wineberry)	0.9	170
Red	0.5	130
Rennet tablets, Junket	38,000	36
Rhubarb		
Frozen in sirup	2	160
Raw	1	70
Rice, dry		
Brown	9	150
Flakes	720	180
Polished and coated	2	130
Puffed	0.9	100
Vitaminized	4	170
Wild (*Zizania*)	7	220
Root beer	8	0.5
Royal Crown Cola	5	2
Rum	2	3
Rutabaga (yellow turnip), less skin and tops, raw	5	260
Ry-Krisp	1500	600
Sage	20	670
Salmon		
Canned	540	300
Raw	48	410
Salt—theoretical value for pure NaCl	39,342	0
Sardines		
Herring, canned in oil	510	560
Pilchard		
Canned in natural sauce	760	260
Canned in tomato sauce	400	320

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm.*
Sauerkraut, canned	630	140
Sausage		
Bologna	1300	230
Frankfurt	1100	220
Pork	740	140
Scallop, frozen	150	420
Shortening, vegetable		
Crisco	4	0
Spry	0.4	0.2
Shrimp, raw	140	220
Sirup		
Chocolate, Hershey	60	130
Maple	14	130
Sorghum	21	600
Table, corn-and-cane, Karo Crystal White	68	4
Soda, baking—theoretical value for pure $NaHCO_3$	27,373	0
Soft drinks		
Carbonated water		
Canada Dry	18	0.6
Made with Sparklet carbon dioxide capsule and distilled water	0	0
White Rock	1	0.6
Coca-Cola	1	52
Ginger ale	8	0.6
Lemon-lime soda	7	33
Orange Crush	2	100
Pepsi-Cola	15	3
Royal Crown Cola	5	2
Root beer	8	0.5
Sorghum sirup	21	600
Soup		
Beef, canned, diluted as served	410	100
Tomato, canned, diluted as served	380	110
Vegetable, canned, diluted as served	380	120
Soybeans		
Dry	4	1000
Flour, solvent-extracted	1	1700
Spaghetti—See macaroni		
Spinach		
Canned	320	260
Frozen	60	380
Raw	82	780
Spry (vegetable shortening)	0.4	0.2
Squash, raw		
Acorn, less rind and seeds	0.4	260
Hubbard, less rind and seeds	0.3	240
White summer, less rind, with seeds	0.2	150
Yellow summer, less rind, with seeds	0.6	200
Squash, cooked, frozen	6	120
Starch, corn	4	4
Strawberries		
Frozen, sweetened	2	180
Raw	0.8	180
Sugar		
Light brown	24	230
White	0.3	0.5

TABLE 1—*Continued*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm.*
Sweetbreads—See pancreas and thymus		
Tangerines		
Juice, sweetened, canned	0.6	170
Pulp and juice	2	110
Tapioca, dry	5	19
Tea, India-Ceylon-Java blend, dry	4	1800
Thyme	38	500
Thymus, beef, raw	96	360
Tobacco, chewing, Spark Plug	1600	1800
Tomatoes		
Canned	18	130
Catchup	1300	800
Juice, canned	230	230
Raw, with skin	3	230
Tongue, beef, raw	100	260
Tripe, pickled	46	19
Tuna, canned	800	240
Turkey, raw		
Breast meat	40	320
Leg meat	92	310
Turmeric	22	2700
Turnips, raw		
Leaves	10	440
White, less skin and tops	37	230
Yellow (rutabaga), less skin and tops	5	260
Vanilla extract	1	74
Veal, lean, raw	48	330
Vinegar		
Cider	1	100
Distilled	0.6	15
Walnuts, raw		
Black	3	460
English	2	450
Water, carbonated—See soft drinks		
Watermelon, pink part of fruit	0.3	110
Wheat		
Beeswing (outermost coats)	4	360
Bran, crude	15	980
Flakes cereal	1300	320
Germ		
Crude	2	780
Malt-flavored, Zing	9	780
Gluten	2	24
Puffed	4	340
Scourings (dirt and fragments)	8	470
Shredded	2	330
Winter, scoured—4 samples	2	370
Wheatena cereal, dry	2	380
Whiskey		
Blended	0.3	1
Bonded	0.1	0.6
Wild rice (*Zizania*), dry	7	220
Wine		
Port	4	75
Sauterne	10	87
Wineberry (oriental raspberry)	0.9	170
Worcestershire sauce	2100	480
Yeast		
Compressed	4	360

TABLE 1—*Concluded*

FOOD	SODIUM	POTASSIUM
	mg./100 gm.	*mg./100 gm*
Yeast, continued		
Debittered brewers', dry	150	1700
Primary cultured, dry—19 samples		
Maximum	320	2200
Minimum	9	1700
Average	115	1860
Zwieback	250	150

content was less than 1 mg. per 100 gm. A sample of fresh coconut showed 29 mg. of sodium and 320 mg. of potassium per 100 gm. The milk of this coconut showed 53 mg. of sodium and 190 mg. of potassium, which reminds one of the rule that in animals sodium predominates in extracellular fluids, potassium in the tissues.

In leaf, stalk, and root vegetables the sodium content varied widely. White potatoes contained but a trace, while celery, beets, and chard contained 100 to 200 mg. per 100 gm. Sodium is usually considered unessential for plant growth, but there are species, particularly the saltworts, which normally grow by the seashore and contain extraordinary amounts of it. Celery is a food plant with an affinity for sodium so marked that farmers add salt to the soil for the purpose of improving the flavor and turgidity of the stalks. Sodium was present in large amounts even in the seed of this species. In analyses of Swiss chard, it was observed that the immature leaves contained 84 mg. sodium per 100 gm. and the mature leaves 210 mg., this being the highest sodium concentration seen in any of the unprocessed plant products.

Throughout the survey it was noted that variations in sodium content were much wider than in potassium content. This was true not only for different products, but for different samples of the same product. To some extent, this was the result of human interference, but it was also evident in purely natural products. It was most evident, percentage-wise, in plant products of low sodium content. For example, three bananas showed 0.2, 0.6, and 0.8 mg. sodium per 100 gm., and similar differences were noted in other products on which several analyses were made.

In the commercial processing of foods, and in domestic cooking, various compounds of sodium are commonly employed. The expression, "salt to taste," implies the addition of 1 or 2 per cent of sodium chloride, so that the product will contain 400 to 800 mg. sodium per 100 gm. This is the usual range for bread. Sharply salted products may contain several thousand milligrams of sodium per 100 gm.

Many manufactured food products which are not intentionally salted contain somewhat more sodium than the natural foods from which they are prepared.

The source of this sodium is found in the numerous special uses of sodium compounds in the food industry. Sodium chloride is employed for many purposes besides seasoning, preserving, pickling, and koshering. As brine it is used (a) in the flotation process of sorting green peas from hardened peas and heavy extraneous matter, (b) for preventing enzymic discoloring of freshly sliced apples and pears which are to be canned, (c) as a heat-transfer and blanching agent in freezing foods, and (d) for regenerating base-exchange water softeners (the sodium appears in the softened water as bicarbonate, and water thus treated is used in canning to prevent toughening of vegetables).

Sodium alginate is employed as a stabilizer for ice cream and chocolate milk drinks. Sodium aluminum sulphate is the alum of baking powders. Sodium benzoate is used as a preservative in fruit juice concentrates, confectionery, margarine, and so on. Sodium bicarbonate is the gas source in baking powder and self-rising flours. It is also used with cocoa in the "Dutching" process, for tenderizing vegetables, and for neutralizing. Sodium carbonate is used for Dutching, neutralizing, and water softening. It has a special use in removing hop resins from brewers' yeast in the debittering process. Sodium citrate is used as a stabilizing agent in evaporated milk and jellies. Sodium glutamate is an important flavoring adjunct. Sodium hydroxide is used for Dutching cocoa, neutralizing corn sirup, debittering ripe olives, and hulling corn in the old lye process of hominy manufacture. It is also widely used in peeling apricots, peaches, grapefruit segments, carrots, and sweet potatoes for canning, and for dipping fruits prior to drying. Sodium nitrate and nitrite are employed for fixing the color of cured and canned meats. Sodium propionate is used to inhibit mold in bread, cake, and cheese. Sodium acid phosphate is used as an acidulating agent. The secondary phosphate is used for emulsifying process cheese, for stabilizing evaporated milk, and as the quickening agent in quick-cooking farina. Sodium acid sulphite is employed for sulphuring fruits prior to drying. These few examples may serve to explain the almost ubiquitous occurrence in processed foods of sodium in amounts greater than are present in the corresponding natural products.

From Table 2 it is evident that public water supplies, as a rule, do not contain much sodium, although there are important exceptions. The most sodium is usually found in well waters, but not all well waters contain much sodium. The water from any given well tends to be seasonally uniform, unless the well is near the ocean and subject to tidal seepage. River and lake waters, from which most large cities derive their supplies, generally contain little sodium, but the amount is subject to seasonal fluctuations. Hardness or softness has no bearing on the sodium content of water, except that when hard water is

TABLE 2

Sodium and potassium content of public water supplies

PLACE	SODIUM	POTASSIUM
	mg./100 cc.	*mg./100 cc.*
Aberdeen, S. D.	20	2
Albany, N. Y.	0.2	0.2
Albuquerque, N. M.	5	0.7
Allentown, Pa.	0.4	0.3
Annapolis, Md.	0.2	0.2
Ann Arbor, Mich.	2	0.5
Atlanta, Ga.	0.2	0.2
Augusta, Me.	0.2	0.2
Austin, Texas	3	0.5
Baltimore, Md.	0.3	0.2
Bangor, Me.	0.2	0.1
Baton Rouge, La.	9	0.2
Beloit, Wis.	0.5	0.2
Billings, Mont.	1	0.2
Biloxi, Miss.	23	0.6
Birmingham, Ala.	2	0.3
Bismarck, N. D.	6	0.6
Boise, Idaho	2	0.3
Boston, Mass.	0.3	0.2
Brownsville, Texas	6	0.3
Brownwood, Texas	2	0.4
Buffalo, N. Y.	0.7	0.3
Burlington, Vt.	0.2	0.1
Butte, Mont.	0.6	0.4
Camden, N. J.	0.9	0.3
Carson City, Nev.	0.4	0.3
Charleston, S. C.	1	0.3
Charleston, W. Va.	0.3	0.2
Charlotte, N. C.	0.3	0.1
Charlottesville, Va.	0.2	0.1
Cheyenne, Wyo.	0.3	0.2
Chicago, Ill.	0.3	0.1
Cincinnati, Ohio	0.7	0.3
Cleveland, Ohio	1	0.3
Columbia, S. C.	0.4	0.2
Columbus, Ohio	5	0.6
Concord, N. H.	0.2	0.1
Corpus Christi, Texas	15	0.6
Crandall, Texas	170*	0.5
Dallas, Texas	3	0.5
Dayton, Ohio	0.7	0.2
Denver, Colo.	3	0.2
Des Moines, Iowa	1	0.4
Detroit, Mich.	0.3	0.1
Dover, Del.	2	0.5
Durham, N. C.	0.4	0.2
El Paso, Texas	7	0.6
Emporia, Kans.	1	0.3
Eureka, Calif.	0.7	0.6
Evansville, Ind.	2	0.5
Fargo, N. D.	5	0.7
Flint, Mich.	2	0.2
Frankfort, Ky.	0.3	0.1
Galesburg, Ill.	30	2
Galveston, Texas	34	0.7

* An extreme example. This water is rarely drunk, but is used for cooking.

TABLE 2—*Continued*

PLACE	SODIUM	POTASSIUM
	mg./100 cc.	*mg./100 cc.*
Grand Forks, N. D.	6	0.4
Hamilton, Ohio	3	0.2
Harrisburg, Pa.	0.2	0.1
Hartford, Conn.	0.2	0.1
Helena, Mont.	0.3	0.2
Houston, Texas	16	0.6
Huntington, W. Va.	3	0.2
Independence, Mo.	9	0.5
Indianapolis, Ind.	1	0.3
Iowa City, Iowa	0.5	0.3
Jackson, Mich.	5	0.3
Jackson, Miss.	0.4	0.2
Jacksonville, Fla.	1	0.2
Jefferson City, Mo.	3	0.4
Jersey City, N. J.	0.3	0.2
Joplin, Mo.	0.5	0.1
Kalamazoo, Mich.	0.7	0.3
Kansas City, Kans.	4	0.4
Kansas City, Mo.	10	3
Lancaster, Pa.	0.4	0.1
Lansing, Mich.	1	0.5
Lawrence, Kans.	4	0.7
Lincoln, Neb.	3	0.7
Little Rock, Ark.	0.1	0.1
Los Angeles, Calif.		
Aqueduct source	6	0.6
Metropolitan source	17	0.5
River source	5	0.5
Louisville, Ky.	2	0.3
Madison, Wis.	0.4	0.2
Manchester, N. H.	0.2	0.1
Marion, Ohio	17	0.7
Memphis, Tenn.	2	0.3
Miami, Fla.	2	0.3
Middletown, Ohio	0.8	0.1
Milwaukee, Wis.	0.3	0.1
Minneapolis, Minn.	0.5	0.3
Minot, N. D.	25	0.6
Moberly, Mo.	0.3	0.2
Montgomery, Ala.	0.8	0.1
Montpelier, Vt.	0.1	0.1
Nashville, Tenn.	0.3	0.2
Newark, N. J.	0.2	0.1
New Haven, Conn.	0.3	0.1
New Orleans, La.	1	0.4
New York, N. Y.	0.3	0.2
Oakland, Calif.	0.3	0.1
Oklahoma City, Okla.	10	0.8
Olympia, Wash.	0.5	0.3
Omaha, Neb.	8	1
Philadelphia, Pa.	2	0.4
Phoenix, Ariz.	11	0.7
Pierre, S. D.	9	0.5
Pittsburgh, Pa.	6	0.5
Portland, Me.	0.2	0.1
Portland, Ore.	0.1	0.1
Providence, R. I.	0.2	0.1
Raleigh, N. C.	0.4	0.1
Reno, Nev.	0.5	0.1

TABLE 2—*Concluded*

PLACE	SODIUM	POTASSIUM
	mg./100 cc.	*mg./100 cc.*
Richmond, Va.	0.7	0.2
Rochester, Minn.	0.7	0.2
Rochester, N. Y.	0.3	0.2
Rockford, Ill.	0.7	0.2
Sacramento, Calif.	0.3	0.2
Santa Fe, N. M.	0.4	0.1
St. Joseph, Mo.	9	0.6
St. Louis, Mo.	5	0.5
St. Paul, Minn.	0.5	0.3
St. Petersburg, Fla.	0.5	0.1
Salem, Ore.	0.2	0.1
Salt Lake City, Utah	0.8	0.2
San Angelo, Texas	5	0.9
San Antonio, Texas	1	0.1
San Diego, Calif.	5	0.5
San Francisco, Calif.	1	0.3
Santa Barbara, Calif.	10	0.3
Seattle, Wash.	0.2	0.1
Sedalia, Mo.	0.3	0.2
Shreveport, La.	2	0.1
Sioux Falls, S. D.	1	0.4
Springfield, Ill.	0.8	0.3
Springfield, Mo.	0.4	0.2
Syracuse, N. Y.	0.2	0.1
Tallahassee, Fla.	0.3	0.1
Tampa, Fla.	0.5	0.1
Texarkana, Ark.	3	0.2
Topeka, Kans.	1	0.5
Trenton, N. J.	0.1	0.1
Tucson, Ariz.	3	0.3
Tulsa, Okla.	0.3	0.4
Uniontown, Pa.	0.2	0.1
Upper Darby, Pa.	0.4	0.2
Washington, D. C.	0.3	0.3
Whittier, Calif.	1	0.2
Wichita, Kans.	5	0.5
Wilkes-Barre, Pa.	0.2	0.1
Wilmington, Del.	0.8	0.1
Winona, Minn.	5	0.4

softened by base-exchange treatment, the calcium and magnesium are replaced by sodium in the ratio of two atoms of sodium for each atom of calcium or magnesium. Softened water is, therefore, always suspect as regards its suitability for low-sodium diets. Additional data on public water supplies can be obtained from the various state and municipal laboratories, and from the federal survey by Collins, Lamar, and Lohr (9).

It is of interest to compare the analyses in Table 1 with those of McCance and Widdowson (4). In spite of the fact that both series cover more than five hundred foods, comparison is possible with only a few items, because most of the foods differ too much in identity, analyzed portion, or previous treatment. Comparison seemed possible in the case of thirty-four low-sodium foods (mostly raw fruits and nuts) and twelve foods of ordinary sodium content.

The McCance and Widdowson values for sodium in the low-sodium foods were, on the average, about three times as large as the flame photometer values, although not nearly as large as corresponding values found in tables in which the chloroplatinate method was involved. The McCance and Widdowson values for potassium and for sodium in foods of ordinary sodium content were also higher than the flame photometer values, but the differences were of lesser degree. To some extent, the higher McCance and Widdowson values for sodium in low-sodium foods were due to large differences found in three of the thirty-four samples. Most of the discrepancy, however, remains to be explained.

SUMMARY

Sodium and potassium determinations on about 500 foods and 150 public water supplies are presented. A discussion of the occurrence of sodium in foods and waters is given, together with incidental comparisons of analytical methods.

REFERENCES

(1) Fresenius, C. R.: Anleitung zur quantitativen chemischen Analyse. 3rd ed. Braunschweig: F. Vieweg und Sohn, 1853, p. 619.

(2) Official and Tentative Methods of Analysis. 6th ed. Washington: Assn. of Official Agricultural Chemists, 1945, p. 121.

(3) Sherman, H. C.: Chemistry of Food and Nutrition. 7th ed. New York: Macmillan Co., 1946, p. 626.

(4) McCance, R. A., and Widdowson, E. M.: The Chemical Composition of Foods. 2nd rev. ed. Brooklyn: Chemical Publishing Co., 1947.

(5) McCance, R. A., and Shipp, H. L.: The colorimetric determination of sodium. Biochem. J. 25: 449, 1931.

(6) McCance, R. A., and Shipp, H. L.: The chemistry of flesh foods and their losses on cooking. Medical Research Council Special Report Series No. 187, November, 1933.

(7) McCance, R. A., Widdowson, E. M., and Shackleton, L. R. B.: The nutritive value of fruits, vegetables and nuts. Medical Research Council Special Report Series No. 213, July, 1936.

(8) Bills, C. E., McDonald, F. G., Niedermeier, W., and Schwartz, M. C.: Reduction of error in flame photometry. Anal. Chem., in press.

(9) Collins, W. D., Lamar, W. L., and Lohr, E. W.: The industrial utility of public water supplies in the United States, 1932. Geological Survey, Water-Supply Paper No. 658. Washington: Govt. Printing Office, 1934.

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028

Sodium Homeostasis, Salt Appetite, and Hypertension

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ABSTRACT

Some principal mechanisms in humoral control of sodium homeostasis have yet to be discovered.

Phylogeny is an important issue in analysis of animal experimental data on hypertension.

The evidence that high-salt intake is one of a group of jointly sufficient and severally necessary conditions in the causation of hypertension is suggestive—but the case is not proven.

The case is sufficiently strong to warrant greatly reducing the amount of salt added to infant food.

The thesis is advanced that chronic concurrence of moderate high-salt intake with central nervous system-induced, metabolically inappropriate, hyperaldosteronism may emerge as one category in the several categories of the causation of hypertension.

ADDITIONAL KEY WORDS sodium aldosterone appetite aversion salt hypertension renin angiotensin comparative experimental neurosis

■ We have been invited by the Chairman to begin this paper by giving a very short general survey of "Humoral Factors in Sodium Homeostasis."

In analyzing present knowledge on the general biology and medical aspects of sodium homeostasis, and humoral factors in the control systems, we can recognize two main processes (fig. 1): Regulation of intake and regulation of output.

Intake involves the question of appetite, and the question of aversion.

There is good evidence that there are innate behavioral mechanisms generating a specific salt hunger or drive as a result of body Na deficiency.[1, 2] Are they primarily stimulated by hormone secretion,—that is, using humoral in a restricted sense?

If a parotid fistula is made in a sheep, it loses 300 to 600 mEq/Na^+/day. If access to Na is withheld for 1 to 3 days, increasing degrees of body Na deficit can be produced; if saliva is returned to the animal at 6-hour intervals, it can be kept near balance. If salt appetite is tested by presenting the animal with a $NaHCO_3$ solution for 15 minutes at the end of each period of depletion, the animal drinks an amount commensurately related to deficit. Each point in figure 2 represents the mean ± SD of 9 to 24 observations over 5 months in each animal.

Similarly, if a parotid fistula is made in an adrenalectomized sheep and a similar experiment is done, the same relation between Na deficit and voluntary Na intake is shown.[2, 3] In the adrenal intact animal aldosterone in blood is roughly proportional to deficit.[4] In the adrenalectomized animal, no mineralocorticoid

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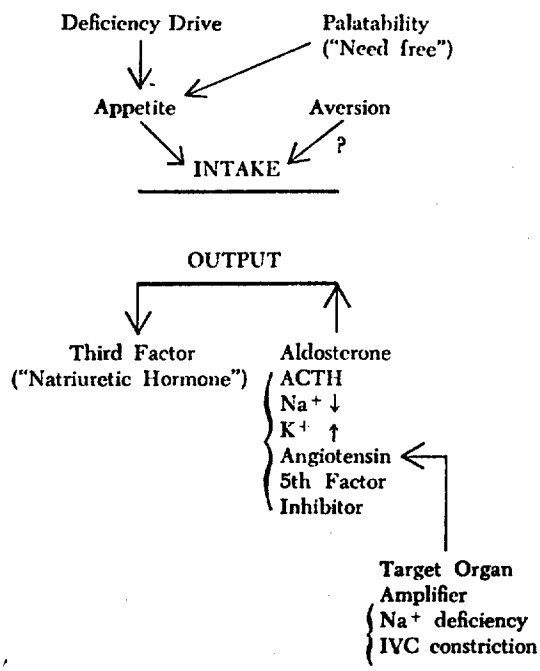
FIGURE 1

Physiological Control of Salt Homeostasis



is present, since the conditions of experiment were that appetite was tested 24 hours after the last dose of mineralcorticoid.[5] Thus, it is difficult to suggest appetite is determined by the contemporary level of aldosterone in blood acting in the brain or in some other fashion. In our view, the evidence is more suggestive that it is generated by change of body balance causing ionic changes within the hypothalamic neuronal systems subserving salt appetite.[2, 6, 7]

However, it is established that in some species, particularly the domestic rat, high doses of hormone—specifically DOCA and aldosterone—will generate salt appetite in the Na-replete animal.[8-11] This paradox may be due to the high doses causing brain ionic changes that parallel those occurring in body Na deficiency.[2, 11]

As another aspect of this question it may be noted that 30 years ago Richter[8] first showed pregnancy and lactation to increase salt appetite in the rat. We have now investigated this phenomenon with external Na balances in wild rabbits. Figure 3 shows the mean results for seven pregnant wild rabbits receiving a diet with abundant Na. Voluntary intake of 500 mEq/L NaCl solution rises 3 to 4 fold in the latter part of pregnancy; during lactation, intake rises spectacularly to amount to daily turnover of the entire extracellular Na content. Balance study shows it to be far in excess of Na need for the products of conception or for

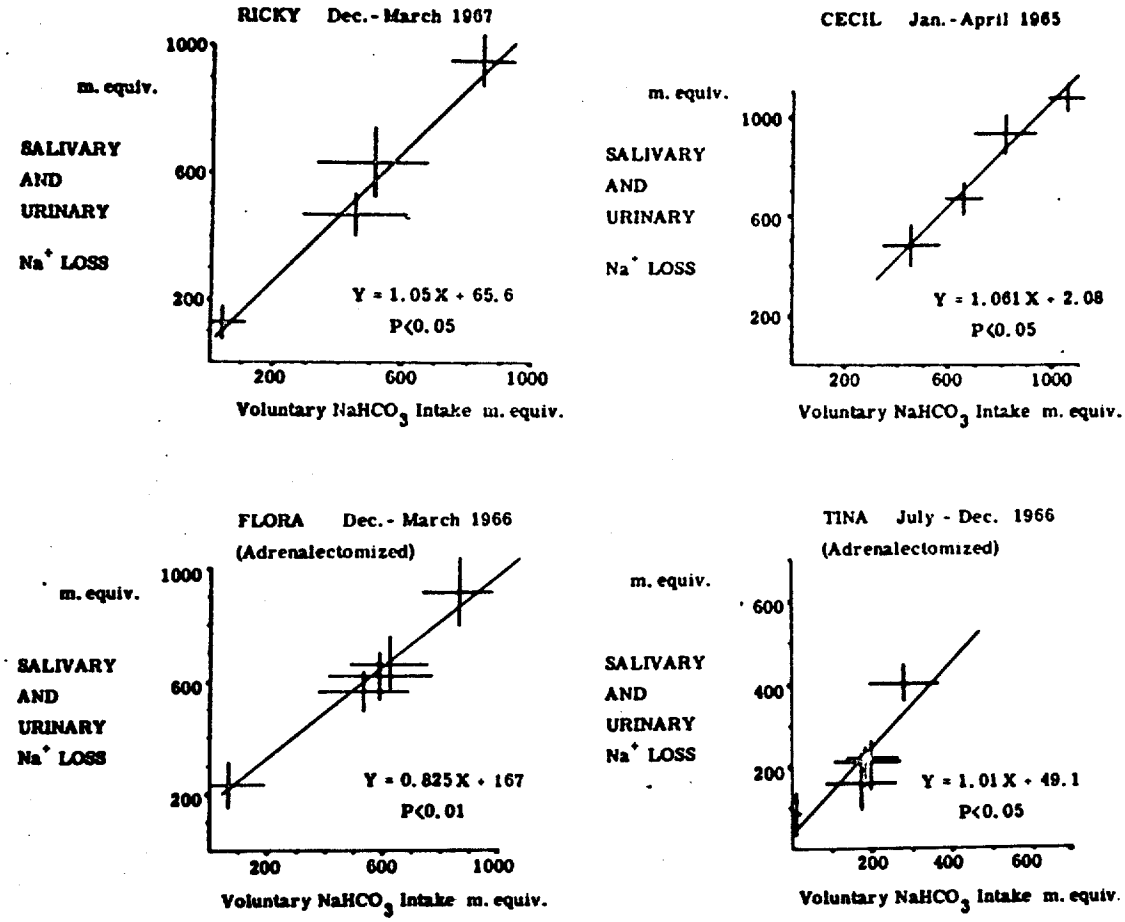


FIGURE 2

The relation between voluntary intake of 300 mEq/L $NaHCO_3$ solution and body Na^+ deficit, as estimated from salivary and urinary Na^+ loss, exhibited by two normal sheep with a parotid fistula, and two adrenalectomized sheep with a parotid fistula. The $NaHCO_3$ solution was presented to the animals for a 15-minute test period following 1 to 3 days of depletion or following a period when Na^+ balance was maintained by returning saliva to the animal episodically by rumen tube. Each point represents the mean ± SD of 9 to 24 observations at each level of Na^+ deficit made over a 5-month period.

Na in milk. The appetite appears to be generated by some aspect of the constellation of hormonic events during the reproductive cycle. In summary, although salt appetite in response to body Na deficit is not primarily hormonically determined, a large salt appetite can be generated by hormones under other physiological conditions. The mechanisms are not yet understood.

The question of whether there is a central nervous mechanism generating an aversion to salt when the body is overloaded is unresolved. This may depend on whether salt appetite control involves a reciprocal excitation-inhibition hypothalamic mechanism similar to food intake—and thus be in contrast to water intake, where no central inhibition mechanism has yet been discovered.[12] There are interesting observations on relative aversion to salt following induction of hyperten-

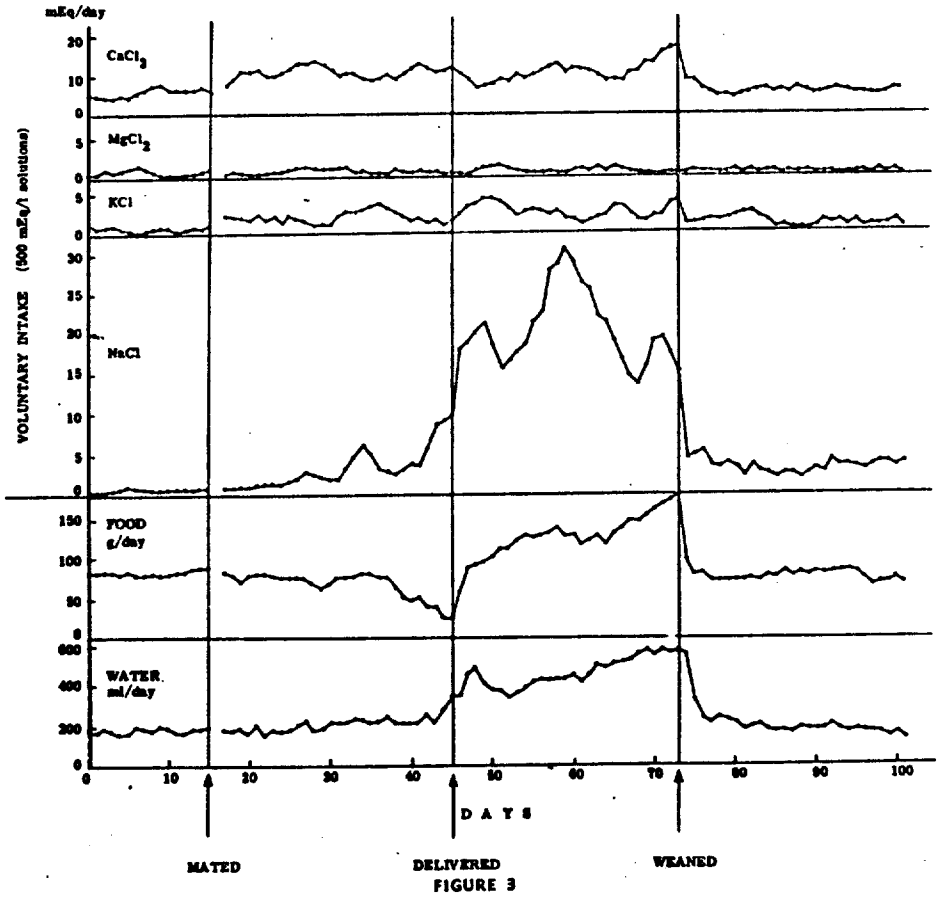


FIGURE 3

The mean intake of food and water, and 500 mEq/L solutions of $CaCl_2$, $MgCl_2$, KCl, and NaCl by seven wild rabbits studied individually in metabolism cages. The food contained abundant Na^+ for normal need (80 mEq/kg). The effect of pregnancy, lactation, and weaning on the voluntary intake is shown.

sion in rats first made by Dr. Landis' group at Harvard[13] but it is not known whether any hormonic influence is involved.

To consider now Na output and hormonic control: The principal hormonic determinant of sodium excretion is aldosterone, and physiologically this involves mode of control and the mechanism of amplification of action. Following isolation of the hormone by the Taits and their colleagues,[14] there is now general agreement that control is not influenced by adrenal denervation,[15-17] and that high levels of ACTH[18-20] and ionic changes—lowered plasma Na and raised K concentration[18]—stimulate aldosterone secretion. In 1958, the cross circulation experiments made concurrently by our group,[17] and Davis' group at the U. S. National Heart Institute,[21] showed that an unidentified humoral stimulus of aldosterone secretion existed. Farrell's data[22] that this originated from the pineal region were not confirmed.[23] Nephrectomy studies by Mulrow and Ganong[24] and Davis' group[19] indicated a renal origin. Genest[25] and Laragh[26] and their associates showed angiotensin stimulated aldosterone and, using closed arterial infusion to the adrenal transplant, we showed the action was directly upon the

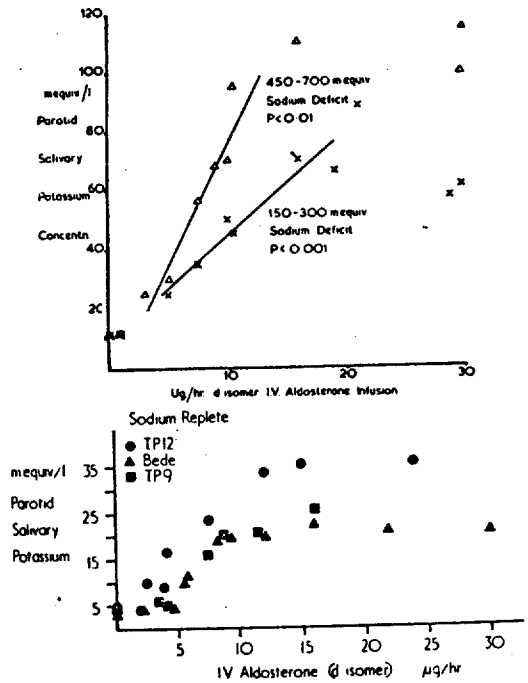


FIGURE 4

The effect of intravenous infusion of d aldosterone at various rates on the maximum K^+ concentration produced in parotid saliva. The lower panel shows the results with Na^+ replete sheep; the upper panel results at two levels of Na^+ deficit in adrenalectomized sheep with a parotid fistula.

adrenal and not via a secondary pathway that released a substance which thereupon acted upon the adrenal.[18, 27]

The idea that—in the absence of overt changes in ionic concentrations or ACTH—the rate of aldosterone secretion is simply and directly controlled by the contemporary level of angiotensin in circulating blood has begun to be extensively tested, the radioimmunoassay of angiotensin having been a critical development in this regard.[28] The present position is controversial.[4, 29] Our group believes the evidence shows other unidentified factors to be involved.[4] This may include a physiological inhibitory system[30] or an unidentified mechanism of stimulation[4, 29] which could possibly be involved in the bilateral hyperplastic form of Conn's syndrome.

Two lines of investigation were reported independently in 1963, indicating that the peripheral action of aldosterone on target organs can be physiologically amplified:

1. Aldosterone acts on the sheep's parotid gland to increase the K concentration of the continuous salivary flow.[17] In the Na-replete normal or adrenalectomized animal, the asymptote of K concentration, with maximum physiological infusion rate of aldosterone (30 µg/hr), is ca. 30 mEq/L (fig. 4). However, if the animal is depleted of 150 to 300 mEq of Na, then the maximum K in the saliva reaches nearer 60 mEq/L with the same dose. If Na is depleted more severely, the same rate of infusion of aldosterone gives a four times higher K - 120 mEq/L.[5] Recent experimental analysis has indicated that with increasing Na deficit, aldosterone acts on the acini as well as the ducts of the parotid, and in this way the physiological action is amplified.[5, 31]

2. Davis' group studied bilaterally adrenalectomized dogs.[32] One kidney was removed; the other was transplanted to the neck. On constant dosage of mineralocorticoid, caval constriction caused Na retention and ascites; this was contrived without any alteration of glomerular filtration rate, renal blood flow, or renal venous pressure. Whether changed oncotic pressure or another physical process could occur and influence tubular Na reabsorption, may have to be taken into account.

Thus, Na deficiency and possibly also caval constriction sensitize target organs to aldosterone action. The mechanism remains unknown.

Finally, is the question of augmentation of Na output and the putative natriuretic hormone. For brevity, consideration will be confined to quoting a distinguished nephrologist. At the recent congress in Stockholm, Berliner stated that if physiologists were to be divided into believers and nonbelievers, he, for one, was thoroughly sceptical of all that had, to that time, purported to demonstrate a natriuretic hormone.[33]

The term third factor was introduced to designate the variable (other than glomerular filtration rate and aldosterone-like activity) that is responsible for the increase in Na

excretion that occurs when saline solutions are infused into the normal animal. Upon this point there appears general agreement.

The attempts to explain saline diuresis are divided generally in two groups: physical and hormonal. Dr. Berliner thinks the evidence that physical factors—changed pressure and flow in peritubular capillaries—play a role is strong. Furthermore, although the existence of additional hormonal factors is likely, he thinks that no suggested demonstration by cross circulation, perfusion of isolated kidney, or extraction of plasma or urine is, as yet, convincing.

In any review of the humoral control of salt homeostasis as a general biomedical field, it is evident that many of the major questions remain to be resolved, and probably many mechanisms are yet to be discovered.

Salt and Hypertension

To move now to consideration of salt and hypertension:

In considering intake of foodstuffs, there are, as Young[34] has pointed out, four main groups of determinants:

1. Constitutional factors depending on innate body mechanisms. They vary from species to species—that is, dogs eat meat, sheep eat grass.
2. Intraorganic conditions produced by deprivation—for example, specific hungers, and as produced by lesions, chemicals, and so forth.
3. Environmental conditions including the complex of sensory stimuli from food.
4. Acquired dispositions—learned appetites —expectancy of effect.

Intake of salt or saline water can be considered in two categories. First that determined by "palatability" or the hedonistic response to taste, and smell, including the learned elements of these responses—groups 3 and 4 of the determinants. Thus, the intake is not metabolically determined. It is "need free" in terms of contemporary sodium balance.

At the experimental level, this is illustrated by the well known preference aversion curve for saline[8] shown by the domesticated rat, *Rattus norvegicus*. When offered water and saline concurrently, the animals commenced to show preference for saline when concentration increased above 12 mEq/L; when the saline concentration reached isotonic, 90% of intake was chosen from that container. The preference for saline decreased substantially as concentration rose above 250 mEq/L. Richter[8] expressed the view that all mammalian species have an instinctive liking for salt and that the capacity to taste nutritious substances confers survival advantage. Bare[35] has suggested, in relation to the biological significance of the need free intake of salt, the simple stimulus-response situation of ingestion if encountered in the wild to some extent protects the animal against deficiency, and it may recall where to find it if deficiency supervenes at a later date.[36]

On the other hand, a comprehensive body of evidence has been assembled by Dahl and Meneely,[37] suggesting that chronic high intake of salt, unrelated to any metabolic requirement, may be important in the genesis of human hypertension. In contrast to earlier periods of history when salt was a relatively scarce and a highly prized commodity for a substantial part of human society, it is now comparatively cheap and abundant in many communities. Social and dietetic factors may operate to condition a high intake. An issue central to the question is the addition of large amounts of salt to baby and infant foods by manufacturers, and the likelihood that the taste of the very young is conditioned to a high salt content from thereon. If it be true that a high salt intake sustained over years is directly causal, or contributory causal with other necessary factors such as genetic predisposition, to development of hypertension in adult life, then this is an important public health issue and manufacturers should be persuaded to stop the practice.

The second broad category of intake, as indicated earlier, is represented by the specific hunger for salt induced by the intraorganic condition of Na deficiency. The physiological analysis of this behavior has been made

mainly in the domesticated *Rattus norvegicus* and various breeds of sheep.

Wolf and colleagues,[1] Nachman,[38] Pfaffmann[39] and others have studied the response to the first experience of Na deficiency in rats that had never had previous access to saline. They showed a specific appetite within 5 to 10 minutes for high—normally aversive—concentrations of saline, commensurate with extent of body Na deficit. This was long before there could be the possibility of learned retroactive benefit from Na drunk and absorbed. Essentially similar results have come from studies of naive sheep with first experience of Na deficiency in the days following the making of a parotid fistula.[30]

Any strict categorization of the basis of intake is, of course, to some extent arbitrary in that the palatability response—the simple unlearned taste preference—is a segment of the integrated physiological mechanisms evoked fully by Na deficiency.

Species Differences

The literature on experiments on renin, aldosterone, hypertension, and salt appetite is derived from studies on a variety of different genera and species of animals. It is clear that some differences in results may be species differences. Since the metabolism of Na is an issue pervading all these questions, we would like to advance briefly a general thesis on the phylogenetic emergence of salt control that may be relevant.[2]

With higher terrestial animals, the ecological impact of salt deficiency falls dominantly on herbivores, and particularly ruminants, with natural selection pressure favoring phylogenetic refinement of salt appetite and mechanisms of aldosterone secretion. This is because the sodium content of grass may be exceedingly low—a trace element—in considerable areas of the earth, particularly in mountains and the interior of continents.[40] A month's food may provide less Na than in 200 to 300 ml of plasma. Furthermore, the large dietetic stress will be aggravated by pregnancy and lactation,[40] and also by any variation in the large volumes of Na-rich fluid involved in digestion.[5]

Much evidence suggests that man evolved under largely tropical conditions during the Miocene, Pliocene, and Pleistocene periods. This tropical mode of existence of feral man, and his evolutionary ancestors, carries attendant stress on temperature control with sweating and salt loss, aggravated again by reproduction, and circulatory effects of the upright posture. For example, Dutch anthropologists recognized that primitive tribes living in the highlands of New Guinea eat a very low salt—vegetarian—diet with K: Na ratio of 400-200:1.[40] In studies in collaboration with Macfarlane and Skinner we have shown these people to have high peripheral blood aldosterone levels—four times that of urban Australians or New Guineans eating western diet—in addition to other evidences of hyperaldosteronism.[44] In this instance, the metabolic situation of primitive man is rather like the herbivore. Coincidentally, there is a highly developed salt trade in some areas of the highlands of New Guinea.[40]

On the other hand, carnivores eat herbivores, and though the latter may be in salt-deficient regions—for example the Rocky Mountains of the U. S.—the permissible variation of the salt content of the tissues of the herbivore compatible with life is relatively small. Thus, carnivores may never receive the impact of a salt-deficient environment as herbivores do. This difference in selection pressure may well be reflected phylogenetically in different salt appetite and hormone mechanisms.

In this ecological context not a great deal is known about the rat, although it is the most extensively used laboratory animal in the whole field of medical investigation. The studies are almost exclusively on the domesticated *Rattus norvegicus*, and to a much lesser extent on the Alexandrine rat. As Richter has documented,[42] the process of domestication has produced radical behavioral, endocrine, and metabolic differences from wild *Rattus norvegicus* captured in the Baltimore back alleys. The point is perhaps put into proper

perspective by noting that the Archbold Expedition of the American Museum of Natural History identified 36 genera, 124 species, and 276 species of Muridae, old and new world rats, on the Continent of Australia and New Guinea.[43] Little is known of the physiology, endocrinology, and appetites of these creatures. When a statement is made, as it is generally and commonly made, that a particular finding is true in the rat, it should be kept in mind, at least if the issue is comparative, that this is a very restricted instance of an inbred domesticated and correspondingly modified, laboratory *Rattus norvegicus*.

Gross[44] has stated that only the rat—and not the cat, rabbit or dog—react to salt overdosage or DOCA administration by developing chronic hypertension.

In man, overdosage of mineralocorticoid or endogenous overproduction will produce hypertension. Extending the comparison to appetite, we can add to this that a specific appetite for sodium salts, under conditions of adequate Na intake in food, can be induced in the laboratory rat by DOCA and aldosterone.[9, 10] The same effect can be produced in the wild rabbit by very large doses of DOCA, but not by physiologically feasible amounts of aldosterone.[11] DOCA does not produce this effect in the sheep, even at toxic level.[41] On the basis of clinical data from patients with primary aldosteronism, high blood levels of aldosterone in man do not induce salt hunger.[41]

This list of interesting species differences could be continued, although we do not suggest that they are necessarily always easily related causally to the postulated phylogenetic history of the different species.

Salt and Human Hypertension

1. There is long-standing evidence that human essential hypertension may be ameliorated by reduction in salt intake, provided intake is reduced to a very low level.[45] Similarly, a high salt intake aggravates existing hypertension.[46]

2. There is a very low incidence of hypertension in several primitive ethnic groups living under differing conditions—Greenland Eskimos,[47] aboriginal tribes in the mountains of China,[48] Cuna Indians of Panama,[49] and aboriginal Australians.[50] Dahl suggests that a common factor of these different ethnic groups, living under widely differing environmental conditions, is a very low salt intake (1 to 2 g/day), relative to that holding generally in western urban civilization (10 to 15 g/day). By contrast, the population of the provinces of northern Honshu in Japan have an extremely high salt intake in their diet (mean 26 g/day), in some instances ranging as high as 50 g/day. The incidence of hypertension and death from cerebral hemorrhage is probably the highest known, approximately 40% of the adult population being hypertensive.[51] Explanations other than salt have been suggested.[52] Dahl has proposed a definite epidemiological correlation between salt intake of communities and the incidence of hypertension, when similar age groups in these communities are compared.

3. Dahl and his colleagues[53] have examined some 900 individuals in the Brookhaven community using the criterion of whether they have a high, average, or low salt intake.[53] This distinction is on the basis of whether the subjects always add salt to food, may add it after tasting, or never add salt to food. They found a significant correlation between the incidence of hypertension and the salt intake. However, in the Framingham survey of some 5000 adult men and women, Dawber and colleagues[54] did not find any relation between salt intake, assessed in a similar fashion, and blood pressure. Furthermore, there was no evidence of blood pressure being related to the 24-hour Na output. Miall,[55] in a study of the population of a Welsh coal mining valley, found no systematic difference between blood pressure and the high and low salt intake groups in males. In the female, surprisingly, the low salt intake group had the higher pressures. There was, however, a low incidence of hypertension in their population.

4. The experimental evidence, accumulated over 30 years, shows a direct, quantitative,

TABLE 1

Na^+ Content of Commercially Available Infant and Baby Foods in Australia

Manufacturer	Product		Na^+/mEq/kg content
	Junior	liver and bacon dinner	128
A	Junior	pineapple and rice	54
	Junior	brains and vegetables	131
	Junior	eggs and bacon	113
	Junior	chicken dinner	140
	Junior	fish dinner	134
	Junior	mixed vegetables	110
	Junior	orange, pineapple and honey	28
	Junior	steak and vegetables	134
	Junior	pears	30
	Junior	fruit dessert	20
	Strained	chicken and noodles	110
	Strained	lamb, liver, and vegetables	123
	Strained	creamed fish	137
	Strained	egg custard	45
	Strained	apple and prune custard	42
	Strained	prunes and cereal	1
	Strained	lamb and vegetables	104
	Strained	lamb and shank broth	128
	Strained	turkey and vegetables	130
B	Junior	lamb, brain and vegetables	120
	Junior	chicken and vegetables	126
	Junior	lamb and vegetables	116
	Junior	mixed vegetables	117
	Junior	beef and vegetables	125
	Junior	fish and vegetables	127
	Junior	chicken dinner	124
	Junior	apricots and rice	1

and unequivocal relation between dietary salt and hypertension in the laboratory rat.[37] Young animals are most susceptible. The longer the salt is eaten, the more severe the effect. The disease varies from a slowly developing benign hypertension to a rapidly fatal malignant type. The hypertension once established is usually self-sustained when salt is removed. Furthermore, from an unselected group of rats of the Sprague-Dawley strain, Dahl has successfully developed inbred strains highly susceptible or resistant to salt-induced hypertension.[56] Neither group develops hypertension on basal nonsalted ration. This demonstration of genetic variability in susceptibility to salt hypertension is proposed as paralleling the variation in human familial predisposition to hypertension. It might also explain why some humans consuming large amounts of salt do not develop hypertension.

The extrapolation of these results from the domesticated rat to the human situation remains controversial. Recently, the debate has centered on the question of the implications of the high salt intake inflicted on the very young because of the addition of salt to baby food by manufacturers.[57, 58]

The argument is as follows:

1. Allowing anabolic requirement, sweat, fecal, and urine losses, daily Na requirement of the healthy infant is met generously by intake of 4 to 8 mEq/day—that is, 1 to 2 mEq/kg.

2. With human breast milk as the sole source of food and fluid, this requirement is met. Daily intake will rarely be more than 10 mEq. Phylogenetically this has been adequate for species survival.

3. Whereas most potential natural foods for the infant would add little salt to diet, the

processors add enough to increase it considerably. It has been shown that an infant receiving various combinations of cow's milk, supplemented by manufacturers solid foods, may receive 4 to 8 mEq Na/kg/day, which is the order of intake of the inhabitants of northern Japan. Analyses by the Howard Florey Laboratories of the infant and junior foods marketed in Australia by two international food processors (table 1) reveal the same procedures as identified in America. There are many figures in the 100 to 180 mEq/kg range (human milk mean, 7 mEq/l; cow's milk 3 to 4-fold higher).

4. There is no benefit accruing from this addition, in that Na cannot be stored for an emergency to any significant extent.

5. Arguments that have been advanced to the effect that this salt addition is a legitimate procedure have been anything but convincing.[59] The fact that the infant has high water, caloric, and protein requirements for growth, which, if extrapolated to the adult on a weight basis, would seem grossly excessive, does not alter the fact that the Na requirements for anabolism and unavoidable loss are accurately known. The suggestion by the apologists that palatability may well be a reasonable means of adjusting intake is specious, since there is no evidence that the human infant finds breast milk unpalatable because of low salt content, and obviously the infant has no choice with baby food since the decision is made by the mother. In fact, there is a suspicion that the salt is added to accommodate to the habituated palate of the mother, and thus her purchasing behavior. Thus, the infant may arrive at a salt content acceptance by 1 or 2 years that the mother required 20 to 30 years to achieve—possibly not the most constructive example of closing the generation gap.

Dahl and others believe there may be a direct link with salt intake and hypertension in later life.[37, 51, 53, 60] The argument is put forward that we are now recognizing the long-term delayed effects of noxious substances, such as benzene and radiation, in causing blood dyscrasias; skin and bladder cancer are linked with occupational exposure to coal tar, emphysema and cancer with cigarettes. Animal experiments are accepted as valid in law and the Food and Drug Administration of the U. S. is guided by them. The manufacturers should be persuaded to stop the practice.

Our view is that it is necessary to take into account the differing susceptibility of different species of animals to salt. It is an open question whether the inbred domesticated laboratory rat has some prerogative as the model of choice. At least much more is known about it than any other model, but this should not narrow the comparative view of the problem. The rat data illustrate beautifully the issue of individual genetic susceptibility within the strain. The parallel with the long-term proven effects of noxious agents can only be drawn with reservation. In dealing with Na, we are dealing with a normal physiological component essential for life. There is an immediate physiological capacity to accommodate to wide variation of intake. The problem, therefore, falls more within the question of the constellation of effects of overnutrition with normal substances, rather than potentially noxious foreign substances. There is good evidence that overnutrition can be detrimental to the organism. The case against salt in the human is rather like the Scottish court verdict of not proven.

However, the evidence is sufficiently strong and suggestive, that, in Australia, for example, the Commonwealth Department of Health, that is, the National Health and Medical Research Council, should at least examine the question of whether it is appropriate to persuade the food producers to reduce or cease the addition of salt to baby and infant foods as a routine practice. In general, it gains nothing and may be dangerous in a subtle long-term fashion. One caveat we would introduce is that proper metabolic examination may justify moderate addition of salt to specified lines recommended for use in physician-guided infant feeding in tropical and subtropical regions of Australia and other regions with long hot summers.

Finally, we would like to introduce some new evidence into this field. Earlier,[30] a thesis

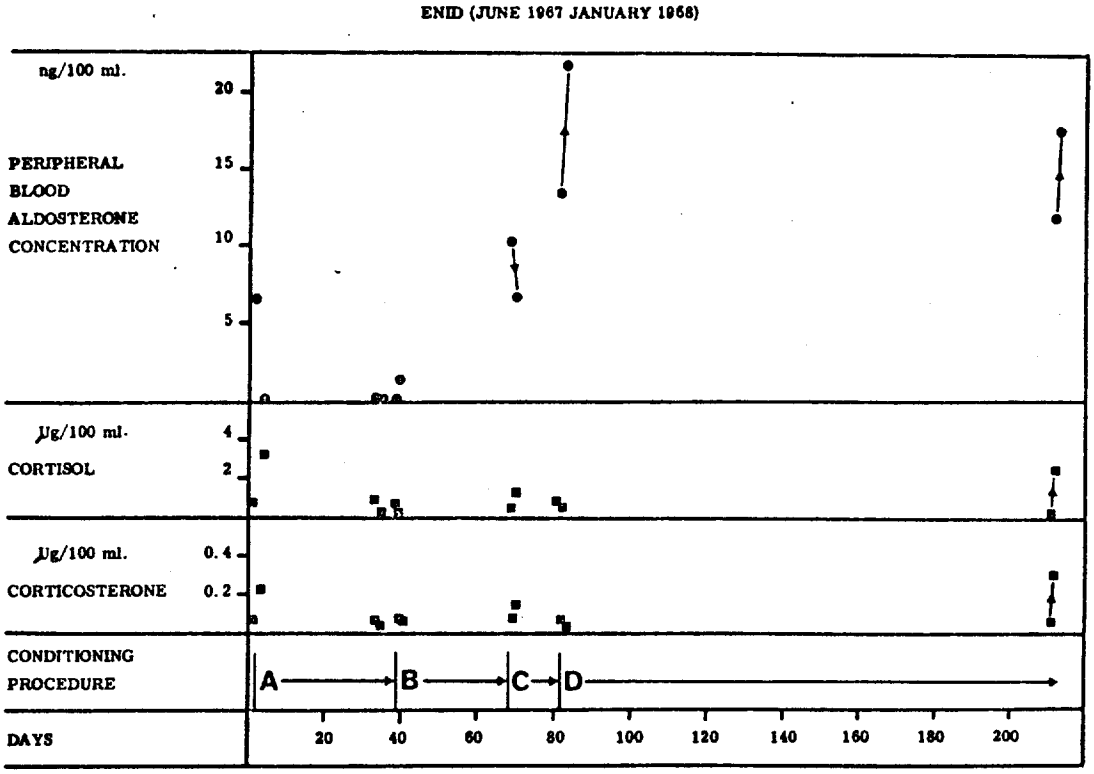


FIGURE 5

The effect of induction of experimental neurosis in a sheep on concentrations of aldosterone, cortisol, and corticosterone as measured in peripheral arterial blood. The measurements were made episodically before, and with the instances joined by an arrow, at the end of a period of stimulation on a particular day. The sequence of the experimental procedure is described in the text.

was advanced of one way in which need free high salt intake in the human might, in the long term, induce hypertension in some individuals and not in others. It could be related to whether personality structure and stress could provide the concomitant condition, integrated over the years, of high peripheral blood aldosterone concentration. The efficacy in animal experiments of the combination of salt and mineralocorticoid in producing hypertension is well established[61] and will not be reviewed here.

One evident condition of experimental approach to this thesis on the mechanism of hypertension would be to demonstrate that central nervous system-induced high blood level of aldosterone can indeed occur, and perhaps, most convincingly, independent of evanescent high ACTH secretion. Figure 5 shows a study over several months in which an experimental neurosis was induced in a sheep. Initially (A), the animal was conditioned to expect mild electric shock to its right leg 5 to 10 seconds after a metronome sounded for 5 seconds. After an interval of 2 minutes a buzzer sounded for 5 seconds. No shock followed; 2 minutes later the metronome sounded again. The cycle was repeated for 2 hours per day, 5 days per week. The experiment was done in isolation in a darkened semi-soundproof room. At the end of (A), the peripheral blood aldosterone sample collected from the sheep in the main cage area without any preliminary disturbance and before the

sheep was taken into the conditioned reflex room was very low normal, as was the value at the end of the conditioning procedure on that day. Peripheral cortisol and corticosterone levels indicative of ACTH secretion were also low. Though the animal, and another animal on the same regime, were lifting their legs in anticipation of the shock, there was no corticosteroid evidence of endocrine disturbance. The control aldosterone some 38 days before when the animal was first brought into the main experimental area had been a little above the normal range, but it was normal after 3 days of the experimental regime. After 36 days of regime A, the signals were reversed in that the animal received no shock after the metronome, but received a shock after the buzzer. At the end of the first days of this regime a similar check showed a neglible change of peripheral blood aldosterone at the end of the 2 hours, and no change of glucocorticoids. From thereon each day the regime was changed, with metronome and buzzer alternating for half-hour periods as the shock signal. Twenty-nine days later the control aldosterone, taken before any procedure, was high (10 ng/100 ml) and of the order seen with mild Na deficiency. On this day the regime was changed to (C) in which the animal received the shocks to the opposite front leg and every third shock was given to the right back leg. The shock signal was alternated over the 2 hours as before. The main point emerging is that after 14 days of this regime, the animal was obviously very apprehensive, and the resting aldosterone was high. It rose over the first day of experimental regime (D) to a level consistent with moderately severe Na deficit without change in glucocorticoid blood levels. On this day, the position of the shocks was changed to two to the back left leg and every third to the original right front leg. Over the following 129 days this regime (D) was continued—except that the interval between signal and shock was lengthened to 2 to 4 minutes—the duration of the time in the conditioned reflex room increased to 4 hours and the regime was only carried out once or twice per week. At the end of this time, resting aldosterone was still very high—and glucocorticoid low. The last experiment involved the animal having a 10-hour period of the regime; glucocorticoid level increased considerably as well as aldosterone. Blood pressure was measured over the 210 days. Daily increases over the course of the experiment were small and inconsistent. There was no definite change in baseline blood pressure. Na intake in food over the experiment was adequate (50 to 70 mEq), and thus the sustained rise of resting aldosterone level was unrelated to any Na deficiency. A similar change in baseline aldosterone was seen in a second sheep on the same regime.

Although the results are purely preliminary, they are interesting and potentially important. They show that induction of anxiety and neurosis will chronically raise resting peripheral blood aldosterone level into the range seen with severe Na deficiency and without comparable alteration in the ACTH-controlled components of adrenal secretion. They are worth following up systematically. At least they suggest that an important physiological component of the thesis proposed is feasible.

In suggesting that the combination of chronic hyperaldosteronism and excess salt intake may require decades to produce its effect on blood pressure, the consideration is in mind that the effect may not require a chronic irreversibly elevated aldosterone level. It may need only to be inappropriately high relative to Na status for an adequate percentage of time over the years for an integrated effect to alter the blood pressure. If blood aldosterone level is consistently high as in Conn's syndrome, an effect on blood pressure may occur over a much shorter period.

References

1. Quartermain, D., Miller, N. E., and Wolf, G.: Role of experience in relationship between sodium deficiency and rate of bar pressing for salt. J Comp Physiol Psychol 63: 417, 1967.
2. Denton, D. A.: Salt appetite. Nutr Abstr Rev 39: 1043, 1969.
3. Denton, D. A., Orchard, E., and Weller, S.: The relation between voluntary sodium intake and body sodium balance in normal and

adrenalectomized sheep. Communications in Behavioural Biology 3: 213, 1969.

4. Blair-West, J. R., Cain, M., Catt, K., Coghlan, J. P., Denton, D. A., Funder, J. W., Scoggins, B. A., and Wright, R. D.: Aldosterone regulation and biosynthesis. Proceedings of the Third International Congress of Endocrinology. Mexico 1968, Excerpta Medica Int. Congress Series No. 184: 276, 1969.
5. Blair-West, J. R., Coghlan, J. P., Denton, D. A., and Wright, R. D.: The effect of endocrines on salivary glands. Handbook of Physiology, Section 6, Chapter 38, Alimentary Canal, Am. Physiol. Soc., Washington, 1967, p. 633.
6. Denton, D. A.: Salt appetite. Handbook of Physiology, Chapter 31, Alimentary Canal, Am. Physiol. Soc., Washington, 1967, p. 433.
7. Denton, D. A.: Some theoretical considerations in relation to innate appetite for salt. Cond Reflex 1: 144, 1966.
8. Richter, C. P.: Salt appetite of mammals: Its dependence on instinct and metabolism. L'instinct dans le comportement des animaux et de l'homme. Paris, Masson, 1956, p. 577.
9. Wolf, G.: Sodium appetite elicited by aldosterone. Psychonomic Sci 1: 211, 1964.
10. Fregly, M. J., and Waters, I. W.: Hormonal regulation of the spontaneous sodium chloride appetite of rats. *In*: Olfaction and Taste II, Proceedings of the Second International Symposium, Tokyo, 1965, p. 439.
11. Denton, D. A., and Nelson, J. F.: The effect of deoxycorticosterone on the salt appetite of wild rabbits (*Oryctolagus Cuniculus* (L)). Endocrinology, 1970. In Press.
12. Brobeck, J. R.: Regulation of feeding and drinking. Handbook of Physiology. Neurophysiology, edited by J. Field, H. W. Magoun, and V. E. Hall. Washington, D. C. Am. Physiol. Soc., 1960, sect. 1, vol. 2, chapt. 47, p. 1197.
13. Tosteson, D. C., DeFriez, A. I. C., Abrams, M., Gottschalk, C. W., and Landis, E. M.: Effects of adrenalectomy, desoxycorticosterone acetate and increased fluid intake on intake of sodium chloride and bicarbonate by hypertensive and normal rats. Amer J Physiol 164: 369, 1951.
14. Simpson, S. A., Tait, J. R., Wettstein, A., Neher, R., von Euw, J., and Reichstein, T.: Isolierung eines neuen kristallisierten Hormons aus Nebennieren mit besonders hoher Wirksamkeit auf den Mineralstoff-wechsel. Experientia 9: 333, 1953.
15. Fleming, R., and Farrell, G.: Aldosterone and hydrocortisone secretion by the denervated adrenal. Endocrinology 59: 360, 1956.
16. McDonald, I. R., Goding, J. R., and Wright, R. D.: Transplantation of the adrenal gland of the sheep to provide access to its blood supply. Aust J Exp Biol Med Sci 36: 83, 1958.
17. Denton, D. A., Goding, J. R., and Wright, R. D.: Control of adrenal secretion of electrolyte-active steroids. Brit Med J 2: 447, 522, 1959.
18. Blair-West, J. R., Coghlan, J. P., Denton, D. A., Goding, J. R., Wintour, M., and Wright, R. D.: The control of aldosterone secretion. Recent Progr Hormone Res 19: 311, 1963.
19. Davis, J. O.: Mechanisms regulating the secretion and metabolism of aldosterone in experimental secondary hyperaldosteronism. Recent Progr Hormone Res 17: 293, 1961.
20. Ganong, W. F., and Mulrow, P. J.: Evidence of secretion of an aldosterone-stimulating substance by the kidney. Nature (London) 190: 115, 1961.
21. Yankopoulos, N. A., Davis, J. O., Kliman, B., and Peterson, R. E.: Evidence that a humoral agent stimulates the adrenal cortex to secrete aldosterone in experimental secondary hyperaldosteronism. J Clin Invest 38: 1278, 1959.
22. Farrell, G.: The physiological factors which influence secretion of aldosterone. Recent Progr Hormone Res 15: 275, 1959.
23. Blair-West, J. R., Coghlan, J. P., Denton, D. A., Goding, J. R., Wintour, Marelyn, and Wright, R. D.: The action of electrolyte changes and some naturally-occurring pharmacological substances on the adrenal cortex. Hormones and the Kidney, edited by Peter C. Williams, Memoirs of the Society for Endocrinology, No. 13, London, Academic Press, 1963, p. 342.
24. Ganong, W. F., and Mulrow, P. J.: Evidence of secretion of an aldosterone stimulating substance by the kidney. Nature (London) 190: 115, 1961.
25. Genest, J. E., Koiw, E., Nowaczynski, W., and Sandor, T.: Study of urinary adrenocortical hormones in human arterial hypertension. Abstracts, First International Congress of Endocrinology. Denmark 1960, p. 173.
26. Laragh, J. H., Angers, M., Kelly, W. G., and Lieberman, S.: Hypotensive agents and pressor substances. JAMA 174: 234, 1960.
27. Peterson, R. E.: Discussion of I. H. Page, and F. M. Bumpus. Angiotensin—a renal hormone. Recent Progr Hormone Res 18: 185, 1962.
28. Catt, K. J., Cain, M. D., and Coghlan, J. P.: Measurement of angiotensin II in blood. Lancet 2: 1005, 1967.
29. Palmore, W. P., Marieb, N. J., and Mulrow, P. J.: Stimulation of aldosterone secretion by sodium depletion in nephrectomized rats. Endocrinology 84: 1342, 1969.
30. Denton, D. A.: Evolutionary aspects of the

emergence of aldosterone secretion and salt appetite. Physiol Rev 45: 245, 1965.

31. Blair-West, J. R., Coghlan, J. P., Denton, D. A., Nelson, J. F., Wright, R. D., and Yamauchi, A.: Ionic, histological and vascular factors in the reaction of the sheep's parotid to high and low mineralocorticoid status. J Physiol (London) 205: 563, 1969.

32. Davis, J. O., Holman, J. E., Carpenter, C. C. J., Urquhart, J., and Higgins, J. T.: An extra-adrenal factor essential for chronic renal sodium retention in presence of increased sodium-retaining hormone. Circ Res 14: 17, 1964.

33. Berliner, R. W.: Which factor is third? Proceedings Fourth International Congress of Nephrology, Stockholm 1969. Basel, S. Karger.

34. Young, P. T.: Palatability: The hedonic response to foodstuffs. *In*: Handbook of Physiology, Chap 26, Alimentary Canal, Am. Physiol. Soc., Washington, 1967, p. 353.

35. Bare, J. K.: The specific hunger for sodium chloride in normal and adrenalectomized white rats. J Comp Physiol Psychol 42: 242, 1949.

36. Krieckhaus, E. E., and Wolf, G.: Acquisition of sodium by rats: interaction of innate mechanisms and latent learning. J Comp Physiol Psychol 56: 1007, 1968.

37. Meneely, G. R., and Dahl, L. W.: Electrolytes in hypertension: the effects of sodium chloride. Med Clin N Am 45: 271, 1961.

38. Nachman, M.: Taste preferences for sodium salts by adrenalectomized rats. J Comp Physiol Psychol 55: 1124, 1962.

39. Nachman, M., and Pfaffmann, C.: Gustatory nerve discharge in normal and sodium-deficient rats. J Comp Physiol Psychol 56: 1007, 1963.

40. Blair-West, J. R., Coghlan, J. P., Denton, D. A., Nelson, J. F., Orchard, E., Scoggins, B. A., Wright, R. D., Myers, K., and Junqueira, C. L.: Physiological, morphological and behavioural adaptation to a sodium deficient environment by wild native Australian and introduced species of animals. Nature (London) 217: 922, 1968.

41. Denton, D. A., Nelson, J. F., Orchard, Elspeth, and Weller, Sigrid: The role of adrenocortical secretion in salt appetite. *In*: Olfaction and Taste, ed. 3, edited by Carl Pfaffmann. New York, Rockefeller Univ. Press, 1968. In Press.

42. Richter, C. P.: The effects of domestication and selection on the behavior of the Norway rat. J Nat Cancer Inst 15: 727, 1954.

43. Tate, G. H. H.: Results of the Archbold expeditions. No. 65. The rodents of Australia and New Guinea. Bull Amer Museum Natural History 97: 183, 1951.

44. Gross, F.: Adrenocortical function and renal pressor mechanisms in experimental hypertension. Essential Hypertension—An International Symposium, Berne 1960, edited by K. D. Bock and P. T. Cottier, Springer-Verlag, 1960, p. 126.

45. Dole, V. P., Dahl, L. K., Cotzias, G. C., Eder, H. A., and Krebs, M. E.: Dietary treatment of hypertension. Clinical and metabolic studies of patients on the rice-fruit diet. J Clin Invest 30: 1189, 1951.

46. Perera, G. A., and Blood, D. W.: The relationship of sodium chloride to hypertension. J Clin Invest 26: 1109, 1947.

47. Thomas, W. A.: Health of a carnivorous race. JAMA 88: 1559, 1927.

48. Morse, W. R., and Beh, Y. T.: Blood pressure amongst Aboriginal ethnic groups of Szechwan Province, West China. Lancet 1: 966, 1937.

49. Kean, B. H.: The blood pressure of Cuna Indians. Amer J Trop Med 24: 341, 1944.

50. Hicks, C. S., and Matters, R. F.: The standard metabolism of the Australian Aborigines. Aust J Exp Biol Med Sci 11: 177, 1933.

51. Dahl, L. K.: Possible role of salt intake in the development of essential hypertension. Essential Hypertension. An International Symposium, Berne 1960, edited by K. D. Bock and P. T. Cottier, Springer-Verlag, 1960, p. 53.

52. Schroeder, H. A.: Discussion of L. K. Dahl's paper (see Ref. 51). Essential Hypertension. An International Symposium, Berne 1960, edited by K. D. Bock and P. T. Cottier, Springer-Verlag, 1960, p. 83.

53. Dahl, L. K., and Love, R. A.: Evidence for relationship between sodium (chloride) intake and human essential hypertension. Archives Intern Med (Chicago) 94: 525,

54. Dawber, T. R., Kannel, W. B., Kagan, A., Donabedian, R. K., McNamara, P. M., and Pearson, G.: Environmental factors in hypertension. The Epidemiology of Hypertension: Proceedings of an International Symposium, edited by J. Stamler, R. Stamler, and T. N. Pullman. New York, Grune & Stratton, 1967, p. 255.

55. Miall, W. E.: Follow-up study of arterial pressure in the population of a Welsh mining valley. Brit Med J 2: 1205, 1959.

56. Iwai, J., Knudsen, K. D., Dahl, L. K., Heine, M., and Leitl, G.: Genetic influence on the development of renal hypertension in parabiotic rats. Evidence of a humoral factor. J Exp Med 130: 507, 1969.

57. Dahl, L. K.: Salt in processed baby foods. Amer J Clin Nutr 21: 787, 1968.

58. Guthrie, H. E.: Infant feeding practices—a predisposing factor in hypertension? Amer J Clin Nutr 21: 863, 1968.
59. Stewart, R. A.: Letter to the Editor, Nutr Rev 25: 254, 1967.
60. Knudsen, K. D., and Dahl, L. K.: Essential hypertension: inborn error of sodium metabolism? Postgrad Med J 42: 148, 1966.
61. Selye, H., Hall, C. E., and Rowley, E. M.: Malignant hypertension produced by treatment with desoxycorticosterone acetate and sodium chloride. Canad Med Ass J 49: 89, 1943.

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STRAIN DIFFERENCES AND THE RELATION OF AGING TO SALT SUSCEPTIBILITY IN MICE[a]

RAYMOND F. BLOUNT AND ISABEL H. BLOUNT[b]

Far-reaching morphological and physiological effects are brought about by the administration of high levels of NaCl, and length of survival is affected. These effects are less pronounced when the NaCl is administered with food than when given in water. In the work of Meneely and associates (10, 11) high salt was administered to rats as part of the diet. Heller (6) gave rats salt in drinking water but in general at low percentages, while Sapirstein, Brandt and Drury (13) used two per cent. Little other work has been done with higher salt administered in this manner.

There are differences in the response to NaCl toxicity by various animals. Dipodomys and Meriones, desert rodents, excrete a very concentrated urine and conserve water, as shown by Howell and Gersh (8), Schmidt-Nielsen; Schmidt-Nielsen and Schneiderman (15) and by Howe and Jewell (7). Schmidt-Neilsen (14) also showed that Dipodomys, the kangaroo rat, is able to drink sea-water. These animals also withstand high salt in the diet somewhat better than does the ordinary laboratory rat, as shown by Nichols (12). As was briefly reported earlier (3, 4) we have had available two strains of mice with contrasting susceptibility to NaCl toxicity. The present study deals with the effect of high NaCl administered in drinking water on the survival time of these two strains at various ages.

Materials and Methods

Mice of two strains were used in the work. One, referred to as the resistant strain is designated IHB, and is a locally developed inbred strain now in more than the twenty-fifth generation of brother-sister matings. The original stock was from the "All Purpose Strain" of mouse from Rockland Farms. The so-called susceptible strain is a substrain of the NH mouse also maintained in our laboratory by brother-sister matings. In all about 1,700 mice have been

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entered in this report. Several hundred additional mice, with the same treatment and showing the same results, have been used for other purposes.

The mice were numbered with ear marks and placed on 4% NaCl as drinking water. Control animals were placed on distilled water. The food was one of the ordinary pellet forms. The animal room had controlled temperature, although a fluctuation of five degrees was sometimes noted. The date of death was recorded for each animal. Test animals and their controls were weighed at intervals and their general appearance and behavior noted.

Results

The general effect of the experiment was a reduction in weight, with debility, and as the terminal stage was approached there was often muscular tremor. A test animal of the resistant strain after a year and 44 days on 4% NaCl is shown in Figure 1 (B) together with its control (A). It weighed 11 grams and its control 35 grams. In contrast an individual of the susceptible strain shown in Figure 2 (B) had been on salt only 11 days and

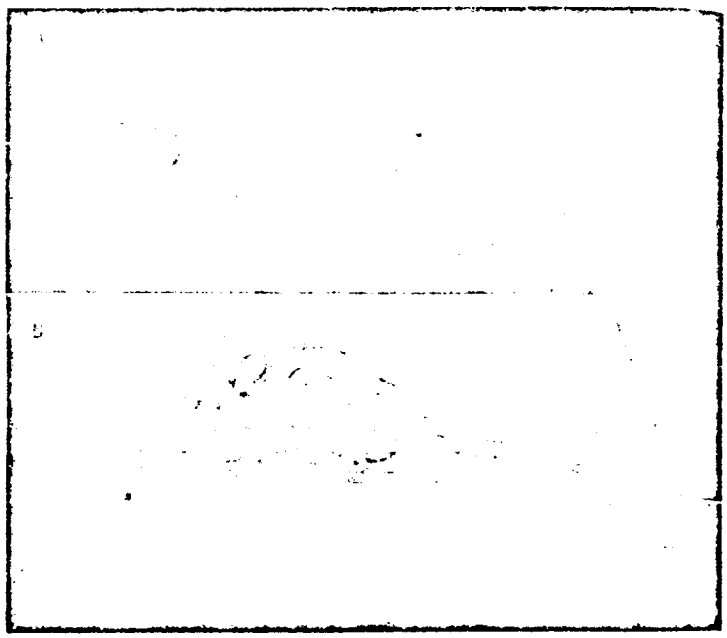

FIG. 1. A set of animals of the resistant strain (IHB). One year and 44 days after the beginning of the experiment. A.—Control animal, weight 35 grams. B.—Test animal on 4% NaCl in drinking water, weight 11 grams.

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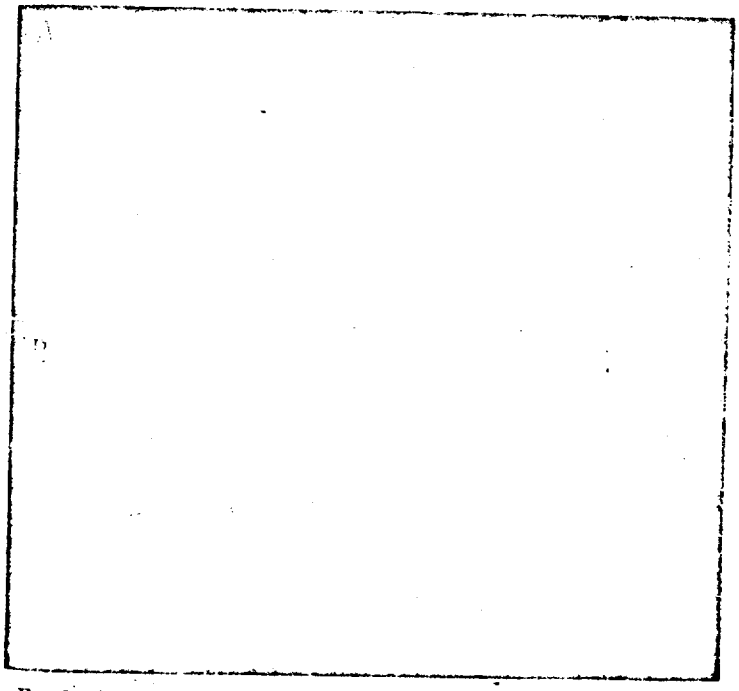

FIG. 2. A set of animals of the susceptible strain (NH). Twelve days after the beginning of the experiment. A.—Control animal, weight 22 grams. B.—Test animal, weight 12 grams. Photograph taken one day previous to death.

weighed 12 grams whereas the control mouse (A) weighed 22 grams. The test animal died on the twelfth day.

Weight curves made for typical test animals of both strains, together with their controls, are shown in Figure 3. For each strain two different starting ages are shown. Most resistant to the NaCl was the IHB animal (curve A) started at 53 days of age and 19 grams in weight. This mouse survived for one year and 34 days, it weighed 13 grams at the time of death. The control (B) by that time had not only attained its adult weight but showed a continued rise through the accumulation of fat. An NH animal (E), started at the same age as the IHB animal and weighing 16 grams, died after only 45 days, at which time its weight was 11 grams. Its control then weighed 24 grams. The older IHB test animal (C) did better than the young NH. Starting on NaCl at 360 days of age and at 35 grams in weight, it de-

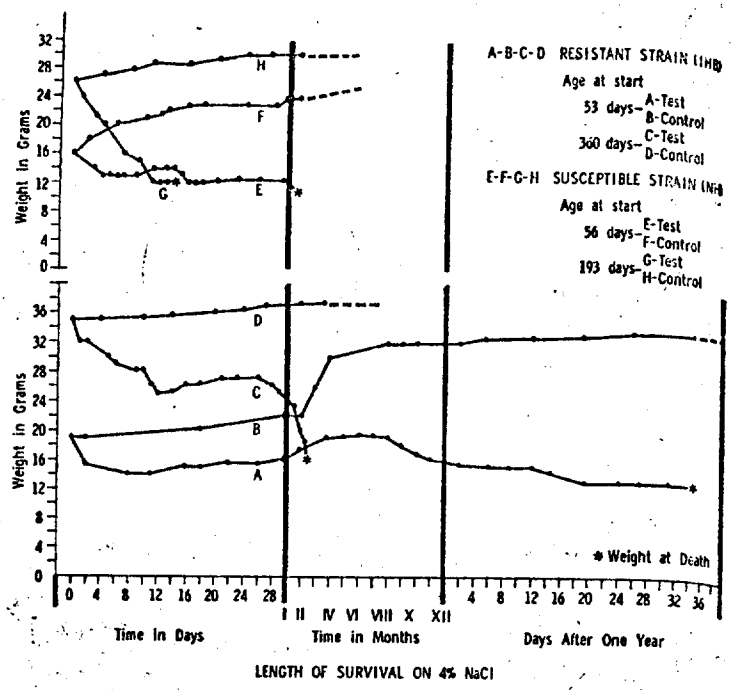

FIG. 3. Weight curves for typical animals. Compare A with E for difference of strain susceptibility. Compare C with A, and G with E for age differences.

clined rather rapidly but survived between 3 and 4 months and weighed 15 grams at death. The growth of the control (4) had levelled off at 37 grams. Shortest-lived of all was the older NH test animal (G) which was put on salt at 193 days of age with a weight of 26 grams. It died on the fourteenth day weighing 11 grams, when its control weighed 28 grams.

For each test animal the weight curve showed a considerable decline at first, while the animal was becoming dehydrated. In all but the older NH (G) a varying amount of adjustment took place, so that the decline became more gradual or there were periods of increased weight, before the final decline. During the latter, animals often shrank to 11 grams or less.

The difference in susceptibility of the two strains of mice is readily evident if the effect upon animals of approximately the same age (Table 1) is compared. One hundred sixty mice of the

TABLE 1

Comparison of the Survival of the Two Strains of Mice

Placed on 4% NaCl between 22 and 40 days of age

Number of Animals: Resistant Strain (IHB)—160; Susceptible Strain (NH)— 50

Days on Salt	Survival in %		Days on Salt	Survival in %	
	IHB	NH		IHB	NH
5	99	80	210	61	0
10	95	38	240	53	0
15	93	34	270	46	0
20	93	18	300	35	0
25	93	14	330	28	0
30	91	12	360	18	0
40	89	8	390	9	0
50	89	6	420	7	0
60	87	6	450	1	0
90	84	0	480	1	0
120	75	0	510	1	0
150	70	0	540	0	0
180	66	0	570	0	0

resistant strain (IHB) were placed on 4% NaCl in drinking water when between 22 and 34 days of age, and 99% were alive after 10 days and 91% after 30 days. Sixty-one per cent of them were still alive after 210 days and 7% lived 420 days. In contrast, of 50 animals of the susceptible strain (NH) placed on salt between the ages of 27 and 40 days, only 38% survived for 10 days, 12% for 30 and 6% for 60 days. None lived for 90 days.

The length of time that animals could survive decreased inversely with the advance in the age at which the salt treatment was started. Thus of 90 animals of the resistant strain put on salt (Table 2) when relatively young (20–30 days), 44 were still living 120 days later and 17 after a year. One lived for 510 days. Of the 103 mice started on salt when they were over a year old (361–450 days) only three were living 60 days afterwards. Between these two groups the results showed a remarkably uniform curve of survival. The older the animals were when the experiment started, the shorter was the average survival.

The susceptible strain showed a similar pattern of survival, but the deleterious effect of the salt appeared at younger age

TABLE 2

Age and Susceptibility of the Resistant Strain

Days Age at Start	21–30	31–60	61–90	91–180	181–270	271–360	361–450	451–540	541–630	631–695
Number Started (1154)	90	345	81	156	147	183	103	45	2	2
Days after Start					Number Surviving					
10	59	341	67	126	82	102	36	16	0	0
20	52	321	50	54	25	24	5	1		
30	51	296	41	37	17	11	3	1		
60	49	281	38	28	12	2	3	0		
90	47	253	35	16	10	1	0			
120	44	240	34	14	8	0				
150	39	205	34	12	6					
180	38	176	30	5	6					
210	37	156	24	2	5					
240	31	138	23	2	2					
270	29	121	18	1	1					
300	29	85	10	1	0					
330	26	58	7	1						
360	17	37	5	0						
390	11	16	4							
420	8	7	4					Control		
450	2	2	0					Median Survival		
480	1	1						Time on H_2O		
510	1	0						547 Days		
540	0									
Median Survival Time	240	225	195	135	105	25	20	15		

levels (Table 3). Thus of 43 animals put on salt when they were between 27 and 40 days old, only one survived for 70 days. Of the 43 started when 121 to 274 days of age (last 3 columns) none survived as long as 40 days.

There is some evidence that animals less than 30 days old are less resistant than those at 30 days of age. A comparison of the figures in the first column of Table 2 with those in the second column shows a smaller proportion of animals surviving for 10 days than might be expected, although the group as a whole lived very well. It appears that if an animal of this age group can survive for 30 days, it has greater tolerance than one started at

TABLE 3

Age and Susceptibility of the Susceptible Strain

Dege Age at Start	27–40	41–50	51–60	61–70	71–80	81–90	91–120	121–150	151–210	211–270
Number Started (299)	43	45	34	37	35	31	31	20	15	8
Days after Start					Number Surviving					
10	17	26	22	25	24	19	21	11	6	3
20	9	15	10	11	10	1	1	1	0	0
30	6	11	9	6	7	1	1	1		
40	4	4	6	0	4	1	0	0		
50	3	0	1		1	0				
60	3		0		1					
70	1				0					
80	0									
Median Survival Time	35	20	25	15	30	20	15	15	6	3

Control—Median Survival Time on distilled H_2O—118 Days

an older age. The same thing is true of the susceptible strain (Table 3).

Discussion

The effect of salt upon animals and upon their survival is dependent upon the method of administration as well as the concentration. The intake can be greater if given as a part of the diet and water is freely available. This has been demonstrated by the work of Meneely *et al.* (10,11) in which there was polydipsia and polyuria. Although the majority of the rats remained in excellent health with the percentage of NaCl in the diet as high as 8.9, growth was decreased and the length of life was reduced in proportion to the various salt levels. There was spontaneous edema in sporadic cases (18%), followed by a rapid decrease in weight, and death (11). It should be noted that we have never found edema in mice with 4% salt in drinking water. Tucker *et al.* (16) administered as high as 21 per cent NaCl in the food. This led to death in about six to eight months as compared to two and a half years in the control animals.

When NaCl was given to rats in the drinking water Heller (6)

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noted deleterious effects on reproduction, and death when the level was above 1.5 or 1.7 per cent, which he considered to be the maximum that could be used with safety. In acute experiments on hypertension Friedman *et al.* (5) used sodium chloride by gavage as a tolerance test, and they pointed out the necessity of keeping the salt load within the capacity of the rat to handle it. Sapirstein, Brandt and Drury (13) also gave rats salt in drinking water, but at only a two per cent concentration and for a period of only six weeks. They noted the relaitvely greater effect when administered in water, in that there was an increase in heart weight and that hypertension was produced. These effects had not been brought about by an even higher intake of salt in food. We have found an increase in the heart weight in our mice on salt (3). As yet blood pressures have not been taken. Binet *et al.* (2) gave 1.6% NaCl in drinking water to rats conditioned by being raised on a high salt intake in food. They noted that these animals were more resistant to high salt than the control animals.

It is clear that the relationship between salt and water intake is an important factor in the toxicity of high NaCl. It is also evident that the two strains of mice used in this work are very different in their reactions. The resistant strain showed examples which lived over 500 days on salt when the normal life span is two years or more, while the susceptible strain showed no survivors over 90 days when the longevity is usually somewhat over a year. Four per cent salt is about the limit that can be tolerated in drinking water, which is above the concentration of sea water. The electrolytic conductivity equivalent of sea water is somewhat above 3% (14). Adolph (1) found that the survival of rats on sea water was 7–26 days, which was only slightly greater than when on no water at all (6–15 days).

The variation as to the susceptibility of littermates, some individuals dying in a relatively short time and others living for extended periods, is in keeping with the experience of others. The differences in tolerance between the two strains of mice are genetically determined. This aspect was studied by McNutt (9) by crossing these two strains and testing the F_1, F_2, F_3 and back-cross generations for tolerance to 4% NaCl. His results suggested that the strains are not genetically homogeneous and that although the difference in tolerance for salt is not the result of a single gene, the number of genes is small.

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The mice show interesting morphological changes when placed on high NaCl. Studies are now underway to determine the factors involved in the mechanism of the resistance or susceptibility to the deleterious effects of NaCl.

Summary and Conclusions

1. Two strains of mice were shown to differ in their resistance to the harmful effects of 4% NaCl administered in drinking water.

2. Weights of animals of both strains decline at first under salt treatment but those of the resistant strain (IHB) and the younger ones of the susceptible strain (NH) are able to adjust themselves for a time, and even regain some weight before shrinkage and death. Older animals of the susceptible strain are least able to maintain weight.

3. In the resistant strain, animals may survive as long as 510 days (17 months) on this regime, while in the susceptible strain none lived for 90 days (3 months).

4. As the age at which animals are placed on salt rises the length of survival decreases.

5. There is evidence that animals started on salt at less than 30 days of age often die within 10 days, but the ones that survive for 30 days behave like those initially placed on salt at 30 days.

6. There is a direct relationship between age and individual susceptibility to salt and this in turn is correlated with the relative longevity of the strain of mouse.

REFERENCES

1. Adolph, Edward F.: Do rats thrive when drinking sea water? *Am. J. Physiol.*, 140: 25–32, 1943.
2. Binet, Léon, Pierre Dejours et A. Lacaisse: Étude du fonctionnement du rein hypertrophié par la surcharge alimentaire en cristalloïdes chez le rat. *Journal de Physiologie*, 43: 365–381, 1951.
3. Blount, Raymond F.: Changes produced in the mouse by prolonged salt treatment particularly in the finer structure of hte kidney. *Anat. Rec.*, 112: 10–11, 1952.
4. Blount, Raymond F., and Isabel H. Blount: Relation of aging to salt susceptibility in mice. *Anat. Rec.*, 124: 389, 1956.
5. Friedman, Sydney M., J. A. M. Hinke, and David F. Hardwick: Sodium tolerance in experimental hypertension. *Circulation Research*, 3: 297–305, 1955.
6. Heller, Victor G.: Saline and alkaline drinking waters. *J. Nutrition.*, 5: 421–429, 1932.

7. Howe, A., and P. A. Jewell: Effects of water deprivation upon the neurosecretory material of the desert rat (Meriones meriones) compared to the laboratory rat. *J. Endrocrinol.*, 18: 118–124, 1959.

8. Howell, A. Brazier, and I. Gersh: Conservation of water by the rodent Dipodomys. *J. Mammalogy*, 16: 1–9, 1935.

9. McNutt, Wallace: A genetic study of tolerance to salt in two strains of mice. *Anat. Rec.*, 121: 338–339, 1955.

10. Meneely, George R., Robert G. Tucker and William J. Darby: Chronic sodium chloride toxicity in the albino rat. I. Growth on a purified diet containing various levels of sodium chloride. *J. Nutrition*, 48: 489–498, 1952.

11. Meneely, George R. Robert G. Tucker, William J. Darby and Stewart H. Auerbach: Chronic sodium chloride toxicity in the albino rat. II Occurrence of hypertension and a syndrome of edema and renal failure. *J. Exp. Med.*, 98: 71–80, 1953.

12. Nichols, John: Effects of high sodium diet and of high potassium diet on the adrenals and kidney of the desert kangaroo rat and domestic albino rat. *Exp. Med. and Surg.*, 7: 366–376, 1947.

13. Saperstein, Leo A., Wilbur L. Brandt, and Douglas R. Drury: Production of hypertension in the rat by substitution of hypertonic sodium chloride solution for drinking water. *Proc. Soc. Exp. Biol. and Med.*, 73: 82–85, 1950.

14. Schmidt-Nielsen, Bodil and Knut Schmidt-Nielsen: Do kangaroo rats thrive when drinking sea water? *Am. J. Physiol.*, 160: 291–294, 1950.

15. Schmidt-Nielsen, Knut, Bodil Schmidt-Nielsen and Howard Schneiderman: Salt excretion in desert mammals. *Am. J. Physiol.*, 154: 163–166, 1948.

16. Tucker, Robert G., Con O. Ball, William J. Darby, Walter R. Earley, Ross C. Cory, John B. Youmans and George R. Meneely: Chronic sodium chloride toxicity in the rat. III. Maturity characteristics, survivorship, and organ weights. *J. of Gerontology*, 12: 182–189, 1957.

Potassium Citrate and Potassium Gluconate Versus Potassium Chloride

Experimental Evaluation of Relative Intestinal Toxicity

Scott J. Boley, MD, Leon Schultz, MD, Solomon Schwartz, MD, Armand Katz, MD, and Arthur C. Allen, MD

IN LATE 1964 a sudden increase in the occurrence of circumferential ulcerating, stenosing lesions of the small bowel was noted both in Europe and the United States. Lindholmer et al[1] in Sweden and Baker et al[2] in this country first suggested the association of these lesions with enteri-coated thiazide diuretic potassium chloride preparations. Extensive experimental and clinical investigations[3-6] quickly identified enteric-coated potassium chloride as the responsible agent, and led to recognition of perforating areas of necrosis as a more severe form of the same lesion. The mechanism of damage is believed to be the release and absorption of high concentrations of potassium chloride over short segments of small bowel acting upon submucosal and mural veins to produce venous spasm or paralysis. The resultant venous stasis causes segmental hemorrhagic infarction of varying severity with ulceration and stenosis.[7]

Recognition of this complication of enteric-coated potassium chloride ingestion has properly led to recommendations that this form of potassium supplement be used only when absolutely necessary. It further has stimulated a search for safe potas-

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Effects of Various Potassium Salts and Sodium Chloride, and Findings at Autopsy

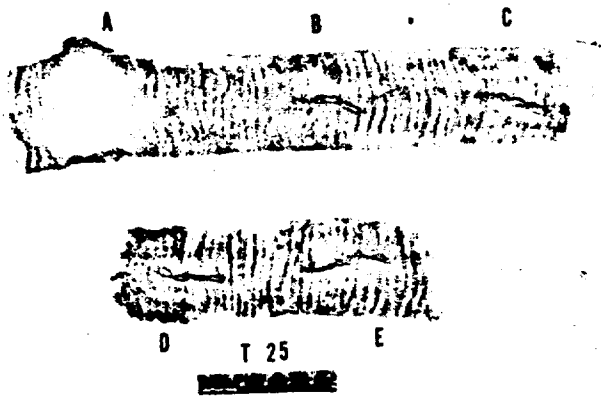
Salt	No. of Sites	Sites With Pathologic Changes	Minimal Superficial Hemorrhage	Segmental Infarction
Potassium citrate*				
13.5 mEq (6 dogs)	15	0	0	0
27 mEq (2 dogs)	5	2	2	0
Potassium gluconate				
10 mEq (2 dogs)	7	0	0	0
15 mEq (3 dogs)	10	1	1†	0
30 mEq (2 dogs)	5	2	2	0
Potassium chloride controls				
13.5 mEq (5 dogs)	5	5	1	4
27 mEq (1 dog)	1	1	0	1
Sodium chloride				
27 mEq (2 dogs)	2	2	2	0

*As hydrochlorothiazide-potassium citrate combination.
†Showed microscopic superficial necrosis.

sium salts and preparations. Liquid, uncoated, and and effervescent preparations are all available but require separate ingestion of the thiazide and potassium salt. Many internists have emphasized the importance of prescribing potassium for patients on long-term thiazide therapy and the desirability of a combination tablet that would obviate the dangers of patient error. In previous papers we have suggested that such a tablet might be in a slow-release form and contain a less toxic potassium salt.

Preliminary studies done in the research laboratories of the Ciba Pharmaceutical Company indicated that three of over 20 potassium salts tested were less apt to produce intestinal lesions (oral communication, Dec, 1965). This impression was based upon results obtained from a smaller number of experiments in monkeys who received the salts orally. In order to evaluate the effects of two of these potassium salts, studies were undertaken with the same experimental model used in our original study of enteric-coated potassium chloride. This preparation was designed to simulate the extreme situation wherein a tablet would be entirely dissolved and its contents absorbed over a short length of intestine.

The effects of potassium gluconate in simple



Specimen from dog. Tablet of potassium chloride containing 13.5 mEq of potassium had been affixed at *A* and tablets of potassium citrate containing 13.5 mEq of potassium had been affixed at *B, C, D,* and *E*. Typical circumferential infarction is seen at site of chloride salt while little or no reaction is present at citrate sites.

tablet form and potassium citrate in combination with hydrochlorothiazide in slow-release form were studied. Tablets of each drug were fixed within the ileum or distal jejunum of mongrel dogs so that dissolution and absorption would occur within a limited segment of the intestine. As many as six implantations were made in a single animal with variations in dosage. The animals were killed five to seven days postoperatively, except where clinical evidence of perforation dictated earlier necropsy. Microscopic examinations were done on all tissue within 4 cm of a tablet site, whether or not gross changes were apparent. In several animals, potassium chloride and sodium chloride tablets were implanted as controls.

Technique

With the dog anesthetized with pentobarbital, the abdomen was opened, and a segment of distal jejunum or ileum isolated. Holes were drilled through the center of the tablets to be implanted and these were strung, singly or in pairs, like beads, on sutures. These sutures were then passed through an enterotomy to the desired site and brought out through the bowel wall. The tablets were thus guided into position and loosely fixed in place by tying the suture. The selected number of tablets were then placed at sites above and below the enterotomy at least 10 cm apart. The enterotomy and abdomen were then closed.

Results

The findings at autopsy are shown in the Table. No remarkable gross changes were seen at any of the sites where potassium gluconate or potassium citrate were implanted. Minimal superficial hemorrhage was seen at two sites where four tablets of citrate (the potassium equivalent of 2 gm of potassium chloride) were affixed, at two sites where six tablets and one site where three tablets of gluconate (the potassium equivalent of 2.25 gm and 1.1 gm of potassium chloride), and at both sites where 2 gm of sodium chloride had been implanted. These changes were very mild and in no way resembled the annular circumferential changes seen with potassium chloride. A mechanical factor due to the bulk of the pills implanted at these sites may explain the minimal findings. In five of the six dogs in whom potassium chloride was implanted as a control, gross circumferential infarction was present at the site of this salt while all other sites of gluconate or citrate implantation were normal (Figure).

Histologic Findings

The histologic changes observed following the application of potassium citrate, potassium gluconate, and sodium chloride correspond to the mildest or initial changes produced by potassium chloride. The changes are confined to the mucosa and inner layer of the muscularis mucosa. They consist of (1) engorgement of the capillaries of the villi, in places as telangiectatic pools; (2) accumulation

of polymorphonuclear leukocytes in the lumens of mucosal capillaries with some spillover into the adjacent stroma and lumens of glands; (3) a concentration of leukocytes at the junction of the mucosa and muscularis mucosa, and (4) a sharply demarcated zone of fibrinoid necrosis of the innermost layer of the muscularis mucosa. In one site of potassium gluconate implantation, a small mucosal erosion developed.

Comment

These studies indicate the apparent safety of the use of potassium citrate and gluconate tablets contrasted with the intestinal lesions that develop following treatment with potassium chloride. Previous experience as well as controls in the present study demonstrate that potassium chloride produces circumferential lesions where the two organic salts do not. These differences are not unexpected since the underlying etiology is the rapid absorption of high concentrations of potassium over a short length of intestine and there is reason to believe that various potassium salts may differ in their rates of absorption. All three potassium salts used in this investigation are ionized almost immediately and totally, but because of the Donnan effect, absorption of a cation across the intestinal wall is at least partially controlled by the rate of absorption of the respective anion. Absorption of polyvalent ions and those of large molecular weight is known to be slower than that of monovalent and small molecular ions.[8] The citrate anion is both large and trivalent and the gluconate ion though monovalent is also a large molecule. These organic anions thus would be more slowly absorbed and would hold potassium within the intestinal lumen diminishing the concentration of potassium in the intestinal wall veins at any one time.

The histologic changes noted though minimal suggest that any high concentration of potassium ions is not completely without physiologic and anatomic effect on the bowel. The marked difference between these findings and those seen with potassium chloride probably is a reflection of the greater concentration of free potassium ion absorbed.

The experimental model used created a situation which will never occur clinically except by error, ie, the arrest of four or six tablets and the simultaneous release of their contents at one point in the intestinal tract. Our failure to produce the typical circumferential lesion attests to the relative safety of the citrate and gluconate salts.

The potassium citrate-hydrochlorothiazide preparation tested in this study provides an additional factor militating against rapid release of potassium over a short segment of intestine. Although uncoated, it is designed to release the medication slowly beginning in the stomach. If so effective, this would further preclude development of intestinal lesions.

Based upon these experiments, potassium citrate and gluconate salts, especially in slow-release-form tablets appear safe for oral administration. No attempt has been made in this study to establish the efficacy of these salts in either maintaining or elevating serum potassium levels.

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Generic and Trade Names of Drug

Hydrochlorothiazide—*Esidrix, Hydrodiuril, Oretic, Hydril, Aquarius.*

References

1. Lindholmer, B.; Nyman, E.; and Raf, L.: Nonspecific Stenosing Ulceration of the Small Bowel, *Acta Chir Scand* 128:310-311 (Sept) 1964.
2. Baker, D.R.; Schrader, W.H.; and Hitchcock, C.R.: Small Bowel Ulceration Apparently Associated With Thiazides and Potassium Therapy, *JAMA* 190:586-590 (Nov 16) 1964.
3. Morgenstern, L.; Freilich, M.S.; and Panish, J.F.: Circumferential Small-Bowel Ulcers: Clinical Aspects in 17 Patients, *JAMA* 191:637-640 (Feb 22) 1965.
4. Boley, S.J., et al: Experimental Evaluation of Thiazides and Potassium as a Cause of Small Bowel Ulcers, *JAMA* 192:763-768 (May 13) 1965.
5. Boley, S.J., et al: Potassium-Induced Lesions of the Small Bowel: I. Clinical Aspects, *JAMA* 193:977-1000 (Sept 20) 1965.
6. Schwartz, S., et al: Some Aspects of Vascular Disease of the Small Intestine, *Radiology* 84:616-623 (April) 1965.
7. Allen, A.C., et al: Potassium-Induced Lesions of the Small Bowel: II. Pathology and Pathogenesis, *JAMA* 193:1001-1006 (Sept 20) 1965.
8. Wilson, T.H.: *Intestinal Absorption*, Philadelphia: W. B. Saunders Co., 1962.

THE ROLE OF FETAL MEMBRANES IN THE EXCHANGE OF SODIUM IN AMNIOTIC FLUID

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It has recently been established that amniotic fluid is not a static accumulation of liquid but is in active circulation resulting in its renewal in a relatively short period of time. In guinea pigs the entire amniotic fluid is completely replaced within 1 hour and in human beings in slightly less than 3 hours.

These investigations have stimulated interest in the rate of exchange of amniotic fluid. The question of where this exchange actually takes place, however, still remains unsettled. Although the debate concerning the origin and the destination of the amniotic fluid started as early as the fourth century B.C., a clear understanding of this process is not yet available. This lack of information concerning the formation and disposal of the amniotic fluid has repeatedly been emphasized since the eighteenth century.

The potential applications of knowledge concerning the circulation of amniotic fluid with regard to fetal nutrition and homeostasis stimulated the investigations reported here.

Our working hypothesis is as follows: If a tracer substance is injected into the amniotic fluid, it is logical that early localization and high concentrations may occur in the tissues responsible for its transfer.

In order to test the validity of this hypothesis, we first conducted experiments in 18 pregnant rabbits. Encouraged by the results obtained, we carried out similar experiments on pregnant ewes, and results are reported herein.

From the Research Institute of the Presbyterian Hospital, Philadelphia. 118: 557-
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METHODS

Eight pregnant ewes in the last month of confinement were anesthetized by intravenous administration of nembutal, 25 milligrams per kilogram of body weight, and atropine, 0.25 milligram. After tracheal intubation and under sterile conditions, the abdomen was opened and the uterus exposed. At a relatively avascular area and away from the palpable cotyledons, a transverse incision was made on the uterus, and its layers were individually dissected and incised. When the uterine cavity was reached and the capsular decidua seen, 25 to 40 microcuries of radiosodium chloride were injected into the amniotic cavity and mixed with the amniotic fluid by repeated aspirations and reinjections with a 50 cubic centimeter syringe.

Arrival and concentration of the radionuclide in the maternal blood stream were continuously monitored by means of a scintillation detector pointing at the maternal heart. Because the sensitivity of this method was found insufficient, the majority of the experiments were monitored by a small tubing and pump arrangement which provided a continuous blood flow of about 10 milliliters per minute from the maternal artery into a well counter and back to the femoral vein. In most cases a detectable concentration of the radioisotope in maternal blood was reached between 4½ and 6¼ minutes. In 1 instance, however, no significant rise in the count rate was ob-

TABLE I.—CONCENTRATIONS OF TRACER

	Experiment No.								
	2	3	4	5	6	7	8	A.M.	Per cent
Fetal membranes	3,490	2,335	8,386	970	3,169	160	13,453	4,566	100
Umbilical cord	1,759	386	8,316	224	1,990	95	3,598	2,110	46
Cotyledon	371	586	114	68	—	0	980	353	7.70
Skin	1,368	30	337	179	45	71	—	338	7.40
Fetal blood	—	35	1,049	82	—	32	165	272	6
Muscle	0	36	308	91	350	68	636	213	4.60
Lung	0	27	154	0	16	11	99	44	0.96
Liver	0	31	51	40	21	0	161	43.3	0.95
Stomach wall	—	16	0	39	4	—	147	41.2	0.90
Stomach contents	0	—	0	8	—	0	—	2	0.04
Small intestine	105	—	0	25	47	40	147	60.7	1.30
Small intestinal contents	43	16	0	—	—	—	—	19.7	0.40
Large intestinal contents	115	11	—	101	36	36	27	58	1.30
Spleen	86	0	0	64	0	0	86	34	0.74
Kidney	—	0	0	93	26	53	240	68.7	1.50
Bladder	—	183	—	29	13	0	208	86.6	1.90
Urine	0	0	—	16	—	0	28	8.8	0.20
Heart	—	144	0	36	4	18	77	46.5	1.20
Brain	47	74	0	10	—	30	2	27.2	0.60
Tongue	7	—	28	—	—	—	—	17.5	0.40
Esophagus	0	241	104	126	126	—	33	105	2.30
Eye	—	22	0	0	—	—	—	7.3	0.16
Amniotic fluid	34,285	32,282	—	20,789	—	41,037	26,149	30,908	677
Maternal uterus	—	499	65	2,157	72	499	0	549	12

Columns 1 to 7 represent actual counts per gram of tissue obtained from experiments 2 to 8. The eighth column represents arithmetic means of samples from each tissue of these 7 experiments. The ninth column is obtained from the eighth by considering the highest concentration of isotope as 100 and calculating the concentrations of other tissues as its percentage.

served over the background, and repeated injections of the radionuclide had to be made; 45 minutes passed before the next step was taken. When a sufficient concentration in the maternal blood was attained, the uterine vessels were suddenly clamped and the uterus was removed en bloc. Hysterectomy was usually completed within approximately 10 to 15 minutes.

Upon death of the fetus in anoxia, samples of approximately 1 gram were taken from all organs. With careful handling to prevent contamination, they were washed in running water, blotted, and weighed to an accuracy of ±2 milligrams. They were then immersed in 5 milliliters of concentrated nitric acid and allowed to dissolve completely. This homogenous solution was counted in a scintillation well system, and the results were corrected for weight and dilution. Liquid samples, blood and urine were measured accurately, and net counts per milliliter were determined.

Maternal blood and tissue samples were taken from the rectus abdominis muscle and the uterine wall and were identically treated.

The resulting values were considered proportional to the concentration of sodium-22 in that particular organ from which the samples were taken.

RESULTS

Fetal membranes revealed by far the highest concentration of the tracer (Table I). Significantly high concentrations were also found in the umbilical cord. The cotyledon, fetal skin, and blood contained more sodium-22 than the rest of the organs, yet less than 8 per cent of that found in fetal membranes. The lungs, stomach, and intestinal wall, which are traditionally believed to be responsible for exchange of amniotic contents, revealed virtually the same amount of the radiotracer as the other viscera, in most instances less than 1 per cent. Fluid samples obtained from the stomach and small intestine showed even lower concentrations.

The variations in the fetal mass and blood volume, quantity of the amniotic fluid, dose of sodium-22, time allowed for transfer, and distance between injection and sampling sites appear to contribute to the rather wide scatter of count rates from 1 experiment to

another. Despite these differences the sequence of comparative tissue concentrations in each instance remained the same. A simple arithmetic mean from which percentages were drawn confirms this conclusion (Table I).

One experiment, because of a 45 minute time lapse following injection, is presented separately in Table II. As can be seen, here also the highest concentration of the tracer occurred in the fetal membranes. The amniotic fluid, fetal blood, cotyledon, kidney, umbilical cord, and skin followed in respective order. Even after this interval each organ contained only a minute amount of sodium-22 compared with that of the amnion. Again, it is interesting to note that the lung, the walls of the stomach, and the small and large intestines revealed only a fraction of the amount found in the fetal membranes.

TABLE II.—CONCENTRATION OF TRACER IN 1 EXPERIMENT

		Per cent
Fetal membranes	164,630	100.00
Amniotic fluid	15,100	9.17
Fetal blood	8,450	5.13
Cotyledon	6,910	4.19
Kidney	5,300	3.27
Umbilical cord	4,533	2.75
Skin	3,764	2.29
Liver	3,500	2.13
Bladder	3,330	2.02
Lung	3,304	2.01
Small intestine	3,280	1.99
Heart	3,200	1.94
Tongue	2,722	1.65
Cord vessels	2,256	1.37
Stomach	2,219	1.35
Diaphragm	2,170	1.33
Spleen	2,120	1.29
Abdominis rectus muscle	2,028	1.23
Muscle	1,457	0.89
Urine	1,304	0.79
Large intestine	780	0.47
Brain	492	0.30
Cord with vessels	417	0.25
Small intestine contents	259	0.16
Stomach contents	230	0.14
Maternal blood	540	0.33
Maternal uterus	3,305	2.010

The first column represents the actual counts per gram of tissue. The figures in the second column are obtained by considering the highest concentration as 100 and expressing the others as its percentage.

DISCUSSION

Influenced by generally accepted theories concerning the site of exchange of amniotic fluid, we thought that the fetal lungs, and alimentary and urinary tracts would most likely reveal the highest concentration of the radiotracer.

On the basis of recovery of lanugo hair from the stomach and intestines, as shown by Jeffcoate and Scott, it is accepted that fetal deglutition occurs. Yet its regularity, rate, and functional significance have never been fully evaluated. In experiments by Setnikar in which India ink was injected into the amniotic cavity it was recovered only irregularly in the digestive tract. From this and other considerations it can be concluded that the swallowing process cannot entirely explain the circulation rate of the amniotic fluid. In studies by Windle and associates in which a contrast medium was injected into the amniotic cavity and frequent roentgenograms were taken, the earliest sign of radiopacity in the fetal stomach was observed in about 60 minutes. In our experiments the time allowed from the injection of the tracer to sampling was approximately 20 minutes. In only 1 instance was it extended to 45 minutes. The low concentration of sodium-22 in the alimentary tract would indicate that the fetus had not swallowed during this period of time. The fetal and maternal tissues, however, had attained a significant concentration of the tracer. It is obvious, then, that the fetal gastrointestinal system did not play a major part in disposal of sodium-22 existing in the amniotic fluid. Whether this would also be the case had the observation period been extended into several hours is a subject for future study.

The role of fetal lungs in absorption or formation of amniotic fluid has been emphasized in the past. Fetal movements reminiscent of respiratory activity have been noted early in fetal life. Detailed descriptions of these movements leave no doubt of their occurrence; however, it is certain that in some instances these do not start unless maternal and therefore fetal anoxia is produced.

Simple observations made by Snyder and

Rosenfeld of the abdomen of the pregnant woman and cinematography have shown that cyclic somatic movements occur in the fetus. The respiratory origin of these movements is an indirect conclusion rather than a proved fact, as indicated by Windle. This statement is well supported by roentgenographic studies of thorotrast injected in front of the muzzle of the fetus in utero. These investigators were able to recover it in the lungs only when maternal asphyxia was produced. Morphologic studies made by Whitehead and associates prove that if the fetus is provided with well oxygenated blood the lungs remain completely "atelectatic" as shown by Potter and Bohlender. The alveoli are collapsed, and their epithelium and that of the bronchioles is cylindric. If fetal anoxia is produced, however, the fetus makes many somatic motions, some of which appear to be of respiratory origin, and amniotic contents are found in its lungs. Atelectasis is no longer complete and the epithelium is flattened. Studies made by Steele and Windle of fetal blood gases indicate that approximately 50 per cent saturation correlates with rhythmic chest motions, but at levels below 36 per cent the respiratory movements are completely absent. In our studies, when fetal anoxia was the cause of fetal death, only extremely small amounts of tracer were found in the lung parenchyma. This finding would indicate that the respiratory system did not contribute to transfer of sodium.

The role of the kidneys in formation of amniotic fluid is documented and well accepted. In one of our experiments, when sufficient time was allowed, the kidneys acquired the highest concentration of radionuclide among fetal viscera (Table II).

Oligohydramnios frequently occurs in cases of congenital bilateral renal agenesis. Although a contributive factor, the renal function of the fetus is not considered adequate to explain the large volume turnover as calculated by several workers.

Amnion has frequently been considered instrumental in production and removal of amniotic fluid. Histologic studies carried out by Danforth and Hull have shown cytoplasmic vacuoles in amnionic epithelial cells to which a secretory function was attributed. Recently, with an electron microscope, channels between microvilli have been observed interconnecting the vacuoles and leading to the interstitial space of the amniotic membrane. It has been proposed that these channels are operative in disposal of amniotic fluid. The demonstration of colloidal gold and trypan blue in the connective tissue of amnion after injection of the same into the amniotic cavity is in favor of this viewpoint.

There is further evidence that fetus and specifically the fetal membranes are responsible for exchange of fluid and at least some antibodies. The latter studies, when considered in the light of our results, suggest that the contribution of membranes to circulation of amniotic fluid constituents may be even more important than is apparent at present.

It is interesting to note that a rather high concentration of sodium-22 was found in the skin, even higher than in the fetal blood (Table I). This finding may indicate that some sodium is taken up by the fetus via the skin even as late in pregnancy as the last few weeks. In vitro studies of Grignon and Lamarche and our findings in rabbit fetus seem to support this function of skin which has been repeatedly mentioned as a possibility in the literature.

SUMMARY

In pregnant ewes radiosodium chloride was injected into the amniotic fluid. Tissue samples were taken after detection of the radioisotope in the maternal blood. The highest concentration occurred in fetal membranes, followed by umbilical cord, cotyledon, and skin. Fetal lungs and gastrointestinal system contained no more of the tracer than the other viscera.

These results indicate that fetal membranes are probably the most important site of exchange of sodium and that absorp-

tion from the lungs and gastrointestinal system apparently does not play an important role in transfer of sodium.

REFERENCES

1. Danforth, D. N., and Hull, R. W. The microscopic anatomy of the fetal membranes with particular reference to the detailed structure of the amnion. Am. J. Obst. Gyn., 1958, 75: 536.
2. Grignon, O. and Lamarche, M. Etude histologique du pouvoir de penetration transcutanee du liquide amniotique. C. rend. Soc. biol., 1959, 153: 680.
3. Jeffcoate, T. N. A., and Scott, J. S. Polyhydramnios and oligohydramnios. Canad. M. Ass. J., 1959, 80: 77.
4. Potter, E. L., and Bohlender, G. P. Intrauterine respiration in relation to development of the fetal lung. Am. J. Obst. Gyn., 1941, 42: 14.
5. Seinikar, I. Ingetion of fetal amniotic fluid of guinea pigs. Boll. Soc. ital. biol. sper., 1959, 35: 2029.
6. Snyder, F. F., and Rosenfeld, M. Intrauterine respiratory movements of the human fetus. J. Am. M. Ass., 1937, 108: 1946.
7. Steele, A. G., and Windle, W. F. A study of some correlations between respiratory movements and blood gases in cat fetuses. J. Physiol., Lond., 1939, 94: 531.
8. Whitehead, W. H., Windle, W. F., and Becker, R. F. Changes in the lung structure during aspiration of amniotic fluid and during air breathing at birth. Anat. Rec., 1942, 83: 255.
9. Windle, W. F. Physiology and anatomy of the respiratory system in the fetus and newborn infant. J. Pediat., S. Louis, 1941, 19: 437.
10. Windle, W. F., Becker, R. F., Barth, E. E., and Schulz, M.D. Aspiration of amniotic fluid by the fetus; experimental roentgenographical study in guinea pig. Surg. Gyn. Obst., 1939, 69: 705.

Sodium Chloride Intoxication in Newborn Infants

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A SERIOUS *epidemic* occurring in newborn infants in the Maternity Ward at Rikshospitalet, Oslo, was first assumed to be due to a pneumonic infection. However, it was discovered later that the content of sodium chloride of the formula they were receiving had accidently been increased markedly.

EPIDEMIOLOGIC CONDITIONS

In the course of one week, 18 of the 71 infants in the Maternity Ward became seriously ill. In 17 of the 18 the pulmonary symptoms were dominant, and one infant had convulsions.

At first this epidemic was thought to have an infectious origin, and numerous bacteriologic examinations were carried out. A variety of bacteria were cultured without throwing any light on the etiology of the epidemic. Virologic examinations gave negative results.

By chance one of the sick infants was included in a control group of normal newborn infants being followed by one of the authors (Knutrud) in connection with a study on postoperative metabolic problems in the neonatal period. All urine, meconium and feces excreted by this infant during the first six days of life had been collected and analyzed, and all intake had been recorded weighed, and analyzed. Up to and including the fifth day of life the infant had received mother's milk, increasing from 170 ml. to 280 ml. daily. On the sixth day a mixture of equal parts of mother's milk and half milk (equal parts of cow's milk and water with 7% of sugar) was given. It is apparent from the balance diagrams for sodium and chloride that something unusual occurred on the sixth day of life. The intake of sodium and chloride rose to 30 mEq. and 34 mEq., respectively; or six times as much as on the previous day. Most of this sodium chloride was retained, as excretion remained practically unchanged. Of 330 ml. fluid administered, 250 ml. was retained. On the eighth day of life an increase in weight of 320 Gm. was observed. The infant was taken ill on the sixth day with pulmonary symptoms and later transferred to the Pediatric Department. At this point the balance study was discontinued.

Unfortunately these striking findings were not discovered until two weeks later when the results of the balance studies were analyzed. An investigation of the circumstances was begun immediately. This soon revealed that the formula preparation department had re-

TABLE 1. *Type of Milk Given the Infants*

	Number of Infants	Number of Sick Infants
I. Mother's milk exclusively	23	0
II. Formula exclusively	4	4
III. Mother's milk formula	42	12
IV. Uncertain*	2	2

* One probably belongs in group II and the other in group III.

cently received a bag of sugar the content of which, according to the nursing staff, was mixed with salt. This bag of sugar, which had been returned to the hospital store and kept there, was now analyzed. The sodium chloride admixture ranged from 2 to 10 per cent in different parts of the bag.

The sugar supply from the hospital store to the formula department typically is enough for one week's consumption—which corresponds to the time the *epidemic* lasted. The formulas for the infants are made up and distributed daily to all infants receiving cow's milk mixtures.

Of the 71 infants staying in the Maternity Department during this week, 18 became ill. No other similar cases appeared at other times. The mothers and staff developed no similar symptoms or indications of other illnesses.

The department has three separate nurseries and all of them had outbreaks of the disease, occurring in each on approximately the same day. No instance of illness occurred in the 23 infants who were fed mother's milk exclusively.

The infants' age at the onset varied somewhat, but 15 of them were from two to six days old. Due to poor maternal health, two of the infants had stayed on in the hospital and their age at the outbreak was 28 days and 58 days respectively. These two infants were on 500 ml. half strength formula and 650 ml. two-thirds strength formula respectively; they became ill after only one day's feeding.

The records indicated that 16 of the 18 sick infants had been given formula prior to the occurrence of symptoms (Table 1), and in one other case this seems probable. The other infant, a Cesarean section baby with a birth weight of 4,900 Gm., became feverish and agitated at two days of age before having been fed formula. However, during the first three days of life this infant had been given a mixture of equal parts of physiologic saline solution and 5 per cent glucose, rising to 260 ml. on the third day. This intake corresponds to an approximate sodium and chloride content of 30 mEq., most of which was given on the third day when 20 mEq. sodium chloride was administered. This will correspond to 128 ml. of physiological saline solution. The increase in weight from the third to the fifth day of life was 120 Gm. Pulmonary symptoms with dyspnea developed on the fourth day of life. After the infant was taken ill it was fed cow's milk mixture, the daily ration being about 25 mEq. sodium in 310 ml. fluid.

COURSE OF ILLNESS

Most of the infants had a fairly similar picture, with acute onset of respiratory symptoms. Many became critically ill, in particular during the first hours, and were transferred to the Pediatric Wards. Their recovery after the transfer was strikingly rapid. The more prominent symptoms disappeared in 24 hours, and by five days most of the infants were clinically well.

Dyspnea with rapid, shallow, sometimes gurgling respirations was the predominant symptom in 14 cases. None had grunting respirations or marked respiratory retractions of the thorax. *Cyanosis* occurred in ten patients, partly intermittent, partly continuously through longer periods. *Abnormal physical findings over the lungs* were present in 14 cases: weak breath sounds, blowing, crepitation or rales. *Fever* to above 37.5 C. was present in 15 infants, in 10 of whom it was more than 38.0 C. The *liver was palpable* in six cases, but in only three was enlargement pronounced. *Pitting edema* was demonstrable in one patient, a mongoloid with congenital heart disease. *Vomiting* was prominent in six and two of these had hematemesis.

Four infants showed signs of *cerebral irritation.* Two were markedly agitated and irritable, and one presented transient opisthotonos. One infant had repeated convulsive spells on the sixth day of life and lumbar puncture revealed bloody spinal fluid. There was nothing to indicate serious birth trauma and the usual 1 mg. dose of vitamin K had been administered after delivery. When he was discharged from hospital at 18 days of age the neurologic examination revealed no abnormalities.

Leukocytosis exceeding 15,000 was found in five cases, and seven infants had a moderate shift to the left in the differential count. *Radiography of the lungs,* carried out in 13 infants revealed infiltrations in 11: bilateral in six, unilateral in five.

COMMENTS

Pneumonia epidemics in fullborn infants are very rare. No etiologic evidence was uncovered by ordinary bacteriologic examinations in these patients. The abortive course of illness in many of the infants is another factor which makes bacterial pneumonia seem unlikely. Epidemics of virus pneumonias in newborn infants have been described by Adams[1] and Kuroya and Istrida.[6] However, in our patients the virologic examinations were negative too.

By chance, it was discovered that one of the sick infants had been given formula with a markedly increased salt content. Later, it was further discovered that sugar mixed with salt had been sent from the hospital store just prior to the outbreak of the epidemic. No new case was recorded after the milk kitchen received a new supply of sugar. No case of illness occurred in the infants who had only been given mother's milk. As a consequence, it seems clear enough that the *epidemic* was caused by sodium chloride intoxication.

It has been demonstrated that in the first week of life infants need several days to rid themselves of a sodium and chloride load.[5] This also applies when a normal amount of 2–5 mEq. per day is given. From the sixth to the eighth day of life, the sodium excretion in the urine will markedly increase, partly with a negative balance despite continued normal administration.[5] McCance and Widdowson[2] have examined the effect of administration of milk containing 0.7 per cent of sodium chloride in three premature infants. A marked weight gain occurred in all cases and the infants immediately fell ill with edemas. As soon as they were put on ordinary milk again the weight decreased and the symptoms disappeared. The same ill effect has been observed as a result of parenteral administration of saline solution postoperatively.[5]

The report on salt intoxication in Binghamton General Hospital[3] dramatically shows the risk of increased administration of sodium chloride to newborn infants: The exact amount or concentration of salt in the formula of any specific infant could here not be determined as salt and sugar were apparently mixed together in the storage container. General irritability, twitching of the eyelids and the extremities were early symptoms. Later general convulsions were common, and respirations were labored, and rales were noted. The temperature was normal or slightly elevated, until pulmonary symptoms intervened, and then it was elevated.

The symptoms in our patients had many points in common with the above described picture. But in most of our patients the cerebral symptoms seem to have been less pronounced and the respiratory symptoms predominated. The reason for this difference may be the degree of intoxication. In Binghamton General Hospital six of the 14 infants died, whereas all our patients survived.

When the kidneys are unable to excrete a sodium load, 6.4 ml. water per mM. salt (or 110 ml. water per Gm. salt) is retained to sustain the osmolarity of the body fluids.[4] It therefore seems strange that edema was only demonstrated in one of the sick infants in the present material. Nor does edema seem to have been a prominent symptom in the sick infants in Binghamton. The most likely explanation seems to be the scanty fluid intake together with the tendency to vomiting.

Editors' Correction

The following changes should be made in the Special Report—The Use of Poliovaccines which appeared in the October 1963 issue of CLINICAL PEDIATRICS.

Page 554A, column two, line 7, should read ". . . which *compromises* complete acceptance by the whole population . . ."

Page 554B, Recommended Schedule, etc., should be:

2 months	DTP and Type 1 OPV	IPV
3 months	DTP and Type 3 OPV	IPV
4 months	DTP and Type 2 OPV	IPV

Also on 554B, column one, fourth line from the bottom, ". . . avoid *chance* of coincident wild paralytic poliomyelitis, . . ."

A conspicuous gain in weight at the onset of illness occurred in ten of the cases and this must be considered as an objective measurement of retained fluid in the form of pre-edema. In the analyzed case and in the infant given saline solution there was good agreement between calculated retained fluid and increase in weight. The respiratory symptoms were probably caused by hydremia, circulatory failure and pulmonary edema. Radiographic pulmonary changes tended to persist for several days, even after symptoms had subsided, and were probably due to secondary atelectasis.

REFERENCES

1. Adams, J. M.: Primary virus pneumonitis with cytoplasmic inclusion bodies: Study of epidemic involving thirty-two infants with nine deaths. J. A. M. A., **116**: 925, 1941.
2. McCance, R. A. and E. M. Widdowson: Mineral metabolism of the foetus and newborn. Brit. Med. Bull. **17**: 132, 1961.
3. Ford, J. H., Jr.: Personal comunication from Binghamton General Hospital, Binghamton, N. Y.
4. Josephson, B.: Chemistry and Therapy of Electrolyte Disorders. Springfield, Ill., Charles C Thomas, 1961.
5. Knutrud, O.: Unpublished material, 1962.
6. Kuroya, M. and N. Istrida: Viral infection of the foetus and of the premature and newborn infant. Advances of pediatrics **XII**: 249, 1962. Ref. Eichenwald, H. F. and H. R. Schinefield.
7. Kvalvik, K.: Sodium chloride intoxication. One case. Tidsskrift for Den norsk lægeforening **77**: 839, 1957.
8. Wilkinson, A. W.: Body Fluids in Surgery. Edinburgh and London, E. & S. Livingstone, Ltd., 1960.
9. Finberg, L.: Overt and cryptic salt poisoning: Recognition and prevention. Clin. Pediat. **2**: 105, 1963.

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Hyperkalemic Cardiac Arrest During Transfusion of Stored Blood

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10

SUMMARY

Cardiac arrest occurred in a boy with pulmonary lacerations during rapid transfusion of stored blood. EKG changes of hyperkalemia were evident which reverted partially after administration of glucose and insulin. The case points to the need for using fresh blood in rapid transfusions.

Stored bank blood undergoes many changes.[1] One of the well-known changes is the leakage of potassium ions from the red blood cells resulting in increased levels of plasma potassium. Pressure pumping may further traumatize red blood cells and add to the leakage of intracellular potassium into the plasma. When such blood is rapidly transfused to a recipient, significant hyperkalemia leading to cardiac arrhythmia may result. The case reported illustrates such an incident.

CASE REPORT

M.D., a 9-year-old boy was admitted to the Emergency Room at 6:30 p.m. after being struck by a taxicab. On admission he was lethargic and had labored respirations. His blood pressure was 100/60, pulse 152/min, and respiration 36/min. There were no gross neurological signs. There were abrasions about the face, forehead, lips, right lower abdomen, right ankle and thigh. There was an obvious fracture of the right femur. Breath sounds were diminished over the right lung field and there were a few rales over the left lower lung field posteriorly. X-rays confirmed a fracture of the right femur as well as fractures of the 6th through 9th ribs on the right side. A thoracotomy tube was inserted in the right lower thorax and by 7:30 p.m. 800 cc of bright red blood had been aspirated. Blood transfusions were immediately started with rapid replacement of blood volume by pressure pumping. As preparations for surgery were carried out, the patient was found to have no spontaneous respiration, pulse, or blood pressure. Cardiac resuscitation was instituted, continuous electrocardiographic monitoring was carried out and ventilation via an endotrachial tube was performed. Over the preceding hour, two units (1000 ml) of bank blood had been given rapidly and a third unit (500 ml) was gradually infused. No accessible veins were found for venipuncture to assess blood electrolytes. However, the electrocardiographic findings were suggestive of hyperkalemia. These will be discussed in detail below. For this reason, an infusion of 500 cc of 10% glucose in water with 15 units of regular insulin was also begun which resulted in improvement of the electrocardiogram and a spontaneous heartbeat resumed. After his condition had stabilized enough, he was taken to the operating room where a thoracotomy was performed which revealed multiple lacerations of the right lung and massive hemmorhage. There was no injury to the heart. Despite attempts to stop the bleeding, and continued direct massage, the patient expired in the operating room.

DISCUSSION

The three units of blood given in the Emergency Room were all over 12 days old and contained increased potassium levels as determined subsequently from the remaining plasma in the transfusion bottles. (Table 1) During the resuscitation efforts no venipunc-

Table 1

BLOOD GIVEN IN EMERGENCY ROOM

TAG #	AMOUNT	AGE (DAYS)	K+ (meq/1)
1852	500 ml	13	10.2
1859	500 ml	13	7.5
95464	500 ml	14	13.0

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ture was possible due to technical difficulties and unfortunately a direct determination of the patient's serum potassium was not obtained. Electrocardiographic strips obtained at approximately 5-min intervals during resuscitation (Fig. 1) showed tall, peaked T waves initially. In the subsequent strips B-F there is AV dissociation with a slow junctional rhythm and again tall, peaked T waves with a short QT interval. The progressive elevation of ST segments simulated a myocardial injury pattern. Strips G-L, obtained after the start of the infusion of glucose and insulin, show a gradual return of the elevated ST segment towards the iso-electric line and improvement of the QRS complexes indicating a decreasing hyperkalemic effect on the heart. In addition, atrial fibrillation with AV dissociation and a junctional rhythm appeared. In strip L the tall, peaked T waves and the short QT interval indicative of hyperkalemia are still quite obvious.

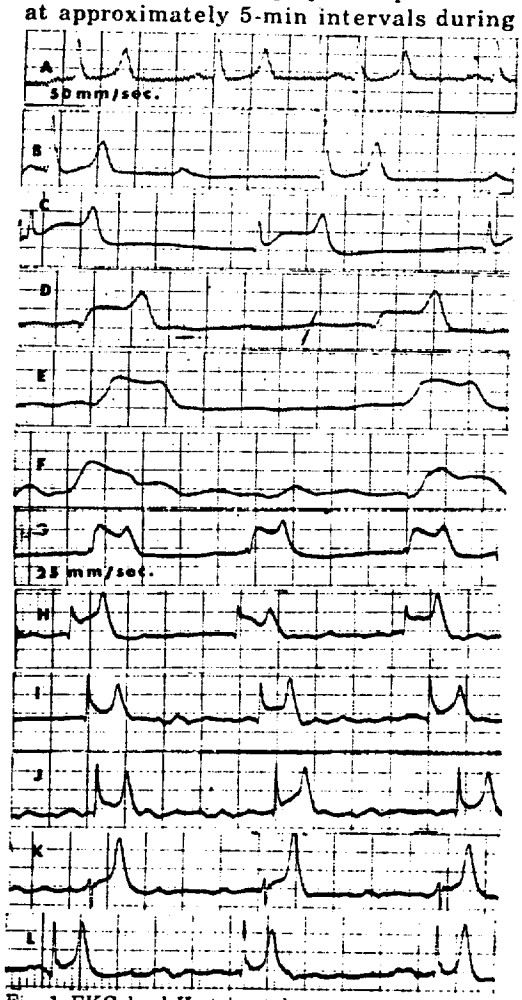

Fig. 1. EKG lead II strips taken at approximately 5-min intervals. Strips A-F were taken at 50 mm/sec, strips G-L at 25 mm/sec. Strip A shows sinus rhythm at 80/min with somewhat peaked T waves. Strip B reveals AV dissociation with a junctional pacemaker at a rate of 42/min and greater peaking of the T waves. In strip C the ventricular rate is slightly faster at 50/min, P waves are absent, probably due to atrial standstill. Strips D-F show further deformity of the QRS complexes and elevation of the ST segment leading to a monophasic complex. Strip G shows some improvement of the QRS complex with emergence of a T wave. The rate is 36/min. Strips H-L show persistence of a slow ventricular rhythm at approximately 30-32/min with atrial fibrillation and AV dissociation. The QRS pattern reverted to normal; however, the peaked, tall T waves indicate persistent hyperkalemia. The regression of these changes occurred during the administration of glucose and insulin.

The electrocardiographic changes seen in hyperkalemia have been well documented.[2-4] Initial changes are reflected by tall, peaked T waves, associated with shortening of the QT interval. Further increase of potassium concentrate results in QRS prolongation. At levels greater than 7 meq/1, the P amplitude decreases and the PR interval lengthens due to slowing of atrial conduction. At about 8.8 meq/1 and above, the P wave is invisible and the pacemaker may be displaced from the SA node to the AV junction.[2] In advanced hyperkalemia, the ST segment may be elevated and resemble an acute injury pattern.[5]

Hyperkalemia may be a cause of death in exchange transfusions in hemolytic disease of the newborn.[6,7] Campbell found that one-week-old blood used in exchange transfusions of infants with hemolytic disease had potassium levels of 6.5-8 meq/1. Although he felt that hyperkalemia might have been the cause of death in some infants, there was no direct relationship between the stored blood potassium level and the potassium level in the child's serum after transfusion.[7] Calcium injections may cause increased tolerance to hyperkalemia.[6] Conversely, hypocalcemia due to the citrate in bank blood may increase the sensitivity to hyperkalemia. Unfortunately, calcium was not administered during the attempted resuscitation of our patient.

We believe this case illustrates the hazards of rapid administration of large volumes of stored bank blood. Hyperkalemia was at least in part responsible for the arrhythmia and cardiac arrest observed in this child. In a situation where rapid replacement of large amounts of blood becomes necessary, fresh blood should be used.

REFERENCES

1. Mollison, P.L.: Blood Transfusion in Clinical Medicine. 4th ed. F. A. Davis Co., Philadelphia, 1967, pp. 28-31.
2. Surawicz, B.: Relationship between electrocardiogram and electrolytes. Am. Heart J. 73: 814, 1967.
3. Braun, H. A., Surawicz, B., and Bellet, S.: T Waves in hyperpotassemia. Am. J. Med. Sc. 230: 147, 1955.
4. Herndon, R. F., Meroney, W. H., and Pearson, C. M.: The Electrocardiographic effects of alterations in concentration of plasma chemicals. Am. Heart J. 50: 188, 1955.
5. Levine, H. D., Wanzer, S. H., and Merrill, J. P.: Dialyzable currents of injury in potassium intoxication resembling acute myocardial infarction or pericarditis. Circulation 13: 29, 1956.
6. Taylor, W. C., Gillis, C. N., Nash, C. W., et al: Experimental observation on cardiac arrhythmias during exchange transfusion of rabbits. J. Pediat. 58: 470, 1961.
7. Campbell, W. A. B.: Potassium levels in exchange transfusion. Arch. Dis. Child. 30: 513, 1955.

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THE CHRONIC ORAL TOXICITY OF SODIUM CHLORIDE AT THE RANGE OF THE $LD_{50(0.1L)}$[1]

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Abstract

The chronic toxicity of sodium chloride was studied in young male albino rats given 2.57–6.14 g/kg in water by stomach tube once daily for 100 days or until half the animals had died, whichever occurred first. The $LD_{50(0.1L)}$, or daily dose which killed 50% of the animals after administration for 100 days, i.e. 1/10 the animal's normal lifespan (0.1L), was 2.69 ± 0.12 g/kg. It is suggested that the $LD_{50(0.1L)}$ expressed as a percentage of the acute LD_{50} would provide a useful index of chronic toxicity; this is termed the C/A $LD_{50(0.1L)}$ index, C meaning chronic and A acute. The value of this index was 72 for sodium chloride, 62 for benzylpenicillin, and 13 for atropine. Rats which survived doses of the order of the $LD_{50(0.1L)}$ had no change in food intake but lost some body weight as daily dose increased. They had a dose-dependent polydipsia and polyuria. In the initial month of drug administration the rats developed a slight fever, proteinuria, and alkalinuria, and in the terminal month a slight hypothermia and aciduria, all of which were statistically significant but not dose-dependent. When deaths occurred within the first week or two, they were similar clinically and pathologically to those seen in studies on the acute oral toxicity of sodium chloride. Other deaths followed a period of hypothermic cachexia and were due to bronchopneumonia, associated with hepatitis, nephritis, arteriolitis, and occasionally encephalopathy, and accompanied by degeneration of the thymus, adrenals, and testes. Animals which survived for 100 days had developed a hypertrophied gastrointestinal mucosa but most other organs had lost weight, and there was some arteriolitis, myocarditis, pulmonary edema, and nephritis.

Introduction

Under section C.08.002-2-g of Division 8 of the Canadian Food and Drug Regulations (1), a new drug submission must contain evidence of safety under conditions of proposed use. Evidence of safety includes studies in animals of the "subacute" or "short-term" and "chronic" or "long-term" toxicity of the new drug. The basic information which such studies should yield is "how much can be given daily for how long without producing what toxic effects" (2). Three fundamental decisions have to be made in planning these costly and time-consuming studies, namely, (*a*) what daily doses should be selected, (*b*) over how long a period should dosage be maintained, and (*c*) what measurements and observations should be made at what intervals (2)?

The limited information to be obtained from standard studies in which two or three fractions of the LD_{50}, or multiples of the probable usual daily therapeutic dose, are given daily has been recognized in this laboratory from work on the chronic toxicity of spiramycin (3), atropine (4), and penicillin (5, 6). It appeared possible that a more useful index of chronic toxicity could be obtained. The higher the fraction of the LD_{50} which kills 50% of animals after a given number of days of daily administration the higher could be considered the

[1]This project was supported by a grant from the Medical Research Council of Canada.

weight per 24 hours, water consumption in milliliters per kilogram per 24 hours, colonic temperature in degrees Fahrenheit, urinary volume in milliliters per kilogram per 24 hours, urinary glucose in milligrams per kilogram per 24 hours, urinary protein in milligrams per kilogram per 24 hours, urinary pH, and other clinical signs as they appeared, quantitated as 1+ to 4+. Premortal signs were recorded in detail when this was possible. Colonic temperature was recorded by a Thermistemp Tele-Thermometer with the probe inserted 8 to 10 cm into the rectum. Urinary glucose, protein, and pH were measured with Ames Combistix Reagent strips.

Survivors were killed with chloroform at 100 days or after 50% of the animals had died, whichever occurred first. Autopsy was performed immediately, to avoid postmortem shifts in fresh weight and water levels. Gross and microscopic appearance, fresh wet weight, and water levels were recorded on the organs listed in Table III. Histopathologic examinations were made upon blocks of tissue fixed in Lillie's buffered formalin and stained with hematoxylin–phloxine–saffron. The sample of skeletal muscle used was the left half of the ventral abdominal wall muscles. The contents of the lumen of the gastrointestinal organs were removed by a standardized washing and milking procedure before the organs were weighed. Water levels were measured by drying a weighed aliquot of fresh tissue to constant weight at 95 °C in a Fisher Forced-Draft Isotemp oven. The sample of skin for water analysis was from the dorsolumbar region. Residual carcass was cut into small pieces and homogenized in a Waring blendor, and an aliquot was selected for measurement of water level. Water levels were calculated as grams water per 100 g dry weight.

The results were analyzed by the t test applied to differences between means in the drug-treated and control groups. These differences were plotted against the daily dose of sodium chloride given, as noted above, for 100 days or until 50% of the animals had died. The statistical methods were those described by Waugh (16).

Death rates were calculated at weekly intervals and plotted against daily dose. By analysis of these regressions, daily doses producing 50% mortality were calculated at weekly intervals and these values were plotted against weeks of administration of sodium chloride. From the estimating equation (Y) ± standard error ($S_{Y \cdot X}$) of the latter regression it was possible to calculate the $LD_{50(0.1L)}$ or daily dose which killed 50% of the rats after administration for 100 days, i.e., 1/10 the normal lifespan (0.1L) of rats used in this study. The calculation is illustrated in Fig. 1 below. The $LD_{0(0.1L)}$ or maximal daily dose producing 0% mortality at 100 days and the $LD_{100(0.1L)}$ or minimal daily dose producing 100% mortality at 100 days were estimated in a similar manner.

The chronic/acute $LD_{50(0.1L)}$ index (C/A $LD_{50(0.1L)}$ index) was calculated by expressing the $LD_{50(0.1L)}$ as a percentage of the acute LD_{50} as determined in the usual way in rats starved overnight, i.e., as $(LD_{50(0.1L)}/LD_{50}) \times 100$. The C/A $LD_{50(0.1L)}$ index of sodium chloride was then compared with the corresponding indices of benzylpenicillin and atropine.

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Results

Lethal Doses

The acute oral $LD_{50} \pm$ S.E. in unstarved rats was 6.14 ± 0.31 g/kg.

As illustrated in Fig. 1, the $LD_{50(0.1L)}$ was 2.69 ± 0.12 g/kg per day. The C/A $LD_{50(0.1L)}$ index was 71.7 ± 3.2 when a value for the acute oral LD_{50} found in this laboratory (7) was used.

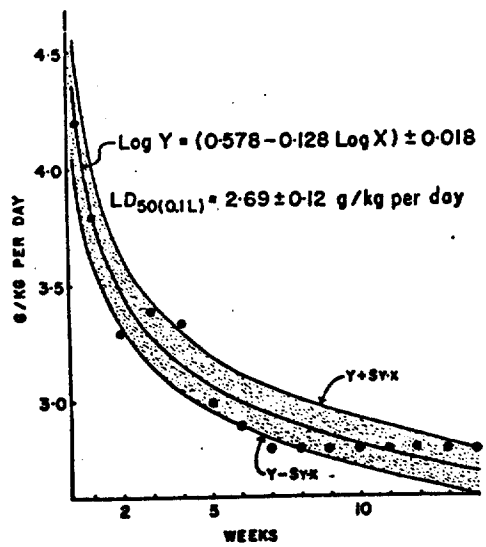

FIG. 1. The regression, on weeks of administration, of the daily dose of sodium chloride which killed 50% of animals.

Determination of a meaningful value for the maximal $LD_{0(0.1L)}$ presented inherent difficulties. Calculated in the same manner as the $LD_{50(0.1L)}$, the value was 2.69 ± 0.21 g/kg per day. At 1 week it was 68% of the corresponding value for 50% mortality, at 4 weeks 87%, at 8 weeks 94%, and at 10 weeks and over 100%. Repetition of the experiment in four groups of 10 rats each, given doses in the range of the estimated $LD_{0(0.1L)} \pm$ S.E. confirmed that the mortality at 100 days could vary between 0 and 50% and was not related to differences of up to 15% in mean body weight. A suggested meaningful value for the $LD_{0(0.1L)}$ is 2.69 g/kg per day minus thrice its standard error, i.e., $2.69 - (3 \times 0.21) = 2.06$ g/kg per day with $P < 0.001$, by available figures, that it would produce deaths.

Similar difficulties were encountered in calculating a meaningful minimal $LD_{100(0.1L)}$. Calculated in the same way as the $LD_{50(0.1L)}$ in Fig. 1, the value was found to be 2.69 ± 0.18 g/kg per day. A suggested meaningful value is $2.69 + (3 \times 0.18)$ or 3.23 g/kg per day.

The total cumulative dose of sodium chloride required to produce 50% deaths was calculated at weekly intervals and found, for example, to be 89, 163,

and 232 g/kg at 4, 8, and 12 weeks respectively. At 100 days the cumulative dose was 269 g/kg, which is approximately 100 times the total body sodium chloride content of a rat, expressed as grams per kilogram of body weight (17).

Growth-inhibiting Doses

The data were analyzed to determine growth-inhibiting daily doses. The normal gain in body weight was calculated from data on control albino rats, and representative mean values of body weight are listed in Table I. The mean

TABLE I

Representative clinical measurements upon albino rats used as controls

Measurement	Days*							
	0	7	14	28	42	56	70	100
Body weight (g)	158±15†	189‡	218‡	277‡	318‡	349‡	366‡	413‡
Food intake (g/kg per day)	104±12	89	79	76	64	66	58	55
Water intake (ml/kg per day)	130±19	109	98	89	76	72	79	68
Colonic temperature (°F)	98.7±1.0	98.5	98.9	99.1	99.6	99.8	99.0	99.4
Urine volume (ml/kg per day)	5.1±4.1	5.7	12.8	9.9	9.7	9.5	12.6	13.1
Urine protein (mg/100 ml)	74±11	89	68	82	114	85	69	87
Urine glucose (g/100 ml)	0±0	0	0	0	0.02	0.05	0	0.02
Urine pH	6.4±0.4	7.2	7.1	6.6	6.8	6.8	7.1	7.1

*The average standard deviation at days 7 to 100 was the same percentage of the mean as at zero day.
†Each value for day zero is expressed as mean ± S.D.
‡Each value for days 7-100 is expressed as $\bar{X}$.

weekly gain in body weight of the controls for each daily dose was subtracted from the corresponding mean for rats given sodium chloride, and the difference was expressed as a percentage of the mean in the controls. The percentage inhibitions of growth were then plotted against dose at weekly intervals, and daily doses producing representative inhibitions of the weekly growth rate of 10%, 25%, and 50% were calculated. These doses were then plotted against the figures for the week during which they were estimated, to obtain a regression.

When the animals which died were assigned a −100% growth rate and included with survivors, the regressions of daily doses producing 10, 25, and 50% inhibition of growth on weeks of treatment were logarithmic and similar to the regression shown in Fig. 1. For example, daily doses producing 10, 25, and 50% inhibition of growth after 100 days of administration were (± S.E.) 2.38 ± 0.086, 2.48 ± 0.087, and 2.61 ± 0.11 g/kg respectively.

Corresponding values were then calculated for survivors only and the regression of daily dose on weeks of treatment was found to be linear. These regression lines are shown in Fig. 2. A dose of 2.89 ± 0.063 g/kg per day inhibited the growth rate of survivors by 50% at the end of 100 days. A dose of 2.71 ± 0.092 g/kg per day inhibited the growth rate of survivors by 10% at the end of 100 days.

Food Intake

With the food intake of animals which died taken as −100% of that of their

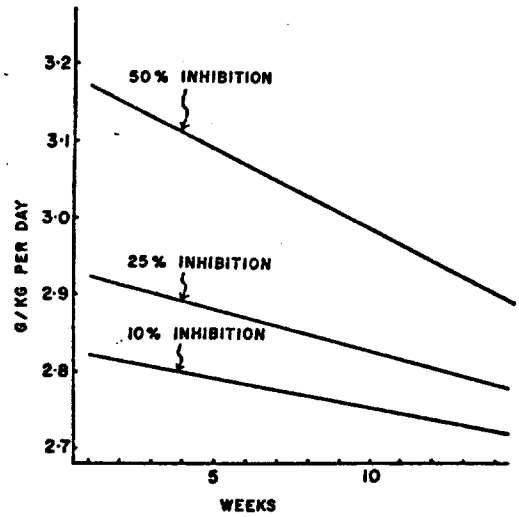

FIG. 2. The regression, on weeks of administration, of the daily dose of sodium chloride which inhibited the weekly growth rate in surviving rats by 50%, 25%, and 10% of that in the controls. The estimating equations ± S.E. were: for 50% inhibition, $Y = (3.19 - 0.021X) \pm 0.063$; for 25%, $Y = (2.93 - 0.011X) \pm 0.035$; and for 10%, $Y = (2.83 - 0.0084X) \pm 0.092$.

controls, the slope of the regression at 100 days of percentage mortality on percentage reduction in food intake was almost unity and the correlation coefficient was 0.946. These results indicated that there was no significant change in the weekly food intake of the surviving rats, irrespective of the daily dose of sodium chloride. This was confirmed by calculating the food intake of survivors as mean differences from that of controls (Table I) at weekly intervals. At no weekly interval was there any significant relation between food intake of survivors and daily dose of sodium chloride.

Water Intake

Water intake increased in all animals except during a period of 1 to 7 days before death. In survivors, the regression, on days of administration, of the daily dose required to augment water intake to four times that of the controls was logarithmic and almost identical with the regression of median lethal doses illustrated in Fig. 1. Corresponding regressions of daily dose which augmented water intake to three times and twice that of the controls lay at progressively lower ordinate levels. All regression lines approached each other and the $LD_{50(0.1L)}$ at 100 days.

These results indicated that a smaller daily dose of sodium chloride was required to increase water intake a given multiple at the end than at the beginning of administration of the salt. This, in turn, suggested that the intake of water per gram of sodium chloride was higher at the end than at the beginning of the 100 day study. To check this, the intake of water of the controls (in

milliliters per kilogram per day) was subtracted from the corresponding value in animals given sodium chloride, to yield a value for water intake due to or associated with sodium chloride intake and thus, presumably, eliminating the food intake as a factor in water intake. This water intake due to sodium chloride was divided by the daily dose of sodium chloride per kilogram body weight to obtain a value for water intake per gram of sodium chloride intake. The figures for water intake per gram of sodium chloride intake were calculated for survivors of each daily dose at intervals up to 100 days. Water intake so expressed bore no significant relationship to daily dose of sodium chloride, within the range of daily doses studied, but did increase linearly with increase in duration of the experiment and with increase in age of the animal. These results are illustrated in Fig. 3.

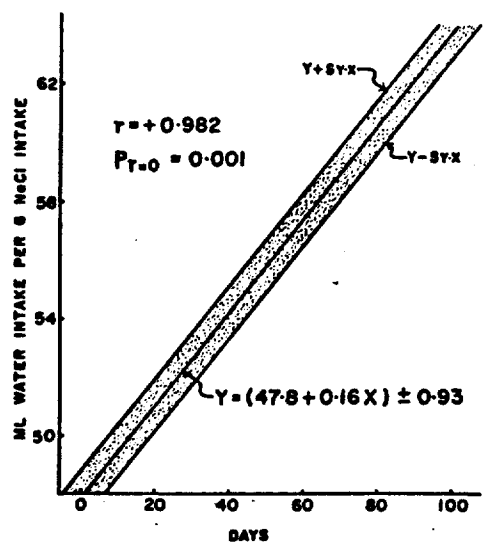

FIG. 3. The regression, on days of daily administration of sodium chloride, of milliliters water intake per gram of sodium chloride intake in surviving rats.

Colonic Temperature

During the first 4 weeks, the rats developed a significant increase in colonic temperature above the normal range shown in Table I. The degree of fever was not dose-dependent over the range of daily doses used. At 5 to 10 weeks colonic temperature returned to the range of the controls, and at 11 to 14 weeks there was a slight, but statistically significant, fall in colonic temperature which again was not related to the doses used. To summarize these changes, mean differences from controls in each dosage group were averaged at nine intervals during the 100 days, and these differences, expressed as percentage changes from their respective controls, are shown in Fig. 4.

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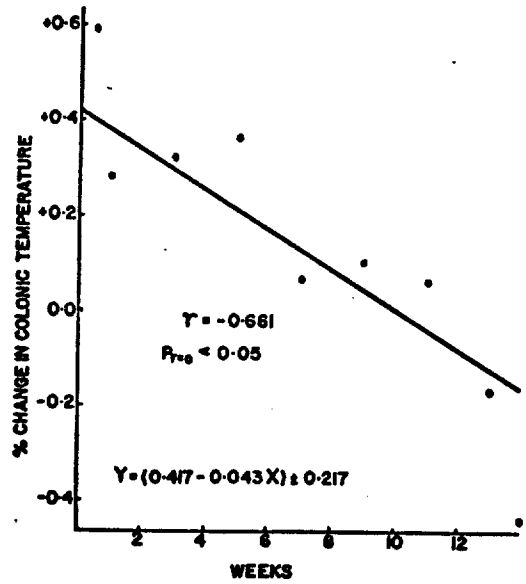

FIG. 4. The regression, on weeks of administration of sodium chloride, of mean changes in colonic temperature from levels in controls.

Diuretic Doses

Diuresis following administration of sodium chloride to rats in drinking water has been recorded by Koletsky (12, 18). In the present experiments, diuresis was expressed as urine volume, in milliliters per kilogram body weight per day, in rats treated with sodium chloride, minus the corresponding value in the controls of the same dosage group. A summary of values in controls is given in Table I. Mean diuresis values in rats which received sodium chloride varied between 69 and 201 ml/kg per day and a dose–response relationship was present. The daily dose of sodium chloride required to produce various selected degrees of diuresis was found to decrease logarithmically with days of administration in a manner similar to that shown in Fig. 1. The daily dose required to produce a selected diuretic volume of urine was always greater than that required to produce the same increase in water intake, and this ratio declined with duration of administration of sodium chloride up to, but not beyond, 6 to 8 weeks.

The volume of diuretic urine per gram of administered sodium chloride increased, in a manner similar to the increase in water intake (Fig. 3), with increasing days of administration of sodium chloride. Per gram of administered sodium chloride, the volume of diuretic urine equalled 0.69 ± 0.04 times the volume of water intake. From these values, the concentration of sodium chloride in urine may be calculated at varying intervals of administration, e.g. at 10 days 2.91%, at 60 days 2.57%, and at 100 days 2.36%, which suggests increasing inability of the animals to concentrate sodium chloride in urine.

Urinary Protein

The concentration of protein in the urine of control rats varied between group averages of 74 and 114 mg/100 ml (Table I), with an average excretion of 8.9 (±2.1) mg/kg body weight per day. The concentration of protein in the diuretic urine of rats given sodium chloride was much less than in that of the controls but the daily excretion averaged 12.2 (± 4.9) mg/kg body weight. An increase in the output of protein in urine, in terms of milligrams per kilogram body weight, occurred mainly in the first 2 weeks of administration of sodium chloride and was not significantly dose-dependent at the range of doses used.

Urinary Glucose

Occasional small amounts of reducing substances, calculated as glucose, were found in normal rat urine as shown in Table I. No change was found in rats which survived administration of sodium chloride.

Urinary pH

The pH of urine varies between 6.0 and 7.5 in normal control rats (Table I). Survivors of daily doses of sodium chloride showed a slight but significant ($P < 0.001$) alkalinuria in the first 5 weeks which regressed to a similarly significant aciduria during the last 4 weeks (Fig. 5). These shifts were not dose-dependent within the range of doses used.

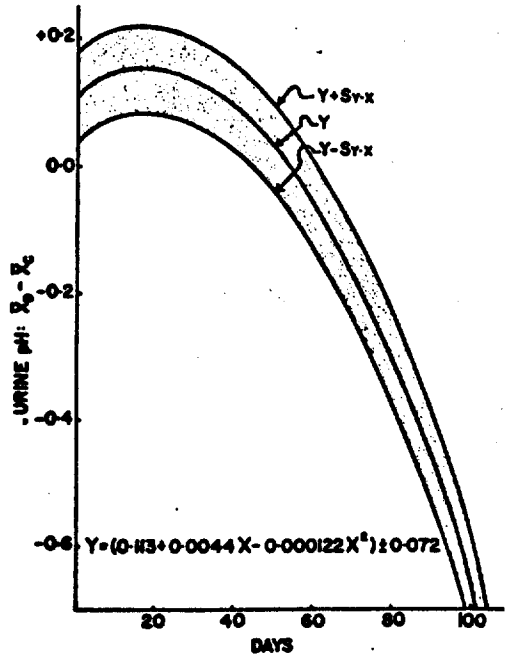

FIG. 5. The quadratic regression, on days of administration of sodium chloride, of shifts in the pH of urine expressed as the mean in the drug-treated group minus the mean of its control at each interval.

Premortal Signs

There were 86 deaths. During the week before death, 74% of these animals lost weight, 87% had a decreased food intake, 84% a decreased water intake, 73% a decrease in colonic temperature, and 67% a decline in the diuresis, and there were no significant changes in urinary protein, glucose, or pH. When death occurred within a week of the start of daily administration of the larger doses of sodium chloride, it was preceded by diarrhea and convulsive movements.

Histopathology

The histopathologic observations are summarized in Table II. In animals which died during the first 2 weeks of treatment, the most common finding was capillary–venous congestion in the lamina propria and the submucosa of the gastrointestinal tract and in many other organs. In some organs, this was accompanied by areas of capillary hemorrhage and edema. The gastroenteritis was accompanied by ulceration in many small areas of the mucosa. In addition, there was some central lobular degeneration of the liver, renal tubular degeneration, inhibition of spermatogenesis, and thymic atrophy. Bronchopneumonia was found in two thirds of the animals.

In animals which died after the first fortnight, the capillary–venous congestion had almost disappeared and the gastrointestinal mucosa was normal or hypertrophied. There was some residual hepatitis, nephritis, inhibition of spermatogenesis, and thymic atrophy. Most arterioles exhibited vacuolation of the tunica media and, to a lesser degree, the tunica intima. This vacuolation was not seen in any veins except in branches of the portal vein. Bronchopneumonia was found in 80% of the animals and a few had small abscesses in the brain. The spleen was contracted and there was some degeneration of the adrenal cortex in the form of vacuolation of the zona fasciculata and zona reticularis.

Causes of both early and late deaths, therefore, were bronchopneumonia, varying degrees of hepatitis and nephritis, and degenerative changes in the thymus and adrenal glands and the testes. Additional factors in the first 2 weeks were encephalitis, acute gastroenteritis, and generalized capillary–venous congestion, hemorrhage, and edema. Other factors after the first 2 weeks were arteriolitis and residual encephalopathy.

Survivors

Animals which survived 100 days of sodium chloride administration exhibited no histopathologic evidence of bronchopneumonia or tubular nephritis. The arteriolitis was present. The mucosa of the cardiac stomach and cecum was hypertrophied and the spleen was contracted. The thymus, adrenals, liver, brain, and testicles were normal. There was capillary congestion in the myocardium, with occasional areas of inflammatory cell (mononuclears and fibroblasts) infiltration. The lungs had areas of perivascular edema. An

TABLE II

Histopathologic changes in the organs of rats which died from daily administration of large doses of sodium chloride

Organ	At death within the first 2 weeks	At death after the first 2 weeks
Adrenal glands	Capillary–venous congestion	Diffuse vacuolation
Brain	Capillary–venous congestion	Occasional abscess
Gastrointestinal tract		
Cardiac stomach	Capillary–venous congestion Capillary hemorrhage	Mild capillary–venous congestion
Pyloric stomach	Capillary–venous congestion Capillary hemorrhage Small ulcers	Mucosal hypertrophy Mild capillary–venous congestion
Small bowel	Capillary–venous congestion Shrunken villi Edema	Mucosal hypertrophy
Cecum	Capillary–venous congestion Small ulcers	Mucosal hypertrophy
Colon	Capillary–venous congestion Capillary hemorrhage Small ulcers	Normal
Heart	Capillary congestion	Mild capillary–venous congestion
Kidneys	Capillary–venous congestion Edema of glomeruli and tubules Areas of tubular necrosis Tubular debris in glomerular space	Tubular edema
Liver	Capillary–venous congestion Mild central lobular vacuolation von Kupffer pyknosis	Diffuse vacuolation
Lungs	Capillary–venous congestion Areas of edema Bronchopneumonic areas	Bronchopneumonia
Meninges	Capillary–venous congestion Capillary hemorrhage	Mild capillary–venous congestion
Muscle (abdominal wall)	Hypovascular	Normal
Salivary glands (submaxillary)	Areas of edema	Normal
Skin	Hypovascular	Normal
Spleen	Edema Billroth cords	Contracted
Testes	Areas of edema of spermatogenic cells Inhibition of spermatogenesis Sertolis and Leydigs normal	Inhibition of spermatogenesis
Thymus gland	Moderate atrophy Capillary–venous congestion	Moderate atrophy
Ureter	Capillary–venous congestion	Normal
Vessels	Normal arterioles	Vacuolation of arteriolar media and portal vein media and intima

occasional reflux of proximal tubular epithelium into the renal glomerular space was noted and found to be accompanied by glomerular edema and hyperemia.

The weight of certain organs of survivors measured at the time at which 50% of the animals had died, varied with the total cumulative dose of sodium chloride which had been administered up to that point (Fig. 6). The quadratic

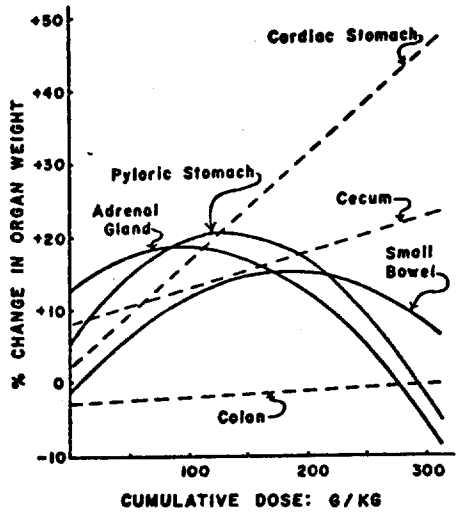

FIG. 6. The regression, on cumulative dose of sodium chloride to 50% mortality, of percentage changes from controls in the fresh weight of the adrenals and gastrointestinal organs of survivors.

regressions for adrenal glands, pyloric stomach, and small bowel, and the linear regression for cardiac stomach shown in Fig. 6, differed from zero correlation at $P < 0.02$.

The fresh weight of other organs in survivors declined with increasing cumulative dose of sodium chloride, the regressions being more or less parallel to that of total body weight. For animals which survived until 100 days, the mean percentage changes from controls are assembled in Table III under two groups: group A, survivors of dosage groups in which about half (35 to 55%) of the rats had previously died, and group B, animals of dosage groups in which there were no previous deaths.

In both groups A and B, the gastrointestinal organs weighed more than in the controls. In group A, the fresh weight of all other organs except adrenals was less than in controls, and in some (lungs, skin, spleen, and thymus) the loss was significantly greater than was the loss of total body weight. In group B, most weight losses were less than in group A, the mean reduction being significant at $P < 0.02$ in kidneys, liver, lungs, and total body weight.

In summary, in survivors loss of weight occurred in most organs except the adrenals and gastrointestinal tract; the loss increased linearly with increasing cumulative dose of sodium chloride and was in general less in dosage groups in which there were no deaths. With increasing cumulative dose of sodium chloride, there was a linear increase in weight of cardiac stomach and cecum, and a quadratic rise and decline in the weight of pyloric stomach, small bowel, and adrenal glands.

There were no significant shifts in the water levels of organs of survivors

TABLE III

The effect of daily administration of sodium chloride upon the fresh weight of organs of survivors at 100 days

Organ	Controls, g wet weight, mean ± S.D. (N = 38)	Group A: survivors in groups with 50% earlier deaths (N = 39)		Group B: groups with no previous deaths, (N = 30)†	
		% change from controls	P	% change from controls	P
Adrenal glands	0.049±0.008	− 0.1	1.00	+ 0.7	0.5
Brain	1.803±0.118	− 5.7	<0.001	− 4.9	0.005
Gastrointestinal tract					
Cardiac stomach	0.406±0.043	+43.2	<0.001	+36.8	<0.001
Pyloric stomach	1.026±0.125	+ 2.5	0.3	+ 3.6	0.01
Small bowel	8.60±0.863	+11.9	0.02	+ 9.3	0.05
Cecum	1.053±0.143	+20.4	<0.001	+47.0	0.05
Colon	2.241±0.241	+ 0.1	1.00	+10.9	0.10
Heart	1.164±0.104	−12.4	<0.001	−10.9	0.02
Kidneys	3.018±0.355	− 7.7	0.001	+ 0.1	0.8*
Liver	16.26±1.992	−16.7	<0.001	−13.8	0.001*
Lungs	1.627±0.249	−23.9	<0.001	+ 1.5	0.7*
Muscle (abdominal wall)	14.63±2.067	−13.8	<0.001	−10.9	0.01
Skin	92.39±11.04	−23.3	<0.001	−16.8	0.02
Spleen	0.825±0.122	−22.4	0.001	−11.1	0.5
Testicles	3.465±0.261	− 8.0	0.001	− 8.8	<0.001
Thymus gland	0.169±0.044	−21.8	<0.001	−18.9	0.1
Residual carcass	225.0±24.1	−15.3	<0.001	−13.7	0.005
Total body weight	413.9±43.1	−11.1	<0.001	− 9.4	<0.001*

†Mean changes significantly different ($P \leq 0.02$) from those in group A are indicated by an asterisk.

autopsied at intervals up to 39 days. In survivors killed at 100 days, there were several minor but significant shifts in organ water levels which were not dose-dependent (except for loss of water in pyloric stomach which increased with increasing cumulative dose) and were the same in group A as in group B animals. The results at 100 days are summarized in Table IV. The small bowel, cecum, and colon were slightly hydrated, but most organs were slightly dehydrated or unchanged in water content.

Discussion

The C/A $LD_{50(0.1L)}$ index ± S.E. of sodium chloride was found to be 71.7 ± 3.2. The corresponding indices for benzylpenicillin (6) and atropine (4) were 61.8 ± 3.0 and 13.2 ± 0.85 respectively. These results indicate that a significantly larger proportion of the acute LD_{50} of sodium chloride than of benzylpenicillin and especially of atropine can be given daily for 100 days without producing 50% mortality.

The acute LD_{50} and the C/A LD_{50} index for a drug might provide a useful summary of its acute and chronic toxicity. The duration of chronic administration would have to be standardized since the index decreases with increasing interval of chronic administration, as may be seen from Fig. 1 and calculated from corresponding data on benzylpenicillin (6) and atropine (4). The decrease

TABLE IV

The effect of daily administration of large doses of sodium chloride upon the water levels of organs of survivors at 100 days

Organ	Controls, mean ± S.D., g/100 g dry weight (N=38)	Rats treated with sodium chloride (N = 69)	
		% change from controls	P
Adrenal glands	211+28	+ 0.1	1.0
Brain	344± 6	− 0.5	0.5
Gastrointestinal tract			
Cardiac stomach	317±17	+ 3.6	0.2
Pyloric stomach	309±12	−10.0	0.001
Small bowel	371±26	+ 6.8	0.02
Cecum	349±19	+12.8	0.001
Colon	409±20	+ 4.4	0.02
Heart	339±11	− 3.9	<0.001
Kidneys	332±10	+ 3.8	0.1
Liver	245±15	− 1.2	0.6
Lungs	359±12	− 4.4	0.01
Muscle (abdominal wall)	261±15	− 0.8	0.7
Skin	135±23	+13.4	0.1
Spleen	319± 6	− 3.2	0.01
Testicles	648±11	− 4.1	<0.001
Thymus gland	327±46	− 7.4	0.05
Residual carcass	185±21	− 3.4	0.3

with increasing interval of administration is not necessarily the same for all drugs. When the C/A LD_{50} indices for benzylpenicillin and atropine are expressed as a percentage of that for sodium chloride, the index for benzylpenicillin remains at 86 to 87% at all intervals from 10 to 100 days while that for atropine decreases logarithmically from 22.4% at 10 days to 18.4% at 100 days.

The C/A LD_{50} index of sodium chloride did not change appreciably during the last 50 days of administration (Fig. 1). The same relation may be calculated for benzylpenicillin (6) and atropine (4). Determination of the C/A LD_{50} index at weekly intervals could provide a means of indicating when daily administration of a drug should be terminated in chronic toxicity studies of this nature.

Difficulties inherent in arriving at a meaningful value for the $LD_{0(0.1L)}$ of sodium chloride were not encountered with benzylpenicillin (6) and atropine (4), for both of which the $LD_{0(0.1L)}$ was about 60% of the $LD_{50(0.1L)}$. In view of the considerable difference between the acute LD_{50} of sodium chloride in unstarved versus starved rats, it is possible that fortuitous differences in food intake could explain why a daily dose of 2.69 g/kg could produce from 0% to 50% deaths at 100 days. The suggested value of 2.06 g/kg per day for the $LD_{0(0.1L)}$ is 77% of the $LD_{50(0.1L)}$.

The growth rate of survivors was not appreciably affected by daily doses equivalent to or less than the $LD_{50(0.1L)}$. No or minor inhibition of growth has been previously reported in rats which survived the addition of large doses of sodium chloride to their food or drinking water (9, 11–14). The food intake of

surviving rats was not affected except for a premortal decline, confirming the findings of Richter and Mosier (15).

The polydipsia reported in rats given large daily doses of sodium chloride in their food (13–15) or drinking water (18) was confirmed. Richter and Mosier (15) calculated that rats drink 50 to 60 ml of water for each gram of sodium chloride added to their diet. This relationship was confirmed and it was found that the water intake per gram of administered sodium chloride bore no relation to daily dose of salt, within the range of daily doses studied, but did increase linearly with days of administration (Fig. 3). The results suggest that during the 100 days of salt administration, the survivors became progressively less able to concentrate urine. In confirmation it was found that the diuretic urine volume per gram of sodium chloride similarly rose and equalled some 70% of the water intake per gram. Alternatively it could be suggested that thirst was more readily stimulated by sodium chloride as the rats grew older during the experiment. Reduced ability of the kidneys to concentrate urine could be due to the cumulative toxicity of sodium chloride unrelated to aging of the animals. The results did explain why the negative regressions on time of daily dose required to increase water intake two-, three-, and four-fold approached each other at 100 days, i.e., it took less sodium chloride at 100 days to double, triple, and quadruple the water intake.

Fever, found in the initial month of administration of sodium chloride, has been reported in rats given large single doses (7) and in children given large single (19) or multiple daily (20) amounts in error, but not in pigs fed lethal doses by mistake over a period of 6 days (21). Failure to record fever in pigs was possibly due to a premortal hypothermia which was also noted in the present study. In rats given doses in excess of the $LD_{50(0.1L)}$, the initial low-grade fever may have been responsible for some of the loss of body weight seen, but probably not a major proportion since it was not dose-dependent. Hypothermia in the terminal month of the experiment probably represents a type of adaptation to pyrogenic doses of sodium chloride.

The fever was accompanied by proteinuria and slight alkalinuria, neither being dose-dependent. Albuminuria has been reported by Koletsky (12, 18) in rats which had large amounts of sodium chloride added to their drinking water. As fever disappeared so did the proteinuria and alkalinuria, and the late mild hypothermia was accompanied by a mild aciduria.

The premortal diarrhea and convulsive movements seen in animals which died within 1 week have been reported in studies of the acute oral toxicity of sodium chloride (7). The hypothermic cachexia and loss of body weight which preceded death after this time have been reported in rats given excess sodium chloride in their drinking water (18), but an edematous gain in body weight sometimes precedes death in rats given the salt in their diet (13).

Histopathologic lesions previously reported in rats given large daily amounts of sodium chloride in their food or drinking water include pneumonia (12, 18–21), hepatitis (11, 19), and nephritis (9, 11–14, 18, 21, 22). Of the additional

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lesions seen in early deaths, encephalitis (20, 21) and gastroenteritis (19) have been previously noted and the generalized capillary–venous congestion has been reported as part of the acute oral toxicity of sodium chloride in the rat (7). The arteriolitis of late death has been extensively described (12, 13, 18, 22–25).

Loss of fresh weight and water in many organs of rats given single doses of sodium chloride at the range of the acute oral LD_{50} has been reported (7). In rats which survived 100 days of administration of sodium chloride in the present study, the gastrointestinal tract appeared to have adapted to the presence of large amounts of salt since it was in general enlarged and partially hydrated. Most other organs had lost some weight and minor amounts of water.

References

1. Department of National Health and Welfare. Office Consolidation of the Food and Drugs Act and of the Food and Drug Regulations with Amendments to June 18, 1964. The Queen's Printer, Ottawa. 1964. p. 126B.
2. E. M. Boyd. J. New Drugs, 1, 104 (1961).
3. E. M. Boyd, S. V. Jarzylo, C. E. Boyd, and W. A. Cassell. Arch. Intern. Pharmacodyn. **115**, 360 (1958).
4. C. E. Boyd and E. M. Boyd. Toxicol. Appl. Pharmacol. **4**, 457 (1962).
5. D. W. Mills. Arch. Intern. Pharmacodyn. **125**, 83 (1960).
6. E. M. Boyd and M. J. Selby. Antibiot. Chemotherapy, **12**, 249 (1962).
7. E. M. Boyd and M. N. Shanas. Arch. Intern. Pharmacodyn. **144**, 86 (1963).
8. L. K. Dahl. J. Exptl. Med. **112**, 635 (1960).
9. L. A. Sapirstein, W. L. Brandt, and D. R. Drury. Proc. Soc. Exptl. Biol. Med. **73**, 82 (1950).
10. S. Koletsky. Arch. Pathol. **68**, 11 (1959).
11. I. S. Kleiner and L. B. Dotti. N.Y. Med. Coll. Flower Hosp. Bull. **3**, 309 (1940).
12. S. Koletsky. Lab. Invest. **7**, 377 (1958).
13. G. R. Meneely, R. G. Tucker, and W. J. Darby. J. Exptl. Med. **98**, 71 (1953).
14. G. R. Meneely, R. G. Tucker, and W. J. Darby. J. Nutr. **48**, 489 (1952).
15. C. P. Richter and H. D. Mosier. Am. J. Physiol. **176**, 213 (1954).
16. A. E. Waugh. Elements of statistical method. 3rd ed. McGraw-Hill Book Co., Inc., New York. 1952.
17. H. H. Ussing, P. Kruhoffer, J. H. Thaysen, and N. A. Thorn. The alkali metal ions in biology. Springer-Verlag, Berlin. 1960.
18. S. Koletsky. Arch. Pathol. **68**, 11 (1959).
19. E. P. Scott and C. C. Rotondo. Kentucky Med. J. **45**, 107 (1947).
20. K. Kualvik. T. Norske Laegeforen, **77**, 839 (1957); Excerpta Med. Sect. II, **11**, 1167 (1958).
21. J. H. Soutter, D. K. Sorenson, and J. J. Clark. J. Am. Vet. Med. Assoc. **130**, 12 (1957).
22. G. R. Meneely and L. K. Dahl. Med. Clin. N. Am. **45**, 271 (1961).
23. L. Tobian. Physiol. Rev. **40**, 280 (1960).
24. F. Gross. Schweiz. Med. Wochschr. **93**, 1065 (1963).
25. L. K. Dahl and E. Schackow. Can. Med. Assoc. J. **90**, 155 (1964).

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THE EFFECT OF SERUM POTASSIUM CONCENTRATION ON QUINIDINE TOXICITY[1]

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ABSTRACT

BRANDFONBRENER, MARTIN, JEAN KRONHOLM AND HARVEY R. JONES: The effect of serum potassium on quinidine toxicity. J. Pharmac. exp. Ther. **154**: 250-254, 1966. Quinidine gluconate was infused intravenously in anesthetized dogs until death, while serum quinidine, serum potassium and electrocardiograph (ECG) and arterial pressure were monitored. Control, hyperkalemic and hypokalemic animals were studied. Hyperkalemia did not significantly affect the intraventricular conduction disturbance produced by quinidine but did decrease survival time. Hypokalemia protected against the conduction prolongation from quinidine, but did not significantly prolong survival when compared to the control group. Despite the greater amounts of quinidine infused in the hypokalemic group than the hyperkalemic or control groups, the serum quinidine levels were not significantly different, suggesting that serum potassium influences the removal of quinidine from the blood. The serum quinidine was found to be a reliable index of severe quinidine intoxication.

Potassium concentration has been reported to affect quinidine action on the heart. Quinidine increases the refractory period and depresses the rate and amplitude of the beat of isolated rabbit atria. This effect is potentiated by an increase and is antagonized by a decrease in potassium concentration in the bath medium (Holland, 1957). In addition, quinidine rapidly inhibits potassium efflux and sodium entry in rabbit atria (Klein *et al.*, 1960). Quinidine decreases potassium permeability (Armitage, 1957). Quinidine also produces a rise in intracellular myocardial potassium concentration (Gertler *et al.*, 1956). According to some (Lee, 1960; Bellet *et al.*, 1959), infusion of quinidine decreases serum potassium: others report different findings (Wasserman *et al.*, 1959). Also, the effects of hyperkalemia and quinidine on intraventricular conduction are similar. The present experiment was performed to study the relationship between serum potassium concentration and the fatal dose of quinidine in the intact dog. The effect of potassium on quinidine-induced intraventricular-conduction prolongation was also studied.

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METHODS. Mongrel dogs, 10 to 17 kg b.wt., were anesthetized with 25 mg/kg of intravenous pentobarbital sodium. A tracheostomy was performed and respiration was maintained with a respirator pump. The inspired gas was 100% O_2. A femoral artery and vein were cannulated for the infusion of drugs and the recording of arterial pressure and blood sampling. Four groups of animals were studied.

Control, group 1. In 10 dogs, quinidine gluconate in isotonic saline, 10 mg/ml, was infused intravenously, 1 mg/kg/min, until death occurred. In three of these animals, saline was infused for 32 min prior to the quinidine infusion. The infusion of saline was carried out for a time equal to the KCl infusion (1.3 mEq/min) in the hyperkalemia group.

Hyperkalemia, group 2. In nine dogs KCl was infused intravenously at 1.3 mEq/min. This was continued until electrocardiographic (ECG) evidence of definite potassium toxicity occurred as manifested by loss of P waves, 50% widening of the QRS complexes or a rhythm disturbance, such as an idioventricular rhythm. This infusion lasted an average of 32 min. A 5-min recovery period resulted in changes of QRS interval toward normal, as evidenced by disappearance of the idioventricular rhythm and a return of atrial activity. Quinidine gluconate was then infused at 1 mg/kg/min until death. In order to prevent the serum potassium level from falling to normal from the level induced by the KCl infusion (1.3 mEq/min), more dilute KCl

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was infused with the quinidine, at the rate of 0.0077 mEq/kg/min.

Hypokalemia, group 3. Eight dogs were treated with 9 α-fluorohydrocortisone, 0.1 mg/day, a meat-free 1.5-g NaCl diet, Na polystyrene sulfonate (Kayexalate) resin, 3 teaspoonsful/day, and chlorothiazide, 750 mg/day, or hydrochlorothiazide, 75 mg/day. After 13 to 30 days of this regimen, the experiment was performed. Normal saline was infused at 0.65 ml/min for 32 min, which was the average duration of the initial high concentration KCl infusion in the hyperkalemic group. Quinidine gluconate in isotonic saline, 1 mg/kg/min, was then infused until death.

Hyperkalemia without quinidine, group 4. In 10 dogs KCl was infused intravenously at 1.3 mEq/min until toxicity, as described in group 2. After a 5-min recovery period, an infusion of KCl, 0.0077 mEq/kg/min, in a normal saline solution, was infused as group 2. No quinidine gluconate was infused. The infusion of KCl was continued for 270 min.

Samples of blood were drawn at 15-min intervals, and the plasma was separated immediately for potassium and quinidine determinations. The potassium was measured in duplicate with an internal standard Baird flame photometer. Serum quinidine was analyzed in duplicate (Brodie and Udenfriend, 1943; Edgar and Sokolow, 1950). The electrocardiogram and aortic blood pressure were recorded on a Sanborn model 150 recorder at 4-min intervals. The ECG was recorded at 25 and 50 mm/sec paper speed.

Quinidine toxicity was defined as occurring when the QRS duration was twice the control value. The control value was that QRS duration immediately prior to the quinidine infusion. When either asystole or ventricular fibrillation occurred, the animal was judged dead. Arterial blood was anaerobically collected between 30 to 60 min after the quinidine infusion was started, and was analyzed in duplicate for oxygen saturation in nine animals and for CO_2 content in 10 animals (Van Slyke and Neill, 1924).

The groups of animals were compared using Student's *t* test (Fisher, 1950) and P values for the differences between the groups were calculated.

Results. *Control, group 1.* Prior to the infusion of quinidine gluconate, the average serum potassium was 3.88 mEq/liter, the QRS was 0.04 sec, the heart rate was 162 beats/min and the blood pressure was 154/111 mm Hg. A QRS duration of twice the control value occurred in 96 min. The average serum potassium was then 3.75 mEq/liter and serum quinidine was 33.6 mg/liter. The blood pressure was 98/60 mm Hg. The average duration of quinidine infusion which produced death was 162 min. The average serum potassium prior to death was 3.65 mEq/liter, the quinidine level was 58.4 mg/liter, the QRS duration was 0.154 sec, the heart rate was 70 beats/min and the blood pressure was 36/16 mm Hg. At death the cardiac rhythm was either a slow idioventricular rhythm with final asystole or ventricular fibrillation.

Hyperkalemia, group 2. Prior to the potassium infusion, serum potassium was 4.08 mEq/liter, the QRS was 0.040 sec, the heart rate was 147 beats/min and the blood pressure was 171/112 mm Hg. ECG evidence of potassium toxicity was noted after an average of 32 min of the high concentration KCl infusion. The average serum potassium was then 9.23 mEq/liter. Five minutes after cessation of the KCl infusion, and just prior to the infusion of quinidine gluconate mixed with dilute KCl, the average QRS was 0.046 sec. The average duration of quinidine infusion which produced a QRS value twice the control was 87.8 min. The serum potassium was then 6.23 mEq/liter and the quinidine level was 35.4 mg/liter. The blood pressure was 115/56 mm Hg. The average duration of quinidine infusion which caused death was 132 min. The average serum potassium prior to death was 6.58 mEq/liter, the quinidine level was 55.3 mg liter, the QRS was 0.163 sec, the heart rate prior to ventricular fibrillation was 75 beats/min and the blood pressure was 39/13 mm Hg. The dogs in this group died with ventricular fibrillation or asystole. This group showed no significant difference from the control group in the QRS duration, pulse rate or blood pressure prior to KCl infusion. In table 1, it may be seen that there was significant hyperkalemia and QRS prolongation prior to the quinidine infusion. There was no significant difference between the control and hyperkalemic groups in the duration of quinidine infusion which produced QRS prolongation or in the serum quinidine levels when the QRS was twice the control duration. The serum potassium remained somewhat elevated throughout the experiment. The hyperkalemic animals died sooner than the control animals, that is, after a smaller total dose of quinidine. This is statistically significant. The serum quinidine at death was not significantly

TABLE 1

	Average Control, Group 1, $n = 10$*	Average Hyperkalemia, Group 2, $n = 9$	Average Hypokalemia, Group 3, $n = 8$	Control vs. Hyperkalemic P value	Control vs. Hypokalemic P Value	Hyperkalemic vs. Hypokalemic P Value
Prior to quinidine infusion						
Serum potassium, mEq/liter	3.88	9.23	3.28	.01	.05 > P > .02	.01
QRS duration, sec	0.040	0.046	0.041	.01	.4 > P > .3	.01
QRS twice control						
Duration of quinidine infusion, min	96	87.8	171.0	.6	.05 > P > .02	.02 > P > .01
Serum potassium, mEq/liter	3.75	6.23	3.32	.01	.05 > P > .02	.01
Serum quinidine, mg/liter	33.6	35.4	43.0	.7 > P > .6	.1 > P > .05	.2 > P > .01
At death						
Duration of quinidine infusion, min	162	132	207	.05 > P > .02	.2 > P > .1	.02 > P > .01
Serum potassium, mEq/liter	3.65	6.58	3.22	.001	.3 > P > .2	.001
Quinidine level, mg/liter	58.4	55.3	49.6	.6 > P > .5	.2 > P > .1	.3 > P > .2
QRS duration, sec	0.154	0.163	0.108	.6	.02 > P > .01	.01

* n = number of observations.

less in the hyperkalemic animals than in the controls. The average oxygen saturation in the nine dogs in groups 1 and 2, drawn prior to the increase in QRS to twice control, was 101% and the average CO_2 content in 10 dogs was 45.39 volume %.

Hypokalemia, group 3. The average initial serum potassium was 3.28 mEq/liter, the QRS was 0.041 sec, the heart rate was 155 beats/min and the blood pressure was 163/104 mm Hg. The average duration of quinidine infusion which prolonged the QRS to twice the control was 171 min. At that time, the serum potassium was 3.32 mEq/liter and the quinidine level was 43.0 mg/liter. The blood pressure was 100/51 mm Hg. The average duration of quinidine infusion which produced death was 207 min. The serum potassium just prior to death was 3.22 mEq/liter, the serum quinidine was 49.6 mg/liter and the QRS was 0.108 sec. The heart rate prior to fibrillation was 84 beats/min and the blood pressure was 42/11 mm Hg. The hypokalemic group showed no significant difference from the controls in the QRS duration, in blood pressure or in heart rate prior to quinidine infusion. The initial serum potassium in the hypokalemic dogs was significantly lower than the controls (table 1). The duration of quinidine infusion was significantly longer, and, therefore, a greater amount of quinidine was required to produce QRS prolongation in the hypokalemic dogs than in the controls or hyperkalemic dogs. The serum quinidine level when the QRS was twice the control duration was not significantly different in the hypokalemic, hyperkalemic and control groups. The average duration of quinidine infusion which caused death, however, was longer in the hypokalemic than in the control and hyperkalemic groups, but this was statistically significant only when the hyperkalemic and hypokalemic groups were compared. The final serum quinidine levels were not significantly different in these three groups, despite the longer duration and therefore greater amount of quinidine infused in the hypokalemic animals. These dogs died with ventricular fibrillation or asystole.

There was no difference between any of the groups in heart rate (all below 100 beats/

min) or blood pressure (all below 75/25 mm Hg) prior to ventricular fibrillation or asystole. The QRS duration just before the terminal arrhythmia was significantly shorter in the hypokalemic group.

Hyperkalemia without quinidine infusion, group 4. None of the animals in this group died. The experiments were discontinued at 270 min. After the KCl infusion (1.3 mEq/liter), serum potassium was 8.9 mEq/liter. At the end of the 5-min recovery period, the QRS duration was 0.043 sec. At 270 min, the average QRS duration was 0.039 sec and the heart rate was unchanged from control. The serum potassium was then 5.60 mEq/liter. There was no arrhythmia noted during the infusion of the dilute (0.0077 mEq/kg/min) KCl solution. Hyperkalemia of this magnitude, *per se*, did not produce the changes in QRS duration, rhythm, blood pressure and heart rate during the 270-min infusion, which were observed in group 2.

Discussion. In various experimental preparations there is a relationship between potassium and quinidine effect. On this basis, these experiments were undertaken to study the relationship of serum potassium concentration to the fatal dose of quinidine and its effect on intraventricular conduction in the intact dog.

In the hyperkalemic group, no less quinidine was required to prolong the QRS than in the control. Bennett *et al.* (1961) found no additive effect of therapeutic doses of quinidine and potassium on intraatrial, atrioventricular or intraventricular conduction. In the present experiments, levels of quinidine in the therapeutic range were maintained for only a short time. Thus, the absence of additive effect of quinidine and hyperkalemia on intraventricular conduction disturbance is confirmed at higher quinidine levels.

Serum potassium elevation was associated with a decreased survival time during quinidine infusion. That is, a smaller total dose of quinidine produced fatal arrhythmias. When the serum potassium was elevated without quinidine infusion, death did not occur and the blood pressure and pulse rate were not significantly different from the preinfusion values after 4 hr of infusion of the dilute (0.0077 mg/kg/min) KCl solution. The ECG abnormalities induced initially by the 1.3 mEq/liter KCl infusion all returned to normal during the dilute KCl infusion. Since elevation of serum potassium, *per se*, does not decrease survival time, the decrease in survival in the hyperkalemia with quinidine group was due to some synergistic action. If the decrease in potassium efflux from cells (Klein *et al.*, 1960) induced by quinidine is related to its toxicity, then an increase in intracellular potassium caused by KCl infusion would decrease the fatal dose of quinidine.

The serum quinidine levels at death were comparable in the control and hyperkalemic groups despite the latter having received less quinidine. At death, serum quinidine is similar in the hypokalemic and hyperkalemic groups despite different amounts of quinidine having been given, which indicates that quinidine removal from the blood might have been influenced in these experiments. Changes in the volume of distribution, differences in quinidine loss in the urine and possible effects of potassium on quinidine conversion to metabolites are possible explanations. However, it is clear that there is a close relationship between quinidine blood level and toxicity.

The hypokalemia produced was not marked, but an increased infusion duration, and, therefore, an increased total quinidine dose, was required to prolong the QRS during hypokalemia. The QRS prolongation just prior to the terminal arrhythmia in the hypokalemic group was significantly less than in the other groups, which supports the possibility that hypokalemia protects against the quinidine-induced intraventricular conduction disturbance. If intracellular potassium depletion accompanied the mild hypokalemia, a quinidine-induced decrease in potassium efflux might be expected to protect against QRS prolongation. Hypokalemia prolonged average survival time, but not significantly, when hypokalemia was compared with the control group. This might have been the result of the mild degree of hypokalemia produced. In fact, the serum potassium in the hypokalemic group was not significantly different from the control group just prior to death. The effect of the drugs given to produce hypokalemia is complex, and in addition to mild hypokalemia, alkalosis and intracellular potassium depletion (Darrow *et al.*, 1948), direct steroid effects could be implicated in the results observed. The hypokalemic ani-

mals were probably alkalotic, but the effect of altered blood hydrogen ion concentration on quinidine toxicity is not agreed upon. Sodium lactate, 1 M, which produces alkalosis as well as providing sodium ion, has been found to protect against quinidine intoxication (Bellet *et al.*, 1959). "No significant beneficial" effect of sodium lactate on severe quinidine intoxication has been reported by others (Lee, 1960).

It is known that a decrease in monovalent cation, either potassium or sodium, increases contractility (Ringer, 1883; Daly and Clark, 1920; and Hajdu, 1953). An increase in potassium in the medium decreases contractility of the frog heart (Ringer, 1883). It is possible that the effect of potassium concentration on quinidine toxicity may be due to the effect of potassium on contractility.

The fall in serum potassium level during quinidine infusion described by others (Lee, 1960; Bellet *et al.*, 1959) is compatible with the observation that quinidine increases the intracellular potassium level (Gertler *et al.*, 1956). Changes in serum ion concentrations in anesthetized animals receiving intravenous infusions are difficult to interpret and may be due to dilution, etc. In these experiments, no significant decrease in serum potassium occurred during quinidine infusion in the control or hypokalemic groups (P .6–.8). The theory that an increase in cardiac intracellular potassium is important in producing quinidine toxicity is only suggestively supported by these experiments.

ACKNOWLEDGMENT. The Eli Lilly & Company, Indianapolis, Indiana, is thanked for providing the quinidine gluconate.

REFERENCES

ARMITAGE, A. K.: The influence of potassium concentration on the action of quinidine and of some antimalarial substances on cardiac muscle. Br. J. Pharmac. Chemother. **12**: 74–78, 1957.

BELLET, S., HAMDEN, G., SOMLYO, A. AND LARA, R.: The reversal of cardiotoxic effects of quinidine by molar sodium lactate: an experimental study. Am. J. med. Sci. **237**: 165–176, 1959.

BENNETT, L. L., RUBIN, M. L., BLEVINS, C. E. AND BAIRD, B.: The effects of infusing quinidine sulfate and potassium chloride separately and combined, on conduction times of the dog heart. Am. Heart J. **61**: 500–503, 1961.

BRODIE, B. B. AND UDENFRIEND, S.: The estimation of quinine in human plasma with a note on the estimation of quinidine. J. Pharmac. exp. Ther. **78**: 154–158, 1943.

DALY, I. D. AND CLARK, A. J.: The action of ions upon the frog's heart. J. Physiol., Lond. **54**: 367–383, 1920.

DARROW, D. C., SCHWARTZ, R., IANNUCCI, J. F. AND COVILLE, F.: The relation of serum bicarbonate concentration to muscle composition. J. clin. Invest. **27**: 198–208, 1948.

EDGAR, A. L. AND SOKOLOW, M.: Experiences with the photofluorometric determination of quinidine in blood. J. Lab. clin. Med. **36**: 478–484, 1950.

FISHER, R. A.: Statistical Methods for Research Workers, 11th ed., pp. 122–125, Oliver and Boyd, London, 1950.

GERTLER, M. M., KREAM, J., HYLIN, J. W., ROBINSON, H. AND NEIDLE, E. G.: Effect of digitoxin and quinidine on intracellular electrolytes of rabbit heart. Fedn Proc. Fedn Am. Socs exp. Biol. **92**: 629–632, 1956.

HAJDU, S.: Mechanism of staircase and contracture in ventricular muscle. Am. J. Physiol. **174**: 371–380, 1953.

HOLLAND, W. C.: A possible mechanism of action of quinidine. Am. J. Physiol. **190**: 492–494, 1957.

KLEIN, R. L., HOLLAND, W. C. AND TINSLEY, B.: Quinidine and undirectional cation fluxes in atria. Circulation Res. **8**: 246–252, 1960.

LEE, Y. C.: Quinidine intoxication: An experimental study of the effect of molar sodium lactate and potassium chloride. Am. Heart J. **60**: 785–798, 1960.

RINGER, S.: A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J. Physiol., Lond. **4**: 29–42, 1883.

VAN SLYKE, D. D. AND NEILL, J. M.: The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. J. biol. Chem. **61**: 523–573, 1924.

WASSERMAN, E., BRODSKY, L., KATHE, J. H., RODENSKY, P. L., DICK, M. M. AND DENTON, P. S.: The effect of molar sodium lactate in quinidine intoxication. Am. J. Cardiol. **3**: 294–299, 1959.

Case Reports

PAROXYSMAL CEREBRAL DYSRHYTHMIA FOLLOWING LARGE DOSES OF POTASSIUM CHLORIDE

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REPORT OF CASE

History.—A man aged 47 was seen first on Sept. 14, 1942. For two months he had staggered when walking, and any movement of the head caused dizziness.

The present illness began in August 1939, when he had his first attack of severe vertigo with vomiting. This came on suddenly when he was standing on the street outside a store. He had several severe attacks after this one and was treated by his local physician for six months. He was seen at a clinic in February 1940, where a low sodium diet and ammonium chloride were prescribed. He had shown much improvement until the summer of 1942, when he consulted his family physician because of staggering and dizziness. He had stopped taking the ammonium chloride for some time before but still maintained the low sodium intake. His blood count was normal, and repeated urinalyses showed nothing abnormal. The Wassermann, Hinton and Kahn tests gave negative reactions. The blood pressure was 140 systolic and 98 to 90 diastolic. The heart and lungs were normal. He was sent to an otolaryngologist, who made the diagnosis of nerve deafness. On July 1, 1942, he was placed under treatment with 6 Gm. of potassium chloride a day. Phenobarbital, up to 3 grains (0.195 Gm.) a day, was prescribed, and he was instructed to continue the restriction of salt intake. His symptoms had improved since he had started the potassium chloride therapy.

The past history revealed that he had had no severe illness. He was married and had no children.

There was no family history of epilepsy, migraine or deafness in the family. One sister was in a psychiatric hospital with a psychosis which had started during the puerperium. His other brothers and sisters were living and well.

Neurologic Examination.—The patient was tense and agitated concerning his symptoms and mentioned the possibility of operation. The fundi and pupils were normal. The extraocular muscles were normal; there was no nystagmus or diplopia. Hearing was slightly reduced on the left. Both air and bone conduction were affected. The Weber test revealed nothing abnormal. He said that he had an occasional crackling sound in the left ear. There was no loss of strength, coordination or sensation in the arms or legs. The deep reflexes were active and equal on the two sides; the plantar response was normal.

Diagnosis.—The diagnosis was Ménière's syndrome, in partial remission.

Treatment and Course.—It was recommended that the dose of potassium chloride be increased to 2 Gm. six times a day for three weeks and that he discontinue the low sodium diet but add no salt to his food at the table and eat no heavily salted foods.

On Oct. 5, 1942, he stated that he felt a great deal steadier on his feet and that he had had no digestive disturbances from taking the 12 Gm. of potassium chloride per day. On November 13 he stated that he was feeling still better but

had had one or two brief attacks of "falling forward" unassociated with dizziness. He had caught himself before he reached the floor.

On March 18, 1943, his dizziness had practically disappeared, but he had had one incident of falling forward while walking on the street, and this time he could not bring himself upright for a minute. He believed he had been momentarily unconscious. His dose of potassium chloride was reduced to 9 Gm. a day, and an electroencephalographic recording was ordered. The report from the Massachusetts General Hospital stated that the record showed irregular, 9.5 per second alpha rhythm and some beta activity. Voltages in the right parietal and occipital regions were higher than those on the left. Overbreathing for three minutes produced no significant changes in the record until the third minute. At that time some relatively prominent slow waves appeared in the occipitoparietal region. The whole appearance of the record was ragged and spiky.

On May 13, 1943, he had had no attacks of any kind, and the dose of potassium chloride was reduced to 1 Gm. six times a day. He was maintained on this dosage without any attacks until Jan. 25, 1944. At this time he had a fainting spell on the sidewalk. He fell on his back but was able to pick himself up and walk off. He was given 0.1 Gm. of phenobarbital per day and was maintained on the 6 Gm. of potassium chloride.

In August 1944 the dose of potassium chloride was reduced to 3 Gm. a day, since he had had no attacks of vertigo. In November 1944 he had had two attacks of falling but stated the belief that he did not completely lose consciousness. At this time treatment with 2 capsules (0.1 Gm. each) of diphenylhydantoin sodium per day was begun. Up to September 1945 he had had no attacks of any kind; the potassium chloride was discontinued but administration of 2 capsules of diphenylhydantoin was maintained.

On June 12, 1947, a second electroencephalogram was made, for it seemed possible that the large doses of potassium chloride had been related to his cerebral dysrhythmia. The dose of potassium chloride had been reduced to 3 Gm. thirty-four months before; he had received none for twenty-one months and had had no loss of consciousness for thirty-one months. The report from the Massachusetts General Hospital stated that the record was normal. Overbreathing for three minutes produced no change in the electroencephalogram. Comparison of this record with the earlier one, of March 19, 1943, showed that the two were much alike except that the later one contained far fewer artefacts, did not break down on overbreathing and showed normal activity in the right parietal region and only questionable disturbances in the right occipital region. After this report, the diphenylhydantoin was discontinued.

On April 4, 1948, the patient stated that he had had no lapses of consciousness and that he had noticed little, if any, dizziness during recent months.

SUMMARY

A man aged 47 had been given large doses of potassium chloride for Ménière's syndrome from July 1942 until August 1944. On Nov. 13, 1942, he had two brief attacks of "falling forward." The electroencephalogram taken after hyperventilation showed slow waves in the occipitoparietal region. He continued to have these brief lapses of consciousness until the dose of potassium chloride was reduced, in August 1944. The electroencephalogram taken twenty-one months after potassium chloride had been stopped showed a normal record, with no breakdown on hyperventilation. It is probable, therefore, that the cerebral dysrhythmia was related to the large doses of potassium chloride.

264 Beacon Street (16).

HAZARDS TO HEALTH

Salt Poisoning*

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THE recent Binghamton disaster, as summarized by Finberg et al.,¶ illustrated the toxic potential of "salt" in infancy. There, the inadvertent substitution of salt for sugar in the preparation of the newborn-nursery formula resulted in a "hyperelectrolyte formula" — and subsequently hypernatremic infants; 6 died. Other isolated reports of salt poisoning consequent to faulty formula preparation have been reported.

We have recently seen a set of fourteen-month-old twins with salt poisoning who acquired their illness more directly. They simply ate salt!

CASE REPORT

At 1:50 p.m. on April 19, 1963, 14-month-old twin Negro girls were admitted to the Columbus Children's Hospital Emergency Room because "they won't drink or eat." The products of their 19-year-old mother's 3d, uncomplicated pregnancy, Marla had weighed 2268 gm. (5 pounds), and Marta 1985 gm. (4 pounds, 6 ounces) at birth. Allegedly, both infants had been entirely well until 2 days before admission, when, in the evening, they both refused to drink their milk. On the next morning, 30 hours before admission, both children ate some egg but again refused to drink. From then until the time of admission the mother stated that neither infant had ingested anything by mouth; allegedly, neither had vomited, had any bowel movement or voided during the 24 hours before admission. Both were lethargic and irritable and refused to play; in each a mild rhinorrhea, but no cough or fever, had developed. At the time of admission no other contributory facts could be elicited.

The vital signs included normal temperatures (99.2 and 98.8°F.), normal respiratory rates (30 and 22) but slightly increased pulse rates (140 and 136). Both infants were lethargic but responded to stimuli. The skins were warm and dry. Subcutaneous turgor was decreased; there was a doughy consistence to the tissue. The fontanels were each slightly depressed, and the eyeballs sunken. Except for mild injection of the nasal mucosas and slight mucoid discharge from the nostrils, both physical examinations were within normal limits, as was neurologic examination — except as noted above. The lengths were 74 and 72 cm. respectively — between the 10th and 25th percentiles according to the Stuart grids. Weights, however, were decreased in both below the 3d percentile, being 5750 and 6350 gm. respectively.

Laboratory studies revealed, respectively, hemoglobins of 9 and 10.8 gm. per 100 ml. and white-cell counts of 13,400 and 19,800, with 78 and 80 per cent neutrophils and 22 and 20 per cent lymphocytes. Admission and follow-up electrolytes are included in Table 1. No urine was passed by either infant for almost 12 hours. Specific gravities then measured 1.030; otherwise — except for a trace of protein in 1 — both urinalyses were within normal limits. A total of 6 stool cultures revealed no pathogens. A single sickle-cell preparation (on Marla) was normal.

The Emergency Room resident immediately suspected hypernatremic dehydration and instituted appropriate diagnostic examinations and intravenous therapy with 0.2 per cent saline in 5 per cent glucose solution. Both children were then admitted to the hospital. Despite repeated questioning no history of either vomiting or diarrhea could be elicited. One twin continued to be treated with saline in glucose solution at a rate of 2000 ml. per square meter of body-surface area per day; the other received levulose (Levugen 75), a polyionic electrolyte solution, beginning 2 hours after arrival in the Emergency Room. The courses of the subsequent falls in serum sodium and related electrolyte concentrations are documented in Table 1. Approximately 24 hours after admission the mother returned to the hospital and, upon further questioning, related that she had, in fact, discovered a box of commercial table salt in the infants' bed and that the salt was distributed throughout the bedclothes.

She postulated that an older sib had made the "toy" available and possibly fed the salt to the twins. Since the 1½-pound box had been full 2 days before and now was almost empty, considerable exposure had taken place.

During the 1st 24 hours in the hospital neither infant vomited; neither had a bowel movement, and each voided but once — the specific gravity of both urines measuring 1.030.

Gradually, the lethargy disappeared, and activity was resumed. In 1 twin slight periorbital edema developed; neither gave other evidence of edema. On the 3d day feedings were resumed by mouth, and both children were subsequently discharged to the outpatient clinic.

A follow-up visit to the home approximately 1 month later was revealing in several respects. Soon after return home, the twins had resumed their previous activity and had remained well. Physical examinations gave no neurologic sequelae; a modified Gessel screening evaluation for developmental maturity showed both infants to be functioning at the 52-week level — within the range of normal. Finally, the examiner noted a fresh box of salt within easy grasp of the twins!

DISCUSSION

A hypernatremic state simply reflects a disproportion between serum sodium and serum water concentrations. It may result from an excessive intake of salt (in proportion to water) — as depicted above or as in cases of erroneously prepared formulas — or, as is most common (and more complex), from a disturbance in the homeostatic balance between oral intake, fecal loss and urinary excretion of both sodium and water that most frequently is associated with infantile diarrhea. Despite an elevated serum sodium, a total body deficit of sodium then actually exists, but this is overshadowed by the more significant body deficit of water. Central-nervous-system sequelae may ensue.

Management involves two factors: the elimination of cause (erroneous diet or diarrhea or both); and specific treatment of the hypernatremic state. Recommendations on the latter point vary considerably; the advantages and disadvantages of contrasting approaches will not be considered here. Suffice it to say that most authors frown on attempts at instantaneous correction.

This episode emphasizes the peculiar appetite of

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¶Finberg, L., Kiley, J., and Luttrell, C. Mass accidental salt poisoning in infancy. *J.A.M.A.* 184:187-190, 1963.

TABLE 1. *Initial and Follow-up Electrolyte Concentrations.*

TIME OF TEST	MARIA				MARTA			
	SODIUM	CHLORIDE	CARBON DIOXIDE	POTASSIUM	SODIUM	CHLORIDE	CARBON DIOXIDE	POTASSIUM
	milliequiv./liter	*milliequiv./liter*	*milliequiv./liter*	*milliequiv./liter*	*milliequiv./liter*	*milliequiv./liter*	*milliequiv./liter*	*milliequiv./liter*
On admission	182	149	14	4.3	179	143	12	4.0
At 8 hr.	180	151	15	4.2	177	144	14	3.9
At 20 hr.	176	146	14	3.0	171	142	18	4.0
At 32 hr.	166	136	15	3.2	162	138	19	4.3
At 44 hr.	155	129	15	4.0	152	126	16	5.0
At 68 hr.	147	116	21	4.7	145	111	22	4.9

the normal toddler. Moreover, the apparent failure of the thirst mechanism deserves mention. Whether as a consequence of curiosity, "oral needs," instinct or contrariness, young children have a tendency to outdo the most outrageous adult gourmet; at times they will eat anything (and at others nothing). Here, a bored and captive audience succumbed to temptation and apparently devoured "straight" salt. It should be noted that there is no record of their having vomited at anytime — hence, the need for skepticism about the emetic efficacy of concentrated sodium chloride solution. Moreover, Ward,* has recently reported a fatal case directly attributable to the use of hypertonic saline solution as an emetic.

We are confident that comparable but unreported episodes have probably occurred in the past: none were detected in our available literature. Moreover, we anticipate that, unfortunately, such cases may be expected to be repeated in the future. Parental awareness of the toxic potential of virtually all household items and of appropriate "prophylactic measures" might militate against such an occurrence.

*Ward, D. J. Fatal hypernatremia after saline emetic. *Brit. M. J.* 2: 432, 1963.

SUMMARY

Confined to their crib and apparently given a box of table salt for entertainment, a set of fourteen-month-old twins consumed an unknown quantity of sodium chloride. Thirty-six hours later — in the absence of either vomiting or diarrhea — they were admitted to the hospital with hypernatremic dehydration. Only after admission, when serum sodium concentrations were determined (179 and 182 milliequiv. per liter) did these historical facts come to light. With parenteral fluid (water and electrolytes) administration recovery was reasonably prompt and seems to have been complete.

Correction. The last sentence of the last paragraph of the right-hand column on page 455 of the Special Article, "Caring for the Patient," by Dr. Herrman L. Blumgart, which appeared in the February 27 issue of the *Journal,* should be changed to read as follows: "For such people, as well as others, the plain unvarnished truth is for them, in the words of John Donne:

> One short sleep past, we wake eternally.
> And death shall be no more: death,
> thou shalt die."

0469

British Medical Journal

Brit. Med. Jour
No. 5494 : 1010-1013

Association of Brain Damage with Therapeutic Abortion Induced by Amniotic-Fluid Replacement: Report of Two Cases

9081

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[WITH SPECIAL PLATE]

Brit. med. J., 1966, 1, 1010–1013

Recently amniotic-fluid replacement by the intrauterine injection of strongly hypertonic solutions has been used increasingly often to produce therapeutic abortions or to induce labour when the foetus is already known to be dead (Csapo, 1961; Wood, Booth, and Pinkerton, 1962). Few complications of the method have been reported, though it has been tried and discarded by other workers because of the risk of intrauterine sepsis (Peel, 1935). Two cases are reported in which widespread cerebral infarction occurred after the procedure and in which it was possible that the strongly hypertonic solutions employed were responsible for the brain damage.

Case 1

A previously healthy 20-year-old secretary was advised, on psychiatric grounds, to have a therapeutic abortion in the 20th week of her first pregnancy. The operation was performed under local anaesthesia by inserting a fine trocar and cannula through the anterior abdominal and uterine walls into the amniotic cavity, removing about 200 ml. of amniotic fluid, which was lightly blood-stained, and injecting manually approximately 400 ml. of sterile 20% sodium chloride solution. She appeared well immediately afterwards, but complained of a burning sensation in her finger-tips. After about four hours her condition deteriorated, and she began to have epileptiform fits. About 12 hours after the operation her blood-pressure was said to be normal. Sedatives were given parenterally as symptomatic treatment, but she suddenly stopped breathing and died some 22 hours after the intrauterine injections. A dead foetus had been delivered 12 hours after the intra-amniotic injection. Twelve hours after the amniocentesis serum electrolytes were: chlorides 143 mEq/l., sodium 174 mEq/l., potassium 3.7 mEq/l., urea 26 mg./100 ml.

Necropsy showed evidence of the recent pregnancy. In the anterior wall of the fundus of the uterus there were two puncture wounds which appeared to have penetrated through the placental bed. The brain on removal appeared to be slightly oedematous, and no subdural or subarachnoid haemorrhages were seen. The lungs showed patchy collapse. The cardiovascular system seemed to be normal and there was no evidence of air embolism. There was no peritonitis, nor any excess of fluid or blood in the peritoneal cavity. The kidneys appeared normal. The foetus and placenta were not available for examination.

Microscopical Examination of Viscera

Sections of heart, lungs, kidneys, liver, and spleen showed no histological abnormality. Pregnancy changes were seen in the pituitary, the breasts were active, and trophoblastic tissue was seen at the placental site in the uterus.

Brain and Spinal Cord

After fixation the brain was cut in coronal slices. These showed symmetrical haemorrhagic softenings of both amygdaloid nuclei, sparing only a thin rim supero-medially, and similar softening of the adjacent parts of both the right and left uncus (Special Plate, Fig. 1). The inferior parts of both claustra and the adjacent insular cortex were haemorrhagic. Other parts of the cerebrum appeared normal. There was a Y-shaped haemorrhage 2 cm. long in the pons, occupying the tegmental region and the ventral part of the median raphe (Special Plate, Fig. 2). The upper medulla appeared normal, but the region of the facial tubercles was congested. The cerebellum appeared normal, as did the spinal cord externally and after cutting transverse sections.

The distribution of lesions seen histologically in the brain is shown diagrammatically in Fig. 3 (Special Plate), where dotted areas represent the damaged parts. The appearances were similar at all the sites examined. Areas of acute selective neuronal necrosis shown by pyknosis and commencing fragmentation were present in sections from the frontal and more anterior parts of the parietal cortex (Special Plate, Fig. 6), but not from the occipital cortex or the posterior parts of the parietal lobes. These lesions were usually found at the crests of gyri and commonly were isolated, though a pseudo-laminar distribution was seen in a few areas. The adjacent white matter appeared normal. In both amygdaloid nuclei there was almost total haemorrhagic infarction (Special Plate, Fig. 7), and similar but less extensive necrosis of neurones and glial cells was seen in the head of the caudate nuclei, ansa lenticularis, and globus pallidus on both sides. The end-plate region and Somner's h_1 section of Ammon's horn were damaged bilaterally, as well as the grey matter of the adjacent anterior end of the lingual gyrus. The tegmentum and median raphe of the pons showed severe haemorrhagic infarction (Special Plate, Fig. 8), and there were less intense symmetrical lesions in the dorsal part of the medulla involving the region of the tractus solitarius and medial vestibular nuclei as far down as the level of the dorsal decussation. In affected areas myelin sheaths appeared to be forming balloons and to be fragmenting, and silver stains showed similar damage to axons. Blood-vessels everywhere appeared normal apart from some margination of polymorphs in capillaries in necrotic areas and pools of protein-rich oedema fluid about capillaries near them.

Sections of the spinal cord and its anterior and posterior roots at all levels appeared normal. No lesions were seen in the spinal dura and extradural venous plexus.

Case 2

A 20-year-old student teacher in good physical health was recommended, after psychiatric examination, for a therapeutic abortion in the 20th week of her first pregnancy. Amniocentesis was carried out under general anaesthesia by aspirating 200 ml. of amniotic fluid and injecting in its place 150 ml. of 30% sodium chloride solution. She never fully regained consciousness, though one hour after the operation she appeared to respond to painful stimuli. One hour later she had the first of several generalized convulsions, which occurred at varying intervals. Her temperature was raised, she was stuporous, responding to painful stimuli, and had a spastic cog-wheel rigidity in all four limbs on passive movement. The plantar reflexes became extensor after 24 hours. There was sludging of red cells in the retinal veins on the right side. The disc margins were distinct and there was no papilloedema. Lumbar puncture, 25 hours post-operatively, showed normal pulsation of a clear

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FIG. 1

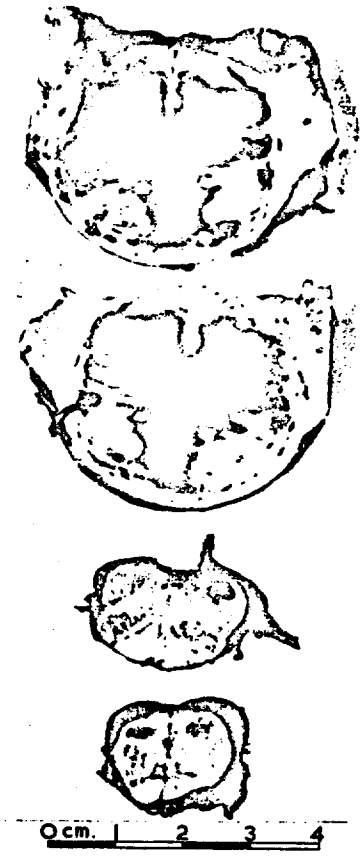


FIG. 2

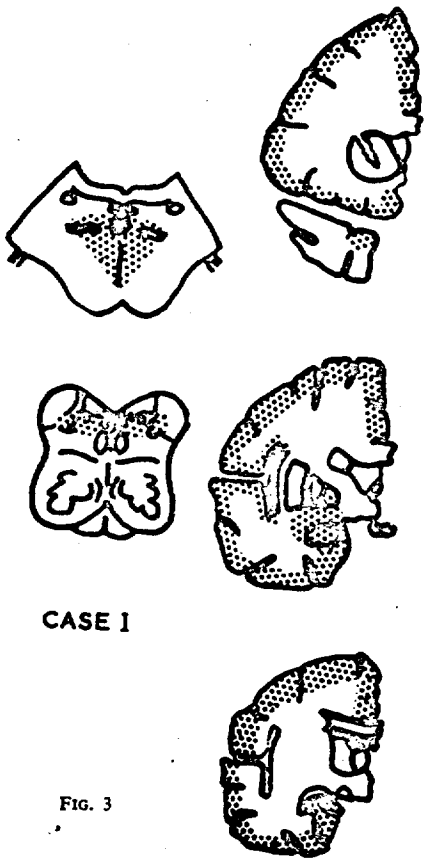


FIG. 3

FIG. 4

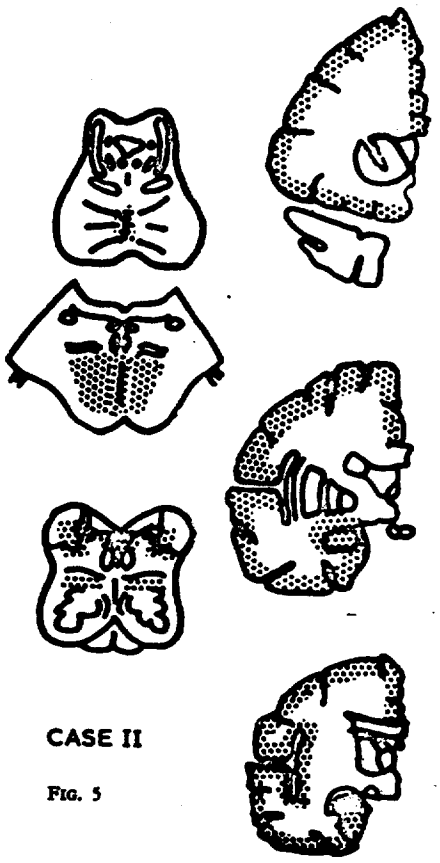


FIG. 5

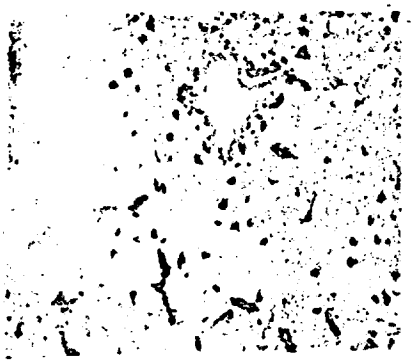
FIG. 1.—Case 1. Coronal slices of brain, showing haemorrhagic infarction of both amygdaloid nuclei and the adjacent parts of the temporal lobes.

FIG. 2.—Case 1. Transverse slices of the pons and medulla. There are haemorrhagic infarcts in the tegmentum and the pes pontis and discrete foci of necrosis in the region of the vestibular nuclei on both sides.

FIG. 3.—Case 1. Diagrammatic representation of the distribution of the lesions in the brain.

FIG. 4.—Case 2. Discoloration about median raphe of pons.

FIG. 5.—Case 2. Diagram to show the distribution of lesions in the brain.



6.—Case 1. Parietal cortex with small hemorrhages and pyknotic neurones. (Haematoxylin and eosin. ×320.)

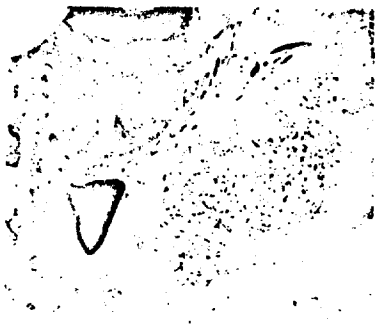
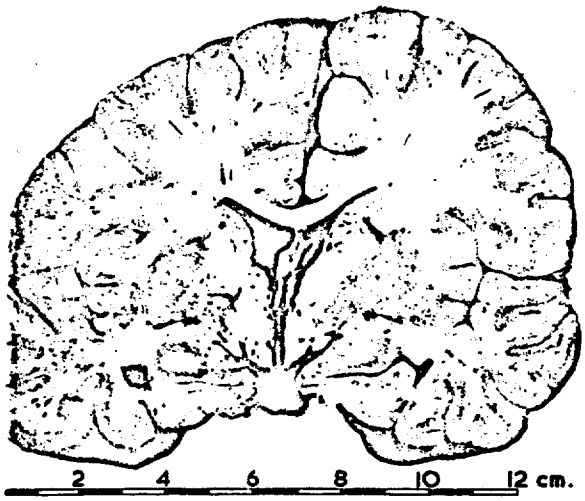


FIG. 7.—Case 1. Haemorrhagic infarction of right amygdaloid nucleus. (Loyez. ×2.2.)

FIG. 8.—Case 1. Infarction of pes pontis. (H. and E. ×2.)



. 9.—Case 2. Discoloration and infarction of both amygdaloid nuclei.

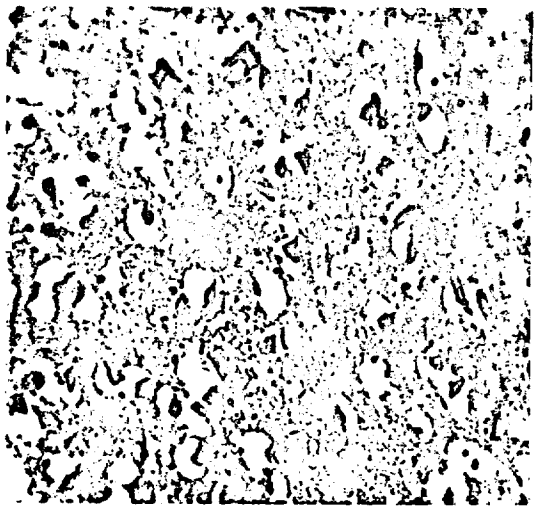


FIG. 10.—Case 2. Acute neuronal necrosis in the temporal cortex. (H. and E. ×400.)

. 11.—Case 2. Polymorphonuclear leucocytes ing a vessel in the median raphe of the pons. (H. and E. ×640.)

FIG. 12.—Case 2. Pallor of staining of myelin in middle of pes pontis. (Loyez. ×6.)

yellow fluid at a pressure of 180 mm. H_2O. It contained 5 lymphocytes/c.mm., and had a protein level of 1,000 mg./100 ml. Slight haematuria and albuminuria was noted in the concentrated urine obtained by catheterization at this time.

Shortly before death the membranes were ruptured and a dead foetus and placenta were delivered. The patient died 45 hours after the operation, her condition having remained unchanged for the previous 24 hours.

Necropsy showed evidence of recent delivery. There were multiple puncture wounds in the anterior wall of the uterus. The brain was slightly oedematous, and no major subdural or subarachnoid haemorrhages were seen. There were bilateral pleural effusions and a peritoneal exudate (approximately 400 ml. in each cavity). The cardiovascular system appeared normal, and there was no evidence of air embolism. The remaining organs appeared healthy.

Microscopical Examination of Viscera

The lungs were congested and oedematous, and there was a small amount of agonal vomitus in the bronchi, with patchy collapse and a low-grade inflammatory reaction. Occasional hyaline casts were seen in the renal tubules. In other viscera, apart from congestion, no abnormality was found.

Brain and Spinal Cord

After fixation the brain was moderately tense and showed some symmetrical uncinate grooving but no definite tonsillar herniation. The leptomeninges were deeply congested, particularly over the convexity of the cerebral hemispheres, and showed a few tiny subarachnoid haemorrhages.

Coronal sections of the cerebral hemispheres showed central discoloration and softening in both amygdaloid nuclei (Special Plate, Fig. 9). Ill-defined discoloration was also present in the uncus and in Ammon's horn on both sides. Patchy softening of the cerebral cortex was most conspicuous in the frontal lobes, particularly on the medial surfaces. Transverse sections of the brain-stem revealed a strip of greyish discoloration in the pes pontis in the neighbourhood of the central raphe (Special Plate, Fig. 4). Dorsally it merged into an ill-defined greyish area in the tegmentum which continued caudally into the medulla. Sagittal sections of the cerebellum showed a discoloration of the deeper parts of the folia, particularly in the vermis. The spinal cord appeared normal externally and on transverse section.

Microscopical examination of the brain showed symmetrical lesions resembling those seen in Case 1. Their distribution is represented in Fig. 5 (Special Plate). The cortex of the frontal, parietal, and temporal lobes showed widespread acute neuronal necrosis, most marked at the crests of gyri (Special Plate, Fig. 10) and often associated with microscopic haemorrhages. In a few areas, such as the parietal cortex, occasional geographical areas of pseudo-laminar necrosis were present, but the commonest findings were of scattered eosinophilic and pyknotic neurones and of congested capillaries, many of which contained marginated polymorphs. The occipital lobes were not affected. In the amygdaloid nuclei on both sides there was very extensive neuronal necrosis and small haemorrhages and perivascular oedema, but no total infarction as seen in Case 1. The adjacent lingual gyri and claustrum were affected to a lesser extent. No damage was found in the basal ganglia. There was marked neuronal destruction and some oedema in the dorso-medial nuclei of the thalamus. In Ammon's horn eosinophilic and pyknotic neurones were found in the end-plate and Sommer's h_1 sector throughout its length. Similar lesions were present in the h_3 sector and the adjacent parts of the dentate gyrus which were associated with capillary haemorrhages.

In the brain-stem most blood-vessels were congested, and midline perforating branches of the basilar artery in the pes pontis at the level of the locus coeruleus were cuffed by polymorphonuclear leucocytes (Special Plate, Fig. 11). Loyez-stained preparations showed pallor of myelin staining in the midline at this level (Special Plate, Fig. 12). There was also acute neuronal necrosis in the nuclei of the pes pontis and reticular formation. At lower levels selective damage to neurones, with the development of vacuoles about the degenerating cell bodies, was seen in the medial and spinal vestibular nuclei and in both hypoglossal nerve nuclei. At the caudal limits of the inferior olives, vacuolation and pyknosis of neurones was marked in the region of the lateral reticular nuclei and in the spinal tract of the trigeminal nerve. In the dorsal part of the inferior medulla several structures were damaged, including the dorsal motor nucleus of the vagus, the cuneate and gracile nuclei, and the spinal tract of the trigeminal nerve. The cerebellum showed oedema of the granular layer, which is often considered to be a post-mortem artefact. The spinal cord appeared normal throughout its length.

Discussion

The cerebral damage found in these patients, in accordance with the clinical history, appears histologically to be of not more than 24 to 48 hours' duration. The lesions have a roughly symmetrical distribution, suggesting that they might have been caused by hypotension or by some systemic metabolic or toxic disturbance.

Systemic hypotension may produce "geographical" and pseudo-laminar necroses in the cerebral cortex which sometimes are associated with damage to the basal ganglia and cerebellum (reviewed by Meyer, 1963). There was no direct evidence of a hypotensive episode in either of the present patients. At necropsy the most marked lesions were in the amygdaloid nuclei, the pons, and the lateral parts of the medulla, whereas the cerebral cortex and basal ganglia were relatively less severely affected. Also, definite lesions were not found in the cerebellum of either patient. There appears to be no record of a proved case of hypotensive brain damage in which the distribution of the lesions has resembled that found in these two patients. It seems unlikely, therefore, that generalized arterial hypotension could have been the cause of the brain damage found in our two patients.

It is more likely that the cerebral infarction was due to some effect on the brain of the large volumes of strongly hypertonic solutions which had been injected into the amniotic cavity. Since the work of Weed and McKibben (1919–20) it has been known that hypertonic solutions of saline or dextrose, when injected intravenously, may cause a fall in the cerebrospinal fluid (C.S.F.) pressure and make the brain shrink. Cushing and Foley (1920) made similar observations in man and showed that oral administration of a concentrated solution had the same effect. The mode of action is thought to be extraction of water from the brain and C.S.F. by the hypertonic blood, from which they are separated by an effectively semipermeable membrane. However, Browder (1930) and Milles and Hurwitz (1932) reported that the abnormally low C.S.F. pressure produced in such a fashion increased after a few hours and might even come to exceed the normal level by a considerable amount. Similar observations have been made by more recent workers, who have observed increases of pressure up to 180% of control levels (Stubbs and Pennybacker, 1960 ; Rosomoff, 1962 ; Nash, Orth, and Smolik, 1965). This "rebound" rise in pressure is attributed to the slow mass movement of solutes across the blood–brain barrier. The brain-fluid compartments are still hypertonic when the blood has returned to its normal tonicity, and the rapid entry of water into the cerebral-fluid compartments causes the rise in pressure.

If this series of events had occurred in our two patients, then some of the lesions could be secondary to the raised intracranial pressure. The time of onset of symptoms is compatible with the delay required for this rebound rise in pressure. It was about four hours in Case 1 and some two hours in Case 2, and both patients might already have been slightly dehydrated by the usual practice of withholding fluids before any general anaesthetic is given. We assume that an intra-amniotic pool of 20% saline would be as effective in rendering the blood hypertonic as is oral or direct intravenous administration. If the brain had become swollen the infarction of the amygdaloid nuclei and adjacent structures would be attributed to compression of the anterior choroidal artery by

downward displacement of the uncus through the tentorial opening (Lindenberg, 1955). Though no deep uncinate grooves were found at necropsy, sufficient time had elapsed before death for the cerebral oedema to have disappeared. Also, both patients had been given intravenous infusions of hypotonic and isotonic fluids, which would tend to relieve the osmotic imbalance. The second patient was probably given such treatment at a much earlier stage than the first, and this may account for the difference in the severity of the lesions in the two cases. The pontine damage is unlikely to be due to such secondary arterial insufficiency, because its Y-shape does not conform to the usual midline distribution of the lesions found in such cases. Extension into the medulla is exceedingly rare for secondary damage of this kind, and even then it does not usually pick out discrete nuclear groups, as was seen in both cases.

Epileptic convulsions may cause diffuse lesions in the amygdaloid nuclei and adjacent structures (Meyer, Beck, and Shepherd, 1955), but these were of selective neuronal necrosis only. They resembled the findings in Case 2, but were quite unlike the total infarction seen in Case 1. The widespread cortical lesions in Case 2 also resemble those described in a fatal case of epilepsy by Small and Woolf (1957). The damage to the pons and medulla in both of the present cases had not been found after epileptic fits. It is unlikely, therefore, that all the damage could be secondary to the convulsions. The second patient was noticed to have "sludging" of the blood in her retinal veins some 24 hours after the amniocentesis. At this late stage in her illness it is impossible to be certain of the significance of this observation, and it cannot be taken to mean increased viscosity of the blood due to hypovolaemia produced by dehydration.

Brain Damage in Other Clinical Conditions

We have been unable to find any reports of cases comparable to the present ones. Other clinical conditions in which brain damage has been associated with hypertonicity of the extracellular-fluid compartment have been reported by Finberg, Kiley, and Lutterell (1963). They described an incident in which 14 infants were given artificial feeds made up with salt instead of sugar. Necropsies were done on five of the six babies who died; these showed widespread capillary haemorrhages in the brain, subdural and subarachnoid haemorrhages, and thrombosis of the major dural sinuses. Finberg (1959) discussed seven other reported cases of salt-poisoning in infants and noted that similar pathological changes had occurred. Cases have been reported in which patients have become dehydrated because of gastro-enteritis or mismanaged régimes of intravenous fluids and gastric suction, so producing more complex deficiencies of water and electrolytes. Crome (1952) found widespread focal scars in the cortex, gliosis in the putamen and outer globus pallidus, and diffuse gliosis throughout the brain-stem of a child aged 25 weeks who died after severe dehydration due to enterocolitis. Hallevorden and Meyer (1956) reported a similar case of a 6-months-old baby who collapsed with enterocolitis and was found later to have diffuse cortical scarring, *état marbré* of the putamen, and lobular sclerosis of the cerebellum.

Mérei (1960), Métraux and Binswanger (1963), and Ludwig (1963) have described adult patients dying in a state of severe dehydration, who were found at necropsy to have widespread petechiae throughout the cerebral hemispheres and cerebellum. Most of these patients also had scattered necroses in the cerebral cortex and sometimes in the basal ganglia and cerebellum. Ludwig's case also had bilaterally symmetrical haemorrhages resembling those found in the medulla of our own cases, and Mérei's patient showed damage in the region of the vestibular nuclei, as in both the present cases. In fatal cases serum sodium levels have been estimated at various times before or after the onset of cerebral symptoms, and the reported levels are shown in the Table.

Plasma Sodium concentrations in Reported Cases of Fatal Dehydration with Brain Damage

Author	Date	Plasma Sodium Concentrations
Skinner and Moll	1956	170–192 mEq/l.
Finberg	1959	142–193 ,,
Ludwig	1963	167 ,,
Métraux and Binswanger	1963	159 ,,
Finberg *et al.*	1963	274 ,,
Present series (Case 1)	1966	174 ,,

Ward (1963) described the case of a patient suffering from overdosage with perphenazine and imipramine to whom 60 g. of sodium chloride had been given to induce vomiting. Most of the salt was retained and the patient died after 24 hours, with a serum sodium level of 174 mEq/l. Lesions in the brain were not described. Alexander and Wu (1935) discussed focal cortical scars and gliosis in the brains of patients dying from a variety of forms of dysentery and enterocolitis. They concluded that the lesions were due to an unspecified vascular cause, and briefly reviewed previous reports of similar lesions in a wide variety of febrile conditions. The latter could well have been associated with dehydration. Clinical accounts of the symptoms and signs of dehydration have also been given by Dodge *et al.* (1962) and Githens (1963).

A further condition in which dehydration and hypertonicity of the body fluids is of clinical importance is heat-stroke. Malamud, Haymaker, and Custer (1946) described pathological findings in the brains of 40 cases of heat-stroke and noted acute changes, cerebral oedema (brain weights were increased up to 1,493 g.), and haemorrhages around the third and fourth ventricles. After 24 hours there was loss of neurones from the cerebral cortex and basal ganglia and a commencing glial reaction. The possible importance of mechanical swelling of the brain in the causation of damage is suggested by the presence of cerebral oedema in the cases of Mérei (1960) (brain weight 1,580 g.) and of Ludwig (1963, Case 1) (brain weight 1,940 g.). Oedema was also noted in Case 6 of the series of seven cases of dehydration in infants due to diarrhoea reported by Skinner and Moll (1956). It is now a commonplace therapeutic manœuvre to treat such disorders as cerebral oedema and incipient acute renal failure by the intravenous or intragastric administration of a hypertonic solution. We have been unable to find any reports of brain damage in such patients which has resembled that described above. Such patients are usually given smaller amounts of solute than were our patients, and their fluid balance is controlled more carefully. Perhaps these factors might account for the lack of reports of brain damage, and if lesions have been observed they may well have been attributed to the primary disease and not to the treatment.

Neither naturally occurring diseases in animals nor experiments have produced morbid anatomical lesions resembling those described in this paper. There are many reports of a disease in pigs called "eosinophilic meningo-encephalitis," which is due to hypernatraemia and dehydration. In this condition there are diffuse cerebral cortical necroses and perivascular infiltration by polymorphs and eosinophils (Innes and Saunders, 1962). In no account of it is there a description of a pattern of lesions comparable to that found in human beings. In the laboratory subdural and subarachnoid haemorrhages have been found in several species of animals after intravenous or intraperitoneal injection of hypertonic solutions of urea, dextrose, or saline (Girard, 1956; Finberg, 1959; Luttrell, Finberg, and Drawdy, 1959; Rush, Finberg, Daviglus, and Cheung, 1961). The intracerebral lesions described by these authors have been punctate haemorrhages in both the grey and the white matter of the cerebral hemispheres and

cerebellum. Luttrell *et al.* (1959) also mention cortical damage, especially in the depths of the sulci, where there was failure of staining of neurones and many pyknotic nuclei were seen.

All these experiments have been of too short a duration for reliable histological signs of the necrosis to have occurred, although E.E.G. records have suggested acute cortical damage (Sotos, Dodge, Meara, and Talbot, 1960). In these experiments it has been shown that, although the systemic blood-pressure may fall, cerebral damage occurs even if it is kept constant (Rush *et al.*, 1961). Luttrell *et al.* (1959) also confirmed the role of mechanical displacement of the brain in causing cerebral damage after dehydration. In their experiments lesions in the brain were completely prevented by the intracisternal injection of sufficient isotonic saline to maintain cerebrospinal fluid pressure at a normal level.

Conclusion

We consider that the cerebral infarction found in the present cases was due to a combination of arterial compression secondary to cerebral oedema, and, perhaps, to some more direct effect of the hypertonic state on sensitive structures. It is also possible that systemic arterial hypotension and anoxia may have aided the production of the diffuse cortical damage noted both in these patients and in the somewhat similar cases reported by Alexander and Wu (1935), Crome (1952), and Hallevorden and Meyer (1956).

Csapo (personal communication, 1964) and Fuchs (personal communication, 1964) state that they and others have used the amniotic-fluid-replacement technique in several thousand patients without any catastrophic results, although Wagatsuma (1965) reported at least 25 deaths following its use in Japan. The explanation for the difference between their findings and our cases is not certain. It is possible that a larger amount of hypertonic saline was injected into the first patient than is usually employed and that less amniotic fluid had been left behind to dilute it. As none of the injected fluid was available for analysis the actual concentration of sodium chloride used could not be checked. It is also possible that some of the hypertonic saline was injected not into the amniotic cavity but directly into the maternal circulation via the enlarged blood-vessels in the placental bed. If this had happened concentrated saline could have reached the brain directly via Batson's vertebral venous plexus. Against this are the delays of two and four hours before definite signs of brain damage appeared. These intervals are comparable with those observed in animal experiments after the intraperitoneal injection of hypertonic solutions, and are considerably longer than those found after intravenous injections (Sotos *et al.*, 1960; Dodge *et al.*, 1962). Although it is less valuable as evidence, the lack of histological damage to the spinal cord and extradural spinal veins also argues against the possible role of Batson's plexus.

We consider that the amniotic-fluid-replacement technique for inducing abortions should be used with caution until further information is available of any potential danger to the mother of the hypertonic state of the body fluids which it may induce. The same problem applies to any therapeutic use of hypertonic solutions for other purposes. As reviewed by Sotos *et al.* (1960), Ward (1963), and Githens (1964), the basic treatment of this disease of blood hypertonicity is the parenteral administration of suitable hypotonic fluids. In an emergency it might be helpful to try the manœuvre employed experimentally by Luttrell *et al.* (1959) and to inject saline into the cisterna magna (or perhaps by an ordinary lumbar puncture) to maintain a normal C.S.F. pressure.

Summary

Two cases are described of 20-year-old women in whom abortions were induced by the intra-amniotic injection of hypertonic sodium chloride solution. Both patients died with acute haemorrhagic infarction of the amygdaloid nuclei and adjacent structures and a Y-shaped infarct in the pons.

From other human cases and animal experiments it is suggested that the cerebral damage was due to excessive dehydration of the brain caused by the intra-amniotic injection of the strongly hypertonic solution.

It is suggested that the amniotic-fluid-replacement technique for inducing abortions should be used with care so as to avoid the production of excessive hypertonicity of the mother's circulation.

ADDENDUM.—Since the submission of this paper one of us (J. M. C.) has been involved with a further case. The almost identical history and post-mortem and microscopic findings were found in a 23-year-old woman following amniocentesis. This case will be discussed in detail at a later date.

We are grateful to H.M. Coroners, Drs. A. G. Davies and R. I. Milne, for permission to report these two cases. We wish to thank Professors F. E. Camps and I. Doniach and Dr. H. Urich for their advice on the preparation of this paper.

REFERENCES

Alexander, L., and Wu, T. T. (1935). *Arch. Neurol. (Chic.)*, **33**, 72.
Browder, J. (1930). *Amer. J. Surg.*, **8**, 1213.
Crome, L. (1952). *Arch. Dis. Childh.*, **27**, 468.
Csapo, A. (1961). In *Progesterone and the Defence Mechanism of Pregnancy, edited by G. E. W. Wolstenholme and M. P. Cameron*, p. 3. Churchill, London.
Cushing, H., and Foley, F. E. B. (1920). *Proc. Soc. exp. Biol. (N.Y.)*, **17**, 217.
Dodge, P. R., Sotos, J. F., Gamstorp, Ingrid, De Vivo, D., Levy, Miriam, and Rabe, T. (1962). *Trans. Amer. neurol. Ass.*, **87**, 33.
Finberg, L. (1959). *Pediatrics*, **23**, 40.
—— Kiley, J., and Luttrell, C. N. (1963). *J. Amer. med. Ass.*, **184**, 187.
Girard, F. (1956). *Acta pediat. Upsala*, **45**, 618.
Githens, J. H. (editor) (1963). *Clin. Pediat. (Pa.)*, **2**, 453.
Hallevorden, J., and Meyer, J. E. (1956). In *Handbuch der speziellen pathologischen Anatomie*, edited by O. Lubarsch, F. Henke, and R. Rössle, section 13, vol. 4, p. 211. Springer, Berlin.
Innes, J. R. M., and Saunders, L. Z. (1962). In *Comparative Neuropathology*, p. 707. Academic Press, London.
Lindenberg, R. (1955). *J. Neuropath. exp. Neurol.*, **14**, 223.
Ludwig, J. (1963). *Schweiz. med. Wschr.*, **93**, 705.
Luttrell, C. N., Finberg, L., and Drawdy, L. P. (1959). *Arch. Neurol. (Chic.)*, **1**, 153.
Malamud, N., Haymaker, W., and Custer, R. P. (1946). *Milit. Surg.*, **99**, 397.
Mérei, F. T. (1960). *Dtsch. Z. Nervenheilk.*, **181**, 174.
Métraux, H. R., and Binswanger, U. (1963). *Schweiz. med. Wschr.*, **93**, 1563.
Meyer, A. (1963). In *Greenfield's Neuropathology*, edited by W. Blackwood, W. H. McMenemey, A. Meyer, R. M. Norman, and Dorothy S. Russell. 2nd ed. Arnold, London.
—— Beck, E., and Shepherd, M. (1955). *J. Neurol. Neurosurg. Psychiat.*, **18**, 24.
Milles, G., and Hurwitz, P. (1932). *Arch. Surg.*, **24**, 591.
Nash, F. P., Orth, G., and Smolik, E. A. (1965). *J. Neurosurg.*, **22**, 264.
Peel, J. (1933). Quoted by Peel, J. (1962). *Brit. med. J.*, **2**, 1397.
Rosemoff, H. L. (1962). *J. Neurosurg.*, **19**, 859.
Rush, B. F., Finberg, L., Davighus, G. F., and Cheung, C.-S. (1961). *Surgery*, **50**, 359.
Skinner, A. L., and Moll, F. C. (1958). *Amer. J. Dis. Child.*, **92**, 562.
Small, J. M., and Woolf, A. L. (1957). *J. Neurol. Neurosurg. Psychiat.*, **20**, 293.
Sotos, J. F., Dodge, P. R., Meara, Pamela, and Talbot, N. B. (1960). *Pediatrics*, **26**, 925.
Stubbs, J., and Pennybacker, J. (1960). *Lancet*, **1**, 1094.
Wagatsuma, T. (1965). *Amer. J. Obstet. Gynec.*, **93**, 743.
Ward, D. T. (1963). *Brit. med. J.*, **2**, 432.
Weed, L. H., and McKibben, P. S. (1919–20). *Amer. J. Physiol.*, **48**, 512.
Wood, C., Booth, R. T., and Pinkerton, J. H. M. (1962). *Brit. med. J.*, **2**, 706.

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BRAIN DAMAGE FOLLOWING THERAPEUTIC ABORTION BY AMNIOTIC FLUID REPLACEMENT: AN EXPERIMENTAL APPROACH

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THREE deaths following the induction of therapeutic abortion using the technique of replacing amniotic fluid by strongly hypertonic saline were reported in 1966 (Cameron and Dayan, 1966; Dayan, Cameron and Phillipp, 1967). The patients were all previously healthy young women who were advised on psychiatric grounds to have therapeutic abortions in the seventeenth to twentieth weeks of their first pregnancies. In each case after inserting a needle through the anterior abdominal wall a volume of amniotic fluid was removed and replaced by hypertonic saline. Volumes of between 150 and 400 ml. were used, containing from 45 to 80 g. of sodium chloride. All three patients developed generalized convulsions within a few hours of the operation and died within 22 to 54 hours. The postmortem findings in each case were similar: symmetrical haemorrhagic infarction of the brain, involving particularly the region of the amygdaloid nuclei and a Y-shaped area in the pons. This was thought to be related to the effect on the brain of severe hypernatraemia. The autopsy findings were consistent with the injected solution having seeped out of the uterine cavity through multiple puncture sites into the peritoneal cavity, or perhaps having been, at least in part, injected directly into either the placental bed or peritoneal cavity. The present experiments attempt to recreate, by the use of intraperitoneal saline, similar conditions in animals.

The animal in which the cerebral effects of hypernatraemia have been most widely investigated is the pig. Animals fed on meal with a high salt content and deprived at the same time of free access to water develop a syndrome known as "salt poisoning". This is characterized by the animals becoming depressed, ataxic, behaving strangely, developing convulsions, and finally dying in coma. It has been established that this syndrome is related to the high osmotic content of the diet (Smith, 1955), and that although this is usually due to the high sodium chloride content a similar picture can also be produced by other osmotically active substances (Done, Harding and Lloyd, 1959). The brain lesions are characteristic, presenting a picture known as "meningo-encephalitis eosinophilica". We have seen no reports of the administration of hypertonic saline to pigs other than by the oral or intravenous routes and it seemed likely that this animal might prove suitable for investigation of the syndrome following amniocentesis.

METHOD

The pigs were all 5- to 6-week-old gilts of average weight 17·5 kg. They were fed on a basic meal diet which contained 0·4 per cent salt, and, except for those animals receiving the high sodium diet, were allowed free access to water.

The pigs were divided into two groups: one received a high salt diet whilst the other received intraperitoneal injections of hypertonic sodium chloride solution. The former group was given the basic diet with added sodium chloride to the

extent of 5·5 per cent and a restricted water allowance of two-and-a-half times the weight of dry food. For intraperitoneal administration a sterile solution containing 32·5 g. sodium chloride per 100 ml. was injected directly into the peritoneal cavity through the anterior abdominal wall without anaesthetic. Volumes of from 20 to 150 ml. were injected at a time, some animals receiving only one such injection whilst others received up to two injections a day for four days. Some of the animals died unobserved during the night; others were killed with light chloroform anaesthesia followed by cutting the throat. Postmortem examination was carried out as soon as possible, samples of all organs being taken for routine histology and the brains being fixed in formalin. The latter were subsequently cut into coronal sections and stained with haematoxylin and eosin. Wherever possible samples of peripheral blood were taken during life for estimation of serum electrolytes. For this purpose blood was aspirated from ear veins, but in some cases it proved impossible to obtain. Postmortem analysis of both blood and peritoneal fluid, when present, was carried out.

Results

Dietary Saline

The administration of a high percentage salt diet to the pigs in the first group was not accompanied by any specific symptoms of salt poisoning, apart from the animals becoming reluctant to feed. After nine days of dietary treatment, one animal was killed, and the two others, which were considered to be clinically normal, were returned to a normal diet and subsequently used in another experiment. When the brain of the sacrificed animal was examined histologically, however, the changes of salt poisoning were seen. Laminar malacia of the middle layers of the cerebral cortex was found, most marked in the sulci of the parietal regions and accompanied by neuronal degeneration and

Table I

Experimental Treatment, Histological Features in Brain and Postmortem Sodium Estimations

Pig Number	Sodium Chloride Injected (g.)	Days of Experiment	Mode of Death	Cortical Spongiosis	Neuronal Necrosis	Purkinje Cell Damage	Subarachnoid Haemorrhage	Peritoneal Exudate	Postmortem Serum Sodium (mEq l.)	Peritoneal Fluid Sodium Concentration (mEq l.)
1	0	0	K						149	
2	0	0	K						138	
3	?	9	K	+++					166	
4	26+?	1+9	D		+++	−		−	152	U
5	26+?	1+9	D	++	+++			−	180	U
6	26	2	K	+++		++		−	U	U
7	26	1	D	+				abscess	146	155
8	45·5	4	K	+++	++	++		+	184	208
9	45·5	2	D		++			+	U	U
10	52	4	D			+			200	
11	52	2	K	++	++		+	abscess	134	155
12	71·5	5	K		+	+		+	160	U
13	71·5	3	K	+++	+++	+	+	+	207	165
14	81·3	1	K						U	
15	97·5	4	D					+	175	223
16	149·5	4	D		++		+	+	182	U
17	156	4	K			++	+	+	151	U

The two animals above the top line were controls; those between the two lines received diet alone (pig 3) or diet and subsequently intraperitoneal saline (pigs 4 and 5). ? indicates unknown dietary saline intake. U = specimen unobtainable, K = killed, D = died.

a glial reaction consistent with a lesion of a number of days standing. Outside the necrotic areas the neurones appeared normal, as did those in the brain stem and cerebellum. Eosinophilic infiltration of the meninges and perivascular spaces was minimal.

Effects of Intraperitoneal Injection

Administration of intraperitoneal saline was accompanied in all instances by symptoms, both immediate and delayed. At the time of injection the animals were obviously upset by the procedure to a much greater extent than when normal saline was injected in similar volume for comparison. The skin became flushed and "goose-pimpled" and the animals often staggered and appeared to be unable to use their rear legs properly. Many became incontinent within fifteen minutes or so and frothed at the mouth. Over a period of 24 hours the pigs became depressed and ataxic, and showed peculiar habits such as walking backwards or trying to climb up the walls of the cage. "Shuddering attacks" and recognizable generalized convulsions occurred. Finally they became comatose and died or were killed as described above.

Postmortem Findings

Five animals had subarachnoid haemorrhages, all of which were largely confined to the posterior fossa. Ten animals had a quantity of free fluid in the peritoneal cavity (the largest amount was 300 ml.) with evidence of mild fibrinous peritonitis; two had suppurative peritonitis without a significant quantity of free fluid. The last two animals had large abscesses at the site of the intraperitoneal injections. The histology of organs other than the brain was normal.

Brain Histology

The distribution of lesions is shown in Table I. Some of the animals showed severe mid-cortical laminar malacia, with a striking spongy appearance in which the vacuoles were generally arranged in rows perpendicular to the brain surface (Fig. 1). This was in most cases confined to the sides and depths of the sulci, although in one case (pig 8) it was most marked in the gyri, and in another (pig 6) was found in both situations. The cortical neurones in these areas stained poorly and showed swelling, vacuolation and fragmentation of the cytoplasm (Fig. 2). In other parts of the cortex neurones tended to be

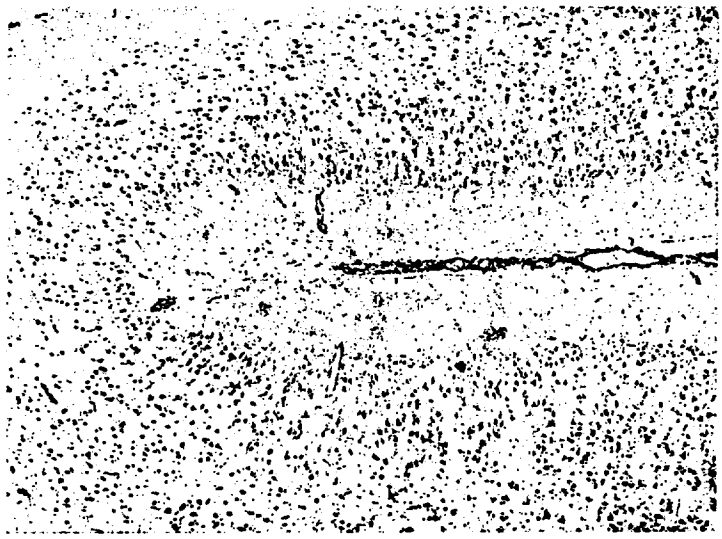

FIG. 1
Coronal section of cerebral cortex showing midcortical spongiosis around the base of a sulcus. (Haematoxylin and eosin, ×48.)

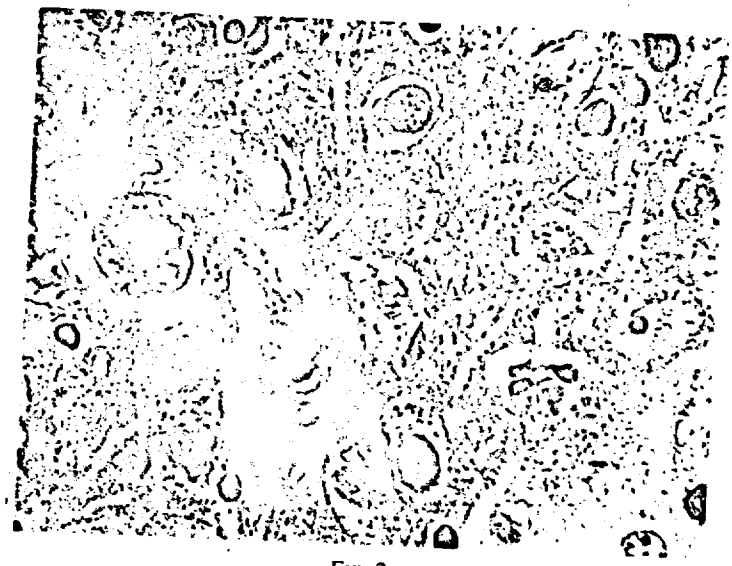

FIG. 2
Cortical neurones in a region of spongiosis showing vacuolation and fragmentation of the cytoplasm. (Haematoxylin and eosin, ×625.)

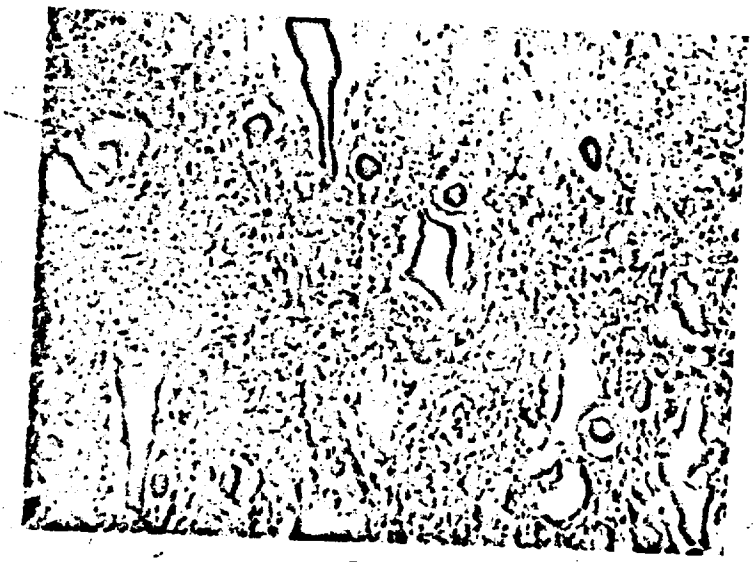

FIG. 3
Section of a gyrus showing neurones with dark cytoplasm and dense, distorted nuclei. (Haematoxylin and eosin, ×625.)

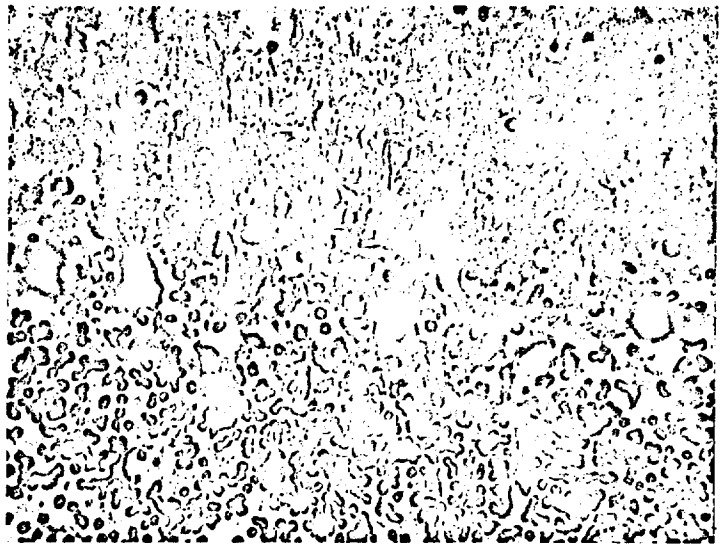

FIG. 4
Section of cerebellum showing pyknotic and distorted Purkinje cells. (Haematoxylin and eosin, ×440.)

dark and shrunken with indistinct pyknotic nuclei. Such appearances were most pronounced in the gyri (Fig. 3) and were, in several cases, confined to them. The white matter, basal ganglia and Ammon's horn were all of normal appearance. In some animals a variable number of the Purkinje cells of the cerebellum were dark and shrunken (Fig. 4). Some pyknotic cells were seen in the mid-brain, pons and medulla, but the appearance of the brain-stem was variable in the control animals and it was thought that these structures were probably normal. In most animals eosinophils could be found in the meninges after diligent searching, but only in the case of pig 6 were they abundant. Perivascular aggregation of inflammatory cells, mainly lymphocytes, was found only as an isolated phenomenon in four animals.

Serum Sodium Estimations

We have accepted values up to 155 mEq. per litre as being within the probable normal range for serum sodium concentration in the pig. This figure is based both on observations in our control animals and on those reported in the literature (Cox and Pitts, 1957; Medway and Kare, 1959; Todd, Lawson and Dow, 1964; Ek, 1965; Ullrey *et al.*, 1967).

The results of estimations on the experimental animals are shown in Table I. Only two of the three animals treated by dietary saline had raised sodium levels (pigs 3 and 5). The third animal (pig 4) had a postmortem serum sodium concentration of 152 mEq. per litre. It is of interest that this is the animal which showed no laminar lesions in the cerebrum, and which may have avoided salt poisoning by refusal to eat the high salt diet. Of the nine satisfactory postmortem blood samples obtained on pigs given only intraperitoneal saline, six had raised sodium levels (160 to 207 mEq. per litre) and three were normal (134 to 151 mEq. per litre). Two of these latter animals had salt poisoning lesions in the brain, and they also had abscesses at the site of the injections. The other animal with a normal serum sodium did not show salt poisoning lesions, but did have Purkinje cell damage and subarachnoid haemorrhage.

Figure 5 shows the results of serial sodium estimations on four animals and a control. Only two antemortem blood samples were obtained on pig 9 (and no satisfactory postmortem sample),

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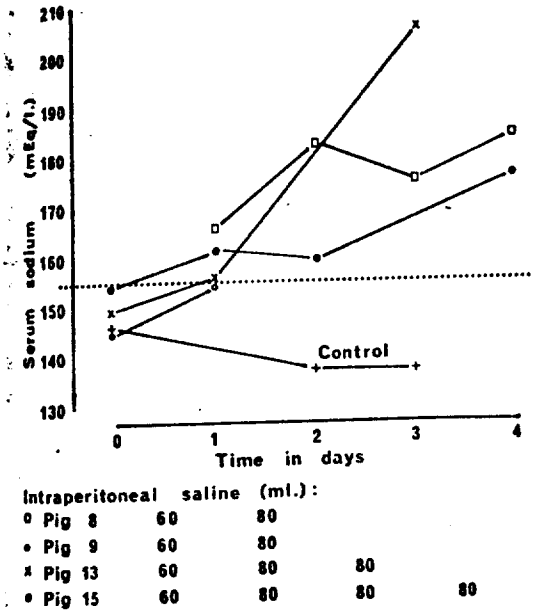

FIG. 5

Serial sodium estimations. Except for pig 9 the last values shown are those of postmortem samples.

but the other animals all showed a steady rise of sodium concentration. Pig 15 showed no salt poisoning lesions in the cerebrum and it is, therefore, of interest that in this animal the sodium levels were least raised (161 mEq. per litre in life; 175 mEq. per litre postmortem).

Peritoneal fluid, when present, was analyzed for its sodium content. Three of the five samples had greater sodium concentrations than normal serum (165 to 223 mEq. per litre); the others were the upper limit of normal—155 mEq. per litre. These two latter samples were from the pigs with abdominal wall abscesses and with normal serum sodium levels.

DISCUSSION

Two points arising from these observations merit discussion, namely:

(1) The relationship of the findings to the clinical and pathological features of classical salt poisoning in pigs, and

(2) The validity of the experiment as a model for the human situation.

Mid-cortical laminar malacia is typical of salt poisoning. We found less marked eosinophilic infiltration than some descriptions led us to expect, but this may be a variable feature and one likely to develop after at least 36 hours of consistent hypernatraemia (Smith, 1955). It has been shown that both the clinical picture of salt poisoning and the findings of mid-cortical laminar malacia can occur in the absence of eosinophilic infiltration when pigs are fed on a diet in which the enhanced osmotic content is largely provided by urea (Done *et al.*, 1959). These authors considered that this cortical damage was due to disturbed water balance rather than to the specific agent causing it. Our results throw some doubt on the relationship of these lesions to the clinical features of the disease. The three pigs which received a high salt diet (which should by all accounts in the literature have readily produced classical salt poisoning) were considered, apart from their reluctance to eat, to have been clinically normal. Even so, two of them showed laminar lesions approximately coeval with the period of dietary treatment. The absence of mid-cortical lesions in one pig of the three may be ascribed to that animal not having consumed sufficient of the unpalatable salty meal, a conclusion supported by the comparatively low level of serum sodium.

Not all the animals receiving intraperitoneal injections of sodium chloride showed recent mid-cortical laminar malacia. However, most of them showed widespread neuronal necrosis affecting the whole cerebral cortex, particularly the gyri, and the Purkinje cells of the cerebellum, features which were absent in the animal which was treated by diet alone. It may be postulated that the acute episode following intraperitoneal injection of saline is reflected by the widespread rather non-specific and possibly post-ictal changes of neuronal necrosis, whilst the chronic effect of sustained hypernatraemia and disturbed water balance associated with classical salt poisoning is reflected by the features of mid-cortical laminar malacia. The occurrence of lesions of the latter type in only some of the animals treated by intraperitoneal injections may be related to the variable water intake.

Comparison of the cerebral lesions in these pigs with those described in women after abortion reveals certain differences. Mid-cortical

laminar malacia was not present in the three human brains and there were no changes in the pigs corresponding to the necrosis of Ammon's horn, amygdaloid nucleus or pons. It is possible that these two sets of lesions are specific to the appropriate species. However, in all three human brains the widespread necrosis affecting the cortical neurones was similar in appearance and distribution to that seen in the pigs. Furthermore, five of the pigs had subarachnoid haemorrhages, a feature found in one of the women dying after amniocentesis and also described in infants after accidental ingestion of large amounts of sodium chloride (Olmstead, 1966). Cameron and Dayan (1966) considered that the widespread neuronal necrosis could have been the result of epileptiform convulsions. Our findings do not invalidate this view, but it is also possible that the neuronal damage was the direct result of hypernatraemia and the cause of the observed convulsions.

The similarity of the clinical picture following intraperitoneal injection in the pig and that following abortion in women may suggest that escape of hypertonic saline into the peritoneal cavity is responsible for the cerebral damage. However, similar pathological features have also been observed in humans after oral administration of large amounts of salt (Dayan, personal communication). It would, therefore, appear that rapid loading of the maternal body fluids with sodium chloride, resulting in a sudden change in osmolality of the plasma, and perhaps of the cerebro-spinal fluid, may be the cause of the acute syndrome. It is possible that injection into the placental bed or escape from a torn amnion might also be mechanisms which would lead to the systemic distribution of saline. On the other hand, the large amounts of fluid which would be involved render diffusion through an intact amnion a highly unlikely possibility. The observed reading of 174 mEq. per litre in one patient supports the hypothesis that a significant proportion of the sodium was quickly distributed throughout the extracellular space.

Experimental evidence suggests that excess of sodium ions may disturb the function of the nervous system. Dodge *et al.* (1962) found that the neurophysiological disturbances induced in rabbits by the intravenous infusion of hypertonic saline were situated at all levels of the nervous system from cortex to spinal cord. It has also been found that intraventricular administration of hypertonic sodium chloride can cause seizures in cats, which could not be produced by sugar solutions of equal hypertonicity or by isotonic saline (Glaser and Wolf, 1963; Glaser, 1964).

The sudden intake of a large volume of osmotically active solution would lead to a rapid expansion of blood volume and of extracellular space, and to dehydration of cells. It is possible that the flushing observed in the pigs immediately after their injections was related to the expansion of blood volume, and that the subarachnoid haemorrhages found at postmortem examination may also be a consequence. At the same time the effect on the kidney will be that of an osmotic diuretic, and the urinary incontinence which was shown by some of our pigs may well have been a result of sudden massive diuresis. The consequence of this effect will be to counteract the expansion of extracellular fluid space. Using rabbits Sotos *et al.* (1960) showed that after a steady intravenous infusion of hypertonic saline, extracellular fluid volume remained fairly constant until the animal came near to death, when this volume rose to reach 110 per cent of normal. This was accompanied by a steady fall of 10 per cent in total body water and a very great reduction of intracellular space to 75 per cent of normal. Thus the saline has two basic effects on fluid volumes. It causes (1) an initial expansion of blood volume, and possibly a second expansion as compensatory mechanisms become exhausted towards death, and (2) a drastic reduction of intracellular volume. Cameron and Dayan (1966) made the suggestion that "rebound cerebral oedema" might occur following cellular dehydration and account for some of the pathological features in their cases: there was no evidence of cerebral oedema in any of the pigs. The pathogenesis of the striking hippocampo-amygdaloid and pontine haemorrhagic necrosis in the human brain thus remains obscure. As it has been impossible to reproduce these lesions in the pig our experiments throw no further light on this problem.

The hypothesis still stands that death following therapeutic abortion by amniotic fluid replacement is a result of the systemic administration of hypertonic sodium chloride solution. The present experiments in pigs have shown that intraperitoneal injection of hypertonic saline in

amounts equivalent to those used in the human cases produces an immediate reaction which may be analogous to the syndrome which led to the patient's death. It is also possible that injection into the placental bed could cause similar effects. Although brain damage occurred in the pigs, the pattern was dissimilar to that found in the human brains. It is not possible, therefore, to add to the explanation of the pathogenesis of this unique pattern. It seems very likely that the disturbance of internal fluid balance induced by the hypertonic saline is primarily responsible, but that the particular pattern of damage is dependent upon anatomical arrangements which are not present in the pig. The clinical syndrome is probably a result of this damage, but disturbances of electrophysiological activity by high sodium ion concentrations may also occur.

Summary

There have been three reports of death following therapeutic abortion by replacing amniotic fluid with hypertonic saline. In each case an identical pattern of cerebral damage was found. In an attempt to produce this syndrome in experimental animals hypertonic sodium chloride was injected into the peritoneal cavities of pigs. The ensuing syndrome of motor disturbances, fits, coma and death, bore resemblances to that following therapeutic abortion. Although the brains showed both the classical changes of salt poisoning in pigs and widespread non-specific damage, the pattern found in the human brains was not reproduced. Acute disturbance of fluid balance between intra- and extracellular compartments results from the intraperitoneal administration of saline, and it seems probable that this is the cause of the cerebral damage. The particular pattern produced may be species specific. The similarity of the clinical picture to that described in women after abortion suggests that death in these cases may have resulted from the accidental intraperitoneal injection of the hypertonic solution, or from seepage out of the amniotic cavity into the maternal tissues through multiple puncture sites.

Acknowledgements

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References

Cameron, J. M., and Dayan, A. D. (1966): *Brit. med. J.*, **1**, 1010.

Cox, D. H., and Pitts, J. C. (1957): *J. Amer. vet. med. Ass.*, **130**, 176.

Dayan, A. D., Cameron, J. M., and Phillipp, E. (1967): *Med. Sci. Law*, **7**, 70.

Dodge, P. R., Sotos, J. F., Gamstrop, I., De Vivo, D., Levy, M., and Rabe, T. (1962): *Trans. Amer. neurol. Ass.*, **87**, 33.

Done, J. T., Harding, J. D. J., and Lloyd, M. K. (1959): *Vet. Rec.*, **71**, 92.

Ek, N. (1965): *Nord. Vet.-Med.*, **17**, 604.

Glaser, G. H. (1964): *Epilipsia*, **5**, 97.

Glaser, G. H., and Wolf, G. (1963): *Nature (Lond.)*, **200**, 44.

Medway, W., and Kare, M. R. (1959): *Cornell Vet.*, **49**, 241.

Olmstead, E. G. (1966): *Mammalian Cell Water*. Kimpton, London.

Smith, D. L. T. (1955): Thesis, Cornell University, No. 15621.

Sotos, J. F., Dodge, P. R., Meara, P., and Talbot, N. B. (1960): *Pediatrics*, **26**, 925.

Todd, J. R., Lawson, G. H. K., and Dow, C. (1964): *J. comp. Path.*, **74**, 331.

Ullrey, D. E., Miller, E. R., Brent, B. E., Bradley, B. L., and Hoefer, J. A. (1967): *J. anim. Sci.*, **26**, 1024.

POTASSIUM LEVELS IN EXCHANGE TRANSFUSION*

BY

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In the Royal Maternity Hospital, Belfast, in common with other centres treating haemolytic disease of the newborn, the number of infants requiring exchange transfusion has been increasing steadily in the past few years. In 1950 there were seven live-born affected infants, of whom five were treated, all successfully, by exchange transfusion, in 1954 there were 42 live-born affected infants of whom 34 were treated by exchange transfusion, 31 surviving.

At the end of 1952 and the beginning of 1953 there were two deaths during the actual transfusion in infants who were comparatively mildly affected. The deaths were sudden and unexpected, and it occurred to us that they might have resulted from an excess of potassium in the bottles of blood used for the exchange. The plasma from several random bottles was therefore analysed, and it was found that levels of 40 mg. % were not uncommon, even though none of the blood had been more than one week in store.

In an attempt to determine whether hyperpotassaemia might in fact be the cause of death in these infants, an investigation was planned on the following lines:—

In every case where it was decided to treat the baby by exchange transfusion, samples of blood were taken from the umbilical vein at the beginning of the tranfusion and after every 100 ml. of the exchange. These samples were taken into heparinized tubes and the plasma separated within four hours. Similar samples were taken from the bottles of stored blood used for the exchange. In general, the criteria for transfusion were those suggested by Mollison (1952), and an exchange of 60 ml. of packed cells per lb. weight was aimed at.

When data had been obtained from 20 infants (21 transfusions) they were examined; the potassium levels for this control group are shown in Fig. 1. It will be seen that 13 of the 104 samples had potassium levels of 28 mg. % or above, and that three of the four deaths in this group had potassium levels above 32 mg. %. (The fourth death was of a premature infant, weighing 4 lb. 6 oz., with a cord blood haemoglobin of 42% (6·2 g.).)

To attempt to correlate the potassium level in the bottles used with the levels reached in the infants, the cases were grouped according to the greatest level in the bottles used, and the maximum level in the babies charted. These results are shown in Table 1.

TABLE 1

POTASSIUM LEVELS (mg. per 100 ml.) IN CONTROL GROUP

Maximum in Bottle	Mean in Bottle	Maximum in Child						Mean of Maximum Levels in Children
		−20	−24	−28	−32	−36	−40	
Below 26	22·34	1	2	1*	2	1*	—	25·83
26–32	29·66	1	1	3	—	—	1*	26·3
Over 32	45·3	1	3	2	1	1*	—	25·95

* Indicates deaths.

In such a group of cases, with many variable factors such as birth weight, cord blood haemoglobin, birth order, maturity, etc., it is impossible to make any valid statistical analysis. It does not appear, however, from the figures shown in this table that there is any simple relation between the potassium level in the bottle of blood used for exchange and the maximum level reached in the child. In this we were unable to confirm the work of Miller, McCord, Joos and Clausen (1952), who found that in a small series of eight cases there was a direct relation between the potassium level in the stored blood and that reached in the child.

Although the results obtained in this control group do not prove that the high potassium levels were the actual cause of death, rather than a coincidental result of some other process in the disease itself or the transfusion, it was felt that an attempt should be made to guard against the rise. It is generally accepted that the most rapid way to remove potassium from the extracellular to the intracellular fluid is to give glucose and insulin

* Based on a paper read to the Ulster Paediatric Society on May 7, 1955.

intravenously. In the absence of any previous reports on this technique as applied to the newborn infant, it was decided in the first instance to give 2 g. of glucose and 2 units of insulin into the umbilical vein after the first 100 ml. of blood had been exchanged. When the first few transfusions had been carried out it was found that the blood sugar levels were high, and as the 50% glucose used tended to make the syringe sticky and thus added slightly to the technical difficulty, it was decided to give the insulin alone. (It should be mentioned that the standard anticoagulant solution used by the Northern Ireland Blood Transfusion Service contains glucose, so that the plasma glucose level of stored blood is generally about 700-800 mg. per 100 ml.)

The potassium levels from 16 infants (17 transfusions) are shown in Fig. 2.

It will be seen that the general trend is lower in this 'insulin' group than in the 'control' group.

The only death in this treated series was a 6-lb. baby with very intense jaundice, who died during a second transfusion, with clinical evidence of kernicterus. (Consent was unfortunately not obtained for a necropsy.) The group of six rather high readings (32 mg. per 100 ml. and above) was examined to see whether any reason could be found for the apparent failure to respond to the insulin. One was the specimen only 100 ml. after giving the insulin, the subsequent samples having normal values, and the infant showing no clinical sign of distress. It is possible that in this case even higher and perhaps fatal potassium levels might have occurred had insulin not been given.

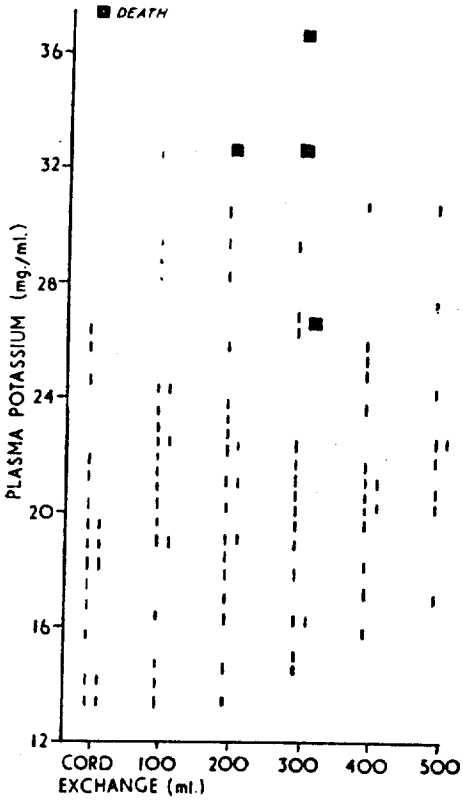

FIG. 1.—Control group.

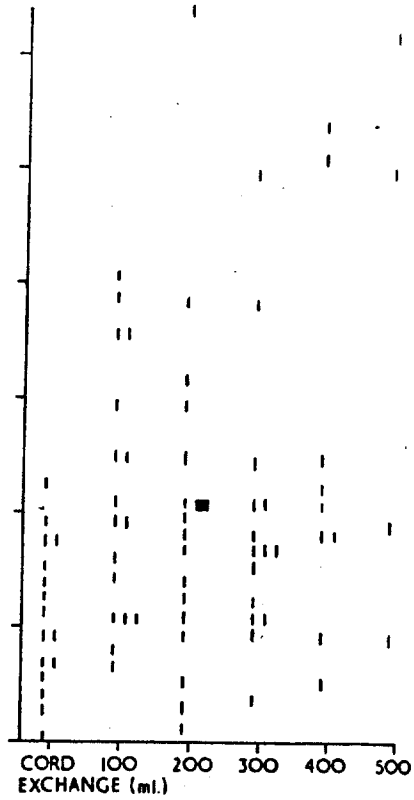

FIG. 2.—Insulin group.

Two were the samples at the end of transfusion in infants showing some collapse, not sufficient to cut short the exchange, and the remaining three were from the one infant, who had intense jaundice and who died at the age of 2 months with evidence of kernicterus.

For comparison with the control group, the relation between the potassium levels in the bottles

used and the maximum levels reached in the infants is shown in Table 2. This shows little difference from Table 1.

TABLE 2

POTASSIUM LEVELS (mg. per 100 ml.) IN INSULIN GROUP

Maximum in Bottle	Mean in Bottle	Maximum in Child						Mean of Maximum Levels in Children
		−20	−24	−28	−32	−36	−40	
Below 26	23·48	2	3	2	—	—	—	21·91
26–32	29·94	3	—	1*	1	—	2	26·6
Over 32	36·86	1	—	1	—	—	1	27·66

* Indicates deaths.

In such small groups of cases, with many variable factors in the individual infants, one cannot make any detailed statistical analysis. Table 3 shows that

TABLE 3

COMPARISON OF CONTROL AND INSULIN GROUPS

	Infants	Transfusions	Mean Birth Weight (oz.)	Mean Cord Haemoglobin (%)	Deaths
Control	20	21	115·6	81·4	4
Insulin	16	17	105·8	81·8	1

as far as birth weight and cord blood haemoglobin were concerned there was no great difference between the two groups.

The actual mean levels of potassium at each stage of the transfusions are shown in Table 4. These are of much less practical importance than the individual readings shown in Figs. 1 and 2, since the occasional high value, associated clinically with collapse or death, does not make an appreciable difference to the mean reading. The mean blood sugar levels are also shown in Table 4, and also the

TABLE 4

MEAN ELECTROLYTE VALUES (mg. per 100 ml.)

		Cord	100	200	300	400	500
Control ..	Potassium	18·6	21·7	21·8	22·4	21·7	22·8
Insulin ..	Potassium	16·4	20·6	20·0	20·0	22·2	25·6
Insulin ..	Sugar	55·0	109	227	252	247	180
Normal infants ..	Potassium	21·4					

mean cord blood potassium in a series of 16 normal infants. The latter was found to agree with the value given by Earle, Bakwin and Hirsch (1951) and not with the much higher values quoted by Smith (1951).

Discussion

From the results obtained in this investigation it appears that hyperpotassaemia is not infrequent in the course of exchange transfusions for haemolytic disease of the newborn, and that the levels reached are potentially lethal. As has been stated, there is no proof that the high potassium content of the plasma has been the cause of death in those infants who died suddenly rather than a coincidental finding. Little work appears to have been done on this aspect of exchange transfusion; apart from a passing reference to one fatal case by Walker and Neligan (1955), the only article that I have been able to trace is that of Miller *et al.* (1952) already referred to. They relate the high potassium levels directly to the high content of the transfusion blood; our results do not confirm this, but it is difficult to offer any explanation for the apparent individual variation in the rate of accumulation of potassium. Although the series is too small to justify detailed subdivision into subgroups according to all the variable factors involved, there does not seem to have been any relation to the severity of the disease as measured by cord blood haemoglobin or bilirubin levels. As far as possible the actual technique of transfusion has been standardized to eliminate possible variations in such factors as rate of exchange, chilling of the baby, etc.

Gustafson (1951) has reported electrocardiographic changes suggesting hypocalcaemia during exchange transfusion, although the total calcium content of the blood did not show any significant change. The plasma calcium levels were estimated in the first few of our series, but as there was no apparent variation, this was discontinued and attention concentrated on the more striking potassium changes. We hope to be able to obtain a direct-reading electrocardiograph in order that a continual check may be kept on the action of the heart muscle during transfusion, as this may prove to be the most sensitive estimate of electrolyte changes and may show the need for appropriate treatment before serious harm can occur.

Our present opinion is that there is sufficient evidence to support the theory that hyperpotassaemia is a cause of death to justify the routine use of insulin in future transfusions. It might be advisable to give a slightly larger dose, e.g., 3 units, before giving the first 10 ml. replacement. (In this connexion, it has been found that a tuberculin syringe is essential for accurate measurement of dosage.)

It will necessarily take a year or more to obtain a really large series of cases, but it is hoped that other centres may consider similar investigations as a check on our findings.

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Summary

An investigation has been carried out into the plasma potassium levels in newborn infants undergoing exchange transfusions for haemolytic disease.

Toxic levels have been found in some infants, and it is suggested that this may be one factor in causing death. The potassium level in the child does not appear to bear a direct relationship to that in the stored blood used.

Intravenous insulin has been used in an attempt to reduce the potassium levels, with some success.

I am very grateful to my obstetric and paediatric colleagues in the Royal Maternity Hospital, Belfast, for much help both in planning and carrying out this trial. Mr. D. W. Neill, M.Sc., A.R.I.C., Senior Scientific Officer, Belfast Hospital Group, has given invaluable advice and much practical assistance at every stage.

REFERENCES

Earle, D. P., Bakwin, H. and Hirsch, D. (1951). *Proc. Soc. exp. Biol., N.Y.*, 76, 756.
Gustafson, J. E. (1951). *J. Pediat.*, 39, 593.
Miller, G., McCord, A. B., Joos, H. A. and Clausen, S. W. (1952). *Amer. J. Dis. Child.*, 84, 637.
Mollison, P. L. (1952). In *The Rh Blood Groups and Their Clinical Effects* (M.R.C. Memo. 27). London.
Smith, C. A. (1951). *The Physiology of the Newborn Infant*, 2nd ed., p. 283. Springfield, Illinois.
Walker, W. and Neligan, G. A. (1955). *Brit. med. J.* i, 681.

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PREFERENCE FOR NaCl IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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SUMMARY

When given free choice between NaCl and water, spontaneous hypertensive (SH) rats exhibited an increased preference for NaCl (0.15 M and 0.30 M) when compared to either Holtzman or Wistar normotensive controls. Similarly, total fluid intake (NaCl and water) was increased in the SH rats compared to Holtzman or Wistar controls. These results are the first to demonstrate a sustained spontaneously increased NaCl preference in a hypertensive rat.

PREFERENCE for NaCl in rats is influenced by a variety of drugs, disease states and experimental procedures. An *increased* preference for NaCl has been demonstrated after adrenalectomy,[1] feeding a diet deficient in sodium,[2] subcutaneous injection of formalin[3] and administration of thiazide diuretic.[4] A *decreased* NaCl preference has been demonstrated after feeding a diet containing high concentrations of NaCl.[2] The effect of hypertension on NaCl preference in the rat has generated considerable interest due to the proposed interrelationship between NaCl intake and hypertension in humans.[5,6] Indeed, *decreased* NaCl preference and increased total fluid consumption has been reported in rats who were made hypertensive by latex encapsulation of the kidney[7,9] or who had a genetic predisposition to hypertension.[10] Deoxycorticosterone acetate administration and subsequent hypertension in rats was associated with a persistent increase in total fluid consumption but only an initial transient increase in salt preference.[11] Rats made hypertensive following unilateral nephroadrenalectomy with contralateral adrenal enucleation exhibited an increased salt intake when compared to controls but these rats were never offered a choice between saline and water.[12]

Because of the well-known relationship between NaCl intake and hypertension the present investigation was undertaken to determine if NaCl preference or total fluid intake in a strain of spontaneously hypertensive rats are different from that of normal, non-hypertensive rats.

Methods

Experimental animals consisted of fourteen male rats of a strain of spontaneously hypertensive (S.H.) Wistar rats.[12,13] Seven male Holtzman rats (Holtzman Co., Madison, Wisconsin) and six male Wistar rats (National Institutes of Health, Bethesda, Maryland) served as controls. Responses of the seven Holtzman controls were always compared with those of a group of eight S.H. rats; responses of the six Wistar controls were always compared with those of another group of six S.H. rats. All animals were individually housed in specially designed stainless steel cages (Acme Metal Products, Chicago, Illinois) to which a feeding dish and 2 graduated fluid bottles (Kontes Glass Co., Vineland, New Jersey) were attached. Animals were kept in a room in which temperature was kept constant at 22°C. All rats were fed, *ad libitum*, a diet of ground Purina rat chow with 15% added dextrose. Triple glass distilled water prefiltered through charcoal and an ion-exchange resin was used alone or as a solvent for reagent grade sodium chloride.

Standard conditions for preference testing with a 24-hour 2 bottle choice experiment were used (15); one bottle contained water, the other, a given concentration of NaCl. Body weights and intake of each fluid were recorded daily during each test period. Intake of NaCl was expressed as a percentage of the total fluid imbibed in the 24-hour period:

$$\% \text{ NaCl preference} = \frac{\text{cc NaCl solution consumed}}{\text{cc total fluid consumed}} \times 100$$

For convenience volume intake was expressed as the ratio of the total daily intake of fluid to the body weight of the animal as cc fluid/gm body weight.

Between test periods animals were maintained on the diet noted above and tap water, *ad libitum*.

Results

Part I

Holtzman control and S.H. rats were presented with a free choice of distilled water or 0.15 M NaCl for days 1 through 7 and 12 through 15, and distilled water or 0.30 M NaCl for days 8 through 11 and 16 and 17. The mean body weight of the S.H. rats over this time period was 303 gm (range: 279-318) while the Holtzman controls was 258 gm (range: 184-308). Wistar control and S.H. rats were presented with a free choice of either distilled water of 0.15 M NaCl for days 1 through 4, distilled water or 0.30 M NaCl for days 5-8, and distilled water or 0.45 M NaCl for days 9-12. Mean body weight of these S.H. rats over this time period was 270 gm (range: 247-303) while the Wistar controls was 273 gm (range: 267-281).

The preference responses of control and S.H. rats are shown in Figs. 1A and 1B. Each point represents the mean plus or minus one standard error of the mean

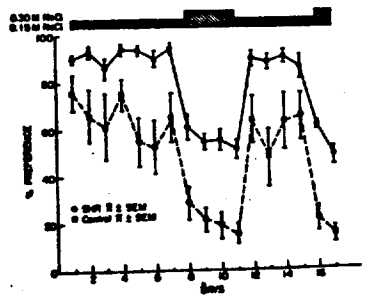

Fig. 1A

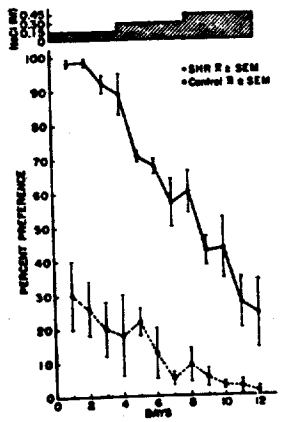

Fig. 1B

Fig. 1A. Comparison of preference for NaCl between Holtzman controls and S.H. rats in Part I. Each symbol represents the mean ± 1 SEM of 7 Holtzman and 8 S.H. rats. % preference is plotted on the abscissa, time in days, on the ordinate.

Fig. 1B. Comparison of preference for NaCl between 6 Wistar controls and 6 S.H. rats. Preference for NaCl among the S.H. rats was greater than that of either control group.

(S.E.M) of each group on each day of the study. When presented with a choice between 0.15 M NaCl and water the mean preference of the control groups varied between 47% and 76% and 18% and 32% for the Holtzman and Wistar rats, respectively, while that of the S.H. rats always remained greater than 85%; preference for 0.15 M NaCl by the S.H. rats was significantly greater ($p < .05$) than that of the Holtzman controls for each day of the study except 1 and 3 and for the Wistar controls for each day ($p < .01$). The difference between Holtsman controls and S.H. rats was present both before and after they were given a choice between 0.30 M NaCl and water.

Given a choice between 0.30 M NaCl or water both control groups exhibited a significant aversion to the salt 13%-28% and 9%-15%, for the Holtzman and Wistar rats, respectively throughout the time that it was presented while the S.H. rats demonstrated a preference (49%-62%). The increased preference of the S.H. rats for 0.30 M NaCl was statistically higher ($p < .01$) than that of the Holtzman controls at each time point tested even after a period during which 0.15 M NaCl was interjected.

Given a choice between distilled water or 0.45 M NaCl Wistar controls exhibited an even greater aversion to the salt than they had for lower concentrations (2%-4%) and the S.H. rats also exhibited aversive behavior for the first time (27%-37%). However, preference of the S.H. rats for the salt was at all times significantly greater than those of the Wistar controls ($p < 0.01$).

Part II

Six weeks after the completion of Part I the Holtzman controls and the S.H. rats were given a choice between 0.30 M NaCl and water on days 1-8 and 20-24 and between 0.45 M NaCl and water on days 9-19. The mean body weight of the S.H. rats over this time period was 400 gm (range: 394-406) and controls 419 gm (range: 414-425).

Given a choice between 0.30 M NaCl and water the S.H. rats demonstrated a significantly higher preference (45%-59%, $p \leq .01$) than did the control rats,

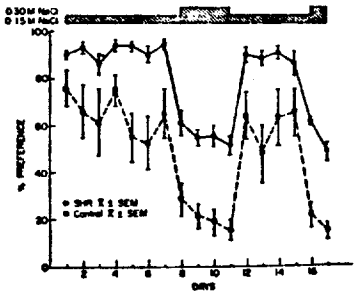

Fig. 2
Comparison of preference for NaCl between Holtzman control and S.H. rats in Part II. Preference for 0.3 M NaCl among the S.H. rats was greater than that for the controls; preference for 0.45 M NaCl was not consistently greater in the S.H. rats.

8 days of this trial with 0.30 M NaCl the S.H. rats preferred essentially the same percentage of NaCl as they did in Part I. However, controls demonstrated a more significant aversion to 0.30 M NaCl than they did in Part I.

Given a choice between 0.45 M NaCl and water both S.H. and control rats demonstrated aversion to the salt. The S.H. rats preferred more NaCl than did the controls, but this difference was significantly different from the Holtzman controls ($p < .05$) on only 4 of the 11 days of the trial (Fig. 2). When then given a choice between 0.30 M NaCl and water there was a significant increase in NaCl preference among the S.H. rats compared to Holtzman controls ($p < .01$); however, the percent preference of NaCl by the S.H. rat was approximately half that which they preferred prior to exposure to 0.45 M NaCl.

The volume intake (ml/gm body weight) of the S.H. rats was not consistently different from the Holtzman strain for either 0.15 M NaCl or 0.30 M NaCl during Part I. However, volume intake of the S.H. rats was significantly greater than that of the Wistar controls ($p < .01$, Fig. 3B). A significant increase in volume intake of the S.H. rats with respect to the Holtzman controls occurred in Part II when given a choice between 0.30 M NaCl and water ($p < .05$, Fig. 3A). This difference persisted throughout the period during which 0.30 M NaCl was given. No differences in volume intake between either the Holtzman or Wistar

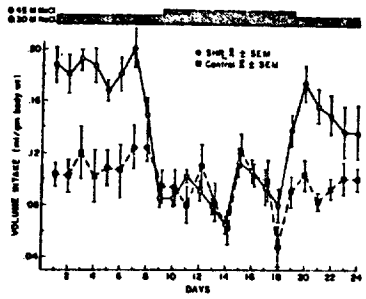

Fig. 3A

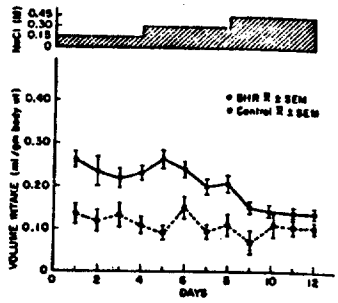

Fig. 3B

Fig. 3A: Comparison of volume intake (NaCl plus water) between Holtzman control and S.H. rats in Part II. Each symbol represents the mean ± 1 SEM of 7 control rats and 8 S.H. rats. Volume intake is plotted on the abscissa, time in days on the ordinate.

Fig. 3B: Comparison of volume intake between 6 Wistar control and 6 S.H. rats. During the time when preference for NaCl was greater in the S.H. rats compared to either control group there was a concomitant increase in volume intake.

controls or the S.H. rats occurred when given 0.45 M NaCl.

Discussion

To our knowledge this is the first study demonstrating a persistent *increased* NaCl preference in a hypertensive rat given a free choice between NaCl and water. Rats made hypertensive through administration of deoxycorticosterone acetate exhibited only a transient increase in salt preference[11] while rats made hypertensive subsequent to adrenal regeneration exhibited an increased salt appetite but only saline was offered to them.[12] Indeed, most previous studies demonstrated that rats with experimentally induced hypertension of several etiologies exhibited a decreased preference for salt.[7-10] Aversive behavior toward NaCl has been previously demonstrated in a genetic strain of hypertensive rats. These animals were predisposed to develop hypertension by administration of large quantities of NaCl and were usually compared to another genetic strain of rats who remained normotensive following administration of similar quantities of NaCl.[10] These hypertensive rats also did not exhibit increased fluid intake.[10] These results suggested to these investigators as well as to us that

the preference behavior and fluid intake responses of these hypertensive rats were not typical of the behavior of the majority of hypertensive animals or of man.

In the present studies responses of two control groups of rats were compared with those of S.H. rats. S.H. rats exhibited consistently significantly increased preference for 0.15 M and 0.30 M NaCl with respect to either control group. However, as the concentration of NaCl was increased the preference of the S.H. rats decreased suggesting that they maintain an ability to distinguish increasing concentrations of NaCl, responding to them in the same manner as do the controls but at significantly higher levels of preference.

Previous investigators demonstrated that rats made hypertensive by several means exhibited an increased volume intake.[7-9,11] Although the S.H. rat did not consistently exhibit this phenomenon increased volume intake occurred when NaCl preference was significantly elevated above controls especially during exposure to 0.30 M NaCl.

It is well-known that hypertension in man is associated with increased salt intake and one of the earliest successful treatments for essential hypertension was dietary restriction of salt intake.[16-18] The increased preference for 0.15 M and 0.30 M NaCl in the S.H. rat may be interpreted to indicate a decreased taste acuity for NaCl.[19] Several investigators have claimed that taste acuity for NaCl in patients with essential hypertension is decreased,[20,21] although this phenomenon has not been consistently observed in hypertensive patients in whom detection and recognition thresholds for NaCl have been measured in our laboratory (unpublished observations). What relationship, if any, does exist in man between NaCl preference and hypertension is not yet clear. However, in the S.H. rat, there is a clear-cut relationship between increased NaCl preference on the one hand and genetically determined hypertension on the other.

References

1. C. P. RICHTER, *Amer. J. Physiol.*, 115, 155 (1936).

2. M. J. FREGLY, J. M. HARPER, JR., and E. R. RADFORD, JR., *Amer. J. Physiol.*

209, 287 (1965).

3. G. WOLF and E. A. STEINBAUM, *J. Comp. Physiol. Psychol.* 59, 334 (1965).

4. M. J. FREGLY, *Proc. Soc. Exp. Biol. Med.*, 125, 1079 (1967).

5. G. R. MENEELY and L. W. DAHL, *Med. Clin. N. Amer.* 45, 271 (1961).

6. L. L. DAHL and R. A. LOVE, *A.M.A. Arch. Int. Med.* 94, 525 (1954).

7. M. ABRAMS, A. I. C. De FREIZ, A. C. TOSTESON and E. M. LANDIS, *Amer. J. Physiol.* 156, 233 (1969).

8. M. J. FREGLY, *Amer. J. Physiol.* 187, 288 (1956).

9. D. M. GREEN, F. J. SAUNDERS, C. G. VAN ARMAN, L. D. CALVIN and F. M. STURTEVANT, *Amer. J. Physiol.* 170, 480 (1952).

10. G. WOLF, L. K. DAHL, and N. E. MILLER, *Proc. Soc. Exp. Biol. Med.*, 120, 301 (1965).

11. D. C. TOSTESON, A. I. C. De FREIZ, M. ABRAMS, C. W. GOTTSCHALK and E. M. LANDIS, *Amer. J. Physiol.* 164, 369 (1951).

12. R. TAKEDA, S. MORIMOTO, S. MIYABO and M. MURAKUMI, *Endocrinol. Japan* 11, 19 (1964).

13. W. J. LOUIS, R. TABEI, A. SJOERDSMA and S. SPECTOR, *Lancet* 1035 (1969).

14. W. J. LOUIS, S. SPECTOR, R. TABEI and A. SJOERDSMA, *Supp. Circ. Res. Supp.* 1, 24, 93 (1969).

15. M. R. Kare, *Physiological and behavior aspects of taste.* M. R. Kare and B. P. Halpern (Eds.), Univ. Chicago Press, Chicago, Illinois (1961).

16. L. AMBARD and E. BEAUJARD, *Semaine Med.*, 25, 133 (1905).

17. F. VOLKARD, *Handbuch der inneren medizin*, 6, Pt. 2, 1753, Ed. 2, Julius Springer, Berlin (1931).

18. F. M. ALLEN, *JAMA*, 74, 652 (1920).

19. R. I. HENKIN and D. F. BRADLEY, *Proc. Nat. Acad. Sci.*, 62, 30 (1969).

20. W. FALLIS, L. LASAGNA and L. TETREAULT, *Nature*, 196, 74 (1962).

21. S. WOTMAN, I. D. MANDEL, R. H. THOMPSON, JR., and J. H. LARAGH, *J. Chron. Dis.* 20, 833 (1967).

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CORRESPONDENCE

Letters must be kept to a reasonable length. Otherwise it may not be possible to find space for them.

DANGERS OF RAPID INFUSION OF POTASSIUM

SIR: I am prompted to comment on the dangers of intravenous potassium administration by a report in *The Australian* (September 6, 1973), of an inquest held the previous day in Sydney. It was stated that a patient who was on intravenous therapy with both an antibiotic and potassium chloride died because she was mistakenly given, by a nursing sister, a direct injection of potassium chloride.

The 15% potassium chloride solution must always be diluted not less than 50 times[1] before injection. A rapid intravenous injection of a bolus of undiluted solution will almost invariably cause cardiac arrest, and I am very aware of this, having had the misfortune to attend a case of irreversible cardiac arrest in a young man with a healthy cardiovascular system who died when a nurse inadvertently injected an ampoule of potassium chloride solution into the tubing of a drip instead of into the drip bottle.

The risk of such a tragic accident can now readily be avoided by the use of standard maintenance fluid for the intravenous therapy of such cases; the need for nurses to mix concentrated potassium chloride solution into other fluids is then obviated. Standard maintenance fluid is available on the pharmaceutical benefits list and contains in each litre 20 mEq sodium, 20 mEq potassium, 20 mEq chlorine and 20 mEq lactate in 5% dextrose, providing 190 kilocalories per litre. Four litres per 24 hours of this solution will provide the fluid, electrolytes and calories recommended by the N.H.M.R.C.[2] for maintenance therapy of the unconscious patient. This can be augmented where necessary with the replacement fluids also available on the Pharmaceutical Benefits list.

The rate of infusion of this standard maintenance fluid should never exceed 1 litre per hour, as the recommended[3] maximum rate of infusion of potassium chloride solution is 20 mEq per hour, if potentially toxic levels of plasma potassium are to be avoided. It should be noted that as Darrow's solution contains 40 mEq potassium per litre, this solution should not be infused at a rate exceeding 500 ml per hour.

As a significant number of doctors are, in my experience, not aware of the dangers of rapid infusion of potassium chloride, it is certainly not surprising that few nurses are. The true incidence of this type of accident is, of course, impossible to ascertain, as nurses and doctors are naturally reluctant to publicize them, but fatalities are, I believe, commoner than is generally realized.

A recent article in the *British Medical Journal*[4] drew attention to the fact that even injecting potassium chloride into large volumes of intravenous fluid is not without hazard. Poor mixing was reported where plastic containers were used instead of glass flasks, with resultant serious potassium toxicity reactions in two cases.

With the ready availability of a variety of dilute potassium solutions, I would suggest that the inherent dangers associated with the use of ampoules of concentrated potassium chloride solution demand that these should no longer be manufactured; at the very least, their use should be only under the strictest medical supervision.

Clare, S.A. 5453. D. G. CHAMBERS.

[1] *British National Formulary*, 1968, London, B.M.A.: 137.

[2] National Health and Medical Research Council, *Aust. Fam. Phycn*, 1972, 1: 489.

[3] *Martindale's Extra Pharmacopœia*, 26th edition, 1972, London: 1516.

[4] Williams, R. H. P., *Brit. med. J.*, 1973, 1: 714.

Effect of potassium chloride on intestinal blood flow

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W. T. CHEN, and FRANCIS J. HADDY *East Lansing, Mich.*

Previous workers have hypothesized that KCl-induced ulcers of the small intestine result from ischemia. The present study was designed to determine whether the luminal placement of KCl does, in fact, reduce intestinal blood flow. While venous outflow was being measured for 60 to 70 min., various combinations of KCl, hydrochlorothiazide-KCl, hydrochlorothiazide, and NaCl tablets and glass beads were placed into 2 in situ adjacent segments (control and experimental) of the dog small bowel. Both ends of the segment were open to the air. In other experiments, luminal pressure was monitored in closed segments containing aqueous solutions of KCl or polyethylene glycol. The placement of KCl or hydrochlorothiazide-KCl tablets caused: (1) an initial transient rise followed by a progressive fall in blood flow, (2) a progressive rise in the potassium concentration of venous plasma from the segment, and (3) tonic contractions of the segment and an increase in luminal pressure. Glass beads or tablets containing only hydrochlorothiazide had little effect. NaCl tablets caused a large increase in blood flow which often remained above the control level for at least one hour with no observed changes in the motility of the segment. It is suggested that the production of ulcers during the chronic administration of tablets containing KCl may result from local mucosal damage by hyperosmolarity coupled with the demonstrated capacity of the potassium ion to reduce blood flow while increasing tonic contractions of the intestine.

The apparent increase in the incidence of circumferential stenosing ulcerations of the small bowel in the last 10 years appears to be related to oral administration of potassium chloride given alone or in combination with diuretics.[2-4] Studies in monkeys and dogs indicate that the diuretic is not ulcerogenic since oral administration of a potassium preparation to these animals causes ulcerations whereas placebo or diuretic tablets do not.[5, 6] Other studies suggest

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that a high concentration of potassium in a localized area is necessary for the development of the lesions. Rapidly disintegrating tablets or syrups containing KCl produce ulcers in the stomach,[6, 7] whereas enteric-coated tablets cause lesions in the small intestine.[5-7] Implantation of KCl tablets on the intestinal mucosa produces ulcerative lesions in an area 2 to 3 cm. distal to the implantation.[8] Combining intestinal stenosis, experimentally produced, with oral administration of KCl tablets results in an increased incidence of intestinal ulcers.[9]

The mechanism by which a high local concentration of KCl causes ulceration is not clear. Although local ischemia has been suggested as a mechanism for the lesions,[8, 10-12] no direct measurements of local intestinal blood flow before and after luminal placement of KCl tablets have been made. The present study was therefore initiated to determine whether local blood flow is, in fact, altered by the presence of KCl in the lumen.

Methods

Blood flow. Mongrel dogs (15 to 18 kilograms) were anesthetized with pentobarbital sodium (30 mg. per kilogram). After a midline incision, a loop of ileum or jejunum was exteriorized and divided into 2 segments such that blood flow from each segment was drained by a single vein (Fig. 1). Short plastic tubes were inserted into each end of both segments, and the cut ends of each segment were tied around the tube to prevent bleeding. Both ends of the segments were left open, and tablets were introduced through these openings. After intravenous administration of heparin sodium (6 mg. per kilogram), the vein draining each

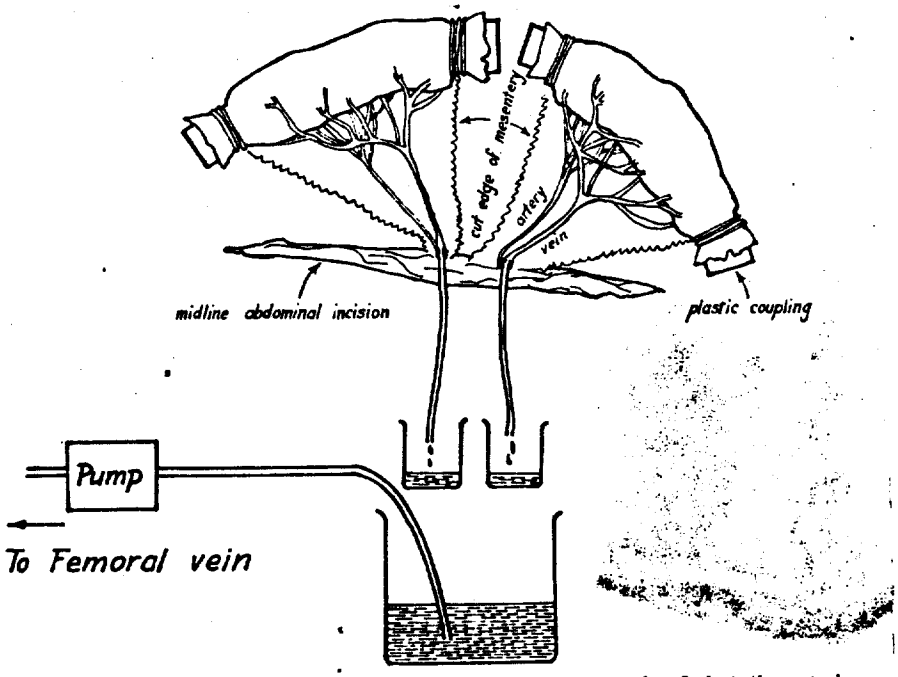

Fig. 1. Schematic drawing of the preparation. The veins were cannulated, but the arteries and nerves remained intact.

loop was cannulated, and the mesentery was cut to exclude collateral flow. Venous blood was collected in a reservoir initially containing 200 ml. of 6 per cent dextran in normal saline, and the blood was continuously pumped back to the dogs via a femoral vein at a rate equal to venous outflow. A femoral artery was cannulated to record systemic pressure. Venous outflows from each segment were collected in small beakers for 5 min. with 1 min. intervals between collection periods. The blood was weighed on a direct reading balance (Top-Loading Precision Balance, P 1200, Mettler Instrument Co., Princeton, N. J.) and then poured into the reservoir. Blood flow was expressed as grams per minute.

The substances under study were divided into the following 5 combinations: (1) KCl tablets (KCl, 975 mg.) vs. glass beads (1 cm. in diameter); (2) hydrochlorothiazide-KCl (Hydrodiuril Ka-50) (KCl, 572 mg.) vs. glass beads; (3) hydrochlorothiazide-KCl vs. hydrochlorothiazide (Hydrodiuril), (4) hydrochlorothiazide-KCl vs. sodium chloride tablets (NaCl, 1 Gm.), and (5) sodium chloride tablets vs. glass beads. Six or seven animals were used for each combination. The enteric coating of the KCl and hydrochlorothiazide-KCl tablets was removed prior to luminal placement; the other tablets have no coating.

After a control period (24 to 30 min.) during which venous outflows became relatively steady, 6 to 8 tablets of one substance (e.g., KCl) were placed into the lumen of one segment, and 6 to 8 tablets of the other substance (e.g., glass beads) were placed into the lumen of the other segment. Their effects on the rate of venous outflow from the 2 segments and on the potassium and/or sodium concentration and osmolarity of this venous plasma were compared. The plasma potassium and sodium concentrations and osmolarity were measured with a flamephotometer (Beckman model 105) and osmometer (Advanced Instruments, Inc.), respectively. After the experiment, the intestinal segments were examined grossly and microscopically for evidence of pathology. Student's t test, modified for a paired comparison,[13] was used to evaluate the changes in blood flow, ion concentrations, and osmolarity. Changes in blood flow were analyzed in 3 ways: (1) comparison of absolute flows (grams per minute) obtained in the same segment before (i.e., control) and at 5 and 60 min. after placement of tablets; (2) comparison of flow change (as per cent of control) which occurred in one segment with the simultaneous change (as per cent of its control) in the other segment; and (3) comparison of flow (grams per minute) from one segment with the corresponding flow from the other segment one hour after luminal placement of tablets. Student's t test modified for unpaired comparison[13] was used for the third comparison. The object of the third comparison was to determine whether blood flow of the segment containing KCl or hydrochlorothiazide-KCl tablets was lower (i.e., more ischemic) than the segment containing glass beads, hydrochlorothiazide, or NaCl.

Motility. KCl tablets in intestinal segments open at both ends produced strong, visible muscular contractions. In an attempt to quantitate the effect of KCl on luminal pressure, we studied the effects of hyperosmotic KCl solutions in similar loops that were tied at both ends. Small rubber tubes, inserted into each loop, allowed for the introduction and removal of solutions and, at all other times, for the monitoring of luminal pressure. A KCl solution (885 mEq. per liter, 1,500 mOsm. per kilogram) was introduced into one loop while the control loop contained a solution of polyethylene glycol (molecular weight, 4,000) of the same osmolarity. This test period was interspersed between control periods during which both loops contained isosmotic polyethylene glycol. Each solution remained in its loop for 15 min. during which blood flow and luminal pressure were measured and samples of the effluent blood were obtained for the determination of plasma potassium concentration. Preliminary studies for longer periods (60 to 90 min.) showed that 15 minutes were sufficient to induce a maximum increase in motility, which persisted thereafter.

Chronic study. Ten dogs (9 to 14 kilograms) were divided into 3 groups. Enteric-coated KCl (975 mg. tablets, 2 tablets every day) was administered orally to the first group (3 dogs); Lomotil (diphenoxylate HCl, 2.5 mg.; and atropine sulfate, 0.025 mg.—2 tablets twice a day) alone to the second group (3 dogs); enteric-coated KCl (2 tablets every day) and Lomotil (2 tablets twice a day) to the third group (4 dogs). The animals received these medications for 2 to 3 weeks with ad libitum feeding. The gastrointestinal tract was examined grossly and microscopically after necropsy.

Results

Blood flow. Systemic blood pressure (100 to 140 mm. Hg) was not significantly altered by any of the experimental procedures except luminal placement of KCl or NaCl tablets. These tablets sometimes caused an initial transient fall in systemic pressure (5 to 10 mm. Hg) as a result of marked rise in venous outflow that transiently exceeded the pump flow returning to the dog. When this occurred, blood flow in the glass beads segment also fell transiently (Figs. 2 and 6). Systemic pressure and blood flow in the bead segment returned to their control levels as soon as pump flow brought the reservoir and thus the animals' blood volumes to their initial levels (Fig. 2).

KCL VS. GLASS BEADS. The KCl tablets caused an initial rise (5 to 20 min.) followed by a fall in blood flow over a 70 min. period (Fig. 2). The initial rise and eventual fall in flow were statistically significant as compared to either the

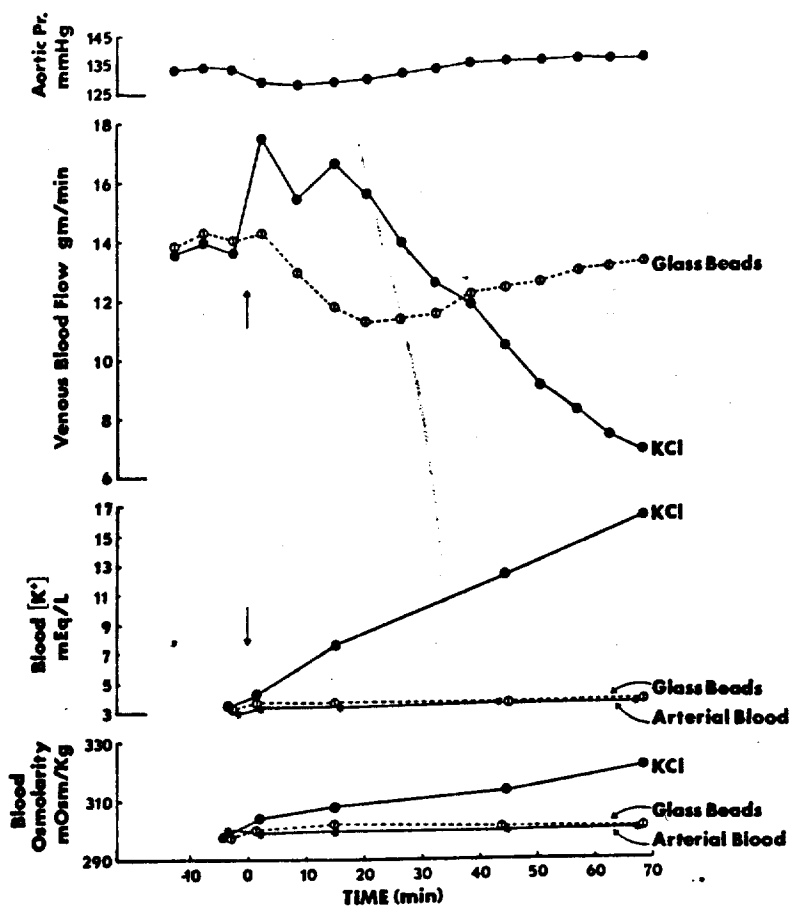

Fig. 2. Average effects over 70 min. when KCl tablets and glass beads were placed in the 2 segments. Arrows indicate time of placement of tablets (N = 6).

flow value in the same segment before placement of KCl (Table I) or to the simultaneous changes in flow that occurred in the segment with beads (Table II). Furthermore, the absolute level of flow one hour after the placement of tablets (Table I) was significantly lower in the KCl segment than in the glass beads segment ($P < 0.01$). In the segment with beads, blood flow fell transiently ($P < 0.02$) from its control at about 20 min. but eventually returned to its control value as systemic blood pressure rose (Fig. 2 and Table I). Venous K^+

Table I. Venous outflow after placing various agents in the gut lumen

No.	Agent	Blood flow (Gm./min.)			Change from control	
		Control	5 min.*	1 hr.*	5 min.	1 hr.
6	KCl	13.62	17.50	6.45	+3.88 ± 1.38†	-7.17 ± 0.16†
	Beads	14.07	15.06	13.38	+0.99 ± 0.63	-0.69 ± 0.66
7	HK	6.54	8.22	3.05	+1.68 ± 0.45†	-3.49 ± 0.43†
	Beads	5.97	5.80	4.88	-0.17 ± 0.36	-1.09 ± 1.17
7	HK	8.74	10.24	5.55	+1.50 ± 0.51†	-3.19 ± 0.45†
	H	8.77	8.81	7.67	+0.04 ± 0.15	-1.10 ± 0.50
6	HK	10.91	13.54	7.31	+2.63 ± 1.00†	-3.60 ± 0.38†
	NaCl	10.33	14.95	10.19	+4.62 ± 1.23†	-0.14 ± 0.39
6	NaCl	17.11	27.68	22.34	+10.57 ± 3.04†	+5.23 ± 1.36†
	Beads	16.64	16.68	15.58	+0.04 ± 0.81	-1.06 ± 1.88

*Flow values at 5 min. and 1 hour after placement of agents.
†Indicates that change from control is statistically significant at P value less than 0.05 (mean ± S.E.).

Table II. Average changes in venous outflow (in per cent of the control) after placement of various agents in the gut lumen

No.	Segment	Agents	Time in minutes after placement of agents										
			0-5	6-11	12-17	18-23	24-29	30-35	36-41	42-47	48-53	54-59	60-65
6	A	KCl	+28*	+13*	+22*	+15*	+3	-7	-13	-22*	-33*	-39*	-45*
	B	Beads	+7	-8	-17	-20	-18	-16	-12	-11	-10	-7	-5
7	A	HK	+26*	+12*	+5	-8	-20	-28	-31*	-45*	-48*	-55*	-54*
	B	Beads	-2	-8	-12	-13	-15	-16	-18	-18	-18	-18	-18
7	A	HK	+17*	+10*	+6	+4	-3	-9	-15	-20*	-24*	-37*	-36*
	B	H	0	-7	-6	-6	-10	-13	-14	-14	-14	-16	-13
6	A	HK	+24*	+19*	-3*	-8*	-11*	-13*	-17*	-23*	-27*	-30*	-33*
	B	NaCl	+46	+43	+19	+17	+13	+13	+12	+10	+4	-1	-1
6	A	NaCl	+61*	+52*	+28*	+29*	+23*	+28*	+28*	+28*	+26*	+30*	+30*
	B	Beads	0	-11	-14	-13	-12	-10	-10	-8	-9	-6	-6

*Indicates that changes in blood flow in segment A are significantly different from simultaneous changes in blood flow that occurred in segment B ($P < 0.05$).

concentration and osmolarity rose significantly in the KCl segment (+13.9 ± 4.2 mEq. per liter and +23.5 ± 5.4 mOsm. per kilogram) (Fig. 2), and about 0.07 to 0.12 mEq. of K^+ appeared in the venous blood in excess of control values per minute. Venous K^+ concentration (+0.6 ± 0.2 mEq. per liter) and osmolarity (+4.0 ± 1.0 mOsm. per kilogram) also rose in the beads segment. These rises, however, were similar to those in arterial blood (Fig. 2).

Vigorous contractions of the segment were observed after luminal placement of KCl tablets. Occasionally the segment became rigid and pale, and this was accompanied by a marked fall in blood flow. Glass beads did not alter gut motility. At necropsy, the segment with KCl showed edema, hemorrhage, and superficial erosion, while the segment with the glass beads appeared normal both grossly and microscopically.

HYDROCHLOROTHIAZIDE-KCL VS. GLASS BEADS OR HYDROCHLOROTHIAZIDE OR NACL. The effects on blood flow of placing hydrochlorothiazide-KCl tablets *(HK)* in the lumen were similar to those produced by KCl tablets (Fig. 2) whether its effect was compared to glass beads (Fig. 3), hydrochlorothiazide tablets *(H)* (Fig. 4), or NaCl tablets (Fig. 5). Blood flow rose initially followed by a progressive fall. The initial rise and eventual fall in flow were statistically significant as compared to either the control values in the same segment (Table I) or to the simultaneous changes in flow occurring in the segment containing beads, *H*, or NaCl (Table II). Furthermore, the absolute level of blood flow (Table I) one hour after luminal placement of tablets was significantly lower in the HK segment than in the glass beads, *H*, or NaCl segment ($P < 0.05$). Venous K^+ concentration significantly rose in the *HK* segment (+5.3 ± 0.5 mEq. per liter, +3.0 ± 0.3 mEq. per liter, and +5.4 ± 1.9 mEq. per liter when paired

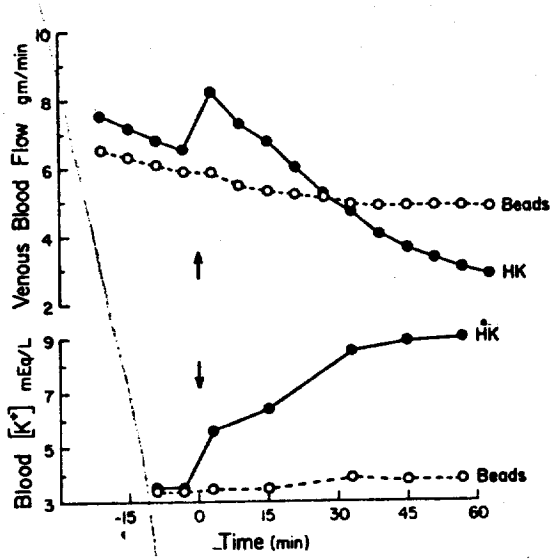

Fig. 3. Average effects over 60 min. when hydrochlorothiazide-KCl *(HK)* tablets and glass beads were placed in the 2 segments (N = 7).

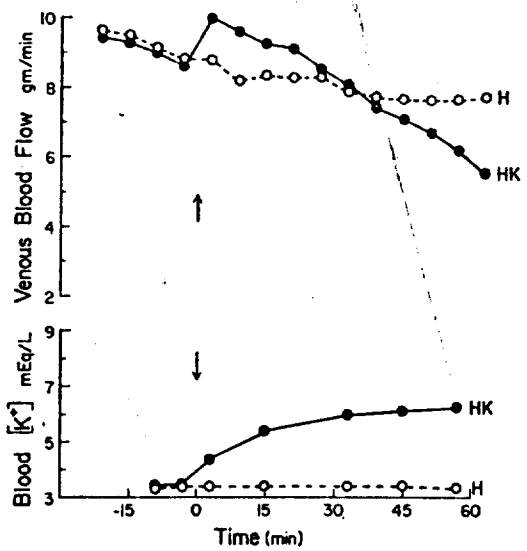

Fig. 4. Average effects over 60 min. when hydrochlorothiazide-KCl *(HK)* and hydrochlorothiazide *(H)* tablets were placed in the 2 segments (N = 7).

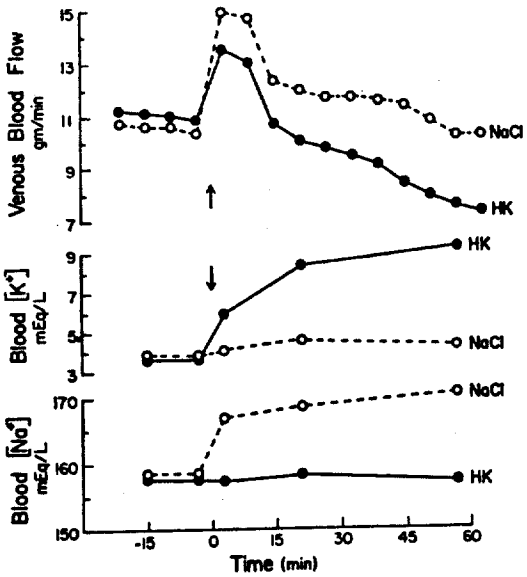

Fig. 5. Average effects over 60 min. when hydrochlorothiazide-KCl *(HK)* and NaCl tablets were placed in the 2 segments (N = 6).

with glass beads, *H*, or NaCl, respectively). The absorption of K^+ from the *HK* segment ranged from 0.01 to 0.05 mEq. per minute.

Blood flow and venous K^+ concentration were not significantly altered by the glass beads (Fig. 3, Table I) or hydrochlorothiazide (Fig. 4, Table I). NaCl caused an initial rise in flow followed by a return to its control value (Fig. 5, Table I). The initial rise in flow in the NaCl segment was significantly greater than the rise in the HK segment (Table II). Plasma Na^+ concentration significantly rose in NaCl segment (+12.5 ± 4.9 mEq. per liter).

At necropsy, the segments with glass beads or hydrochlorothiazide appeared normal both grossly and microscopically. In contrast, hydrochlorothiazide-KCl or NaCl caused edema, hemorrhage, and superficial erosions of the mucosa. These erosions were confined to the villi, and lesions caused by NaCl and *HK* were indistinguishable histologically. Grossly, however, the hemorrhage with NaCl appeared bright red and less extensive than that produced by KCl or *HK*. The hemorrhage with KCl or HK was dark red and extended over the entire segment. Like KCl, *HK* caused vigorous contractions of the segment. Hydrochlorothiazide or NaCl did not affect gut motility.

NaCl vs. glass beads. In contrast to glass beads, NaCl tablets caused a sustained rise in venous outflow (Fig. 6, Table II). Blood flow was still above control even one hour after placement of NaCl (Table I). On the average, the initial rise with NaCl tablets (+61 per cent) was greater than that with KCl tablets (+28 per cent), glass beads being the control in both instances (Table II). Glass beads did not significantly alter blood flow (Table I) excepting a transient fall ($P < 0.02$) that occurred at 15 min. (Fig. 6). The sodium concentration in the venous plasma did not change significantly in either segment.

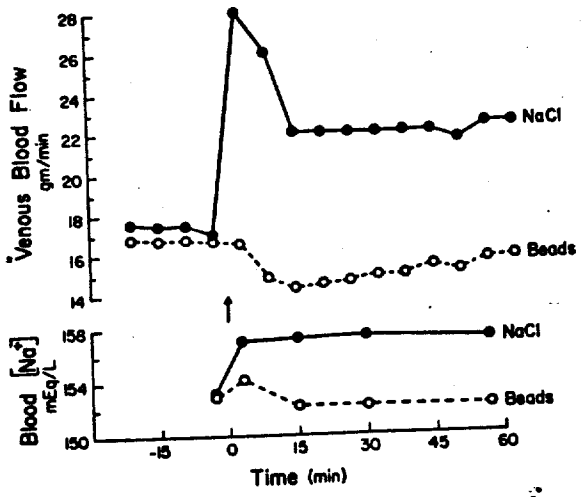

Fig. 6. Average effects over 60 min. when NaCl tablets and glass beads were placed in the 2 segments (N = 6).

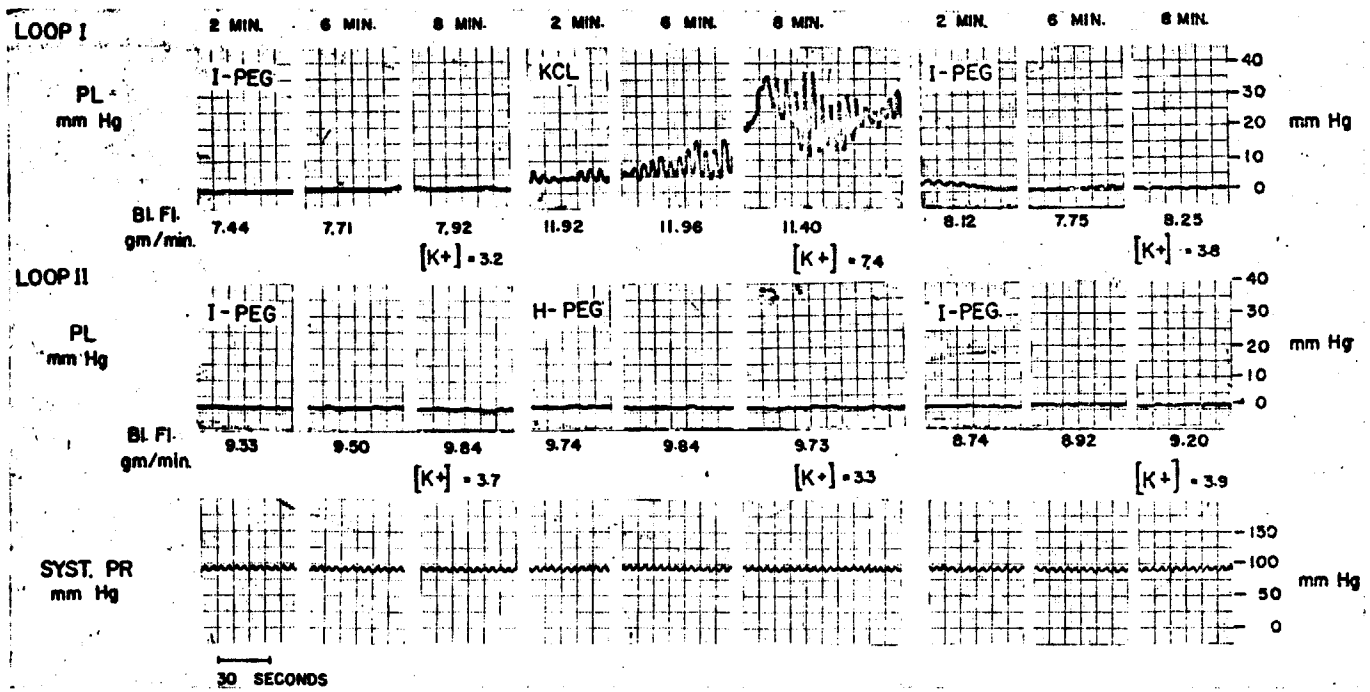

Fig. 7. Effects on intestinal luminal pressure *(PL)*, blood flow *(Bl. Fl.)*, and venous plasma potassium concentration of isosmotic polyethylene glycol *(I-PEG)*, hyperosmotic polyethylene glycol *(II-PEG)*, and hyperosmotic KCl solutions.

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Intestinal motility. Luminal placement of a 1,500 mOsm. per kilogram of KCl solution (10 ml.) into a closed intestinal segment (N = 9) for 15 min. raised blood flow (+5.5 ± 0.8 Gm. per minute), luminal volume (+6.4 ± 0.6 ml. per 15 min.), venous K^+ concentration (+3.8 ± 1.0 mEq. per liter), venous osmolarity (+8.0 ± 2.0 mOsm. per kilogram), and luminal pressure. Luminal pressure increased progressively to 20 to 50 mm. Hg by the eighth minute and remained at this level thereafter. A 1,500 mOsm. per kilogram of polyethlene glycol *(H-PEG)* on the other hand did not significantly alter blood flow, K^+ concentration, or luminal pressure but raised luminal volume (+2.0 ± 0.3 ml. per 15 min.) and venous osmolarity (+5.0 ± 2.0 mOsm. per kilogram). Isosmotic polyethylene glycol *(I-PEG)* did not significantly alter any of these parameters. A typical luminal pressure record from an experiment is shown in Fig. 7.

Chronic study. During the course of medication, all 10 dogs remained in apparent good health except one given enteric-coated KCl tablets which developed diarrhea. The gastrointestinal tracts of the 3 dogs medicated with enteric-coated KCl tablets appeared normal at necropsy. One of the 3 dogs medicated with Lomotil alone had slightly hyperemic duodenal mucosa. The gastrointestinal (GI) tracts of the other 2 dogs given Lomotil appeared normal. Two of the 4 dogs medicated with KCl and Lomotil showed hemorrhage, hyperemia, and edema of the duodenum and ileum. Microscopic examination, however, failed to reveal erosions or ulcers in these areas. The GI tracts of the other 2 similarly treated dogs appeared normal.

Discussion

In the past, virtually all circulatory studies of the exteriorized intestine have been done in single segments. With the single-segment technique, the change due to treatment may be difficult to separate from those occurring spontaneously with time. By the simultaneous use of 2 similarly prepared adjacent segments, we feel that this problem has been essentially solved. With the double-segments technique, any difference in the response of the segments can logically be attributed to the treatment (e.g., the luminal contents in the present study).

These studies show that the placement of KCl tablets or tablets containing KCl into the lumen of the exteriorized dog gut for 60 to 70 minutes causes: (1) an initial transient rise followed by a progressive fall in total blood flow, (2) a progressive rise in the potassium concentration of venous plasma from the segment, (3) a tonic contraction of the segment and an increase in its luminal pressure, and (4) mucosal erosion and hemorrhage. Tablets containing hydrochlorothiazide alone are without effect. The studies also show that NaCl tablets cause a greater rise in flow than KCl tablets and that the flow remains above or at the control value for at least an hour.

We have shown previously that an intra-arterial infusion of KCl which slightly raises the plasma potassium level raises intestinal blood flow but infusion rates which raise the concentration above 10 mEq. per liter decrease blood flow.[14, 15] Studies by other workers demonstrate that hyperosmolarity produced by intra-arterial administration also has a biphasic effect on blood flow. Increase in osmolarity to about 800 mOsm. per liter increases flow whereas further increase tends to reduce flow.[16, 17]

It seems likely that placement of KCl into the gut lumen would produce vascular effects similar to those produced by intra-arterial infusion, if K^+ is readily absorbed and diffuses into the tissue fluid bathing intramural vessels of the gut wall. Absorption of K^+ has been shown to increase in a linear fashion as the function of luminal K^+ concentration.[18, 19] Furthermore, it has also been shown that with K^+ in the gut lumen for 10 min., 0.7 per cent of the K^+ appeared in the venous blood while 9.3 per cent resided in the gut wall. Ten minutes post absorption, 8.5 per cent of the original K^+ was still in the gut wall.[20] These studies show that K^+ is readily absorbed, diffuses into, and remains for some time in the intestinal tissue. If mucosal cells are damaged, as observed in this study, diffusion of K^+ into the gut tissue will likely increase and tissue fluid K^+ concentration will be higher than that in the normal gut. Venous K^+ concentration increased with time (Figs. 2 to 5) indicating a progressive rise in K^+ absorption from the gut lumen.

The initial rise in blood flow caused by KCl or HK tablets was most likely due to vasodilator action of slight to moderate rises in K^+ concentration and osmolarity in the tissue fluid bathing intramural vessels of the segment. As tissue K^+ concentration increased with time and reached its constrictor range, total blood flow began to fall (Fig. 2). High concentration of K^+ increases the contractions of both vascular and visceral smooth muscle.[14] These contractions act synergistically to reduce blood flow through active vasoconstriction and passive compression.[21] Vigorous contractions associated with blanching of the intestine were observed. The compressive effect of visceral muscle contraction, however, must have been masked by the vasodilator action of K^+ and osmolarity during the initial 20 min.

The rise in flow caused by NaCl tablets is most likely due to a rise in osmolarity since much data support the concept that NaCl is not vasoactive except nonspecificially through osmolarity.[17, 22] It was interesting that the blood flow in NaCl segment remained above control value for the entire experimental period in NaCl-beads experiments, while flow fell to its control value at 48 to 65 min. in NaCl-HK experiments (Figs. 5 and 6, Table II). The difference in flow changes in the 2 types of experiments (+30 vs. −1 per cent at 60 min.) is significant ($P < 0.02$).* This finding may be related to the presence of K^+ in the opposite segment since we have observed a similar phenomenon when studying hyperosmolar $MgCl_2$ solution (1,500 mOsm. per liter) and KCl in the double-segment preparation. When one segment contained hyperosmolar NaCl (1,500 mOsm. per liter), $MgCl_2$ in the other segment raised blood flow by 43.2 ± 9.8 per cent of its control value. However, when one segment contained hyperosmotic KCl (1,500 mOsm. per liter), $MgCl_2$ in the other segment raised blood flow by only 7.4 ± 6.6 per cent. The difference in the effects of $MgCl_2$ on blood flow in these 2 types of experiments was significant ($P < 0.02$).* Thus, the presence of KCl in one segment appears to reduce the vasodilator effect of hyperosmolarity in the other segment. The mechanisms are currently under investigation but appear not to be related to the absorption of Na^+, Mg^{++}, or K^+.

The mechanism by which a high concentration of KCl causes ulceration has

*Comparison made by Student's t test modified for unpaired comparison.[13]

not been clearly delineated. Two possibilities are: (1) a direct necrotizing action on the mucosa, and (2) an indirect necrotizing action on the mucosa via ischemia.

The superficial lesions caused by KCl, NaCl, or HK tablets may be attributed to hyperosmolarity. Luminal placement of various hyperosmotic solutions, e.g., glucose or urea, has been shown to produce superficial erosion of the mucosa, and the causative factor appears to be hyperosmolarity.[23] Hyperosmolarity may damage mucosal cells by a direct irritant action (osmotic removal of intracellular water, coagulation of cell protein, inhibition of the sodium pump?). However, mucosal ischemia may also contribute to the production of lesions. If tissue osmolarity near the points of contact between tablets and mucosa rises to 800 mOsm. per liter or more, local mucosal blood flow might fall.[16]

Although lesions caused by KCl and NaCl were indistinguishable microscopically in the present acute study, others have shown in long-term experiments that NaCl tablets do not cause lesions under conditions in which KCl causes lesions.[5, 10] The present study and our previous studies[24] show that, in contrast to NaCl tablets or solutions, KCl decreases blood flow and causes tonic contraction of the segment. These 2 distinctive actions of the potassium ion may play a critical role in the induction of KCl lesions seen in long-term experiments. The basic pathological feature of KCl-induced lesions in man and experimental animals is an hemorrhagic infarct.[8, 11] Intestinal ischemia caused by occlusion of a mesenteric artery produces superficial mucosal lesions in 2 hours.[25] Injection of microspheres into an intestinal artery in the dog produces, in 2 weeks, lesions similar to those seen in patients receiving diuretic-KCl medication.[12] Tonic contraction may delay the passage of tablets along the gut lumen and thus prolong exposure of a localized area of the gut mucosa to a high concentration of potassium. The latter has been shown to be a necessary condition for the lesions.[5-7]

Partial mechanical obstruction of the bowel, experimentally produced, increases the incidence of KCl-induced bowel lesions,[9] perhaps by prolonging the exposure of the mucosa to the KCl tablets. Similar conditions may result clinically during simultaneous administration of KCl tablets and antidiarrheal medications which prolong GI transit time. In the present study, ulcerative lesions were not observed during administration of enteric-coated KCl tablets alone or in combination with an antidiarrheal drug, Lomotil. Perhaps the KCl tablets did not localize to a discrete area either in the absence or in the presence of Lomotil. Since the primate appears to be more susceptible to KCl-induced ulcers than the dog,[5] study of the relationship of KCl ulcers to antidiarrheal medication in this species might be valuable.

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REFERENCES

1. Chou, C. C., Baker, T. A., and Dabney, J. M.: The role of blood flow in the induction of intestinal ulcer by diuretic-KCl tablets, Clin. Res. 15: 416, 1967.
2. Baker, D. R., Schrader, W. H., and Hitchcock, C. R.: Small-bowel ulceration apparently associated with thiazide and potassium therapy, J. A. M. A. 190: 586, 1964.
3. Lindholmer, B., Nyman, E., and Raf, L.: Nonspecific stenosing ulceration of small bowel: A preliminary report, Acta. Chir. Scand. 128: 310, 1964.

4. Morgenstern, L., Freilich, M., and Panish, J. F.: The circumferential small-bowel ulcer, J. A. M. A. 191: 637, 1965.
5. Lawrason, F. D., Alpert, E., Mohr, F. L., and McMahon, F. G.: Ulcerative-obstructive lesions of the small intestine, J. A. M. A. 191: 641, 1965.
6. Bokelman, D., Bagdon, W., Zwickey, R. E., and Mattis, P. A.: Ulcerogenic effect of potassium chloride on the gastrointestinal tract of the monkey, Fed. Proc. 24: 715, 1965.
7. Diener, R. M., Shoffstall, D. H., Earl, A. E., and Gaunt, R.: The production of potassium induced gastrointestinal lesions in monkeys, Fed. Proc. 24: 714, 1965.
8. Boley, S. J., Schultz, L., Krieger, H., Schwartz, S., Elguezabal, A., and Allen, A. C.: Experimental evaluation of thiazides and potassium as a cause of small-bowel ulcer, J. A. M. A. 192: 763, 1965.
9. Stahlgren, L. H., Dapena, A., and Roy, R. L.: Ulcerogenic properties of enteric coated compounds in dogs, Surg. Forum 196: 367, 1965.
10. Mansfield, J. B., Schoenfeld, F. B., Suwa, M., Geurkink, R. E., and Anderson, M. C.: Role of vascular insufficiency in drug-induced small bowel ulceration, Amer. J. Surg. 113: 608, 1967.
11. Allen, A. C., Boley, S. J., Schultz, L., and Schwartz, S.: Potassium-induced lesions of the small bowel, J. A. M. A. 193: 1001, 1965.
12. Boley, S. J., Krieger, H., Schultz, L., Robinson, K., Siew, F. P., Allen, A. C., and Schwartz, S.: Experimental aspects of peripheral vascular occlusion of the intestine, Surg. Gynec. Obstet. 121: 789, 1965.
13. Steel, R. G. D., and Torrie, J. H.: Principles and procedures of statistics, New York, 1960, McGraw-Hill Book Company.
14. Dabney, J. M., Scott, J. B., and Chou, C. C.: Effects of cations on ileal compliance and blood flow, Amer. J. Physiol. 212: 835, 1967.
15. Texter, E. C., Jr., Laureta, H. C., Frohlich, E. D., and Chou, C. C.: Effects of major cations on gastric and mesenteric vascular resistances, Amer. J. Physiol. 212: 569, 1967.
16. Read, R. C., Johnson, J. A., Vick, J. A., and Meyer, M. W.: Vascular effects of hypertonic solutions, Circ. Res. 8: 538, 1960.
17. Haddy, F. J., and Scott, J. B.: Effects of electrolytes and water upon resistance to blood flow through intact vascular beds, *in* Bajusz, E., editor: Electrolytes and cardiovascular disease, Basel, 1965, S. Karger, A. G., pp. 383-400.
18. Phillips, S. F., and Code, C. F.: Sorption of potassium in the small and the large intestine, Amer. J. Physiol. 211: 607, 1966.
19. Phillips, S. F., and Summerskill, W. H. J.: Water and electrolyte transport during maintenance of isotonicity in human jejunum and ileum, J. LAB. CLIN. MED. 70: 686, 1967.
20. Williams, L. F., Jr., and Davies, M. W.: In vivo measurement of the 3 compartmental components of intestinal transport of Na and K, Fed. Proc. 28: 324, 1969.
21. Chou, C. C., and Dabney, J. M.: Interrelation of ileal wall compliance and vascular resistance, Amer. J. Dig. Dis. 12: 1198, 1967.
22. Overbeck, H. W., Molnar, J. I., and Haddy, F. J.: Resistance to blood flow through the vascular bed of the dog forelimb. Local effects of sodium, potassium, calcium, magnesium, acetate, hypertonicity, and hypotonicity, Amer. J. Cardiol. 8: 533, 1961.
23. Kameda, H., Aber, T., Nasrallah, S., and Iber, F. L.: Functional and histological injury to intestinal mucosa produced by hypertonicity, Amer. J. Physiol. 214: 1090, 1968.
24. Chou, C. C., Chen, W. T., and Dabney, J. M.: Ileal blood flow and motility with cations in the lumen, Proc. Int. Union Physiol. Sci. 7: 85, 1968.
25. Glotzer, D. J., Villegas, A. H., Anekamaya, S., and Shaw, R. S.: Healing of the intestine in experimental bowel infarction, Ann. Surg. 155: 183, 1962.

Slow Sodium: An Oral Slowly Released Sodium Chloride Preparation

10017

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Summary

The use of a slowly released oral preparation of sodium chloride is described. It was given to patients and athletes to treat or prevent acute and chronic sodium chloride deficiency. Gastrointestinal side effects were not encountered after the ingestion of up to 500 mEq in one day or 200 mEq in 10 minutes.

Introduction

Sodium depletion, with the fluid loss with which it is usually accompanied, produces symptoms which are well described (King and Barry, 1962; Leithead and Lind, 1964): headache, thirst, anorexia and nausea, apathy, drowsiness, postural giddiness, and muscle cramps. Eventually the blood pressure falls and death may ensue from peripheral circulatory failure.

In temperate climates this picture is commonly seen only in patients with uncompensated loss of gastrointestinal secretions, in patients with renal damage subjected to dietary salt restriction, or with over-enthusiastic use of diuretic drugs. Occasionally it may appear in Addisonian crisis, in uncontrolled diabetes, or with cerebral lesions. Treatment is usually by intravenous saline infusion after the patient has been admitted to hospital. This, however, does not give a true picture of the extent of the problem of sodium depletion throughout the world. For many people in certain climatic conditions or in certain occupations, such as coal miners and gold miners (King and Barry, 1962), seamen in tropical waters (Stenning, 1945), or the armed Forces abroad, the fight against incipient salt and water depletion may be continuous.

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In theory an increased salt and water intake should prevent depletion. But though people regularly subjected to prodigious sweat loss eventually achieve overall sodium and water balance, they may still suffer from a steady state of sodium depletion or the daily recurrence of a transient salt depletion syndrome. This problem is particularly acute in individuals who are not acclimatized (Leithead, Leithead, and Lee, 1958). It is then easy to acquire a pronounced initial salt and water deficit which may take days to correct. During this time physical perfomance will be much lowered. This was strikingly illustrated in the 1970 World Cup football competition in Mexico. Training for and playing football in temperatures approaching 100°F (37·8°C) produced a noticeable weight reduction due to sweat loss, which was not necessarily regained in spite of deliberate attention to salt and water replacement. For this reason a few players of some of the national teams in the competition were incapacitated for several days.

There has been no effective way to administer large quantities of sodium chloride orally (Leithead and Lind, 1964). Saline solutions are unpalatable. Enteric-coated tablets are notorious in that they are excreted unchanged in the faeces (Leithead *et al.*, 1958). Some years ago therefore we asked the Ciba pharmaceutical company to prepare slow release sodium chloride tablets using the wax sponge formulation they had introduced for their potassium supplement preparation Slow-K. We here report the results of some of our experimental work with these tablets and clinical experience over several years. This preparation, which has been named Slow Sodium (sodium chloride 600 mg, equivalent to 10 mEq Na^+ and Cl^-) was also used by the English international football team in Mexico in 1970 where it proved very successful in solving the problem of salt and water depletion.

Balance Studies

Tests were first carried out in vitro to ensure that if the tablets should pass through the gut unchanged they would be detected in the faeces by our analytical technique. Tablets of Slow Sodium were added to a sample of homogenized faeces. The faeces and tablets were then homogenized for a further 20 to 30 minutes, which is the time normally taken to homogenize a five-day faecal collection. Sodium in the faeces both before and after the

addition of the tablets was then estimated and the recovery of sodium added in the form of tablets was calculated. Recovery of the sodium chloride contained in the Slow Sodium tablets was 99%.

Two sodium balance studies were then carried out in the metabolic ward according to the principles of Reifenstein, Albright, and Wells (1946). The balances were performed on one normal subject and one patient with chronic renal failure. The subjects received the same diet throughout the balance. They were studied during a control period of 5 to 13 days and were then given sodium supplements in the form of Slow Sodium for 4 to 40 days. Chromium sesquioxide was used as a continuous faecal marker in both subjects. Details of the subjects and the results of the balance studies are shown in the Table. The results show that the sodium chloride content of the Slow Sodium tablets is totally absorbed.

Balance Data before and during an Increase in Sodium Chloride Intake in the Form of Slow Sodium in a Patient with Chronic Renal Failure and a Normal Individual

Details of Subject	Faecal Collection period (Days)	Average Plasma Na (mEq/L)	Intake	Urine	Faeces	Absorption	Balance
			mEq/day				
Chronic renal failure	12·7	137·0	22·0	50·0	1·9	+20·1	−29·9
	3·7	137·0	78·0	76·9	2·0	+76·0	−0.9
Normal	5·0	—	20·0	16·5	0·5	+19·5	+3·0
	14·3	—	120·0	106·6	1·4	+118·6	+12·0
	9·2	—	120·0	110·2	1·5	+118·5	+8·3
	16·7	—	120·0	110·0	0·2	+119·8	+9·8

Rate of Absorption

In-vitro dissolution tests of Slow Sodium tablets show that the release of sodium chloride is exponential, with about half being released in the first hour (Fig. 1). Absorption was then studied in vivo. The Slow Sodium tablets were rendered radioactive by neutron bombardment in a nuclear reactor and were given to volunteers at a time when the ^{24}Na activity was about 5 μCi per tablet. Absorption was followed by measurements of radio-

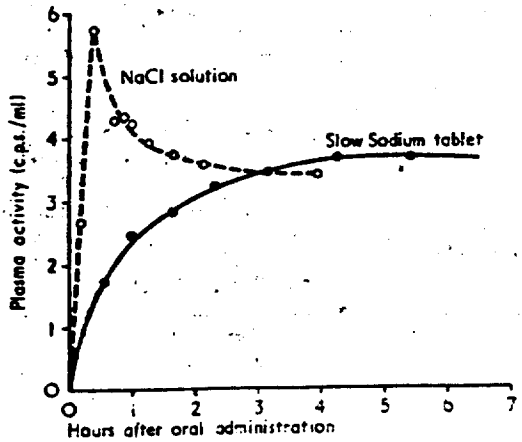

FIG. 1—Plasma radiosodium levels on two occasions in the same fasting subject, after ^{24}NaCl solution and after a ^{24}NaCl slow release tablet.

activity in successive plasma samples and by the disappearance of radioactivity from the abdomen with a surface counting technique.

The results show that Slow Sodium tablets have a predictable pattern of absorption at rest and during exercise (Fig. 2). If taken without food most of the NaCl is absorbed over the first two to three hours but absorption is not complete for six hours. If taken with food the absorption pattern is similar but subject to an initial delay of up to two hours.

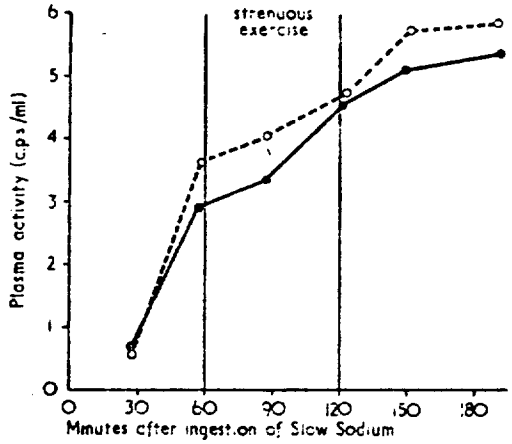

FIG. 2—Radiosodium absorption from ^{24}NaCl slow release tablet in two subjects. Plasma levels before, during, and after strenuous exercise.

Slow Sodium in Patients with Chronic Renal Failure

A few patients with chronic renal failure have a spontaneous and persistent high urinary excretion of sodium which may cause severe sodium depletion, profound hypotension, and a rapid deterioration in residual renal function. This syndrome is often referred to as "salt-losing nephritis." In these patients rapid correction of sodium and water depletion with sodium supplements thereafter is essential to correct the acute deterioration in renal function and preserve residual renal function. It is also well known that patients with chronic renal failure in a stable state may be precipitated into terminal uraemia by acute depletion of sodium and water due to vomiting and/or diarrhoea.

It is less widely appreciated that a number of patients with chronic renal failure have a less obvious insidious chronic urinary sodium-losing tendency which may cause a progressive deterioration of residual renal function. These patients have a normal blood pressure. Indeed it is a useful approximation to consider that any patient with severe chronic renal failure and a normal blood pressure is probably suffering from sodium depletion. These patients often have a progressive loss of weight and therefore daily weighing is a most important guide to diagnosis and treatment with a high salt intake.

In these various sodium chloride deficiency states of chronic renal failure intravenous sodium chloride must be given if the patient is vomiting. When the patient can take drugs orally, however, Slow Sodium has proved to be extremely valuable. Until the introduction of Slow Sodium it was usual to give sodium chloride capsules or enteric-coated tablets. These preparations, however, often proved unpalatable and if given in large amounts commonly resulted in nausea, vomiting, and abdominal pains. In contrast, it has been possible to give up to 200 mEq of sodium chloride as Slow Sodium by mouth in 10 minutes to patients without causing gastrointestinal side effects.

Slow Sodium in Patients on Maintenance Haemodialysis

Slow Sodium has been found useful to correct the hypotension which may occur during haemodialysis. Hypotension during haemodialysis may occur if the rate of ultrafiltration is too great. Salt and water depletion then causes profound hypotension and cramps which can be corrected by reducing the negative pressure across the membrane, or by applying a positive pressure, or by

the intravenous administration of 0·9% saline. We have found that it is usually unnecessary to administer saline intravenously for the same results can be obtained by giving Slow Sodium and water orally. Lesser degrees of excessive ultrafiltration may become apparent only at the end of dialysis when the patient is found to have lost an excessive amount of weight and to have pronounced postural hypotension. Again in this situation we have found that the rapid administration of 10 to 20 Slow Sodium tablets and a litre of water by mouth is effective.

Some patients on maintenance haemodialysis remain persistently hypotensive both during and between dialyses. This is a potentially dangerous situation which may precipitate shunt clotting and will also result in poor blood flows during dialysis. A few of these patients have a persisting urinary sodium leak which it has been possible to correct by the regular administration of Slow Sodium between dialyses. Usually in such patients, however, it is still necessary to remove water by ultrafiltration during each dialysis. This manœuvre may be vitiated by the development of hypotension owing to the simultaneous removal of sodium. In this situation the additional oral administration of large amounts of Slow Sodium without water during haemodialysis has corrected the hypotension and made it possible to remove 2 to 3 kg of excess water (Fig. 3).

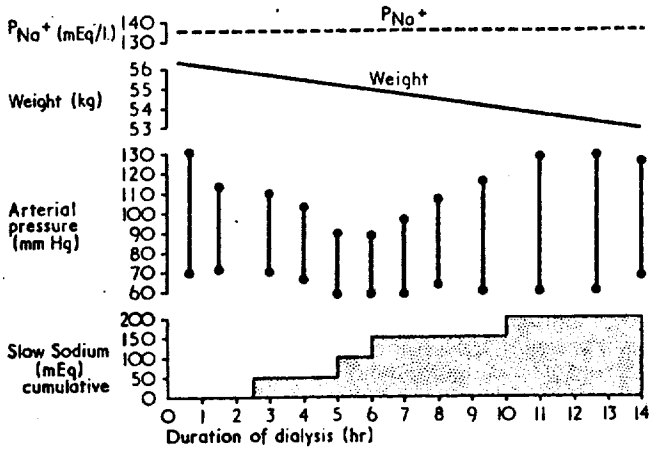

FIG. 3—Use of Slow Sodium during a 24-hour haemodialysis with a dialysate sodium concentration of 130 mEq/l. Hypotension corrected by the oral administration of Slow Sodium during the removal of 3 kg of the patient's weight by ultrafiltration.

Slow Sodium in Athletes

Slow Sodium tablets were used by the England football team during the World Cup competition in Mexico in 1970. The team trained and played in environmental temperatures approaching 100°F (37·8°C) with a humidity of 20-30% and were thus exposed to two problems due to water and salt loss. Firstly, a cumulative salt and water deficiency due to the increased amounts of sweat production occurring daily with acclimatization to the heat (Robinson, Turrell, Belding, and Horvath, 1944). Secondly, an acute salt and water deficiency occurred during training and playing from the considerable sweat loss associated with hard physical work in the heat. Slow Sodium tablets were used to overcome both of these problems. In the year before the World Cup competition 1970 Slow Sodium tablets had been given in increasing amounts to the players before training sessions and international matches. No detectable symptoms or side effects were reported by any of the players, and they were therefore prepared to take as many tablets as might be considered necessary.

The management of the England players in the heat of Mexico was along the lines described by Leithead and Lind (1964). If only one measurement is used as an index of physiological strain when working in the heat, then theoretically the sweat loss, as estimated by the change in nude weight over a known exposure, has much to commend it (Hatch, 1963). In Mexico it was found that a 100-minute training session was associated with a weight loss of up to 3·2 kg, and the aim was to regain this weight loss by sodium and water replacement daily. Players were advised to use liberal quantities of salt on their food, to drink until their thirst was quenched, and then to drink an additional 1,500 ml of fluid daily. In addition, the players were given a daily maintenance dose of eight Slow Sodium tablets (80 mEq) four tablets being taken morning and evening.

In order to minimize the extent of acute sodium chloride deficiency which might occur during training or playing, an anticipatory dose of Slow Sodium was given before each training session or match. The timing of this dose was based on the data which had been obtained about the rate of absorption of Slow Sodium. Four Slow Sodium tablets (40 mEq) were given three hours before all training sessions and matches. For one match, in which it was considered possible that extra time might be played, an additional four Slow Sodium tablets were given two hours before the game. In addition, the players were formally given 500 ml of a lemon-flavoured drink (Gatarade) containing 54 g of glucose and 21 mEq of sodium chloride per litre in the two hours before training and playing, a further 400 ml at half-time, and freely at the end of the game. This fluid was drunk at a temperature of between 55 and 60°F (12·8 and 15·6°C) to minimize side effects (Leithead and Lind, 1964).

In spite of these measures some weight reduction from sweat loss occurred during training sessions and matches. The aim was that this loss of weight should be regained so that the daily pre-breakfast weight should remain unchanged. If nevertheless a fall in this pre-breakfast weight occurred the player's urine was tested for chloride content by the method of Fantus (1936). If the chloride content was found to be low additional Slow Sodium tablets were given together with an increased intake of fluids to correct the weight loss.

Results

In 26 of the 28 players it was found that the body weight was maintained with supplements of 80-120 mEq of Slow Sodium per day—that is, 8 to 12 tablets. One of the other two players, however, lost 4·5 kg over three days in spite of these supplements, his urinary chloride concentration falling to 3 g per litre. He was then given an additional supplement of 280 mEq of sodium chloride as Slow Sodium (28 tablets) for one day and an additional 120 mEq (12 tablets) per day for the next two days. His water intake was also increased. He regained his normal weight in 60 hours. The other player was away from the main party for four days, during which he ceased to take any Slow Sodium and his intake of dietary salt was reduced. On his return he had lost 5 kg in weight and his urine did not contain any chloride. He was given an additional supplement of 280 mEq of sodium chloride as Slow Sodium for the first day, and an additional 120 mEq per day for the next two days, together with an increased intake of fluids. He regained his normal weight in 48 hours. Both these players took 200 of the 280 mEq of additional Slow Sodium on the first day over a period of two hours without any side effects. One of the players had a 100-minute training session two hours later during which he felt well.

Discussion

There is no doubt that this oral preparation of sodium chloride is acceptable and well tolerated. Its acceptability is particularly well illustrated by the way the English football team consumed large amounts in Mexico. Like all athletes, professional footballers have very conservative views of what they should eat and drink and are very suspicious of any tampering with their fixed habits. A year before the World Cup competition the England team had played a series of matches in South America. A few

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players suffered acutely from "dehydration" after a game. The impression gained from this experience was that the main disability was a cumulative sodium chloride depletion. Three players suffered from severe giddiness, fatigue, nausea, and vomiting for up to 48 hours and would not have been fit to play again for several days. Attempts to use enteric-coated tablets of sodium chloride were singularly unsuccessful, and the use of compressed salt tablets had caused nausea and vomiting. By the time the same players returned to Mexico they were aware of the need for sodium chloride replacement. During the World Cup competition they were prepared to take as many Slow Sodium tablets as were considered necessary. One player took 200 mEq of Slow Sodium two hours before an intensive training session. In Mexico no player suffered from any clinical symptoms suggestive of either acute or chronic salt and water depletion.

Cramp is a common symptom reported in subjects suffering salt depletion. Collings, Shoudy, and Shaffer (1943) reported an incidence of 60% in his series and King and Barry (1962) indicate a higher incidence in gold miners. Despite their exceptional physical fitness, football players not infrequently become temporarily incapacitated by muscle cramps, particularly near the end of a match. The frequency and severity of the cramp is clearly exacerbated by the physical exercise which fatigues the muscles. Such players who have received Slow Sodium as salt supplementation before matches claim that muscle discomfort and cramps have been eliminated.

Slow Sodium is not only acceptable but it is also a convenient way of giving sodium chloride. We have given up to 500 mEq per day and up to 200 mEq in 10 minutes without any gastrointestinal side effect except thirst if insufficient amounts of water are consumed at the same time. It can therefore be used to treat severe salt and water depletion in any individual who can swallow. For this reason the consumption of intravenous saline in the wards and in the maintenance haemodialysis unit of this hospital has considerably diminished. The price of Slow Sodium has not yet been determined but on the assumption that it will be about the same as Slow-K (Ciba) 140 mEq of Slow Sodium should cost about 2·5p. In contrast, 1 litre of intravenous saline and a disposable administration set cost 50p. The oral route of administration also avoids all the disadvantages of intravenous therapy such as thrombophlebitis, local infections, air embolism, etc. Economy is always welcome, but in some parts of the world where intravenous preparations are difficult to obtain the use of an acceptable oral sodium chloride preparation should make it possible to treat many more patients who suffer from acute sodium chloride depletion.

The use of an acceptable oral sodium chloride preparation should also be of great value in some industrial workers. There are certain industries, such as the South African gold mines, in which the problem of adequate sodium chloride replacement has never been satisfactorily answered. We have also been told that sodium depletion is a great problem in the oil industry. Its use in other athletes who perform strenuous activities over a long period, such as tennis players and marathon runners, is obvious. The principle advantage of Slow Sodium for the healthy individual, apart from its freedom from side effects, is that not only can it correct a loss of sodium chloride after the loss has occurred but it can anticipate such a loss. Most of the absorption from the gut of the sodium chloride contained in Slow Sodium tablets is achieved at two and a half hours but continues for some time thereafter. Therefore, if a supply of Slow Sodium tablets is ingested at a suitable time before an acute sweat loss is about to take place it is possible for the loss to be at least partially replaced from the gut as it is occurring. In most prolonged industrial and athletic activity it is usually possible to have access to water from time to time. With a continuous absorption of sodium chloride from the Slow Sodium tablets in the intestine and the intermittent oral ingestion of water, the stultifying weariness and muscular weakness of extracellular fluid volume depletion need never occur.

We wish to thank Drs. D. M. Burley and D. A. Richards and the Ciba Laboratories for the supply of Slow Sodium; and Hill and Knowton, Ltd. for the Gatarade. We are also grateful to the many persons who helped in the study of the absorptive properties of the preparation, particularly Mr. D. N. Sillince and the other members of the Charing Cross Hospital Medical School football team.

References

Collings, G. H., Shoudy, L. A., and Shaffer, F. E. (1943). *Industrial Medicine*, 12, 728.
Fantus, B. (1936). *Journal of the American Medical Association*, 107, 14.
Hatch, T. F. (1963). In *Temperature: Its Measurement and Control in Science and Industry*, vol. 3, part 3, ed. J. D. Hardy. New York, Chapman, for the American Institute of Physics.
King, B. A., and Barry, M. E. (1962). *South African Medical Journal*, 36, 451.
Leithead, C. S., Leithead, L. A., and Lee, F. D. (1958). *Annals of Tropical Medicine and Parasitology*, 52, 456.
Leithead, C. S., and Lind, A. R. (1964). *Heat Stress and Heat Disorders*. London, Baillière, Tindall and Cassell.
Reifenstein, E. C., Albright, F., and Wells, S. L. (1946). *Journal of Clinical Endocrinology and Metabolism*, 6, 232.
Robinson, S., Turrell, E. S., Belding, H. F., and Horvath, S. M. (1944). *American Journal of Physiology*, 140, 168.
Stenning, J. C. (1945). *Journal of the Royal Naval Medical Service*, 31, 129.

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The role of salt in experimental and human hypertension

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Abstract: This paper reviews evidence that salt and water have an important role in determining blood pressure, particularly in instances of normal plasma renin activity. Data from patients and experimental animals show that hypertension follows fluid retention when the fluid retention is due to either increased salt intake and/or decreased renal function. Salt-induced, experimental hypertension is characterized by early increases in fluid volumes and cardiac output and late increases in peripheral resistance. The temporal relationship that is observed between blood flow and resistance supports the theory that the autoregulation of blood flow is continuously attempting to maintain flow at metabolically needed levels; consequently, the elevated arterial pressure of salt-induced hypertension will, in time, be maintained by elevated peripheral resistance. This thesis is consistent with data from many other types of experimental hypertension and many clinical situations in which plasma renin levels are not excessively elevated.

KEY INDEXING TERMS

Salt-induced hypertension *Hypertension* *Experimental hypertension*

Due mainly to Dr. Goldblatt's efforts in the 1930s, hypertension research during the past four decades has focused on vasoconstrictors and, specifically, the renin-angiotensin system. Traditionally, salt has been assigned a ubiquitous yet minor role in various schemes of blood pressure control. Several recent developments have stimulated interest in the role of salt and water in the control of arterial pressure, although presently the importance of salt in the control or arterial pressure is not well understood, particularly in the case of essential hypertension.

There is some clinical evidence suggesting that in uncomplicated hypertension increased salt intake and decreased salt excretion will increase arterial pressure while, on the other hand, decreased intake and increased excretion will lower pressure. Evidence for the latter includes the frequently hypotensive (and beneficial) effects of a low salt diet, such as the Kempner rice diet. Evidence comes also from the effectiveness of diuretics in lowering blood pressure in many instances. The initial response to administration of a diuretic is a marked diuresis and natriuresis. Plasma volume and extracellular volume are decreased by these losses and there is an accompanying decrease in cardiac output.[1,2] Early decreases in arterial pressure are primarily due to decreases in cardiac output with peripheral resistance remaining unchanged. During chronic diuretic administration the hemodynamic picture reverts to one of nearly normal fluid volumes, normal cardiac

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output, salt and water balance, and decreased peripheral resistance.[1,2] The decreased arterial pressure is initially caused by decreased cardiac output, but is subsequently maintained by decreased peripheral resistance. This sequence of events suggests that the acute loss of salt and water causes a chronic reduction in peripheral resistance, due either to direct effects on the vasculature or to indirect effects that are a function of the transient decrease in fluid volumes and cardiac output. These observations show that salt is involved in a very real way in the control of blood pressure although the specific mechanisms involved are not demonstrated. Animal experimentation has supplemented these observations with a substantial amount of additional information.

The Combined Effects of Reduction of Renal Mass and Salt Loading

The effect of increased salt intake on blood pressure is modified by changes in renal mass. A combination of salt loading and reduction of renal mass (or renal function) is more effective in elevating blood pressure than either taken separately. Effectiveness, in this case, means both an increase in the incidence of hypertension and a decrease in the time until the occurrence of elevated blood pressure. This phenomena was demonstrated by Koletsky and Goodsitt[3,4] in rats with varying amounts of renal mass, maintained on either normal diets or 1 per cent saline. Their data, summarized in Table I, show the synergistic relationship between salt loading and reduction of renal mass, in which in each instance the incidence of hypertension following subtotal nephrectomy and salt loading is greater than the incidence following each of these events occurring separately.

TABLE I

INCIDENCE OF HYPERTENSION IN RATS AS A FUNCTION OF RENAL MASS AND DIETARY SALT*

	Incidence of Hypertension	
Renal Mass	Normal Diet	1% Saline
100%	2.5%	66%
50%	16 %	94%
25%	56 %	100%

*Koletsky and Goodsitt.[3,4]

Intact rats (that is with 100 per cent renal mass) had a very low incidence of hypertension and the time to onset of this hypertension was several years. If 1 per cent saline was substituted for drinking water, the incidence increased markedly to 66 per cent and the duration of time until the onset of hypertension was reduced substantially. Following unilateral nephrectomy the natural incidence of hypertension was 16 per cent with high salt intake. The time until occurrence of hypertension was considerably shortened. Finally, three-quarters nephrectomy increased the natural incidence of hypertension to 56 per cent. Salt loading increased the incidence to 100 per cent and reduced the time until occurrence to several weeks or less. These data demonstrated that either a reduction of renal function or an increase in salt and water intake can lead to elevated arterial pressures, but that in combination these two factors are particularly effective.

We have observed the same relationships in studies on dogs over shorter periods of observation, usually two to three weeks to several months. In general, dogs, like humans, appear to be more resistant to salt loading than rats. With kidneys intact, salt loading has increased blood pressure 5 to 10 mm Hg over a period of three months.[5] Vogel[6] observed a 20 to 30 mm Hg increase in mean pressure in less than a month's time on salt-loaded, intact dogs in which salt loading was achieved by administering both saline and salt supplements in the animal's food. We have observed modest, generally about 20 mm Hg, increases in arterial pressure when unilaterally nephrectomized dogs are salt-loaded over a period of approximately two weeks.

Unilateral nephrectomy in conjunction with contralateral subtotal nephrectomy or unilateral nephrectomy alone leads to very modest though significant increases in pressure (less than 10 mm Hg) when the animals are maintained on normal salt diets. When reduction of renal mass to approximately 30 per cent of control is combined with saline loading, very

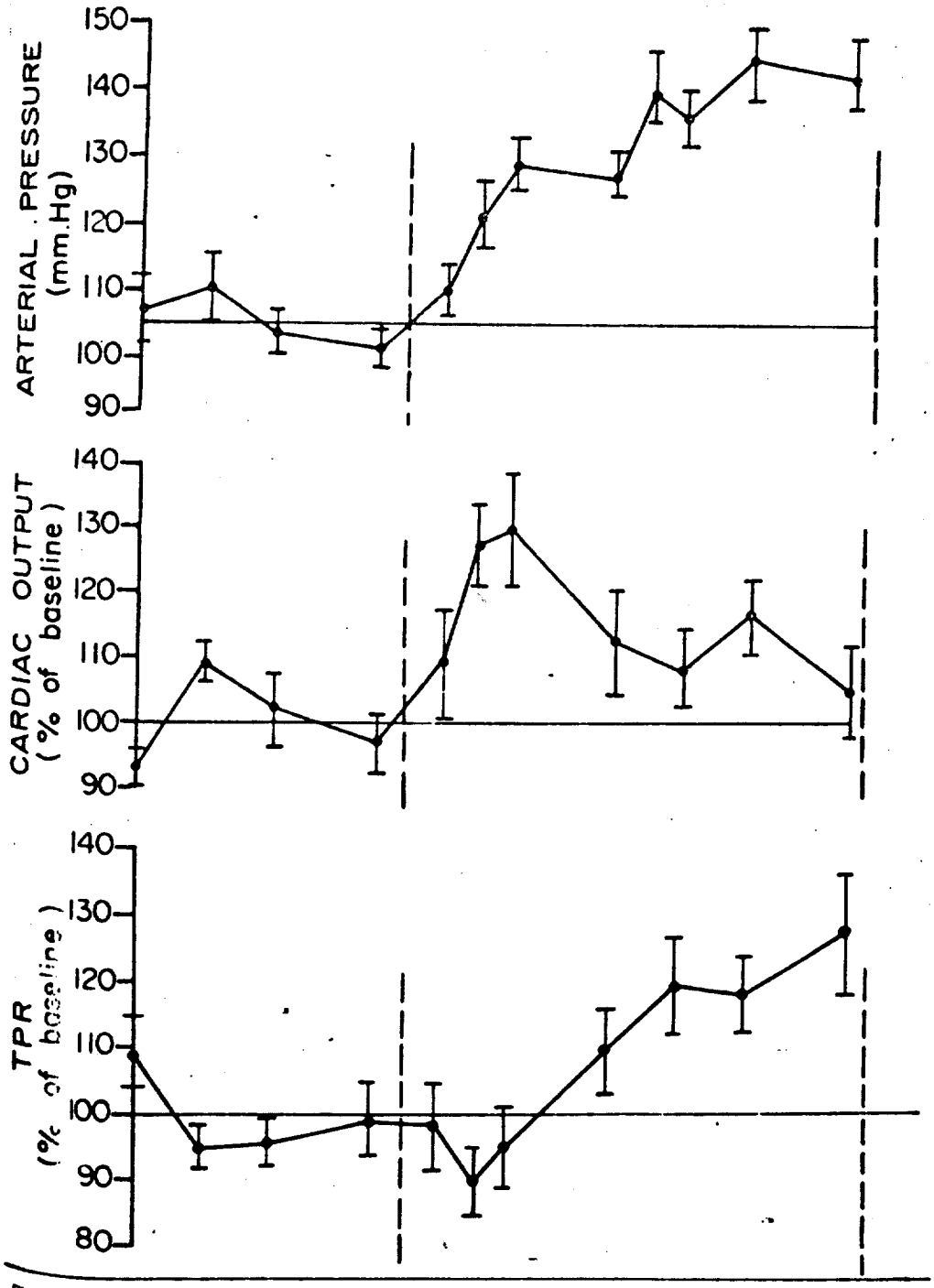

Fig. 1: Blood pressure, cardiac output, and peripheral resistance of six subtotally nephrectomized dogs during a one-week control period and a two-week saline infusion period.

marked hypertension occurs. Mean arterial pressure increases from 30 to 40 mm Hg or sometimes greater after one to two weeks. This particular surgical and dietary protocol is very effective in elevating arterial pressure and has become a standard experimental preparation in our laboratories.

There is considerable evidence that salt and water retention and fluid volume expansion in conjunction with an early increase in cardiac output play an important role in this type of hypertension. Douglas[7] used the preparation mentioned above and found an elevated arterial pressure during the duration of the salt-loading period. He found, though, that the animal's fluid volumes were temporarily expanded and returned to nearly normal, while the saline loading and hypertension persisted. Specifically, he found that the blood volume was transiently elevated, that the sodium space was transiently elevated, and that the interstitial pressure changes, which are a good indicator of extracellular volume changes, followed the changes in sodium space. Examination of the time course of these events reveals that maxima were reached in about one week after the initiation of saline loading, and that after two weeks of loading, volumes had returned nearly to normal again.

Another series of experiments was designed to quantitate the hemodynamic response to salt loading.[8] The preparation used was roughly the same as before, that is, subtotal nephrectomy plus saline loading, but in this case we used a constant infusion of 0.9 per cent saline to obtain a somewhat smoother response. The hemodynamic response of these animals is shown in Figure 1. We observed a 30 to 35 mm Hg increase in mean arterial pressure during a two-week infusion period (occurring between the two vertical dashed lines). The increase in blood pressure was correlated with changes in cardiac output and total peripheral resistance. The essence of this analysis is that arterial pressure is the mathematical product of cardiac output and total peripheral resistance, and changes in pressure are due either to changes in flow or changes in resistance, or a combination of both. Sequential measurements of cardiac output showed an early elevation in flow with cardiac output increasing to 29 per cent above normal on the third day of infusion, and subsequently reverting back toward normal. The early elevation in arterial pressure, then, was due to increased cardiac output. Peripheral resistance, the bottom curve of Figure 1, initially decreased slightly and then, for the duration of the experiment, continued to increase. The arterial pressure was subsequently maintained by this elevated peripheral resistance. This temporal sequence, *ie*, first an increase in flow and subsequently an increase in peripheral resistance, suggested that autoregulation of blood flow was involved in the elevation of pressure. A postulated sequence of events is as follows: (1) salt loading causes increased fluid volume; (2) increased fluid volume increases cardiac output; (3) the autoregulatory response is triggered; (4) flow is autoregulated back toward normal, while peripheral resistance increases; and (5) a pressure-induced diuresis decreases fluid volumes toward normal.

Several additional observations made on this series of dogs are of interest. Heart rate decreased as the arterial pressure increased, and we interpreted this to mean that the baroreceptors were opposing the increase in pressure. Minimum heart rate was observed on the sixth day of infusion, and heart rate then increased throughout the remainder of the infusion period, remaining somewhat depressed at the end of two weeks. The combination of increased cardiac output and decreased heart rate caused the calculated variable, stroke volume, to become significantly, though transiently, elevated. Finally, measurements of right atrial pressure very closely paralleled the changes in cardiac output. We interpreted this to mean that the increased blood flow was due to increased venous return rather than cardiac factors. Atrial pressure was elevated approximately 3.5 mm Hg above control at the same time that cardiac output reached a maximum: These experiments show that the hypertension that accompanies salt loading after subtotal nephrectomy is maintained by an increased peripheral resistance, but

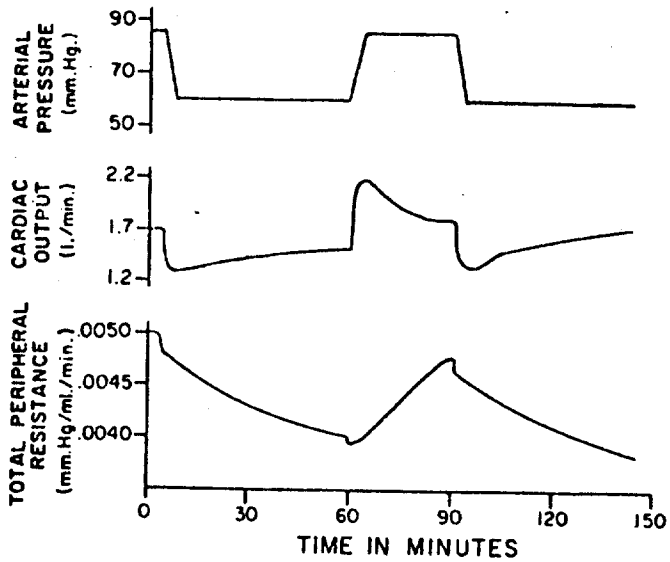

Fig. 2: Flow and resistance response of an areflexic dog to abrupt changes in arterial pressure.

that early increases in arterial pressure are due to an increased cardiac output.

Autoregulation of Blood Flow

One interpretation of the sequence of hemodynamic events at the onset of hypertension (described in the previous section) involves autoregulation of blood flow. Autoregulation of blood flow has been demonstrated in almost every tissue of the body, leading to the contention that each tissue regulates its own blood flow at some metabolically required level. Local changes in blood flow are implemented by changes in regional resistance. An increase in perfusing pressure would initially cause an increased blood flow; subsequently, the autoregulatory response would cause increased local resistance and flow would decrease back toward the metabolically proper level. Since this response is common to most tissues of the body, it is reasonable to expect that the summated response of all of the tissues would be reflected in changes in cardiac output and total peripheral resistance. Granger and Guyton[9] have demonstrated this phenomena in areflexic dogs, prepared by spinal anesthesia and decapitation. Figure 2 shows the interrelationship of pressure, flow, and resistance in a typical experiment in which arterial pressure is changed in a stepwise fashion by raising and lowering a blood infusion bottle connected to the animal's femoral artery. It can be seen that the whole-body response of this animal to an increase in arterial pressure (the center of Figure 2) is initially an increase in flow with little change in resistance. Then, subsequently, resistance increases while flow decreases back toward, but not entirely to, the control value. This response is symmetrical in that a decrease in pressure leads to a decrease in flow that precedes a decrease in peripheral resistance. The average gain of this response expressed in the terminology of control theory was shown to be 3.3, meaning that the response is moderately powerful. With this gain, whole-body compensation for a step disturbance would be approximately 75 per cent of the magnitude of the disturbance. This amount of compensation is entirely reasonable when we consider that some vascular beds are passive.

Correspondence between the response just described and the events observed during the onset of hypertension suggests that, in a hierarchy of control schema within the body, the control of blood flow is more important than the control or arterial pressure. Consistent with this concept is the contention that a disturbance tending to increase blood flow would be

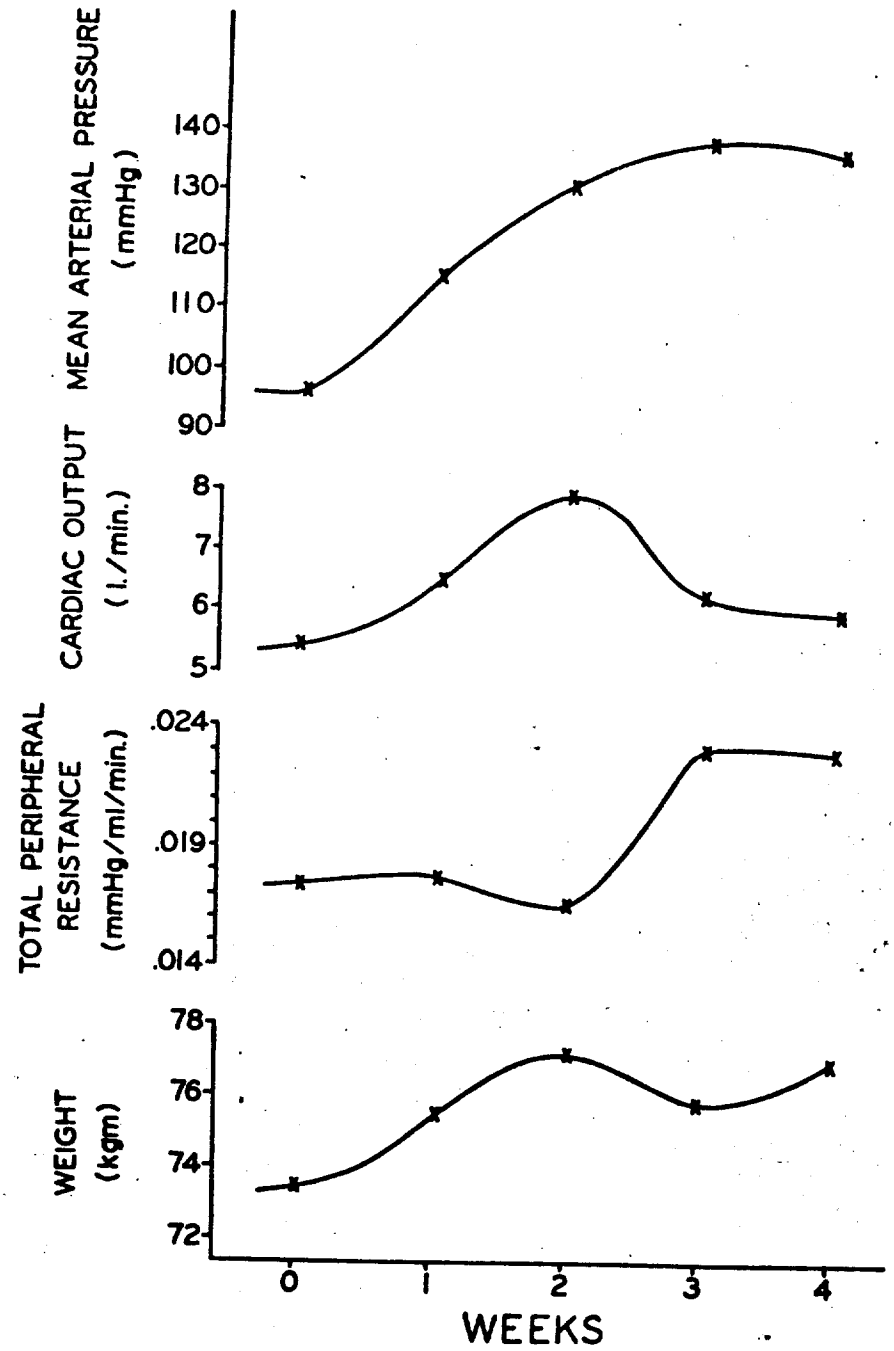

Fig. 3: Hemodynamic response of an anephric patient (female, 22 years old) to overhydration. Each data point represents a weekly average.

opposed by an increase in resistance tending to return blood flow back toward metabolically needed levels. Homeostasis would be re-established with arterial pressure at a new, elevated level, the price that has to be paid for maintaining flow at the proper value. All the determinants of the time course of the response are not known, but in all probability every system that is involved in the control of blood pressure (baroreceptors, renin-angiotensin, aldosterone, and others) can alter the apparent time course of the response. The stimulus for an increase in cardiac output can come either from (a) a sequence of events that includes increased fluid volumes, increased mean systemic pressure, increased venous return, and, subsequently, increased cardiac output, or (b) from decreased venous compliance, increased mean circulatory pressure, increased venous return, and, again, increased cardiac output.

The postulated role of autoregulation of blood flow in the hypertensive response is to elevate peripheral resistance and, subsequently, arterial pressure in response to conditions of increased flow, which are in turn the result of increased salt intake or decreased renal function.

Control of Blood Pressure In Anephrics

Unfortunately, the hemodynamic response to increased salt and water intake is modified, and potentially obscured, in studies in which the renin-angiotensin system is very active or, in some cases, dominant. We have had the unique opportunity to observe pressure, flow, and resistance changes in anephric patients maintained on chronic hemodialysis. The special features of these observations are: (a) the renin-angiotensin system is not operative since the patients are anephric; (b) fluid volume changes can be easily regulated by the hemodialysis protocols; and (c) sequential cardiac output determinations have been possible with the minimum of risk. Cardiac output was determined using dye-dilution techniques. Cardiogreen dye was injected into the venous end of the patient's flowing chronically implanted shunt and blood samples were withdrawn from the arterial end of the same shunt through a standard cuvette densitometer.[10] These techniques have permitted us to do extensive, sequential studies on selected patients with a minimum of inconvenience to the patient.

Patients were allowed to undergo sequential periods of normal hydration, overhydration, and then normal hydration. Overhydration was the result of reduced ultrafiltration during several hemodialysis periods, while reversion back to normal hydration was achieved by aggressive ultrafiltration during the necessary number of hemodialysis periods. Arterial pressure, cardiac output, heart rate, patient weight, and several other variables were measured before dialysis and total peripheral resistance and stroke volume were calculated from these measurements. Transient changes in circulatory function were anticipated and the protocol was designed with sequential measurements to identify these transients as they occurred.

Figure 3 shows the hemodynamic response of one patient to overhydration. The stimulus for the increased mean arterial pressure was increased fluid volume, as estimated in the bottom curve of the figure by a 3 kg increase in weight. Cardiac output increased initially and was the cause of the elevated blood pressure; then flow reverted toward normal. Peripheral resistance was initially unchanged but then increased, eventually maintaining the elevated pressure. The magnitude and time course of this change in flow and resistance are very similar to changes observed in subtotally nephrectomized animals given a salt load as previously described.

A composite analysis of serial observations of three anephric patients undergoing both overhydration and then subsequent dehydration back to normal volumes[11] showed that cardiac output initially increased to 22 per cent above control values. As the patients were returned to normal hydration by ultrafiltration, cardiac output decreased initially to 6 per cent below control (not significant) and then increased to the initial, control values. Decreasing flow preceded decreasing resistance, a temporal sequence that is similar to the response that follows the administration of diuretics.

Interpretation

Our interpretation of these studies is that renal dysfunction (used in a purposely vague sense) can require increased arterial pressure in order to re-establish adequate renal function and salt and water balance—in short, in order to re-establish homeostasis. Several different mechanisms can be responsible for the increase in pressure. For instance, increased pressure can be achieved by an increase in peripheral resistance, mediated by circulating vasoconstrictors, particularly those from an active renin-angiotensin system. This appears to be the case in instances of accelerated or malignant hypertension and in some instances of renal artery stenosis. It certainly appears to be the case in the early phases of Goldblatt hypertension. In each of these instances, renal dysfunction includes renal hypoperfusion.

In the presence of renal dysfunction and in the absence of increased plasma renin activity, homeostasis can be re-established by fluid retention, temporarily increased cardiac output, and subsequently increased peripheral resistance due to long-term autoregulation of blood flow. This appears to be the case in salt-induced hypertension, the latter stages of Goldblatt hypertension, primary aldosteronism, hypertension in anephrics, experimental renal hypertension in conjunction with high titers of angiotensin antibodies, and other conditions. The increased blood pressure in each instance is characterized by an absence of renal hypoperfusion, by low (effective) plasma renin activity, and by early fluid volume expansion.

The direct effect of salt and water retention on the vasculature is not clear, although there is little evidence that salt has an important direct effect. Studies of changes in tissue composition above and below coarctation of the aorta (such as those by Hollander) do not support the contention that salt has a direct causal role in elevating arterial resistance.

In summary, then, it appears to us that salt (and subsequently water) plays an important role in the long-term control of blood pressure. Salt and water retention can be due either to an increase in sodium intake or to a decrease in the ability of the kidney to excrete sodium, and most effectively to a combination of both. In these instances, increased arterial pressure re-establishes homeostasis.

References

1. Conway J, Loawers R: Hemodynamic and hypotensive effects of long-term therapy with chlorothiazide. Circulation 21:21, 1960.
2. Wilson IM, Fries ED: Relationship between plasma and extracellular fluid volume depletion and the antihypertensive effect of chlorothiazide. Circulation 20:1028, 1959.
3. Koletsky S: Role of salt and renal mass in experimental hypertension. Arch Path 68:11, 1959.
4. Koletsky S, Goodsitt AM: Natural history and pathogenesis of renal ablation hypertension. Arch Path 69:654, 1960.
5. Langston JB, Guyton AC, Douglas BH et al: Effects of changes in salt intake on arterial pressure and renal function in partially nephrectomized dogs. Circ Res 12:508, 1963.
6. Vogel JA: Salt-induced hypertension in the dog. Am J Physiol 210:186, 1966.
7. Douglas BH, Guyton AC, Langston JB et al: Hypertension caused by salt loading. II: Fluid volume and tissue pressure changes. Am J Physiol 207:669, 1964.
8. Coleman TG, Guyton AC: Hypertension caused by salt loading in the dog. III. Onset transients of cardiac output and other circulatory variables. Circ Res 25:153, 1969.
9. Granger HJ, Guyton AC: Autoregulation of the total systemic circulation following destruction of the central nervous system in the dog. Circ Res 25:379, 1969.
10. Bower JD, Coleman TG: Circulatory function during chronic hemodialysis. Trans Am Soc Artif Intern Organs 15:373, 1969.
11. Coleman TG, Bower JD, Langford HG et al: Regulation of arterial pressure in the anephric state. Circulation 42:509, 1970.

Zeitschr. Krebsforsch.
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Influence of sodium chloride and lime on the predisposition to malignancy of rabbit carcinoma

by

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The Brown-Pearce rabbit tumor, a genuine eptihelioma, is excellently suited to the study of the influence of various agents on the predisposition to malignancy. If a group of rabbits is intravenously identically infected, the tumor does not develop in all the animals in the same manner. Some animals remain completely healthy. Others develop weak cancerization of the kidneys and liver, these organs revealing numerous tumors ranging in size from pinhead to millet seed. In other animals again, such a pronounced spread of cancerization is observed that the kidneys, for instance, grow to five times their original size, the liver is completely filled with nodules the size of a pea to a bean, the ovaries are swollen to more than the size of a walnut and the lungs are filled with numerous small metastases.

If any kind of chemical or physical force is made to act on a group of identically infected animals and if the same number of animals are left as controls under otherwise

identical living conditions, it is possible to establish the cancer promoting or inhibiting effect of the treatment on the predisposition to malignancy by comparing the intensity of cancer growth in the treated and untreated animals.

A report is given below in which the influence of sodium chloride and lime is studied on the predisposition to malignancy. Our procedure was as follows:

A group of rabbits was intravenously infected with a fine suspension of tumor cells filtered through gauze (from a primary testicular carcinoma). Half the animals were kept as controls, while the other half were regularly treated daily (except Sundays). After ca. 4-6 weeks the animals that had not died spontaneously were killed and dissected. As a rule 10 rabbits were used for each experiment, so as not to have too many animals in one and the same group. However, the experiments were repeated several times, to exclude individual errors as far as possible.

Influence of sodium chloride

The influence of sodium chloride on cancer growth has not been experimentally investigated so far, to our knowledge. Earlier, J. Braithwaite, on the basis of empirical and statistical data, came to the conclusion that an excess of NaCl could cause cancer growth. This view was immediately challenged by Sticker, and Omega indeed claimed the opposite, namely that NaCl had a beneficial influence on cancer.

In our experiments NaCl was not administered to the rabbits

through a stomach tube, but injected subcutaneously as a 10% solution. In addition to ordinary sodium chloride we also used a table salt to which potassium, calcium and magnesium had been added. The results of the experiments carried out from August to November are shown in Table 1.

In the table, "sp" after the day of death means that the animal died spontaneously, while "get" means that it was killed. In the autopsy findings "1" means slight cancerous growth, "4" means very strong cancer growth, while "3" and "2" are intermediate.

If we study the table, it will be seen that treatment with NaCl distinctly enhanced cancer growth. While for instance among the controls 33% animals were negative, only 16% of animals treated with NaCl were negative. Thus under NaCl treatment half as many rabbits remained negative as among the controls. The same was observed upon comparison of very pronounced carcinoma growth. While among the control animals only 13% revealed very strong carcinoma growth, 28% of the animals treated with NaCl revealed very strong carcinoma growth. Here again we distinctly observed an increased predisposition to malignancy as a result of NaCl treatment.

A striking feature of these findings was the relatively small quantity of sodium chloride capable of intensifying malignancy. The daily dose never exceeded 0.3 g per animal (or 3.0 cc of 10% solution) and the total dose was a maximum of 6.5 g per animal. That such small quantities of NaCl already caused the strong increase in malignancy observed may be worth

noting.

Unfortunately these experiments were carried out on only 40 rabbits and we must consider the possibility that the percentages might diverge in one direction or the other in a study on a considerably larger number of animals. It nevertheless seems to us certain on the basis of our experiments that an increased predisposition to malignancy can be achieved by means of sodium chloride.

Here we would like to note briefly that we always refer to the months in which the experiments were carried out, since it appears that biological experiments of the type described are considerably influenced by the food given in the months in question.

The influence of lime

The influence of lime on cancer growth has not so far been experimentally studied. Some authors believe that carcinoma growth is inhibited by lime. At the time of the lymph theory of cancer, lime was administered by Boerhave in the form of lime water and was considered for some time as a specific remedy. Even later, it was recommended as medication by Roerig.

The procedure in our experiments was to add organically bound lime to a small part of the animals' food and only once the animals had consumed the lime-containing food did they receive the remainder of their food. Some of the animals received 0.5 g lime per day, while others received 2.5 g per day.

The results of the experiments carried out from December to March are shown in Table 2.

Study of this table shows that of the animals fed with lime, 5, or 33%, were negative, while 3, or 20% of the controls were negative. Of the animals treated with lime, 3 (20%) developed very extensive carcinoma, as did 3 (20%) of the control animals.

If we disregard the somewhat higher number of animals fed with lime that remained negative, these experiments lead to the conclusion that lime has no marked influence on the predisposition to malignancy.

Summary

In experiments with Brown-Pearce rabbit carcinoma it was found that the peroral administration of lime had no marked effect on the predisposition to malignancy.

On the other hand the predisposition to malignancy was increased by relatively small quantities of sodium chloride (daily dose 0.2-0.3 g; total dose maximum of 6.5 g).

Table 1. Influence of sodium chloride on rabbit carcinoma

Treated	How often	Total g	dead after - days	Autopsy findings
NaCl			33 killed	Kidneys 2, ovaries 2
				Normal
				kidneys 2
				ovaries 2, kidneys 1, adrenals 1 omentum 1
				ovaries 2
				normal
			12 spont.	liver 1
				liver 2, lungs 2
				liver 2, lungs 1
				kidneys 2
				kidneys 2, lungs 1
				normal
				normal
				kidneys 2
				liver 4, kidneys 4, ovaries 3 lungs 2
				kidneys 1, right ovary 1
				kidneys 2, ovaries 1
				right ovary 1
				left ovary 1
				liver 3, lungs 3, kidneys 2, ovaries 2, omentum 1
				ovaries 2, abdominal muscles 2, dorsal muscles 2
				kidneys 3, ovaries 3
				kidneys 2
				kidneys 3, ovaries 2
				kidneys 3, ovaries 2, peritoneum 1
				normal
				kidneys 3, right ovary 1
				normal
				normal
				kidneys 2, right ovary 1
				kidneys 2, ovaries 1
				kidneys 2, lungs 2, liver 1
				kidneys 2
				kidneys 2
				kidneys 1, right ovary 1
				normal
				normal
				kidneys 2
				ovaries 1
				ovaries 1, kidneys 1

Table 2. Influence of lime on rabbit carcinoma

'reated	How often	Total g	dead after - days	Autopsy findings
,ime			30 spont.	Kidneys 2, ovaries 3, lungs 1, peritoneum 1
			37 killed	kidneys 1, right eye 2
				ovaries 1, peritoneum 1
				normal (pneumonia)
				ovaries 4, kidneys 2, eyes 2
				normal (intercurrent pneumonia)
				normal
				liver 3, kidneys 2, ovaries 4
				kidneys 2, ovaries 2
				right ovary 2
				kidneys 1, ovaries 3-4
				normal
				kidneys 2, left ovary 2
				normal
				liver 4, lungs 1
				eyes 2
				kidneys 1
				normal
				left lung 1 (pneumonia)
				kidneys 4, liver 4, lungs 4, adrenals 4, ovaries 4, pancreas 4, abdominal wall 4
				normal
				left kidney 1, right ovary 2
				kidneys 1, left ovary 2, right lung 1
				normal
				right kidney 2, right ovary 2, right oviduct 1, right adrenal 3
				kidneys 2, lungs 1, ovaries 2-3
				kidneys 4, ovaries 3-4, lungs 1 (?)
				ovaries 4, kidneys 1
				kidneys 1, right ovary 4, left ovary 2, lung 1, thymus 1, right eye 3
				kidneys 1, ovaries 2

Bibliography

See p. 295.

(Aus dem Institut Robert Koch Berlin. Chemotherapeutische Abteilung. Direktor: Geheimrat Prof. Dr. *F. K. Kleine*.)

Der Einfluß von Kochsalz und Kalk auf die Malignitätsdisposition des Kaninchencarcinoms.

Von

W. A. Collier und A. Cohn.

(*Eingegangen am 29. November 1932.*)

Der *Brown-Pearcesche* Kaninchentumor, ein echtes Epitheliom, eignet sich ganz vorzüglich für Untersuchungen über den Einfluß verschiedener Agentien auf die Malignitätsdisposition. Wird eine Serie Kaninchen gleichmäßig intravenös infiziert, so entwickelt sich der Tumor nicht bei allen Tieren in gleicher Weise. Einzelne Tiere bleiben völlig gesund. Bei anderen Tieren kommt es zu einer schwachen Krebsentwicklung in den Nieren und der Leber, so daß man in diesen Organen zahlreiche stecknadelkopfgroße bis hirsekorngroße Tumoren findet. Bei anderen Tieren wieder beobachtet man eine so starke Ausdehnung des Krebswachstums, daß beispielsweise die Nieren bis auf die fünffache Größe anwachsen, daß die Leber völlig von erbsen- bis bohnengroßen Knötchen durchsetzt ist, die Ovarien bis über Walnußgröße angeschwollen sind und die Lungen mit zahlreichen kleinen Metastasen durchsetzt sind.

Läßt man auf eine Serie gleichmäßig infizierter Tiere irgendwelche chemische oder physikalische Kräfte einwirken und läßt die gleiche Anzahl Tiere als Kontrollen unbehandelt unter sonst genau den gleichen Lebensbedingungen, so läßt sich aus dem Vergleich der Intensität des Krebswachstums bei den behandelten und unbehandelten Tieren unter Umständen ein Urteil über den fördernden bzw. hemmenden Einfluß der Behandlung auf die Malignitätsdisposition bilden.

Im folgenden sei über Versuche berichtet, bei denen der Einfluß von Kochsalz und von Kalk auf die Malignitätsdisposition untersucht wurde. Hierbei wurde folgendermaßen vorgegangen:

Eine Serie Kaninchen wurde intravenös mit einer feinen, durch Gaze filtrierten Suspension von Tumorzellen (von einem primären Hodencarcinom) infiziert. Hierauf wurde die eine Hälfte der Tiere als Kontrollen gelassen, die andere Hälfte wurde anschließend regelmäßig täglich (mit Ausnahme der Sonntage)

behandelt. Nach Verlauf von etwa 4—6 Wochen wurden die noch nicht spontan eingegangenen Tiere getötet und seziert. Für jeden Versuch wurden in der Regel etwa 10 Kaninchen genommen, um in der gleichen Serie nicht zu viele Tiere zu haben. Dafür wurden aber die Versuche stets mehrmals wiederholt, um individuelle Fehler tunlichst auszuschalten.

Der Einfluß von Kochsalz.

Der Einfluß von Kochsalz auf das Krebswachstum wurde unseres Wissens bisher noch nicht experimentell untersucht. In früheren Zeiten kam allerdings *J. Braithwaite* auf Grund empirischer und statistischer Belege zu der Ansicht, daß ein Übermaß von NaCl die Krebsbildung verursachen sollte. Dieser Ansicht trat sofort *Sticker* entgegen, und *Omega* behauptete sogar das Gegenteil, daß man nämlich durch NaCl den Krebs günstig beeinflussen könne.

Bei unseren Versuchen wurde das NaCl den Kaninchen nicht mittels Schlundsonde gegeben, sondern täglich als 10proz. Lösung subcutan injiziert. Neben gewöhnlichem Kochsalz benutzten wir ein Speisesalz, dem Kalium, Calcium und Magnesium zugesetzt wurden. Das Ergebnis der im August—November durchgeführten Versuche findet sich in Tab. I.

In der Tabelle bedeutet „sp." hinter dem Tag des Todes, daß das Tier spontan gestorben ist, „get." bedeutet, daß es getötet wurde. Bei den Sektionsbefunden bedeutet „1" schwaches Carcinomwachstum, „4" sehr starkes, „3" und „2" liegen dazwischen.

Überblickt man die Tabelle, so sieht man, daß durch die Behandlung mit Kochsalz eine deutliche Förderung des Krebswachstums erzielt wurde. Während beispielsweise bei den Kontrollen 33% der Tiere negativ waren, war dies bei den mit Kochsalz behandelten Tieren in nur 16% der Fall. Bei der Kochsalzbehandlung blieben somit halb soviel Kaninchen negativ als bei den Kontrolltieren. Das gleiche Bild findet sich auch beim Vergleich des besonders starken Carcinomwachstums. Während hier bei den Kontrolltieren nur 13% ein sehr starkes Carcinomwachstum zeigten, war dies bei den mit Kochsalz behandelten Tieren in 28% der Fall. Auch hier sieht man deutlich eine Steigerung der Malignitätsdisposition infolge der Kochsalzbehandlung.

Auffällig ist bei diesen Befunden die verhältnismäßig geringe Menge Kochsalz, die bereits die Malignität zu steigern vermag. Die tägliche Dosis überstieg nie 0,3 g pro Tier (d. h. 3,0 ccm der 10proz. Lösung), und die Gesamtdosis betrug höchstens 6,5 g pro Tier. Daß derartig geringe Mengen Kochsalz bereits die beobachtete starke Steigerung der Malignität verursachten, dürfte beachtenswert sein.

Leider wurden diese Versuche an nur 40 Kaninchen durchgeführt, und man muß mit der Möglichkeit rechnen, daß diese Prozentzahlen bei Untersuchung einer wesentlich höheren Tierserie vielleicht eine gewisse Verschiebung nach der einen oder anderen Seite erfahren werden. Als sicher scheint uns aber trotzdem aus unseren Versuchen hervorzugehen, daß *durch Kochsalz eine Steigerung der Malignitätsdisposition* zu erzielen ist.

Tabelle 1. *Einfluß des Kochsalzes auf das Kaninchencarcinom.*

Behandelt	Wie oft	Total g	Tot nach Tagen	Sektionsbefund
NaCl	16× 0,2 11× 0,3	6,5	33 get.	Nieren 2, Ovarien 2
„	16× 0,2 11× 0,3	6,5	33 „	o. B.
„	16× 0,2 11× 0,3	6,5	34 „	Nieren 2
„	16× 0,2 11× 0,3	6,5	34 „	Ovarien 2, Nieren 1, Nebennieren 1, Netz 1
„	16× 0,2 2× 0,3	3,8	23 „	Ovarien 2
„	22× 0,2	4,4	28 „	o. B.
„	9× 0,2	1,8	12 sp.	Leber 1
„	10× 0,2	2,0	14 „	Leber 2, Lunge 2
„	21× 0,2	4,2	26 „	Leber 2, Lunge 1
„	22× 0,2	4,4	28 get.	Nieren 2
„	22× 0,2	4,4	31 „	Nieren 2, Lunge 1
„	22× 0,2	4,4	31 „	o. B.
„	22× 0,2	4,4	31 „	o. B.
„	22× 0,2	4,4	31 „	Nieren 2
„	21× 0,2	4,2	26 „	Leber 4, Nieren 4, Ovarien 3, Lunge 2
Salz	16× 0,2 11× 0,3	6,5	33 „	Nieren 1, rechtes Ovar 1
„	16× 0,2 11× 0,3	6,5	46 „	Nieren 2, Ovarien 2
„	16× 0,2 11× 0,3	6,5	33 „	Rechtes Ovar 1
„	16× 0,2 11× 0,3	6,5	34 „	Linkes Ovar 1
„	16× 0,2 3× 0,3	4,1	23 sp.	Leber 3, Lunge 3, Nieren 2, Ovarien 2, Netz 1
„	22×0,2	4,4	28 get.	Ovarien 2, Bauchmuskeln 2, Rückenmuskeln 2
„	22× 0,2	4,4	28 „	Nieren 3, Ovarien 3
„	22× 0,2	4,4	28 „	Nieren 2
„	22× 0,2	4,4	28 „	Nieren 3, Ovarien 2
„	22× 0,2	4,4	28 „	Nieren 3, Ovarien 2, Peritoneum 1
—	—	—	23 „	o. B.
—	—	—	33 sp.	Nieren 3, rechtes Ovar 1
—	—	—	33 get.	o. B.
—	—	—	34 „	o. B.
—	—	—	34 „	Nieren 2, rechtes Ovar 1
—	—	—	28 „	Nieren 2, Ovarien 1
—	—	—	26 get.	Nieren 2, Lungen 2, Leber 1
—	—	—	28 „	Nieren 2
—	—	—	28 „	Nieren 2
—	—	—	28 „	Nieren 1, rechtes Ovar 1
—	—	—	29 „	o. B.
—	—	—	31 „	o. B.
—	—	—	31 „	Nieren 2
—	—	—	29 „	Ovarien 1
—			29 „	Ovarien 1, Nieren 1

Hier möchten wir kurz bemerken, daß wir stets die Monate angeben, in denen die Versuche durchgeführt wurden, da allem Anschein nach biologische Versuche der hier geschilderten Art weitestgehend durch das in den betreffenden Monaten gereichte Futter beeinflußt werden.

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Der Einfluß von Kalk.

Der Einfluß des Kalkes auf das Krebswachstum wurde bisher noch nicht experimentell untersucht. Einzelne Autoren glauben, daß das Carcinomwachstum durch Kalk gehemmt wird. In der Zeit der Lymphtheorie des Krebses wurde Kalk in Form von Kalkwasser schon von *Boerhaave* angewandt und galt einige Zeit hindurch als spezifisches Heilmittel. Auch später noch empfahl *Roerig* dieses alte Medikament.

Bei unseren Versuchen gingen wir derart vor, daß wir organisch gebundenen Kalk einem kleinen Teil der Nahrung zusetzten, und erst nach Feststellung, daß die Tiere die kalkhaltige Nahrung verzehrt hatten, das übrige Futter gaben. Ein Teil der Tiere erhielt 0,5 g Kalk täglich, ein anderer Teil erhielt 2,5 g Kalk täglich.

Das Ergebnis der im Dezember bis März durchgeführten Versuche ist in Tab. 2 zusammengestellt.

Tabelle 2. *Einfluß von Kalk auf das Kaninchencarcinom.*

Behandelt	Dosis	Total g	Tot nach Tagen	Sektionsbefund
Kalk	12 × 0,5	6,0	30 sp.	Nieren 3, Ovarien 3, Lungen 1, Peritoneum 1
„	12 × 0,5	6,0	37 get.	Nieren 1, rechtes Auge 2
„	12 × 0,5	6,0	34 sp.	Ovarien 1, Peritoneum 1
„	12 × 0,5	6,0	26 „	o. B. (Pneumonie)
„	12 × 0,5	6,0	38 get.	Ovarien 4, Nieren 2, Augen 2
„	10 × 0,5	5,0	14 sp.	o. B. (Pneumonie, interkurrent)
„	17 × 2,5	42,5	31 get.	o. B.
„	23 × 2,5	57,5	27 sp.	Leber 3, Nieren 2, Ovarien 4
„	23 × 2,5	57,5	30 get.	Nieren 2, Ovarien 2
„	23 × 2,5	57,5	30 „	Rechtes Ovar 2
„	21 × 0,5	52,5	35 „	Nieren 1, Ovarien 3—4
„	21 × 0,5	52,5	35 sp.	o. B.
„	21 × 0,5	52,5	35 „	Nieren 2, linkes Ovar 2
„	21 × 0,5	52,5	35 „	o. B.
„	15 × 2,5	37,5	19 sp.	Leber 4, Lunge 1
—	—	—	36 get.	Augen 2
—	—	—	36 „	Nieren 1
—	—	—	37 „	o. B.
—	—	—	17 sp.	Linke Lunge 1 (Pneumonie)
—	—	—	33 „	Nieren 4, Leber 4, Lunge 4, Nebennieren 4, Ovarien 4, Pankreas 4, Bauchwand 4
—	—	—	37 get.	o. B.
—	—	—	31 „	Linke Niere 1, rechtes Ovar 2
—	—	—	31 „	Nieren 1, linkes Ovar 2, rechte Lunge 1
—	—	—	31 „	o. B.
—	—	—	31 „	Rechte Niere 2, rechtes Ovar 2, rechtes Ovidukt 1, rechte Nebenniere 3
			34 „	Nieren 2, Lungen 1, Ovarien 2—3
			34 „	Nieren 4, Ovarien 3—4, Lunge 1 (?)
			35 sp.	Ovarien 4, Nieren 1
			36 get.	Nieren 1, rechtes Ovar 4, linkes Ovar 2, Lunge 1, Thymus 1, rechtes Auge 3
			36 „	Nieren 1, Ovarien 2

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Überblickt man diese Tabelle, so zeigt sich, daß von den mit Kalk gefütterten Tieren 5, d. h. 33%, negativ waren, während dies bei den Kontrolltieren in 3 Fällen, d. h. in 20%, der Fall war. Von den mit Kalk behandelten Kaninchen hatten 3 (20%) eine sehr starke Carcinomentwicklung, von den Kontrolltieren ebenfalls 3 (20%).

Wenn man von der etwas höheren Zahl der negativ gebliebenen, mit Kalk gefütterten Kaninchen absieht, so kommt man aus diesen Versuchen zu dem Schluß, daß Kalk auf die Malignitätsdisposition ohne merklichen Einfluß ist.

Zusammenfassung.

Bei Versuchen mit dem *Brown-Pearce*schen *Kaninchencarcinom* zeigte es sich, daß perorale *Kalk*zufuhr *ohne* nennenswerten Einfluß auf die Malignitätsdisposition ist.

Demgegenüber wurde die Malignitätsdisposition durch verhältnismäßig *geringe Mengen* (tägliche Dosis 0,2—0,3; Gesamtdosis höchstens 6,5 g) *von Kochsalz gesteigert.*

Literaturverzeichnis.

Braithwaite, J., Lancet **1901** — Excess of salt in the diet with three other factors the probable causes of cancer. London 1902. — *Omega* (Pseudonym), Cancer its probable cause etc. London 1903. — *Roerig*, Dtsch. med. Presse **1911**, Nr 22. — *Sticker, A.*, Dtsch. Medizinalztg **1902**.

Committee on Specifications. 1972

Food Chemicals Codex, 2nd edition

Comm. Food Prot., Natl. Acad. Sci., Natl. Res. Coun., Washington, D.C.
pp. 645-647; 734-735

Experientia 20:282, 1964

Effect of a Physiologic Solution of Sodium Chloride on the Development of the Chick Embryo

By: G. Conti and G. Milio[1]

The results reported in this article come from the series of research having for its aim the identification of the effects of different solvents on the development of the chick embryo. In a preceding article published in this journal, we referred to the effect exerted by injection and distilled water. In this article we will illustrate by means of three tables, the results obtained by injection of a physiologic solution of sodium chloride (8.5 g% of NaCl in distilled water). The tests were done on a total of 1000 chicken eggs divided into four series of experiments; the technique, the period of injections, as well as the quantity of injected substance are the same as described in our previous article.

From all of these experiments, we can draw the following conclusions:

(a) The physiologic solution of sodium chloride injected into the egg white at different stages of incubation has a lethal effect which is evident either by the early death of the embryos, or by a later death which strikes the embryos during the period of incubation. This lethal effect is quite, and sometimes very significant with amounts higher than 0.5 and 1 cm of physiologic solution; it is slight with weak doses from 1/20 to 1/10 cm^3. However, it must be emphasized that weak doses cause a significant lethal effect when they are injected before, or 5 days after incubation. Before incubation, the chicken embryo is particularly sensitive to different stimulants because at that time it is in an unstable state of equilibrium. At the fifth day of incubation, the embryo again becomes very sensitive and this fact is seemingly related to the development of its vascular system which can absorb injected substances very rapidly (hydrous disequilibrium poorly tolerated).

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[1]This research was carried out thanks to a subsidy from the Swiss National Fund for scientific research and the E. Barell Foundation.

[2]G. Conti and G. Milio, Exper. 20, 110 (1964).

Table II

Number of embryos (%) at the 21st Day.

Date of Treatment	Control	Injection of Physiologic Solution (NaCl 8.5%) 0.05 cm^3	0.1 cm^3	0.5 cm^3	1 cm^3
Before incubation	75.8	56.4	57.8	37.1	10.8
After 24 hours		76.0	71.4	61.4	32.5
After 48 hours		76.6	86.0	54.1	23.5
After 3 days		84.7	84.4	40.6	14.8
After 4 days		68.0	67.3	44.4	14.1
After 5 days		10.0	30.0	68.9	17.8

Table III

Number (%) of Malformed Embryos

Date of Treatment	Control	Injection of Physiologic Solution (NaCl 8.5%) 0.05 cm^3	0.1 cm^3	0.5 cm^3	1 cm^3
Before incubation	6.3	10.9	8.9	25.7	5.4
After 24 hours		2.0	7.1	4.5	2.5
After 48 hours		4.3	6.9	2.7	8.8
After 3 days		2.2	4.5	3.4	2.9
After 4 days		10.0	4.1	4.4	2.2
After 5 days		8.0	2.0	4.4	2.2

(Institute of Histology and General Embryology of the University of Fribourg (Switzerland), November 25, 1963)

(b) The physiologic solution is not a teratogenic agent: the number of malformations is completely normal if weak doses are injected, slightly higher with the injection of strong doses.

(c) From looking at the three tables, it is evident that the most favorable period for injecting the chick embryo with weak doses of physiologic solution is between the 24th and 48th hour of incubation. A logical conclusion arises as a result: if it is desired to verify the effect of a certain substance on the organism of the chick embryo and this substance is dissolved in physiologic solution, it is necessary to inject small doses after 24 hours of incubation. It is better to inject the substance after 24 hours than after 2, 3, or 4 days of incubation because even if during these four days, the lethal effect of the solvent is nearly the same, the injected substance risks not arriving to influence the development of certain organs which by the third or fourth day are already well defined.

(d) The results obtained with the injection of physiologic solution in regard to the early lethal effect, the late lethal effect and the number of malformations, correspond to the results obtained with the injection of distilled water: as a result, a hydrosoluble substance which must be injected into the chick embryo may be dissolved equally in distilled water or physiologic solution of sodium chloride.

Table I

Number of embryo deaths (%) in the first three days after treatment.

Date of Treatment	Control	Injection of Physiologic Solution ($NaCl$ 8.5%)			
		0.05 cm^3	0.1 cm^3	0.5 cm^3	1 cm^3
Before incubation	10.2	26.1	20.0	17.1	43.2
After 24 hours		8.0	14.3	4.5	32.5
After 48 hours		12.8	9.3	27.0	61.8
After 3 days		8.7	4.7	43.7	50.0
After 4 days		12.0	16.3	37.8	73.3
After 5 days		10.0	56.0	26.7	82.2

Action de la solution physiologique de chlorure de sodium sur le développement de l'embryon de poulet[1]

Les résultats rapportés dans ce mémoire rentrent dans le cycle des recherches ayant pour but de préciser l'action de différents solvants sur le développement de l'embryon de poulet. Dans une communication précédente, publiée dans cette revue[2], nous nous sommes référés à l'action exercée par la piqûre et l'eau distillée. Dans cette note, nous illustrons au moyen de trois Tableaux les résultats obtenus par l'injection d'une solution physiologique de chlorure de sodium (8,5 g ‰ de NaCl dans de l'eau distillée). Les résultats portent sur un ensemble de 1000 oeufs de poule, partagés en quatre séries d'expériences; la technique et la période des injections, comme d'ailleurs la quantité de substance injectée, sont les mêmes que celles décrites dans la note précédente.

Tableau I. Nombre des embryons (%) morts dans les 3 premiers jours après le traitement

Date du traitement	Contrôle	Injection de solution physiologique (NaCl 8,5 ‰)			
		0,05 cm³	0,1 cm³	0,5 cm³	1 cm³
Avant l'incubation	10,2	26,1	20,0	17,1	43,2
Après 24 h		8,0	14,3	4,5	32,5
Après 48 h		12,8	9,3	27,0	61,8
Après 3 jours		8,7	4,7	43,7	50,0
Après 4 jours		12,0	16,3	37,8	73,3
Après 5 jours		10,0	56,0	26,7	82,2

Tableau II. Nombre des embryons (%) arrivés au 21e jour

Date du traitement	Contrôle	Injection de solution physiologique (NaCl 8,5 ‰)			
		0,05 cm³	0,1 cm³	0,5 cm³	1 cm³
Avant l'incubation	75,8	56,4	57,8	37,1	10,8
Après 24 h		76,0	71,4	61,4	32,5
Après 48 h		76,6	86,0	54,1	23,5
Après 3 jours		84,7	81,1	40,6	11,8
Après 4 jours		68,0	67,3	41,4	11,1
Après 5 jours		40,0	30,0	68,9	17,8

Tableau III. Nombre (%) des embryons malformés

Date du traitement	Contrôle	Injection de solution physiologique (NaCl 8,5 ‰)			
		0,05 cm³	0,1 cm³	0,5 cm³	1 cm³
Avant l'incubation	6,3	10,9	8,9	25,7	5,4
Après 24 h		2,0	7,1	4,5	2,5
Après 48 h		4,3	6,9	2,7	8,8
Après 3 jours		2,2	4,5	3,1	2,9
Après 4 jours		10,0	4,1	4,4	2,2
Après 5 jours		8,0	2,0	4,4	2,2

De l'ensemble de ces expériences, nous pouvons tirer les conclusions suivantes:

(a) La solution physiologique de chlorure de sodium, injectée dans le blanc de l'oeuf à des stades différents de l'incubation, provoque un effet léthal qui se rend évident soit par la mort précoce des embryons, soit par une mort plus tardive qui frappe les embryons au cours de l'incubation. Cette action léthale est assez, et parfois même très importante, avec les doses élevées de 0,5 et 1 cm³ de solution physiologique; elle est modeste avec les doses faibles de $^1/_{20}$ et $^1/_{10}$ème de cm³. Il est toutefois à souligner que même les doses faibles provoquent un effet léthal important lorsqu'elles sont injectées avant l'incubation ou après 5 jours. Avant l'incubation, l'embryon de poulet est particulièrement sensible aux différentes stimulations puisque à ce moment, il se trouve dans un état d'équilibre instable. Au 5ème jour de l'incubation, l'embryon redevient de nouveau très sensible, et ce fait tient vraisemblablement au développement de son système vasculaire qui peut absorber les substances injectées avec une grande rapidité (déséquilibre hydrique mal supporté).

(b) La solution physiologique n'est pas un agent tératogène: le nombre des malformés est tout à fait superposable à la normale si l'on injecte des doses faibles, un peu plus élevé avec l'injection de doses fortes.

(c) Si l'on observe dans l'ensemble les trois Tableaux, il ressort d'une manière évidente que la période la plus favorable pour injecter à l'embryon de poulet de faibles doses de solution physiologique se situe entre la 24ème et la 48ème h d'incubation. Une conclusion d'ordre pratique s'impose par conséquent: si l'on veut éprouver l'action d'une substance déterminée sur l'organisme de l'embryon de poulet et que cette substance doit être dissoute dans la solution physiologique, il faut injecter des petites doses après 24 h d'incubation. Il vaut mieux injecter la substance après 24 h qu'après 2, 3 ou 4 jours d'incubation, car même si pendant ces quatre jours, l'action léthale du solvant est à peu près la même, la substance injectée risque de ne pas arriver à influencer le développement de certains organes qui, au 3ème ou 4ème jour, sont déjà bien ébauchés.

(d) Les résultats obtenus avec l'injection de solution physiologique correspondent, en ce qui concerne l'action léthale précoce, l'action léthale tardive et le nombre de malformés, aux résultats obtenus avec les injections d'eau distillée: par conséquent, une substance hydrosoluble qui doit être injectée à l'embryon de poulet, peut être indifféremment dissoute dans de l'eau distillée ou de la solution physiologique de chlorure de sodium.

Summary. The authors have tested the action of the physiological solution of natrium chloride on the development of the chick embryo: no teratogenic action, no early lethal action with small doses, and an important lethal action with large doses.

G. CONTI et G. MILIO

Institut d'Histologie et d'Embryologie générale de l'Université de Fribourg (Suisse), le 25 novembre 1963.

[1] Ces recherches ont été faites grâce à un subside du Fonds National Suisse de la recherche scientifique et de la Fondation E. Barell.

[2] G. CONTI et G. MILIO, Exper. 20, 110 (1964).

Dietary Sodium in the Development of Renal Hypertension[1] (34205)

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Sodium retention has been shown to aggravate renoprival hypertension (1); it is essential for the development of DCA hypertension (2); and balance studies have shown that it plays a part in the early stages of the development of hypertension after the application of the Goldblatt clamp to the renal artery (3).

The object of the present study was to investigate the role that sodium retention might play in the maintenance of hypertension over a longer period of time. For this purpose, the evolution of hypertension was compared in rats maintained on a normal sodium intake with those on a virtually sodium-free diet.

Material and Methods. One hundred young Wistar rats, of approximately 200 g, were used in this experiment. Blood pressure was recorded from the tail by the microphonic technique (4) twice weekly before and after the application of the silver clip to one renal artery, with contralateral nephrectomy. After the operation half the animals were placed on a synthetic diet, low in sodium (50 mg/kg)[2] (5); while the other half were fed the same diet to which 0.6% sodium chloride was added. Distilled water was permitted *ad libitum*. After 8 weeks on the respective diets, the sodium intake of the two diets was switched for a further period of 5 weeks.

In addition, two groups of control animals were followed concurrently. They were subjected to a sham operation in which a unilateral nephrectomy was performed and a clip was placed on the opposite renal artery and then immediately removed. Twenty-six of these received the low sodium and 22, the higher sodium intake. The diet of the control groups was not switched at 8 weeks to permit evaluation of growth and blood pressure trends in these animals over the entire 14-week period.

Results. Hypertension developed over a period of 4–6 weeks in both groups of experimental animals as is illustrated (Fig. 1); but those on the low sodium diet maintained a lower blood pressure throughout the initial period of 8 weeks, at the end of which the mean systolic blood pressure of 158 (SD 28) mm Hg was significantly lower than those with a normal sodium intake, 176 (SD 26) mm Hg. $p<0.05$. When the diets of the experimental groups were changed, the difference between their blood pressures disappeared. This was partly attributable to a rise in blood pressure in those formerly on a low sodium intake and a slight fall in those who had the normal sodium intake.

In the control groups, the systolic pressures were virtually identical for those on a low or normal sodium intake. The weight curves were also identical but, in spite of this, the weight gain in the experimental group on the normal sodium intake was lower than it should have been. This difference reached 47 gm at 8 weeks. With the restoration of a normal sodium intake, the weight in this group promptly rose to the normal level; on the other hand, the experimental group which had been on a normal sodium intake ceased to gain weight as soon as they were placed on the low sodium diet.

Conclusions. Renal hypertension in rats appears to be moderated but not prevented by sodium restriction. This finding is analagous to that obtained in short-term balance studies in the dog (3). Thus indicating that this type of hypertension can be maintained in the face of a very low sodium diet. It has been suggested that sodium restriction does

[1] This work was supported by USPHS Grant HE 07764-07.

[2] General Biochemicals, Cleveland, Ohio.

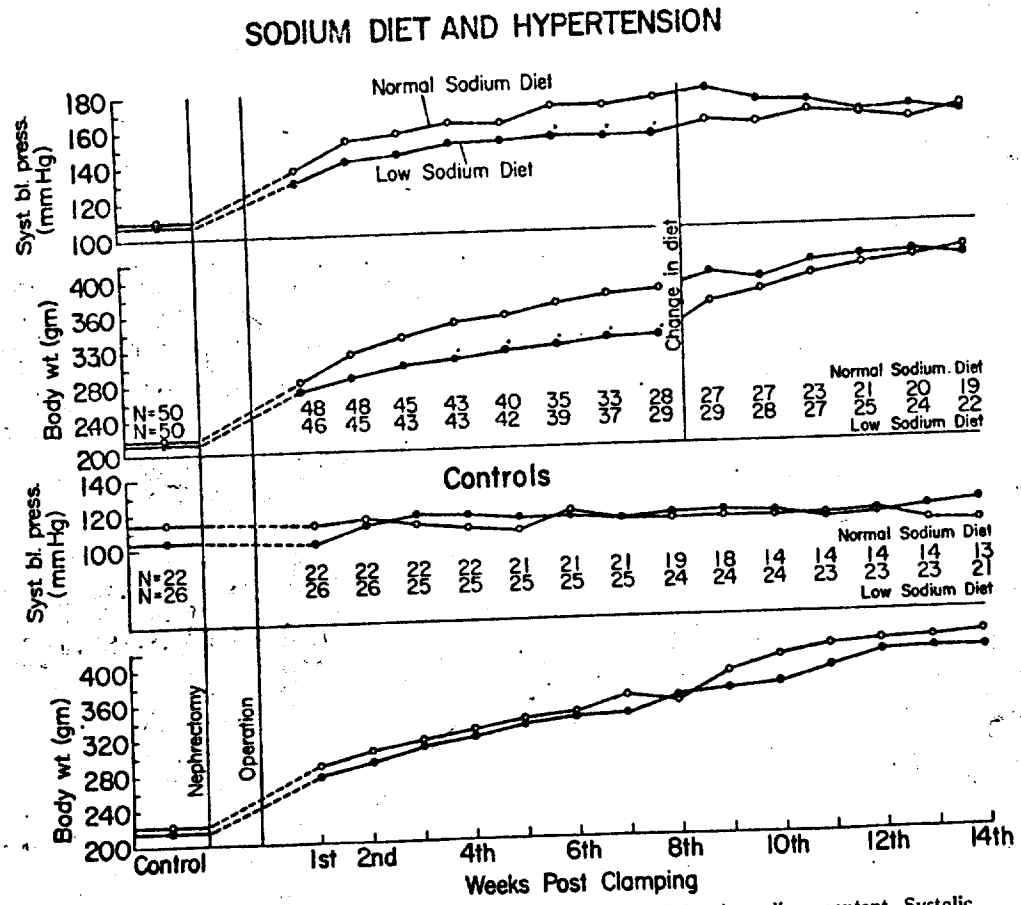

FIG. 1. Evolution of hypertension in animals fed on diets differing in sodium content. Systolic blood pressure and weight gain in rats on a normal (○) or a low sodium intake (●). All animals were subjected to nephrectomy; (upper), a clip was applied to the opposite renal artery; and (lower), a sham operation on that side; N indicates the numbers of animals in the study during each week of observation; *Indicates a "significant difference" $p<0.05$ between rats on the sodium deficient diet as compared to those on a normal diet.

not affect the level of blood pressure in hypertensive rats (6, 7); although if a low sodium diet was administered before clipping the renal artery, the subsequent level of blood pressure was lower (7). The satisfactory survival rate and weight gain in the control animals on a low sodium diet in the present study attest to its adequacy in this respect. The clipped rats on a low sodium diet failed to gain weight normally. This unexpected finding is without explanation, but it may be related to the polydypsia which is known to develop after the application of a clip to the renal artery (8). In conclusion, severe sodium restriction did not prevent development of experimental renal hypertension even though free access to sodium aggravated the condition.

Summary. The evolution of experimental renal hypertension was studied in rats on a sodium deficient diet (50 mg/kg) as compared to those on the same diet with 0.6% sodium added and also with sham operated controls on the two diets. On the low sodium diet, hypertension emerged more gradually and at 8 weeks, the blood pressure was sig-

nificantly lower than those with the normal intake. The mean difference, however, was only 18 mm Hg. The experimental animals on the sodium-deficient diet weighed less than those on the normal diet. In control animals without a clip on the renal artery, there was no difference, according to diet, in weight gain or indeed in blood pressure.

1. Orbison, J. L., Christian, C. L., and Peters, E., A. M. A. Arch. Pathol. 54, 185 (1952).

2. Friedman, S. M., Friedman, C. L., and Polley, J. R., Am. J. Physiol. 153, 226 (1948).

3. Conway, J., Circulation Res. 22, 763 (1968).

4. Friedman, M. and Freed, S. C., Methods Med. Res. 5, 251 (1952).

5. Hartroft, P. M. and Eisenstein, A. B., Endocrinology 60, 641 (1957).

6. Fisher, E. R. and Klein, H. L., Proc. Soc. Exptl. Biol. Med. 113, 37 (1963).

7. Redleaf, P. D. and Tobian, L., Circulation Res. 6, 343 (1958).

8. Fregly, M. J., Am. J. Physiol. 182, 143 (1955).

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Sodium Content of Drinking Water in Nebraska

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THE treatment of certain cardiac, renal, and hepatic diseases with low salt diets is recognized as an often effective measure. In theory, in a person with alteration of sodium metabolism, a reduced sodium intake will result in a smaller total body sodium with less retained water.

There has been much emphasis placed on the sodium content of natural and prepared foods, and many diets have been formulated to aid in the preparation of palatable low sodium diets. In 1947 Cole introduced the possible significance of sodium in drinking water, an often unsuspected source of considerable sodium.[1] Since that time there have been several articles concerned with the sodium content of drinking water in many states.

There is a recent report of two cases of congestive heart failure that were not controlled until it was recognized that their drinking water contributed more sodium than the restriction for the total diet allowed.[2] While these were extreme examples of high sodium water, similar problems are possible in many towns in Nebraska.

The Nebraska State Health Department has routinely analyzed public water supplies and has compiled average values representing several samples of water from July, 1960, to July, 1963.[3] The majority of these samples were from the supplying wells; a few samples were taken from the distribution-system. The sodium values were determined by flame spectrophotometry. Table 1 gives the sodium content in milligrams per liter of water and the hardness expressed as milligrams of calcium carbonate per liter of water. These representative values may not reflect seasonal variation. Only communities with 50 mg sodium per liter or more were included; this limit was chosen arbitrarily since 50 mg per liter will give a person drinking 3 to 4 liters a day a total of 150 to 200 mg of sodium. Lincoln and Omaha were also included. Obviously, the higher the sodium content or the stricter the sodium restriction, the more important is the sodium content of the drinking water. Also, low sodium foods may have their sodium content increased when boiled in relatively high sodium water even if the water is discarded, and higher sodium foods will lose less sodium when boiled under the same circumstance.[4]

Figure 1 shows the distribution of the towns with over 50 mg sodium per liter. No general statement can be made about the pattern.

Water softening is another problem. The municipal treatment plants usually use the lime-soda ash method.[5] Columbus, Falls City, Lyons, Nebraska City, and Superior use this method or a variation. The calcium and magnesium ions, which are principally responsible for hardness, are precipitated with calcium hydroxide and sodium carbonate, primarily as magnesium hydroxide and calcium carbonate. Sodium is thus added to the water; the amount added depends upon the degree of softening desired.

The ion-exchange process is more popular for home use for softening hard water, al-

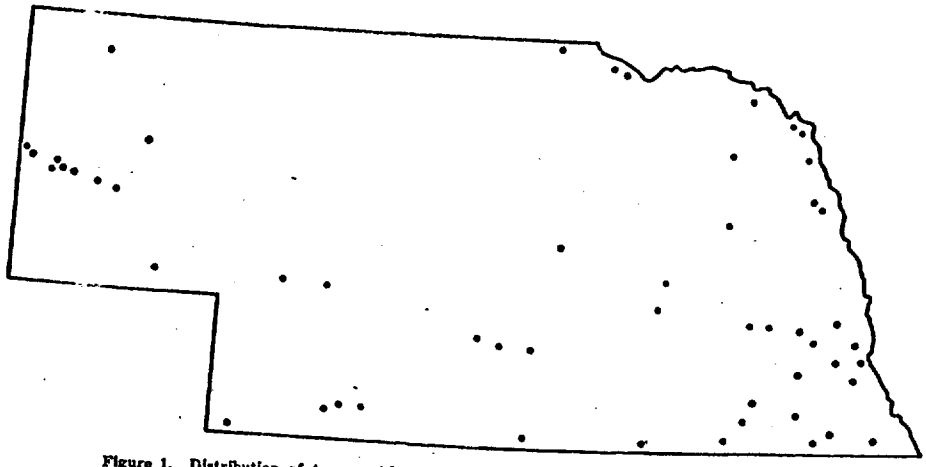

Figure 1. Distribution of towns with 50 mg. or more sodium per liter of drinking water.

Table 1

Community	Sodium mg/liter	Hardness mg CaCO3/liter
*Alliance	78	294
Anoka	53	740
Bridgeport	112	292
Burchard	85	2600
Clarks	58	324
Craig	70	392
Culbertson	59	528
†*Dakota City	78	556
Dawson	58	516
DeWitt	175	260
Eagle	60	380
Elm Creek	63	364
†*Fairbury	62	208
*Filley	58	408
Garland	92	192
Gering	150	262
Haigler	300	1480
Hardy	57	256
Henry	68	320
Hershey	110	426
Hordville	53	372
Kearney	68	428
Leigh	73	392
†*Lincoln	26	196
Lodgepole	101	232
Louisville	55	350
McCook	68	304
*Macy	103	492
Maskell	102	640
Minatare	112	150
*Monowi	460	80
Morrill	87	338
Naponee	71	572
‡*Nebraska City	50	196
‡‡*Nehawka	110	76
North Loup	65	434
†Oakland	69	476
†*Omaha	48	153
Overton	61	454
Panama	63	300
Paxton	270	568
Plymouth	60	452
Raymond	52	304
Scottsbluff	188	328
†South Sioux City	80	600
Steinauer	78	548
Syracuse	52	290
†Talmage	52	152
Terry Town	110	368
Trenton	70	304
*Verdel	300	72
Waverly	110	316
*Whitney	270	380
Winnebago	122	644
Winside	62	492

*—samples from distribution system
†—water treatment plant used

‡—water treatment plant used with lime softening
‡‡—water treatment plant used with ion-exchange softening

though there is one municipal ion-exchange-treatment plant at Nehawka and one being constructed at Macy. This process uses a resin, usually Zeolite, which exchanges two ions of sodium for each ion of calcium or magnesium; the sodium that was originally attached to the resin goes into the water and the calcium or magnesium is bound to the resin. The resin is recharged at intervals by adding salt to the resin tank. By this process it is possible to remove essentially all the hardness. Actually, the maximum softening is regulated by the efficiency of the system and the degree of replacement of the resin-bound sodium; 1 mg hardness as calcium carbonate is approximately equal to 0.46 mg sodium in this softening process. It can be seen that considerable sodium could be introduced in water such as at Burchard with a total hardness of 2600 mg per liter or at Haigler with 1480 mg per liter. If maximum exchange were achieved, the sodium content would be 1280 mg sodium per liter at Burchard and 980 sodium per liter at Haigler.

If the sodium content of the drinking water is too high to maintain a desirable sodium restriction, distilled, deionized, or other low sodium water is advisable. If softening of the water is necessary with a home unit, the plumbing should be connected so that only the bath and washing water is softened and the drinking and cooking water is not altered.

Summary

The sodium in drinking water may furnish a considerable and unsuspected quantity of sodium to the cardiac, renal, or hepatic patient on a low salt diet. The average sodium content of water is presented for Nebraska towns with 50 mg per liter or over. The possible increase of sodium content by ion-exchange softening is discussed.

References

1. Cole, S. L.: Tap water sodium in the low salt diet. JAMA 140:19, 1949.

2. Elliott, G. B., and Alexander, E. A.: Sodium from drinking water as an unsuspected cause of cardiac decompensation. Circulation 23:562, 1961.

3. Nebraska Health Department: Chemical analyses of Nebraska municipal water supplies. Unpublished data.

4. Bills, C. E.; McDonald, F. G.; Niedermeier, W., and Schwartz, M. C.: Sodium and potassium in foods and waters. J Amer Diet Ass 25:304, 1949.

5. Nebraska Health Department: List of water treatment plants. Unpublished data.

". . . the federal government had no right 'to assume and assert a priority of interest in the problems of youth and aged, the ill and the poor, and the temporarily unemployed'." (Ex-president Eisenhower as quoted by Chicago Tribune, December 10, 1964).

DISTRIBUTION AND EXCRETION OF WATER AND CHLORIDES AFTER MASSIVE SALINE INFUSIONS

AN EXPERIMENTAL STUDY

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Massive intravenous infusion has become an accepted therapeutic measure of wide application among practitioners of medicine and surgery, and present trends in medical practice seem to indicate even more extensive use of the method in the future. Nevertheless, comparatively little is definitely known about the physiologic responses of the body to the direct artificial addition of solutions of inorganic substances to the circulation. This is because the procedure has developed primarily at the bedside rather than in the experimental laboratory. Moreover, as ordinarily practiced, it has proved almost completely devoid of danger to life.

Because of the simplicity of the common vehicles of infusion—watery solutions of sodium chloride and dextrose usually—the consideration of intermediate metabolism has not seemed to most practitioners a serious issue, and the attention of experimentalists has turned, rather, to such matters as the causation and prophylaxis of the postinfusion reaction. It is true that attention has been paid to the assimilation and elimination of dextrose when used in infusions, and some consideration has been given to the factor of hypertonicity. By and large, however, the fundamental proposition that parenterally administered fluids are the physiologic equivalents of the same fluids administered by mouth has never been subjected to thorough experimental investigation.

The experiments to be reported in this communication were originally designed to determine the maximum rate of infusion compatible with survival and the lethal dose of essentially isotonic solutions of sodium chloride. It was moreover proposed to discover the cause of death after such lethal infusions and to trace the intermediate assimilation, trans-

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location and excretion of both the sodium chloride and the water when given in just sublethal amounts. As so frequently happens in experimental work, the original aims have not been entirely fulfilled, but important facts have, we believe, been collected. This investigation is, of course, but part of a much larger one, involving parenteral administration of solutions in other concentrations, of other substances and by other methods than intravenous injection. Until the general problem has been studied in all of these aspects, it cannot be assumed that the results of this study are applicable to all types of parenteral administration.

TECHNIC

In the experiments now to be reported, cats exclusively were used as experimental animals. No particular selection of animals was made with respect either to size or to physical condition. They were stock animals, which for varying periods had been fed ordinary laboratory rations, including as much water as they would drink.

Intraperitoneal injection of solution of dial and ethyl carbamate (urethane)[1] (0.9 grains [0.054 Gm.] of diallylmalonylurea per kilogram of body weight) was practiced routinely for anesthesia. In a few instances, in which the initial level of anesthesia produced by this agent was not deemed sufficiently deep, a little ether was temporarily given by inhalation to facilitate the primary operative procedures.

The intravenous infusions were given from a standard graduated arsphenamine buret holding 300 cc., which was connected by means of a short piece of rubber tubing to a glass cannula. This was inserted into the jugular vein (usually on the right) except in some cases in which the femoral vein was selected for special reasons. The arsphenamine tube was attached to a buret stand at such a level that the height of the column of fluid above the cannula varied from about 25 inches (63 cm.), when the tube was full, to about 16 inches (40 cm.), when the level of fluid had dropped to the last graduation.

In such a gravity system the speed of injection depends not only on the head of hydrostatic pressure but on the available caliber of the injectional system at its narrowest point, which ordinarily is the point of junction between the venous cannula and the vein. This narrowest part of the system is subject to variations in caliber from moment to moment because of such factors as twisting or change in angulation of the cannula with respect to the vein. These variations cannot be altogether avoided because living animals cannot be absolutely immobilized. Because we were anxious to maintain strictly constant rates of injection, we originally used the principle of the Mariotte flask in order to stabilize the hydrostatic pressure. We soon found, however, that the variability which resulted from fluctuations in the pressure was of little importance compared with that due to changes in caliber. Therefore, we ultimately fell back on the simple expedient of keeping the system adjusted practically to the full caliber of the vein. Minor variations were compensated by slight manipulation of a screw clamp and all our adjustments gaged by constant checking of the rate of fall of the meniscus in the buret.

We sought to avoid as much as possible the debatable point as to how strong a solution of sodium chloride constitutes an exactly isotonic or physiologic solution

1. Obtained through the courtesy of the Ciba Company.

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for the cat. Therefore, we have arbitrarily selected the strength of 1 per cent as being sufficiently close to the proper value for practical purposes. This figure is nevertheless not so exactly the proper value as to make it appear that we consider isotonicity actually critical.

In most of the acute experiments tracheal cannulation was performed, and blood pressure and respiration were continuously recorded on a long paper kymograph. The blood pressure was taken by direct cannulation of the common carotid artery and the respiration recorded from the tracheal cannula through a rubber tube connecting with a sensitive tambour.

PRELIMINARY EXPERIMENTS TO DETERMINE THE "LETHAL DOSE" OF SOLUTIONS OF SODIUM CHLORIDE

Our recorded tabulation (table 1) includes results from 10 cats. A considerably larger number of animals was actually used, omissions in

TABLE 1.—*Rates of Injection and Total Amounts of Sodium Chloride and Water in Lethal Doses of Saline Solutions Administered Intravenously* *

Cat Number	Strength of Sodium Chloride Solution, %	Weight of Animal in Kg.	Rate of Injection in Cc. per Kg. of Body Weight per Min.	Total Volume in Cc. per Kg. of Body Weight	Total Sodium Chloride in Cc. per Kg. of Body Weight per Min.
2A	1	1.53	7.71	503	5.03
9A	1	2.95	6.68	517	5.17
10A	1	2.77	4.04	639	6.39
11A	1	2.88	4.44	446	4.46
3A	2	2.50	5.68	320	6.41
4A	2	2.78	4.52	241	4.82
7A	2	2.90	3.93	196	3.93
8A	2	3.45	3.24	362	7.24
6A	5	2.40	1.66	170	8.50
5A	10	1.76	4.23	55	5.51

* The injections were continued at a constant rate until death occurred.

the final record being made in order to avoid unnecessary compounding of evidence. Four cats received a 1 per cent solution of sodium chloride, 4 others a 2 per cent solution and the 2 remaining a 5 per cent and a 10 per cent solution, respectively.

The death of the animals in all cases was obviously due to the injection of sodium chloride in water, and the fatal dose is a matter of record. It is not, however, strictly accurate to speak of this as the lethal dose in the same sense as that in which the term is applied to intrinsically toxic substances. Both water and sodium chloride are, of course, normal constituents of the blood stream, and they become toxic only fortuitously, when their relative concentrations are grossly disturbed. The data for the first and the last animal of the preceding table clearly exemplify this statement, since both received approximately the same amount of sodium chloride, yet the first tolerated approximately nine times as much water as the last before death occurred, the solution which the last received being ten times as concentrated. Intermediately placed in the

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series are 2 animals, 9A and 10A, which received considerably greater amounts of water than either of the other 2 animals. From these observations it may be concluded that water is not the lethal agent.

Furthermore, sodium chloride does not appear to be the lethal agent either, for there are animals intermediate in the series, 3A, 6A, 8A and 10A, which tolerated before death occurred considerably greater amounts of sodium chloride than did either the first or the last animal.

The logical conclusion is, of course, that neither of these agents is toxic in itself and that separately, therefore, they cannot be said to have any assignable lethal dose. In combination, however, they do appear to produce death after certain amounts have been given. The necessary amount depends on the concentration of the salt in the solution and undoubtedly also on the rate of its administration. The lethal dose of any given solution injected at any given constant speed is undoubtedly constant for any given concentration.

That hypertonicity (or hypotonicity) is a factor of great importance in determining the lethal dose is strongly indicated by the reactions of the animals in this short series. They appeared definitely less tolerant of a 2 per cent solution than of a 1 per cent solution. In still higher ranges of concentration (5 per cent and 10 per cent) it appears that much smaller amounts are fatal. At such concentrations rates of injection must be greatly reduced or death ensues rapidly.

The only conclusion for our present purposes which we wish to draw from this short series of animals is that cats tolerate the intravenous injection of tremendously large amounts of solution of sodium chloride at a rapid rate, provided the solution is not more concentrated than 2 per cent. The lethal amount is of the order of 500 cc. per kilogram of body weight for 1 per cent solutions and 275 cc. for 2 per cent solutions when the injection is made at the speed of approximately 5 cc. per kilogram of body weight per minute. Translated into terms of a human being weighing 70 Kg., or 154 pounds, this would be a total of 35 liters of 1 per cent solution, given at the rate of 350 cc. per minute. This is, of course, a much more massive volume and a much more rapid rate of injection than is actually used in clinical practice. It is therefore questionable whether death has ever resulted in a human being merely from the injection of the weaker solutions of sodium chloride, as such.

Milbert[2] said that he felt he had grossly exceeded what are customarily considered the limits of safety when he used in a series of clinical cases a rate of injection between 0.83 cc. and 2.5 cc. per kilogram of body weight per minute, though the total amount of solution was

2. Milbert, A. H.: Infusion Reactions with Special Reference to "Speed Shock," Am. J. Surg. 26:479-485, 1934.

in many cases less than 1,000 cc. To be sure, he used some solutions which were hypertonic (2 per cent and 5 per cent solutions of sodium chloride and 10 per cent solutions of dextrose). The fact that Milbert's patients suffered no ill effects attributable to the rate of infusion corroborates the belief that such quantities and rates are conservative rather than radical.

It is not a new or recent observation that experimental animals tolerate the intravenous infusion of huge quantities of saline solution. Dastre and Loye[3] published in 1888 a convincing communication describing the introduction of suitable fluids (0.7 per cent solutions of sodium chloride) into the circulation of rabbits and dogs. They injected amounts totaling as much as three quarters of the body weight within the relatively short period of three to seven hours. These injections produced no unfavorable effects, either immediate or remote, in two months of observation. We can corroborate this result unreservedly for postinfusion periods of several days to two weeks, though we have seen no occasion to prolong the observation of recovery beyond such limits.

A. Effect of Massive Infusions on Blood Pressure.—The capacity of the vascular system is constantly shifting by virtue both of (1) its vasomotor reactions, which tend to adapt its capacity to the cubic measure of its contents, and of (2) the permeability of its immense capillary system, which seems to change both locally and generally in accordance with a number of different physical and chemical factors. It is therefore impossible by any means at present available to determine how long any artificially injected soluble substance remains in the circulation. This is particularly true of solutions of sodium chloride because of their extreme diffusibility.

The best that can be done is to attack the problem indirectly. A long series of tracings of blood pressure, taken during the large, rapidly administered infusions which we have employed, serves to show conclusively that the blood pressure is neither constantly nor greatly affected. A representative tracing, somewhat condensed, is reproduced in figure 1.

In general, if the blood pressure is somewhat low at the time the infusion is started a temporary rise is produced, which tends to be well sustained. If, on the contrary, the blood pressure is moderately high at the beginning of the infusion, it customarily decreases slightly within a short time and thereafter remains at about the same level throughout the infusion. In general, the blood pressure is maintained at a satisfactory level throughout a fatally large infusion and falls abruptly just

3. Dastre, A., and Loye, P.: Le lavage du sang, Arch. de physiol. norm. et path. 2:93-114, 1888.

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as death is occurring. A slight progressive decrease in blood pressure throughout the entire course of the infusion is the rule.

Every indication points to a marked increase in the volume of circulating blood during such infusions. Hematocrit estimations show increasing preponderance of fluid over cells and increase in the water content of the blood is revealed by drying successive samples to constant weight. Therefore a fairly stationary blood pressure at a relatively high level can mean only that great compensatory vasodilatation occurs.

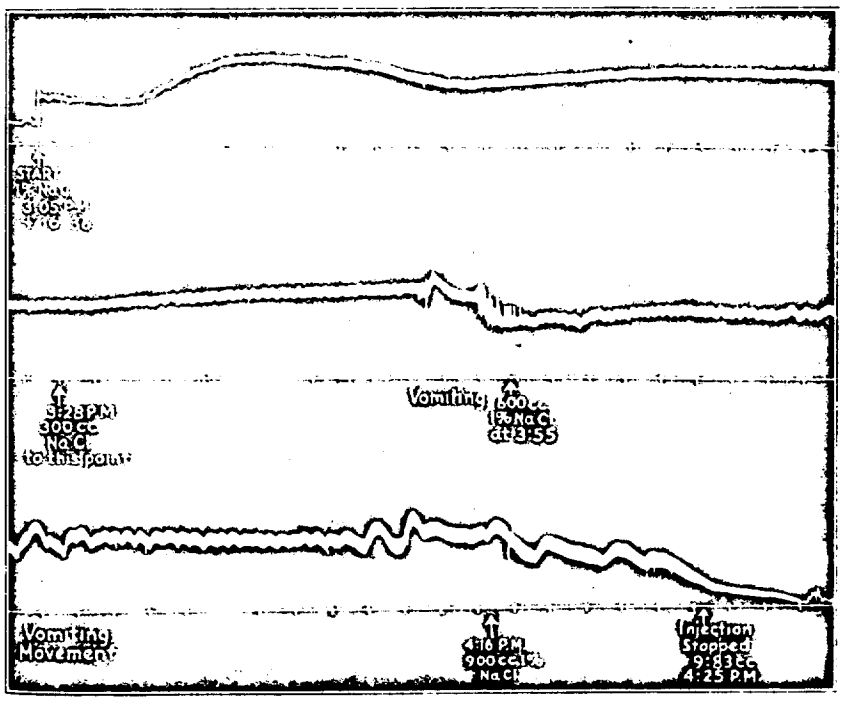

Fig. 1.—A characteristic tracing of blood pressure; the kymogram has been cut in three sections, which are reproduced one under the other. Note the relatively constant level sustained throughout a continuous massive infusion of 1 per cent solution of sodium chloride (983 cc. in a cat weighing 1.76 Kg.).

B. Gross Pathologic Observations.—It was hoped that examination of the various tissues and organs of the cats at autopsy would give some definite clue as to the cause of death after massive infusions. This hope was by no means completely fulfilled, for although definite changes were evident they were diffuse, and it was therefore impossible to attribute the fatality to the failure of any particular organ or system.

For the animals which died from the effects of massive doses of 1 per cent saline solution typical postmortem observations were as follows:

General Appearance.—The animal's abdomen was considerably distended; watery yellow fecal material was streaming from the rectum; urine was trickling from the urethra, and watery mucoid material was dripping from mouth and nose.

Peritoneal Cavity.—The peritoneal cavity contained some slightly blood-stained free watery fluid, the amount of which was estimated at 100 cc. (The staining with blood was probably the result of the action of dial on the peritoneum.)

Gastrointestinal Tract.—The walls of the stomach were thick, white and edematous; the cavity of the organ contained a small amount of bile-stained watery material. Both large and small intestine were thick, white and edematous; both were moderately filled with watery fecal material. The amount of material which the gastrointestinal tract contained was much less than one would suppose from the appearance of its various parts before incision. Most of the apparent distention which was seen from the peritoneal surface was actually due to the great thickness of the walls of the intestine.

Pancreas.—The pancreas was completely water logged, pale pink and friable; the structural elements of the organ were widely separated by gelatinous material. Altogether, the organ was several times its normal size. The cut surface did not exude much fluid unless squeezed or minced with a knife.

Kidney.—The organ showed no particular enlargement. The capsule stripped easily. Thus exposed, the organ was pale and tense and bled freely from its cut surface.

Adrenal Glands.—Except for some possible enlargement, the adrenal glands were apparently normal.

Urinary Bladder.—The bladder appeared distended, but when it was opened the appearance seemed to be due not so much to the quantity of urine it contained as to the thick, edematous condition of its walls. Such urine as it did contain was clear and colorless.

Gallbladder.—The gallbladder was dark bluish green and was distended with bile.

Liver.—Aside from some questionable enlargement, the liver presented a normal appearance.

Retroperitoneal Space and Space Between the Leaves of the Mesentery.—These spaces presented a remarkable appearance, as if widely distended with fluid. Cutting into the tissues, however, revealed that the appearance was due to a gelatinous edema rather than to an accumulation of free watery material. Little fluid escaped spontaneously from the cut surface.

Thoracic Cavity.—There was judged to be a slight excess of free fluid in the thoracic cavity. It was clear and watery in appearance, and its amount was estimated at 10 cc.

Lungs.—The dependent portions of both lungs were dark red and mottled; the upper lobes were pink and crepitant. The entire organ showed a frothy edema when cut and squeezed, and the lower lobes exuded a clear, serous fluid from the cut surface, which dripped when this part of the organ was compressed. Squeezing the entire organ caused frothy exudate to appear at the opening in the neck formed by tracheotomy.

Salivary Glands.—The salivary glands were swollen, pale and edematous.

Other Tissues and Organs.—All other organs, including the thyroid gland, the brain and the heart, appeared normal. In general, the tissues were diffusely pale, swollen and edematous, but they did not pit on pressure.

The observations on the animals which died from the infusion of 2 per cent solution of sodium chloride were superficially similar, but different in certain details. The elements of similarity included the abnormal amounts of free fluid in the abdominal cavity, the characteristic and striking gelatinous edema between the leaves of the mesentery and in the retroperitoneal space, the gelatinous edema of the pancreas and the watery edema of the stomach, intestine, and salivary glands. Among the most characteristic respects in which the latter group of animals differed from the former were the following:

1. Although the intestine was white and edematous, the small intestine was tightly contracted and beaded, the large intestine only being dilated.

2. The kidneys, though somewhat swollen, instead of being pale were dark and brownish, and the cut surface showed hemorrhagic areas about the pyramids.

3. The heart showed extreme contraction, with complete emptying of all its chambers.

4. The lungs showed much less edema, but scattered areas of punctate hemorrhage were noted.

Comment.—All in all, comparison of the postmortem observations on the animals which died from the effects of the 1 per cent solution and those on the animals which died from the effects of the 2 per cent solution tends to support the view that the physiologic mechanisms which produced death in the two groups were probably not the same. If the animals which died from the effects of less concentrated solutions succumbed to the bulk of the fluid injected, the animals which died from the effects of more concentrated solution probably succumbed to osmotic derangements.

Survival experiments in which just sublethal amounts of 1 per cent solutions of sodium chloride were administered apparently demonstrate that the pathologic changes just described are all reversible up to a sharp end point beyond which death inevitably occurs. If the infusion is stopped at any stage prior to the actual onset of death, the animal invariably recovers completely. If representative animals are killed at various intervals during the period of recovery, which lasts twenty-four hours or slightly longer, the changes in the various tissues and organs are seen to be progressively disappearing. At the end of about twenty-four hours all tissues and organs appear normal again, and no vestige of the former gross changes is to be observed.

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At the risk of anticipating confirmatory physical and chemical evidence, it is suggested that after the massive infusion of essentially isotonic saline solutions death is due to no single factor. In such cases the cause of death is therefore not to be found in changes in any particular organ or system. Death probably results, rather, from a generalized physiochemical imbalance, the exact nature of which it is at present impossible to determine. It seems likely that normally reversible changes of a diffuse nature are gradually pushed further and further when the rate of infusion greatly exceeds the rate of excretion. Ultimately the organism is more or less suddenly overwhelmed, and death comes as a widespread physiochemical collapse.

This was the opinion of Dastre and Loye,[3] who concluded that tolerance to large intravenous infusions depended primarily on the rate of injection. If it did not exceed the combined capacities of the emunctories and the mechanisms for storage, part of the injected fluid was excreted, and the rest remained in the tissues. In such a case, they declared, accommodation was complete, and the net result was nothing more than a washing of the blood, a *lavage du sang*. When, on the contrary, the rate of injection was excessive, the emunctories could no longer increase their rate of elimination to such an extent as to keep pace with it. When this occurred, accumulation in the tissues soon exceeded the capacity for physiologic storage, and ultimately mechanical effects due to the inflow of the fluid overwhelmed the animal.

Before the discussion proceeds to other matters, the subject of accidental or adventitious death should receive some special consideration. Unless certain precautions are observed and sometimes in spite of the observance of these precautions, animals may succumb as the result of much smaller intravenous infusions of entirely compatible fluids. This is likely to occur particularly when solutions are hypertonic and when rates of injection are rapid.

The most frequent cause of these accidental or adventitious deaths is vomiting or regurgitation under conditions in which the swallowing reflex is abolished or obtunded. Under such conditions the regurgitated material may never appear at the mouth to give a hint of what is actually happening, and the usual convulsive muscular movements of the vomiting act may be in abeyance. Death occurs, nevertheless, because of aspiration of the regurgitated material into the respiratory tract, and the animal drowns in its own secretion. Doubtless the activity of the bronchial, pulmonary and salivary secretory mechanisms under the stimulation of intravenous infusion acts as a predisposing cause of this suffocation. Perhaps also the acidity of the gastric secretion which is inspired as a result of regurgitation is important as a precipitating cause. At all events, autopsy of an animal which has died for no apparent cause

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during intravenous infusion almost invariably reveals the trachea and both bronchi full of frothy mucous and gastric secretion. Rarely frothy exudate appears at the nose and mouth before death; it can almost invariably be expressed mechanically after death by squeezing the exposed lungs. Two facts sufficiently confirm this statement of the cause of death: 1. If preliminary tracheal cannulation is performed death does not occur, in spite of the fact that such a procedure presumably does not interfere with the tracheal, bronchial and pulmonary secretory mechanisms. In short, death does not occur as a result of pulmonary edema as such. 2. If records of blood pressure and respiration are traced in animals not subjected to preliminary tracheal cannulation, they always show that such deaths are shortly preceded by the increased respiratory excursions and the profound depression of blood pressure which are characteristic of occult vomiting.

We are convinced that this is the explanation of some of those sudden deaths which occur during infusions. Such deaths previously have been assigned to other causes; thus Hirshfeld, Hyman and Wanger [4] described under the title of "speed shock" a condition which seems to fall within this category. They stated, as a result of a series of experiments, that the condition of speed shock could be produced by introducing intravenously as little as 1 or 2 cc. of solution, provided the solutions were introduced by hypodermic syringe and needle "as rapidly as the plunger could be pressed." The reaction which they described occurred within from forty to sixty seconds and included salivation, vomiting, diarrhea, dyspnea, muscular atony or at times muscular spasm and incoagulability of the blood. It was preceded by or associated with abrupt depression of the blood pressure. "At one time or another . . . in whole or in part" they were able to produce the condition with a wide variety of substances in solution as well as with insoluble substances, though "inconsistency and inconstancy" of symptoms were the rule.

In spite of many trials we have never been able to produce this phenomenon by using essentially isotonic solutions of sodium chloride, even in 10 cc. amounts and violently forceful injections. Hypertonic solutions, however, especially after animals have already received considerable amounts of fluid, are very likely to produce the vomiting reaction which we are convinced is the basis of the phenomenon. Presumably many of the substances used by Hirshfeld and his co-workers are particularly likely to stimulate the vomiting reflex.

Incidentally, it may be of interest to note that workers like Dastre and Loye [3] who have extensively employed rabbits in their experimental

4. Hirshfeld, S.; Hyman, H. T., and Wanger, J. J.: Influence of Velocity on the Response to Intravenous Injections, Arch. Int. Med. 47:259-287 (Feb.) 1931.

work are not likely to have noted the phenomenon, for rabbits have a poorly developed vomiting mechanism. Experimenters who perform preliminary tracheotomy do not see death from this cause for the obvious reason that tracheotomized animals cannot inspire what they regurgitate. Furthermore, if the vomiting mechanism is not productive of actual regurgitation death does not ensue, and the only result is transitory hyperpnea and depression of the blood pressure, which would correspond to nonfatal "speed shock" (fig. 2).

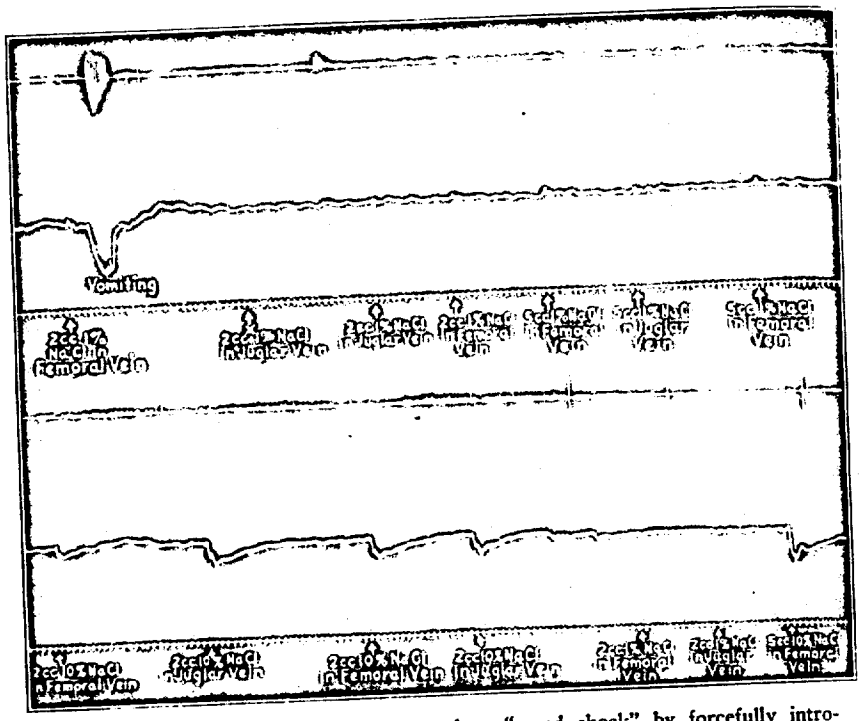

Fig. 2.—A series of attempts to produce "speed shock" by forcefully introducing small amounts of saline solution into the circulation. The very first injection (2 cc. of 1 per cent solution of sodium chloride) was followed by vomiting—extreme upper left corner. This was accidental, as indicated by the fact that repetitions of the same dose, injections of larger amounts of the same solution, and injection of small amounts of various hypertonic solutions failed to elicit the phenomenon a second time. Very hypertonic solutions (see lower panel) do produce variable momentary reductions in the blood pressure.

It is important also to avoid "pyrogenic" products in the distilled water employed in the compounding of solutions for injections and contamination of the paraphernalia used in such injections by "pyrogenic substances." The role of these substances in the production of post-

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infusion reactions has been investigated by Rademaker,[5] following the lead of Seibert.[6] Although the only precaution against the inclusion of pyrogenic substances in our solutions has been fresh single distillation of water through an ordinary laboratory still and immediate sterilization by boiling, we have only occasionally encountered effects attributable to such a factor. On several occasions, however, we have attempted the use of solutions which had previously stood in the laboratory for several days under conditions which were compatible with the development of pyrogenic substances and have encountered deaths attributable to no other cause that we could determine. The use of a freshly and accurately compounded solution has obviated all difficulty from this source. On this basis we are convinced, though admittedly on insufficient evidence, that the possible presence of pyrogenic substances in infusions given to laboratory animals cannot be overlooked with impunity.

TRANSLOCATION OF WATER AND CHLORIDES

A moment's preliminary consideration of the wide fluctuations in the intake of water to which the body is accustomed should lead one to expect a well developed and probably complex mechanism for its translocation after absorption.

It should be emphasized that ordinarily water is not rejected by the gastrointestinal tract, regardless of the quantity consumed. On the contrary, all the water taken by mouth is rather speedily absorbed into the blood stream. Exceptions to this rule occur, first, when the absorbing surface of the intestine, mainly the small intestine, is the seat of some inflammatory process which interferes with its normal function. This condition produces those diarrheas which are due to abnormal products of digestion or to the presence of bacteria and their toxins. In the second place, exceptions occur when there is a disordered blood supply to the intestine, for example, in ileus. Finally, exceptions occur rarely when huge quantities of water are ingested.

That the body is capable of absorbing large quantities of water without necessarily suffering therefrom is amply demonstrated in cases of diabetes insipidus, in which the daily absorption may amount to as much as 8 to 10 liters or more over long periods. Apparently as long as the elimination keeps reasonable pace with the intake no symptoms develop.

That the absorptive surface of the intestine does not refuse water even when the latter is presented in harmfully large quantities has been

5. Rademaker, L.: The Cause and Elimination of Reactions After Intravenous Infusions, Ann. Surg. **92**:195-201, 1930; Reactions After Intravenous Infusions: A Further Study on Their Elimination, Surg., Gynec. & Obst. **56**:956-958, 1933.

6. Seibert, F. B.: Fever-Producing Substances Found in Some Distilled Waters, Am. J. Physiol. **67**:90-104, 1923.

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observed by a number of investigators, Priestley,[7] MacCallum and Benson,[8] Miller and Williams[9] and Amberg and Austin.[10]

Rowntree[11] first duplicated the symptoms of water intoxication—asthenia, muscular irritability, convulsions, salivation and ultimately death—in a series of animals by administering large quantities of distilled water by mouth. He was able to relieve the symptoms both by administering saline solution and by trephining the skull. From this he concluded that the cause of death was edema, especially edema of the brain.

Helwig, Schutz and Curry[12] recently reported a clinical case in which they believed the death of a 50 year old woman was caused by absorption of some 9,000 cc. of tap water from the bowel within the first thirty hours after an operation for gallstones. In a series of 7 healthy young rabbits these authors were able to produce water intoxication and death by the repeated administration of 50 cc. of tap water by rectal catheter at intervals of thirty minutes. The first indication of water intoxication was the appearance of salivation after about 500 cc. of water had been given. This was followed by fine fibrillary twitchings of the ear and muscles of the extremities after the administration of about 600 cc. After about 700 cc. had been administered clonic convulsions and marked opisthotonos developed; these signs were associated with dilatation of the pupils and decreased urinary output. Death ultimately occurred in a generalized convulsion. These authors believe that the cause of death in water intoxication is cerebral edema, though Smyth, Deamer and Phatak[13] in 1933 explained water intoxication on the basis of alkalosis and loss of chlorides.

Of course it is a matter of everyday observation that increasing the intake of water increases the output of urine, and it is also a truism to remark that ultimately elimination must balance intake. The point

7. Priestley, J. G.: The Regulation of Excretion of Water by the Kidneys, J. Physiol. **1**:304-311, 1916.

8. MacCallum, J. B., and Benson, C. C.: On the Composition of Dilute Renal Excretions, J. Biol. Chem. **6**:87-104, 1909.

9. Miller, J. L., and Williams, J. L.: The Effect on Blood Pressure and Nonprotein Nitrogen in the Blood of Excessive Fluid Intake, Am. J. M. Sc. **161**:327-334, 1921.

10. Amberg, S., and Austin, R. S., cited by Rowntree.[11]

11. Rowntree, L. G.: (*a*) Water Intoxication, Arch. Int. Med. **32**:157-174 (Aug.) 1923; (*b*) The Effect on Mammals of the Administration of Excessive Quantities of Water, J. Pharmacol. & Exper. Therap. **29**:135-159, 1926.

12. Helwig, F. C.: Schutz, C. B., and Curry, D. E.: Water Intoxication: Report of a Fatal Human Case, with Clinical, Pathologic and Experimental Studies, J. A. M. A. **104**:1569-1575 (May 4) 1935.

13. Smyth, F. S.; Deamer, W. C., and Phatak, N. M.: Studies in So-Called Water Intoxication, J. Clin. Investigation **12**:55-65, 1933.

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is that elimination certainly does not occur at equal pace with absorption under ordinary conditions but at first definitely lags behind it. Ultimately, therefore, if a balance is to result, the rate of elimination not only must equal the rate of intake but must exceed it, in order to counterbalance the initial lag. In the meantime, no less certainly, the tissues of the body must accommodate the excess of water.

That simple dilution of the blood stream is not the only mechanism involved in the storage of water is almost axiomatic. Indeed, all clinical and experimental observers agree that a portion of any considerable amount of water added to the blood stream almost immediately leaves the circulation, lodging temporarily in the fixed tissues. This is true whether the water is absorbed into the blood stream from the gastrointestinal tract or is artificially added by direct infusion of a watery solution. Clinically, such an egress of water from the blood stream to the fixed tissues of the skin and to the subcutaneous connective tissue is recognized as edema. It shows the elementary physical sign of "pitting on pressure." One would certainly surmise, even though no experimental or other evidence were available, that the skin and subcutaneous tissues are not the only tissues involved. As a matter of fact, there is much evidence that other tissues are actually involved.

It is universally agreed that after the infusion of considerable quantities of saline solution the blood volume does not long remain augmented but tends soon to return to normal. Unfortunately there exists at present no really satisfactory and accurate method of measuring the true total volume of the blood, or the true volume of either of its two constituent parts, the plasma or the cells. This is because no known method is capable of differentiating between changes due to vasomotor adjustments and changes due to transfer of fluid to or from extravascular spaces. The methods based on the use of dye are, of course, subject to gross errors, due most probably to the influence of the vasomotor mechanism on the distribution of the dye.

Simple dilution of the blood and consequent increase in its circulating volume are the first changes that follow the addition of fluid to the blood stream. Almost immediately these changes begin to be compensated by vasomotor changes tending to augment the cubic capacity of the circulatory system. As soon as dilution of the blood has occurred to any appreciable extent, however, osmotic and other relationships are undoubtedly upset. Mechanisms are then brought into play which effect a redistribution of electrolytes between the blood stream and the tissue spaces. Thus three essentially different compensatory mechanisms are operative almost immediately after an intravenous infusion has been started. To these, moreover, is very soon added a fourth, the elimination of water and salts by the kidneys and the other emunctories.

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In the absence of any accurate method for examining these several mechanisms individually, conclusions as to their interrelationships and relative importance must be somewhat conjectural. Large or even huge intravenous injections fail to produce sustained elevation of the blood pressure. Moreover, the ordinary methods of computing the total blood volume fail to show an increase for moderate additions of inorganic solutions to the blood stream. Accordingly, investigators in general believe that the escape of most artificially introduced solutions from the actual blood stream is rapid.

Thus Dastre and Loye [3] as early as 1888 computed experimentally that 75 per cent of fluids intravenously injected in large quantities passed after a short time into the tissue spaces and serous cavities of dogs and rabbits. They adduced evidence that the tissues can store with impunity amounts of such fluids equal to from one tenth to one quarter of the total body weight.

MacCallum [14] in 1904 obtained up to 15 per cent of fluids intravenously injected in large volumes into experimental animals from a cannula tied into the lumen of the intestine. He thus apparently demonstrated the importance of the intestine as a water emunctory under unusual conditions.

Bogert, Underhill and Mendel [15] in 1916 concluded as a result of experiment that the blood volume returned to normal within thirty minutes after the injection of a quantity of saline solution equal to its computed total and within so short a time as five minutes when smaller volumes were injected.

Smith and Mendel [16] in 1920 injected into rabbits an amount of fluid equal to the computed normal volume of blood and observed that the bulk of the injected fluid had left the blood stream within the first five minutes. They expressed the belief that the loss of this amount of fluid could not be explained on the basis of passage of water into the muscles or cellular tissues. It must, therefore, they stated, have been extravasated into the serous cavities, the stomach and the intestine, so far as it was not excreted in the urine.

Until much more is known about the normal movements of water and chlorides in the body, and especially about the forces concerned in the movement of water between the vascular tree and the extra-

14. MacCallum, J. B.: On the Action of Saline Purgatives in Rabbits and the Counteraction of Their Effect by Calcium, Am. J. Physiol. **10**:101-110, 1904.

15. Bogert, L. J.; Underhill, F. P., and Mendel, L. B.: The Regulation of the Blood Volume After Injections of Saline Solutions: Studies of the Permeability of Cellular Membranes, Am. J. Physiol. **41**:189-218, 1916.

16. Smith, A. H., and Mendel, L. B.: The Adjustment of Blood Volume After Injection of Isotonic Solutions of Varied Composition, Am. J. Physiol. **53**:323-344, 1920.

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vascular spaces and between the intracellular spaces and the extracellular spaces, the ultimate significance of the portion of our experimental observations about to be described will necessarily remain in some doubt.

We have, however, estimated the distribution of water and chlorides in many of the soft tissues after the administration of an amount of 1 per cent solution of sodium chloride almost, but not quite, sufficient to cause death. The injection was stopped and the animals killed at the first suggestion of the precipitous decline in blood pressure which experience had shown immediately preceded death when an infusion was fatally large. Tissues were removed for analysis thereafter, as rapidly and gently as possible in order to minimize the factor of postmortem translocation of fluids and salts. At the same time all possible precautions were taken to prevent mechanical redistribution of blood from organ to organ or mechanical draining away of blood from cut surfaces.

The water content of the tissues was determined by drying excised, accurately weighed, minced tissues to constant weight in an oven at a temperature of 125 C.

It should be emphasized at this point that the condition of the tissues and organs which were analyzed for water and salt content was not necessarily incompatible with continued life. Though the condition was that which exists just preceding death, it was nevertheless one from which full and complete recovery is possible if the infusion is stopped exactly at this point. Repeatedly animals subjected to infusions of similar or almost similar magnitudes have recovered when the infusion has been interrupted just short of a presumably fatal dose.

As Skelton[17] has shown, and as appears from table 2, the water content and the mass relative to body weight of the various tissues in man and in the cat are closely similar. Presumably, therefore, similar changes in distribution of water occur in both species after massive infusions.

We have used the figures quoted by Skelton representing the weight of each organ in percentage of the total body weight, except for the various parts of the gastrointestinal tract. In this case we have used our own figures, for we have found no values in other sources. We have derived our own figures also for the normal water content of the tissues and organs, including the several portions of the gastrointestinal tract.

Our values for the gastrointestinal tract are based on analyses of 12 control cats. From these we removed the small intestine, the large intestine and the stomach. Having weighed these organs, we computed the relative weights with respect to the total body weight and thereafter

17. Skelton, H.: Storage of Water by Various Body Tissues, Arch. Int. Med. 40:140-152 (Aug.) 1927.

determined the normal water content. In table 3 is summarized the relationship of the weights.

In table 4 are summarized the changes in water content of a number of tissues and organs after the infusion of a 1 per cent solution of sodium chloride in just sublethal amount. The figures presented in the first

TABLE 2.—*Water Content and Mass Relative to Body Weight of Various Tissues in Man and the Cat*

Organ	Percentage of Water in Organ by Weight		Weight of Organ in Percentage of Total Body Weight	
	Man	Cat	Man	Cat
Skin	72.03	68.3	18.0	13.94
Muscle	75.67	74.5	41.7	45.36
Skeleton	22.04	32.8	15.9	12.67
Brain	74.84	78.0	2.01	1.04
Liver	68.25	67.9	2.26	2.96
Heart	79.21	78.46	0.47	0.37
Lungs	78.96	76.3	0.69	0.51
Kidney	82.68	75.3	0.37	0.81
Spleen	75.77	78.2	0.18	0.28
Blood	83.00	86.7	4.90	4.46
Intestine	74.54	78.3	1.81	3.80

TABLE 3.—*Weight of the Separate Portions of the Gastrointestinal Tract Relative to the Total Body Weight in Cats*

Cat Number	Body Weight	Percentage of Body Weight		
		Stomach	Small Intestine	Large Intestine
1	2,610	1.30	3.32	0.58
3	2,590	1.08	3.87	0.55
4	2,680	1.41	4.80	0.92
5	2,900	0.97	2.90	0.71
6	3,010	1.16	4.50	0.61
7	3,150	0.99	2.86	0.64
8	2,380	1.48	3.70	0.94
9	2,480	0.82	2.86	0.55
10	2,480	1.49	4.24	0.75
11	2,540	1.05	3.45	0.67
12	2,760	0.76	2.63	0.63
13	1,860	0.83	3.44	0.73
Mean percentage of body weight		1.11	3.55	0.69
Mean percentage of body weight represented by the whole gastrointestinal tract			5.35	

* This table shows great variation in the weight of all three parts of the gastrointestinal tract, stomach, small intestine and large intestine, expressed as percentage of the total body weight. In individual cases the values vary from as much as 30 per cent below to as much as 30 per cent above the mean for the entire group.

column are our own and are therefore not identical with the values quoted in table 2, though it will be noted that the agreement is very close.

As previously stated, the figures given in table 4 represent the distribution of water after virtually maximal intravenous infusions. It is a question whether this distribution of water is characteristic after infusions of various magnitudes or only after extremely large infusions. We have no direct evidence, but comparison of our results with those of Skelton, who estimated the distribution of water among some of these

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tissues after the intravenous administration of much smaller amounts of 0.9 per cent solution of sodium chloride, shows that the order of the tissues when arranged according to their increase in water content is essentially the same for the smaller and the larger infusions. This is shown in table 5.

TABLE 4.—*Change in Water Content of Organs After Massive Infusion of 1 per Cent Solution of Sodium Chloride*

Organ	Normal Percentage of Water	Percentage of Increase in Water After Infusion
Brain	78.7	— 3.9 (decrease)*
Spleen	77.8	10.0
Skeletal muscle	75.0	18.4
Cardiac muscle	76.8	21.0
Duodenum	77.8	29.0
Liver	70.3	29.0
Kidney	76.6	43.5
Skin	57.0	57.0†
Lung	79.5	111.0
Pancreas	73.9	109.0
Salivary glands	72.0	119.0
Colon	77.0	215.0
Stomach	77.0	254.0

* Under the conditions of the experiment it is questionable whether a variation of 3.9 per cent, as in the water content of the cerebrum, is significant. Nevertheless, being in the direction of decrease rather than increase, it makes it possible to state with some confidence that the cerebral tissue does not store water.

† The value for water in the skin is uncertain; not only is there a considerable normal variation in the water content of this tissue, but its fat content introduces a sizable error. The presence of fat operates to minimize any actual increase in water content, for on drying the fat does not evaporate. It thus adds to the ultimate weight of the dried tissue even though it has been entirely passive with respect to ability to hold water. The value recorded—57 per cent—was computed on the same basis as those for the other tissues. The tissue's fat content was disregarded, all possible care being exercised not to include any gross particles of fatty tissue.

In 5 cases values for water were calculated for the same animal both before and after infusion. The increase of water averaged 49.5 per cent, but this figure was not corrected for fat content.

In 2 cases in which the fat content was excluded by preliminary extraction of the tissues with ether the increase of water amounted to 38.8 per cent. In these 2 cases, however, there were no normal values as controls for the individual animals used.

TABLE 5.—*Percentage of Increase in Water Content of Organs After Large and Small Infusions of Solution of Sodium Chloride*

After Massive Infusion (500 Cc. or More per Kg. of Body Weight)—Cutting et al		After Smaller Infusion (20 Cc. per Kg. of Body Weight)—Skelton	
Blood	102	Blood	27.7
Skin	57	Skin	4.5
Liver	29	Liver	4.3
Cardiac muscle	21	Muscle	0.0
Skeletal muscle	18		

Although the magnitude of water stored increases with the size of the infusion as one would expect, the four tissues for which data are available store water in the same relative order. This would seem to indicate that each of these particular tissues, at least, shows a characteristic capacity for storing water, which increases to relatively the same extent in each case as more and more water is supplied.

Perhaps the gastrointestinal tract is an exception to this rule. Skelton[17] observed that it increased its water content by only 1.9 per cent after the smaller infusion which he used. He did not state, however, which portion of the tract was used for actual analysis. As has been previously explained, we were guided by differences in the gross appearance of the various portions of the tract at autopsy. We accordingly determined the values for separate portions of the tract and observed that the behavior of the stomach and colon differs markedly from that of the small intestine. The stomach and colon contain or store water in large amounts, whereas the small intestine contains or stores relatively little. If Skelton's figure for the gastrointestinal tract represents the small intestine only, this fact may be cited as another instance of close correlation between our findings and his. It then offers further evidence that the various tissues show characteristic behavior in the amount of water they store or contain, regardless of the absolute amount injected.

If, on the other hand, Skelton's figures represent the average capacity for storage of the gastrointestinal tract as a whole, our figures for the stomach and the colon are very much out of line. Such an interpretation would suggest that these tissues show an increasing affinity for water as the amount of infused solution increases. If the latter hypothesis is correct, there is a strong presumption that the stomach and colon take up the added water as a step in a process of excretion into the lumen of the intestinal canal, which thus acts as a safety valve. Certainly the fact that massive infusions frequently produce copious vomiting and watery purging may be considered to lend support to such a hypothesis.

The fact that the cerebrum definitely stores no water is of particular importance in view of the opinion of some investigators that when death results from water intoxication its immediate cause is cerebral edema.

Rowntree[11a] noted that cerebral edema was an outstanding feature in experimental animals in which water intoxication had been produced. The convulsive seizures and ultimate death of such animals could be prevented, he found, by decompressing the brain by means of openings made with a trephine in the skull.

Helwig, Schutz, and Curry[12] based their opinion that the dramatic features of water intoxication were due to cerebral edema largely on microscopic changes in the tissue. These changes were confined to the lungs, liver, brain, kidneys and bowel, and the most striking alteration was an acute swelling of the brain. The cerebral changes included definite widening of the perineural and perivascular spaces; vacuolation of the intercellular stroma; vacuolation, swelling and subependymal edema of the choroid plexus; vacuolation, swelling and desquamation of the ependymal lining of the ventricles, and vacuolation of the stroma of the cerebral substance.

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Our observation that the cerebral substance after massive infusions does not increase its water content seems to demonstrate conclusively that whatever may be the cause of death after such infusions it is not cerebral edema. This can mean only that if the conclusions of the preceding authors are correct our animals did not die of water intoxication. Such a conclusion is not necessarily illogical, for saline solutions are not physiologically equivalent to water, even though the actual amounts of water and of saline solution which cause death may be practically identical. In all candor, however, the idea of cerebral edema as a cause of death under either set of circumstances does not seem particularly easy to reconcile with the Monro-Kellie doctrine, even in its most recently modified form.

Probably the most impressive feature observed relative to the water content of tissues after massive infusions is that it is without exception the glandular organs which show the greatest increase in water. All of these organs, the lungs, pancreas, salivary glands, colon and stomach, apparently are able to store and eliminate water against osmotic pressure gradients as at present understood. Nothing is more evident than that the distribution of water in the body after massive infusions of essentially isotonic saline solutions is not governed by any simple applications of the laws of osmosis and diffusion.

The amount of chloride in the blood and tissue was determined in these experiments by the method of Van Slyke as modified by Sunderman and Williams.[18] This method consists essentially of dissolving the tissues in a solution of potassium hydroxide, neutralizing this solution with nitric acid to the end point of methyl red, precpitating the chlorides with silver nitrate, digesting the mixture with strong nitric acid until all organic material has been destroyed and then titrating against potassium thiocyanate, using ferric alum as an indicator. All data on chlorides are reported in terms of grams of sodium chloride per kilogram of wet tissue.

Because the values for chloride in the tissues which we could find in the literature were unsatisfactory, we have computed all of our own values, both normal and after infusion. In table 6 are recorded the sodium chloride content of normal cat tissues, the sodium chloride content of these same tissues after massive infusions of 1 per cent solution of sodium chloride and the percentage of increase.

Of all the tissues, the skin shows the greatest variability in capacity for storing both water and sodium chloride. Its absolute value is intermediate among those for the other tissues of the body.

18. Sunderman, F. W., and Williams, P. J.: The Analysis of Chlorides in Tissues, J. Biol. Chem. **102**:279-285, 1933.

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Careful comparison of table 6 with table 4 shows that many of the tissues have a difference in capacity for storing the two substances. If the various tissues stored the infused solution as presented to them, viz., in the form of a 1 per cent solution of sodium chloride, the order in which the tissues appear in the two lists should be the same. The actual results are expressed mathematically in table 7.

TABLE 6.—*Normal Sodium Chloride Content of Cat Tissues and Content After Massive Intravenous Infusion of 1 per Cent Solution of Sodium Chloride*

Organ	Content in Gm. of Sodium Chloride per Kg. of Wet Organ		
	Normal	After Infusion	Percentage of Increase
Kidney	4.21	4.00	5.2
Liver	3.41	3.74	9.7
Lung	4.31	5.31	23.2
Duodenum	3.26	4.48	37.0
Pancreas	3.00	4.38	46.0
Skin	3.22	5.30	65.0
Muscle, cardiac	1.78	2.95	66.3
Salivary glands	2.75	4.86	76.0
Stomach	3.36	5.99	78.0
Spleen	2.51	4.86	94.0
Colon	2.69	5.58	107.0
Muscle, skeletal	1.05	2.42	130.0

TABLE 7.—*Evaluation of the Data Contained in Table 4 and Table 6 on the Assumption that Storage in the Tissues Is in the Form of a 1 per Cent Solution*

Organ	Gm. of Sodium Chloride per Kg. of Tissue		Ratio
	Theoretical	Observed	
Cerebrum	2.31	2.82	
Duodenum	4.80	4.48	1 : 1.06
Muscle, skeletal	2.60	2.42	1 : 1.08
Muscle, cardiac	3.20	2.95	1 : 1.08
Spleen	5.32	4.86	1 : 1:09
Skin	5.90	5.30	1 : 1.10
Liver	4.87	3.74	1 : 1.30
Lung	7.28	5.31	1 : 1.38
Kidney	6.00	4.00	1 : 1.50
Pancreas	6.90	4.38	1 : 1.57
Stomach	10.90	5.99	1 : 1.82
Colon	10.85	5.58	1 : 1.86
Salivary glands	11.30	4.86	1 : 2.31

As will be noted, the cerebrum acts anomalously in that it alone appears to store sodium chloride but no water. Of the remaining tissues, the duodenum, skeletal and cardiac muscles, spleen and skin store the solution most nearly in the concentration in which it is presented to them, viz., as a 1 per cent solution. Incidentally, none of these tissues are among those which take up the most water. All the other tissues store relatively far more water than sodium chloride.

ELIMINATION OF SODIUM CHLORIDE AND WATER

The effect of massive infusions of 1 per cent solution of sodium chloride is to increase the elimination of water by the water emunctories. Direct visible evidence occurs as follows: 1. Mucoid material accumulates in the tracheal cannula, showing excess secretion of the tracheal and other glands of the respiratory tract. 2. Saliva drips from the mouth, nasal secretion from the nose and tears from the eyes, showing increased activity of salivary, buccal, nasal and lacrimal glands. 3. There may be copious watery dejections from the bowels, especially if the infusion is large, showing increased secretory activity of the glands of the gastrointestinal tract. 4. Vomiting or attempted vomiting almost always occurs, probably because of stimulation by excess secretion into the stomach and upper portion of the small intestine. 5. Urine is invariably evacuated in quantity, showing evidence of increased filtration by the kidneys.

Probably the other avenues of water excretion, particularly the mechanism of sensible and insensible perspiration, are affected, though we have made no attempt to collect evidence to demonstrate such activity. Although presumably all the water emunctories contribute to the elimination of the added water of a large intravenous infusion, the actual amount eliminated by all the avenues except the kidneys is not great.

During the course of our experiments, whenever an opportunity presented itself to collect vomitus, saliva or dejections, this was done, and the volumes were measured and recorded. In a number of cases in which the volumes were sufficient, chemical analyses were made for chloride content. Such observations, however, have proved difficult to evaluate for several reasons:

1. Except for occasional cases of excessive vomiting or large dejections, the amounts of secretion which have been collected have been actually very small.

2. Animals which have neither vomited nor passed stools have recovered like other animals; though we feel sure, from autopsies performed in acute experiments on other animals, that their gastrointestinal tracts contained increased amounts of secretion during the infusion, this material must have been reabsorbed during recovery and ultimately evacuated by other channels. This phenomenon apparently shows that vomiting and defecation constitute overflow mechanisms rather than essential avenues of excretion.

3. Careful analyses of the urine eliminated during the period of recovery have shown that the kidneys eliminate virtually all the sodium

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chloride and by far the largest part of the water of a massive infusion within about twenty-four hours. Actually, within such a period the kidneys may eliminate more sodium chloride than was injected, and they usually eliminate more than three quarters of all the water.

TABLE 8.—*Rate of Early Elimination of Infused Sodium Chloride and Water (During the Period of Infusion)*

Cat Number	Size of Infusion (Cc. per Kg. of Body Weight)	Time in Min.	Water Eliminated (Expressed as Percentage of Amount Infused)	Sodium Chloride Eliminated (Expressed as Percentage of Amount Infused)
2B	432	85	27.0*	....
4B	550	172	32.06	....
5B	550	156	16.0	28.0
6B	725	157	31.8*	....
7B	411	74	25.0	32.0
8B	561	130	35.0*	....
9B†	427	58	9.0	10.0
10B†	439	42	7.0	9.0
16B	545	120	2.0	4.4
17B	690	150	15.7	20.5
18B	500	120	20.0	25.0
19B‡	500	122	10.0	14.0
20B	500	124	20.0	25.3
21B	408	122	14.0	16.7

* In these animals the values were calculated on the basis of difference in weight before and after infusion; the urine was not collected.

† Animals 9B and 10B both passed copious watery stools, but the values are based solely on the urine collected.

‡ Animal 19B evacuated 130 cc. of fluid by bowel; the figures express only the elimination by kidney.

TABLE 9.—*Rate of Elimination of Infused Sodium Chloride and Water During the First Twenty-Four Hours*

Cat Number	Size of Infusion (Cc. per Kg. of Body Weight)	Time in Hours	Water Eliminated (Expressed as Percentage of Amount Infused)	Sodium Chloride Eliminated (Expressed as Percentage of Amount Infused)
5B	550	5	32	54
		20	63	95
		24.5	78	115
7B	411	5	70	95
		24	77	108
9B	427	6	47	60
		18	73	99
10B	439	5	38	48
		17	62	72
16B	500	7	47	58
		24	78	80
21B	408	7	48	59
		24	90	100

We have made a series of careful analyses of the rates of elimination of sodium chloride and water by the kidneys, which are summarized in tables 8 and 9. In table 8 appears an account of the rate of elimination of these two substances during the course of the actual infusion.

The diuretic effect of a massive infusion is shown by the kidneys within a short time after the infusion has been started, and during the actual period of administration considerable elimination occurs. During

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this period the elimination of both salt and water is variable, averaging 19 per cent of all the water injected and 18.5 per cent of all the salt.

In table 9 is recorded an itemized account of the elimination of sodium chloride and water by the kidneys during the first twenty-four hours following massive infusions of 1 per cent solutions of sodium chloride.

Interesting in connection with the figures recorded in this table also is the variability of excretion of both water and sodium chloride from animal to animal, though it is by no means as great as the variability in the earlier period. The easiest explanation is to attribute such differences in rate of excretion to differences in the functioning of the kidneys, in which case we should seem to be dealing with another example of a test of renal function. We assured ourselves that the variability is actually due not to the functioning of the kidneys alone by plotting the half-hourly excretion of water and sodium chloride of three representative animals. Not only do the rates of excretion of both substances vary considerably from one half-hour to the next, but the variation is of the haphazard, unpredictable or scatter type, which indicates that the rate of excretion is governed by not one but several factors.

It is of some special interest that within twenty-four hours all the injected sodium chloride but only about 80 per cent of the water has been eliminated by the kidneys. Coller, Dick and Maddock[19] recently observed that when surgical patients were given moderate amounts of dextrose in saline solution intravenously for several days they showed a gain in weight. This was obviously due to a retention of water, though clinical edema did not necessarily appear. When the salt was omitted, even though the same amount of fluid as before was given, in the form of 5 per cent dextrose in distilled water, the retention disappeared, and weight was lost. Coller and his co-workers apparently made no determinations to discover whether the retention of water was associated with, or due to, a concomitant retention of sodium chloride; indeed, they failed even to discuss this matter at all. Most clinicians, in the absence of data to the contrary, probably would assume that the retention of water was secondary to the retention of chloride. The results we obtained by using massive doses of solutions of sodium chloride without dextrose seem to indicate definitely that retention of water is not dependent on retention of chloride. Indeed, the retention occurs at a time when the kidneys have excreted not only all the sodium chloride which was artificially injected but some of the salt previously present in the body. Half the animals excreted more than 100 per cent of the injected chloride within twenty-four hours.

19. Coller, F. A.; Dick, V. S., and Maddock, W. G.: Maintenance of Normal Water Exchange with Intravenous Fluids, J. A. M. A. **107**:1522-1527 (Nov. 7) 1936.

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GENERAL ACCOUNT OF THE DISTRIBUTION AND ELIMINATION OF MASSIVE INFUSIONS OF SODIUM CHLORIDE AND WATER

If it now is attempted, on the basis of the figures already derived, to account for the entire bulk of solutions known to have been infused into the animals, the results are at first rather surprising.

The more bulky tissues of the body, muscles, skin, intestine, liver and skeleton, together account for about 80 per cent of the total weight of the cat, as can readily be computed from table 2. It may reasonably be assumed that the skeleton, which accounts for only about 13 per

TABLE 10.—*Storage of Water and Sodium Chloride in Muscles, Skin and Gastrointestinal Tract After Massive Infusions* *

	Muscle		Skin		Gastrointestinal Tract		Liver		Total	
Cat No.	Sodium Chloride	Water	Sodium Chloride	Water	Sodium Chloride	Water	Sodium Chloride	Water	Sodium Chloride	Water
2B	....	13.8	....	4.7	....	...	....	....	.....	.....
4B	14.4	10.8	11.3	14.0	2.56	7.6	....	....	.....	.....
6B	8.4	7.1	4.0	4.4	1.22	8.7	....	....	.....	.....
8B	7.3	11.3	7.9	11.0	1.44	6.9	....	....	.....	.....
16B	15.2	10.0	....	11.7	1.24	9.7	....	....	.....	.....
17B	7.6	7.2	5.3	4.4	1.46	5.5	....	....	.....	.....
Mean	10.57	10.03	7.12	8.56	1.58	7.7	1.14†	0.15†	20.41	20.24

* All values are expressed in percentage of the total amount infused.
† Derived from previous tables.

TABLE 11.—*The Abdominal Cavity as a Reservoir for Excess Sodium Chloride and Water*

Cat Number	Quantity Infused, Cc. per Kg.	Quantity of Abdominal Fluid at End of Infusion	Sodium Chloride Content, %	Percentage of Total Infused	
				Sodium Chloride	Water
1B	500	160 cc.	.....	.....	9.0
2B	492	132 cc.	.....	.....	13.4
3B	692	110 cc.	1.000	12.20	12.2
4B	550	130 cc.	0.950	7.50	7.8
6B	725	160 cc.	0.710	9.00	13.7
8B	561	140 cc.	0.835	7.90	9.5
17B	600	115 cc.	0.780	9.06	12.7

cent of the total body weight, is incapable of storing infused solutions because of its mechanical inexpansibility. On this assumption it appears that 80 per cent of the tissues of the body account for only about 20 per cent of the infused sodium chloride and only about 26 per cent of the infused water. The derivation of these values is shown in detail in table 10.

The only other tissue which might contribute any particular addition would be the blood, but it normally accounts for little more of the total body weight than the liver. Unfortunately, the ultimate volume after infusion being unknown, its contribution cannot be computed. However, actually the value would be negligible, for the simple reason that the figures already derived include the blood content of so great a percentage of the total mass of the body.

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There are, of course, other possible storage places besides the fixed tissues, viz. the body cavities. That the abdominal cavity is an important storage place is indicated by the figures recorded in table 11. The thoracic cavity and the pericardial cavity contain only relatively insignificant amounts of fluid.

In the general accounting, of course, must be included the sodium chloride and water that have already been eliminated from the body by the kidneys. When all this has been done the balance sheet appears as follows:

	Sodium Chloride	Water
Percentage contained in 80 per cent of the fixed tissues of the body	19.27	26.09
Percentage contained in the abdominal cavity	9.31	11.20
Percentage excreted by the kidneys	20.30	15.10
Total	48.88	52.19

Half of the injected fluid and salt is all that can be accounted for. The remaining 50 per cent must either have been stored in the 20 per cent of fixed tissues not subjected to analysis or have accumulated in the various body cavities other than the abdominal cavity. As previously stated the gross examination of such cavities as the intestinal canal and thoracic and pericardial cavities indicates that not much of the remaining 50 per cent of the infusion could have found lodgment in these locations. The capacity of the unanalyzed 20 per cent of the tissues of the body may be much greater than at first seems logical to suppose. The distensible connective tissues about the pancreas, between the leaves of the mesentery and in the retroperitoneal space have been reported as presenting a remarkable appearance due to gelatinous edema. Doubtless other loose, areolar connective tissues throughout the body present a similar condition which is not so evident to the naked eye. Thus the subcutaneous tissues are extensive enough to contain much of the injected fluid and sodium chloride.

It is suggested that as a result of infusion the extravascular tissue spaces are distended with water and its dissolved sodium chloride. In that case this, rather than the swelling of the cells of the tissue themselves, is the important factor in the increase in size and apparent edema of the various tissues and organs. Wherever the tissues are mechanically distensible a characteristic gelatinous edema develops. In these locations, the tissues look as if they had been simply distended with water in its ordinary free-flowing form, i. e., as if they had been infiltrated by a hypodermic needle and syringe. Nevertheless, incising them reveals the existence of the water in a peculiar jellylike state, the cut surface of the tissue presenting a sticky, viscid appearance. Such a gelatinous state is said to characterize tissue fluids normally, in contradistinction to the free-flowing character of, for instance, lymph.

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CONCLUSIONS

1. The lethal value for infusions of 1 per cent solution of sodium chloride in cats is of the order of 500 cc. per kilogram of body weight when the rate of injection is 5 cc. per kilogram of body weight per minute. This would correspond in a man weighing 154 pounds (70 Kg.) to 35 liters at a rate of 350 cc. per minute. Hypertonic solutions are lethal in smaller bulk and at slower rates of infusion.

2. During such massive infusions the blood pressure is neither greatly nor constantly affected. Vasodilatation and, particularly, diffusion of the solution into the tissue spaces serve to stabilize the blood pressure at an essentially normal level. At the time of death the blood pressure abruptly declines.

3. The important changes observed at autopsy are (*a*) evidences of watery vomiting and purging, (*b*) swelling of the entire animal, especially the abdomen, (*c*) the presence of a considerable amount of free fluid in the abdominal cavity, (*d*) edematous thickening of the stomach, the colon, and the urinary bladder, (*e*) gelatinous edema of the pancreas, the space between the leaves of the mesentery and the retroperitoneal space and (*f*) edema of the lungs and the salivary glands.

4. Unless pushed to a rather sharp end point of decompensation at which the blood pressure rapidly declines, infusions are not incompatible with rapid and complete recovery. The sharp end point is believed to represent some physiochemical imbalance rather than failure of any particular organ or system. Accidental death will occur in perhaps half the cases before any considerable amount of solution has been infused if precautions are not taken to prevent regurgitation, aspiration of the regurgitated material and ultimate drowning of the animal in its own vomitus. Pyrogenic substances contaminating the water used in compounding infusions may account for some premature deaths.

5. The relative weights of the various organs and tissues of the body and the water content of these tissues and organs are closely comparable as between man and the animals commonly used in the laboratory, specifically the cat. Experiments on the translocation of water following the administration of large intravenous infusions in the lower animals should therefore presumably be comparable with what occurs in man under similar conditions.

6. During and after a massive infusion both the water and the salt tend to leave the blood stream rapidly; they accumulate presumably in the tissue spaces. Relatively little water can be accommodated in such closely knit organs as the muscles and the skin, though because of their bulk their actual capacity for storage is considerable. Presumably the salt contained in the water thus stored is in essentially isotonic concentration. Organs such as the alimentary tract (except the duodenum),

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the salivary glands and the lungs, which act to some extent as natural water emunctories, seem to store water differently. They probably do not store it passively in their tissue spaces as in the case of the organs previously mentioned, but actively within their parenchymatous cells, where they are preparing it for excretion. Accordingly, analyses show that duodenum, muscle, spleen and skin store sodium chloride and water in the proper proportions to make a 1 per cent solution. Liver, lungs, kidneys, pancreas, stomach, colon, and salivary glands, all organs concerned with the elimination of water, store water considerably in excess of the amount necessary to make a 1 per cent solution with their contained sodium chloride.

7. The cerebrum is the one tissue in the body which stores no water, though it seems to store some salt. Cerebral edema definitely does not occur after massive infusions of 1 per cent solution of sodium chloride and therefore cannot be the cause of death when the limits of toleration are exceeded.

8. Even during the course of the actual infusion of a massive quantity of 1 per cent solution of sodium chloride the kidneys eliminate a considerable amount of both the water and the salt. Before the infusion is complete nearly 20 per cent of both the water and the salt has been eliminated by the kidneys.

9. The amount of salt and water stored in tissues, amounting to approximately 80 per cent of the total body weight, for which analyses were available and the amount eliminated by the kidneys during the infusion equal about half of the total amount infused. The remaining half must have been stored in tissues constituting not more than 20 per cent of the total body weight. The most logical conclusion, in view of the spectacular gelatinous edema which was seen in the loose areolar connective tissues about the pancreas and in the retroperitoneal space, is that enormous quantities of water and salt are probably stored in these and other loose, areolar connective tissues throughout the body. These tissues, for obvious reasons, were not collected for analysis.

10. Within twenty-four hours after the administration of a just sublethal massive infusion of 1 per cent solution of sodium chloride all of the salt and about 80 per cent of the water have been eliminated by the kidneys. The rate of elimination is variable from hour to hour.

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CAUSE OF DEATH RESULTING FROM MASSIVE INFUSIONS OF ISOTONIC SOLUTIONS

AN EXPERIMENTAL STUDY

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The therapeutic status of intravenous infusion has undergone an important revision in the last few years. Just as the discovery of blood groups and their interrelations early in the present century made possible the extensive use of blood transfusions, so, more recently, has the discovery of the so-called "pyrogenic factor" in the causation of "infusion reactions" and of methods for its elimination made feasible the extensive use of intravenous infusions.

Prior to about ten years ago it was commonly held by well informed practicing physicians that intravenous injections were essentially dangerous because they always involved the risk of disrupting such compensatory mechanisms as those which maintain the acid-base balance, the osmotic tension and the viscosity of the blood. These mechanisms were conceived to be delicately balanced and easily upset. Accordingly, solutions for intravenous use were carefully compounded from "triple-distilled water" and if they were to be given in any considerable quantity were also carefully "buffered."

Physiologists had long known that experimental animals survive infusions of physiologic solution of sodium chloride, even though the solution is prepared without benefit of complicated physicochemical theories, in volumes far exceeding any ever used clinically;[1] but when using these solutions clinicians encountered "infusion reactions" in man which are not recognized, or at least are encountered only in very mild forms, in the lower animals. In spite of the fact that the outstanding clinical features of such reactions were chills and fever, almost every conceivable theory of their causation was entertained except the obvious

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1. Dastre, A., and Loye, P.: Le lavage du sang, Arch. de physiol. norm. et path. 2:93-114, 1888.

one that something in certain solutions, not present in others, affected the temperature-regulating mechanism of the body. Man, possessing a more unstable temperature-regulating mechanism than other animals, would naturally be particularly susceptible to the effects of such an entity.

Not until the painstaking experimental investigations of Rademaker,[2] based on preceding observations by Seibert,[3] were published did the real source of the "infusion reaction" come to be appreciated. As a result of these investigations and others substantiating them [4] has come the installation of "solutions laboratories" in the larger hospitals. In these laboratories scrupulous care is exercised in the preparation of solutions and apparatus so that they are free from reaction-producing contaminations. Another result is the commercial production by medical supply houses of complete infusion outfits containing any of the solutions at present considered useful, together with the necessary rubber tubing and needles. These are prepared in convenient sterile packages, ready for immediate use and conforming with the highest laboratory standards.

Such developments in technic have reduced the incidence of infusion reactions so near to the vanishing point that hypodermoclysis and proctoclysis are rapidly becoming obsolete in many institutions. It has recently been felt by many physicians that this release from what amounted to a superstition, accomplished without introducing into the solutions themselves any substance except the chemically pure dextrose, sodium chloride or other simple chemical compounds and the distilled water in which they are dissolved, opens the way to new dangers which are likely to be engendered by undue confidence.[5] Because of the natural tendency to overdo any form of therapy which is relatively free from immediate danger, it seems that the time is now ripe for a more extensive laboratory appraisal than has yet been made of the basic responses to massive infusions. To assume that such simple solutions of dextrose and sodium chloride as are commonly used as vehicles are assimilated and utilized in precisely the same manner as they would be if they were absorbed from the intestinal tract and that

2. Rademaker, L.: The Cause and Elimination of Reactions After Intravenous Infusions, Ann. Surg. **92**:195-201, 1930; Reactions After Intravenous Infusions: A Further Study on Their Elimination, Surg., Gynec. & Obst. **56**:956-958, 1933.

3. Seibert, F. B.: Fever-Producing Substances Found in Some Distilled Waters, Am. J. Physiol. **67**:90-104, 1923.

4. Co Tui; McCloskey, K. L.; Schrift, M., and Yates, A. L.: A New Method of Preparing Infusion Fluids Based on the Removal of Pyrogen by Filtration, J. A. M. A. **109**:250-252 (July 24) 1937.

5. (*a*) de Takáts, G.: Push Fluids: Surgeons' Postoperative Order, Am. J. Surg. **11**:39-44, 1931. (*b*) Fantus, B.: Fluid Postoperatively: A Statistical Study, J. A. M. A. **107**:14-17 (July 4) 1936.

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the reactions of the circulation to the effects of bulk or volume can be predicted or explained on the basis of what is already known of hemodynamics, until checked by laboratory data, is distinctly gratuitous.

In our laboratory we have been performing certain simple but fundamental experiments designed to test the intermediate and ultimate fate of the simpler solutions, particularly of sodium chloride and dextrose, which are so commonly used clinically in considerable volumes for intravenous infusion.

In a previous report [6] we described some of the physiologic responses of the cat to massive infusions of 1 per cent sodium chloride solution. In the present communication we have to report some observations on the infusion of 5 per cent dextrose solution. These observations, in conjunction with previously reported and as yet unreported additional data on sodium chloride solution, seem to present a virtually complete picture of the cause of death when isotonic solutions of these two substances are injected at excessive rates and in excessive amounts.

METHOD

As in the previous series of studies, cats were used as experimental animals. The solutions were injected by gravity from an arsphenamine buret, which was ordinarily raised to such a level that the height of the column of fluid above the cannula varied from about 25 inches (63 cm.) when the tube was full to about 16 inches (40.5 cm.) when the level of the fluid had dropped to the last graduation. Additionally, however, we have on a number of occasions used a gravity system in which the height of the fluid reservoir above the animal was about 6 feet (182.8 cm.) and with which the rate of flow was adjusted to a constant volume output by a screw clamp and a glass dropping tube without any vent; this was in an attempt to stabilize the rate of delivery of fluid against minute variations by increasing the head of pressure. This modification of technic was found to offer no particular advantages over the original method, but as a check it is perhaps of some importance. Solutions were injected at approximately body temperature in all instances. Some experiments have been performed in which the temperature was accurately controlled by an electric heating device and thermometer placed just proximal to the cannula inserted into the vein of the animal. This refinement of technic, however, was found to be of little importance except as a check on the ordinary procedure of preheating the solution to a temperature sufficiently higher than that of the body to allow for the unavoidable loss of heat as the solution flows through the delivery system.

RATE AND VOLUME RELATIONS OF LETHAL DOSES OF ESSENTIALLY ISOTONIC SOLUTIONS OF SODIUM CHLORIDE AND DEXTROSE

In tables 1 and 2 are recorded representative data from experiments designed to determine the maximal rates at which prolonged infusions

6. Cutting, R. A.; Lands, A. M., and Larson, P. S.: Distribution and Excretion of Water and Chlorides After Massive Saline Infusions: An Experimental Study, Arch. Surg. 36:586-613 (April) 1938.

can be given and the total amounts which can be infused before death supervenes. Only observations obtained under the most carefully controlled conditions have been included in the tables. Although the number of cases shown in table 1 is less than the number shown in table 2, the average value is probably as reliable in the one instance as in the

TABLE 1.—*Lethal Doses of 1 Per Cent Sodium Chloride Solution*

Animal Number	Weight in Kilograms	Rate of Infusion, Cc./Kg./Min.	Total Amount Infused, Cc./Kg.	Retained Fluid, Cc./Kg. Including Peritoneal Fluid	Retained Fluid, Cc./Kg. Not Including Peritoneal Fluid
S-35	2.10	3.7	679	575	468
S-36	1.70	5.6	727	495	423
S-37	1.90	5.8	606	522	450
S-38	1.70	4.8	742	519	446
S-39	1.75	5.7	777	666	540
Mean		5.1	706.2	555.5	465.4
Mean variation			7.2 per cent	9.4 per cent	6.7 per cent

TABLE 2.—*Lethal Doses of 5 Per Cent Dextrose Solution*

Animal Number	Weight in Kilograms	Rate of Infusion, Cc./Kg./Min.	Total Amount Infused, Cc./Kg.	Retained Fluid, Cc./Kg. Including Peritoneal Fluid	Retained Fluid, Cc./Kg. Not Including Peritoneal Fluid
S-13	2.57	3.8	535	352	294
S-14	2.15	4.1	405	358	299
S-16	2.35	3.5	511	345	286
S-17	2.15	3.9	368	300	280
S-18	2.30	3.3	391	370	319
S-19	2.60	4.0	433	291	255
S-22	2.45	4.2	531	409	384
S-25	2.50	4.5	516	339	296
S-26	2.10	4.8	350	309	283
S-27	2.40	4.6	406	358	322
S-31	1.95	4.8	559	328	274
S-32	2.05	4.5	450	387	328
S-33	2.00	4.8	393	320	279
S-40	2.60	4.6	574	344	311
S-41	2.37	4.0	557	376	339
Mean		4.2	465.3	345.7	303.2
Mean variation			15.1 per cent	7.33 per cent	8.05 per cent

other, for in both instances the averages have been checked against considerably larger series of not quite so well controlled experiments.

In explanation of the recorded values, it should be emphasized that the rates called "maximal" refer to prolonged injections, not to speeds of injection which are initially tolerated or which can be maintained at odd intervals for short periods. It should be obvious that compensatory mechanisms which may be adequate during the first minutes of a massive infusion may fail as the cumulative strain of infusion during many

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minutes becomes felt, or that repeated surges of strain of short duration may fail to break down compensatory mechanisms which would succumb under continuous pressure of similar magnitude.

As will immediately be seen, it is possible to give 1 per cent saline solution considerably more rapidly and in considerably larger total amounts than 5 per cent dextrose solution. With cats, it is possible to infuse 1 per cent saline solution at rates averaging 5.1 cc. per kilogram of body weight per minute, whereas for 5 per cent dextrose solution the maximal rate of infusion averages only 4.2 cc. per kilogram per minute. Cats tolerate saline solution in total amounts up to 706 cc., whereas they tolerate dextrose solution in total amounts up to only 465 cc. per kilogram of body weight.

All the infusions represented in these two tables were carried to the actual death of the animals. The volumes of retained fluid, tabulated in the last two columns, were calculated by subtracting from the total volume of fluid infused the volume of urine which, during the infusion, drained away through a retention catheter introduced into the bladder. The mathematical difference between the figures in the last two columns for each animal represents the amount of peritoneal fluid collected from the abdominal cavity directly after death. As might reasonably be expected, the amount of retained fluid for animals given lethal doses of saline solution was considerably greater than that for animals to which the dextrose solution had been administered (in the ratio of 555 to 345 cc. per kilogram of body weight, including peritoneal fluid).

With only such values before one, it might seem logical to conclude that because the rate and volume tolerated in the case of saline solutions are so definitely greater than the rate and volume in the case of dextrose solutions, the chemical composition of the infused fluid must be the important factor in causing death. As a matter of fact, a study of the changes in blood pressure associated with massive infusions seems to indicate otherwise, at least as far as the immediate cause of death is concerned.

BLOOD PRESSURE CHANGES RESULTING FROM MASSIVE INFUSIONS

Chart 1 is a kymographic record of respiratory and arterial blood pressure responses to a lethal infusion of 5 per cent dextrose solution. In every essential respect, the tracing, which is representative for dextrose solutions, conforms to tracings obtained during infusions of 1 per cent saline solutions.[6] The respiratory portion of the kymogram shows nothing of particular importance except for a period of hyperpnea associated with vomiting, which the animal showed relatively early in the infusion, and a gradual progressive increase in the amplitude of respirations as more and more of the solution accumulated in the body

of the animal, especially (toward the end of the infusion) in the abdominal cavity; as the animal became overwhelmed, the amplitude of the respirations again diminished to the point of final apnea.

The tracing of the arterial pressure shows in the first fifteen minutes a slight increase in mean systolic pressure; this, however, did not exceed 10 mm. of mercury. With the attack of vomiting the pressure decreased in typical fashion. As the animal became overwhelmed by the infusion the pulse pressure increased progressively, whereas the mean arterial blood pressure decreased to the terminal stages of failure of the

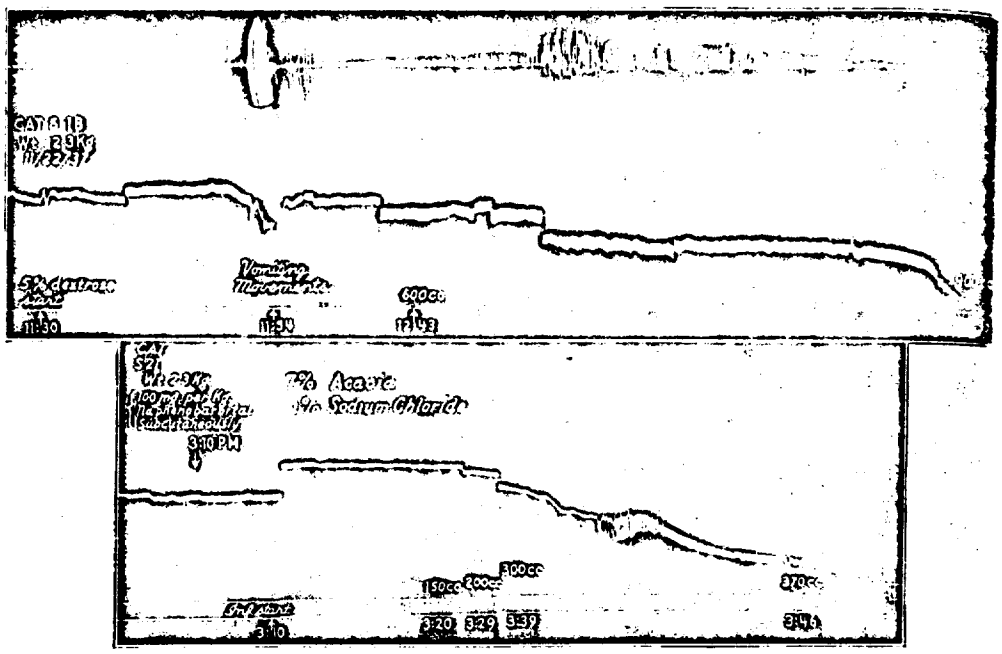

Chart 1.—The upper portion of the chart represents the response of the blood pressure of the cat to a lethal infusion of 5 per cent dextrose solution. The lower portion represents the response of the blood pressure to a lethal infusion of "gum-saline" solution (7 per cent acacia in 1 per cent solution of sodium chloride).

circulation. Though the condition is not well shown in this particular tracing, the last few minutes of infusion was characteristically associated with bradycardia.

The kymogram at the bottom of the figure represents a typical response of the arterial blood pressure to a lethal infusion of 7 per cent acacia in 1 per cent sodium chloride solution, the familiar "gum-saline" solution used clinically. Both the upper tracing and the lower one have been considerably telescoped for purposes of reproduction, but not in

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exactly the same proportion: the lower tracing, in order to be strictly comparable, should have been shortened to only a little more than one-third the length of the upper one. It takes approximately two and one-half times as much 5 per cent dextrose solution as gum-saline solution to cause death when the two are given at comparable rates, and, accordingly, an animal given 5 per cent dextrose solution survived almost three times as long as one given gum-saline solution. Except for this, however, the changes in the arterial blood pressure are similar; the early increase in mean arterial pressure is in evidence, amounting in this instance to about 36 mm. of mercury, and as the animal becomes overwhelmed by the solution the progressive decrease in mean arterial pressure, the increase in pulse pressure and the terminal bradycardia are all well shown.

The suggestion is obvious that all three solutions, physiologic sodium chloride, physiologic dextrose and physiologic acacia, when infused in massive amounts cause death by overloading the circulation. Further evidence of this is afforded by a study of responses of the circulating blood volume and by estimations of venous pressure.

EFFECT OF MASSIVE INFUSIONS ON CIRCULATING BLOOD VOLUME AND VENOUS PRESSURE

It seems highly improbable that any method now in use for the estimation of true blood volume, plasma volume or red cell volume is capable of close or accurate interpretation. This is, without doubt, particularly true under conditions which tend to change blood volume profoundly, such as severe hemorrhage or massive infusion. The reason seems to lie in the impossibility of differentiating between vascular changes due to gain or loss of fluid and those due to vasomotor or circulatory changes.[7] No special comment need be made on the well recognized fact that under changing conditions the total bulk of the blood within the entire vascular tree is capable of relatively rapid gains or losses by virtue of the passage of water and certain other substances in solution from the terminal ramification of the vessels to the extravascular tissue spaces and vice versa. The effect of vasomotor or circulatory changes is not quite so obvious. There is some evidence that at least two important mechanisms may be operative. One of these leads to the stagnation of concentrated blood in certain areas of the body. Krogh, cited by Hooper and his associates,[8] illustrated this par-

7. Lampson, P. D., and Rosenthal, S. M.: Inadequacy of Our Present Blood Volume Methods, Am. J. Physiol. **63**:358-367, 1923.

8. Hooper, C. W.; Smith, H. P.; Belt, A. E., and Whipple, G. H.: Blood Volume Studies: Experimental Control of a Dye Blood Volume Method, Am. J. Physiol. **51**:205-220, 1920.

ticular mechanism in his description of the physiologic effect of applying a small drop of ethyl carbamate (urethane) solution to a single narrow capillary and arteriole in the mucous membrane of the frog's tongue:

> While the arteriole remains narrow, the capillary dilates more and more as the blood flows into it and little or no blood flows out from the venous end, but at the same time, the concentration of the blood is visibly increased from moment to moment. The corpuscles become packed together, forming a solid clump near the venous end; this clump grows into a long column by fresh corpuscles being added to it. One is able to watch quite distinctly how the plasma disappears from the normal blood entering the capillary, while the corpuscles are piled up until, finally, the whole strongly dilated capillary is completely filled and we have the typical stasis. The entire plasma, with all its colloids, is filtered off through the capillary wall. . . . A number of other observations and experiments show that the increased permeability cannot be due to any specific action of the ethyl carbamate but is a regular accompaniment of dilatation quite irrespective of the manner in which the dilatation is brought about.[9]

The other mechanism seems to provide for local dilution of the blood in certain areas and thus is the antithesis of the mechanism just described. Illustrative of the evidence for its existence is the following: Miller and Poindexter[10] found no demonstrable increase in circulating blood volume, as measured by the dye method, following rapid intravenous administration of rather large quantities of 0.9 per cent sodium chloride solution; hematocrit readings, on the other hand, indicated an average dilution of about 20 per cent. Crandall and Roberts[11] found the same discrepancy between the two methods of determining circulating volume following injections of 5 per cent dextrose solutions. They attempted to explain this on the basis of the hypothesis that the added fluid dilutes the blood in certain vessels, particularly those in the splanchnic area, to a greater extent than is the case elsewhere in the body, for they found that if they infused 50 to 100 cc. per kilogram of body weight of isotonic dextrose or saline solution into dogs with Eck fistulas, estimations of the blood volume by the dye method showed a definite increase in 4 out of 5 animals, accounting for as much as 40 to 50 per cent of the injected fluid.

Meek and Eyster[12] have shown, by means of photomicrographs of the capillaries and venules of the ear of the dog after intravenous

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9. Krogh, A.: The Anatomy and Physiology of Capillaries, New Haven, Yale University Press, 1923.

10. Miller, J. R., and Poindexter, C. A.: The Effects Observed Following the Intravenous and Subcutaneous Administration of Fluid: An Experimental Study on Dogs, J. Lab. & Clin. Med. **18**:287-297, 1932.

11. Crandall, L. A., Jr., and Roberts, G. M.: Blood Volume After Intravenous Fluid in Normal and Eck Fistula Dogs, Am. J. Physiol. **101**:24-25, 1932.

12. Meek, W. J., and Eyster, J. A. E.: Reactions to Hemorrhage, Am. J. Physiol. **56**:1-15, 1921.

injection of acacia-saline solution, that these vessels act as reservoirs preventing any increase in the volume of the effective circulation. Thaysen [13] stated that if the erythrocytes were counted systematically over long periods in cases of polycythemia, remarkable fluctuations in the count would probably be found. He reported a case in which the erythrocyte count ran in the course of twelve hours from 5,300,000 to 10,600,000. His investigations and tests showed that the fluctuations were caused by varying contraction and dilatation of the capillaries and precapillaries of the skin.

Hooker [14] summarized the situation as follows:

> If the body has at its control such a highly organized device for the disposition and partition of the blood by which extensive capillary beds may be largely emptied of or packed with corpuscular elements or plasma, an explanation is readily found for the uncertainties of blood cell counts and blood volume determinations. Individual capillaries may be opened up or closed or they may be gorged with stagnant inactive corpuscles, as is possible to demonstrate on the finger. Mediation of these and similar changes would be accomplished by activation of the arteriole, capillary or venule functioning individually or collectively. It is natural to infer that normally such forces are well balanced and counteract one another so that the volume and corpuscular composition of the blood is held relatively stable, but in time of physiological stress or in disease the alterations which develop might assume considerable proportions.

Our experiments confirm the observation of others that although repeated estimations of the hemoglobin content during the course of a massive infusion seem to indicate that the circulating blood is undergoing considerable dilution, estimations of the blood volume according to "dye methods" give evidence that the increase in volume may be only moderate. Thus, in a typical experiment, assuming the normal circulating blood volume of the cat to be 63 cc. per kilogram of body weight (actual average for 11 animals), we found that after infusion of 200 cc. of 1 per cent saline solution per kilogram of body weight, the circulating volume as determined by the dye method was 78.7 cc. per kilogram, whereas hemoglobin dilution indicated a circulating volume of 104 cc. per kilogram.

With the reservations implied in the foregoing discussion of the value of hemoglobin dilution as a measure of circulating blood volume, the following data are presented:

Chart 2 shows simultaneous comparisons of "blood volume" estimated by the hemoglobin dilution method and estimations of venous pressure in animals given lethal infusions of 1 per cent saline solutions, and chart 3 shows similar comparisons for animals given lethal infusions of 5 per cent dextrose solutions.

13. Thaysen, T. E. H.: Polycythemia, abstracted, J. A. M. A. **75**:72 (July 3) 1920.

14. Hooker, D. R.: The Functional Activity of the Capillaries and Venules, Am. J. Physiol. **54**:30-53, 1920.

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The upper portion of each figure is a graph in which a large number of estimations of blood dilution have been plotted on the basis of changes in hemoglobin concentration of the blood. The hemoglobin dilution is plotted vertically, and the volume of infused solution per kilogram of body weight of the animal is plotted horizontally; the horizontal ordinate can, of course, be used as an approximate measure of the duration of infusion, inasmuch as rates of infusion were comparable in all cases.

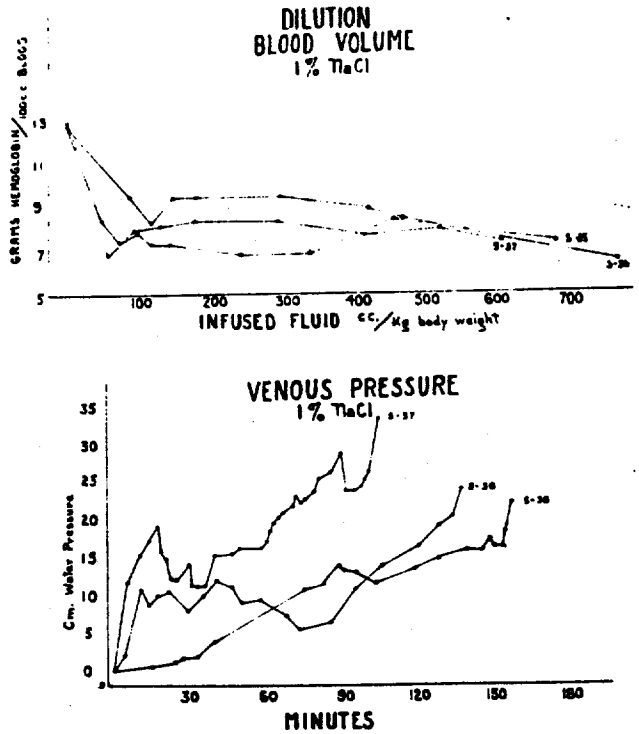

Chart 2.—The upper curve is a graph showing dilution of hemoglobin. For reasons explained in the text the hemoglobin dilution may not be taken as a direct measure of the magnitude of changes in the blood volume, but the conclusion seems unavoidable that some increase in circulating blood volume does occur as a large infusion progresses, as suggested by this curve, and explains at least in part the increase in venous pressure which is graphically shown in the lower curve. Such a view is certainly in accord with the observation of Meek and Eyster[16] that increase in stroke volume and increase in diastolic size of the heart results from intravenous infusions and our own observation (fig. 1) that increased pulse pressure without bradycardia (except terminally) occurs.

The lower graph in each of the two figures represents venous pressure in centimeters of water, as measured by a simple single-armed water manometer filled with physiologic solution of sodium chloride and connected by a short piece of rubber tubing to a cannula introduced into one of the external jugular veins of the animal. The error introduced by treating the pressure of the saline solution as pure water pressure is negligible.

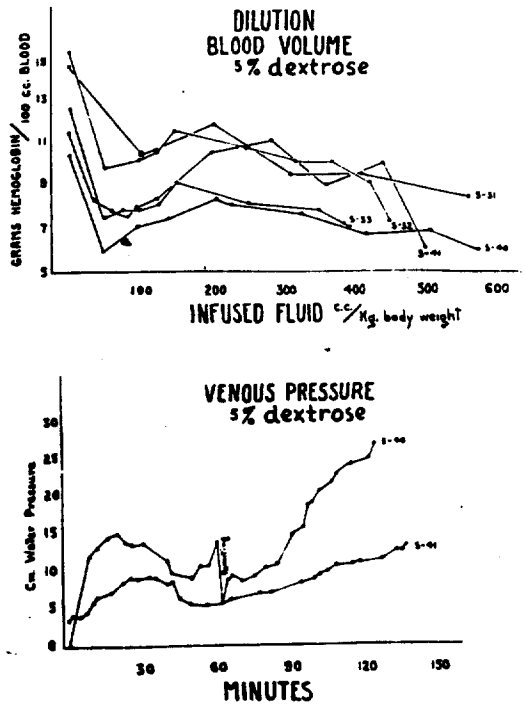

Chart 3.—The treatment and interpretation are the same as for figure 2. Dextrose instead of saline solution was used.

The most important feature of the venous pressure curves is the progressive increase as more and more fluid is infused. In most cases more than a third of the entire increase in venous pressure occurs within the first fifteen minutes of the infusion period; in other words, the curves commence with an abrupt rise.

The obvious explanation for this is that the added fluid increases the bulk of the circulating blood, and the venous blood pressure therefore rises.

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Following the initial period characterized by an increase in the venous pressure there occurs a phase of reversal which we are rather at a loss to explain. During the ensuing period of approximately thirty minutes, i. e., in the interval from fifteen to forty-five minutes, the hemoglobin concentration increases slightly, while the venous pressure declines slightly. Inasmuch as during this period the infusion was continued at the same regular rate as before, the explanation must obviously be sought either in an unusually rapid diffusion of water out of the vascular system (such that diffusion actually occurred faster than fluid was added to the circulation) or in some relative addition to the circulation of hemoglobin in the form of red blood cells, such as might occur as a result of contraction of the spleen. Of the two possible explanations the former seems the more probable, especially in view of the fact that the phenomenon occupies a considerable period, during which additional water is being rapidly poured into the circulation. Although we have no evidence to indicate that such is the case, it is possible that during this period some special storage reservoir of the body, such as the peritoneal cavity, is rapidly opened. Perhaps, on the other hand, the mechanical dilation of the smaller blood vessels is associated at a certain point with a sudden marked increase in permeability the effect of which dominates the picture for a time.

At all events, whatever this intermediate mechanism may be, it ultimately fails to provide the necessary differential, and thereafter the hemoglobin concentration progressively declines while the venous pressure progressively increases until death supervenes.

It thus appears that increasing venous pressure is the immediate cause of death. Wiggers and Katz [15] and Meek and Eyster [16] have shown that in "good hearts" a circulatory crisis of decompensation occurs when the pressure of blood in the atrium reaches 250 to 310 mm. of water. The venous pressure just before death for 2 of our 5 animals definitely fell within this range, and values for 2 of the remaining 3 at death rather closely approached it. In 1 of the 5 animals, on the other hand, the venous pressure at death was considerably less. It may be that this discrepancy was due to the fact that this animal did not have a "good heart" to begin with. At all events, Wiggers' experiments and ours are not exactly comparable. Apparently, increases in venous pressure were produced very rapidly in Wiggers' experiments, even though they were produced by infusions of saline solution, whereas in our experiments the increase in venous pressure was gradually built up

15. Wiggers, C. J., and Katz, L. N.: The Contour of the Ventricular Volume Curves Under Different Conditions, Am. J. Physiol. **58**:439-475, 1922.

16. Meek, W. J., and Eyster, J. A. E.: The Effect of Plethora and Variations in Venous Pressure on Diastolic Size and Output of the Heart, Am. J. Physiol. **61**:187-202, 1922.

during more than two hours. Thus the duration of the exposure to increased venous pressure may have weakened the hearts of certain of our animals to such a degree that they actually succumbed to a relatively low intra-atrial pressure.

We have been interested in the question whether at the time when the circulating blood volume is at its maximum the vascular system is actually dilated to its utmost physiologic capacity. That this is probably not true is suggested by the results shown in chart 4. This is a kymogram showing the effect of allowing animals to inhale amyl nitrite at intervals throughout a massive infusion of 1 per cent sodium chloride solution. The depressions in the blood pressure that consistently result from this procedure suggest that the vasodilator mechanism is still

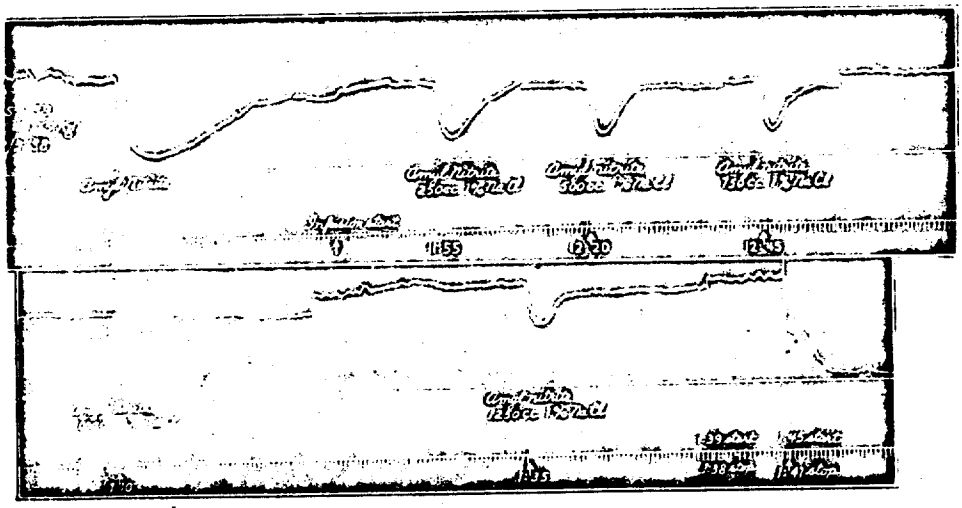

Chart 4.—Characteristic response to administration of amyl nitrite throughout a lethal infusion of 1 per cent saline solution.

active even up to about four minutes before death in an infusion which lasts nearly two hours. The drug, being in gaseous form, could not conveniently be given in measured doses, and for this reason the responses cannot be considered as quantitatively comparable. The fact, however, that all of the inhalations during the actual infusion showed responses of approximately the same magnitude somewhat suggests that vasodilator mechanisms play no more than a minor role in the accommodation of the circulatory system to increased volumes or, from another point of view, may be taken as evidence that there is little actual increase of blood volume to be accommodated.

Considering the evidence afforded by arterial pressure, venous pressure and blood volume and the apparent similarities of response evoked

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by saline, dextrose and acacia infusions, we believe there can be little doubt that the cause of death induced by massive infusions of isotonic solutions of these substances is the same in all cases. The animals die as a result of circulatory failure. Terminally the respiratory center fails first, and directly thereafter the heart undergoes complete decompensation, with the values for blood volume and venous pressure at the maximum.

GROSS OBSERVATIONS AT AUTOPSY

In general, the gross observations at autopsy on a cat dying from massive infusions of 5 per cent dextrose solution are closely similar to those shown by an animal dying from a massive infusion of 1 per cent saline solution. The following is a typical protocol recording all the features usually noted in the case of dextrose solutions.

External Examination.—There is distention of the abdomen, but little edema either of the extremities or of the body wall. No other features of interest are seen.

Peritoneal Cavity.—When the peritoneal cavity is opened it is seen to be filled with clear serous fluid, which measures 70 cc. The stomach and intestine are both moderately edematous. The former contains 15 cc. of clear yellow bile-stained fluid. The latter contains 35 cc. of clear nonmucoid, nonbile-stained fluid. The large intestine presents a normal appearance externally and on being incised is found to contain a moderate amount of formed feces covered with mucus. The liver is perhaps slightly enlarged but is otherwise normal in color and general appearance. The gallbladder is pale, contracted and virtually empty. The pancreas, the retroperitoneal space and the adjacent spaces between the leaves of the mesentery present a remarkable appearance; a gelatinous edema pervades this area and has widely permeated the pancreas, so that the latter appears broken up into a series of lobulations, the fragmented organ being very pale.

The kidneys are somewhat enlarged and pale; their capsules are under tension, and when incision is made a small amount of clear serous fluid is discharged through the incision. The cut surface of these organs is pale and drips clear, colorless fluid. The spleen is normal in size and appearance.

Thoracic Cavity.—The pleural cavities together contain 10 cc. of clear serous fluid. The surfaces of the lungs are spotted diffusely with minute petechial hemorrhages. The upper lobes are pink, crepitant and apparently normal. The lower lobes are congested, dark bluish red and definitely edematous; clear, frothy fluid can be squeezed from their cut surfaces. The chambers of the heart are collapsed, but otherwise the organ is normal in appearance; the pericardium contains no extraordinary amount of fluid.

Cranial Cavity.—The cerebrum appears normal; the convolutions are well marked, and there are no evidences of either compression or shrinkage of the organ. Only a normal amount of cerebrospinal fluid is found.

As has been stated, the variations from normal seen in these animals are similar in many respects to those seen in animals succumbing to the effects of massive infusions of saline solution. The pancreatic and retroperitoneal gelatinous edema, which is the most remarkable anatomic feature at autopsy in both cases, is, however, definitely less spectacular after dextrose infusions. In both cases there

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is considerable free colorless watery fluid in the peritoneal cavity—less after dextrose infusions, as might be expected. Pulmonary edema, present in both cases, is less marked after dextrose infusions. Gross evidence of cerebral edema is seen in neither, but most of the other tissues are pale and watery under both conditions. The characteristic differences are: (1) Buccal and nasal secretions are more copious in the case of saline infusions; (2) animals receiving such infusions show diarrhea, which is absent in the case of dextrose infusions (colonic contents are formed and almost normally inspissated); (3) the gall-bladder is distended and full after saline infusions, contracted and empty after dextrose infusions; and (4) the heart is full and dilated in the former case, empty and flabby in the latter. We have not observed in these experiments in which death was more or less suddenly produced the gross lesions described by Jacobs and Colwell [17] which apparently are seen after prolonged dextrose injections in dogs, namely, congestion of the serous membranes and meninges, hyperemia, swelling and softening of the hypophysis, pallor, enlargement and friability of the liver, congestion, gross hemorrhage and even frank gangrene of the pancreas.

The changes which we have observed apparently are not inconsistent with complete and relatively rapid return to normal, for if the infusion is stopped somewhat short of actual failure of the heart, some animals make an uneventful recovery within from twenty-four to thirty-six hours. If such animals are killed and autopsy is performed after five or six hours, most of the gross abnormalities are found to have abated. Except for the presence of some clear peritoneal fluid and a condition of the retroperitoneal tissues and the pancreas which is approximately midway between the intense edematous condition found at the end of the infusion and the normal appearance of these parts, there is little evidence of abnormality within the abdominal cavity. Within the thorax the pathologic changes of the lungs have entirely disappeared except at the extreme bases, and the heart appears normal.

At the end of about twenty-four hours the gross appearance of the contents of the abdominal and thoracic cavities may be normal.

INTRINSIC TOXICITY OF DEXTROSE

Although we believe that the foregoing is an accurate description of the cause of sudden death resulting from infusions of isotonic solutions of either dextrose or sodium chloride at rapid rates, we have reason for believing that if such infusions are interrupted just short of death the subsequent course of events may not be exactly the same with both solutions. Animals given infusions of just sublethal doses of saline

17. Jacobs, H. R., and Colwell, A. R.: Lesions in the Pancreas and in the Anterior Hypophysis with Fatal Acidosis Following Prolonged Intravenous Administration of Glucose (in Dogs), Am. J. Physiol. **116**:194-200, 1936.

solution recover without exception, and, as far as clinical indications go, their recovery is virtually complete within twenty-four hours. Such animals live indefinitely and show no complications or sequelae as far as can be clinically observed. Animals given infusions of just sublethal doses of dextrose solution, on the other hand, are quite as likely to die within a few hours as to live, and if they live they are likely to remain drowsy and depressed for a period varying from twenty-four to forty-eight hours. The depressed animals sometimes present peculiar clinical manifestations ranging all the way from a sort of general stupidity to muscular twitchings; the latter amount at times to mild tonic and clonic convulsions. In a single instance the animal's behavior was that characteristically seen after decerebration. In this case there were blindness, general muscular weakness (affecting particularly the hindlegs, though not to the extent of preventing the animal from standing) and a definite tendency to develop cataleptic postures, which were maintained for minutes at a time. Animals showing moderate depression usually recover and ultimately live indefinitely in an entirely normal condition. Those showing severe degrees of toxicity die after variable intervals.

Such convalescent peculiarities of animals infused with massive doses of 5 per cent dextrose solutions, which are never seen in animals infused with 1 per cent saline solutions, suggest that dextrose in the concentrations in which it occurs terminally as a result of such infusions is itself toxic. The terminal blood sugar levels of the animals with which we carried the infusion to actual death were determined and found to range generally above 2,000 mg. per hundred cubic centimeters of blood, the actual range for 6 animals being from 1,980 to 2,620 mg. On the other hand, in the case of survival experiments, because of the impossibility of predicting the exact moment at which death will occur it is necessary to estimate on the basis of clinical manifestations when the lethal point is approaching and stop the infusion just in advance of it. In such cases, of course, one does not have the guidance of blood pressure tracings, and the tendency is naturally to err in the direction of conservatism. As a matter of fact, estimations of the blood sugar at the point of cessation of infusion in our survival experiments show that 2,000 mg. per hundred cubic centimeters of blood has never been exceeded. From the research which has appeared to date on the subject of dextrose metabolism under conditions of artificial addition of dextrose to the circulation, it appears that dogs do not survive blood sugar levels of more than 2,000 mg. per hundred cubic centimeters for much more than two hours, for at this level disturbances of renal function appear, and toxic manifestations are shown by unanesthetized animals.[18]

18. Wierzuchowski, M.: Overflow Diabetes and Toxic Phenomena Due to the Infusion of Glucose in Normal Dogs, J. Physiol. 87:85P-86P, 1936.

CONCLUSIONS

Animals tolerate intravenous infusion of isotonic solutions of sodium chloride or dextrose in much larger quantities and at much higher rates of speed than seems to be generally appreciated. If animals (cats) are not allowed to fall victim to either of the two common varieties of accidental death, aspiration drowning following regurgitation or fatal intoxication with fever-producing contaminants of water, infusions of isotonic sodium chloride can usually be given at rates as high as 5.1 cc. per kilogram of body weight per minute without causing death or interfering with complete clinical recovery and indefinitely continued health. Isotonic dextrose solutions may be equally well tolerated at a somewhat decreased speed of injection and in a somewhat smaller total amount, but still in relatively huge doses, that is, at rates up to 4.2 cc. per kilogram of body weight per minute and in volumes up to 455 cc. per kilogram of body weight per minute.

When such total doses or rates of infusion are exceeded, death occurs from cardiac decompensation. The physiologic mechanisms of blood dilution, capillary filtration into the tissue spaces, urinary excretion and secretion into the peritoneal cavity and gastrointestinal tract act as efficient compensating mechanisms up to a certain point. Beyond this, however, the vascular load gradually produces a progressively increasing venous pressure which, when it attains a value somewhere between 150 and 250 mm. of water in the great veins draining into the right side of the heart causes acute cardiac decompensation and death. The respiratory center undergoes paralysis due to anoxia a few minutes before the terminal abrupt fall in blood pressure which signalizes cardiac failure.

Gross pathologic observations on animals dying suddenly from the effects of such massive infusions fail to show evidence of cerebral edema. Pulmonary edema, especially at the bases of the lungs, ranges from moderate to severe. The peritoneal cavity contains relatively large collections of clear serous fluid, though the pleural and pericardial cavities contain little secretion. Secretion into the gastrointestinal tract is voluminous in the case of saline solutions and relatively slight in the case of dextrose solutions. The retroperitoneal space and the pancreas show a gelatinous edema which is the most spectacular feature of the pathologic picture. None of these changes is inconsistent with complete clinical recovery of the animal and an indefinitely prolonged life thereafter; in about twenty-four hours all gross evidence of change has disappeared in animals which are allowed to survive.

Dextrose solutions infused at the concentration and at the rate reported produce a blood sugar level of some 2,000 mg. per hundred cubic centimeters. Apparently this level is a critical one, below which the

intrinsic toxicity of dextrose is not so likely to be acutely felt but above which specific lesions are rapidly produced which themselves are fatal. Because of the peculiarities of individual susceptibility there is probably about an equal chance that animals in which the blood sugar levels have been brought to such a value for short periods, though they may survive for a while, will die within a period varying from twelve to twenty-four hours. In the remaining animals, however, no change may occur which is inconsistent with complete clinical recovery. The failure of animals to tolerate as great a speed of injection or as large total quantities of dextrose as of saline solution for short periods is probably associated not so much with this phenomenon as with the fact that dextrose solutions do not undergo diffusion from the blood stream or elimination either by the kidney or the gastrointestinal tract with the same facility as do saline solutions.

Experimental Hypercholesterolemia from Chronic Excess Salt Feeding*

Am. J. of Cardiol. 8: 705-713

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THE PATHOGENESIS OF both hypertension and atherosclerosis remains unsettled; therefore, it is not surprising that the interrelationship of the two diseases is obscure. Most observers would probably agree that the diseases share no common etiology but that hypertension both hastens the onset of atherosclerosis and speeds its progress.[1]

My interest in salt intake in the etiology of human essential hypertension has been summarized in another paper in this seminar.[2] As part of the program to explore the relationship between dietary salt and hypertension, we have been studying the effects of chronic excess salt feeding in man and several species of animal for some years. Recent observations on rats and dogs indicate that such a feeding regimen is capable of elevating plasma lipids to a significant degree.[3] I am aware of no evidence which suggests that this mechanism is operative in man, or that it is involved in the pathogenesis of either human or experimental atherosclerosis. Nonetheless, in view of the widespread curiosity concerning factors which affect lipid metabolism in atherosclerosis, these observations may be of interest. Furthermore, if dietary salt is involved in the pathogenesis of hypertension[4-7] the present studies provide at least speculation on a basic link between hypertension and atherosclerosis.

EXPERIMENTAL PROCEDURE

Details are presented in the original paper on this subject.[8] Only a brief summary is included here, sufficient to clarify the general experimental procedure.

Animals and Diets: All of the rats were Sprague-Dawley females. They were started on the experimental regimen at an age of from 3 weeks to 2½ months and subsequently continued without change for periods ranging from 2 to 15½ months. Each experimental group had appropriate control animals, the basic diet of which consisted of Ralston Purina fox chow pellets without added sodium chloride: our analyses indicated that this food had 0.50 to 0.75 per cent sodium chloride. Test animals received the same chow to which one of two supplementary sodium-containing salts had been added by the manufacturer so that the final concentration calculated as sodium chloride approximated 8 per cent by weight. One of the salts was chemically pure sodium chloride, whereas the other was a mixture derived by evaporating sea water and hence called "sea salt" in this paper.† Our analyses indicated that the first of these salt-containing foods ("8 per cent NaCl food") contained an average of 8.1 per cent NaCl, whereas the other ("11.6 per cent sea salt food") contained about 7.3 per cent. The rationale of using sea salt to induce experimental hypertension is treated in another paper in this seminar.[8] All animals were housed in air-conditioned rooms, two to six animals per cage, with free access to drinking water and food. Weights and blood pressures were measured at approximately monthly intervals. Food consumption was checked over 4 day periods from time to time and, in association with parallel weight and growth curves, suggested similar amounts of food were being consumed by all groups.

The dog colony consisted of seventeen purebred beagles including sixteen females and one castrated male. Each animal was fed daily and consumed an average of 200 gm. of kibble, 100 gm. canned horse meat and 1 teaspoonful of a low sodium mineral-vitamin supplement. The five *control* animals received this diet plus tap water *ad libitum*. The four "1 per cent NaCl-H_2O" animals ate the same diet but drank 1 per cent saline *ad libitum* in place of tap water. The four "2 per cent NaCl food" animals and the four "6 per cent NaCl food" animals were fed kibble to which NaCl had been added in amounts calculated to be 2 and 6 per cent, respectively.

† I am indebted to the Trace Elements Corporation, Houston, for a supply of Admiral brand trace element sea salt sufficient to initiate these experiments.

* From The Medical Research Center, Brookhaven National Laboratory, Upton, New York. Supported by the United States Atomic Energy Commission.

TABLE I

Group I. Female Rats, Eighteen Months of Age at Sacrifice

Rat No.	Food	Months on Diet	Body Weight (gm.)	Systolic Blood Pressure (mm. Hg)	Blood Urea Nitrogen (mg. per cent)	Total Cholesterol (mg. per cent)
1	Control	17½	306	123	...	95
2	Control	17½	302	109	9.1	95
3	Control	17½	342	125	10.2	75
4	Control	17½	270	96	...	92
5	Control	17½	312	109	19.4	106
Average (and S.D.)			*300.1 (±25.7)*	*112.4 (±11.9)*	*12.9*	*92.6 (±11.2)*
6	11.6 per cent sea salt	15½	268	160 (3)*	11.1	121 (2)
7	11.6 per cent sea salt	15½	326	177 (3)	...	193 (3)
8	11.6 per cent sea salt	15½	235 (2)	157 (3)	12.7	400 (3)
9	11.6 per cent sea salt	15½	252 (2)	125	...	115 (3)
10	11.6 per cent sea salt	15½	314	159 (3)	10.3	160 (3)
11	11.6 per cent sea salt	15½	272	130	...	198 (3)
12	11.6 per cent sea salt	15½	236 (3)	166 (3)	10.8	100
13	11.6 per cent sea salt	15½	267	144 (2)	10.3	210 (3)
14	11.6 per cent sea salt	15½	332	133	...	125 (2)
15	11.6 per cent sea salt	15½	223 (3)	162 (3)	8.6	441 (3)
16	11.6 per cent sea salt	15½	243 (2)	151 (3)	17.2	380 (3)
Average (and S.D.)			*269.8 (±38.2)*	*151.3 (±16.4)*	*11.6 (±2.8)*	*222.1 (±74.8)*

Systolic blood pressures are the average of last three values, obtained at monthly intervals.

* Differs from mean value in control groups by 2 or 3 S.D., respectively. Salt-fed group had significantly ($p < 0.01$) higher mean systolic blood pressure and plasma cholesterol than control group.

FROM: DAHL, L. K. *J. Exper. Med.*, 112: 635, 1960.[3]

Analyses of these foods in our laboratory gave the following sodium concentrations, calculated as NaCl: unmodified kibble, 1 per cent; 2 per cent NaCl kibble, 2.5 per cent; 6 per cent kibble, 6 per cent; horse meat, 0.5 per cent. The animals receiving added sodium chloride began eating it at 2½ to 4½ months of age and at the time of the blood lipid and blood pressure measurements upon which this report is based, they had completed from 45 to 48 months on this regimen. The four animals in the 6 per cent NaCl food group received 2 per cent NaCl food during the first 7½ months of the experiment after which they received the 6 per cent food.

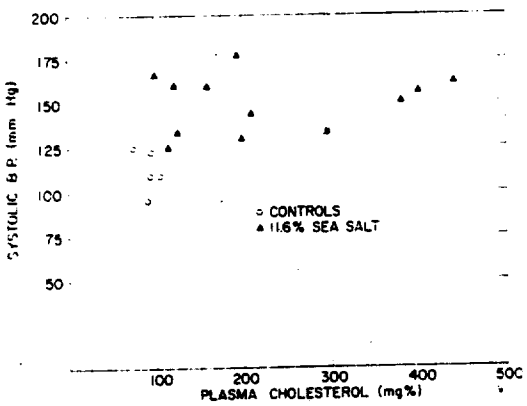

FIG. 1. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female rats. Results after 15½ months. From: DAHL, L. K. *J. Exper. Med.*, 112: 635, 1960.[3]

Blood Pressure Measurements: For both rats and dogs, the microphonic method of Friedman and Freed[9] was used with the modifications as described.[3]

Miscellaneous Methods: Blood was obtained from rats by nicking the tail, from dogs by venepuncture, and in both instances was put directly into heparinized tubes which were immediately centrifuged and the plasma removed. Cholesterol was measured by the technic of Abell et al.[10] and blood urea nitrogen by Conway's microdiffusion technic.[11] Statistical significance of the difference between means of groups was calculated by Student's test using the modern tables of Fisher and Yates.[12]

RESULTS

RATS

Group 1 (Table I, Fig. 1): The salt-fed animals had received extra salt for about 15½ months when the final measurements shown in Table

TABLE II

Group 2. Female Rats, Seven and One-Half Months of Age at Time of Bleeding for Plasma Cholesterol

Rat	Food	Months on Diet	Body Weight (gm.)	Systolic Blood Pressure (mm. Hg)	Total Cholesterol (mg. per cent)
16	Control	7	297	114	113
17	Control	7	297	124	63
18	Control	7	232	124	81
19	Control	7	268	126	81
20	Control	7	242	120	99
21	Control	7	269	126	125
22	Control	7	255	123	113
23	Control	7	292	132	90
24	Control	7	263	124	89
25	Control	7	260	137	63
26	Control	7	285	138	94
Average (and S.D.)			*269.1 (±21.8)*	*126.2 (±7.1)*	*91.9 (±19.9)*
27	11.6 per cent sea salt	6½	262	154 (3)*	125
28	11.6 per cent sea salt	6½	249	122	138 (2)
29	11.6 per cent sea salt	6½	263	180 (3)	125
30	11.6 per cent sea salt	6½	239	120	119
31	11.6 per cent sea salt	6½	283	166 (3)	113
32	11.6 per cent sea salt	6½	249	172 (3)	71
33	11.6 per cent sea salt	6½	281	162 (3)	123
34	11.6 per cent sea salt	6½	245	182 (3)	132 (2)
35	11.6 per cent sea salt	6½	263	136	106
36	11.6 per cent sea salt	6½	275	162 (3)	123
37	11.6 per cent sea salt	6½	265	170 (3)	113
38	11.6 per cent sea salt	6½	239	135	85
39	11.6 per cent sea salt	6½	271	194 (3)	143 (2)
40	11.6 per cent sea salt	6½	264	120	96
41	11.6 per cent sea salt	6½	256	130	125
Average (and S.D.)			*260.3 (±14.0)*	*153.7 (±24.6)*	*115.8 (±19.5)*
42	8 per cent NaCl	6½	277	140 (2)	109
43	8 per cent NaCl	6½	270	190 (3)	155 (3)
44	8 per cent NaCl	6½	254	145 (2)	90
45	8 per cent NaCl	6½	270	148 (3)	106
46	8 per cent NaCl	6½	258	138	113
47	8 per cent NaCl	6½	263	142 (2)	90
48	8 per cent NaCl	6½	253	150 (3)	104
49	8 per cent NaCl	6½	271	138	113
50	8 per cent NaCl	6½	247	124	99
51	8 per cent NaCl	6½	268	116	[illegible]
52	8 per cent NaCl	6½	268	134	106
53	8 per cent NaCl	6½	222 (2)	126	90
54	8 per cent NaCl	6½	245	126	99
55	8 per cent NaCl	6½	267	132	91
56	8 per cent NaCl	6½	229 (2)	153 (3)	99
Average (and S.D.)			*257.2 (±16.5)*	*140.1 (±17.3)*	*101.8 (±18.6)*

Systolic blood pressures are those for the last month of the experiment.

* Differs from mean value in control group by 2 or 3 S.D., respectively. Both salt fed groups had higher mean blood pressure (sea salt < 0.01, NaCl $< 0.05 > 0.01$) than control group. Sea salt group only had higher ($p < 0.01$) mean plasma cholesterol than control group.

FROM: DAHL, L. K. *J. Exper. Med.*, 112: 635, 1960.[3]

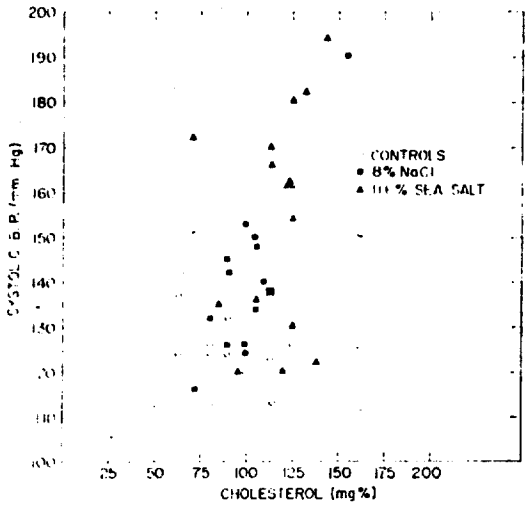

FIG. 2. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female rats. Results after $6^1/_2$ months. FROM: DAHL, L. K. *J. Exper. Med.*, 112: 635, 1960.[3]

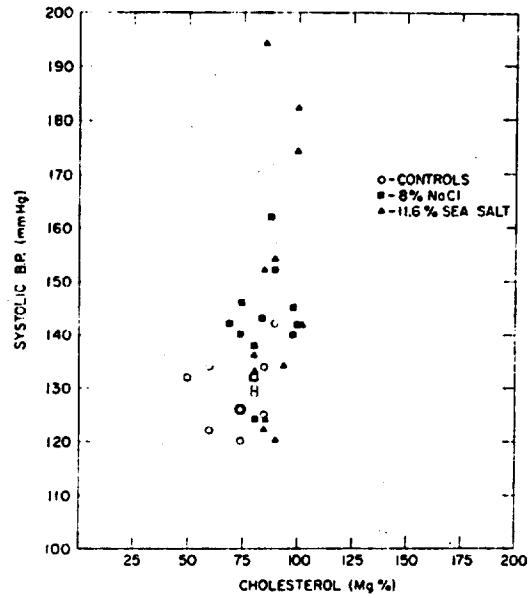

FIG. 3. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female rats. Results after 2 months. FROM: DAHL, L. K. *J. Exper. Med.*, 112: 635, 1960.[3]

I were obtained. The average blood pressure and systolic pressure were higher in the salt-fed group (p less than 0.01) than in the control group. However, in individual animals there did not appear to be a direct correlation between increase in pressure and increase in plasma cholesterol, as exemplified by rats 11 and 14 which had nearly identical blood pressures but rat 14 had more than four times the plasma cholesterol concentration of 11. Inspection of the graph (Fig. 1) shows similar if lesser disparities. Neither blood pressure nor cholesterol concentration was correlated with weight or blood urea nitrogen.

Group 2 (Table II, Fig. 2): The two test groups had been fed extra salt for about $6^1/_2$ months at the time of the determination of blood pressure and plasma cholesterol concentration shown in Table II. Among individual salt-fed animals, elevations in systolic pressure were common whereas elevations in plasma cholesterol were not. Among the sixteen rats with significantly elevated blood pressures only three also had increased plasma cholesterol; a single animal (No. 28) had normal pressure but a slightly increased plasma cholesterol (p less than 0.05). These data suggested that if salt-feeding were to result frequently in hypercholesterolemia, a period longer than 6 months was commonly required. No correlations with weight were evident.

Group 3 (Table III, Fig. 3); The test animals in this group had received extra salt for only 2 months and, in support of this deduction relative to length of time required to elevate plasma cholesterol concentration, individual significant elevations were present in only four animals, all of which were included among the thirteen salt-fed animals with elevated systolic pressures. Again, no correlations could be established with weight or blood urea nitrogen.

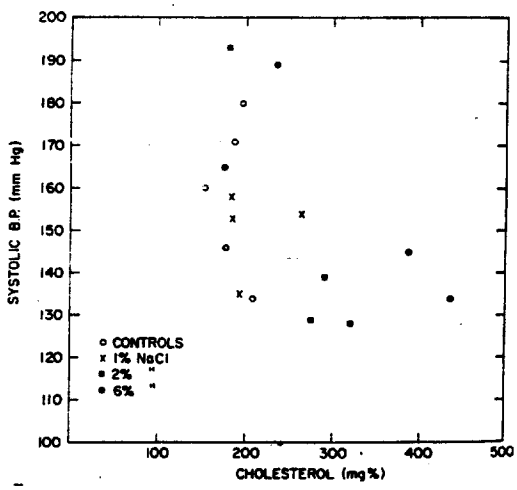

FIG. 4. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female dogs. Results after 4 years. FROM: DAHL, L. K. *J. Exper. Med.*, 112: 635, 1960.[3]

TABLE III

Group 3. Female Rats, Eleven Weeks of Age When Blood for Plasma Cholesterol Obtained and Systolic Blood Pressures Measured

Rat	Food	Body Weight (gm.)	Systolic Blood Pressure (mm. Hg)	Blood Urea Nitrogen (mg. per cent)	Total Cholesterol (mg. per cent)
57	Control	191	122	18.7	60
58	Control	225	126	17.4	74
59	Control	204	125	29.7	85
60	Control	210	126	20.9	74
61	Control	200	130	19.6	81
62	Control	215	142 (2)*	18.6	90
63	Control	209	129	...	81
64	Control	222	132	...	50
65	Control	223	120	...	74
66	Control	225	134	...	85
67	Control	235	132	...	81
68	Control	240	134	...	60
Average (and S.D.)		*216.6 (±14.4)*	*129.3 (±6.0)*	*20.8 (±4.5)*	*74.6 (±.121)*
69	11.6 per cent sea salt	210	194 (3)	13.0	85
70	11.6 per cent sea salt	181 (2)	133	21.6	81
71	11.6 per cent sea salt	218	134	18.4	94
72	11.6 per cent sea salt	201	152 (3)	20.9	85
73	11.6 per cent sea salt	214	182 (3)	23.4	100 (2)
74	11.6 per cent sea salt	215	154 (3)	17.8	90
75	11.6 per cent sea salt	189	122	...	85
76	11.6 per cent sea salt	211	174 (3)	...	100 (2)
77	11.6 per cent sea salt	205	136	...	81
78	11.6 per cent sea salt	175 (2)	120	...	90
79	11.6 per cent sea salt	201	124	...	81
80	11.6 per cent sea salt	197	142 (2)	...	101 (2)
Average (and S.D.)		*201.4 (±13.8)*	*147.3 (±24.6)*	*19.2 (±3.7)*	*89.4 (±7.7)*
81	8 per cent NaCl	190	124	19.3	81
82	8 per cent NaCl	203	152 (3)	26.8	90
83	8 per cent NaCl	233	138	19.5	81
84	8 per cent NaCl	208	143 (2)	40.4	84
85	8 per cent NaCl	204	146 (2)	41.6	74
86	8 per cent NaCl	241	132	31.0	81
87	8 per cent NaCl	220	140	...	98
88	8 per cent NaCl	213	142 (2)	...	100 (2)
89	8 per cent NaCl	212	142 (2)	...	69
90	8 per cent NaCl	179 (2)	162 (3)	...	88
91	8 per cent NaCl	225	145 (2)	...	98
92	8 per cent NaCl	215	140	...	74
Average (and S.D.)		*211.9 (±17.2)*	*142.2 (±9.4)*	*29.8 (±9.8)*	*84.8 (±10.2)*

All rats were on the diets for 2 months.

* Differs from control mean value by 2 or 3 S.D. respectively. Both salt-fed groups had higher mean blood pressures and plasma cholesterol concentrations compared with the control group: *blood pressure*, sea salt, $p < 0.05 > 0.01$; NaCl, $p < 0.01$; *cholesterol*, sea salt, $p < 0.01$; NaCl, $p < 0.05 > 0.01$. The mean *weight* of the group fed sea salt was significantly less than the control group: $p < 0.05 > 0.01$.

FROM: DAHL, L. K. *J. Exper. Med.*, 112: 635. 1960.[3]

DOGS

The control and salt-fed groups did not differ significantly in mean systolic blood pressures; furthermore, among individual members with elevated pressure, no correlation with plasma cholesterol concentration was apparent (Table IV, Fig. 4). Among the twelve animals which were fed extra salt seven had an increase in cholesterol of which all but one exceeded three standard deviations of the

TABLE IV

Seventeen Beagles Fed Diets Containing Different Amounts of NaCl for Four Years

Dog	Food	Age (mo.)	Mo. on Diet	Body Weight (kg.)	Systolic Blood Pressure (mm. Hg)	Blood Urea Nitrogen (mg. per cent)	Total Cholesterol (mg. per cent)	Phospholipids (mg. per cent)	Total Cholesterol/Phospholipid	Triglycerides (mg. per cent)
1	Control	52	50	8.5	160	17.5	153	...	...	...
2	Control	52	50	10.6	134	10.3	208	...	...	...
3*	Control	52	50	10.2	180	12.1	195	440.0	0.45	18.1
4	Control	52	50	13.9	146	9.4	177	358.0	0.49	20.4
5†	Control	41	40	9.6	171	11.6	186	...	...	...
Average (and S.D.)				*10.6 (±2.0)*	*158 (±19)*	*12.2 (±3.2)*	*184 (±21)*	...	...	...
6	6 per cent NaCl	49	45‡	7.5	165	9.3	175	...	...	...
7	6 per cent NaCl	49	45	11.8	145	12.7	386 (3)§	618.0	0.63	18.7
8	6 per cent NaCl	49	45	11.0	134	30.4	435 (3)	687.8	0.63	29.3
9	6 per cent NaCl	49	45	16.8	189	11.5	233 (2)	...	...	...
Average (and S.D.)				*11.8 (±3.8)*	*157 (±24)*	*16.0 (±8.7)*	*307 (±123)*	...	...	...
10	2 per cent NaCl	50	45	13.4	193	10.8	180	...	...	...
11	2 per cent NaCl	49	45	11.4	129	12.3	275 (3)	...	...	...
12	2 per cent NaCl	49	45	13.1	128	10.3	320 (3)	602.5	0.51	70.4
13	2 per cent NaCl	49	45	9.3	139	11.5	290 (3)	476.0	0.61	19.9
Average (and S.D.)				*11.8 (±1.9)*	*147 (±30)*	*11.2 (±0.9)*	*266 (±61)*	...	...	...
14	1 per cent NaCl	52	48	8.4	135	12.7	193	...	...	...
15	1 per cent NaCl	52	48	9.1	153	11.2	185	...	...	...
16	1 per cent NaCl	52	48	14.2	154	12.3	264 (3)	479.0	0.55	17.3
17‖	1 per cent NaCl	52	48	13.6	158	9.1	183	385.2	0.48	11.6
Average (and S.D.)				*11.3 (±3.0)*	*150 (±10)*	*11.3 (±1.6)*	*208 (±39)*	...	...	...

All dogs females except 17 (1 per cent NaCl).

* Dog 3 impregnated by No. 17 and delivered two pups at age 11 months.

† Offspring of No. 3.

‡ First 7½ months, all 6 per cent NaCl dogs received 2 per cent NaCl food, so time on 6 per cent NaCl food was 37½ months.

‖ Male, castrated at age of 9 months.

§ Differs from mean control value by 2 or 3 S.D., respectively. Blood pressures not significantly different among groups. Mean cholesterol of 2 per cent NaCl group significantly higher ($p < 0.05 > 0.01$) than control group. The large coefficient of variation (40 per cent) in 6 per cent NaCl group probably contributes to the mean cholesterol level not being significantly greater than that of control group.

FROM: DAHL, L. K. *J. Exper. Med.*, 112: 635, 1960.[3]

mean value in the control animals. In two animals from each group, plasma phospholipids and triglycerides were measured in addition to cholesterol.* These data as well as the calculated total cholesterol/phospholipid (TC/PL) ratios are shown in the last three columns of Table IV. By the same method for analysis of phospholipids, normal dogs were observed to have serum concentrations of 362 mg. per cent[13] and a TC/PL ratio of 0.41. In that study, total cholesterol was analyzed by a method[14] which yields slightly lower values than that used in our study so that it would be

* For these determinations, I am greatly indebted to Drs. Howard A. Eder and Lewis Gidez of the Department of Medicine, Albert Einstein College of Medicine.

expected, as found, that the normal TC/PL ratio derived from the present technics would be somewhat higher than 0.41. One dog (No. 12, 2 per cent NaCl) was found to have a markedly elevated triglyceride value of 70.4 mg. per cent. Although this is a limited sample, there is suggestive evidence that other plasma lipids may increase in response to the same stimulus that resulted in hypercholesterolemia.

Comments

The present observations indicate that experimentally produced chronic excess salt feeding may result in hypercholesterolemia; there is suggestive evidence that other plasma lipids may share in this response. Chronicity of feeding is clearly important: rats which had eaten extra salt for periods up to 6 months had relatively infrequent and only modest elevations, whereas rats and dogs fed added salt for more than 1 and 4 years, respectively, frequently had marked elevations in plasma cholesterol. Further confirmation has been observed in studies now being prepared for publication. In one such study, by making consecutive measurements on the same animals over a period of about 1 year it was found that after 9 or 10 months of salt feeding, 40 per cent of the members of a large colony of rats had plasma cholesterol values ranging from 150 to 516 mg. per cent.

It is of some interest that plasma lipids were not increased equally among members of two of the groups of dogs which were ingesting roughly equivalent amounts of salt, as 1 per cent NaCl drinking solution for one group and, in the other, as 6 per cent NaCl food. Animals which obtained their salt primarily as 1 per cent saline solution seemed to have less significant elevations than those which consumed it in their food. The latter animals drank about 50 per cent more water than did those drinking 1 per cent saline but whether this difference is important cannot be said at present.

The role of hypertension *per se* in the elevation of plasma lipids observed in some of these animals is not entirely clear. In the rats, separation of elevated pressure from elevated lipids is made particularly difficult because of the frequency with which salt feeding is associated with some degree of hypertension. In our experience, about four rats in five will have hypertension of mild to severe degree when they are chronically on one of the salt regimens used by us. Therefore in such a group the probability of any single animal with hypercholesterolemia also having hypertension would be high. In this case, although a high correlation would exist it would not necessarily indicate a causal relationship between the two observed phenomena; it is also possible that both were the result of a common stimulus (excess salt consumption) in which case the elevated pressure would not be the *cause* of the elevated lipids.

Fortunately, some clarification of this problem is provided by observations on the dogs. In our animals, salt-feeding did not appear to induce hypertension, presumably because of the profound capacity of dogs to excrete oral salt loads quickly.[15] Among the seven dogs with significantly elevated plasma cholesterol concentrations, only one was definitely hypertensive (No. 9, 6 per cent NaCl group) and a second was borderline (No. 16, 1 per cent NaCl group): these two animals had the lowest cholesterol levels among the seven with elevations.

Whether hypertension can be a primary cause of elevated serum lipids is not clear from a recent extensive review of the literature.[16] On the whole, it is fair to say that most of the available experimental data suggest that hypertension *per se*, probably will not *cause* an elevation in plasma lipids,[17-20] although some of the data are equivocal. Thus, Lewis, Green and Page [21] found that changes did not develop in dogs with neurogenic hypertension, whereas comparable animals with nephrogenic hypertension had elevated serum lipoproteins; the latter elevations were interpreted to be related to the kidney damage associated with the renal hypertension rather than the hypertension itself. Katz and Stamler[22] induced hypertension in cockerels by substituting 0.9 per cent saline for drinking water or by feeding up to 8 per cent NaCl in the food; they observed neither hypercholesterolemia nor enhanced atherosclerosis in the animals receiving extra salt. Meneely and his associates[23,24] observed elevated serum cholesterol levels in the rats chronically fed excess salt, but concluded that the correlation was more striking with blood pressure than with salt consumption.

Deming et al.[25] studied the effect of experimental hypertension on induced atherosclerosis in the rat. Among animals on an atherogenic regimen, there was a positive correlation between increase in blood pressure and increase

in serum cholesterol concentration. However, in evaluating the effect of salt, they found that it was "not possible to conclude from these results that salt alone has no effect on cholesterol concentration . . ." (Experiment 3). Donomae et al.[26] induced atherosclerosis in rabbits by feeding lanolin which in six instances was supplemented with added NaCl. Among these six animals were those with the highest concentration of serum cholesterol in the series of fifty-two rabbits.

At the present time no explanation is at hand for the mechanism by which hypercholesterolemia is induced with chronic salt-feeding. The possible role of the kidney is suggested by the nephrotic syndrome in man; furthermore, Meneely found that some of his salt-fed rats had a clear-cut nephrotic-like syndrome.[23,24] However, this was not observed in any of the animals in this series although we have seen it occasionally in others. Furthermore, as judged by the blood urea nitrogen concentration in our animals, significant kidney disease was not present. At the present time an extensive histologic and histochemical review of tissues from these and other rats on the same regimen is being made. Preliminary observation suggests no clear-cut correlation between pathologic changes and hypercholesterolemia, although final judgment must await conclusion of the study. In any event, the kidney cannot be ruled out at the present time.

In the absence of facts to the contrary, it is permissible to speculate on possible mechanisms. It should be clearly stated in advance that our own prejudices are against rather than for kidney damage being the cause of the hyperlipemia here. Variation in exogenous supplies of cholesterol probably need not be seriously considered since the only known variable was the added salt and this did not affect food consumption significantly. It is more intriguing to me, at least, to account for these lipid changes on the basis of enhanced intestinal absorption, increased synthesis or decreased degradation, or possibly some combination of these. In line with such an interpretation, the instillation of water or salt solution into the gastrointestinal tract is used by lymph physiologists to enhance intestinal lymphatic flow.[27,28] Theoretically it is possible that increased lymphatic flow could result in an increase in effective absorption from the gut as well as an increased hepatic lymph flow, possibly with an attending increase in synthesis of cholesterol.

From these studies it cannot be concluded, or even inferred, that chronic excess salt consumption is involved in the pathogenesis of atherosclerosis either in experimental animals or in man. In fact, in the experimental studies mentioned,[22,25,26] the authors specifically stated that there was no increase in the atherosclerosis as a result of salt-feeding. Nonetheless, since chronic excess salt consumption is capable of elevating plasma lipids, it could conceivably affect the atherosclerotic process as well. It is rather unlikely that salt consumption could play a primary role in atherosclerosis, but it might play an accessory role in an organism on an atherogenic regimen such as that of the inhabitants of most western societies. It is important to remember that slight abnormalities operating over decades can result in significant disease. Such a potential may exist here.

Summary and Conclusions

Female rats and dogs which had consumed excess salt for periods of about 1 and 4 years, respectively, frequently had increases in plasma cholesterol concentration, sometimes quite marked.

Chronicity of feeding appeared to be critical for development of the hypercholesterolemia since rats on the same regimen for only 2 or 6 months had less frequent and less marked elevations.

The evidence at hand suggests that the lipid changes were not related primarily to increased blood pressure, kidney disease, differences in food consumption or in body weight.

There is no indication that excess salt consumption is related to atherogenesis although a factor capable of elevating plasma lipids conceivably could affect the atherosclerotic process.

References

1. Katz, L. N. and Stamler, J. Experimental Atherosclerosis, p. 59. Springfield, Ill. 1953. Charles C Thomas.
2. Dahl, L. K. The possible role of chronic excess salt consumption in the pathogenesis of essential hypertension. *Am. J. Cardiol.*, 8: 571, 1961.
3. Dahl, L. K. Effects of chronic excess salt feeding. Elevation of plasma cholesterol in rats and dogs. *J. Exper. Med.*, 112: 635, 1960.
4. Dahl, L. K. and Love, R. A. Evidence for relationship between sodium (chloride) intake and human essential hypertension. *Arch. Int. Med.*, 94: 525, 1954.
5. Dahl, L. K. and Love, R. A. Etiological role of sodium chloride intake in essential hypertension in humans. *J.A.M.A.*, 164: 397, 1957.
6. Dahl, L. K. Salt intake, adrenocortical function

and hypertension. *Nature, London*, 181: 989, 1958.

7. Dahl, L. K. Salt intake and salt need. *New England J. Med.*, 258: 1152, 1205, 1958.
8. Dahl, L. K. and Heine, M. The enhanced hypertensogenic effect of sea salt over sodium chloride. *Am. J. Cardiol.*, 8: 726, 1961.
9. Friedman, M. and Freed, S. C. Microphonic manometer for the indirect determination of systolic blood pressure in the rat. *Proc. Soc. Exper. Biol. & Med*, 70: 670, 1949.
10. Abell, L. L., Levy, B. B., Brodie, B. B. and Kendall, F. E. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.*, 195: 357, 1952.
11. Conway, E. J. Microdiffusion Analysis and Volumetric Error, ed. 3 (revised), pp. 152–164. London, 1950. Crosby Lockwood and Son, Ltd.
12. Fisher, R. A. and Yates, F. Statistical Tables, ed. 3, p. 1. New York, 1948. Hafner Publishing Co., Inc.
13. Havel, H. J., Eder, H. A. and Bragdon, J. H. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.*, 34: 1345, 1955.
14. Sperry, W. M. and Webb, M. A revision of the Schoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.*, 187: 97, 1950.
15. Ladd, M. and Raisz, L. G. Response of the normal dog to dietary sodium chloride. *Am. J. Physiol.*, 159: 149, 1949.
16. Waris, E. Studies on serum lipids and lipoproteins in hypertension. *Acta med. Scandinav., suppl.* 337, 161: 79, 1958.
17. Page, I. H. The relationship of the extrinsic renal nerves to the origin of experimental hypertension. *Am. J. Physiol.*, 112: 166, 1935.
18. Dill, L. V. and Isenhour, C. E. Occurrence of atheroma in the aorta in rabbits with renal hypertension. *Arch. Path.*, 33: 655, 1942.
19. Rodbard, S. and Freed, S. C. The effect of desoxycorticosterone acetate on the blood pressure of the dog. *Endocrinology*, 30: 365, 1942.
20. Wilens, S. L. The effect of postural hypertension on the development of atheromatosis in rabbits fed cholesterol. *Am. J. Path.*, 19: 293, 1943.
21. Lewis, L. A., Green, A. A. and Page, I. H. Ultracentrifuge lipoprotein pattern of serum of normal, hypertensive and hypothyroid animals. *Am. J. Physiol.*, 171: 391, 1952.
22. Stamler, J. and Katz, L. N. The effect of "salt" hypertension on atherosclerosis in chicks fed mash without a cholesterol supplement. *Circulation*, 3: 859, 1951.
23. Meneely, G. R., Tucker, R. G., Darby, W. J. and Auerbach, S. Chronic sodium chloride toxicity in the albino rat. II. Occurrence of hypertension and of a syndrome of edema and renal failure. *J. Exper. Med.*, 98: 71, 1953.
24. Meneely, G. R. and Ball, C. O. T. Experimental epidemiology of chronic sodium chloride toxicity and the protective effect of potassium chloride. *Am. J. Med.*, 25: 713, 1958.
25. Deming, Q. B., Mosbach, E. H., Bevans, M., Daly, M. M., Abell, L. L., Martin, E., Brum, L. M., Halpern, E. and Kaplan, R. Blood pressure, cholesterol content of serum and tissues, and atherogenesis in the rat. *J. Exper. Med.*, 107: 581, 1958.
26. Donomae, I., Matsumoto, Y., Kokubu, T. and Koide, R. Production of coronary heart disease in the rabbit by lanolin feeding. *Circulation Res.*, 5: 645, 1957.
27. Watkins, A. L. and Fulton, M. N. The effect of fluids given intraperitoneally, intravenously and by mouth on the volume of thoracic duct lymph in dogs. *Am. J. Physiol.*, 122: 281, 1938.
28. Simmonds, W. J. The effect of fluid, electrolyte and food intake on thoracic duct lymph flow in unanesthetized rats. *Australian J. Exper. Biol. Med. Sc.*, 32: 295 1954.

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Guest Editorial

Salt in Processed Baby Foods[1,2]

L. K. DAHL, M.D.

IN A PAPER elsewhere in this issue (1), Dr. Helen Guthrie reports that among 89 infants studied, solid foods were consumed by 35 within 3 weeks, and by 82 within 7 weeks, from birth. Dr. Guthrie is worried that the high sodium content of these baby foods may be a predisposing factor to hypertension. Such concern was also expressed recently by Puyau and Hampton (2) and by Fomon (3).

The subject of salt, as an etiological factor in hypertension, has been my main scientific interest for nearly two decades. It was only natural, therefore, that I should have had the same concern for some years (4). It seems particularly timely to explore this matter now, since there is increasing awareness of the baneful effects that may develop from chronic exposure to apparently benign environmental influences.

There can be no question about the high concentrations of NaCl in processed baby foods; except for fruits, they contain concentrations of NaCl, added by the processors, that range from about 5 to more than 100 times that of the original natural products (4–7). I shall examine this practice from the general standpoints of whether it is necessary, possibly beneficial, or possibly harmful. Arbitrarily, the infants of reference are so-called "normal" ones, less than 1 year of age. Specifically, they are not premature, have no overt evidence of cardiovascular-renal disease, and have no unusual NaCl losses from the skin or gastrointestinal tract. Finally, although the chloride ion is not unimportant, it is only the sodium ion that I will discuss. When the terms sodium or salt are used, sodium chloride is implied.

SALT NEEDS

The primary purposes of foodstuffs are to provide *a*) building material for new, or repairing old, tissues, and *b*) energy. I know of no method by which the precise NaCl requirements may be calculated for the body's energetics, but neither do I see an immediate reason to develop such a method; sufficient NaCl is available when growth and repair are provided for. Daily NaCl requirements for the infant under discussion appear to be remarkably small. There is probably no more convincing indicator of the low requirement for NaCl than the fact that human milk, a low sodium food, obviously satisfied infants' needs for millenia. It seems preposterous to dismiss as "unscientific" a feeding practice that led to the survival of uncounted generations under a remarkable variety of environmental conditions. Furthermore, the continued use of breast feeding in this, and other similar modern societies, confirms the earlier experience of the human race. Several years ago we analyzed 124 samples of milk taken on different days from 26 healthy nursing North American mothers whose salt intakes were not controlled. We found a median concentration

[1] From the Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973.
[2] Supported by the United States Atomic Energy Commission.

of 7 mEq Na/liter, with only one sample below 2, and only four above 20 mEq/liter; these figures agree with those from other series (5, 8). Therefore, when human milk is used as the sole source of food and fluid, the total daily intake of sodium will rarely be more than 5–10 mEq but often substantially less.

McCance and Widdowson (9) estimated that a 3.5-kg infant would contain 243 mEq Na and a 70-kg man, 5,150, a net gain of approximately 4,900 mEq from infancy to adulthood or, in round terms, about 75 mEq Na for each kilogram of new tissue. If it were assumed that growth ceased at age 16, the average daily requirement for growth alone would be less than 1 mEq. Even assuming a doubling of the birth weight at 5 months and a tripling at 12, the growth rate would be readily satisfied by 1.3–1.8 mEq/day.

Other requirements for sodium are imposed by obligatory losses from skin, urine, and feces. Cooke, Pratt, and Darrow (10) reported that the concentrations of sodium in the sweat of three normal babies, aged 5–16 months, ranged from 1.8 to 7.6 mEq/liter with an average of 4.8; at an environmental temperature of 91 F, the calculated sweat volume was 45–65 ml/kg body wt per day. Assuming a constant ambient temperature of 91 F and a maximal sweating rate, a 5-month-old, 7-kg infant would lose an average of 2.2 mEq/Na per day at this rate. Under more temperate conditions the loss would probably approximate 1 mEq/day. Gamble et al. (11) found sodium content of stool to be small in two healthy infants about 2 and 4 months of age. Despite wide variations in their electrolyte intake, daily stool losses ranged from 0.2 to 1.7 mEq. A daily allowance of 1–2 mEq for stool loss is probably ample and is in line with adult figures. I have been unable to find data on the minimal urinary sodium loss of infants after prolonged sodium restriction. The remarkable capacity of the adult kidney to conserve sodium is well known; in our experience (12), 5 adults with good renal function excreted only 0.21 to 1.52 mEq/day in the urine on prolonged daily sodium intakes that averaged 2.17 mEq. Equal effectiveness of the infant kidney is indicated by the fact that breast-fed infants do not develop sodium deficiency.

In sum then, the foregoing reasoning suggests that, during the 1st year of life, requirements for losses plus growth would be generously met by daily intakes of 4–8 mEq. These calculations are superfluous since breast milk is a demonstrably adequate source of sodium for normal infants.

SALT INTAKES

How do these low sodium requirements during the 1st year of life compare with actual intakes in our society? The average infant on only breast milk, consuming 150–200 ml/kg per day (3), would have an intake of about 1–1.4 mEq/kg. Feeding such an infant undiluted cow's milk would increase its sodium intake three- to fourfold (8). The addition of strained foods, to which salt was not added by the processor, would supplement the sodium intake approximately as follows (mEq Na/100 g food): fresh fruits, 0.1 mEq (apples, apricots, bananas, cherries, grapefruit, oranges, peaches, pears, plums); fresh vegetables (except carrots, 1.3 mEq), 0.1–0.6 mEq (green beans, lima beans, peas, white potatoes, squash, tomatoes); dry cereals, 0.1 mEq (Cream-of-Wheat, oat meal, Wheatena); meats (cooked), 2–4 mEq (beef, chicken, lamb, liver, pork, veal); eggs, 5–6 mEq (5, 13, 14). Consumed in the usual permutations, these foods would contribute to the sodium intake only small amounts of NaCl, namely of the order of 1 mEq Na/kg body wt, or less. This would be true even if several hundred grams of these foods were eaten every day.

With the exception of fruits, the processed baby foods widely distributed to the

American markets contain concentrations of sodium far in excess of those cited above for the natural product (4–7). Furthermore, the processors add water, in amounts that may sometimes exceed 50%, and often also starch. The finished product, except for dry cereals, is thus a diluted one. To such attenuated mixtures, the processors add NaCl in quantities that may exceed 1% of the final product. This percentage would be significantly higher if calculated on the basis used for the unsalted foods (above), i.e., relative to the undiluted "solids" present. In our analyses (4) of 16 different samples of strained meats, the sodium content ranged from 293 to 510 mg (12.7 to 22.2 mEq)/100 g contents with an average of 359 mg (15.6 mEq); among 14 samples of vegetables and miscellaneous mixtures, sodium ranged from 144 to 357 mg/100 g contents (6.2 to 15.5 mEq), with an average of 244 mg (16.6 mEq). These figures are similar to those compiled by the Department of Agriculture (5). We did not analyze cereals processed for baby foods but the data from the Department of Agriculture analyses (5) indicate even higher average concentrations of sodium, i.e., 421–653 mg (28–35 mEq)/100 g dry cereal. The sodium content of processed baby foods, therefore, exceeds that of natural, unsalted foods by factors of about 5–6 for meats, 6–60 for vegetables, and in excess of 100 for dry cereals. Calculated on the basis of equivalent solids, the disparity would be actually greater for those processed meats and vegetables to which water and starch had been added.

In sum, a hypothetical 5-kg breast-fed infant consuming a maximum of 200 ml milk/kg and one jar containing 100 g unsalted mixed vegetables and meat in equal proportions, would have an average daily sodium intake of not more than 1.8 mEq/kg body wt and, often, much less. Changing the foregoing by substituting cow's milk for breast milk only, would roughly increase this to about 4.4–6 mEq/kg. The use of both cow's milk and processed vegetables and meat would yield an intake of about 7–8 mEq/kg. The actual intake could be substantially higher, however, if the heavily salted cereals were also consumed. This practice is common in current infant nutrition. These high calculated intakes are not merely theoretical, as is shown by the data of Puyau and Hampton (2), referred to by Dr. Guthrie: one 3-month old child was estimated to have a daily sodium intake of 10.9 mEq/kg body wt, namely between 500 and 1,000% in excess of probable requirements.

IS THERE POSSIBLE BENEFIT FROM ADDING SALT TO BABY FOODS?

There is no metabolic need for the high sodium diets current in American infants. Yet, is there some subtle benefit from intakes that grossly exceed requirement? There is no evidence to suggest this. Unlike body stores of fat, which can be increased or decreased drastically, total body sodium is normally maintained within very narrow limits. These can be expanded or contracted only slightly before the emergence of trouble, often serious trouble. A high sodium intake, therefore, does not provide a sodium reservoir upon which the organism can draw in time of need. During need, the supply must come from exogenous sources, whatever the level of salt intake might be prior to need.

On one occasion it was proposed that a high intake of salt was somehow necessary and beneficial in that salt stimulated the adrenal cortex. This was supposed to set in motion adaptive mechanisms that made the individual more responsive to the complex demands of our modern society (15). I have dealt with this possibility in some detail elsewhere (16–19). It will suffice to say here that there is no evidence—physical, physiological, biochemical, or psychological—to support such a theory relative to adults and I do not think that infants differ in this respect.

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Should salt appetite be considered? I am not aware of evidence indicating that the normal infant has an innate appetite for salt. The available evidence (17) suggests rather that, in the absence of frank sodium deficiency, salt appetite is an acquired taste which can adapt itself readily to the salt level in the diet. Furthermore, failure to satisfy this mythical innate salt appetite does not appear to harm modern breast-fed infants.

I would conclude by saying that the benefits of added salt are putative, at best.

IS THERE POSSIBLE HARM IN ADDING SALT TO BABY FOODS?

The high infant mortality rate in the United States (20), when compared with that in other medically advanced countries, is not among the causes for national self-congratulation. Data allowing one to evaluate the precise role of current infant feeding practices in this unenviable record are lacking. I suggest that this lack is regrettable.

The evidence indicating that the renal clearance of sodium is "impaired" in the newborn has been reviewed by Smith (21) and by McCance and Widdowson (22, 23). It seems unlikely that what applies to the newborn would not apply to at least some older infants during the 1st year of life. As a practical matter, however, in the absence of overt cardiovascular or renal disease, the infant—like the adult—seems capable of handling high NaCl intakes without immediate injury if sufficient water is provided as well. It may not be enough, however, to consider only immediate ill effects. It is possible that a high NaCl intake, particularly in the young, could have delayed effects that become apparent only after prolonged ingestion. The problems associated with the modern experience of widespread overnutrition are not yet fully understood, but there is increasing recognition of their seriousness. The experimental studies of Berg and Simms (24, 25) showed that rats on restricted food intakes from weaning had significantly greater longevity as well as a lower incidence of cardiac, renal, and vascular lesions than did their controls on ad libitum feeding. Could the price of enhanced growth in our infants include enhanced probability of cardiovascular-renal disease in our adults? The delayed appearance of blood dyscrasias from benzene and radiation, thyroid cancer in adolescents from thymic radiation in infants, skin and bladder cancer from occupational exposure to coal tars, emphysema and cancer from cigarettes, et cetera, need not be emphasized here. There is ample precedent for the possibility that excess salt ingestion in infants could manifest its injurious effects in adults.

There is impressive evidence suggesting that sodium is involved in the pathogenesis of essential hypertension. In man, the evidence that implicates dietary salt as an etiological factor (17, 26–29) is indirect, but in the rat it is direct and unequivocal (30). The hypertension induced in rats has a remarkable similarity to "essential" hypertension in man. Indeed, the clinical picture runs from slowly developing, benign hypertension to early, rapidly fatal "malignant" hypertension (31, 32). Furthermore, it is usually self-sustained after the excess dietary salt is removed (33). When salt consumption is increased, blood pressure increases and, in general, stepwise increases in daily salt intake will be reflected in corresponding increases in average blood pressures (31, 34). Not only are young animals more susceptible than older ones, but the longer the salt is eaten, the more severe are its effects (34).

Genetic factors play a decisive role in determining whether a rat will develop hypertension after exposure to salt. Two strains of rats have been evolved of which one rapidly and predictably develops fatal hypertension from the same salt intake to which the other does not respond (29, 35, 36). In the hypertension-prone strain, if

dietary salt is restricted at the time of weaning the animals remain normotensive or develop only mild, benign elevations: by contrast, if a high salt diet is begun at this time, a malignant hypertension is evoked from which most animals will be dead within 3–4 months (34). If the high salt intake is delayed for 3–6 months, hypertension develops much more slowly although, ultimately, all will die with it. Finally, even a transient high salt intake early in life will induce permanent, often fatal, hypertension in these genetically susceptible animals (34).

In 1963, we found the high concentrations of NaCl in vegetable and meat mixtures processed for baby foods. Thereafter, we induced hypertension in some members of the hypertension-prone strain simply by feeding them such mixtures (4). The purpose of this study was merely to confirm our suspicion that these foods contained sufficient NaCl to induce hypertension in genetically susceptible individuals. When the experiment was terminated after only 4 months, 5 of the 7 test rats had developed hypertension whereas their 7 controls remained normotensive. Based on current experience, had this experiment been continued a few months more, all 7 rats on the baby foods would have developed severe hypertension which would have been lethal in at least 3 of the 7.

As I have said elsewhere, "... it is *necessary* to transfer such experimental results to man: Federal law admits the validity of animal experimentation and the FDA is guided by such experiments" (37). Until proved otherwise, these data must be taken to indicate that: the younger the organism, the greater will be the susceptibility to hypertension from salt; as the amount and duration of salt consumption increase, the probability of hypertension will increase; in sensitive individuals, even transient high salt intakes may induce hypertension; and, finally, the genetic constitution is a prime determinant in susceptibility to developing hypertension from salt.

One might say that it is only the hypertension-prone child that we need worry about here. There are no genetic markers available by which to identify him any more than there are for most other diseases. In my opinion, therefore, salt should be decreased for all. Added salt in baby foods is neither necessary nor beneficial. It can be harmful. The evidence in rats is so straightforward that, it seems to me, the burden of proof lies with those who add salt to their baby foods rather than with those of us who question this practice.

REFERENCES

1. Gerhart, H. A. Infant feeding practices: a predisposing factor in hypertension. *Am. J. Clin. Natr.* 21: 863, 1968.
2. Pryor, F. A., and L. P. Hamilton. Infant feeding practices, 1966. *Am. J. Diseases Children* 111: 370, 1966.
3. Fomon, S. J. *Infant Nutrition.* Philadelphia: Saunders, 1967, p. 160.
4. Dahl, L. K., M. Heine and L. Tassinari. High salt content of Western infant's diet: Possible relationship to hypertension in the adult. *Nature* 198: 1204, 1963.
5. *Composition of Foods—Raw, Processed, Prepared*, edited by B. K. Watt and A. C. Merrill. *U. S. Dept. Agr. Handbook* no. 8. Washington, D. C.: U. S. Dept. Agr., 1963.
6. Heinz Nutritional Data (5th ed.). Pittsburgh, Pa.: H. J. Heinz Co., 1964, p. 124–139.
7. Gerber Marketing Services Dept. Fremont, Mich.: Gerber Products Co.
8. The Composition of Milks. *Natl. Acad. Sci. Natl. Res. Council Publ.* 254, Washington, D. C., 1953, p. 26.
9. McCance, R. A., and E. M. Widdowson. Mineral metabolism of the foetus and new born. *Brit. Med. Bull.* 17: 132, 1961.
10. Cooke, R. E., E. L. Pratt and D. C. Darrow. The metabolic response of infants to heat stress. *Yale J. Biol. Med.* 22: 227, 1950.
11. Gamble, J. L., W. M. Wallace, E. Eaton, M. V. Holloway, M. Cushman, J. Appleton, A. Shenkin and J. Pollit. Effects of large loads of electrolytes. *Pediatrics* 30: 1964.
12. Dole, V. P., L. K. Dahl, G. C. Cotzias, H. A. Eder and M. E. Krebs. Dietary treatment of hypertension. Clinical and metabolic studies

of patients on the rice-fruit diet. *J. Clin. Invest.* 29: 1189, 1950.

13. Davidson, C. S., L. E. Chiecorn, P. A. Clifford, G. J. Garuzda and C. Robinson. Sodium restricted diets. *Natl. Acad. Sci. Natl. Res. Council Publ.* 325. Washington, D. C., 1954.
14. Dahl, L. K., L. M. Tassinari and N. Gillespie. Sodium in foods for a 100 mg diet, analyzed vs. calculated values. *J. Am. Dietet. Assoc.* 34: 717, 1958.
15. Kaunitz, H. Causes and consequences of salt consumption. *Nature* 178: 1141, 1956.
16. Dahl, L. K. Salt intake, adrenocortical function, and hypertension. *Nature* 181: 989, 1958.
17. Dahl, L. K. Salt intake and salt need. *New Engl. J. Med.* 258: 1152, 1205, 1958.
18. Dahl, L. K., L. Silver, R. W. Christie and J. Genest. Adrenocortical function after prolonged salt restriction in hypertension. *Nature* 185: 110, 1960.
19. Dahl, L. K., L. Silver, S. Spraragen and M. Smilay. The effect of chronic high and low salt intakes on adrenocortical function. *Nature* 190: 348, 1961.
20. Metropolitan Life Insurance Co. Infant mortality in the United States and abroad. *Statist. Bull.* 48: 3, May, 1967.
21. Smith, H. W. *The Kidney—Structure and Function in Health and Disease.* New York: Oxford Univ. Press, 1951, p. 492.
22. McCance, R. A. Renal function in early life. *Physiol. Rev.* 28: 331, 1948.
23. McCance, R. A., and E. M. Widdowson. Physiology of the newborn animal. *Lancet* 2: 585, 1957.
24. Berg, B. J., and H. S. Simms. Nutrition and longevity in the rat. II. Longevity and onset of disease with different levels of food intake. *J. Nutr.* 71: 255, 1960.
25. Berg, B. J., and H. S. Simms. Nutrition, onset of disease, and longevity in the rat. *Can. Med. Assoc. J.* 93: 911, 1965.
26. Dahl, L. K., and R. A. Love. Evidence for relationship between sodium (chloride) intake and human essential hypertension. *A.M.A. Arch. Internal Med.* 94: 525, 1954.
27. Dahl, L. K., L. Silver and R. W. Christie. The role of salt in the fall of blood pressure accompanying reduction in obesity. *New Eng. J. Med.* 258: 1186, 1958.
28. Dahl, L. K. Posible role of salt intake in the development of essential hypertension. *Essential Hypertension, An International Symposium*, edited by P. T. Cottier and K. D. Bock. Heidelberg: Springer, 1960, p. 53–65.
29. Knudsen, K. D., and L. K. Dahl. Essential hypertension: Inborn error of sodium metabolism? *Postgrad. Med.* 42: 148, 1966.
30. Meneely, G. R., and L. K. Dahl. Electrolytes in hypertension: The effects of sodium chloride—the evidence from animal and human studies. Hypertension and its treatment. *Med. Clin. N. Am.* 45: 271, 1961.
31. Meneely, G. R., R. G. Tucker, W. J. Darby and S. H. Auerbach. Chronic sodium chloride toxicity in albino rat. II. Occurrence of hypertension and syndrome of edema and renal failure. *J. Exptl. Med.* 98: 71, 1953.
32. Dahl, L. K., and E. Schackow. Effects of chronic excess salt ingestion: Experimental hypertension in the rat. *Can. Med. Assoc. J.* 90: 155, 1964.
33. Dahl, L. K. Effects of chronic excess salt feeding. Induction of self-sustaining hypertension in rats. *J. Exptl. Med.* 114: 231, 1961.
34. Dahl, L. K., K. D. Knudsen, M. A. Heine and G. J. Leitl. Effects of chronic excess salt ingestion. Modification of experimental hypertension in the rat by variations in the diet. *Circulation Res.* 22: 11, 1968.
35. Dahl, L. K., M. Heine and L. Tassinari. Effects of chronic excess salt ingestion. Evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J. Exptl. Med.* 115: 1173, 1962.
36. Dahl, L. K. Effects of chronic excess salt ingestion—experimental hypertension in the rat: Correlation with human hypertension. *The Epidemiology of Hypertension. Proc. Intern. Symp.* Sponsored by Chicago Heart Assoc. and Am. Heart Assoc., Chicago, 1964, edited by J. Stamler, R. Stamler and T. N. Pullman. New York: Grune & Stratton, 1967, p. 218–227.
37. Dahl, L. K. Letter to the editor. *Nutr. Rev.* 26: 124, 1968.

EFFECTS OF CHRONIC EXCESS SALT FEEDING

ELEVATION OF PLASMA CHOLESTEROL IN RATS AND DOGS*

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The interrelationship of hypertension and atherosclerosis has been the subject of repeated investigation and a voluminous literature. At the present time it is generally thought that there is no basic etiological relationship between the two diseases; however, it appears that hypertension accelerates the progress of established atherosclerosis and that the inception of atherosclerosis is hastened by the presence of antecedent hypertension (1).

For some years we have been studying the effects of chronic excess salt feeding in man and animals in an effort to explore the etiologic relationship between salt ingestion and human essential hypertension (2–5). We recently concluded such a study on dogs and rats. The results suggest that chronic salt ingestion is capable of elevating the concentration of plasma lipids. The available evidence is insufficient to conclude that such a factor operates in man to raise plasma lipid levels or that it is involved in the production of atherosclerosis, either human or experimental. Nonetheless, since salt has been invoked in the pathogenesis of human hypertension (4), the present studies suggest one possible biochemical basis for a link between atherosclerosis and hypertension.

EXPERIMENTAL

Animals and Feeding Programs:

1. Rats.—All animals were Sprague-Dawley females.

Group I consisted of 16 animals of which 11 were salt-fed and 5 were controls; salt feeding was begun at age 2½ months and continued up to the time of sacrifice, more than 15 months later. Group II included 41 animals, 11 controls and 15 on each of the two salt regimens described below; salt feeding was started at age 1 month and blood for cholesterols was obtained 6½ months later. Group III with 36 animals, of which 12 were controls, was similar to Group II except that salt feeding began at 3 weeks of age and blood for cholesterols was obtained 8 weeks later. All animals were housed in air-conditioned rooms, 2 to 6 animals per cage, and were allowed free access to drinking (tap) water and food. Repeated analyses of the tap water showed it to contain only 0.5 to 0.7 m.eq. of sodium per liter. The basic diet that all control animals received in unmodified form was Ralston Purina fox chow pellets, which by our analyses contained sodium in amounts ranging from 0.51 to 0.74 per cent calculated as NaCl.

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Test animals received the same chow to which one of two supplementary sodium-containing salts had been added by the manufacturer so that the final concentration calculated as NaCl approximated 8 per cent by weight. In one food ("8 per cent NaCl food") the supplementary salt was sodium chloride: our analyses indicated an average of 8.09 per cent NaCl.

The other food ("sea-salt food") had 11.6 per cent "sea-salt" added, an amount which was calculated to give a sodium concentration equal to that in the 8 per cent NaCl food; our analyses showed this food to have less than calculated, namely an average of 7.28 per cent (as NaCl). The rationale of using sea-salt for inducing hypertension is not pertinent to the current studies and therefore discussion of its use will be deferred to another report. Information from the producers of this special salt[1] indicate that it is obtained by evaporating sea water and is finally dried at an air temperature of 1100°F. All rats were weighed monthly when blood pressures were measured and equivalent food consumption among the various groups was indicated by: (*a*) similar weight and growth curves, and (*b*) at intervals the food actually consumed was carefully checked by weighing food dishes daily over 4-day periods.

2. Dogs.—Care and housing: The colony consisted of 17 pure bred beagles, 16 females and one castrated male. The original colony had 16 animals born between November 16, 1955, and February 7, 1956, purchased as weanling females but found to contain one male by error. It was elected to keep this male and castrate him when it became technically feasible; through oversight this was delayed until the age of 9 months by which time impregnation of one female (No. 8-5—control) had occurred. This female delivered 2 normal pups on October 13, 1956, of which a female (No. 3-24—control) was added to the experimental group. At the beginning of the salt feeding program animals were distributed by weight as uniformly as possible among the 4 groups. The animals were housed in large screened pens with cement floors in a heated building, by groups according to the salt feeding regimen. These pens were scrubbed and cleaned thoroughly every day. All dogs were put outside in special runs for several hours each day except in the most inclement weather. Their care, handling, and health were supervised by a staff veterinarian and each animal received the usual routine immunizations and semiannual deworming procedures. On this regimen they remained uniformly healthy and active.

Feeding: All beagles received the same basic diet of kibble, canned horse meat, a sodium-free mineral-vitamin supplement, and water. During the first months of the study when they were still young, animals were fed 3 times a day and received milk; the frequency of feeding as well as the amount of milk were gradually decreased and after 6 months of age they were fed only once a day without milk. Each animal had its own food dish but members of a group were fed simultaneously; feedings were supervised by an animal attendant to prevent a dog from eating food other than its own and in the occasional cases where this occurred animals were fed separately as long as necessary to prevent it. The stable weights suggested that food consumption was at a constant level.

Each animal daily was fed and consumed an average of 200 gm. kibble, 100 gm. canned horse meat, and 1 teaspoon of the mineral-vitamin supplement. The 5 *control* animals received this diet, plus tap water *ad lib.* The 4 "1 per cent NaCl-H_2O" animals were fed this diet but drank 1 per cent saline in place of tap water, consuming an average of about 0.9 liter per dog per day. The 4 "2 per cent NaCl food" animals were fed kibble calculated to contain 2 per cent NaCl, and otherwise as the control animals. The 4 "6 per cent NaCl food" animals differed from the preceding group only in that their kibble contained 6 per cent NaCl. Analysis of the various foods for sodium concentration in the author's laboratory gave the following average concentrations, calculated as NaCl: unmodified kibble 1.00 per cent; 2 per cent NaCl kibble, 2.46 per cent; 6 per cent NaCl kibble, 5.97 per cent; canned horse meat, 0.48 per cent. The

[1] The author is indebted to the Trace Elements Corporation, Houston, for a supply of admiral brand trace element sea salt sufficient to initiate these experiments.

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daily average NaCl intake for the groups was estimated to have been as follows: Control, 2.5 gm.; 1 per cent NaCl-H_2O, 11.5 gm.; 2 per cent NaCl food, 5.5 gm.; 6 per cent NaCl food, 12.5 gm. The 3 groups on extra salt began receiving it when the members ranged from 2½ to 4½ months of age and at the time of the latest blood lipid and blood pressure measurements had completed from 45 to 48 months on this regimen. The 4 animals in the 6 per cent NaCl food group, received 2 per cent NaCl food during the first 7½ months of the experiment, after which they received the 6 per cent NaCl food continuously.

Blood Pressure Measurements:

1. Rats.—The microphonic method of Friedman and Freed (6) was used; measurements were made under light ether anesthesia, using a flowing oxygen-ether mixture, in a special box with temperature thermostatically controlled at 38°C. At least 4 systolic readings were recorded with each measurement and the average of these 4 was used. This technique has given highly reproducible readings for several years and many successive monthly measurements on control animals up to 21 months of age have never shown systolic pressures consistently in excess of 130 mm. Hg. For this reason, animals with pressures of 140 mm. Hg or above have been considered hypertensive. Blood pressure measurements were made at monthly intervals except among the animals of Group III, on which measurements were made only once, just prior to the bleeding for plasma cholesterol concentrations after 8 weeks of salt feeding.

2. Dogs.—Measurements were made in one of two ways on unanesthetized animals; (*a*) direct, intra-arterial femoral mean pressures were recorded using a Tycos aneroid manometer (calibrated against a mercury manometer with each use); (*b*) indirect, by means of the Friedman and Freed technique (6) with which accurate systolic pressures were recorded easily. Since this latter method is simple, appears to be accurate, requires little training of animals, and has not been described for dogs, a description of and justification for its use are presented briefly.

The technique for dogs is basically similar to the original description for rats and only the pertinent modifications are noted. The tail is clipped closely for 6-to 8 inches from the base towards the tip, after which a depilatory of 20 per cent Na_2S in water is applied for several minutes to the entire circumference of the tail over an area 2 to 3 inches in length beginning about 3 inches from the base of the tail; ordinarily at the end of this time the stubble has turned yellow and soft and is easily wiped off after which the area is thoroughly rinsed with 70 per cent alcohol. To the area which is now smooth and clean of hair a small amount of vaseline is applied where the microphonic attachment is placed—generally best in a lateral position. The signal to noise ratio is significantly increased by the foregoing procedures, and the pulse sounds usually come through clearly. A small rubber cuff 2 inches wide, made up like a standard cuff used for measuring human blood pressure is applied above the microphone near the base of the tail and connected to a mercury manometer and bulb. Systolic pressure is recorded as for the rat tail technique. Interestingly, in some animals, a diastolic muffling occurs without disappearance of the sound. It is important that the animals be warm and comfortable. Therefore, we ordinarily measure pressures in a room temperature of about 27–30°C., and do not allow the animals outdoors beforehand because of possible cooling with constriction of the tail vessels. A thick foam rubber mattress beneath the animals increased their comfort with resulting greater relaxation. As judged by the pulse rate, most animals are not excited by this procedure, although the usual precautions concerning noise, motion, strangers, etc., must be observed. Results obtained with this technique were compared with those obtained by simultaneous direct iliac artery cannulation in two anesthetized dogs using a Sanborn transducer and multichannel recorder. Various manipulations of blood pressure including intravenous epinephrine indicated remarkably good correlation in systolic readings,

and in one animal in which diastolic muffling occurred the values for diastolic pressure were almost identical with those obtained using the direct technique. In one animal, systolic pressure by the tail technique ranged from 0 to 6 mm. Hg higher than direct iliac pressures; in the other dog the indirect systolic pressures were recorded −1 to +20 mm. different from the iliac pressures, with an average of +11 mm. Hg. It was thought that the slightly higher pressures recorded with the tail technique were due to the sensitivity of the microphone resulting in faint pulse sounds prior to true systolic pressures. By using as routine direct and indirect methods simultaneously, it is likely that the small differences recorded could be decreased by learning the point at which these early sounds of the tail pulse become significant.

TABLE I

Group I. Female Rats, 18 Months of Age at Sacrifice

Rat	Food	Mos. on diet	Body weight	Systolic B.P.	BUN	Total cholesterol
			gm.	*mm. Hg*	*mg. per cent*	*mg. per cent*
427	Control	17½	306	123	N.D.	95
429	"	"	302	109	9.1	95
430	"	"	342	125	10.2	75
433	"	"	270	96	N.D.	92
435	"	"	312	109	19.4	106
Average (and S.D.)			306.4 (±25.7)	112.4 (±11.9)	12.9	92.6 (±11.2)
401	11.6 per cent sea salt	15½	268	160***	11.1	121**
402	" " " " "	"	326	177***	N.D.	193***
403	" " " " "	"	235**	157***	12.7	400***
407	" " " " "	"	252**	125	N.D.	115**
410	" " " " "	"	314	159***	10.3	160***
411	" " " " "	"	272	130	N.D.	198***
412	" " " " "	"	236**	166***	10.8	100
413	" " " " "	"	267	144**	10.3	210***
414	" " " " "	"	332	133	N.D.	125**
417	" " " " "	"	223***	162***	8.6	441***
424	" " " " "	"	243**	151***	17.2	380***
Average (and S.D.)			269.8 (±38.2)	151.3 (±16.4)	11.6 (±2.8)	222.1 (±74.8)

Systolic blood pressures are the average of last 3 values, obtained at monthly intervals.
BUN, blood urea nitrogen.
N.D., not done.
** and ***, differs from mean value in control groups by 2 or 3 S.D., respectively. Salt-fed group had significantly ($p < 0.01$) higher mean systolic blood pressure and plasma cholesterol than control group.

We have not attempted this refinement but in view of the correspondence between the direct and indirect methods, we have adopted the latter for routine measurements and the blood pressures recorded in this paper are those obtained by the tail method. When it became apparent that salt feeding was not resulting in significant elevation of blood pressure, measurements were recorded at irregular intervals. The values used in this report are those obtained during the month in which blood was removed for estimation of cholesterol concentration, and agree with earlier values, within expected limits of variation. About 10 to 15 readings were made on each animal during a single measurement and the final 4 values were averaged. Variations of pressure among these 4 readings were insignificant.

Miscellaneous Methods:

Blood was obtained from rats by nicking the tail and bleeding directly into heparinized tubes; dogs were bled from an extremity by venepuncture, and the blood put into heparinized

tubes. Bloods were centrifuged immediately, plasma removed and refrigerated during the 1 to 2 hours before extraction of cholesterol was carried out.

Cholesterol was measured by the method of Abell, Levy, Brodie, and Kendall (7) and blood urea nitrogen by Conway's microdiffusion technique (8); statistical significance of the difference between the means of groups was calculated by Student's t test using the modern tables of Fisher and Yates (9); p values of <0.05 were required for differences to be considered significant, and use of the phrase "significant difference" in this paper implies $p<0.05$.

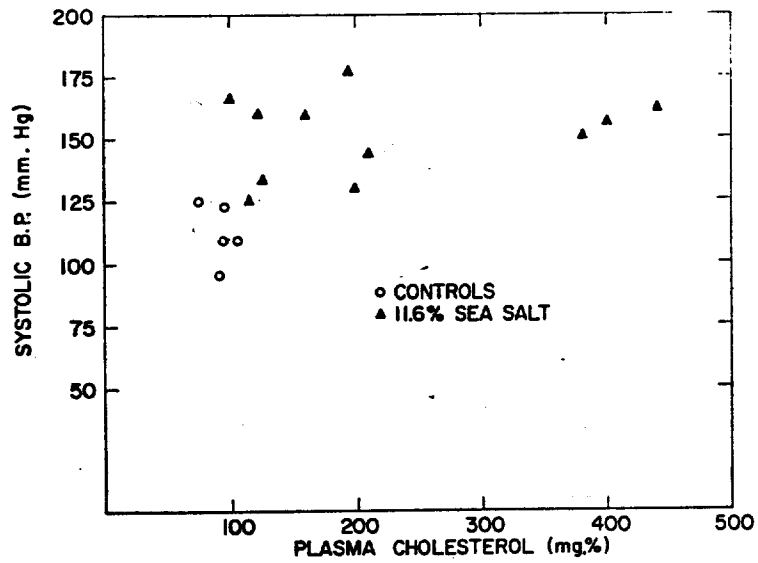

FIG. 1. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female rats. Results after 15½ months.

RESULTS

Rats.—Group I (Table I). The salt-fed animals in this group had been on supplemental salt for over 15 months when blood was obtained for cholesterol estimation. Both mean systolic blood pressure and plasma cholesterol were higher in the salt-fed than in the control group. However, among individual members fed salt, the correlation between an increase in cholesterol and in blood pressure was not constant or direct. For instance rats 412 and 417 with similar pressures of 166 and 162 mm. Hg, respectively, had, in the former case a plasma cholesterol of 100 mg. per cent, and in the latter instance 441 mg. per cent. Similarly rat 401 had a pressure of 160 mm. Hg with a plasma cholesterol of 121 mg. per cent while No. 403 with a pressure of 157 mm. Hg had a plasma cholesterol of 400 mg. per cent. Inspection of the table indicates other, if lesser, disparities. The data are presented graphically in Fig. 1 which emphasizes that while there is a trend toward higher blood pressures being associated

TABLE II

Group II. Female Rats, 7½ Months of Age at Time of Bleeding for Plasma Cholesterol

Rat	Food	Mos. on diet	Body weight	Systolic B. P.	Total cholesterol
			gm.	*mm. Hg*	*mg. per cent*
557	Control	7	297	114	113
558	"	"	297	124	63
559	"	"	232	124	81
561	"	"	268	126	81
562	"	"	242	120	99
563	"	"	269	126	125
564	"	"	255	123	113
565	"	"	292	132	90
566	"	"	263	124	89
567	"	"	260	137	63
568	"	"	285	138	94
Average (and S.D.)			269.1 (±21.8)	126.2 (±7.1)	91.9 (±19.9)
501	11.6 per cent sea salt	6½	262	154***	125
502	" " " " "	"	249	122	138**
508	" " " " "	"	263	180***	125
511	" " " " "	"	239	120	119
514	" " " " "	"	283	166***	113
518	" " " " "	"	249	172***	71
520	" " " " "	"	281	162***	123
522	" " " " "	"	245	182***	132**
523	" " " " "	"	263	136	106
524	" " " " "	"	275	162***	123
525	" " " " "	"	265	170***	113
526	" " " " "	"	239	135	85
527	" " " " "	"	271	194***	143**
531	" " " " "	"	264	120	96
532	" " " " "	"	256	130	125
Average (and S.D.)			260.3 (±14.0)	153.7 (±24.6)	115.8 (±19.5
533	8 per cent NaCl	6½	277	140**	109
534	" " " "	"	270	190***	155***
535	" " " "	"	254	145**	90
536	" " " "	"	270	148***	106
537	" " " "	"	258	138	113
538	" " " "	"	263	142**	90
540	" " " "	"	253	150***	104
541	" " " "	"	271	138	113
546	" " " "	"	247	124	99

TABLE II (*continued*)

Rat	Food	Mos. on diet	Body weight	Systolic B. P.	Total cholesterol
			gm.	*mm. Hg*	*mg. per cent*
547	8 per cent Na Cl	6½	268	116	73
549	" " " "	"	268	134	106
551	" " " "	"	222**	126	90
552	" " " "	"	245	126	99
554	" " " "	"	267	132	81
555	" " " "	"	225**	153***	99
Average (and S.D.)			257.2 (±16.5)	140.1 (±17.3)	101.8 (±18.6)

Systolic blood pressures are those for the last month of the experiment.

** and ***, differs from mean value in control group by 2 or 3 S.D., respectively. Both salt fed groups had higher mean B.P. (sea salt < 0.01, NaCl $< 0.05 > 0.01$) than control group. Sea salt group only had higher ($p < 0.01$) mean plasma cholesterol than control group.

with higher cholesterols, there are significant exceptions. Neither blood pressure nor cholesterol concentration was correlated with weight.

Group II (Table II). Supplemental salt had been supplied to the test animals in this group for 6½ months at the time of the plasma cholesterol and blood pressure measurements. Both salt-fed groups had mean blood pressure elevations compared with the control group (the sea salt group being higher) but only the group fed sea salt had a significant increase in cholesterol. Among individual salt-fed animals in this group elevations in blood pressure were common but elevations in cholesterol were not. Among the 4 rats with significant elevation in plasma cholesterol (Nos. 502, 522, 527, 534) 3 had significant elevations in blood pressure. By contrast, among the 16 animals with elevated blood pressure, there were only 3 with increased plasma cholesterol. These data could be interpreted to indicate that (*a*) if an elevated blood pressure *per se* will *cause* an elevation in plasma cholesterol, the pressure must exist for a longer period than was present here; or (*b*) both the elevated pressure and increase in cholesterol were the result of a common stimulus but the pressure had no causal relationship to the cholesterol levels; or (*c*) the few, and modest, elevations in plasma cholesterol found here were entirely fortuitous. Body weight again showed no correlation with either blood pressure or cholesterol concentration. See Figure 2.

Group III (Table III). The salt-fed animals in this group had been on salt food for only 2 months. Both series fed added salt had higher mean blood pressures and plasma cholesterol concentrations than their controls (Table III). Individual significant elevations in cholesterol concentration were present in only 4 animals, all of which had elevated pressures as well; among the 24 salt-fed animals, 13 had elevated pressures (as did 1 control). Comparison of

the mean blood pressures in animals fed the same foods in groups II and III were not different; however, all 3 series in the older animals of group II had significantly higher plasma cholesterol concentrations: calculation of values for t gave 4.40 ($p \ll 0.01$), 2.85 ($p < 0.01$) and 2.54 ($p < 0.05 > 0.01$) as estimates for significance of the difference between the mean cholesterol concentrations in the sea salt, NaCl, and controls in these 2 groups, respectively.

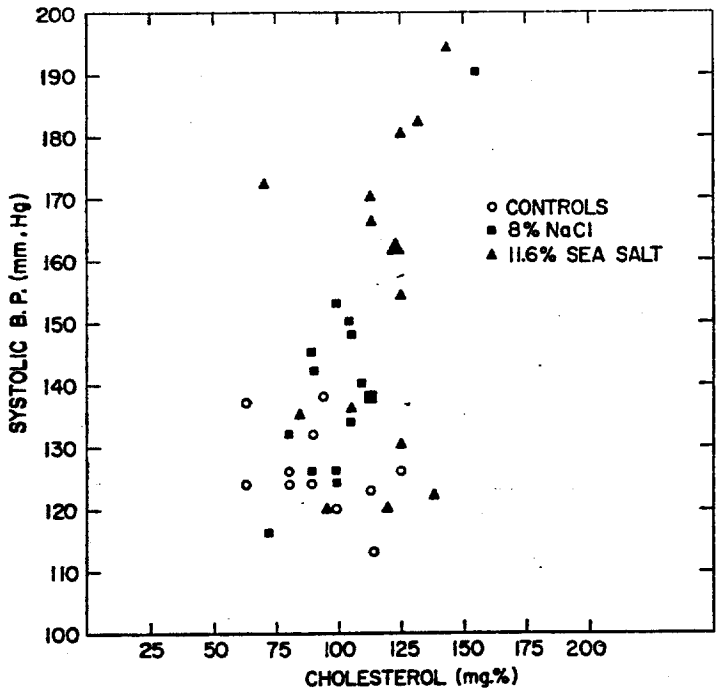

FIG. 2. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female rats. Results after 6½ months.

Since the control animals of group II at 7½ months of age, had increased plasma cholesterol concentration compared with the controls in group III at 3 months, some of the elevation in plasma cholesterol found in the animals fed salt for 6½ months must be ascribed to aging. However, it is doubtful that aging alone is a cause of the significant increase in plasma cholesterol levels found in this study for control animals in groups I and II, had similar plasma cholesterol levels at 17½ and 7½ months of age, respectively. No correlations could be established with weight. See Figure 3.

Dogs.—(Table IV, Fig. 4). The control and salt-fed groups did not differ

TABLE III

Group III. Female Rats, 11 Weeks of Age When Blood for Plasma Cholesterol Obtained and Systolic Blood Pressures Measured

Rat	Food	Mos. on diet	Body weight	Systolic B. P.	BUN	Total cholesterol
			gm.	*mm. Hg*	*mg. per cent*	*mg. per cent*
765	Control	2	191	122	18.7	60
766	"	2	225	126	17.4	74
767	"	2	204	125	29.7	85
768	"	2	210	126	20.9	74
769	"	2	200	130	19.6	81
770	"	2	215	142**	18.6	90
773	"	2	209	129	N.D.	81
774	"	2	222	132	"	50
775	"	2	223	120	"	74
776	"	2	225	134	"	85
777	"	2	235	132	"	81
778	"	2	240	134	"	60
Average (and S.D.)			216.6 (±14.4)	129.3 (±6.0)	20.8 (±4.5)	74.6 (± 12.1)
701	11.6 per cent sea salt	2	210	194***	13.0	85
702	" " " " "	2	181**	133	21.6	81
703	" " " " "	2	218	134	18.4	94
704	" " " " "	2	201	152***	20.9	85
705	" " " " "	2	214	182***	23.4	100**
706	" " " " "	2	215	154***	17.8	90
709	" " " " "	2	189	122	N.D.	85
710	" " " " "	2	211	174***	"	100**
711	" " " " "	2	205	136	"	81
712	" " " " "	2	175**	120	"	90
713	" " " " "	2	201	124	"	81
714	" " " " "	2	197	142**	"	101**
Average (and S.D.)			201.4 (±13.8)	147.3 (±24.6)	19.2 (±3.7)	89.4 (±7.7)
733	8 per cent NaCl	2	190	124	19.3	81
734	" " " "	2	203	152***	26.8	90
735	" " " "	2	233	138	19.5	81
736	" " " "	2	208	143**	40.4	84
737	" " " "	2	204	146**	41.6	74
738	" " " "	2	241	132	31.0	81
739	" " " "	2	220	140	N.D.	98
740	" " " "	2	213	142**	"	100**
741	" " " "	2	212	142**	"	69
742	" " " "	2	179**	162***	"	88
743	" " " "	2	225	145**	"	98
744	" " " "	2	215	140	"	74
Average (and S.D.)			211.9 (±17.2)	142.2 (±9.4)	29.8 (±9.8)	84.8 (±10.2)

** and ***, differs from control mean value by 2 or 3 S.D. respectively. Both salt-fed groups had higher mean blood pressures and plasma cholesterol concentrations compared with the control group: *blood pressure*, sea salt, $p < 0.05 > 0.01$; NaCl, $p < 0.01$; *cholesterol*, sea salt, $p < 0.01$; NaCl, $p < 0.05 > 0.01$. The mean *weight* of the group fed sea salt was significantly less than the control group: $p < 0.05 > 0.01$.

N.D., not done.

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significantly in mean systolic blood pressures, and among individuals with higher pressures no correlation with plasma cholesterol concentration was present. Marked elevations in cholesterol were present in some of the salt-fed animals although among the groups only the mean level in the 2 per cent NaCl animals was significantly higher than that of the control group. The high coefficient of variation of the mean (40 per cent) in the 6 per cent NaCl group presumably accounted for lack of a statistically significant difference from the

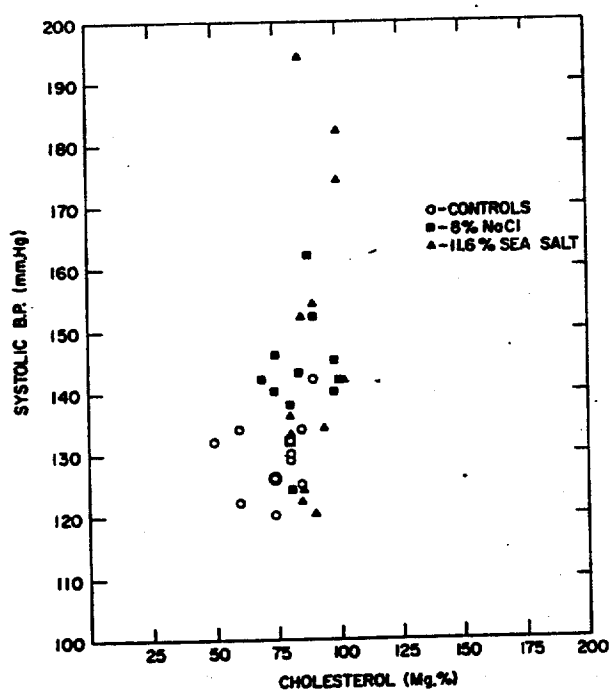

FIG. 3. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female rats. Results after 2 months.

mean cholesterol value in the control group; in this 6 per cent NaCl group were two dogs with the greatest elevations in cholesterol namely 386 and 435 mg. per cent. Among the 12 animals fed added salt, 7 had an increase in cholesterol which exceeded 2 standard deviations of the mean value in the control animals, and of these 7, 4 were in excess of 5 standard deviations from this mean. In 8 of the 17 dogs studied, 2 from each group, plasma phospholipids and triglycerides were measured in addition to the cholesterols.[2] These values

[2] The author is indebted to Drs. Howard A. Eder and Lewis Gidez of the Department of Medicine, Albert Einstein College of Medicine, for these determinations.

TABLE IV

17 Beagles Fed Diets Containing Different Amounts of NaCl for 4 Years

Dog	Food	Age	Mos. on diet	Body weight	Systolic B.P.	BUN	Total cholesterol	Phospholipids	Total cholesterol / Phospholipid	Triglycerides
		mos.		*kg.*		*mg. per cent*	*mg. per cent*	*mg. per cent*		*mg. per cent*
1-1	Control	52	50	8.5	160	17.5	153			
8-3	"	52	50	10.6	134	10.3	208			
8-5*	"	52	50	10.2	180	12.2	195	440.0	0.45	18.1
9-3	"	52	50	13.9	146	9.4	177	358.0	0.49	20.4
3-24‡	"	41	40	9.6	171	11.6	186			
Average (and s.d.)				10.6 (±2.0)	158 (±19)	12.2 (±3.2)	184 (±21)			
4-6	6 per cent NaCl	49	45§	7.5	165	9.3	175			
5-2	" " " "	49	45	11.8	145	12.7	386***	618.0	0.63	18.7
5-3	" " " "	49	45	11.0	134	30.4	435***	687.8	0.63	29.3
5-5	" " " "	49	45	16.8	189	11.5	233**			
Average (and s.d.)				11.8 (±3.8)	157 (±24)	16.0 (±9.7)	307 (±123)			
4-8	2 per cent NaCl	50	45	13.4	193	10.8	180			
5-1	" " " "	49	45	11.4	129	12.3	275***			
5-4	" " " "	49	45	13.1	128	10.3	320***	602.5	0.51	70.4
5-6	" " " "	49	45	9.3	139	11.5	290***	476.0	0.61	19.9
Average (and s.d.)				11.8 (±1.9)	147 (±30)	11.2 (±0.9)	266 (±61)			
8-1	1 per cent NaCl	52	48	8.4	135	12.7	193			
8-4	" " " "	52	48	9.1	153	11.2	185			
9-2	" " " "	52	48	14.2	154	12.3	264***	479.0	0.55	17.3
9-5‖	" " " "	52	48	13.6	158	9.1	183	385.2	0.48	11.6
Average (and s.d.)				11.3 (±3.0)	150 (±10)	11.3 (±1.6)	208 (±39)			

All dogs females except No. 9-5 (1 per cent NaCl).

* Dog 8-5 impregnated by No. 9-5 and delivered 2 pups at age 11 months.

‡ Offspring of No. 8-5.

§ First 7½ months, all 6 per cent NaCl dogs received 2 per cent NaCl food, so time on 6 per cent NaCl food was 37½ months.

‖ Male, castrated at age of 9 months.

** and ***, differs from mean control value by 2 or 3 s.d., respectively. Blood pressures not significantly different among groups. Mean cholesterol of 2 per cent NaCl group significantly higher ($p < 0.05 > 0.01$) than control group. The large coefficient of variation (40 per cent) in 6 per cent NaCl group probably contributes to the mean cholesterol level not being significantly greater than that of control group.

and the derived total cholesterol/phospholipid ratio (TC/PL) are shown in the last 3 columns of Table IV. With the same method for phospholipid analysis in another study (10), normal dogs were found to have a serum value of 362 mg. per cent, and a total cholesterol/phospholipid ratio of 0.41. In that work, total cholesterol was analyzed by the Sehoenheimer-Sperry method (11), a technique which yields lower values than that of Abell *et al.* used in our study;

therefore the normal TC/PL ratio could be expected to be lower in the earlier study than in the currently reported one. From this very limited sample, there is highly suggestive evidence that other lipids may increase under the same circumstances which lead to an increase in plasma cholesterol in these animals. It is of some interest that animals in the 1 per cent NaCl-H_2O and 6 per cent NaCl food graphs which were ingesting roughly equivalent amounts of NaCl,

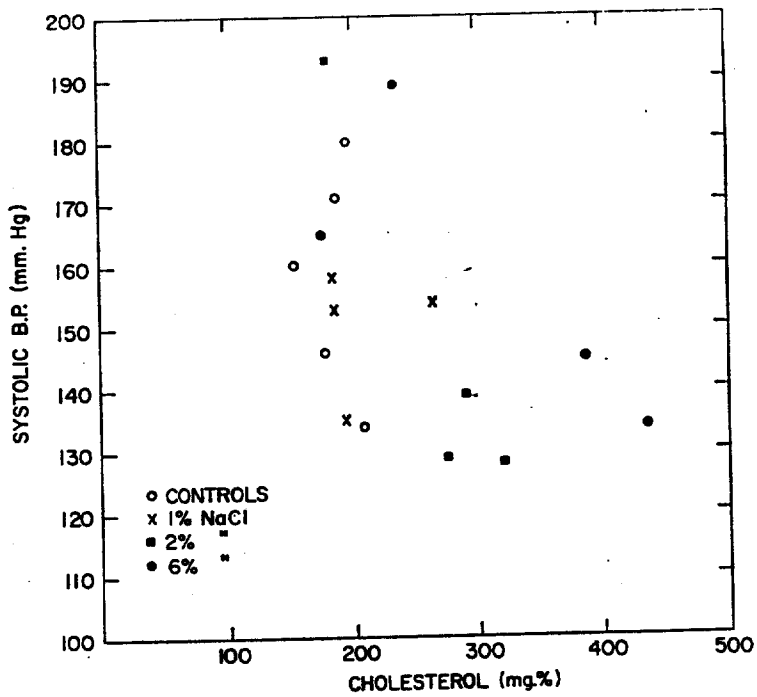

FIG. 4. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female dogs. Results after 4 years.

probably differed in the degree to which plasma lipids were increased: the dogs which received salt mainly as a 1 per cent saline drinking solution appeared to respond less dramatically than those which consumed the salt in their food.

DISCUSSION

The present observations indicate that chronic excess salt feeding can be associated with an elevation in plasma cholesterol, and possibly other lipids as well. The data do not suggest that an elevation in blood pressure is necessary

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for this phenomenon although they do not rule out the possibility that it may be accentuated by hypertension. It seems clear that chronicity of salt feeding is an important factor in the observation of this abnormality since rats fed extra salt for periods from 2 to 6 months had very modest elevation of plasma cholesterol. In contrast, rats and dogs fed added salt for 15 month and 4 years, respectively, often had quite marked increases in plasma cholesterol concentration.

Dissociation of hypercholesterolemia from elevated blood pressure is made difficult in rats chronically fed excess salt because of the frequency with which salt feeding is followed by some degree of hypertension in these animals. For instance, in one colony of 60 animals now under observation, after 7 months of added salt 78 per cent had hypertension with systolic pressures ranging from 142 to 236 mm. Hg. This means that in such a series, an animal with hypercholesterolemia would have about 4 chances in 5 of also having hypertension, a relatively high correlation but not indicating any necessary causal relationship.

Fortunately, separation of hypertension from hyperlipemia appears to be more definite in the dogs which were fed salt in this study. In these animals, there was no evidence that chronic excess salt feeding resulted in hypertension, possibly because of the great capacity of dogs to excrete oral salt loads rapidly (12). Indeed, among the 7 salt-fed animals with increased plasma cholesterol levels, 5 were non-hypertensive, the 6th was borderline, and the 7th was definitely hypertensive: these last 2 animals also had the lowest cholesterol levels of the 7 with elevations.

Whether hypertension does or can play a primary role in elevating serum lipids is not settled (13). There is evidence derived from animals with experimental hypertension which fails to indicate that hypertension results in elevated serum lipids (14–17). In a study by Lewis, Green, and Page (18), dogs with nephrogenic hypertension had elevated serum lipoprotein values which were thought to be related to kidney damage rather than hypertension: animals with neurogenic hypertension failed to develop these changes. There are several reports of serum lipid concentrations in salt-fed animals which are pertinent. Katz and Stamler (19) produced hypertension in cockerels by the substitution of 0.9 per cent saline solution for drinking water or by the addition of up to 8 per cent sodium chloride to the food and found neither an increase in serum cholesterol or more atherosclerosis as compared with the control chicks. Meneely and coworkers (20–21) have made extensive studies on rats chronically fed excess salt in the manner used in the current studies. They also found a number of animals with elevated serum cholesterols and noted that "serum cholesterol levels tended to increase with increasing dietary sodium chloride, but more striking was the correlation between serum cholesterol and the observed level of the blood pressure. Elevated cholesterol did

not occur in normotensive animals, although not all hypertensive animals were hypercholesterolemic" (21). The summarizing report (21) of their work in which blood pressure and serum cholesterol are correlated, indicates on Fig. VIII that the relationship was similar to that shown in Fig. 1 of the present report. The exceptions are frequent enough to question whether the elevation in pressure was causal to the elevation in cholesterol or not.

Deming *et al.* (22), in studying the effect of experimental hypertension on atherogenesis in the rat, concluded that among animals on an atherogenic diet there was a positive correlation between elevation in blood pressure and serum cholesterol concentration. In attempting to assess the role of salt as distinct from that of desoxycorticosterone they concluded that salt was without effect on plasma lipids. However, the data were not unequivocal on this point: in one set of experiments (Experiment 3) the statement was made that it was "not possible to conclude from these results that salt alone has no effect on cholesterol concentration . . ." . Donomae *et al.* (23) produced experimental atherosclerosis in 52 rabbits by lanolin feeding supplemented in 6 instances with added NaCl: among the 6 animals so treated were those with the highest serum cholesterol in the series. Blood pressures were not measured.

Thus viewed in the fashion indicated, the data from the experiments being reported are not necessarily in discord with earlier experience. There are two considerations which invite speculation: (*a*) what the mechanism of production of this hypercholesterolemia may be and (*b*) whether it plays a role in atherogenesis.

The possible role of the kidney in the elevation of lipids is suggested by the well known nephrotic syndrome in man; Meneely (20–21) reported that some of his salt-fed animals developed a well documented nephrotic-like syndrome. None of the animals in our report presented evidence of such a syndrome at any time. One dog (No. 5-3, Table IV) which had plasma cholesterol concentration of 435 mg. per cent also had a slight elevation of blood urea nitrogen, namely 30.4 mg. per cent; however dog 5-2 in this same group had a normal blood urea nitrogen although the plasma cholesterol was 386 mg. per cent; similarly the 3 rats (Table I) with plasma cholesterols of 380, 400, and 441 mg. per cent respectively, had no nitrogen retention. Nonetheless, more subtle effects on the kidney cannot be ruled out as a cause of this phenomenon.

It seems improbable that the elevated plasma cholesterol concentrations were the result of increased *exogenous* supplies, since the only known variable was salt and this did not influence food consumption. Therefore increases in either intestinal absorption or synthesis of cholesterol or a decrease in the rate of its elimination, are the logical mechanisms which would account for the plasma lipid changes noted here. No data are at hand which would allow a choice from among these possibilities.

It is well established that instillation of water or salt solutions in the gastro-

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intestinal tract markedly enhance lymphatic flow (24, 25), and this is a common maneuver in the hands of lymph physiologists. It is intriguing to speculate that these lipid levels might be the result of effects from the chronically increased intestinal lymphatic flow associated with the high salt intake. In this regard it should be recalled that the animals receiving their salt as 1 per cent solution in the drinking fluid had generally lower plasma cholesterol levels than those fed 6 per cent salt with food. The latter consumed about 50 per cent more fluid than did the animals drinking 1 per cent saline. Therefore it is possible that the enhanced fluid intake is the primary factor. It would be interesting to study correlations of uncontrolled diabetes insipidus, clinical or experimental, with hypercholesterolemia.

At the present time, we are aware of no evidence which would suggest that salt plays a role in atherogenesis either in experimental animals or man. Indeed, in the experimental studies of atherosclerosis alluded to above (19, 22, 23) the authors noted specifically that the excess salt ingestion failed to increase the atherosclerotic lesions. Nonetheless, if excessive salt ingestion is capable of elevating plasma lipids it may conceivably affect atherogenesis as well. None of the experimental studies compares in chronicity with the natural history of atherosclerosis in man, in whom slight abnormalities in lipid metabolism operating over decades might result in significant vascular disease.

SUMMARY AND CONCLUSIONS

Among female rats and dogs which were fed excess salt for periods of about 1 and 4 years, respectively, elevations in plasma cholesterol concentrations were frequent and in some cases, quite marked.

Rats on a similar regimen for only 2 and 6 months had less frequent and significantly lesser elevations of cholesterol.

The lipid changes did not appear to be related primarily to increased blood pressure, kidney disease, differences in body weight or food consumption.

There is no present evidence which suggests that this phenomenon is related to atherogenesis although it was speculated that this was possible.

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BIBLIOGRAPHY

1. Katz, L. N., and Stamler, J., Experimental Atherosclerosis, Springfield, Illinois, Charles C Thomas, 1953, 59.
2. Dole, V. P., Dahl, L. K., Cotzias, G. C., Eder, H. A., and Krebs, M. D., Dietary treatment of hypertension. Clinical and metabolic studies of patients on the rice-fruit diet, *J. Clin. Inv.*, 1950, **29**, 1189.

3. Dole, V. P., Dahl, L. K., Cotzias, G. C., Dziewiatkowski, D. D., Harris, C., Dietary treatment of hypertension. II. Sodium depletion as related to the therapeutic effect, *J. Clin. Inv.*, 1951, **30**, 584.
4. Dahl, L. K., and Love, R. A., Evidence for relationship between sodium (chloride) intake and human essential hypertension, *A.M.A. Arch. Int. Med.*, 1951, **94**, 525.
5. Dahl, L. K., Salt intake and salt need, *New England J. Med.*, 1958, **258**, 1152, 1205.
6. Friedman, M., and Freed, S. C., Microphonic manometer for the indirect determination of systolic blood pressure in the rat, *Proc. Soc. Exp. Biol. and Med.*, 1949, **70**, 670.
7. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity, *J. Biol. Chem.*, 1952, **195**, 357.
8. Conway, E. J., Microdiffusion analysis and volumetric error, London, Crosby Lockwood and Son, Ltd., 3rd revised edition, 1950, 152–164.
9. Fisher, R. A., and Yates, F., Statistical Tables, New York, Hafner Publishing Co., Inc., 3rd edition, 1948.
10. Havel, H. J., Eder, H. A., and Bragdon, J. H., The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, *J. Clin. Inv.*, 1955, **34**, 1345.
11. Sperry, W. M., and Webb, M., A revision of the Schoenheimer-Sperry method for cholesterol determination, *J. Biol. Chem.*, 1950, **187**, 97.
12. Ladd, M., and Raisz, L. G., Response of the normal dog to dietary sodium chloride, *Am. J. Physiol.*, 1949, **159**, 149.
13. Waris, E., Studies on serum lipids and lipoproteins in hypertension, *Acta Med. Scand. Suppl. No. 337.*, 1958, **161**, 79.
14. Page, I. H., The relationship of the extrinsic renal nerves to the origin of experimental hypertension, *Am. J. Physiol.*, 1935, **112**, 166.
15. Dill, L. V., and Isenhour, C. E., Occurrence of atheroma in the aorta in rabbits with renal hypertension, *Arch. Path.*, 1942, **33**, 655.
16. Rodbard, S., and Freed, S. C., The effect of desoxycorticosterone acetate on the blood pressure of the d, *Endocrinology*, 1942, **30**, 365.
17. Wilens, S. L., The effect of postural hypertension on the development of atheromatosis in rabbits fed cholesterol, *Am. J. Path.*, 1943, **19**, 293.
18. Lewis, L. A., Green, A. A., and Page, I. H., Ultracentrifuge lipoprotein pattern of serum of normal, hypertensive and hypothyroid animals, *Am. J. Physiol.*, 1952, **171**, 391.
19. Stamler, J., and Katz, L. N., The effect of "salt" hypertension on atherosclerosis in chicks fed mash without a cholesterol supplement, *Circulation*, 1951, **3**, 859.
20. Meneely, G. R., Tucker, R. G., Darby, W. J., and Auerbach, S., Chronic sodium chloride toxicity in the albino rat. II. Occurrence of hypertension and of a syndrome of edema and renal failure, *J. Exp. Med.*, 1953, **98**, 71.
21. Meneely, G. R., and Ball, C. O. T., Experimental epidemiology of chronic sodium chloride toxicity and the protective effect of potassium chloride, *Am. J. Med.*, 1958, **25**, 713.

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22. Deming, Q. B., Mosbach, E. H. Bevans, M., Daly, M. M., Abell, L. L., Martin, E., Brun, L. M., Halpern, E. and Kaplan, R., Blood pressure, cholesterol content of serum and tissues, and atherogenesis in the rat. *J. Exp. Med.*, 1958, **107**, 581.
23. Donomae, I., Matsumoto, Y., Kokubu, T., and Koide, R., Production of coronary heart disease in the rabbit by lanolin feeding, *Circ. Research*, 1957, **5**, 645.
24. Watkins, A. L., and Fulton, M. N., The effect of fluids given intra peritoneally intravenously and by mouth on the volume of thoracic duct lymph in dogs, *Am. J. Physiol.*, 1938, **122**, 281.
25. Simmonds, W. J., The effect of fluid, electrolyte and food intake on thoracic duct lymph flow in unanaesthetized rats, *Australian J. Exp. Biol. Med. Sc.*, 1954, **32**, 285.

THE ROLE OF SALT IN THE FALL OF BLOOD PRESSURE ACCOMPANYING REDUCTION IN OBESITY*

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THERE is considerable evidence that the incidence of hypertension is significantly greater among obese adults than among similar nonobese persons.[1,2] Numerous studies have been published indicating that when weight reduction is brought about in such cases a fall in the elevated blood pressure frequently follows.[3-13] Although this extensive list suggests a remarkable agreement among observers, at least two authors have expressed doubt that a decline in weight is responsible for a decline in the high blood pressure of obese subjects.[14,15]

For some years one of us (L.K.D.) has been interested in the relation of sodium to the hypertensive process in human beings, not only in the mechanism by which sodium restriction produces its blood-pressure-lowering effect but also, more particularly, in the accumulated evidence that excess sodium ingestion is the primary etiologic factor in the develop-

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ment of essential hypertension.[16-23] During the course of repeated prolonged twenty-four-hour collections of urine on ambulatory subjects for the purpose of estimating sodium intake by measurement of urinary sodium output,[24] several persons were found whose sodium intakes had changed sharply. On one such subject in whom the collections were made approximately a year apart, the average level dropped from over 20 gm. to about 5 gm. of sodium chloride per day. Inquiry revealed that he had placed himself on a moderate reducing diet in the interval, because of obesity. This marked fall in salt intake with only moderate decrease in caloric intake suggested that with the drastic caloric restriction commonly used in the studies alluded to above (400 to 600 calories per day), salt intake might be lowered correspondingly. We were aware of the reports summarized by Keys et al.[25] indicating that in some situations of semi-starvation, salt intake, instead of decreasing, appeared to have increased. However, the abnormal social, psychologic and physiologic conditions of famine bear no necessary relation to the condition of individual obesity reduction. In reviewing the reports cited above relating weight reduction to blood-pressure reduction, it was noted that many of the authors had found that the blood pressure often fell strikingly during the first few weeks on the low-calorie diet, long before significant weight loss had occurred. The fall in blood pressure was generally assigned to "caloric deficit." However, from the descriptions, this was the precise pattern frequently observed by the authors in hypertensive subjects after salt (that is, sodium) limitation under otherwise stable conditions.

As a result of these considerations, it was postulated that not weight loss but curtailment in salt intake was the effective means of lowering blood pressure in obesity-reduction programs. The data presented in this paper suggest that this is highly probable.

Material

The 12 ambulatory adult patients in this study were hospitalized on a metabolic ward of the Brookhaven Laboratory research hospital throughout the entire investigations reported here. Eleven patients had "essential" hypertension, which had been present for two to fourteen years. The diagnosis was confirmed at this hospital after prolonged examination and evaluation. Details of the general clinical and laboratory evaluation have been omitted except as seemed pertinent. No patient had evidence of serious renal disease, and intravenous pyelograms were normal in all; none had had heart failure or edema although some variable degree of cardiac enlargement was evident by x-ray as well as electrocardiographic changes, primarily in T waves; all had negative phentolamine tests for pheochromocytoma. All these patients were about 20 to 100 per cent in excess of their ideal weight.

The 12th subject (C.H., Table 1) was a grossly obese woman of fifty-two who weighed 179 kg. (394 pounds) during the control period, a weight more than three times her ideal. Although mild hypertension (blood pressure of 164 systolic, 102 diastolic)

Table 1. *Data in Obese Patients with Essential Hypertension Who Had Drastic Caloric Restriction but No Salt Restriction.*

Patient	Age	Sex	Study	Hospital Day (Ended)	Weight: Control	Weight: Ideal	Weight: Control/Ideal	Weight: At end of study	Weight: Difference from control weight	Weight: % control	Weight: % ideal	Blood Pressure*: Systolic	p	Blood Pressure*: Diastolic	p
	yr.				kg.	kg.	%	kg.	difference from control weight	% control	% ideal	mm. Hg.	p	mm. Hg.	p
C.H.†	52	F	Control, high sodium chloride	28	179.4	56.9	315					164(15)		102(14)	
			Low calorie, high sodium chloride	410				86.5	92.9	48	152	135(6)	<0.01	70(5)	<0.01
W.I.	48	F	Control, high sodium chloride	40	106.2	52.4	203					141(10)		90(10)	
			Low calorie, high sodium chloride	168				80.2	26.0	75	153	161(9)‡	<0.01	103(5)	<0.01
P.A.	58	M	Control, high sodium chloride	40	74.7	60.0	125					242(13)		122(8)	
			Low calorie, high sodium chloride	88				66.6	8.1	89	111	249(10)	<0.1	124(8)	>0.5
S.E.	53	M	Control, high sodium chloride	40	78.8	66.0	119					162(9)		105(8)	
			Low calorie, high sodium chloride	107				66.0	12.8	84	100	172(13)	<0.05	113(6)	<0.05

*Mean blood pressures calculated from last 10 daily values at end of each study period. Figures in parentheses standard deviation from mean. p = probability that successive mean values differ from each other (calculated from Fisher's[31] "t" test).
†Diagnosis of hypertension open to question in this grossly obese woman (see text).
‡Significant *elevation* of blood pressure over preceding period.

was found during the four weeks of the control period, the diagnosis of essential hypertension is clearly open to doubt, in view of the problem of measuring of blood pressure in such a patient. Several investigators have shown that in obese arms the auscultatory technic may give higher values than the intra-arterial measurement.[26,27] For obese patients we have used especially designed cuffs, embodying the principles suggested by Ragan and Bordley,[26] with greater width and length in the rubber bag. We have found that the use of a snug elastic bandage after the cuff is applied is helpful in keeping the cuff from moving downward on the conical arm of the obese person. In nonobese subjects this additional bandage does not affect the reading obtained.

The 4 patients in Table 2 were selected from among 13 similar overweight hypertensive women, all of whom were studied in the four years before the present work when the effect of sodium limitation rather than weight loss was being investigated. These 4 women are representative of the group. Other investigators who have worked with overweight subjects will appreciate the difficulty in maintaining admission weights exactly; despite all feasible efforts to ensure eating sufficient food to prevent weight loss, in our experience the usual obese patient complains so loudly and frequently of being "overfed" that occasional decreases in intake must be allowed, with resulting slight loss of weight. In the light of present experience, these losses, which generally were of the order of 1 to 3 per cent of admission weight, were so small that they may be disregarded as having any effect on blood pressure. The other 8 subjects studied here represent sequential admissions selected only on the basis of obesity and (supposed) hypertension.

TABLE 2. *Data in Obese Patients with Essential Hypertension Who Had Drastic Salt Restriction without Caloric Restriction.*

Patient	Age	Sex	Study	Hospital Day (Ended)	Weight: Control	Weight: Ideal	Weight: Control/Ideal	Weight: At end of study	Weight: Difference from control weight	Weight: % control	Weight: % ideal	Blood Pressure*: Systolic	Systolic p	Diastolic	Diastolic p
	yr.				kg.	kg.	%	kg.				mm. Hg.		mm. Hg.	
D.I.	45	F	Control, high sodium chloride†	49	69.9	58.2	120					161(8)		101(7)	
			Low sodium chloride‡	104				69.5	0.4	99	119	126(11)	<0.01	83(8)	<0.01
C.O.	39	F	Control, high sodium chloride†	35	69.8	56.0	125					162(10)		111(9)	
			Low sodium chloride‡	95				67.1	2.7	96	120	130(6)	<0.01	94(6)	<0.01
H.O.	54	F	Control, high sodium chloride†	38	71.5	55.0	130					207(11)		108(6)	
			Low sodium chloride‡	96				70.0	1.5	98	127	143(10)	<0.01	84(7)	<0.01
K.R.	52	F	Control, high sodium chloride†	46	81.3	56.9	143					190(20)		90(9)	
			Low sodium chloride‡	101				76.0	5.3	93	134	140(19)	<0.01	73(8)	<0.01

*Mean blood pressures calculated from last 10 daily values at end of each study period. Figures in parentheses standard deviations from mean. p = probability that successive mean values differ from each other (calculated from Fisher's[21] "t" test).
†10 gm. of enteric-coated sodium chloride/day (4000 mg. of sodium) plus basic low-sodium diet.
‡115 mg. of sodium/day (by analysis).

METHODS

Diet

On admission all patients were placed on a basic control low-sodium diet, which was weighed and calculated daily and which contained (by analysis) approximately 5 milliequiv. (115 mg.) of sodium per day. This amount of sodium restricted protein in the food to 45 to 55 gm. per day so that supplemental low-sodium protein was given as either Lesofac* or Lonalac† to make a total of 1.5 gm. of protein per kilogram of body weight. Carbohydrates and fats were added in sufficient amounts to satisfy appetite and maintain admission weight. Supplemental salt was taken as enteric-coated tablets, 10 gm. (170 milliequiv.) daily during the "high-sodium" phases of this program. This could be added or subtracted without otherwise changing the food intake.

The low-sodium, low-calorie reducing diets ordinarily contained 600 to 800 calories per day and 85 to 105 mg. of sodium, with a minimum of 1 gm. of protein per kilogram of ideal weight and the remaining calories in fat and carbohydrate. The low-sodium, high-calorie diets consisted of the basic control diet,

*Kindly supplied by Wyeth Laboratories, Incorporated, Philadelphia.
†Available from Mead Johnson and Company, Evansville, Indiana.

to which were added sodium-free carbohydrates and fats in quantities sufficient to ensure a slow, steady weight gain.

It was our aim to make but a single change at a time in the regimen — that is, calories or salt — so that the effects of changes in weight and salt could be separated clearly. In 2 subjects this was not possible. Patient J.E. (Table 3) was reduced 11 per cent of his control weight on high salt intake; but since this weight was still above his ideal weight, he requested to be allowed to reduce further during the period of salt limitation. The additional weight loss after salt restriction was 5.1 kg. as compared with a 10.6-kg. loss before salt limitation. Patient O'L. (Table 3) underwent the same program as the preceding patient, but several weeks before salt restriction was begun, after she had lost more than 20 per cent of her admission weight, she became depressed and complained of excessive fatigue. This continued unchanged when salt was restricted. In view of the recent report by Stunkard[28] that dieting precipitated severe emotional disorders in 9 of 25 obese subjects, it was thought to be in the best interests of the patient to augment the caloric intake. Therefore, on the hundred and seventy-eighth hospital day, fifteen days after she had gone on a low salt intake, her caloric intake was increased from 600 to 1600 calories, but her salt was not augmented: with this additional food her weight loss stopped and the fatigue and depression disappeared rapidly.

Three of the patients in Table 1 left the hospital earlier than planned so that salt restriction could not be carried out after weight had been reduced. The 4th patient (S.E.) is being studied as part of a continuing program to determine the effects of semi-starvation on the blood pressure of hypertensive persons; a separate report of the effects of drastic weight reduction (to well below ideal levels) will be made on this man and on 2 other patients investigated in similar fashion.

TABLE 3. *Data in Obese Patients with Essential Hypertension Who Had Drastic Salt Restriction, Preceded or Followed by Caloric Restriction.*

Patient	Age	Sex	Study	Hospital Day (Ended)	Weight: Control	Weight: Ideal	Weight: Control/Ideal	Weight at end of study				Blood Pressure*: Systolic		Blood Pressure*: Diastolic	
	yr.				kg.	kg.	%	kg.	difference from control weight	% control	% ideal	mm. Hg.	p	mm. Hg.	p
D.R. (1st admission, 1954)	54	F	Control, high sodium chloride	25	107.1	56.9	189					255(23)		135(20)	
			Low sodium chloride	97				102.3	4.8	96	180	157(11)	<0.01	83(9)	<0.01
(2d admission, 1957)			Control, high sodium chloride	34	114.7	56.9	201					173(17)		90(7)	
			Low calorie, high sodium chloride	121				95.3	19.4	83	168	182(22)	0.3	95(8)	<0.2
J.E.	42	M	Control, high sodium chloride	30	93.5	72.4	129					164(14)		110(6)	
			Low calorie, high sodium chloride	81				82.9	10.6	89	114	158(10)	>0.3	110(4)	>0.5
			Low calorie, low sodium chloride	117				77.8	5.1	83	108	145(9)	<0.01	101(6)	<0.01
J.A.	56	M	Control, high sodium chloride	50	82.6	51.4	161					197(13)		103(8)	
			Low sodium chloride	106				82.6	± 0	100	161	163(9)	<0.01	92(5)	<0.01
			Low sodium chloride, low calorie	198				73.0	9.6	88	142	183(13)	<0.01	94(7)	>0.1
O'L.	49	F	Control, high sodium chloride	29	84.9	55.0	154					196(9)		98(7)	
			Low calorie, high sodium chloride	163				63.9	21.0	75	116	181(5)	<0.01	94(5)	>0.1
			Low calorie, low sodium chloride	191				59.8	4.1	70	109	124(6)	<0.01	73(6)	<0.01

*Mean blood pressures calculated from last 10 daily values at end of each study period. Figures in parentheses standard deviation from mean. p=probability that successive mean values differ from each other (calculated from Fisher's[31] "t" test).

Hospital Regimen

All patients were ambulatory throughout their period in the hospital except during a few hours for special studies or occasional intercurrent illnesses. They were weighed daily, nude, after voiding, and

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before breakfast on a special balance sensitive and accurate to 25 gm. Blood pressures were measured by us six mornings a week under standard conditions.

Experimental Procedure

During the preliminary control periods, which lasted approximately four to seven weeks, all patients were on a "high sodium" intake (10 gm. per day), with sufficient calories to maintain admission weight. After both blood pressure and weight seemed to have become stabilized, either salt or food intakes were altered as described earlier. The patients were continued at the new levels usually for periods of several months although occasionally less, when time did not permit.

Calculations

The study weights shown in Tables 1-3 were those recorded in the morning of the hospital day indicated. Ideal weights were derived from tables of ideal weights for men and women.[29,30] For both sexes, the ideal weights were calculated for the mid-point of the range for the body build and height of the person under consideration; since these published "ideal" weights were obtained from clothed persons whereas our subjects were weighed nude, we arbitrarily subtracted 1.3 kg. (3 pounds) from the values derived from the tables.

The figures for blood pressure represent the average of the last ten morning values recorded in a regimen; P values for the significance of the difference between two successive means was calculated from the student's "t" test.[31] The average pressures at the end of two consecutive regimens were considered here as being significantly different when p values of less than 0.01 were obtained; many of the p values derived from the t test were far less than 0.01, but these all were assigned nominal values of less than 0.01 in the tables; p values of less than 0.05 were interpreted to be of doubtful significance. Therefore, in this paper if a change in blood pressure is said to be significant without further qualification, this may be assumed to indicate that p is less than 0.01.

Results

The results are summarized in Tables 1-3, in which the patients have been divided into three groups on the basis of similar hospital experience.

Obese Patients Who Had Drastic Salt Restriction without Caloric Restriction (Table 2)

As stated earlier these 4 women were chosen from a group of 13 studied in the same fashion before the current investigations began. They are presented primarily to demonstrate that in obese hypertensive subjects, salt restriction without significant weight loss is capable of producing a significant decline in blood pressure. It may be of interest that among the 13 women studied similarly, 8 had a fall in both systolic and diastolic pressures ($p < 0.01$) after salt limitation; a 9th had attained normal pressure by the end of her control period; the remaining 4 patients had no fall or a decline in only the systolic or diastolic pressures. Such a high percentage of responses is unusual in our considerable experience with salt limitation in hypertension: ordinarily we expect between a quarter and a third of the subjects to have a significant drop in both systolic and diastolic pressures. In their sensitivity to salt restriction these obese patients resembled those described by Schroeder[32] as having the "endocrine hypertensive syndrome." We did not attempt to evaluate the patients from this standpoint during the course of our studies, but re-examination of the records and laboratory data suggests that although some might be so classified, others could not; yet there was no clear-cut difference in sensitivity to salt restriction. Since our numbers are relatively small elaboration of this point does not seem warranted at present.

Obese Patients Who Had Drastic Caloric Restriction but no Salt Restriction (Table 1)

After losing 93 kg. during her thirteen months in the hospital patient C.H. had a lower blood pressure than during her control period. As stated earlier the original diagnosis of hypertension must remain in doubt in view of the gross obesity of her arms before weight loss. One of the other patients (W.I.) had a clear-cut rise in pressure despite a 26-kg. weight loss (25 per cent of control weight); the 3d subject (P.A.) had no change in blood pressure after losing 8.1 kg. (11 per cent of control weight). Patient S.E. was reduced to his ideal weight by the loss of 12.8 kg. (19 per cent of control weight), with no fall in pressure. Thus, in this group in which salt was not restricted, the 3 subjects with unequivocal hypertension failed to have a reduction in blood pressure associated with a reduction in weight.

Obese Patients Who Had Drastic Salt Restriction, Preceded or Followed by Caloric Restriction (Table 3)

Patient D.R., who had two admissions approximately two years apart, responded dramatically to salt limitation in 1954 with a weight loss of only 4.8 kg. (4 per cent of control). She was readmitted to the hospital two years later, weighing more than on her first visit; despite her high caloric intake while at home, she maintained that she had stayed on a salt-restricted diet, and the blood pressure during the first week or two in the hospital was similar to that upon discharge two years before. By the end of her control period, after five weeks on "high sodium," the level had risen significantly. Three months later, while she was still on salt but after she

had lost 17 per cent (19.4 kg.) of her admission weight, the blood pressure may have risen further and certainly had not fallen. Patient J.E., during fifty-one days of the "high-sodium," low-calorie regimen, had no fall in blood pressure despite an 11 per cent (10.6 kg.) weight loss; during the subsequent thirty-six days, when he was allowed both to reduce (as described above under Methods) further (17 per cent of control) and to have salt restricted, the blood pressure dropped. In this case although the effect of salt restriction and weight loss cannot be separated unequivocally, the blood pressure did not fall until salt was restricted.

Patient L.A., after salt restriction without weight loss, had a significant fall in blood pressure. The continued restriction of salt with a weight loss of 9.6 kg. (12 per cent of control) was associated with a rise in systolic pressure whereas the diastolic pressure was unchanged.

Patient O'L. had a modest but statistically significant fall in systolic pressure with a weight loss of 21 kg. (25 per cent of control), while on the "high-sodium regimen." After salt limitation, however, both systolic and diastolic pressures fell dramatically to normal and continued during a period when weight reduction had ceased (as described above under Methods). The clinically significant blood-pressure changes here clearly resulted from the salt restriction and not from the weight loss.

In sum, only 1 of these 4 patients had a significant fall in blood pressure (systolic only) with weight loss. All subjects had significant declines in blood pressure (systolic and diastolic) after salt intake was restricted.

The results obtained in this study suggest that in obese hypertensive patients reduction of weight uncommonly leads to reduction of blood pressure unless salt intake (in the form of sodium chloride) is also reduced. Weight reduction alone was associated with a decline in both systolic and diastolic pressures in only 1 patient in this study (C.H., Table 1). This woman was so grossly obese (three times the ideal weight) on admission as to make the original diagnosis of essential hypertension suspect, because of the difficulty in measuring the blood pressure in obese arms. We suggest that when hypertensive patients who are also obese go on a weight-reduction diet, salt intake ordinarily is reduced *pari passu* with food intake. When a fall in blood pressure follows the institution of a weight-reduction program, it is due to this restriction of salt and not the restriction of calories.

As mentioned earlier, the term "caloric deficit" has been used to explain the fall in blood pressure frequently observed before significant weight loss in the obese hypertensive patient subjected to caloric restriction. The present study lends no support to this thesis. However, it is fair to ask whether other items than restriction of salt — by which restriction of sodium is actually implied — might have effected the changes observed here. The lowering of blood pressure in these patients bore a temporal relation to antecedent salt restriction that resembled precisely the response that we have observed in nonobese hypertensive patients studied during the past ten years.[16-20] Furthermore, prolonged metabolic observations[16-18,33] indicated that wide variations in the intake of chloride, protein, fat and water were ineffective in decreasing the blood pressure of patients with hypertension, in the absence of sodium restriction. Although it is possible that other, unknown, factors were restricted in the present study simultaneously with restriction of sodium chloride (for example, trace metals) no data are presently at hand to indicate the need for invoking such a mechanism.

The evidence here suggests that the hypertension of the obese person is essentially the disease that exists in the nonobese. Green and Beckman[11] reached a similar conclusion from a study of obesity and hypertension; in their judgment the hypertensive process was caused by the same factors in obese and nonobese persons, and the obesity per se was not a causative factor in the hypertension. We are in accord with this position. Our interpretation of the relation between obesity and hypertension is that in eating more food generally, the obese person regularly consumes more salt. It is the amount of salt consumed and not the obesity that is the primary etiologic agent in producing the hypertension. The high percentage of obese hypertensive persons who respond with a fall in blood pressure after salt limitation found by Schroeder[32] and ourselves suggests that the obese hypertensive subject may be somewhat more sensitive to the effects of salt restriction than the nonobese. If this is true, it might follow that the obese hypertensive patient may not require drastic limitation of sodium to gain the desired effect, as the nonobese subject does, and that the obese person may be more sensitive to the hypertensive-producing effects of excess salt ingestion. These problems will be investigated.

Summary and Conclusions

Reduction of weight in 8 obese hypertensive persons was uncommonly associated with a fall of blood pressure. The single subject whose blood pressure appeared to fall with weight reduction alone was so obese as to make the original diagnosis of hypertension in doubt. In this study, fall of blood pressure appeared to be closely correlated with curtailment of salt intake (in the form of sodium chloride). There was some evidence that the obese hypertensive subject was more sensitive to salt restriction than the nonobese. It was suggested that the obese person, in eating more food, also ate more salt and hence had more hypertension; in obesity reduction, salt as well

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as calories ordinarily were reduced, and it was the salt restriction, not the caloric restriction, that was the effective agent in lowering blood pressure.

This work could not have been done without the cooperation of the Misses J. O'Connell, R.N., and N. Gillespie, dietitian.

References

1. Robinson, S. C., Brucer, M., and Mass, J. Hypertension and obesity: statistical and clinical study of 10,883 individuals. *J. Lab. & Clin. Med.* **25**:807-822, 1940.
2. Levy, R. L., White, P. D., Stroud, W. D., and Hillman, C. C. Overweight: its prognostic significance in relation to hypertension and cardiovascular-renal diseases. *J.A.M.A.* **131**:951-953, 1946.
3. Rose, R. H. Weight reduction and its remarkable effect on high blood pressure. *New York M. J.* **115**:752-755, 1922.
4. Preble, W. E. Obesity: observations on one thousand cases. *Boston M. & S. J.* **188**:617-621, 1923.
5. Bauman, L. Obesity: recent reports in literature and results of treatment. *J.A.M.A.* **90**:22-24, 1928.
6. Spencer, H. J. Clinical observations concerning obese. *M. Clin. North America* **12**:603-610, 1928-1929.
7. Master, A. M., and Oppenheimer, E. T. Study of obesity: circulatory, roentgen-ray and electrocardiographic investigations. *J.A.M.A.* **92**:1652-1656, 1929.
8. Evans, F. A., and Strang, J. M. Treatment of obesity with low caloric diets. *J.A.M.A.* **97**:1063-1069, 1931.
9. Adlersberg, D., Coler, H. R., and Laval, J. Effect of weight reduction on course of arterial hypertension. *J. Mt. Sinai Hosp.* **12**:984-992, 1946.
10. Braun, I. Complications of obesity with special reference to high blood pressure: report on 125 cases. *M. Rec.* **160**:350-353, 1947.
11. Green, M. B., and Beckman, M. Obesity and hypertension. *New York State J. Med.* **48**:1250-1253, 1948.
12. Terry, A. H., Jr. Obesity and hypertension. *J.A.M.A.* **81**:1283, 1923.
13. Fletcher, A. P. Effect of weight reduction upon blood-pressure of obese hypertensive women. *Quart. J. Med.* **23**:331-345, 1954.
14. Martin, L. Effect of weight-reduction on normal and raised blood-pressures in obesity. *Lancet* **2**:1051-1053, 1952.
15. Pomeranze, J. Recurrent obesity. *New York State J. Med.* **56**:3017-3020, 1956.
16. Dole, V. P., Dahl, L. K., Cotzias, G. C., Eder, H. A., and Krebs, M. E. Dietary treatment of hypertension: clinical and metabolic studies of patients on rice-fruit diet. *J. Clin. Investigation* **29**:1189-1206, 1950.
17. Dole, V. P., Dahl, L. K., Cotzias, G. C., Dziewiatkowski, D. D., and Harris, C. Dietary treatment of hypertension. II. Sodium depletion as related to therapeutic effect. *J. Clin. Investigation* **30**:584-595, 1951.
18. Dole, V. P., et al. Dietary treatment of hypertension. III. Effect of protein on appetite and weight. *J. Clin. Investigation* **32**:185-191, 1953.
19. Dahl, L. K., Stall, B. G., III, and Cotzias, G. C. Metabolic effects of marked sodium restriction in hypertensive patients: changes in total exchangeable sodium and potassium. *J. Clin. Investigation* **33**:1397-1406, 1954.
20. Dahl, L. K. Pressor effects of norepinephrine after drastic reduction of sodium intake. *Circulation* **15**:231-236, 1957.
21. Dahl, L. K., and Love, R. A. Evidence for relationship between sodium (chloride) intake and human essential hypertension. *Arch. Int. Med.* **94**:525-531, 1954.
22. Dahl, L. K. Evidence for increased intake of sodium in hypertension based on urinary excretion of sodium. *Proc. Soc. Exper. Biol. & Med.* **94**:23-26, 1957.
23. Dahl, L. K., and Love, R. A. Etiological role of sodium chloride intake in essential hypertension in humans. *J.A.M.A.* **164**:397-400, 1957.
24. Dahl, L. K. Sodium intake of American male: implications on etiology of essential hypertension. *Am. J. Clin. Nutrition* **6**:1-7, 1958.
25. Keys, A., Brožek, J., Henschel, A., Mickelsen, O., and Taylor, H. L. *The Biology of Human Starvation*. Vol. I. 763 pp. Minneapolis: University of Minnesota Press, 1950. P. 666.
26. Ragan, C., and Bordley, J., III. Accuracy of clinical measurements of arterial blood pressure: with note on auscultatory gap. *Bull. J. Hopkins Hosp.* **69**:504-528, 1941.
27. Trout, K. W., Bertrand, C. A., and Williams, M. H. Measurement of blood pressure in obese persons. *J.A.M.A.* **162**:970, 1956.
28. Stunkard, A. J. "Dieting depression": incidence and clinical characteristics of untoward responses to weight reduction regimens. *Am. J. Med.* **23**:77-86, 1957.
29. Ideal weights for men. *Statist. Bull. Metropolitan Life Ins.* **24**:6, 1943.
30. Ideal weights for women. *Statist. Bull. Metropolitan Life Ins.* **23**:6, 1942.
31. Fisher, R. A. *Statistical Methods for Research Workers*. Ninth edition. 354 pp. London: Oliver & Boyd, 1944. P. 116.
32. Schroeder, H. A. *Hypertensive Diseases: Causes and control*. 610 pp. Philadelphia: Lea, 1953. P. 295.
33. Hatch, F. T., et al. Effects of diet in essential hypertension. III. Alterations in sodium chloride, protein and fat intake. *Am. J. Med.* **17**:499-513, 1954.

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THE RELATION OF WATER AND ELECTROLYTES TO METABOLISM

A CONSIDERATION of the views held regarding the regulation of water by the living organism reveals an interesting fact. Most workers in this field have stressed the physico-chemical aspects of the problem and have adopted what is essentially a non-vitalistic concept of water balance. Thus Fischer[1] regarded acidosis as being the primary factor in water retention. Schade[2] has emphasized alkalosis. Gamble[3] and his co-workers considered that the total amount of water was determined by the total electrolytes present in the organisms. Starling[4] pointed out the importance of the colloid osmotic pressure of the blood. Davis and Dragstedt[5] have shown how acidosis diminished the ability of the body to retain water. All this evidence points to a mechanistic concept of water regulation. In order to examine further the truth of this purely physico-chemical explanation of

[6] Aided by a research grant from the Penrose Fund of the American Philosophical Society.

[1] N. H. Fischer, "Oedema," pp. 209, New York, 1910.

[2] H. Schade, *Ergebn. inn. Med.*, 32: 425, 1927.

[3] J. L. Gamble, N. C. Putnam and C. F. McKhann, *Am. Jour. Physiol.*, Vol. 88, p. 571.

[4] E. H. Starling, "Fluids of the Body," pp. 186, 1909.

[5] H. A. Davis and L. R. Dragstedt, *Am. Jour. Physiol.*, 109: 88, 1934.

water balance, this investigation was undertaken. The very essence of the non-vitalistic theories is exemplified by the fact that salt water is retained much better by the body than is salt-free water.

A preliminary series of over 25 dogs was used. Isotonic solutions of 0.9 per cent. NaCl solution and 5 per cent. glucose solutions were injected intravenously in amounts from 2,500 cc to 4,000 cc. The oxygen consumption of the animal was measured before, during and after the injection at standard intervals. In all the animals a considerable increase in the oxygen consumption occurred from 100 per cent. to 500 per cent. above the basal level. The increase in O_2 consumption produced by equal amounts of 5 per cent. glucose solution was greater than that produced by 0.9 per cent. sodium chloride solutions. The amount of water lost was proportional to the increase in the oxygen consumption and metabolic rate. In 3 animals in whom an increased oxygen consumption did not occur during the injection death occurred. Water given by the alimentary tract increased the consumption but slightly. This is interesting in view of the fact that fluid by mouth is retained for longer periods than is fluid given by vein. Conversely, it was found that repeated blood-letting lowered the metabolic rate. In such animals, isotonic solutions were retained for longer periods.

Moreover, the diuresis was quite limited. In the light of these findings, it might be suggested that one of the fundamental factors concerned in water regulation is the oxygen consumption rate of the body tissues. Further work is proceeding along these lines.

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Placebo Effect of Saline on Locomotor Activity in Several Strains of Mice

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The effect of intraperitoneal injection of 0.9 per cent saline solution upon locomotor activity of six strains of mice was observed in actometer cages. Whether tested singly or in groups of four, a depressant effect of saline upon activity was seen. The strains differed significantly in activity levels, but not in degree of effect of saline on activity. Group testing had differing degrees of effect on activity counts compared to single-mouse tests in the several strains.

A REDUCTION in the locomotor activity of mice which received an injection of physiological saline in comparison to the activity of uninjected mice has been reported by Schnitzer and Ross (1). They found that this effect was dependent upon more than the act of handling the mouse or handling plus intraperitoneal insertion of a hypodermic needle. Meier (2) confirmed their findings with 0.9% saline group compared to a sham-injected group even while using only one-half the volume (0.005 ml./Gm.) administered by the former workers. Schnitzer and Ross (3) later failed to observe an inhibitory effect of saline when they used younger mice than those of their first report; however, testing at a higher room temperature may have been responsible for the difference. Meier's confirmation was by means of younger mice than were used in either of the other reports.

Meier *et al.* again reported inhibition of locomotion by saline injection incidental to other observations involving four inbred strains of mice (4). They stated that the ranking of post-saline activities of the strains was significantly different from the ranking of activities in a previous noninjection test, but without presenting fully the data to describe this finding. Further evidence regarding the genetic factor as a possible variable in the "placebo effect" of saline in-

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TABLE I—EFFECTS OF SALINE INJECTION ON LOCOMOTOR ACTIVITY OF SIX STRAINS OF MICE TESTED SINGLY OR IN GROUPS OF FOUR

Mean 30 min Activity Count[a] (Log Transformed)					
Single Mice			Grouped Mice		
Strain	No Inject.	Saline	Strain	No Inject.	Saline
NLW	2.990	2.810	NLW	3.522	3.109
C57BL/6	2.948	2.901	C57BL/6	3.452	3.360
DBA/2	2.931	2.855	C3H/An	3.381	3.331
BALB/c	2.913	2.905	CBA	3.335	3.353
C3H/An	2.847	2.796	DBA/2	3.324	3.235
CBA	2.778	2.714	BALB/c	3.322	3.300

[a] N = 12 in all cases.

RESULTS

Locomotor activity values under the several treatment conditions are shown in Table I. Results of the analysis of variance performed on the log-transformed data[4] are shown in Table II. The analysis revealed significant main effects of both saline injection ($p < 0.005$) and of strains ($p < 0.005$), and quite expectedly, of grouping ($p < 0.005$).

In view of the nonsignificant interaction of injection and strains, further analysis of the injection effects within strains was unwarranted. Further details of the between-strains differences contributing to the significant strains effect were determined by means of Duncan's multiple range test (8). This test was performed separately on the data for singly-tested and group-tested mice because of the significant interaction of strains and groupings, but after combining injected and noninjected groups in view of the nonsignificant interaction of injection with strains. Results of these multiple comparisons between strains are given in Table III. The significant interaction between grouping and strains, along with obvious difference in ordering of the strains under single and group tested conditions (Table III) suggested also the application of Duncan's test to the differences for each strain between values of single and grouped mice. The results of this test (Table IV) indicate that in addition to the differences in motility between strains as shown in Table III there is a significant difference in the effect of grouping on motility in different strains. The obvious and expected increase in counts when four mice are tested rather than one was in fact significantly more pronounced in the CBA, NLW, and C3H/An strains than in the DBA/2 and BALB/c. This suggests that the increase of counts with grouping is, for some strains at least, not merely a physical phenomenon as a consequence of the increased number of mice in the actometer, but also a behavioral phenomenon involving social factors.

TABLE II—ANALYSIS OF VARIANCE ON LOG-TRANSFORMED ACTIVITY DATA

Term	Degrees of Freedom	Sum of Sq.	Mean Sq.	F	p
Treatments	(23)	19.381	0.842	30.07	<.005
Injection	1	0.280	0.280	10.00	<.005
Grouping	1	17.763	17.763	634.39	<.005
Strains	5	0.695	0.139	4.96	<.005
Injection × grouping	1	0.001	0.001	<1	N.S.
Injection × strains	5	0.115	0.023	<1	N.S.
Grouping × strains	5	0.493	0.098	3.50	<.01
Injection × grouping × strains	5	0.034	0.006	<1	N.S.
Error	264	7.482	0.028	...	...
Total	287	26.863	0.093	...	...

jection on locomotor activity of mice seemed desirable. Since spontaneous locomotor activity is well known as a behavioral phenotype which is influenced by genetic differences between strains of laboratory mice (5, 6), it is of particular interest whether the effect of saline injection would occur independently of genotype. As all the previous reports of this phenomenon were based on observations of individual mice, it also seemed desirable to test for the effect in the case of small groups as are often used in studying locomotor effects of drugs. In summary, locomotor activity of groups of four mice and of single mice from six strains under noninjection and saline-injection conditions have been compared.

EXPERIMENTAL

Observations of locomotor activity were made in three commercially manufactured[1] photocell activity devices (7). All actometer units were exposed to each level of all the experimental variables. The number of counts, each representing the interruption of one of the six light beams distributed symmetrically around the ring-shaped track, was recorded by digital counters.

Inbred male mice of the C57BL/6, DBA/2, C3H/An, CBA, and BALB/c strains[2] and random-bred male mice of a Swiss-Webster albino strain (NLW)[3] were received at about 30 days of age and were between 8 and 12 weeks old when 60 from each strain were used in these experiments. A 0.9% saline solution in distilled water was administered intraperitoneally in a volume of 0.01 ml./Gm. Locomotor activity recording was begun immediately after injection and continued for 30 min. The handling of noninjected mice was approximately the same as for the saline group, but sham-injection (*i.e.*, needle penetration) was not performed as Schnitzer and Ross (1) had found needle insertion had little or nothing to do with reduction of activity.

[1] Woodard Research Corp., Herndon, Va.
[2] Cumberland View Farms, Clinton, Tenn.
[3] National Laboratory Animal Co., Creve Couer, Mo.

DISCUSSION

The earlier reports (1, 2, 4) of the activity-inhibiting effect of intraperitoneal saline treatment in mice are further confirmed and extended by these positive results. The question of age as a factor in the effect which was suggested by one negative report (3) is contradicted since the mice used in this case were also considerably younger than those of the initial report (1). An additional attribute of this

[4] Log-transformation was performed in order to eliminate the proportionality between the means and variances of single mice *versus* groups of mice that occurred in the untransformed data.

TABLE III—RANKING AND COMPARISON OF MEAN LOCOMOTOR ACTIVITY OF SIX STRAINS OF MICE COMBINED ACROSS NONINJECTION AND SALINE TREATMENTS

	Mice Tested Singly*					
Strain	CBA	C3H/An	DBA/2	NLW	BALB/c	C57BL/6
Activity	2.733	2.821	2.894	2.900	2.908	2.926

	Mice Tested in Groups*					
Strain	DBA/2	BALB/c	CBA	C3H/An	C57BL/6	NLW
Activity	3.820	3.311	3.344	3.356	3.406	3.465

* Values not sharing underline differ significantly ($p < 0.05$).

TABLE IV—COMPARATIVE EFFECTS OF GROUPING ON ACTIVITY IN SIX STRAINS MEASURED AS MEAN DIFFERENCES FROM SINGLY TESTED MICE*

Strain	DBA/2	BALB/c	C57BL/6	C3H/An	NLW	CBA
Difference	0.386	0.403	0.480	0.535	0.565	0.611

* Values not sharing underline differ significantly ($p < 0.05$).

phenomenon indicated by these data is its occurrence in mice tested in groups, as well as those tested singly. Although the present observations include four of the same inbred strains utilized in the study by Meier *et al.* (4), the data do not confirm their implication that the effect of saline treatment varies between strains. While there was a superficial suggestion in the untransformed data that the degree of response to saline might differ between strains, the nonsignificant injection x strain interaction term failed to support such an inference. Any such trend surely was not consistent enough to be detected by the analysis.

The pharmacological basis for the phenomenon of saline inhibition of activity remains quite unknown. The authors have termed it a placebo effect following the definition of Wolf (9), "...any effect attributable to a pill, potion or procedure, but not to its pharmacodynamic or specific properties." It surely cannot have the same explanation as that given for a placebo effect of saline injection upon locomotor activity of rats (10), *i.e.*, simple Pavlovian conditioning of the depressant effect of a previously administered pharmacologically active agent, scopolamine.

REFERENCES

(1) Schnitzer, S. B., and Ross, S., *Psychol. Rept.*, **6**, 351 (1960).
(2) Meier, G. W., *ibid.*, **11**, 333(1962).
(3) Schnitzer, S. B., and Ross, S., *ibid.*, **8**, 142(1961).
(4) Meier, G. W., Hatfield, J. L., and Foshee, D. P., *Psychopharmacologia*, **4**, 81(1963).
(5) Thompson, W. R., *Can. J. Psychol.*, **7**, 145(1953).
(6) Thompson, W. R., *J. Heredity*, **47**, 147(1956).
(7) Wright, L. S., Jr., Horn, H. J., and Woodard, G., *Federation Proc.*, **21**, 420(1962).
(8) Duncan, D. B., *Biometrics*, **11**, 1(1955).
(9) Wolf, S., *Pharmacol. Rev.*, **11**, 689(1959).
(10) Herrnstein, R. J., *Science*, **138**, 677(1962).

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Response of New-born Children to Hypertonic Solutions of Sodium Chloride and of Urea

FROM all that is known about the renal function of new-born infants, it is now clear that their glomerular filtration-rates are considerably lower than those of adults, and also their clearances of urea and sodium chloride[1]. These discoveries have an important bearing on clinical practice, but much more information is required about the way in which the kidney of an infant reacts to changes in the internal environment. Accordingly, some experiments have been made to study the response of the kidney in new-born infants to a considerable rise in the osmotic pressure of the body. The children used for these experiments had all been born with inoperable meningo-myelocœles. Healthy adults have been used as controls.

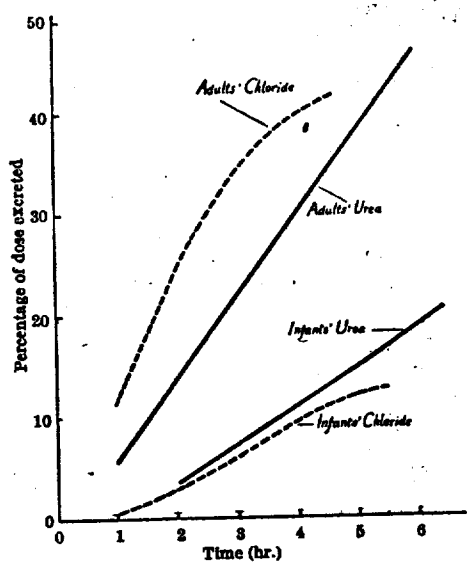
All the subjects were deprived of water for about 12 hours before the tests began. Sufficient sodium chloride was then given intravenously as a 10 per cent solution to raise the serum concentration of chlorine to about 135 m.eq./l. in the adults and to about 147 m.eq./l. in the infants. The results may be summarized as follows:

(1) A diuresis was produced in both infants and adults.

(2) On a basis of surface area, this diuresis was much smaller in infants than in adults. The greatest rate of flow in an infant has so far been 2·7 c.c./1·73 sq. m./min. All adults have exceeded a rate of 7·6 c.c./1·73 sq. m./min., and one has reached a value of 19·1 c.c./1·73 sq. m./min.

(3) The administration of sodium chloride tended to raise rather than lower the osmotic pressure of the urine in infancy, but it greatly reduced the osmotic pressure of the urine in adults[2].

(4) The infants excreted the administered sodium chloride much more slowly than adults (see graph). In one hour they excreted less than ·1 per cent of the dose given, and in four hours 9·5 per cent. In the same times adults excreted 11 and 40 per cent of the dose. When comparisons were made on the basis of surface area, it was found that the maximum rate of excretion of chloride by adults was eight times that of infants. A feature of the experiments in both adults and infants has been that the diuresis produced by the salt solutions fell off while the concentration in the plasma was still high. No satisfactory explanation of this can be suggested at present.



Urea has been given by mouth as a concentrated solution to adults and infants in quantities sufficient to raise the blood urea to about 30 m.mol./l. The response at both ages has had the same general characters as the response to sodium chloride (see graph), but the differences between the adults and the infants have not been so great and the maximum output of urea by the adults per unit of surface area has not been more than six times as great as that of the infants. The diuresis, moreover, which at its peak was only about one third as great as that produced by the salt solutions, declined more slowly and the rate of excretion of the urea was better maintained.

It is evident that the kidneys of human infants respond better than those of new-born rats to the administration of strong solutions of sodium chloride and urea[3], but that the responses are essentially similar in nature. It is likely that these signs of immaturity will be demonstrable in all young mammals, but that no two species will show them to the same degree.

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[1] McCance, R. A., and Young, W. F., *J. Physiol.*, 99, 265 (1941).
[2] Hervey, G. R., McCance, R. A., and Taylor, R. G. O., *Nature*, 157, 338 (1946).
[3] McCance, R. A., and Wilkinson, E., *J. Physiol.*, 106, 256 (1947).

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Production of Potassium-Induced Gastrointestinal Lesions in Monkeys[1]

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Primary nonspecific ulceration of the small intestine is an infrequent lesion in the human. A review by Watson (1963) produced only 170 case reports. However, during the summer of 1964, additional cases of nonspecific stenosing ulceration were reported from the Hennepin County General Hospital in Minnesota. Shortly thereafter two publications appeared, one by Lindholmer *et al.* (1964) and the other by Baker *et al.* (1964), which drew attention to the fact that small bowel ulcerations might be associated with the oral administration of enteric-coated potassium chloride or thiazides with enteric-coated potassium chloride.

Animal studies were begun immediately in our laboratory in an effort to find a counterpart of the lesions seen in the humans. Dogs were used first, but results in this species were only suggestive, not conclusive. However, experiments in the rhesus monkey in which large doses of potassium chloride were administered orally produced intestinal lesions similar to those seen in the human. These lesions could be reproduced so consistently that a screening procedure was set up for the testing of various potassium formulations which might be less irritating to the intestinal tract than those commonly used. Factors such as tablet size, dose-response, and possible synergistic effects of thiazides and tablet coatings were studied. The results of these experiments are reported here.

METHOD

Acclimated rhesus monkeys (*Macaca mulata*) weighing 4–6 kg were housed in individual cages in an air-conditioned room at a temperature of 80°F and a relative humidity of 50%. The animals had free access to water and were fed commercial monkey crackers supplemented with vitamins and fruit once a day. All monkeys were medicated twice daily, at 10:30 A.M. and again at 3:30 P.M. Tablets were placed in the back of the mouth near the glottis by means of forceps; liquids were administered by stomach tube. Medication was continued for 4½ days, and the monkeys were sacrificed at the end of the fifth day. At necropsy all gross lesions were noted; the entire gastrointestinal tract was first examined *in situ*, then removed from the carcass, slit open, and again examined. Multiple sections of the stomach, duodenum, jejunum, ileum, ileocecal valve, cecum, and colon were fixed in Bouin's solution for histopathologic examination. Tissues were embedded in paraffin and stained with hematoxylin and eosin.

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RESULTS

Since most of the lesions described in humans were supposedly caused by enteric-coated tablets[2] (Lachman and Cooper, 1963) containing thiazides plus potassium chloride, the specific ingredient causing the ulcerations had to be determined first. The results achieved in monkeys are summarized in Table 1, and it can be seen that gross

TABLE 1
RESPONSE OF THE GASTROINTESTINAL TRACT OF MONKEYS TO THE ADMINISTRATION OF ENTERIC-COATED TABLETS OF VARIOUS COMPOSITION

Number of monkeys	Medication[a]	Occurrence of gross lesions		
		Stomach	Small intestine	Cecum, large intestine
2	No medication	None	None	None
2	Enteric-coated placebo	None	None	None
2	Enteric-coated thiazide	None	None	None
2	Enteric-coated potassium chloride (1000 mg)	None	Ulceration	Hyperemia, hemorrhage
2	Enteric-coated thiazide (50 mg) and potassium chloride (1000 mg)	None	Ulceration	None

[a] Two tablets were administered twice daily for 4½ days. Necropsy was performed 2–4 hours after last medication.

intestinal lesions resulted only in those animals that received tablets containing potassium chloride. Enteric-coated placebo tablets and enteric-coated thiazide tablets produced no demonstrable lesions. The size and coatings of the tablets were identical. The severity of the lesions produced by the potassium chloride tablets did not differ significantly from those produced by the tablets containing potassium chloride and thiazide (Figs. 1 and 2).

After potassium chloride had been identified as the causative agent of the intestinal ulcers, it was necessary to establish a dose-response relationship. A summary of the results from this phase of the work is given in Table 2. By using enteric-coated tablets of a constant size in which the amount of potassium chloride had been varied (the quantity of lactose filler was increased as potassium chloride decreased), as well as making use of potassium chloride-thiazide preparations already on the market, it was possible to establish a direct relationship between the amount of potassium ingested and the severity of the lesions produced in the gastrointestinal tract (Figs. 3–5).

The relationship between tablet size and the incidence of intestinal lesions was also investigated. One group of 6 monkeys was medicated with 12-mm diameter (commercial size) enteric-coated tablets containing 100, 250, or 1000 mg of potassium chloride, while another group of 10 monkeys received tablets reduced to 6.5 and 7.3 mm in diameter but which still contained 100 and 250 mg of potassium chloride, respectively. Results from these two groups of monkeys indicated that equal doses of potassium chloride generally produced lesions of similar severity regardless of tablet size. Furthermore, intestinal lesions could be produced equally well with one 1000-mg

[2] Tablets which resist disintegration in simulated gastric fluid for 1 hour but dissolve completely within 2 hours in simulated intestinal fluid in accordance with U.S.P. XVI.

FIG. 1. Circumscribed ulceration of the monkey ileum produced by enteric-coated tablets of potassium chloride. Two tablets, each containing 1000 mg of potassium chloride were administered twice daily for 4 1/2 days.

FIG. 2. Circumscribed ulceration of the monkey ileum produced by enteric-coated tablets containing 50 mg of hydrochlorothiazide and 1000 mg of potassium chloride. Two tablets were administered twice daily for 4 1/2 days.

tablet, four 250-mg tablets, or ten 100-mg tablets, although the severity of the ulcerations was reduced considerably by the 100-mg tablets.

During the course of testing various proprietary and experimental potassium-containing preparations, it was noted that lesions were not consistently produced in the small intestine. Sometimes the stomach, the cecum, or the colon was affected (Figs. 6 and 7). Multiple lesions occurred in more than half of the animals tested. The number of abnormal sites per monkey usually ranged from 2 to 5, although much individual variation existed.

It soon became evident, after observing tablets in various states of disintegration near the lesion sites, that ulceration usually occurred where the greatest amount of potassium chloride was released from the tablet. By determining the disintegration

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TABLE 2

RESPONSE OF THE GASTROINTESTINAL TRACT OF MONKEYS TO THE ADMINISTRATION OF VARIOUS AMOUNTS OF POTASSIUM CHLORIDE IN ENTERIC-COATED TABLETS

Potassium chloride (mg)	Occurrence of gross lesions[b]		
	Stomach	Small intestine	Cecum, large intestine
100[a]	None (2/2)	None (2/2)	None (2/2)
250[a]	None (2/2)	Hyperemia (2/2)	None (2/2)
500[a]	None (3/4)	Necrosis (3/4)	None (3/4)
	Necrosis (1/4)	None (1/4)	Ulceration (1/4)
572[a]	None (2/2)	Necrosis (2/2)	None (2/2)
1000[c]	None (5/5)	Necrosis (2/5)	None (3/5)
		Ulceration (3/5)	Necrosis (2/5)
2000[c]	None (6/6)	Ulceration (6/6)	None (6/6)

[a] One tablet twice a day for 4½ days. Necropsy was performed 2–4 hours after last medication.

[b] The ratio in parentheses is the number of animals in which the lesion was found divided by the number of animals dosed.

[c] Two tablets twice a day for 4½ days. Necropsy was performed 2–4 hours after last medication.

FIG. 3. Typical lesions in the monkey ileum produced by the daily administration of one enteric-coated tablet containing 50 mg of hydrochlorothiazide and 250 mg of potassium chloride for 4 1/2 days. Note leukocytic infiltration (*a*) in the mucosa and dilated vessel (*b*). × 138.

times for the various tablets *in vitro*, it was possible to correlate short disintegration times with stomach or upper intestinal lesions while tablets with long disintegration times produced ulceration in the lower intestinal tract. A summary of these observations is presented in Table 3.

As expected, liquid potassium chloride preparations containing approximately 13.5 meq of potassium per 5-ml dose (the equivalent of 1000 mg of potassium chloride) were irritating chiefly to the stomach (Fig. 6).

FIG. 4. Mucosal necrosis (*a*) and submucosal edema (*b*) of the monkey ileum produced by enteric-coated tablets containing 50 mg of hydrochlorothiazide and 500 mg of potassium chloride. One tablet administered twice daily for 4 1/2 days. Note also dilation of vessels in villi. × 60.

FIG. 5. Perforating ulceration of the monkey ileum. Note ulcer crater (*a*), submucosal edema and inflammation (*b*), and perforation of tunica muscularis (*c*). One enteric-coated tablet containing 50 mg of hydrochlorothiazide and 1000 mg of potassium chloride was administered twice daily for 4 1/2 days. × 92.

Gradual-release tablets which dissolved slowly but steadily over a prolonged period of time in either gastric or intestinal fluid produced lesions located predominantly in the stomach or duodenum depending on where the longer contact between tablet and mucosa was maintained. Results from various types of gradual-release

FIG. 6. Ulceration (dark area) of the gastric mucosa near the pylorus. This monkey was administered 5 ml of a solution containing 1000 mg of potassium chloride twice daily for 4 1/2 days.

FIG. 7. Hyperemia of the ileocecal valve of a monkey. Two enteric-coated tablets containing a total of 1000 mg of potassium chloride were administered twice daily for 4 1/2 days.

TABLE 3

RELATIONSHIP OF TABLET DISINTEGRATION TIME TO INCIDENCE OF GROSS LESIONS IN THE GASTROINTESTINAL TRACT OF MONKEYS RECEIVING POTASSIUM CHLORIDE PREPARATIONS

Type of preparation[a]	Disintegration time		Incidence of gross lesions[b]		
	Gastric fluid (min)	Intestinal fluid (min)	Stomach	Small intestine	Cecum, large intestine
Liquid	—	—	4/4	1/4	0/4
Gradual release tablet[c]	—	—	2/2	0/2	0/2
Gradual release tablet	> 44	4	5/5	4/5	1/5
Enteric-coated tablet	> 60	24	0/1	1/1	1/1
Enteric-coated tablet	> 240	24	0/2	2/2	0/2
Enteric-coated tablet	> 240	47	0/2	2/2	0/2
Enteric-coated tablet	> 240	64	0/2	1/2	1/2
Enteric-coated tablet	63	91	0/2	0/2	1/2

[a] Medication was given twice a day for 4½ days. Each dose contained 1000 mg of potassium chloride. Necropsy was performed 2–4 hours after last medication.

[b] Incidence is expressed as the ratio of the number of animals in which lesions were found to the number dosed.

[c] 52% of potassium chloride released in 1 hour, and 72% in 2 hours, in either gastric or intestinal fluid.

tablets indicated that a release of 30% or more of the potassium chloride from a 1000-mg tablet was irritating to gastric mucosa and produced a high incidence of gross ulceration.

Enteric-coated potassium chloride tablets produced lesions which predominated in the jejunum and ileum (Fig. 8). No gastric lesions were generally noted with these preparations because the enteric coatings of the tablets resisted dissolution for at least an hour in simulated gastric juice. On the other hand, if the tablets resisted disintegration in intestinal fluid beyond an hour, lesions were often found in the cecum and colon.

How potassium chloride-induced intestinal ulcerations are resolved after medication

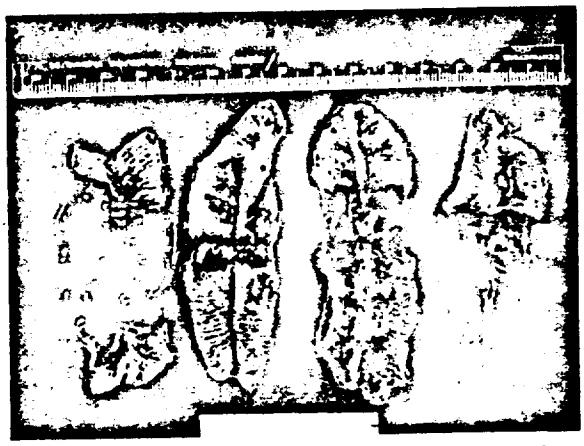

FIG. 8. Multiple annular ulcerations in the monkey ileum. Two enteric-coated tablets, each containing 50 mg of hydrochlorothiazide and 1000 mg of potassium chloride, were administered twice daily for 4 1/2 days.

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is withdrawn has been studied in only one monkey to date. One enteric-coated tablet containing 50 mg of hydrochlorothiazide and 1000 mg of potassium chloride was administered twice a day for 5 days. Four weeks after the last medication, the monkey was necropsied and an area of gross stenosis was found in the ileum (Figs. 9 and 10). The stenosed intestine pictured in Fig. 9 corresponds to lesions observed in the human and is described by Allen (1965) as being dilated proximal to the narrow annular constriction while the segment distal is of normal caliber. Furthermore, the constriction, which resulted from the inflammation and adhesions of the annular ulcer, was most

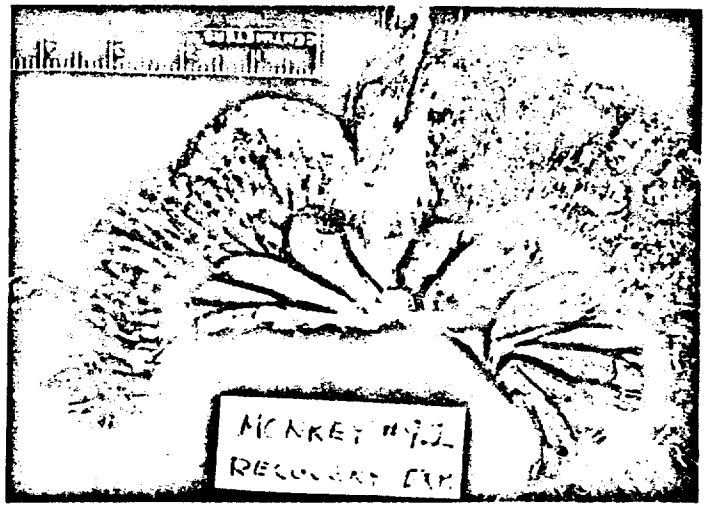

FIG. 9. Monkey ileum with an area of stenosis. Note adhesion of omentum to intestine. One enteric-coated tablet containing 50 mg of hydrochlorothiazide and 1000 mg of potassium chloride was administered twice daily for 5 days. The animal was sacrificed and autopsied 1 month after the last dose.

FIG. 10. Mucosal surface of the stenosed area pictured in Fig. 9. Note the annular constriction of the intestinal lumen.

severe on the anti-mesenteric border of the bowel, an observation which is common also in human cases.

DISCUSSION

Lawrason *et al.* (1965) in an exhaustive retrospective study of clinical records from 488 hospitals essentially verified the conclusions of Lindholmer *et al.* (1964) and Baker *et al.* (1964) that the nonspecific intestinal ulcers seen in man were in fact due to enteric-coated tablets containing potassium chloride and a thiazide diuretic. However, the incidence of these lesions was so small, approximately 1 in 35,000 cases (personal communication), that statistical methods and large numbers of case reports had to be used to establish a reliable cause-effect relationship. Only after ulcerations could be produced repeatedly in monkeys was it possible to state with some assurance that potassium chloride alone, and not the thiazide diuretic or the enteric coating of the tablet, was responsible for the injury to the intestinal tract.

The monkey thereby became a test animal which could be used to investigate the possible pathogenicity of the iatrogenic ulcers, as well as to screen for less-irritating forms of potassium. The fact that approximately 14 times the human dosage on a milligram per kilogram basis had to be employed to produce ulcers consistently in monkeys does not detract from the validity of the test procedure, since the release of potassium in amounts comparable to those used in monkeys would most probably increase the incidence of intestinal ulcers in humans, especially if predisposing factors such as cardiovascular changes are also found to play a part in this syndrome.

Doses of approximately 1000 mg of enteric-coated potassium chloride given twice daily to 6- to 8-week-old swine produced intestinal ulcerations similar to those noted in monkeys and man.[3] Yet, in the dog, doses ranging from 500 to 2000 mg twice daily (4–14 times the human dose) for 5 days produced no ulcerations in the gastrointestinal tract. Intestinal ulcerations were achieved, however, in dogs by Boley *et al.* (1965) by suspending tablets of potassium chloride on sutures in the intestine, thus restricting the area wherein medication was dissolved and absorbed. Inherent physiological or anatomical differences between the intestinal tracts of carnivores and omnivores may account for the marked resistance of the dog to potassium-induced lesions, although these differences are not immediately apparent upon histologic examination and remain to be clarified.

The exact pathogenesis of the intestinal ulcerations, whether in man or experimental animals, has not yet been determined, but two hypotheses have been advanced. One concept is that an ulcer results from the corrosive action of the potassium which is rather suddenly released from its tablet, producing an initial eschar in the intestinal mucosa that in turn progresses to ulceration. In contrast to this theory, Allen (1965) proposes that ulcerations are in fact secondary to hemorrhagic infarcts caused by the sudden absorption of high concentrations of potassium in a localized area. To date neither theory has been disproved.

SUMMARY

Intestinal ulcerations, similar to those reported in humans, were produced in the rhesus monkey within 5 days by the administration of 1000 mg of potassium chloride in enteric-coated tablets

[3] Personal communication from Dr. Herbert A. Wendel, Warren-Teed Pharmaceuticals, Inc., Columbus, Ohio.

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twice daily. Doses of 250 mg of potassium chloride on the same schedule produced milder lesions which could not be predictably reproduced. Tablets of 100 mg were without effect. The dimensions of the tablet were not the determining factor in the production of lesions. The specific locations of lesions appeared to coincide with the areas where potassium chloride was released or came into contact with the gastric or intestinal mucosa. Various solutions of potassium salts produced erythema and ulcerations of the stomach. A number of commercially available enteric-coated tablets containing various concentrations of potassium chloride and thiazide diuretic produced intestinal lesions ranging from hyperemia to perforating ulcerations. Thiazide diuretics did not influence the severity of the pathology noted nor cause lesions when given alone.

REFERENCES

ALLEN, A. C. (1965). The pathology and pathogenesis of clinical and experimental potassium induced lesions of the small intestine. *J. Am. Med. Assoc.* in press.

BAKER, R., SCHRADER, W. H., and HITCHCOCK, C. R. (1964). I. Thiazides and potassium: Small bowel lesions, *J. Am. Med. Assoc.* **190**, 586-590.

BOLEY, S. J., SCHULTZ, L., KRIEGER, H., SCHWARTZ, S., ELGUEZABEL, A., and ALLEN, A. C. (1965). Experimental evaluation of thiazides and potassium as a cause of small-bowel ulcer. *J. Am. Med. Assoc.* **192**, 763-768.

LACHMAN, L., and COOPER, J. (1963). A programmed automated film-coating process. *J. Pharm. Sci.* **52**, 490-496.

LAWRASON, F. D., ALPERT, E., MOHR, F. L., and MCMAHON, F. G. (1965). Ulcerative-obstructive lesions of the small intestine. *J. Am. Med. Assoc.* **191**, 641-644.

LINDHOLMER, B., NYMAN, E., and RAF, L. (1964). Nonspecific stenosing ulceration of small bowel: Preliminary report. *Acta Chir. Scand.* **128**, 310-311.

WATSON, M. R. (1963). Primary nonspecific ulceration of small bowel. *Arch. Surg.* **87**, 600-603.

DIETARY TREATMENT OF HYPERTENSION. II. SODIUM DEPLETION AS RELATED TO THE THERAPEUTIC EFFECT [1]

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Abnormal accumulations of sodium have been demonstrated by direct analysis of the tissues of dogs (1) and rats (2) that had been made hypertensive by renal ischemia. These observations are of special interest because of the reductions of blood pressure in hypertensive patients that may be induced by means of extreme restriction of the dietary sodium. Taken together, these facts suggest that the benefit experienced by the patients might be due to the removal of excess sodium.

On the other hand, it must be recognized that hypertension is not a necessary, or even a usual, consequence of sodium retention. Accumulations of extracellular fluid in association with normal levels of blood pressure are of frequent occurrence in a variety of diseases, including renal disorders. Therefore, if the retention of sodium is to be regarded as contributory, rather than as merely incidental, to the development of hypertension, it must be linked to some other metabolic disturbance in this particular disease.

In the present study five hypertensive patients were subjected to large changes in their rations of dietary sodium. Resultant changes in the sodium content of their bodies were measured by isotope dilution. These observations have been correlated with the changes in their clinical status and with inferred changes in the degree of cellular hydration. It is suggested that the latter may be the important factor that is linked to the depletion of sodium.

PROCEDURE

Five patients with benign and uncomplicated hypertension were chosen for a study. Apart from Grade I changes in their ocular fundi and slight enlargements of the heart shadow in their chest films, the results of physical and laboratory examinations were negative. In particular all patients had normal urea clearances and absence from the urine of significant quantities of protein, casts and cells. Further clinical data are summarized in Table I; similar data also are given for the three normal subjects who served as controls in the measurement of exchangeable sodium.

During the five to seven months of this study the patients remained entirely on the metabolic ward, except for a vacation of three days during the Christmas season. Confinement to a hospital ward necessarily limited the physical activity of each patient; at no time, however, were the patients restricted to bed.

A central feature in the design of the investigation was the provision of an extended time during which the sodium intake was maintained at a high normal value (180

[1] The radiosodium used in this investigation was supplied by Oak Ridge on allocation from the Isotopes Division, United States Atomic Energy Commission.

TABLE I

Clinical data

Patient	R. I. H. No.	Age	Sex	Family history of hypertension	Known duration of hypertension	Disease other than hypertension	Ratio initial wt. / ideal wt.*
		years			*years*		
A	12,450	26	female	+++	4	0	1.12
B	12,447	44	female	0	7	0	1.07
C	12,451	27	male	+	5	migraine	1.12
D	12,453	39	female	0	19	0	1.06
E	12,465	41	male	0	10	0	1.12
F	normal control	35	male	0	0	0	1.01
G	normal control	37	male	0	0	0	1.06
H	normal control	28	male	+	0	0	0.85

* "Ideal weight" for each patient was calculated from normal values which had been derived from life insurance statistics (7).

meq./day) by the addition of salt tablets to a diet otherwise low in sodium (less than 7 meq./day). During this preliminary time, changes in the daily intakes of water and of protein were imposed in order to demonstrate any clinical effects of these factors in the absence of sodium restriction. After from two to four months had elapsed, the intake of sodium was reduced to the 7 meq./day level by omission of the supplementary tablets. Ten weeks later the sodium intake was returned to the higher level by resumption of the supplement in order to test the reversibility of the clinical and metabolic changes which had followed the restriction of this element.

The detailed timing of these manipulations may be seen in the figures. The total study was divided into six periods, which are separated in all the figures by vertical lines. These periods were differentiated by changes in the water or the sodium intake; one of these two factors remained constant throughout any consecutive pair of periods while the other was changed. An upward direction of the arrow in relation to the symbols in the figures indicates that this factor was administered in high dosage during the period; a downward direction indicates the converse. For sodium the high dosage level was 180 meq./day and the low dosage was the residual amount in the diet alone, namely a value less than 7 meq./day. During the periods of high water intake, 4,500 cc./day of fluid were administered in addition to the preformed and metabolic water of the diet; as a result the urinary volumes were maintained at high average values (Table II). Water restriction in the low dosage periods was carried to the point of slight thirst; an intake of 800 to 1,000 cc./day of supplementary fluid, which maintained the urinary volumes in the range 600 to 900 cc./day, appeared to be the minimum tolerable fluid supplement when sodium was concurrently administered in high dosage. In the figures a symbol for water in Period I is absent because the patients were allowed uncontrolled access to water; during this time their urine volumes ranged between 800 and 1,800 cc./day.

The changes in level of protein intake are indicated in Figure 3. It may be seen that the daily consumption was 30–40 grams until the middle of Period III, when the amount was approximately doubled. Prior to this time the patients had felt dissatisfied with the diet despite the fact that it was as diversified as was compatible with the requirement of sodium restriction, and was unlimited in quantity except with respect to the foods of moderate sodium content. Since the usual high protein foods fell into the latter category, the protein intake was curtailed until Period III, when a special low sodium, high protein food[2] was made available for the patients to take in amounts regulated by their appetites. During Period IV they continued to take the increased amount of protein; with the restriction of water in Periods V and VI the amount of this supplement that could be conveniently given was limited by the lack of fluid for its suspension.

One exception to the plan of deferred sodium restriction should be noted: through oversight, the supplementary salt tablets were not ordered for Patient C. When the omission was discovered he had already shown a reduction in blood pressure below the value on admission and the earlier values that had been observed in the out-patient clinic. Therefore, it was decided to continue the study of his response without the supplement until the final period.

METHODS

Daily measurements of blood pressure and of body weight were made each morning except Sundays under standardized conditions. The blood pressure was determined by the same physician for each patient; at least 20 minutes of bed rest preceded the reading. Body weight was measured after urination and before breakfast on a calibrated scale that was accurate to 100 grams; the patients were nude except for a covering sheet of known weight.

At biweekly intervals a standard 2 meter chest film was obtained for measurement of heart size. At monthly intervals the basal metabolic rates were measured, and electrocardiographic tracings taken.

A total collection of urine was made, and divided into 24 hour pools; analyses of sodium, potassium and chloride concentrations were made upon each, using methods previously discussed (3). During two intervals of five days each, at the ends of Periods IV and VI, collections of stools were made; the total output from each interval was pooled and analyzed for sodium and potassium (3).

The determination of exchangeable sodium was based on the dilution of a measured dose of radiosodium. An infusion fluid containing $0.8 - 1.0\,\mu c$ of Na^{24} Cl/cc. of 5 per cent dextrose in water was injected intravenously from a calibrated 100 cc. burette at a dose of just 1 cc./kg.[3] Samples of blood were taken at hourly intervals for seven hours; in some tests additional samples were taken after 24 and 48 hours.

With a Tracerlab β-γ dip-counter (TGC-5) and autoscaler, measurements of the radioactivity of the samples were made after dilutions that were sufficient to bring the counting rates within the range of linear response of the tube. An equilibrium value (a) of the specific activity of the serum (ratio of radioactivity in arbitrary units to the sodium concentration) as attained in the five to seven hour interval was estimated graphically. The quantity (q) of sodium with which the injected dose (d) of tracer had been mixed was calculated from the relation $q = d/a$. This quantity is about 10 per cent less than that which would be calculated from the 24 hour equilibration of specific activity and therefore does not include the

[2] "Low sodium food," Wyeth: protein, 50 per cent; carbohydrate, 19.5 per cent; fat, 25 per cent; total ash, 3.5 per cent; sodium, 0.02 per cent. A generous supply of this product was donated by Wyeth, Inc.

[3] Calculation based on the data given by Marinelli and associates (4) indicated that the patients were exposed by this test to less than 0.1 equivalent roentgen of radiation during the first 24 hours and to negligible amounts thereafter.

TABLE II—*Measurements of clinical status and body sodium content*

Subject	Period		Average sodium intake	Average urine volume	Blood pressure means (and standard deviation)		Heart size		Exchangeable sodium measurements					
									Time of measurement		Body weight		Sodium	
	No.	Duration			Systolic	Diastolic	Maximum diameter	Internal diameter of chest	After sodium restriction	After sodium increase	Actual wt.	Actual wt. / Ideal wt.	Per kg.	Total
		days	*meq./day*	*cc./day*	*mm. Hg*	*mm. Hg*	*mm.*	*mm.*	*days*	*days*	*kg.*		*meq./kg.*	*meq.*
A	I	36	180	780	168 (7)	108 (11)	141	272			76.2	1.12		
	II	47	180	660	169 (10)	109 (7)	138	271			70.9	1.04		
	III	34	180	3660	170 (4)	106 (4)	130	272	−1		71.3	1.05	39.9	2850
	IV	45	7	3770	*	*	*		28		70.0	1.03	39.0	2730
					136 (7)	81 (7)	115	268	43		70.2	1.03	* 34.8	* 2440
	V	24	7	900	131 (6)	* 73 (8)	118	272	64	−5	69.4	1.02	35.7	2480
	VI	18	180	1160	* 158 (6)	* 96 (2)	* 138	272		16	70.2	1.03	* 40.4	* 2830
B	I	41	180	800	233 (13)	132 (8)	139	260			60.7	1.07		
	II	47	180	785	233 (17)	131 (11)	139	263			57.0	1.00		
	III	34	180	3775	227 (15)	127 (8)	139	258	−1		57.6	1.01	38.1	2195
	IV	45	7	3720	*	*			28		56.6	0.99	* 31.7	* 1826
					188 (15)	110 (7)	132	258	43		56.6	0.99	32.1	1817
	V	24	7	3835	184 (14)	* 95 (9)	135	262	64	−5	55.8	0.98	33.8	1886
	VI	18	180	4000	* 220 (18)	* 116 (7)	140	257		16	55.7	0.98	* 42.4	* 2362
C	I	35	7	590	145 (12)	83 (8)	146	298			69.1	1.12		
	II	47	7	430	140 (15)	85 (7)	142	295			66.2	1.07		
	III	34	7	3050	141 (11)	88 (8)	139	285	119		65.0	1.05	34.3	2230
	IV	44	7	3890					147		63.7	1.03	33.6	2140
					142 (7)	87 (6)	146	289	162		64.4	1.04	32.5	2093
	V	25	7	810	140 (9)	* 78 (9)	140	295	183	−5	63.7	1.03	35.3	2249
	VI	18	180	990	148 (3)	* 91 (4)	* 150	295		16	65.1	1.05	* 39.6	* 2578

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TABLE II—*Continued*

Subject	Period		Average sodium intake	Average urine volume	Blood pressure means (and standard deviation)		Heart size		Exchangeable sodium measurements					
									Time of measurement		Body weight		Sodium	
	No.	Duration			Systolic	Diastolic	Maximum diameter	Internal diameter of chest	After sodium restriction	After sodium increase	Actual wt.	Actual wt. / Ideal wt.	Per kg.	Total
		days	*meq./day*	*cc./day*	*mm. Hg*	*mm. Hg*	*mm.*	*mm.*	*days*	*days*	*kg.*		*meq./kg.*	*meq.*
D	I	26	180	1585	177 (11)	108 (11)	139	293			69.7	1.06		
	II	47	180	665	173 (9)	103 (9)	142	285			64.9	0.99		
	III	34	180	3960	170 (9)	103 (8)	142	290	−1		65.2	1.00	38.1	2484
	IV	45	7	3940	* 145 (6)	* 90 (6)	135	288	28 43		63.2 62.8	0.97 0.96	* 32.6 31.8	* 2060 1997
	V	24	7	860	140 (8)	88 (8)	* 125	287	64	−5	61.7	0.94	35.3	2178
	VI	18	180	1255	150 (12)	93 (10)	* 138	281		16	62.6	0.96	* 39.6	* 2479
E	I													
	II	26	180	930	204 (15)	111 (10)	165	338			92.6	1.12		
	III	34	180	3670	215 (9)	114 (11)	166	328	−1		92.0	1.11	44.5	4094
	IV	45	7	3155	213 (15)	105 (10)	162	329	28 43		90.5 90.6	1.09 1.09	* 35.8 36.5	* 3239 3307
	V	24	7	470	206 (23)	* 93 (9)	169	331	64		88.2	1.06	38.8	3422
	VI	18	7	430	218 (20)	88 (8)	163	329	75		87.7	1.06	39.3	3447
F	Normal controls										77.2	1.01	35.3	2725
G											82.7	1.06	35.0	2894
H											57.7	0.85	40.7	2348

*Asterisk indicates a significant difference between consecutive values.

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most slowly equilibrating sodium in the body. It is to be expected, however, that equilibration would have been complete in the soft tissues which have been shown (1, 2) to accumulate sodium in the animals with hypertension.

Criteria of Significance

Average values of blood pressure were calculated from the consecutive readings obtained during the last two weeks of each period except Period VI for which only the last week was taken. Fisher's "t test" (5) was used to calculate the probability that the differences between successive averages might occur by chance alone. Changes were considered significant when this probability was less than 0.01; these changes are indicated by an asterisk in Table II.

Heart diameter on a given chest film could be measured reproducibly within 2 mm. In order to exclude the effects of differences in projection and heart cycle it was taken as necessary that changes must exceed 10 mm. to be significant. These are indicated in Table II.

The exchangeable sodium is calculated from four primary measurements: the counting rates of solution injected and of the serum at equilibrium, the volume of fluid injected and the sodium concentration of the serum sample. Since the fluid was delivered into the subject from a calibrated burette which was accurate to less than 0.1 cc., the error in this factor may be neglected. The average counting rates of the diluted infusion fluid, diluted serum and the background were 5,000, 400, and 44 cpm., respectively, and were counted for 20,480, 2,048, and 4,096 total counts. From these values it may be estimated that the coefficients of variation (ratio of standard deviation to mean value) were 0.007 and 0.022, respectively, for the infusion fluid and the serum samples (6). For the variation in the determination of total sodium concentration the coefficient 0.020 was assumed. These factors contribute to the uncertainty of estimation of their product according to the formula $E = (F^2 + S^2 + C^2)^{1/2}$ where the symbols indicate the coefficients of variation of the exchangeable sodium, the infusion fluid counting rate, the serum counting rate, and the serum sodium concentration, respectively. Substitution of the values above give $E = 0.031$. Since the final value for body sodium was based on at least three independent serum samples the coefficient for the final result is reduced by the factor $(1/\sqrt{3})$ and thus was approximately 0.02. In the case of an adult of average weight this value corresponds to a standard deviation of less than 60 meq. The changes discussed in this paper were of considerably greater magnitude; in Table II, under the heading of "Exchangeable sodium," the asterisks signify a change of five standard deviations or more.

OBSERVATIONS

The blood pressures of all patients remained elevated during the periods in which the intake of sodium was maintained at 180 meq./day (Figure 1). During the two to four months which were spanned by these three periods, large variations of water intake were imposed and considerable losses of weight occurred; there were no significant changes in the average values of blood pressure or in the heart sizes (Table II).

When the sodium was reduced to the dietary level of about 7 meq./day by omission of the tablets, a statistically significant reduction of blood pressure was observed in each patient. Of the five patients three became normotensive (or nearly so), while the other two remained in the hypertensive range. The latter patients had the most marked elevations of blood pressure during the control periods and, therefore, would have had to experience greater reductions in order to approach the normal range.

Three of the four patients who were given salt tablets in the final period showed increases in their average blood pressures. Since the trend of the daily blood pressure values was upward in Period VI (Figure 3), it is probable that even higher averages would have been obtained in a more extended period. With the return of hypertension there were increases of the heart size which abolished earlier reductions (Table II).

Weight losses were observed in all patients (Figure 2). The graphical form of the weight curves was similar to that previously reported for a group of patients who received the rice-fruit diet (3); with both diets the weight losses proceeded at a rate which was rapid at first but which decreased with time. The rates of adaptation were so slow, however, that several additional months probably would have been required for attainment of definite weight equilibrium.

Since the caloric intakes of the patients remained under their control, it is evident that some factor in the regimen must have reduced their appetites. This factor certainly was not restriction of sodium since four of the five patients of the present study received 10 grams NaCl/day during the initial phase of rapid loss, and the remaining patient (C), who received no salt supplement, experienced a weight loss which was indistinguishable from the others. Moreover, the weight changes were not related to adaptation of the basal metabolic rates; these were measured at monthly intervals and were found to remain within normal limits (± 10 per cent) in all patients.

The explanation which seems most probable is that the limitation of protein intake caused the

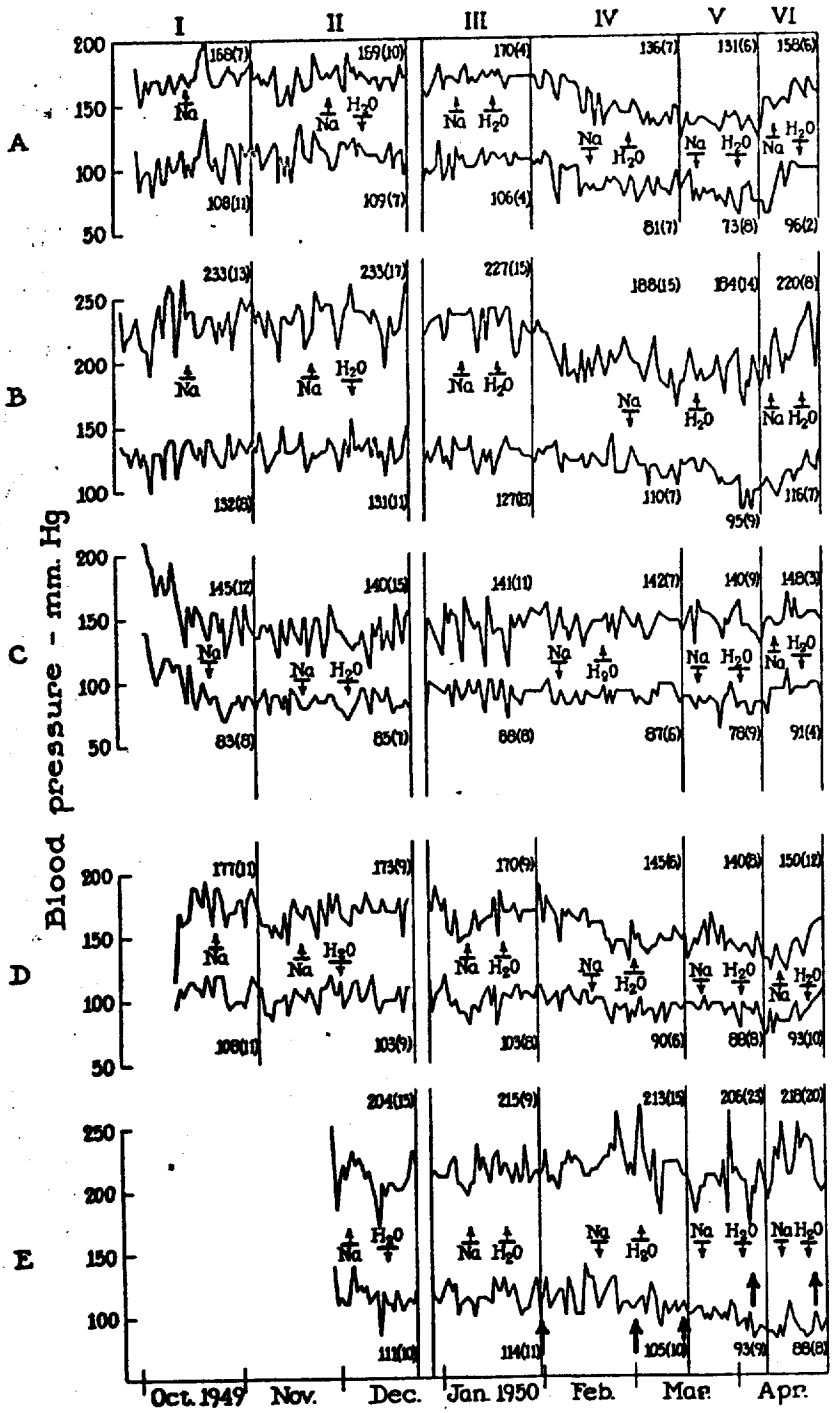

FIG. 1. MORNING VALUES OF THE SYSTOLIC AND DIASTOLIC BLOOD PRESSURES

The numbers near the end of each period are the averages and the standard deviations for the last two weeks of each period, except the last for which a one week average was taken. Sodium intake was 180 meq./day during periods with the arrow up, and 7 meq./day during those with arrow down. High and low water intakes, indicated similarly, were 4,500 cc./day and 800 to 1,000 cc./day, respectively.

weight losses. The basic diet was deficient in protein, as compared to the standard which was established by the patients themselves when given free choice in the matter, since each patient nearly doubled his protein intake when the protein supplement was made available for unlimited consumption (Figure 3, Periods III and IV). With the increased intakes of protein there were associated

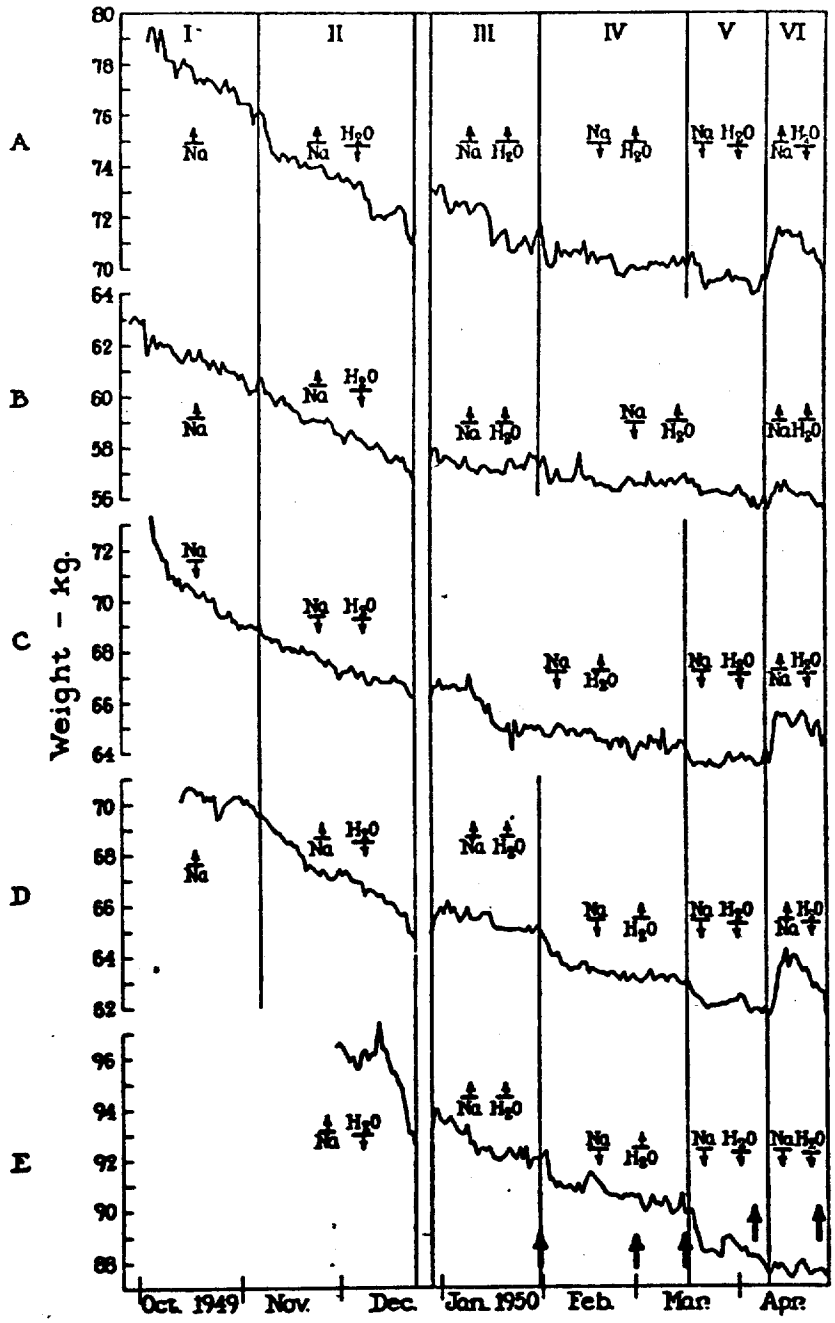

FIG. 2. MORNING WEIGHTS

Symbols are defined in the legend of Figure 1.

increases of non-protein calories, amounting to 100 to 300 calories per day.

A more direct demonstration of the relation between protein intake and change in body weight under the conditions of this study has been obtained in subsequent work. Three other patients, who were given the same diet under the same circumstances except that their protein intakes were maintained from the onset at 70–90 grams/day by a prescribed administration of the supplement, not only failed to lose weight but actually gained 1 to 4 kg. during a three month period of observation.

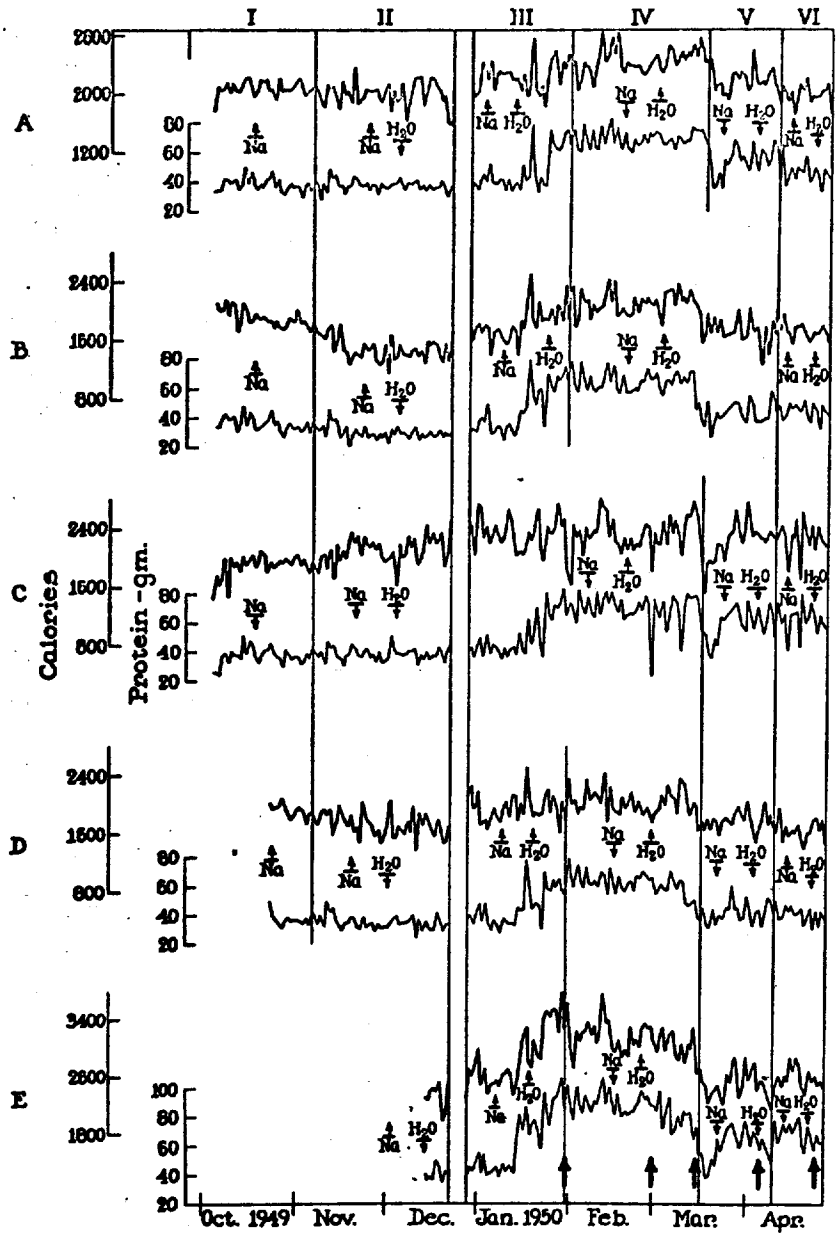

FIG. 3. CALCULATED DAILY CONSUMPTIONS OF TOTAL CALORIES AND OF PROTEIN
Symbols are defined in the legend of Figure 1.

The changes in exchangeable sodium are shown in Table II. The hypertensive patients, before omission of the salt supplement, did not differ from the three normal subjects with respect to the quantity of exchangeable sodium per unit body weight. These data do not exclude the possibility that a small difference between the average values of hypertensives and normals might be demonstrable in a larger series, but they indicate that the hypertensive subjects are not distinguished from the normals by a consistent or gross discrepancy.

The first measurements of exchangeable sodium were made at the end of Period III, two days before reduction of intake from 180 to 7 meq./day; the second were made one month later. In three of the four patients a reduction of 14–20 per cent was found. The explanation for the failure of Patient A to conform is not apparent; however, at the time of the third measurement two weeks later she was found to have a comparable reduction, while the other patients were unchanged. The fourth measurements were made three weeks later (a total of nine weeks from reduction of the sodium intake) and showed no further change in any of the patients.

The final measurements were made 16 days after the sodium intake had been returned to 180 meq./day. In the three patients (A, B, D) for whom the comparison could be made it was found that the exchangeable sodium had been returned to the control level.

The concentration of sodium in the serum was measured with each determination of exchangeable sodium. In contrast to the large changes which occurred in the latter, the concentration was not significantly affected by the variations of sodium intake.

The urinary excretions of sodium are shown graphically in Figure 4. Following reduction of their sodium intakes, the patients reduced their urinary excretions quite slowly: at least a week was required for reduction to the level of dietary intake. By the third week the urinary loss had fallen to 1 meq./day or lower, the output at this extremely low level being somewhat accelerated by a large urine volume and reduced with restriction of fluids.

After increase of the sodium intake in Period VI, the urinary excretions rose. Again there was a lag in the renal response to the changed situation.

When the fecal excretions of sodium were measured during the last five days of Period IV and of Period VI, it was found that the daily losses by this avenue averaged less than 1.5 and 2.2 meq./day, respectively. Losses of this magnitude, although important with respect to a long-term balance under severe restriction of intake (3), evidently are negligible by comparison with the acute imbalances between intake and urinary excretion that were observed to follow a radical change in the level of intake. Therefore, since no gross sweating occurred during these observations, it is probable that the changes in the exchangeable sodium were due mostly to the lags in adjustment of the urinary excretion. Given time, the patients were able to resist further depletion, as shown by the constant values of the exchangeable sodium in the second, third, and fourth measurements; conversely, in the periods of high sodium intake they maintained a constant urinary excretion despite wide variations in their urine volumes.

It will be observed that the decreases in exchangeable sodium (Table II) were greater than the urinary excretions of the element during the first five days after reduction of intake (Table III). The probable reason for this is that the isotope equilibrations were attained in the afternoon, when the sodium ration of the day had been absorbed but not yet excreted (unpublished observations). The data for the excretions during the 14 hours that followed the last dose of NaCl were omitted from the calculations of Table III in order to present the ratios of sodium to chloride excretions on the subsequent days when no salt was administered.

The urinary excretions of chloride tended to parallel those of sodium. In particular it was noted that the net urinary losses of the two ele-

TABLE III

Comparison of the net urinary losses of sodium and chloride during the five days following omission of salt tablets

Patient	Urinary output less dietary intake		Na/Cl
	Na meq./5 days	Cl meq./5 days	
A	131	82	1.60
B	144	119	1.21
D	146	132	1.11
E	166	115	1.44

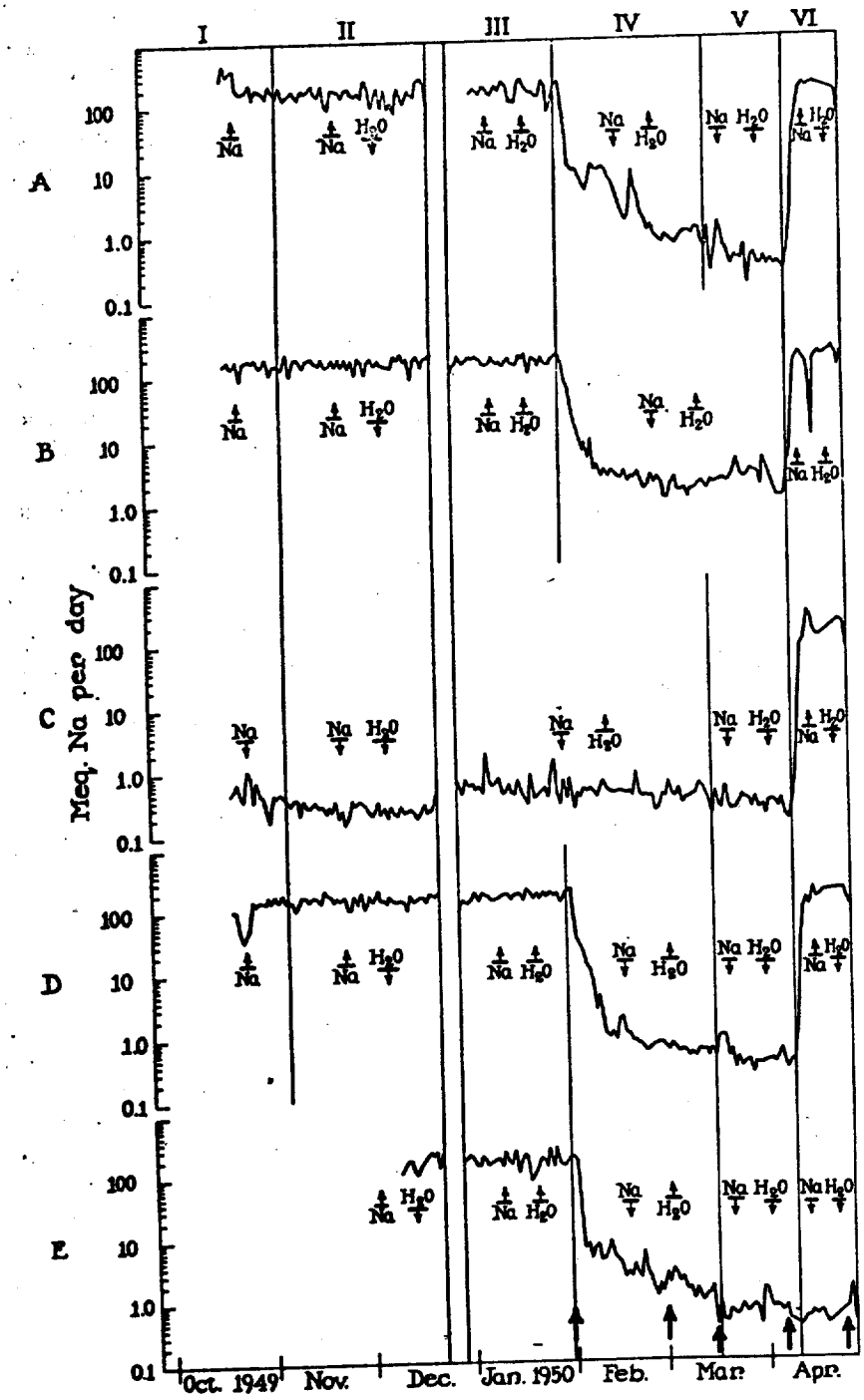

FIG. 4. URINE SODIUM EXCRETIONS

Note that the ordinate scales are logarithmic. Symbols are defined in the legend of Figure 1.

ments during the five days following reduction of intake occurred in ratios that approximated their ratios in extracellular fluid (Table III).

The urinary excretions of potassium tended to be greater during the early periods of rapid weight loss than subsequently. There was no clear relation of the urinary or fecal excretion of potassium to the changes in sodium intake.

INTERPRETATION

The proportional relation between the sodium and the chloride excretions on the days that followed reduction in intake (Table III) suggests that the sodium, which was lost from the body early in Period IV, was derived from a source with the composition of extracellular fluid. A similar inference with respect to reaccumulation may be drawn from the immediate weight changes that follow readministration of salt. It was observed in an earlier study (3), and confirmed in the present one, that the patients for a time gain weight at a rate which is just sufficient to account for the formation of extracellular fluid from the newly-available sodium.

It will be assumed, therefore, that the component of the body which gained and lost sodium as a result of the dietary changes was the extracellular fluid. If 20 per cent of the sodium of the extracellular fluid were lost, it would cause a profound drop in the concentration of sodium in the serum (*e.g.*, to 115 meq./L or lower), unless the loss of sodium were compensated by a parallel loss of water. Therefore, since there were no significant changes in the concentration of sodium in the serum, it follows that water, as well as sodium, must have left the extracellular fluid at the time of the sodium loss, and conversely, that the extracellular volume must have expanded during reaccumulation.

It now becomes of interest to inquire into the fate of this extracellular water. If it had been excreted from the body, there would have been a weight loss of about 1 kg. for every 140 meq. of sodium which were lost, providing that an immediate large increase in the solid matter of the body had not occurred. The latter possibility seems quite unlikely, partly because the antecedent weight curves show that body tissue was being lost rather than gained at the time of the sodium depletion, and partly because this hypothesis leads to the unlikely supposition that this solid matter was abruptly expelled following the subsequent reaccumulation of sodium. Since the weight losses of all patients fell significantly short of equivalence with their losses of exchangeable sodium (Table II), the simplest hypothesis is that part or all of the water which left the extracellular space did not leave the body. Under this interpretation the average cellular hydration was increased by about 5 per cent as a result of the sodium depletion.

This hypothesis also simplifies the explanation of the biphasic nature of the weight change (Figure 2) following readministration of sodium. The initial effect, as mentioned above, probably is an expansion of the extracellular fluid. The secondary effect is a loss of the newly-gained weight but, as shown by the elevated final value of exchangeable sodium, not of the sodium which was accumulated. Therefore it appears that the water which entered the cells during the previous adaptation to a low sodium intake was removed from them and excreted during the secondary phase of readjustment to a high intake of sodium.

The general interpretation which may be given to these changes is that there is a reciprocal relation between the volume of the extracellular fluid and the volume of part or all of the cellular fluids. A radical reduction in the level of dietary sodium shifts the balance slightly in the direction of cellular hydration and a return to a high intake level reverses the shift.

CLINICAL-METABOLIC CORRELATION

The persistent hypertension of all patients during the periods of high sodium intake was an impressive control for the reductions in blood pressure that occurred after omission of the salt tablets. Whether or not the antecedent two to four months of hospitalization, with the weight losses and the changes in protein metabolism, may have contributed to the ultimate therapeutic response cannot be said at present, but it is clear that these factors were not sufficient to reduce the blood pressure in the absence of sodium restriction.

The most obvious metabolic consequence of the reduction in sodium intake was the reduction in exchangeable sodium. However, this fact alone may not be a sufficient explanation of the clinical effect since there was no consistent correlation between the clinical status of the subjects and the quantity of exchangeable sodium per unit body

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weight. Moreover, the reductions in blood pressure and in exchangeable sodium were separated in time; the latter was mostly completed before the former became manifest.

Evidence has been brought forward to support the conclusion that the reduction of sodium intake caused an increase of intracellular water in addition to the contraction of extracellular volume. An indication that the change in intracellular water might be more closely related to the change in blood pressure than the change in extracellular volume is given by the delay in rise of blood pressure when salt was readministered; in this process it appears that the immediate effect is the expansion of extracellular volume and the delayed effect, which coincides with the blood pressure rise, is the expulsion of cellular water.

It is obvious that this hypothesis needs to be tested by direct measurements. Such test, however, must await the development of more precise methods for the measurement of small changes in the quantity of intracellular water in human subjects.

SUMMARY

1. Restriction of dietary sodium to 7 meq./day caused statistically significant reductions in the average blood pressures of five patients with uncomplicated hypertension. These reductions were due to the limitation of dietary sodium, since no blood pressure changes had been observed during a preliminary equilibration stage of two to four months duration in which the low sodium diet was supplemented with salt tablets (10 grams/day). Weight losses and large variations in the intakes of protein and of water during this time were without significant effects on the blood pressure.

2. Following withdrawal of the salt supplement, the patients lost 14 to 20 per cent of their exchangeable sodium during the first month of adaptation and then apparently attained balance. On readministration of salt 10 weeks later, the patients reaccumulated sodium approximately to the control level.

3. On the supposition that the lost sodium was derived from extracellular fluid, the losses of body water appeared to be disproportionately small. The interpretation offered was that the surplus extracellular water, not excreted with its sodium, shifted into an intracellular position and thereby increased cellular hydration by about 5 per cent. The reverse effects were observed following readministration of salt. The hypothesis was advanced that the low sodium diet exerted its beneficial effect in the hypertensive patients by causing an increase in cellular hydration.

REFERENCES

1. Eichelberger, L., The distribution of water and electrolytes between blood and skeletal muscle in experimental hypertension. J. Exper. Med., 1943, 77, 205.
2. Laramore, D. C., and Grollman, A., Water and electrolyte content of tissues in normal and hypertensive rats. Am. J. Physiol., 1950, 161, 278.
3. Dole, V. P., Dahl, L. K., Cotzias, G. C., Eder, H. A., and Krebs, M. E., Dietary treatment of hypertension. Clinical and metabolic studies of patients on the rice-fruit diet. J. Clin. Invest., 1950, 29, 1189.
4. Marinelli, L. D., Quimby, E. H., and Hine, G. J., Dosage determination with radioactive isotopes. II. Practical considerations in therapy and protection. Am. J. Roentgenol., 1948, 59, 260.
5. Fisher, R. A., Statistical Methods for Research Workers. Oliver & Boyd, Ltd., London, 1944, 9th Ed., p. 114.
6. Jarvett, A. A., Statistical methods used in the measurement of radioactivity. U. S. Atomic Energy Commission, AECU-262, Mon P-126, June 17, 1946.
7. Duncan, G. G., Diseases of Metabolism: Detailed Methods of Diagnosis and Treatment. W. B. Saunders Co., Philadelphia, 1942, p. 938.

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Meningo-encephalitis Eosinophilica of Swine

II. Studies on the Experimental Reproduction of the Lesions by Feeding Sodium Chloride and Urea

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BY

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SUMMARY.—Pigs fed on diets containing 5·3 and 9·4 per cent. of sodium chloride, with water restricted to about 2½ times the weight of dry food, developed typical signs and lesions of meningo-encephalitis eosinophilica.

The syndrome was also produced by feeding 3 per cent. sodium chloride + 7·5 per cent. urea with restricted water, but when a further 7·5 per cent. urea was substituted for the sodium chloride (giving a total of 15 per cent. urea), perivascular eosinophile infiltration of the cerebrum did not appear though the histopathological picture was otherwise similar. The feeding of 3 per cent. sodium chloride with the same allowance of water produced no significant clinical or histological abnormality.

It is suggested that cerebral midcortical laminar malacia with vascular endothelial proliferation and perivascular lymphocytic infiltration may result from disturbed water balance arising from various causes, but that the pronounced eosinophile infiltration in meningo-encephalitis eosinophilica is a characteristic effect of the sodium ion.

It is recommended that sodium chloride should not be added to the compounded diets of pigs and that unrestricted water should always be available.

Introduction

It is now well recognised that meningo-encephalitis eosinophilica of swine is closely associated with poisoning by sodium salts while on a restricted water intake (Smith, 1955; Done, Brooksbank & Buntain, 1957; Hjärre & Öbel, 1956; Smith, 1957). The authors' interest in the role of sodium chloride in the pathogenesis of the disease was aroused by the outbreaks described by Done, Brooksbank & Buntain (*op. cit.*). Following the experimental reproduction of the condition with sodium chloride, some attempt was made to investigate the specificity of this agent for the production of the various lesions making up the complete picture. The literature contains many references to the typical signs and lesions of meningo-encephalitis eosinophilica (Michalka, 1941; Larsen, Moller & Thordal-Christensen, 1955; Smith, 1957) so only a brief account is given of the early experiments using sodium chloride alone. The later proceedings are given in more detail.

Material and Methods

Most of the pigs used in the experiments were Large Whites or Large White crosses which were purchased from commercial sources. Those used in Experiment IIIc were the progeny of a sow kept at the laboratory, who had had contact with Talfan disease with her preceding litter. The pigs used in Experiment I had been vaccinated against swine fever with crystal violet vaccine and had survived challenge with virulent virus 3 weeks previously. The sodium chloride used in Experiment I was a vacuum-dried salt obtained from the same source as a batch of meal which had caused poisoning in the field. Analysis of this product did not reveal significant quantities of salts other than sodium chloride. Analytical grade sodium chloride and urea were used in the other experiments. In order to provide competition as an inducement to consume the rather unpalatable feed, trough space inadequate to the number of pigs was provided; initially 2 lb. of meal per pig were given daily, but in view of the animals' reluctance to eat their full allowance they were later fed to appetite at each meal and the total weight of food consumed was calculated at the end of the experiment. Figures of total salt consumed per pig each day assume that appetite was the same in each pig and that the amount eaten varied as the number of feeds given, and are probably very approximate.

Pigs were killed by the intracardiac injection of pentobarbitone sodium, followed by exsanguination, and a sample of cardiac blood was usually taken immediately before this operation. Urine samples were taken at necropsy. Serum chloride was estimated by the method of Whitehorn (Hawk, Oser & Summerson, 1949). For histopathological examination of the central nervous system, standard blocks were taken as follows :—

(1) One or more transverse sections of one or both cerebral hemispheres.

(2) Transverse section through the anterior corpora quadrigemina.

(3) Longitudinal section through the cerebellar vermis.

(4) Transverse section through the medulla oblongata at the level of the obex.

(5) Transverse section through the lumbar spinal cord.

Tissues were fixed in 10 per cent. formol saline, embedded in paraffin, sectioned at 5 to 7 microns, and stained by Ehrlich's haematoxylin and eosin and in some cases by Masson's trichrome stain.

Experiment I

Vacuum dried salt was thoroughly mixed with a proprietary fattening meal to give a final concentration

of 5·3 per cent. by weight. A 2-gallon bucket was half filled with meal and water added till the bucket was nearly full.

Feeding of the experimental mixture to 7 pigs commenced on the evening of 19.7.55. Slight abnormalities of behaviour were evident in P.1397 on 22.7.55, and in the remaining pigs on 23.7.55.

On 24.7.55, P.1397 began to show typical signs of meningo-encephalitis eosinophilica. The remaining pigs were dull and showed some intention tremor.

Owing to an unforeseen change of attendant the pigs were then given an additional supply of water and their signs diminished. On 25.7.55 P.1397 was killed.

On 26.7.55, the error in water supply was discovered and rectified, but none of the surviving pigs subsequently showed more than depression with some trembling and ataxia, and they were killed on 28.7.55 and 29.7.55.

The macroscopic appearance at necropsy was similar in all pigs, the only abnormalities being moistness and congestion of the brain, a few " milk spots " in the liver and about 100 c.c. of urine in the bladder.

Typical vascular lesions of meningo-encephalitis eosinophilica were present in the cerebra of 4 out of the 7 pigs, but none showed the regular mid-cortical band of malacia which is a characteristic but inconstant lesion of the disease.

This experiment is summarised in Table I.

Experiment II

Analytical grade sodium chloride was mixed with meal to give a final concentration of 9·4 per cent. by weight. Immediately before feeding, the meal was mixed with 2½ times its weight of water.

Four pigs were fasted on the evening of 8.9.55, and started on the experimental diet on 9.9.55. On the morning of 11.9.55, typical signs of meningo-encephalitis eosinophilica had appeared in 3 of the pigs, and 2 died that day. P.1636 appeared normal, but developed fine tremors on the next day.

By 14.9.55 P.1632 was *in extremis* and was killed. P.1636 continued to feed moderately, but showed tremors and seemed a little hyperaesthetic, and was killed on 15.9.55.

Histologically, typical vascular lesions of meningo-encephalitis eosinophilica were present to varying degrees in the cerebra of all 4 pigs, and the regular midcortical zone of malacia was demonstrable in the cerebrum of P.1634, the first pig to die.

The kidney of P.1632 showed considerable vacuolation of the epithelial cells of the convoluted tubules, which contained eosinophilic detritus and globules. Possibly these changes are indicative of some degree of tubular nephrosis, which may explain the low chloride content of this pig's urine.

This experiment is summarised in Table II.

In Experiments I and II, abnormalities of the central nervous system were detected only in the cerebrum.

Experiment III

In view of the above results and those of Smith using sodium proprionate the question arose whether the syndrome was due to direct toxicity of the sodium ion or to disturbances of water balance occasioned by the high osmotic tension of the diet. It was therefore decided to feed groups of pigs on diets containing urea alone, or urea with sodium chloride, at concentrations calculated to give an osmotic effect equivalent

TABLE I

EXPERIMENT I. EFFECTS OF FEEDING MEAL CONTAINING 5·3 PER CENT. NaCl

Number of pigs	Mode of death	Days to death	Salt eaten, gm. per kilo. bodyweight per day	White blood cell counts at time of death						Plasma chloride mg. per cent.	Lesions	
				Total	L per cent.	N per cent.	E per cent.	M per cent.	B per cent.		Perivascular eosinophile infiltration	Malacia
1397	K	5½	4·7	19,750	34·0	53	4·0	9	0	505	++++	−
1400	K	8½	3·3	12,200	65·0	27	1·0	7	0	480	−	−
1402	K	8½	1·8	24,300	63·0	25	6·0	6	0	575	+++	−
1403	K	8½	2·4	22,600	74·0	17	2·0	7	0	482	+	−
1398	K	9½	2·1	25,150	64·5	29	1·5	5	0	502	−	−
1399	K	9½	2·3	20,200	50·5	33	5·5	11	0	475	+	−
1401	K	9½	2·4	19,100	48·5	36	5·5	10	0	535	−	−

TABLE II

EXPERIMENT II. EFFECTS OF FEEDING MEAL CONTAINING 9·4 PER CENT. NaCl

Number of pig	Days to signs	Days to death	Mode of death	Salt eaten gm. per kilo. bodyweight per day	White blood cell counts at time of death						Urine chloride mg. per cent.	Serum chloride mg. per cent.	Lesions	
					Total	L per cent.	N per cent.	E per cent.	M per cent.	B per cent.			Perivascular eosinophile infiltration	Malacia
1634	2	2	D	4·8	Not done						Not done		+++	++
1631	2	2	D	4·3							615	Not done	+++	−
1632	2	5	K	4·9	4,050	12	79	2	7	0	142	425	+++	−
1636	4	6	K	3·7	15,600	40	50	2	8	0	1,133	473	++	−

to that of a concentration of about 7 per cent. sodium chloride.

In this experiment the calculated quantity of urea and/or sodium chloride was dissolved in the water which was mixed with the meal in a proportion of 2½ : 1 by weight.

Group A

Four pigs were placed on a diet of proprietary fattening meal (which contained 0·8 per cent. sodium chloride when purchased) and 15 per cent. urea.

The pigs were fasted on the evening of 2.10.56 and the experimental diet introduced on the morning of 3.10.56. On 4.10.56 all the animals seemed depressed and tended to shiver.

On the morning of 5.10.56, all were depressed and 3 had variable ataxia and tended to push their heads into the corners of the box. By the evening of the same day, the 3 affected pigs were recumbent whenever possible, and if roused, wandered aimlessly about, bumping into the walls and each other. P.4174 was depressed but only slightly ataxic.

On 6.10.56 P.4194 was recumbent and semi-comatose, P.4189 was recumbent with intermittent head-nodding, P.4174 and P.4180 were standing but markedly depressed and somewhat ataxic. P.4194 died at about midday following cardiac puncture.

On the morning of 7.10.56, P.4189 was found dead. P.4180 was semi-comatose with slight muscular tremors, and P.4174 was depressed with slight ataxia, amaurosis and constipation.

On 8.10.56 the condition of P.4180 was unchanged, and P. 4174 was also recumbent and semicomatose. Both pigs were killed about midday.

Necropsy revealed no consistent picture. The stomachs of all the pigs were well filled, and the livers showed various degrees of "milk spot." The alimentary contents of P.4194 and P.4189 were abnormally dry, and the arcuate vessels in the kidneys of P.4189 and P.4180 were congested. Urine volume varied from 150 ml. in P.4194 to 5 ml. in P.4174.

Histopathology. P.4194. Slight endothelial proliferation and perivascular lymphocytic infiltration affected the vessels of the cerebrum, and there were typical zones and foci of midcortical malacia.

P.4189. The vessels of the cerebrum had undergone moderate endothelial proliferation and lymphocytic infiltration, and traces of vacuolation were visible in the ground substance in the regions usually affected by malacia.

P.4174. A mild degree of endothelial proliferation and lymphocytic infiltration involved a number of cerebral vessels, and there were large zones of typical midcortical malacia.

P.4180. A number of cerebral vessels showed moderate perivascular infiltration and slight endothelial proliferation, and patches of midcortical malacia were widespread.

In all 4 pigs, the central nervous system other than the cerebrum showed no obvious abnormality, save for a moderate hyperaemia.

Group B

On the evening of 2.10.56, 4 pigs were fasted, and on the morning of 3.10.56 were introduced to a diet of the same meal (containing 0·8 per cent. sodium chloride) together with 7·5 per cent. urea and 3 per cent. additional sodium chloride. By the evening of 4.10.56, all the pigs seemed depressed and tended to shiver. On the morning of 5.10.56 P.4183 had typical signs of acute meningo-encephalitis, with head nodding, walking backwards and occasional convulsions, and died early in the afternoon. P.4188 and P.4193 showed typical less acute signs, amaurosis and aimless wandering, which became more severe later in the day when they pressed their heads against the walls and tripped over objects. P.4192 was merely depressed. On 6.10.56 P.4188 and P.4193 were recumbent with continual head nodding and occasional convulsions. P.4192 could walk but was very depressed, amaurotic and had occasional convulsions.

On 7.10.56, P.4188 was recumbent with continuous clonic spasms of the head and neck, P.4193 was semi-comatose with diffuse muscular spasms, P.4192 was recumbent with constant paddling movements of the limbs, staring eyes and occasional convulsions and was killed.

On 8.10.56, P.4188 was alive in the early morning but was found dead at noon.

P.4193 was recumbent and semicomatose, with constant clonic contractions of the muscles, and was killed.

Necropsy revealed well-filled stomachs in all pigs, with some hyperaemia of the mucous membrane. There was some fatty change in the liver of P.4183, and the arcuate vessels in the kidneys of P.4193 were congested.

Histopathology. P. 4183. Only minimal endothelial proliferation was detected in a few of the cerebral vessels, but numerous foci of malacia extended in a regular band round the midcortical zone of the cerebrum.

P.4192. Moderate endothelial proliferation and eosinophile infiltration was present in a number of cerebral vessels, and the ground substance of the cortex was considerably vacuolated in the region where malacia usually occurs.

P.4188. Large areas of malacia and vacuolation were visible in the cerebrum not only in the usual midcortical zone but also in the plexiform and outer granule layers. The vessels had developed only slight endothelial proliferation and lymphocytic infiltration with an occasional single eosinophile present in the vessel wall, but adjacent to a few of them were small areas of rather diffuse microgliosis.

P.4193. Many cerebral vessels showed considerable endothelial hyperplasia, sometimes accompanied by a little lymphocyte and eosinophile infiltration. A chain of foci of malacia ran around the midcortical zone, and areas of diffuse gliosis were scattered about the grey matter. In these 4 pigs, no abnormalities other than moderate hyperaemia was detected in the central nervous system outside the cerebrum.

Group C

In order to assess the effect of 3 per cent. added sodium chloride alone at this level of water intake, 3 pigs were subjected to a similar process to that

J. T. DONE, J. D. J. HARDING & M. K. LLOYD.—MENINGO-ENCEPHALITIS EOSINOPHILICA OF SWINE

FIG. 1.—Pig. 4174 (15 per cent. urea). Cerebrum showing cortical laminar malacia. Masson's trichrome × 20 approx.

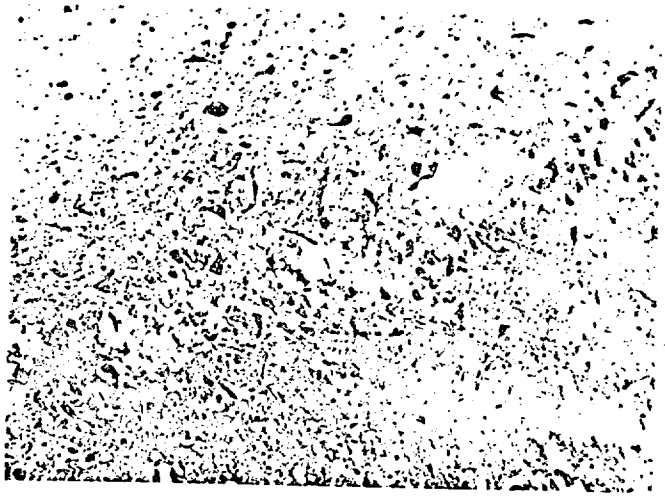

FIG. 4. Pig 1634 (9·4 per cent. NaCl). Detail from Fig. 2, showing necrotic neurones and vacuolation of ground substance. Masson's trichrome × 90 approx.

FIG. 2.—Pig 1634 (9·4 per cent. NaCl). Cerebrum showing cortical laminar malacia. Masson's trichrome × 20 approx.

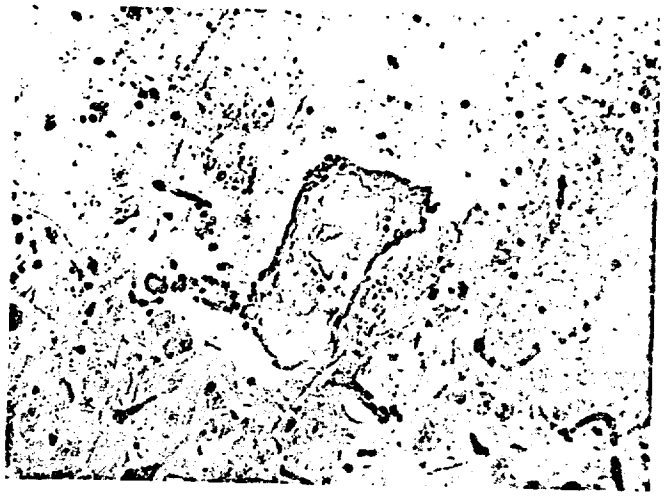

FIG. 5.—Pig 4174. (15 per cent. urea). Vessel in cerebral cortex showing mild perivascular lymphocytic infiltration and endothelial proliferation. H. & E. × 220 approx.

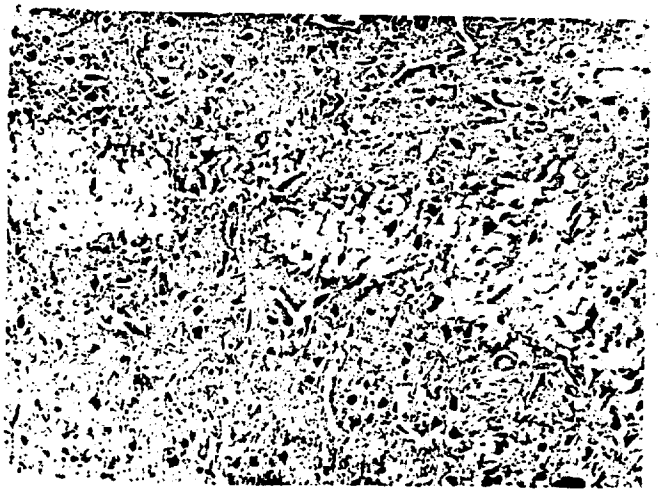

FIG. 3 - Pig. 4174 (15 per cent. urea). Detail from Fig. 1, showing necrotic neurones and vacuolation of ground sub-

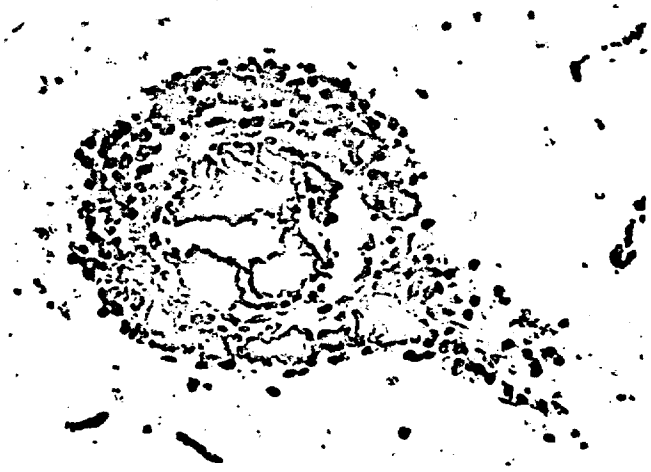

FIG. 6. Pig 1634 (9·4 per cent. NaCl). Vessel in cerebral

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TABLE III

EXPERIMENT III. EFFECTS OF FEEDING MEAL CONTAINING (A) 15 PER CENT. UREA; (B) 3 PER CENT. NaCl + 7·5 PER CENT UREA; (C) 3 PER CENT. NaCl

	Number of pigs	Days to signs	Days to death	Mode of death	Lesions: Perivascular eosinophile infiltration	Perivascular lymphocytic infiltration	Vascular endothelial proliferation	Microgliosis of cerebrum	Malacia of cerebrum	Serum chloride mg. per cent.	Remarks
A. 15 per cent. urea	4194	2	3½	D/K	—	+	+	—	+++	Not done	
	4189	2	4	D	—	+	++	++	±	,,	
	4174	3	5	K	—	+	+	—	+++	445	
	4180	2	5	K	—	+	±	—	++	500	
B. 3 per cent. NaCl 7·5 per cent. urea	4183	2	2	D	—	—	±	++	++	490	Malacia irregularly distributed
	4192	3	4	K	++	—	++	—	+	500	
	4188	2	5	D	—	—	+	+	+++	Not done	
	4193	2	5	K	+	+	++	++	++	490	
C. 3 per cent. NaCl	4106	—	11	K	—	+	—	—	—	Not done	
	4107	—	11	K	—	±	—	—	—	,,	
	4108	—	11	K	—	—	—	—	—	,,	

D = Died. K = Killed. ± = Doubtful. — = Negative. + to +++ = ascending degrees of severity

used in A and B, save that only 3 per cent. of sodium chloride was added to the diet. The experiment was carried on for 11 days, in the course of which time the pigs became slightly dull and reluctant to eat and P.4106 occasionally showed slight ataxia and fine muscular tremors over the flanks. The pigs were killed on the morning of the 12th day.

Histopathology. P.4106 had slight perivascular round cell infiltration in a number of vessels of the cerebrum, with a little sponginess of the ground substance around a few of them. There was also a slight patchy non-suppurative leptomeningitis over the cerebellum.

P.4108 had traces of round cell infiltration in the cerebral leptomeninges and around a few vessels in the cerebral white matter and in the corpora quadrigemina.

P.4109 had slight, patchy round cell infiltration of the cerebellar leptomeninges and around one or two vessels in the corpora, where there was also one small focus of microgliosis.

None of these pigs demonstrated any of the typical lesions of meningo-encephalitis eosinophilica.

The results of Experiment III are summarised in Table III.

Discussion

Experiments I and II confirm that the classic lesions of meningo-encephalitis eosinophilica may be reproduced by feeding sodium chloride with restricted water, particularly when inadequate trough space encourages competitive eating. The wealth of literature recording difficulties in reproducing salt poisoning experimentally serves to emphasise the importance of water restriction in the pathogenesis of the disease and it is clearly meaningless to speak of a toxic dose of sodium chloride as if it were an absolute figure, without reference to the quantity of water available to the animal. At the same time it must be realised that the water supplied in these experiments (*i.e.* 2½ times the weight of dry meal) falls within the limits advocated as good pig husbandry (Watson & More, 1945 ; Braude, 1954).

Since sodium chloride, sodium proprionate (Smith, 1957) and sodium lactate (Hjärre & Öbel, 1956) have all been used successfully to reproduce meningo-encephalitis eosinophilica, it is often assumed that all the lesions of the disease are specific to sodium salt poisoning. Experiment III suggests that, while the perivascular eosinophile infiltration is so specific, mid-cortical laminar malacia and vascular endothelial proliferation, sometimes accompanied by a degree of perivascular lymphocytic infiltration and microgliosis, may be occasioned by other substances in high osmotic concentration in the diet ; indeed, that those lesions are probably more indicative of disturbed water balance in the individual than of any specific poisoning. In this respect the observations of Hjärre & Öbel (1956) and of Gellatly (1957) are of considerable interest. The former authors attempted to poison 6 pigs with ammonium chloride, and found in one a " lymphocytic encephalitis " of which they do not give details. Gellatly (1957) described a syndrome of depression, circling and opisthotonus in pigs under 2 months old, characterised pathologically by "laminar cerebral cortical necrosis," and usually associated with dehydration and anoxia. He also drew attention to a report by Douglas (1949) of rather similar lesions in man, occasioned by anoxia.

Hjärre & Öbel (1956) and Smith (1957) also noted that some of their experimental pigs died after sudden heavy water consumption, suggesting that a disturbance of water balance rather than a specific toxic agent was the operative factor.

Morrill (1957) was quoted as saying that lesions resembling those of meningo-encephalitis eosinophilica are seen in pigs poisoned with smartweed but, in a private communication (1958) he indicates that the statement was a verbal one made from memory and he does not care to state unequivocally that the lesions are identical.

It must, of course, be agreed that in field outbreaks

of meningo-encephalitis eosinophilica, as in experiments described by several authors, individual cases occur in which eosinophile infiltration is absent but some of the other typical lesions are invariably present. It would seem sound practice to make a firm diagnosis of sodium salt poisoning on neuropathological evidence alone when typical eosinophile infiltration is present; in its absence the other lesions should only suggest a disturbed water balance.

Malacic foci with or without eosinophile infiltration, similar to those occurring in the cerebrum are sometimes seen in other parts of the brains from clinically similar cases of field disease (Hjärre & Öbel, 1956; Michalka, 1941) but no author has recorded lesions in experimental pigs elsewhere than in the cerebrum, and such extracerebral lesions should be interpreted with caution.

The precise mechanism by which the lesions arise remains obscure, but present observations suggest that dehydration plays a large part in the process. In man there is a maximal osmotic concentration of urine which varies with the rate of output, being higher for low minute volumes (Harvey, McCance & Taylor, 1946) so that 2 osmotically active substances cannot exist simultaneously in urine each at its maximum concentration. Furthermore, even in severe dehydration urine continues to be formed—the "urine obligatoire" of Ambard & Papin (1909). Assuming similar limits in pigs, the feeding of high osmotic concentrations of any solute which is absorbed and excreted, unchanged, by the kidney, could result in more water being required for their excretion than was available to the pig, causing the osmotic pressure of the body fluids to rise. Thus, in Experiment III, the feeding of urea occasioned disease in pigs receiving quantities of sodium chloride and water normally incapable of causing poisoning, the critical value being the total quantity of osmotically active substances being excreted by the kidney, relative to the quantity of water available from all sources.

Though limited in number the chloride values of the serum and plasma samples examined compare fairly closely with those of other workers, where only sodium chloride poisoning was involved. (Cox & Pitts (1957) found the average plasma chloride level from 3 normal pigs to be 354 mg. per cent., and the levels in 3 poisoned pigs to range from 441 to 486 mg. per cent. Smith (1957) found serum chloride levels in 14 pigs before being placed on experiment ranged from 350 to 408 mg. per cent., and levels in 10 pigs showing signs of sodium chloride poisoning ranged from 442 to 507 mg. per cent.) However, the results of Experiment III suggest that similar elevation of blood chloride may occur in the absence of high dietary concentrations of sodium salts, possibly due to the preferential excretion of urea preventing sodium chloride from reaching its normal osmotic concentration in the urine. The present work did not cover the estimation of sodium levels, which may be a more reliable diagnostic aid.

Smith (1957) noted an eosinopenia in the blood of his experimental pigs. Michalka (1942) mentions "a circulating eosinophilia" but gives no figures and may have intended to refer only to eosinophiles visible in the lumina of vessels seen in histological sections. The present authors found no obviously abnormal blood picture, but counts were performed only at the time of death and transient changes may have been missed.

Most diets fed to swine in this country probably contain adequate amounts of sodium and chloride (Done, 1957) and, in the light of our present knowledge of meningo-encephalitis eosinophilica and similar conditions, it seems wise that no sodium chloride should be added to the food, and that pigs should always have access to unlimited water supply.

References

AMBARD, L., & PAPIN, E. (1909). *Arch. Int. Physiol.* **8.** 432.
BRAUDE, R. (1954). "Progress in the Physiology of Farm Animals." Ed. J. Hammond. Vol. 1, page 73. Butterworth Scientific Publications, London, quoting Evvard, J. N. (1929). *Iowa Res. Bull.* 118.
COX, D. H., & PITTS, J. C. (1957). *J.A.V.M.A.* **130.** 176.
DONE, J. T. (1957). *Vet. Rec.* **69.** 212.
———, BROOKSBANK, N. H., & BUNTAIN, D. (1957). *Ibid.* **69.** 67.
DOUGLAS, C. J. (1949). *Canad. Med. Ass. J.* **61.** 123.
GELLATLY, J. B. M. (1957). *Vet. Rec.* **69.** 1,351.
HARVEY, G. R., MCCANCE, R. A., & TAYLOR, R. G. O. (1946). *Nature.* **157.** 338.
HAWK, P. B., OSER, B. L., & SUMMERSON, W. H. (1949). "Practical Physiological Chemistry." 12th ed., page 573. J. A. Churchill, London.
HJÄRRE, A., & ÖBEL, A.-L. (1956). *Mh. Vet. Med.* **11.** 690.
LARSEN, N. B., MOLLER, T., & THORDAL-CHRISTENSEN, A. (1955). *Nord. VetMed.* **7.** 653.
MICHALKA, J. (1941). *Wien. tierarztl. Mschr.* **28.** 295.
MORRILL, C. C. (1957). *J.A.V.M.A.* **130.** 13.
———. (1958). Private communication.
SMITH, D. L. T. (1955). Thesis, Cornell University Publication No. 15621.
———. (1957). *Am. J. Vet. Res.* **18.** 825.
WATSON, J. A. S., & MORE, J. A. (1945). "Agriculture." 8th ed., page 684. Oliver & Boyd, Edinburgh.

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THE ASSOCIATION OF VETERINARY STUDENTS' ANNUAL CONFERENCE

The XIth Annual Conference of the Association of Veterinary Students was opened in London by the Minister of Agriculture on Friday, January 23rd. There was a large attendance of veterinary students from the 7 veterinary colleges in the United Kingdom and Eire, and the occasion was also marked by the presence of some distinguished members of the profession, including Professor T. J. Bosworth (President of the Royal College of Veterinary Surgeons), Mr. H. F. Hebeler (President of the British Veterinary Association), and Mr. J. N. Ritchie (Chief Veterinary Officer of the Ministry of Agriculture). The Principal of the Royal Veterinary College, Dr. R. E. Glover, replied to the Minister's speech. A full report of the proceedings will be published in our next issue.

Sodium Balance, Plasma Renin, and Aldosterone in Hypertension

By Austin E. Doyle, M.D., F.R.A.C.P., F.R.C.P., and George Jérums, M.B., B.S., M.R.A.C.P.

ABSTRACT

The effects of dietary sodium depletion and the effects of frusemide on plasma renin concentration have been studied in patients with essential hypertension and in normotensive controls.

There was no significant difference in renal sodium excretion between the two groups during sodium loading, dietary sodium restriction, or after the diuretic.

Peripheral plasma levels of aldosterone were similar in both groups during sodium loading and rose to an approximately equal degree during sodium depletion.

Plasma renin concentration was consistently lower in the hypertensive patients in all circumstances, but although some hypertensive patients had persistently very low and unresponsive renin levels, they did not appear to form a separate group from the remaining patients.

It is concluded that the renin angiotensin system is quantitatively less important in controlling aldosterone secretion in hypertensive patients than in normal man, and that in hypertension, sodium conservation and aldosterone secretion may be under the control of a different mechanism.

ADDITIONAL KEY WORDS plasma aldosterone plasma renin hypertension sodium depletion diet diuretics

■ Differences in sodium metabolism between hypertensive patients and normal subjects have been frequently described. Hypertensive patients excrete an infused sodium load faster than patients with normal blood pressure, and have also been found to conserve sodium more efficiently than normal during dietary sodium restriction.[1, 2]

In most normal animals and man, dietary sodium restriction leads to a rise in plasma renin concentration, a rise in urinary and plasma levels of aldosterone, and to a fall in urinary sodium excretion.[3–7] Likewise, the administration of diuretics causes increased urinary sodium excretion followed by a rise in plasma renin concentration and a rise in aldosterone excretion rate.[8, 9]

Recently, it has been observed that both dietary sodium restriction and the intravenous administration of diuretics fail to cause a rise in plasma renin concentration in 20 to 30% of hypertensive patients studied. These unresponsive patients have been regarded as forming a separate subgroup of hypertensive patients.[10–17]

It is clearly important to attempt to define whether this lack of response of renin is a characteristic of certain distinctive hypertensive patients or whether a diminished responsiveness of plasma renin levels is a graded characteristic present in most hypertensive patients.

To study this, a comparison of the effects of dietary sodium restriction and two dose levels of the diuretic drug frusemide has been made in hypertensive and normotensive patients.

Materials and Methods

The 50 hypertensive patients studied were

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attending the Hypertensive Clinic at the University Department of Medicine, Austin Hospital. Their mean age was 50 years, and the male:female ratio was 1:1. When first seen, none of the patients had had the cause of their hypertension defined, and they were included in this study only after investigations, including intravenous pyelography in all cases and renal angiography when indicated (24 patients) had shown no renal cause for the hypertension. Eight of the patients were being treated when first seen, and in these the drugs were stopped 1 week before admission to hospital.

Thirty selected normotensive volunteers were also studied. Their mean age was 54 years and the male:female ratio was 5:1. They were chosen from a group of asymptomatic ambulant patients admitted for investigation of radiological pulmonary abnormalities and were all free from cardiovascular disease. No patients were receiving treatment known to affect plasma renin levels or sodium metabolism, such as progestational agents, diuretics, or sodium-containing drugs.

Plasma renin concentrations were measured in these 80 patients in the sodium-loaded and sodium-depleted state. Measurements of urinary aldosterone excretion were carried out after sodium loading and after sodium depletion in 21 hypertensive patients and in nine normotensive subjects. Measurements of plasma aldosterone, cortisol, and corticosterone from peripheral venous blood were performed in the 15 hypertensive patients and in the 11 normotensive patients from whom sufficient plasma was available. No other selection was made.

Methods

At the start of the study, each patient was given a daily supplement of 100 mEq of sodium as salt tablets for 5 days, in addition to a normal diet. On the last day of salt supplements, the patient was admitted to hospital. Twenty-four hour collections of urine were commenced immediately and continued for 5 consecutive days.

At 09.00 hours on the second day of admission, after 12-hour recumbency, a sample of venous blood was drawn, and the patient's blood pressure and weight were measured. The patient then received a low-sodium diet containing about 10 mEq of sodium per day for 4 days. Patients were allowed up during this period, and the blood pressure was recorded daily. During this period the blood pressure fell in most patients but did not return to normal. At 09.00 hours on the sixth day of admission, after 4 days of sodium restriction and after being out of bed for 2 hours, the supine blood pressure was measured, a venous blood sample was drawn, and the patient was again weighed. Plasma urea, sodium, potassium and renin and steroid levels were measured in the two blood samples. Urinary sodium and potassium were determined in the five 24-hour urine specimens and the first and last urine specimens were also assayed for levels of creatinine and aldosterone. The plasma renin concentration (PRC) was measured by a selective substrate denaturation technique.[18] The concentration of renin is expressed in terms of nanograms of angiotensin produced per milliliter per hour of incubation (ng/ml/hr). Urinary aldosterone and plasma aldosterone, cortisol, and corticosterone were measured by double-isotope dilution methods.[19, 20]

Eight hypertensive and nine normotensive patients were given 40 mg of frusemide intravenously, and 15 hypertensive and 12 normotensive patients were given 60 mg of frusemide intravenously. These studies were carried out on an outpatient basis. The patients attended fasting at 09.00 hours, a venous blood sample was drawn, and the bladder emptied. Following the intravenous injection of frusemide, the patients were allowed up but remained fasting. All urine passed for the next 3 hours was collected, and 3 hours after the injection a second venous blood sample was drawn for measurement of plasma renin. In three normotensive volunteers, urine collections were continued for 3 further hours, and a third blood sample was collected 6 hours after administration of 60 mg of frusemide.

Results

DIETARY SODIUM RESTRICTION

At the end of the period of sodium loading

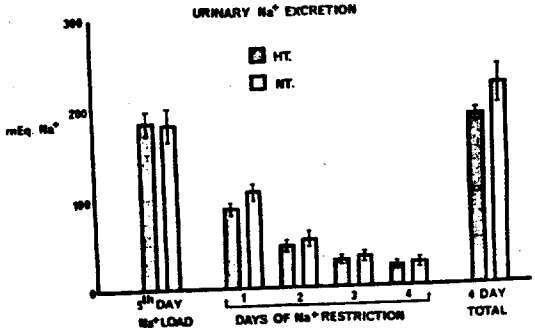

FIGURE 1

Pattern of urinary sodium excretion in the fifth day of sodium loading and during the course of dietary sodium deprivation in hypertensive (shaded columns) and normotensive (open columns) subjects. The mean and standard error of the mean are represented for each day. The last columns represent the cumulative sodium loss over the 4 days of sodium depletion.

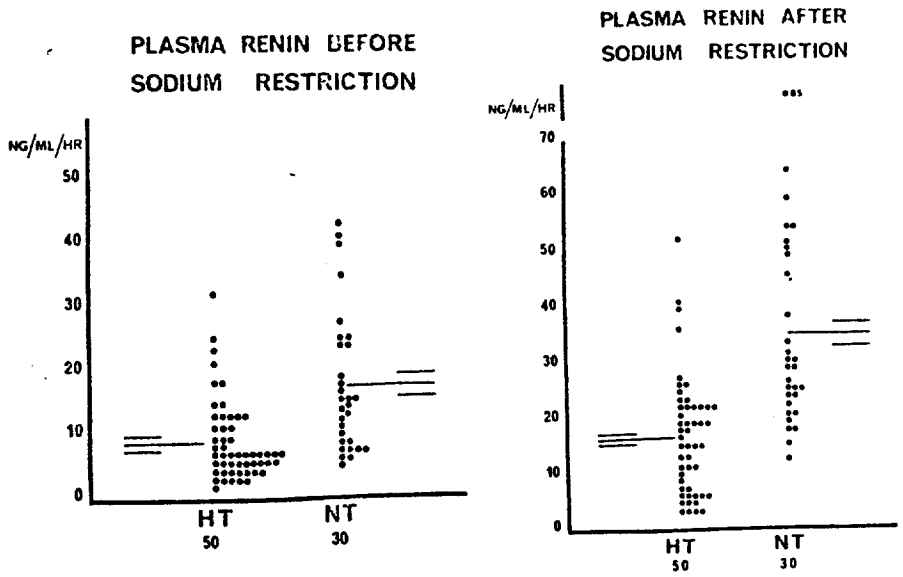

FIGURE 2

Plasma renin concentration in hypertensive (HT) and normotensive (NT) patients before sodium restriction (left) and after 4 days of sodium restriction and 2 hr of upright posture (right).

the urinary sodium excretion varied considerably for individuals, but there was no significant difference in the urinary sodium excretion between the hypertensive or the control groups. In both groups, the low-sodium diet sharply reduced sodium excretion to an equal degree in both groups (fig. 1).

The major differences of the hypertensive from the control groups was in the plasma renin concentration (PRC). The hypertensive patients had significantly lower levels of PRC than the control group during sodium loading. After the low-sodium diet, the difference between the groups was exaggerated. The hypertensive patients approximately doubled the mean PRC from 8.6 to 16.3 ng/ml/hr; the mean PRC in the normotensive control group also approximately doubled, rising from 17.3 to 35.0 ng/ml/hr. Thus in absolute terms, the hypertensive group had lower levels of PRC and a smaller rise with sodium depletion (figs. 2 and 3).

Fourteen of the 50 hypertensive patients had either no rise in PRC or a rise of less than 4.0 ng/ml/hr on sodium depletion. In these 14, the highest level of PRC reached was 7.3 ng/ml/hr. These 14 patients were arbitrarily separated from the remaining hypertensive patients to attempt to decide whether the two groups differed in any other respect. As can be seen from table 1, there were no statistically significant differences between the responsive and nonresponsive hypertensive groups in respect of urinary sodium or potassium excretion, plasma sodium or potassium levels, plasma urea, urinary creatine, or blood pressure. Moreover, clinically, the two groups were indistinguishable.

Urinary aldosterone excretion was measured in 21 of the hypertensive patients and nine of the control patients during sodium loading and after sodium depletion. Peripheral venous blood levels of aldosterone, cortisol and corticosterone were measured in 15 hypertensive and nine normotensive patients both during sodium loading and sodium depletion. There was no significant difference in any of these measurements between the hypertensive

TABLE 1 (a)

Clinical and Biochemical Data in Normotensive and Hypertensive Subjects. The Latter Are also Analyzed According to Whether or Not They Responded to Sodium Restriction by a Rise in PRC

Group	Plasma renin concentration ($ng\ ml^{-1}\ hr^{-1}$)		Increase in plasma renin concentration ($ng\ ml^{-1}\ hr^{-1}$)	Total urinary sodium (mEq/96 hr)	Plasma sodium (mEq/L)		Plasma potassium (mEq/L)	
	Pre	Post			Pre	Post	Pre	Post
Normotensive	17.3 ± 2.0	35.5 ± 2.0	17.6 ± 2.1	223 ± 20.5	141 ± 0.9	139 ± 0.6	4.0 ± 0.1	4.1 ± 0.1
(n = 30)	(5.5 – 41.3)	(31.1 – 58.0)	(4.5 – 60.0)	(30 – 449)	(128 – 150)	(126 – 142)	(3.3 – 47)	(3.4 – 4.8)
Total hypertensive	8.6 ± 0.8*	16.3 ± 1.5	7.8 ± 1.0	189 ± 9.8	142 ± 0.8	140 ± 0.7	3.8 ± 0.1	4.0 ± 0.1
(n = 50)	(1.9 – 32.0)	(2.5 – 51.5)	(– 6 to 30.0)	(57 – 376)	(133 – 160)	(125 – 148)	(2.9 – 4.9)	(3.3 – 5.8)
Responsive renin	10.2 ± 1.0	21.2 ± 1.5	11.0 ± 0.9	176 ± 10.1	141 ± 0.7	140 ± 0.6	3.9 ± 0.1	4.0 ± 0.1
(n = 36)	(1.9 – 32.0)	(8.7 – 51.5)	(4.0 to 30.0)	(57 – 295)	(134 – 151)	(134 – 151)	(2.9 – 4.9)	(3.3 – 5.8)
Unresponsive renin	4.8 ± 0.6	5.0 ± 0.6	0.2 ± 0.5	222 ± 21.6	144 ± 2.2	141 ± 2.2	3.7 ± 0.1	3.9 ± 0.1
(n = 14)	(2.5 – 13.3)	(2.5 – 7.3)	(– 6.6 to 2.0)	(102 – 376)	(133 – 160)	(125 – 148)	(3.3 – 4.4)	(3.5 – 4.6)

TABLE 1 (b)

Group	Plasma urea (mg/100ml blood)		% Change in urinary creatinine excretion	Change in supine systolic blood pressure (mm Hg)	Change in supine diastolic blood pressure (mm Hg)	Urinary potassium (mEq/24 hr)		Total urinary potassium (mEq/96 hr)	Change in body weight (kg)
	Pre	Post				Pre	Post		
Normotensive	28 ± 1.8	34 ± 1.9	– 1.7 ± 5.7%	– 3 ± 2.8	– 2 ± 1.8	46 ± 4.4	48 ± 2.6	175 ± 12.1	– 1.3 ± 0.1
(n = 30)	(15 – 46)	(18 – 49)	(+ 41 to + 23%)	(+ 40 to – 40)	(+ 25 to – 10)	(18 – 79)	(28 – 63)	(170 – 233)	(+ 0.3 to – 2.7)
Total hypertensive	34 ± 1.9	39 ± 2.2*	– 7.6 ± 6.4%	– 17 ± 3.6*	– 8 ± 1.5*	42 ± 3.4	40 ± 2.8	153 ± 9.3	– 1.4 ± 0.1
(n = 50)	(18 – 80)	(17 – 74)	(+ 69 to – 76%)	(+ 30 to – 65)	(+ 20 to – 30)	(12 – 101)	(16 – 72)	(87 – 238)	(+ 0.2 to – 3.1)
Responsive renin	35 ± 2.5	39 ± 2.6	– 7.3 ± 7.3	– 18 ± 4.0	– 8 ± 1.6	38 ± 3.1	39 ± 3.0	145 ± 11.6	– 1.4 ± 1.3
(n = 36)	(18 – 80)	(17 – 74)	(+ 69 to – 76%)	(+ 10 to – 65)	(+ 10 to – 25)	(12 – 54)	(16 – 61)	(87 – 238)	(+ 0.2 to – 2.8)
Unresponsive renin	30 ± 1.7	37 ± 2.3	– 8.5 ± 12.9	– 13 ± 7.4	– 9 ± 3.0	53 – 6.5	48 ± 6.5	184 ± 16.9	– 1.4 ± 2.8
(n = 14)	(20 – 37)	(25 – 70)	(+ 59 to – 69%)	(+ 3 to – 95)	(+ 20 to – 30)	(28 – 101)	(26 – 72)	(124 – 217)	(+ 0.4 to – 2.5)

All values represent the mean ± SEM, with the range of results in parentheses.

Pre = sodium loaded, supine state; Post = sodium depleted, ambulant state.

Unresponsive renin, refers to the group of hypertensive patients who showed an increase of less than 4.0 ng $ml^{-1}hr^{-1}$ in plasma renin levels following sodium restriction and ambulant. Responsive renin, includes the remaining hypertensive patients.

Total urinary sodium, refers to the cumulative urinary excretion of sodium during 4 days of sodium restriction.

*Indicates a significant difference from results in the normotensive group ($P < 0.05$).

†Values for urinary potassium excretion represent results from 30/50 hypertensive patients and 17/30 normotensive patients.

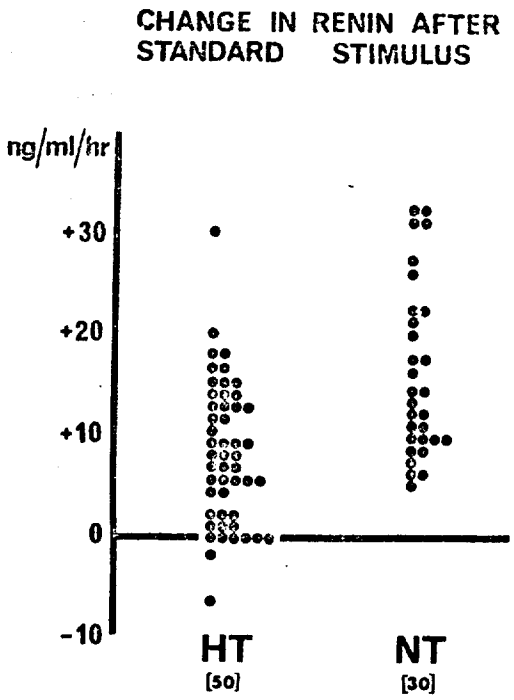

FIGURE 3

Change in plasma renin concentration produced by a standard stimulus in which the subjects were first sodium loaded and blood was obtained in the supine state and then after 4 days of sodium restriction, blood taken in the upright state.

and control group, and the levels in the responsive and nonresponsive group of hypertensive patients were not significantly different from each other. The mean changes in PRC and plasma aldosterone for the hypertensive and control group are shown in figure 4.

THE EFFECTS OF FRUSEMIDE

A dose level of 40 mg intravenously of frusemide produced a rise in PRC in only two of the eight hypertensive patients, but the same dose produced a rise in PRC in five of the nine normal controls (fig. 5).

Sixty milligrams of frusemide induced a rise in PRC in six of the 14 hypertensive patients, whereas the same dose induced a rise in 11 of the 12 control patients (fig. 6).

The total urinary sodium deficit and rise in PRC induced by dietary restriction and by the two doses of frusemide are shown in table 2. It is evident firstly that under all circumstances, the rise in PRC is smaller in hypertensive than in control subjects; secondly, that in both groups the diuretic induced a much smaller rise in PRC than dietary restriction; and lastly, that the degree of sodium depletion produced by the two procedures is of the same magnitude. That this small rise is not simply due to the short time involved is demonstrated in figure 7, in which in three control subjects the study was prolonged to 6 hours. In only one subject did the renin rise further in the second 3 hours. These data also suggest that the high urinary sodium concentration is not responsible for inhibiting the rise in PRC since during the second 3 hours, with an increased sodium deficit and a sharp fall in urinary sodium concentration, there was little additional rise in PRC. However, even during the second 3-hour period, the urinary sodium excretion is still higher than that present during chronic dietary sodium depletion.

Discussion

The results presented here confirm the results of other workers[10–17] that a substantial proportion of hypertensive patients fail to

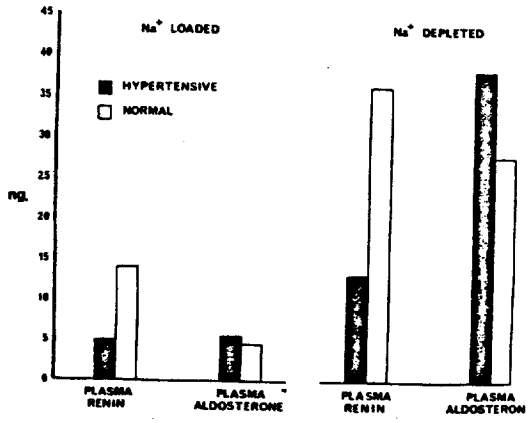

FIGURE 4

The mean levels of PRC and peripheral plasma aldosterone in 15 hypertensive patients (shaded columns) and nine control patients (open columns) during sodium loading and sodium depletion.

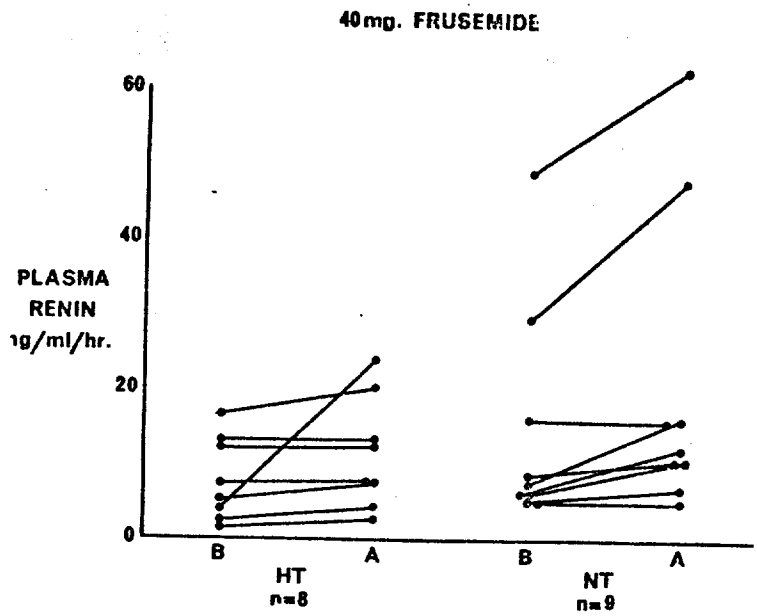

FIGURE 5

The responses of PRC to the intravenous administration of 40 mg of frusemide in eight hypertensive (HT) patients and nine normotensive (NT) controls. B is before and A 3 hours after frusemide.

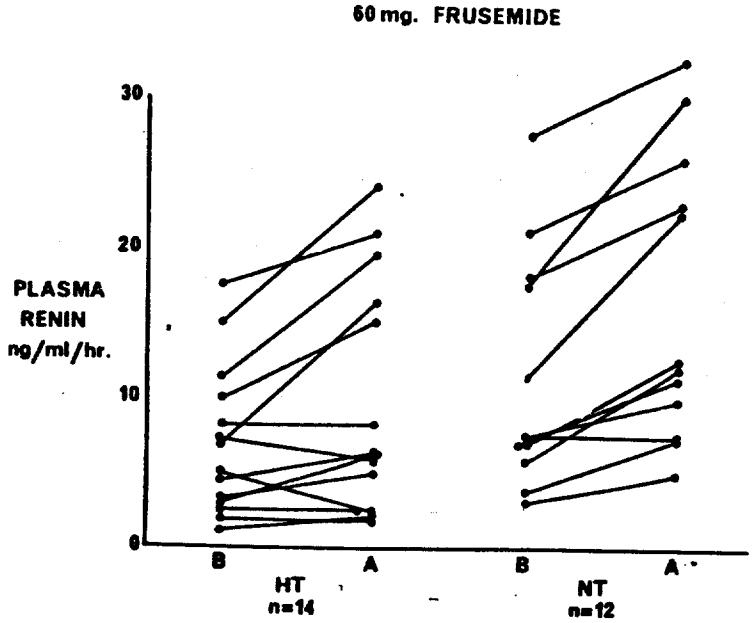

FIGURE 6

The responses of PRC to the intravenous administration of 60 mg of frusemide in 14 hypertensive (HT) patients and 12 normotensive (NT) controls. B is before and A 3 hours after frusemide.

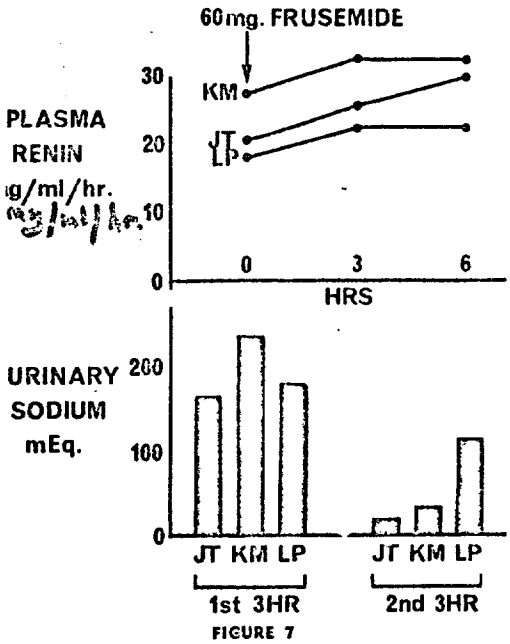

FIGURE 7

The effects of 60 mg of frusemide in three individual control patients on PRC and urinary sodium excretion 3 and 6 hours after the dose was given.

respond to a low-sodium diet by a rise in PRC. Originally, it was postulated that such patients might have primary aldosteronism. Although this might be the explanation in a few patients, the evidence presented here and by others,[19] that aldosterone levels are not abnormally high and serum and urinary potassium levels are within normal limits, make it most unlikely that the majority of these patients have Conn's syndrome.

Our results illustrate the difficulty of deciding whether the patients who fail to raise their levels of PRC on sodium depletion form a separate discrete group or whether they are merely extreme examples of a situation that exists to a graded extent in most if not all hypertensive patients. Two thirds of the hypertensive patients studied had a rise in PRC of less than 10 ng/ml/hr whereas less than one third of the control patients had responses of this order. Only one hypertensive patient had a rise of PRC greater than 20 ng/ml/hr whereas 10 of the 30 control patients studied had changes of this order. It seems reasonable to conclude that the hypertensive group as a whole had blunted responses in terms of rise in PRC and that the 30% who failed to give a significant rise in PRC represent the lower end of the distribution curve rather than a discrete entity.

Using the less powerful stimulus of intravenous frusemide the same conclusions can be drawn, namely, that at both dose levels the hypertensive patients gave smaller responses in PRC than the controls and the proportion of patients not responding is greater at smaller dose levels.

One explanation may be that in hypertension, the adrenal zona glomerulosa is more sensitive to the effects of angiotensin than in normal patients. Such a situation would explain the normal rise of aldosterone found in hypertensive patients after sodium depletion in spite of the smaller and often insignificant rises in PRC. The suppression of PRC may be a feedback suppression via aldosterone.[21] Adrenal hypersensitivity preceding the onset of hypertension might lead in the early stages of the disease to excessive rises in aldosterone

TABLE 2

A Comparison of the Urinary Sodium Loss and the Rise in PRC Induced by the Low-Sodium Diet and Two Intravenously Administered Doses of Frusemide

	Urinary sodium loss (mEq)		Mean rise in plasma renin concentration (ng/ml/hr)	
	Hypertensives	Controls	Hypertensives	Controls
Diet	193 mEq	218 mEq	6.7	22.6
Frusemide 40 mg	138 mEq ± 15.6	129 mEq ± 17.1	3.5	7.3
Frusemide 60 mg	146 mEq ± 15.3	183 mEq ± 13.3	2.7	5.3

production in response to normal rises in PRC, and normal angiotensin levels thus leading to inappropriate sodium retention which could initiate hypertension.

An alternative explanation is that in hypertension the control of aldosterone secretion is genetically less directly controlled by the renin-angiotensin system. If this were so, and if another stimulus were responsible for controlling aldosterone output, a high level of aldosterone production might occur unrelated to renal ischemia or under perfusion, so that sodium retention might also occur at a level inappropriate to renal and cardiovascular hemodynamics. Such a situation might lead to both suppression of renin production and the development of a secondary natriuretic mechanism, either hormonal affecting proximal tubular sodium absorption, or mechanical by a rise in blood pressure.

Finally, the suppression of PRC in hypertension may be due to a natriuretic process occurring proximal to the macula densa. The increased distal tubular sodium load might suppress PRC and blunt the response both to sodium depletion.

Further studies involving the control of aldosterone secretion of normal and hypertensive man seem necessary to clarify these possibilities.

References

1. Farnsworth, E. B., and Barker, M. H.: Tubular resorption of chloride in hypertensive and in normal individuals. Proc Soc Exp Biol 52: 74, 1943.
2. Perera, G. A., and Blood, D. W.: Disturbance in salt and water balance in hypertension. Amer J Med 1: 602, 1946.
3. Blair-West, J. R., Coghlan, J. P., Denton, D. A., Goding, J. R., Munro, J. A., Peterson, R. E., and Wintour, M.: Humoral stimulation of adrenal cortical secretion. J Clin Invest 41: 1606, 1962.
4. Binnion, P. F., Davis, J. O., Brown, T. C., and Olichney, M. J.: Mechanisms regulating aldosterone secretion during sodium depletion. Amer J Physiol 208: 655, 1965.
5. Laragh, J. H., Angers, M., Kelly, W. G., and Lieberman, S.: Hypertensive agents and pressor substances: The effects of epinephrine, norepinephrine, angiotensin II, and others on the secretory rate of aldosterone in man. JAMA 174: 234, 1960.
6. Biron, P., Koiw, E., Nowaczyski, W., Brouillet, J., and Genest, J.: The effects of intravenous infusion of valine-5-angiotensin II, and other pressor agents on urinary electrolytes and corticosteroids including aldosterone. J Clin Invest 40: 338, 1961.
7. Davis, J. O., Ayers, C. R., and Carpenter, C. C. J.: Renal origin of an aldosterone-stimulating hormone in dogs with thoracic caval constriction and in sodium depleted dogs. J Clin Invest 40: 1466, 1961.
8. Weinberger, M. H., Dowdy, A. J., Nokes, G. W., and Luetscher, J. A.: Plasma renin activity and aldosterone secretion in hypertensive patients during high and low sodium intake and administration of diuretic. J Clin Endocr 28: 359, 1968.
9. De Champlain, J., Genest, J., Veyrath, R., and Boucher, R.: Factors controlling renin in man. Arch Intern Med (Chicago) 117: 355, 1966.
10. Jerums, G., and Doyle, A. E.: Renal sodium handling and responsiveness of plasma renin levels in hypertension. Clin Sci 37: 79, 1969.
11. Gunnells, J. C., Grim, C. E., Robinson, R. R., and Wilderman, N. M.: Plasma renin activity in healthy subjects and patients with hypertension. Arch Intern Med (Chicago) 119: 232, 1967.
12. Kuchel, O., Fishman, L. M., Liddle, G. W., and Michelakis, A.: Effect of diazoxide on plasma renin activity in hypertensive patients. Ann Intern Med 67: 791, 1967.
13. Creditor, M. C., and Loschky, U. K.: Plasma renin activity in hypertension. Amer J Med 43: 371, 1967.
14. Kaneko, Y., Ikeda, T., Takeda, T., Inoue, G., Tagawa, H., and Ueda, H.: Renin release in patients with benign essential hypertension. Circulation 38: 353, 1968.
15. Creditor, M. C., and Loschky, U. K.: Incidence of suppressed renin activity and of normokalemic primary aldosteronism in hypertensive negro patients. Circulation 37: 1027, 1968.
16. Helmer, O. M., and Judson, W. E.: Metabolic studies on hypertensive patients with suppressed plasma renin activity not due to hyperaldosteronism. Circulation 38: 965, 1968.
17. Channick, B. J., Adlin, E. V., and Marks, A. D.: Suppressed plasma renin activity in hypertension. Arch Intern Med (Chicago) 123: 131, 1969.
18. Skinner, S. L.: Improved assay methods for renin "concentration" and "activity" in human plasma. Circ Res 20: 391, 1967.
19. Coghlan, J. P., Wintour, M., and Scoggins, B. A.: The measurement of corticosteroids in adrenal vein blood of sheep. Aust J Exp Biol Med Sci 44: 639, 1966.
20. Coghlan, J. P., and Scoggins, B. A.: Aldosterone, its measurement in peripheral blood of men and sheep. J Clin Endocr 27: 1470, 1967.
21. Vander, A. J.: Control of renin release. Physiol Rev 47: 359, 1967.

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A STUDY OF THE EFFECTS OF EXCESSIVE POTASSIUM INTAKE UPON BODY POTASSIUM STORES[1]

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There is extensive evidence that provision of potassium as well as sodium may be of vital importance in the treatment of a wide variety of metabolically disturbed patients (1–3). Physicians sometimes are hesitant to administer potassium, however, because they are concerned that such therapy may result in potassium intoxication. The present studies were undertaken in an effort to gather information which would facilitate considerations of this problem.

Specifically, the aims of this study were threefold: The first was to determine the upper limit of potassium intake compatible with body potassium homeostasis. The second was to determine the nature of the variables which the body's homeostatic systems are attempting to protect under conditions of potassium loading. The third was to find out to what extent, if any, the chronically loaded individual can store surplus potassium in innocuous form when intake exceeds excretory capacity. Because these investigations of necessity involved the administration of potentially lethal doses of potassium, they have been carried out on rats rather than human beings.

MATERIALS AND METHODS

All experiments were performed on healthy male Sprague-Dawley rats weighing between 150 and 200 Gm. The animals were individually caged and provided with tap water *ad lib.* Control animals received approximately 7 Gm. of Purina rat chow per 100 Gm. of body weight per day. This provided the animal with approximately 1.6 mEq. of potassium and 0.8 mEq. of sodium per 100 Gm. of body weight per day. Potassium loaded rats were allowed 6 to 7 Gm. of the same feed in powdered form to which was added a solution containing 3.54 mEq. of potassium, 2.66 mEq. chloride and 0.88 mEq. bicarbonate per ml. This mixture was used in an effort to avoid deviations in extracellular pH which may of themselves cause shifts of potassium within the body (4). Each animal was caged separately and due attention was given to physical cleanliness. Acceptance of feed was estimated within 1 Gm. daily and potassium dosages were adjusted for changes in rat size at five day intervals. All animals were anaesthetized with intraperitoneal pentobarbital sodium prior to sacrifice. Electrocardiograms were obtained during light anaesthesia (30 mg. per Kg. body weight) with a Sanborn Twin-Beam photographic apparatus at a paper speed of 75 mm. per second and internal calibration of 1 cm. equal to 1 mV. The tracings were measured by caliper. Blood specimens for determination of plasma pH (5), carbon dioxide content (6), sodium (7), potassium (7) and chloride (8) were collected from the vena cava in heparinized syringes and delivered beneath oil for centrifugation. Immediately thereafter a representative sample of the vigorously contracting cardiac ventricles was excised, blotted free of blood and subjected to separate analysis. The intestinal tract from the esophago-gastric junction to the rectum was then removed and discarded. The heart muscle sample and the remainder of the carcass were weighed, dried to constant weight, defatted in equal parts of ethyl and petroleum ethers, dried again to constant weight and extracted with 0.75 N nitric acid for determination of sodium (7), potassium (7) and chloride (9). Cardiac chloride was determined polarographically (8) on an 0.75 N nitric acid extract of 0.3 to 0.5 Gm. of dry, defatted tissue made up to a volume of 10 ml. prior to filtering and analysis.[2]

Data for the extracellular and intracellular volumes and electrolyte distribution were calculated assuming that all chloride is extracellular and present at a concentration equal to that of an ultrafiltrate of serum.[3] In

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[2] The titration and polarographic methods for chloride were compared by application to eight samples of filtered, nitric acid carcass extract in which chloride concentration ranged between 13 and 17 mEq. per liter and to eight samples of serum in which the concentration of chloride ranged between 96 and 114 mEq. per liter. Analysis of the variance yielded a standard error of 0.358, a t value of 0.183 and a p value of 0.5.

[3] Ultrafiltrate concentration values were derived from serum concentration values by application of an assumed constant serum water value of 0.93 and a Donnan equilibrium factor of 0.95 (10).

calculating intracellular potassium concentrations, correction was made for the small extracellular potassium content of the tissues. The control electrocardiograms and serum and heart analyses carried out during the present study were supplemented by carcass analyses of normal rats of the same strain, sex, weight and dietary intake performed in this laboratory in the course of a previous study (11). In group comparisons, differences have been considered significant where the value for p is equal to or less than 0.05 using the t test.

OBSERVATIONS

Figure 1 demonstrates the relationship of the animals' potassium intake (abscissa) to the concentration of potassium in the extracellular and to the concentration and content of potassium in the total carcass and cardiac intracellular compartments. The individual control data are shown at the ordinary intake level of 1.6 mEq. of potassium per 100 Gm. body weight per day and the normal 95 per cent distribution as found for 25 animals (1.97 standard deviations) is represented by the horizontal shaded bars.

When the potassium intake was altered by 8 mEq. per 100 Gm. body weight per day stepwise increments at five day intervals, the animals refused small portions of feed on the first day of each new intake level, but otherwise remained apparently well at intakes of 9.6, 17.6 and 25.6 mEq. per 100 Gm. body weight per day. Marked diuresis and polydipsia were noted with the increasing osmotic load, but no other changes in appearance or activity were observed. A seven per cent mean weight gain occurred in these experimental animals during the course of the 15 days. Plasma potassium concentration determined in four or five animals which were sacrificed for this purpose at the end of each five day period remained within the normal range as noted in the top section of Figure 1.

When rats were subjected to the next higher dose, *i.e.*, 33.6 mEq. per 100 Gm. body weight per day, continuing partial refusal of the potassium-containing feed occurred with the result that the net potassium intake of certain rats was less than the dose offered. The animals were therefore maintained on this feeding for ten days, and were then offered a diet containing 38.4 mEq. per 100 Gm. body weight per day in an effort to attain the 33.6 mEq. per 100 Gm. body weight per day net intake level in all animals. By the 14th day of potassium loading in excess of 25.6 mEq. per 100 Gm. body weight per day, half of the 16 animals had died, each within three days of attaining its individual maximum intake (27.5 to 37.9 mEq. per 100 Gm. per day or about 60 mEq. per rat per day). The experiment was terminated at this predetermined end-point of 50 per cent deaths (LD_{50}) by the sacrifice of the eight remaining animals. These sacrificed animals had lost weight terminally to a mean of 92 per cent of initial weight and evidenced the same symptoms noted in the animals that died, namely, a progressive tendency toward roughening of coat, irritability, lethargy and eventually generalized weakness. The maximum potassium intakes in the sacrificed animals (29.4 to 37.9 mEq. per 100 Gm. per day) had also been attained between one and three days prior to sacrifice. At sacrifice, these animals had gross evidence of adrenal enlargement.

Table 1 shows that the plasma potassium and chloride concentration values of the LD_{50} survivors were significantly higher than those of animals on the 1.6 mEq. per 100 Gm. body weight

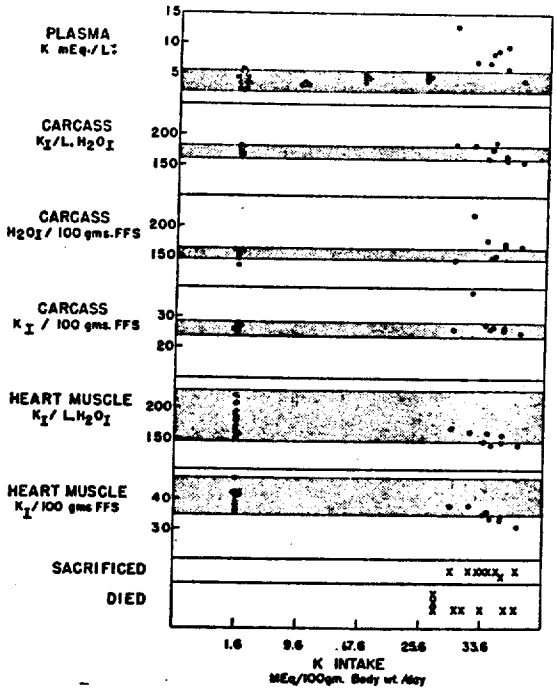

FIG. 1. RELATIONS BETWEEN K INTAKE AND EXTRACELLULAR AND INTRACELLULAR CONCENTRATION AND CONTENT VALUES

I stands for intracellular, FFS for fat free solids.

TABLE I

*Plasma concentrations**

		pH	CO_2	Cl	K	Na
Controls	M	7.43	26.7	101	4.15	143
	SE	0.0092	0.679	0.753	0.147	0.472
	N	8	8	19	25	19
LD_{50} survivors	M	7.42	27.5	108	8.0	143
	SE	0.0187	0.522	1.875	0.684	2.067
	N	8	6	8	8	8
p				0.01	0.01	

* CO_2 content, chloride, potassium and sodium are expressed as mEq. per liter of plasma. M = mean; SE = standard error of mean; N = number of animals; p = significance level. Plasma CO_2 was not determined in two LD_{50} survivors with plasma pH of 7.45.

per day potassium intake. However, as can be seen in the upper half of Table II, there was no significant difference between the LD_{50} survivors and the controls with respect to the total potassium content or to the concentration of potassium in cell water of the carcass as a whole.

The lower half of Table II shows that the cardiac muscle of the LD_{50} survivors contained significantly less total potassium both per 100 Gm. of fat free wet weight and per 100 Gm. of fat free solids than did the controls. The cardiac muscle of the LD_{50} animals also differed slightly from the controls with respect to water, sodium and fat content.

Electrocardiograms were made in each of the LD_{50} survivors immediately prior to sacrifice. These were compared with control electrocardiograms obtained on 10 normal rats as well as with a series gathered on rats subjected to acute potassium intoxication by intraperitoneal loading. The acute loading had resulted sequentially in bradycardia, P wave voltage depression, T wave voltage *depression* or *inversion*, PR and QT interval prolongation, QRS widening and, terminally, sine wave formation; Table III shows that the chronically loaded LD_{50} animals also showed significant changes in the first three of these variables. As can be seen in Figure 2, the changes in T wave voltage bore an inverse relation to plasma potassium concentration; there also was a statistically significant correlation between T wave voltage and potassium concentration per liter of cardiac intracellular water.

COMMENTS

In defining the ceiling, or upper limit of body tolerance for a substance, two levels may be considered: *a*) an upper lethal limit, and *b*) a lower physiologic ceiling at which pathologic alteration of body composition or concentration first occurs. It might be anticipated that the location of the intoxication zone defined by these parameters would differ among individuals as a result of biologic variability. Thus it appears from Figure 1 that the upper lethal limit for rats under the circumstances of the present experiments is between 27.2 and some quantity in excess of 38.4 mEq. per 100 Gm. of body weight per day. The fact that all but one of the LD_{50} survivors showed both hyperkalemia and characteristic electrocardiographic changes indicates that these individuals were ingesting po-

TABLE II

Potassium concentration in total carcass and cardiac muscle

			Fat % total weight	H_2O_T FFWW	H_2O_E FFWW	H_2O_I FFWW	H_2O_T FFS	Cl_T FFWW	K_T FFWW	K_E FFWW	K_I FFS	K_I LICW	Na_T FFWW	Na_E FFWW	Na_L FFS	Na_I LICW
Total Carcass	Control	M	2.62	72.1	29.2	42.6	154	3.28	7.46	0.13	26.2	175	5.02	4.24	2.78	18.1
	N=8	SE	0.277	0.322	0.336	0.456	3.32	0.0445	0.0883	0.0068	0.430	1.91	0.0671	0.0421	0.225	1.4
	LD_{50} survivors	M	1.11	73.1	29.9	43.2	162.8	3.52	7.67	0.23	28.0	172	5.29	4.36	3.53	21.5
	N=8	SE	0.325	0.859	0.742	0.714	7.88	0.0940	0.194	0.0270	1.56	4.60	0.1059	0.0750	0.369	1.88
		p	<0.01													
Cardiac Muscle	Control	M	0.092	78.1	28.7	49.4	226	3.30	8.88	0.11	40.6	178.4	4.63	4.19	2.14	9.20
	N=11	SE	0.0181	0.299	0.650	0.702	4.21	0.0760	0.226	0.0078	1.044	7.20	0.1189	0.0800	0.602	2.39
	LD_{50} survivors	M	0.288	77.2	25.2	52.1	229	2.99	8.10	0.20	35.6	152	3.90	3.56	1.49	6.38
	N=8	SE	0.0781	0.164	0.698	0.669	3.35	0.1017	0.166	0.0287	0.828	3.90	0.1321	0.0731	0.442	2.61
		p	<0.05	<0.05		<0.05			<0.05		<0.01	<0.025	<0.01	<0.005		

* Values for chloride, potassium and sodium (MEq.) and water (Gm.) are expressed per 100 Gm. of fat free wet weight (FFWW), per 100 Gm. fat free solids (FFS) and per liter of intracellular water (LICW). Subscript T = total; E = extracellular; I = intracellular.

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TABLE III

Electrocardiographic standard in 10 normal male Sprague-Dawley rats weighing 150 to 200 Gm. and in the present LD_{50} survivors during light pentobarbital anaesthesia

	Controls		LD_{50} survivors		
	Mean	SD	Mean	SD	p
Ventricular rate per min.	390	14	321.9	+37.0	<0.001
P voltage, lead II, mV.	0.16	0.03	0.095	+ 0.04	<0.01
T voltage, lead II, mV.	+0.16	0.03	+0.08	+ 0.07	<0.025
PR interval, msec.	46.5	2.8	48.0	+15.0	>0.5
QT interval, msec.	56.4	3.6	51.5	+18.3	>0.5
QRS, msec.	13.8	0.7	16.8	+ 6.9	<0.4

tassium at rates which were in excess of their respective physiologic ceiling levels. The absence of diarrhea in these animals suggests that the measured intake ceilings were, in fact, indirect estimations of renal excretory capacity for potassium ion. In confirmation of this, one animal accepting 32 mEq. per 100 Gm. body weight per day was found to be excreting 97.5 per cent of this potassium in the urine.

The LD_{50} survivors appeared to be limiting their intake somewhat in accordance with their degree of hyperkalemia (*i.e.*, intoxication). Though this reaction might be considered protective in nature, it failed to prevent the spontaneous ingestion of lethal doses of potassium. Inasmuch as this phenomenon has been observed also in patients with renal failure, it appears that one cannot depend upon instinct to protect human beings against taking a lethal load of potassium by mouth in the form of fruit juice or other ordinary nutrient. Fortunately, it is possible to estimate an individual's tolerance and to prescribe accordingly (12).

The data obtained on animals taking between 1.6 and 27.2 mEq. of potassium per 100 Gm. body weight per 24 hours reveal that extracellular concentration values are strongly defended over a wide intake range. The close similarity in the carcass and cardiac values obtained on the control and LD_{50} animals indicates that the same is true also with respect to intracellular content and concentration values. It may be inferred therefore that the body's potassium homeostatic system is designed to prevent appreciable deviations in the potassium status of either the extracellular or the intracellular compartment. On the other hand, the fact that animals chronically overloaded with potassium develop electrocardiographic and other evidences of potassium poisoning in association with elevated extracellular, but normal total carcass and slightly lowered cardiac intracellular concentration and content values suggests that potassium intoxication as it occurs in normal individuals is largely an extracellular phenomenon, a conclusion reached by others on the basis of plasma and isolated muscle tissue measurements (13).

It is conceivable that these rats might have shown more of an increase in cellular potassium content had their caloric intake and hence glycogen stores been sustained by forced feeding immediately prior to death (14). Even so, however, it can be appreciated that such retention could afford only extremely limited protection under conditions of prolonged potassium loading. Moreover, the behaviour of these animals was quite characteristic of potassium intoxicated individuals who are apt to be weak and anorexic. It follows

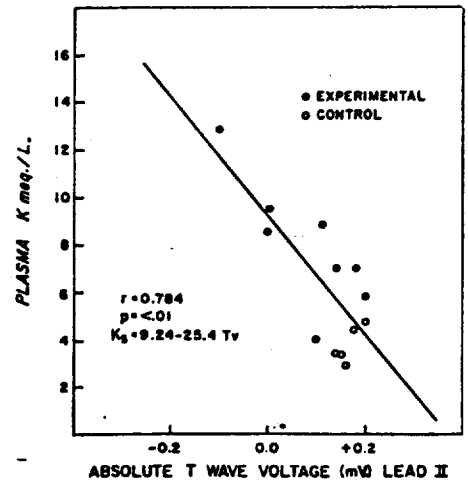

FIG. 2. RELATIONS BETWEEN ELECTROCARDIOGRAPHIC T WAVE VOLTAGE AND THE CONCENTRATION OF POTASSIUM IN PLASMA

	AUTHORS	ANIMAL	EXTRACELL. pH	DATA	COMMENT
ACUTE K LOAD	BOURDILLON (15)	MAN	NOT REPORTED	EXTERNAL BALANCE	EXP. 6 + 7
	WINKLER + SMITH (18)	DOG	NOT REPORTED	EXTERNAL BALANCE	REPORTED Δ K'S
	SCRIBNER ET AL (17)	DOG	NORMAL	EXTERNAL BALANCE	CONTROL GROUP
	MILLER - DARROW (13)	RAT	NOT REPORTED	MUSCLE ANALYSIS	GROUPS 6, 7 + 8
	X PRESENT AUTHORS	RAT	NORMAL	EXTERNAL BALANCE	LIGATED URETERS
ADRENALECTOMY	□ DARROW ET AL (18)	CAT	NOT REPORTED	"	MARKED INSUFF.
	● DARROW ET AL (18)	DOG	NOT REPORTED	"	MOD. INSUFF.
	○ HARRISON + DARROW (19)	RAT	NOT REPORTED	"	MARKED INSUFF.
	▼ ROLF + WHITE (20)	RAT	NOT REPORTED	"	MARKED INSUFF.
	◊ CONWAY + HINGERTY (21)	RAT	7.14	"	MEAN DATA

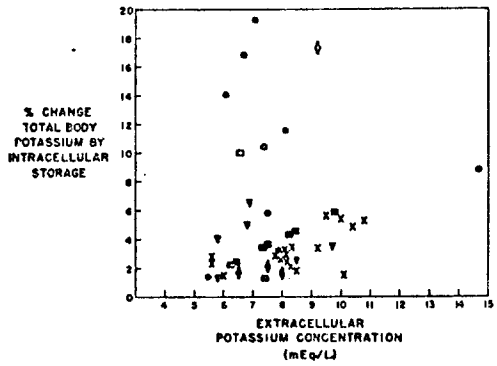

FIG. 3. PER CENT INCREASE IN CELLULAR POTASSIUM ABOVE AVERAGE NORMAL FOLLOWING ELEVATION IN EXTRACELLULAR POTASSIUM CONCENTRATION BY *ACUTE* POTASSIUM LOADING

that prevention of hyperkalemia under these circumstances depends almost exclusively upon homeostatic adjustment of renal potassium output. While this mechanism works admirably over a much wider range of intake than the normal individual is apt ever to experience, there is an upper limit to renal excretory capacity. Once this has been reached, even small increments in potassium intake in excess of maximal bodily excretory capacity can result in the production of hyperkalemia of toxic degree (12). From this interpretation, it may be concluded that the ceiling zone for potassium under conditions of chronic loading is narrow in a given individual.

The absence of intracellular potassium storage in the present chronic loading experiments is in contrast to the storage which may be observed immediately following an acute potassium load. Thus Figure 3, which presents observations on rats, dogs and man, shows that intracellular storage amounting to as much as 6 per cent of normal body potassium content may occur when the extracellular potassium concentration is abruptly elevated to 10 mEq. per liter (13, 15–22).[4] In the rat, the quantity thus stored is equal to about one quarter of the animal's usual daily intake (about 2.5 mEq. per 180 Gm. rat); in the adult human, it is equivalent to about 150 per cent of a commonplace total daily intake (about 120 mEq.). It has been found that the extra potassium stored in cells following such an acute load normally is eliminated within one to three hours (13, 25, 26). Perhaps this is fortunate, for otherwise the capacity of the cellular compartment to buffer tendencies to hyperkalemia after high potassium meals might soon become saturated.

Figure 3 also shows that the cell potassium is much higher relative to the extracellular concentration in adrenalectomized animals than in normal animals. This suggests that adrenal hormones may have a direct effect on the extracellular intracellular concentration gradient for potassium (27, 28). In view of the evidence that potassium loading is a potent stimulus to aldosterone secretion (29), the possibility arises that such adrenocortical activity may have been the factor which minimized cellular potassium storage in the body as a whole and actually caused lowering of the cardiac cellular potassium in the chronically loaded animals here reported. Because some time must elapse before the adrenals can react fully to a

[4] Data from the literature were calculated to standard units utilizing the following assumptions: *1.* Normal initial body potassium 45 mEq. per Kg. in man and appropriate normal values for dog, cat and rat tissue (23, 24). *2.* Per cent change in muscle composition reflects per cent change in total body intracellular potassium. *3.* Initial serum potassium concentration (Ks) equals 4.0 mEq. per liter when Δ concentration reported. In addition to the data shown in Figure 3 are data derived from rats identical to the control group reported in the main body of the experiment. These animals underwent ureteral ligation after a control blood sample was drawn by cardiac puncture. Within five minutes of ureter ligation, the abdomen was closed tightly and potassium was administered intraperitoneally as a 4 KCl : 1 $KHCO_3$ solution containing 0.58 mEq. potassium per ml. Dosage ranged from 2 to 6 mEq. per Kg. body weight. The animals were sacrificed at one or two hours after potassium administration by exsanguination as described. Intracellular potassium storage was calculated from the known dosages and initial and final plasma concentrations of chloride and potassium, correcting for minimal changes in weight and assuming the initial body water composition shown for control animals in Tables I and II. Plasma pH was not altered in the final blood specimen when determined in representative animals. Control animals subjected to the same procedure without the potassium load showed unaltered pH values and changes in plasma potassium concentration ranging from −0.6 to +0.4 mEq. per liter.

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potassium load, such a mechanism would permit body cells to store potassium temporarily following an acute load, but would prevent the storage of potassium under conditions of sustained hyperkalemia. These findings also suggest that cardiac muscle may differ from other body protoplasm in its responsiveness to these adrenocortical hormones.

SUMMARY AND CONCLUSIONS

Rats subjected to loads of potassium which were increased stepwise from a normal intake level of 1.6 to as high as 36.8 mEq. per 100 Gm. body weight per day showed no signs of toxicity, electrocardiographic changes or elevation of plasma potassium concentration values until the intake exceeded 25.6 mEq. per 100 Gm. body weight per day. Of the animals taking more than this amount, one half died with signs of potassium intoxication within three days after attaining intake levels ranging between 28.8 and 38.4 mEq. per 100 Gm. body weight per day. Measurements carried out on the surviving half of the group disclosed pathologic elevation of plasma potassium concentration and characteristic electrocardiographic changes, but no significant change in total carcass potassium content or concentration values and a significant decrease in cardiac intracellular potassium concentration. These findings were in contrast to data obtained on animals on an ordinary dietary intake in which some intracellular "storage" of potassium may be observed transiently following the administration of an acute load. It is suggested that the restoration of cellular potassium values to normal levels following an acute load and the maintenance of these values at normal levels even in the presence of sustained hyperkalemia under conditions of chronic loading may be mediated by adrenocortical hormones.

It is concluded that the occurrence of sustained hyperkalemia means that the individual has surpassed the upper limit of his individual tolerance for potassium and that he probably has very little capacity to store additional potassium within the body in innocuous form.

REFERENCES

1. Darrow, D. C., Pratt, E. L., Flett, J., Jr., Gamble, A. H., and Wiese, H. F. Disturbances of water and electrolytes in infantile diarrhea. Pediatrics 1949, 3, 129.
2. Butler, A. M. Diabetic coma. New Engl. J. Med. 1950, 243, 648.
3. Eliel, L. P., Pearson, O. H., and Rawson, R. W. Postoperative potassium deficit and metabolic alkalosis. New Engl. J. Med. 1950, 243, 471 and 518.
4. Scribner, B. H., and Burnell, J. M. Interpretation of the serum potassium concentration. Metabolism 1956, 5, 468.
5. Hastings, A. B., and Sendroy, J., Jr. Studies of acidosis. XX. The colorimetric determination of blood pH at body temperature without buffer standards. J. biol. Chem. 1924, 61, 695.
6. Van Slyke, D. D., and Neill, J. M. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. J. biol. Chem. 1924, 61, 523.
7. Wallace, W. M., Holliday, M., Cushman, M., and Elkinton, J. R. The application of the internal standard flame photometer to the analysis of biologic material. J. Lab. clin. Med. 1951, 37, 621.
8. Zimmerman, W. J., and Layton, W. M., Jr. A polarographic micromethod for the determination of blood chloride. J. biol. Chem. 1949, 181, 141.
9. Wilson, D. W., and Ball, E. G. A study of the estimation of chloride in blood and serum. J. biol. Chem. 1928, 79, 221.
10. Hastings, A. B., and Eichelberger, L. The exchange of salt and water between muscle and blood. J. biol. Chem. 1937, 117, 73.
11. Spater, H., Hunt, A., Todd, R., Meara, P., Terry, M., Crawford, J. D., and Talbot, N. B. The development and therapy of cellular sodium intoxication in potassium deficiency. Submitted for publication.
12. Talbot, N. B., Crawford, J. D., Kerrigan, G. A., Hillman, D., Bertucio, M., and Terry, M. Application of homeostatic principles to the management of nephritic patients. New Engl. J. Med. 1956, 255, 655.
13. Miller, H. C., and Darrow, D. C. Relation of muscle electrolyte to alterations in serum potassium and to the toxic effects of injected potassium chloride. Amer. J. Physiol. 1940, 130, 747.
14. Grob, D., Liljestrand, A., and Johns, R. J. Potassium movement in normal subjects. Amer. J. Med. 1957, 23, 340.
15. Bourdillon, J. The distribution in body fluids and excretion of ingested ammonium chloride, potassium chloride, and sodium chloride. Amer. J. Physiol. 1937, 120, 411.
16. Winkler, A. W., and Smith, P. Z. The apparent volume of distribution of potassium injected intravenously. J. biol. Chem. 1938, 124, 589.
17. Scribner, B. H., Fremont-Smith, K., and Burnell, J. M. The effect of acute respiratory acidosis on the internal equilibrium of potassium. J. clin. Invest. 1955, 34, 1276.

18. Darrow, D. C., Harrison, H. E., and Taffel, M. Tissure electrolytes in adrenal insufficiency. J. biol. Chem. 1939, 130, 487.
19. Harrison, H. E., and Darrow, D. C. The distribution of body water and electrolytes in adrenal insufficiency. J. clin. Invest. 1938, 17, 77.
20. Rolf, D., and White, H. L. Tissue potassium retention in adrenal insufficiency. Amer. J. Physiol. 1955, 182, 393.
21. Conway, E. J., and Eingerty, D. The influence of adrenalectomy on muscle constituents. Biochem. J. 1946, 40, 561.
22. Crismon, J. M., Crismon, C. S., Calabresi, M., and Darrow, D. C. The electrolyte redistribution in cat heart and skeletal muscle in potassium poisoning. Amer. J. Physiol. 1943, 139, 667.
23. Corsa, L. J., Jr., Olney, J. M., Jr., Steenburg, R. W., Ball, M. R., and Moore, F. D. The measurement of exchangeable potassium in man by isotope dilution. J. clin. Invest. 1950, 29, 1280.
24. Elkinton, J. R., and Danowski, T. S. The Body Fluids. Baltimore, Williams & Wilkins Co., 1955.
25. Keith, N. M., and Osterberg, A. E. Human tolerance for potassium. Proc. Mayo Clin. 1946, 21, 385.
26. Neyzi, O., Hillman, D., Terry, M., and Talbot, N. B. Studies of body water and electrolyte homeostasis in persons receiving maintenance fluid therapy. New Engl. J. Med. In press.
27. Knight, R. P., Jr., Kornfeld, D. S., Glaser, G. H., and Bondy, P. K. Effects of intravenous hydrocortisone on electrolytes of serum and urine in man. J. clin. Endocr. 1955, 15, 176.
28. Danowski, T. S., and Elkinton, J. R. Exchanges of potassium related to organs and systems. Pharmacol. Rev. 1951, 3, 42.
29. Laragh, J. H., and Stoerk, H. C. A study of the mechanism of secretion of the sodium-retaining hormone (aldosterone). J. clin. Invest. 1957, 36, 383.

Sodium Chloride

Does the Sodium Chloride Content of Industrially Prepared Infant Foods Fulfill the Requirements of Infants During the First Half Year of Life?

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Summary and Key Words (See original, page 70)

During the years 1905 to 1920, numerous authors (2) have carried out extensive clinical and experimental studies on sodium chloride and its importance for nursing infants. In these studies, it has already been shown that unfavorable effects of sodium chloride on the water balance and circulation are a function of the sodium ion. Since that time, the rule adopted in German pediatrics is to avoid adding sodium chloride to milk and supplementary food of healthy infants during the first half year of life.

Since several years, the majority of infants in West Germany are raised, during their first 6 months of life, on industrially prepared milk products and on an industrially processed supplementary diet. This type of nutrition presents many advantages over a home-made diet. In view of this change in the form of nutrition, we were interested in finding out whether the sodium chloride content of industrially prepared milk and supplementary food products fulfills the requirements of healthy infants during their first 6 months of life. We have investigated, therefore, the sodium, potassium and chloride content of milk and supplementary food products commonly used in West Germany. The results were compared with the salt content of similar home-made milk and supplementary food products.

Salt Content in Milk Diets

In Table 1, the average amounts of sodium, potassium and chloride present in mature women's milk (11) and cow's milk (11,5) are compared.

Table 1 (See p. 71) Sodium, potassium and chloride content in women's milk and cow's milk, mg/100 ml.

According to this table, a nursing infant would receive, while drinking an average amount of 800 g in 24 hrs, 120 mg Na at the end of his 2nd month of life, and 135 mg Na at the end of the 5th month of life, while drinking an average amount of 900 g in 24 hrs. When calculated per kg body weight (BW), these figures are equal to 25 and 20 mg Na, resp.

If the infant is fed cow's milk, then he would get, with a 2/3 milk mixture and drinking the same amounts, 240 mg Na in 24 hrs. at the end of the 2nd month of life, and 270 mg in 24 hrs. at the end of the 5th month of life, namely twice as much sodium as the breast-fed infant. Calculated per kg BW, these figures are equal to 50 and 40 mg Na. This rough estimate is valid provided that commonly used cereal products, such as flaked oats (Quaker oats), cornstarch meal or fine grits are used in the preparation of milk mixtures. These cereal products have an average content of 1-5 mg Na in 100 g (11).

Milk diets are prepared today predominantly with a so-called dry meal preparation specially made for feeding infants, or a flour, semolina (grits) or groats product. Table 2 shows that individual products differ considerably in their salt content. In most products listed in this table, the sodium and chloride content is higher than in commonly used cereal consumer products.

Table 2 (See p. 71) - Milk-free carbohydrate additives and milk-free supplementary diets.

When using a product with a sodium content of 10 mg in 100g, the sodium content in the milk diet would be increased by 1% if the product is boiled down to a 3% concentration. With a product containing 100 mg Na in 100 g, the sodium content in the milk diet would increase by 10%. If Gerber oatmeal or Gerber children grits are added to the milk diet, the sodium supply in 2/3 milk would be 50-60% higher. In this case, an infant would then receive with a 2/3 milk at the end of 2nd month of life 360-390 mg sodium instead of 240 mg Na, and at the end of the 5th month of life it would receive 390-420 mg Na instead of 270 mg. Calculated as sodium chloride the infant would then receive exactly 1.0 g NaCl instead of 0.6-0.7 g.

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Table 3 shows the sodium, potassium and chloride conte
of milk products commonly used by an infant during its firs
The sodium and chloride content in most of these products i
of a 2/3 milk mixture. Only the products Aptamil and Nan,
to the group of adjusted milk diets, have a lower sodium an
In the products Nektarmil Honey milk 1 and Black Forest mil
sodium and chloride content in the batches investigated by
more or less to that of whole milk. In the products Aletemi
Humana 1 initial diet, the sodium content is the same as tha
milk, while the chloride content corresponds to that of a 1/
mixture.

Table 3 (See p. 72) Milk products for healthy infants.

Salt Content in Fruit, Fruit and Vegetable Juices and F

The sodium chloride supply of infants is not increased b
feeding of fruit juices prepared from oranges, strawberries,
tangerines, black currant or lemons (11). Carrots, with an a
45 mg Na in 100 g, contain ten times as much Na as fruit (11)
supply of sodium chloride provided by carrot juice is only 5%
industrially prepared fruit and carrot juices do not differ s
in their sodium and chloride content from household preparatio

In the case of fruit meals (pulps), there are differences
industrial and common household preparations. In the fruit pr
made by the Hipp firm, the sodium and chloride content corresp
average values found in fresh fruit. In the fruit preparation
Alete and Gerber (German Maizena Works) the sodium content, eq
in 100 g, is 10 ten times higher than in fresh fruit. When fr
made by these firms are used to feed infants during their firs
the sodium supply is increased by less than 10%.

Salt Content in Vegetable Meats (Pulps) without Addition o
Fish or Meat and in Finely Mashed Diets.

Carrots and spinach have an average sodium content of 45 t
in 100 g. In cauliflower, French beans, peas, kale and potatoe
sodium content ranges from 2 to 20 mg in 100 g (11).

When 20-25 g veal, beef, chicken meat or calf's liver is added to a household meal made from carrots and potatoes (11), the sodium content of the meal is increased from 50 mg to 65-70 mg. Instead of fresh meat or liver, canned mashed meat (puree) made by the Hipp firm can also be used. With 20-25 g meat of this canned product, an average of 150 mg Na can be supplied, as compared to 15-20 g Na in the corresponding amount of fresh meat (11).

Table 5 lists a number of finely mashed diets (purees), consisting of vegetables and/or nutrients with additions of egg or meat, that can be used in infants starting at the 5th month of age. The sodium content in these diets varies from 100 to 240 mg per 100 g. According to the type of diet selected, an infant receives, with 150 g of the diet, between 150 and 360 mg Na. Thus, one such meal diet contains practically as much sodium as the amount present in the mashed vegetables without any egg, fish or meat addition listed in Table 4.

Table 5 (See p. 73) Supplementary diets used for feeding infants.

Salt Content in Milk Porridges (Paps)

During the 6th month of life, instead of 2/3 milk bottle, the infant is generally fed 200 g whole milk porridge (consisting mostly of 10 g grits per 100 ml milk). Fine wheat grits contain, on the average, 1 mg Na per 100 g (11). The sodium content of grits thus can be considered as insignificant during calculation. In case of 45 mg Na/100 ml of fresh milk (Table 1), the infant receives ab. 90 mg sodium with 200 g of a whole milk grits mash.

For the preparation of the mash, one of the flour (meal), grits, groats or cereal flake products listed in Table 2 is generally used. The sodium content of these products ranges from 4 to 744 mg/100 g. When a product with a sodium content of up to 50 mg/100 g is used, the sodium content in 200 g of whole milk mash increases from 90 mg to 100 mg, with products with a sodium content of up to 250 mg/100 g it increases from 90 mg to 140 mg, and when a Gerber 7-corn mash is used, the Na content increases from 90 mg to 240 mg.

A large number of milk-containing ready to use mashed diets for infants are also supplied by the dietetic food industry, and most of these products consist of 1/2 and 2/3 milk porridges. The sodium content in 200 g of such mash products lies mostly in the range of 50 to 90 mg. In some cases, the sodium content is as high as 160 mg per 200 g mash product.

Discussion

For a normal development (growth) during the first year of life, the sodium content of mother's milk ought to be sufficient. In this case, the average daily sodium requirement of 120 mg of a healthy infant at the end of the 2nd month of life sould be fulfilled, and the average daily Na requirement of 150 mg at the end of the 5th month would also be met. A sodium supply at this level should also be sufficient to compensate for short-term losses of sodium chloride, for example due to vomiting, diarrhea or loss with sweat.

When fed with a cow's milk mixture, an infant always gets more sodium than a breast-fed infant. In healthy infants, fed a 1/3 and 2/3 milk with and without addition of 2% germ oil, we found (5) an average daily sodium intake of 195 mg between the 8th and 10th week of life (Table 6). An average daily amount of 152 mg Na was eliminated in the stool, urine and skin. An average of 43 mg sodium was retained daily, which amounts to 22% of the sodium intake. Between the 20th and 22nd week of life, when the infants were fed a 1/3 and 2/3 milk plus 2% germ oil and a supplementary diet according to their age, we observed (5) (Table 6) an average daily sodium intake of 220 mg. Of this amount, 45 mg came from the usual household supplementary diet (carrots, potatoes, fruit mash, fruit and vegetable juices). An average of 190 mg Na was excreted through the stool, urine and skin, while 50 mg Na was retained daily, equal to 14% of the sodium intake. On the basis of calculation, Gamble (3) and McCance and Widdowson (10) consider that an average daily sodium retention of 1.3-1.8 milliequiv., or 30-40 mg, is sufficient to meet the sodium requirements of healthy infants during the 1st half year of life. According to our studies, a daily sodium uptake of 7-8 milliequiv., or 160-180 mg, such as Dahl(3) and also Fomon (6) recommend for artificially fed infants during the 1st half year of life, is always exceeded during feeding with cow's milk diet.

Table 6 (see p. 74) Sodium balance of maturely born health infants.

A healthy infant, fed with a milk diet appropriate to its age and with a supplementary diet prepared according to household procedures without addition of salt, receives during its first 3 months of life a daily amount of about 200-250 mg Na, corresponding to 0.51-0.63 g sodium chloride (Table 7).

Table 7 (See p. 74). Sodium uptake during 1st half year of life.

During the 2nd quarter year of life, the daily sodium intake lies between 300 and 350 mg, corresponding to 0.76-0.88 g sodium chloride. When products made by the dietetic food industry are used, such as milk products, with free carbohydrate additives, mashed vegetables, and finely dispersed (puree) diets, then it is possible, at least during the first 3 months of life, not to exceed significantly the above-mentioned amounts of sodium and sodium chloride if the above products are selected only according to their salt content. If, during the selection of these products, the salt content is not taken into account, then the sodium and salt uptake can be up to 150% higher (Table 7). In this case, an infant would receive about 1 g salt per day at the end of the 2nd month of life, 2 g at the end of the 4th month of life, and about 3 g at the end of the 6th month of life.

According to our investigations, almost all industrially manufactured supplementary diet products used in infant nutrition contain more or less large amounts of added sodium and sodium chloride. Additions of salt to the food are not necessary (required) for the growth and development of infants. Based on experience, infants consume equally well food with or without addition of salt. This pediatric experience has been recently confirmed in comparative studies by the Fomon working group (1,7). A supplementary diet for the infant without addition of salt is tasteless to the adult. Apparently, the addition of salt to industrially manufactured supplementary diets is a concession to the taste of the mother on the part of the manufacturer.

Since the addition of salt to infant food is useless, the question arises as to whether such addition is indifferent or perhaps harmful. According to our knowledge, direct injuries in infants after regular consumption

of industrially manufactured supplementary diets containing added salt have not been described sofar. It should be noted in this connection that considerably greater amounts of sodium and sodium chloride are added in industrially prepared supplementary diets made in the U.S.A. than in the corresponding products available on the German market (1).

Excess amounts of salts taken up with the food must later be eliminated (excreted). Compared to the breast-fed infant, large amounts of protein decomposition products and minerals are excreted by the artifically fed infant. Additions of salt to the food place an additional load (burden) on the water balance and thus may lower the reserves of "free body water." The connection between the regular consumption of foods with a high salt content and the development of hypertension has been often discussed. Dahl (4) has drawn attention to the fact that young rats, after being fed for many months a diet consisting of industrially prepared mashed vegetables and a finely dispersed infant diet with a sodium content of 150 to 500 mg per 100 g canned goods, already develop a hypertension more frequently and have a higher mortality rate than control animals. These observations have not remained undisputed in the case of man (9). On the basis of the studies available sofar, a decision cannot be made as to whether the amounts of sodium present in industrially prepared finished products for infants are harmful or not for the growth of the infant. For this reason, we consider it advisable to use milk and supplementary diets without the addition of sodium chloride and other sodium salts.

Bibliography

1. Anderson, Th.A., Fomon, S.J.: Commercially prepared strained and junior foods for infants. J.Amer. diet. Ass. 58,620 (1971).

2. Czemy A., Keller A.: Child nutrition, nutrition disorders and nutrition therapy. Vol. II, 2nd ed., Leipzig. Vienna, Franz Deuticke, 1928, (in German).

3. Dahl, I.K.: Salt in processed baby foods Amer. J. clin. Nutr. 21, 787 (1968)

4. Heine, M., Leitl.G., Tassinari, L.: Hypertension and death from consumption of processed baby foods. Proc. Soc. exp. Biol. (N.Y.) 188, 1405 (1970)

5. Droese, W., Stolley H.: unpublished figures

6. Fomon, S.J.: Infant nutrition. Philadelphia-London: W.B. Saunders Comp. 1967

7. Thomas, L.N., Fifer, L.J., jr.: Acceptance of unsalted foods by normal infants. J. Pediat. 76, 242 (1970).

8. Gamble, J.L. et al.: Effects of large loads of electrolytes. Pediatrics 7, 305 (1951)

9. Gros.G., Weller, I.M., Hoobler, S.W.: Relationship of sodium and potassium intake to blood pressure. Amer. J. clin. Nutr. 24, 605 (1971)

10. McCance, R.A., Widdowson, E.M.: Mineral metabolism of the foetus and the newborn. Brit. med. Bull. 17, 132 (1961)

11. Souci S.W., Fachmann W., Kraut H.: Composition of foods, nutritional value tables, Vols. I and II, Stuttgart, Scientific Publishing Society MBH, 1969 (in German)

Table 1. Sodium, potassium and chloride content in women's milk and cow's milk, mg/100ml

	Women's milk (11)	Cow's milk (11)	Cow's milk (11)
Sodium	15	47	42 ± 4
Potassium	53	155	155 ± 9
Chloride	36	90	101 ± 6

Table 2. Milk free carbohydrate additives and milk-free supplementary diets.

Present in 100 g	Sodium mg	Potassium mg	Chloride mg
1. Prepared farinas for the preparation of corn meal, the so-called dry meal preparations (precooked farinas, farinas with addition of mucous products)			
2. Honey meal with honey, sugar, vitamins	11	67	32
3. Rice milk	16	232	53
4. Three-corn meal	228	357	398
5. Oatmeal with B-vitamins and mineral salts	479	307	345
6. Milana whole wheat flakes	7	365	140
7. Oat dry meal	4	331	63
8. Rice flakes	8	93	44
9. Corn Vita meal with vitamins and mineral salts	404	114	533
10. Flour (farina) products, grits, and groats			
11. Children grits with bee honey and vitamins	10	174	68
12. Children grits enriched with 10% Dextropur and B vitamins	612	145	458
13. Children grits with honey and vitamins	4	158	70

Table 2 (Cont'd)

Present in 100 g	Sodium	Potassium	Chloride
14. Miluvit, honey children grits	30	223	69
15. 5 Corn Vita mush (meal) with 10 vitamins	192	58	397
16. Fennel Vita mush (meal) with honey and 10 vitamins	25	56	97
17. Chocolate Vita mush (meal) with powdered chocolate, sugar, nutritive sugar and 10 vitamins.	26	334	84
18. Children grits, enriched with vitamins B_1 and B_2	6	121	71
19. Multiple corn meal with bee honey and sugar	92	139	192
20. Grain (corn) flakes			
21. Mixtures of different corn flakes			
22. 6-corn flakes with vitamins & iron	10	297	56
23. Apple mush (meal) from wheat, oats, rice, with bee honey and 10 vitamins			
24. Bircher meal (gruel), with apples, bananas, nuts, raisins and 10 vitamins	142	419	43
25. Oat meal (gruel), oat flour, cornstarch, cracker flour and B-vitamins	93	234	171
26. Cocoa-nut meal, with bee honey, cocoa powder, sugar, hazelnuts, almonds, B vitamins	19	536	38
27. Rice flakes with bee honey and B vitamins	11	142	29
28. Children's "Kostli" (delight) with oranges, apples, grits, crackers, sugar, vitamins and minerals	221	184	365
29. 7-corn meal with B vitamins, mineral salts and 10% dextropur	744	431	657
30. Apple meal with honey, apples, wheat, rice, corn, oats, honey,	45	250	93
31. 7-corn flakes	158	361	310

Table 3. Milk products for healthy infants

Present in 100 ml of diet ready for drinking	Sodium mg	Potassium mg	Chloride mg
Nonadjusted milk products			
Milumil, liquid, sweet milk infant diet, Noktarmil honey milk	36	110	75
Partially adjusted milk products			
Powder (Aletemil)	45	145	60
Powder (Aletana)	49	162	72
Liquid (Aletemil)	38	136	50
Liquid (Aletana)	41	105	56
Black Forest milk diet 2	43	133	83
Adjusted milk products			
Black Forest milk diet 1	31	99	62
Multival initial diet 1 (powder)	37	111	49
Humana 1 initial diet	53	99	64
Humana 2 permanent diet	38	82	57
Liquid (Aptamil)	26	88	56
Liquid (Nan)	30	86	47
Powder (Nan)	26	94	49
Infant diet	32	71	42

Table 4. Supplementary diets used in feeding infants

Mashed vegetables canned in glass jars without addition of egg, fish or meat. present in 100 g	Sodium mg	Potassium mg	Chloride mg
Carrot preparation (6th week)	32	204	36
Tender garden vegetables (9th wk)	259	193	395
Mixed vegetables (9th wk)	87	283	135
Summer vegetables with fennel (9th wk)	246	152	348
Spinach preparation (4th month)	160	400	249
Carrots (6th wk)	97	176	152
Garden vegetables (9th wk)	107	194	188
Tender vegetables (9th wk)	138	181	257
Spinach (4th month)	104	194	164
Early carrots (6th wk)	119	221	192
Mashed salad, from tender garden lettuce with bananas (6th wk)	108	91	209
Carrots (9th wk)	148	180	208
Tender garden vegetables (9th wk)	241	136	345
Mixed vegetables (9th wk)	136	145	231
Spinach (4th months)	139	170	238
Dry vegetable products, 100 g powder contain			
Aponti carrot flakes (6th wk)	898	1430	1420
Humana mashed carrots and tomatoes (6th wk)	72	499	129
Humana mashed mixed vegetables (6th wk)	100	729	92

Table 5. Supplementary diets used for feeding infants

Finely mashed diets (purees), canned in glass jars, used for infants at 4-5 months of age (vegetables and/or nutrients with addition of egg or meat)

100 g contain:	Sodium mg	Potassium mg	Chloride mg
Chicken, vegetables, egg noodles	183	210	315
Chicken, vegetables, egg noodles	203	101	308
Veal in vegetables, spaghetti	226	101	366
Liver diet from carrots, pig's liver, fresh egg noodles	222	237	329
Beef in spinach, mashed potatoes	181	138	277
Beef in tender peas	236	134	300
Carrot cream with egg yolk	145	167	236
Turkey in vegetables and rice	218	121	327
Veal in garden vegetables	125	82	219
Chicken in carrots	107	87	180
Liver in carrots	123	112	204
Beef in tender vegetables	158	190	252
Puree with veal and egg yolk	135	58	189
Beef in noodles	129	50	214
Chicken in rice	101	68	185
Chicken in rice, cream of vegetables	191	98	341
Beef in tomatoes and rice	140	126	254
Liver in carrots	175	151	293
Liver in spinach	131	102	249

Table 6. Sodium balance of maturely born healthy infants

	8th to 10th week of life; 1/2 and 2/3 milk with and without addition of 2% germ oil; number of infants: 23; number of balance days per infant: 21	20th to 22nd week of life 1/2 and 2/3 milk with addition of 2% germ oil and supplementary diet; number of infants: 7; number of balance days per infant: 21.
	m ± s	m ± s
Sodium intake (mg/24 hrs)	195,0 ± 7,3	220,9 ± 11,5
Sodium excretion (mg/24 hrs)		
Stool	16,6 ± 9,4	48,8 ± 35,7
Urine	118,2 ± 34,2	125,1 ± 35,7
Skin	16,9 ± 8,2	16,7 ± 8,0
Sodium retention (mg/24 hrs)	43,3	30,3
Sodium retention (% of uptake)	22,2%	13,7%

m = Average value
s = Standard deviation

Table 7. Sodium uptake during 1st half year of life

	Sodium uptake				calculated as sodium chloride	
	mg/Day		mg/kg x Day	mg/Day		
At the end of the 2nd month of life						
Mothers milk	120		25		300	
2/3 milk + milk-free carbohydrate additives	240	375	50	78	605	945
Milk finished products	210	450	45	93	530	1125
At the end of the 4th month of life						
Mother's milk and vegetables-potatoes	190		30		480	
Mother's milk and canned vegetables (industrial products)	200	540	31	85	505	1380
2/3 milk and milk-free carbohydrate additives and veg.-potatoes (household procedure)	310	510	49	80	780	1280
2/3 milk and milk-free carbohydrate additives and canned vegetables (industrial products)	320	812	50	127	806	2050
At the end of the 6th month of life						
Mother's milk	120		25		300	
2/3 milk, whole milk mash + milk-free carbohydrate additives, vegetables and potatoes + meat (household procedure)	350	450	48	62	883	1135
2/3 mk. whole milk mash+milk-free carbohydrate addit. veg.-potatoes (Ind.)	530	1100	74	153	1333	2780

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Entspricht der Kochsalzgehalt in den industriell hergestellten Fertignahrungen den Bedürfnissen von Säuglingen im 1. Lebenshalbjahr?***

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Does the sodium chloride content of industrially prepared infant foods confirm to requirements of infants during the first half year of life?

Summary. The sodium, potassium and chloride contents of infant foods available in West Germany, as obtained by chemical analysis, are reported. Precooked and other cereal products contain 4 to 740 mg Na/100 gm as compared to 1 to 5 mg/100 gm in the original cereals. Most milk formulas contain sodium, potassium and chloride similar to home made milk formulas for infants (2 parts of cows' milk, 1 part of water). Strained fruits contain 2 to 40 mg Na/100 gm. This is the same as or up to 10 times the content of the original fruits (2 to 4 mg Na/100 gm). Strained vegetables and menues contain 100 to 250 mg Na/100 gm, which is 2 to 5 fold that of the original vegetables.

Sodium intakes are calculated for infants fed on industrially prepared infant foods and are compared to sodium balances of healthy infants fed on a home made diet without sodium chloride added. The sodium intake may double at the end of the second month and triple at the end of the sixth month of life by feeding ready made infant foods.

The question is discussed, wether the higher sodium intakes may be beneficial, irrelevant, or even dangerous for the development of infants. It is suggested that according to pediatric experience sodium chloride and other sodium salts should not be added to infant foods.

Key words: Infant nutrition, infant food, sodium content, potassium content, chloride content, sodium balance.

Zusammenfassung. Auf Grund eigener Analysen wird über den Natrium-, Kalium- und Chlorid-Gehalt in einer Anzahl in der Bundesrepublik gebräuchlicher Milch- und Beikostpräparate für Säuglinge im 1. Lebenshalbjahr berichtet.

Sog. Trockenschleimpräparate und Mehl-, Grieß- oder Schroterzeugnisse enthalten 4–740 mg Natrium/100 g, verglichen mit 1–5 mg/100 g in vergleichbaren handelsüblichen Produkten.

In der Mehrzahl der Milchpräparate liegt der Natrium-, Kalium- und Chloridgehalt in der trinkfertigen Mischung in Höhe einer $^2/_3$-Milch.

Obstbreie enthalten 2–40 mg Natrium pro 100 g, verglichen mit 2–4 mg/100 g in entsprechendem Obst.

In den Gemüsekonserven ohne Zusatz von Ei, Fisch oder Fleisch und in den feinpürierten Menüs ist der Natriumgehalt mit 100–250 mg/100 g 2–5fach größer als in der Ausgangsware.

Es wird die Natriumaufnahme von Säuglingen bei ausschließlicher Ernährung mit industriell hergestellten Milch- und Beikostpräparaten berechnet und mit der Natriumbilanz von Säuglingen verglichen, die altersgemäß mit haushaltsüblich zubereiteter Milchnahrung und Beikost ohne Kochsalzzusatz aufgezogen wurden. Bei Gebrauch von industriell hergestellten Säuglingsnahrungen kann die Natriumaufnahme am Ende des 2. Lebensmonates auf das Doppelte, am Ende des 6. Lebensmonates auf das Dreifache vergrößert werden.

Es wird diskutiert, ob es für den Säugling im 1. Lebenshalbjahr nützlich, gleichgültig oder schädlich ist, Nahrungen mit Kochsalzzusatz zu erhalten. Gemäß der kinderärztlichen Erfahrung sollte der Milch- und Beikostnahrung kein Kochsalz und keine anderen Natriumsalze zugesetzt werden.

Schlüsselwörter: Säuglingsernährung, Säuglingsnahrung, Natriumgehalt, Kaliumgehalt, Chloridgehalt, Natriumbilanz.

In den Jahren 1905 bis 1920 haben sich zahlreiche Autoren [2] in klinisch-experimentellen Untersuchungen eingehend mit dem Kochsalz und seiner Bedeutung für den Säugling befaßt. In diesen Untersuchungen konnte bereits nachgewiesen werden, daß ungünstige Wirkungen von Kochsalz auf den Wasserhaushalt und auf den Kreislauf eine Funktion des Natriumions sind. Seit dieser Zeit gilt in der Deutschen Kinderheilkunde die Regel, Zusätze von Kochsalz zur Milchnahrung und zur Beikost von gesunden Säuglingen im 1. Lebenshalbjahr zu vermeiden.

Die Mehrzahl der Säuglinge im 1. Lebenshalbjahr wird seit einigen Jahren in der Bundesrepublik mit industriell hergestellten Milchnahrungen und mit industriell verarbeiteter Beikost aufgezogen. Diese Ernährungsform bietet gegenüber einer haushaltsmäßig hergestellten Nahrung manche Vorteile. Bei diesem Wandel in der Ernährungsweise interessierte uns, ob in den industriell hergestellten Milch- und Beikostnahrungen der Kochsalzgehalt den Bedürfnissen des gesunden Säuglings im 1. Lebenshalbjahr entspricht. Wir untersuchten deshalb in einer Anzahl in der Bundesrepublik gebräuchlicher Milch- und Beikostpräparate den Natrium-, Kalium- und Chloridgehalt. Die Ergebnisse wurden mit dem Salzgehalt haushaltmäßig hergestellter Milch- und Beikostnahrungen verglichen.

* Die Untersuchungen wurden mit Mitteln der Deutschen Forschungsgemeinschaft in Bad Godesberg und des Ministeriums für Wissenschaft und Forschung — Landesamt für Forschung — des Landes Nordrhein-Westfalen durchgeführt.

** In memoriam Prof. Dr. E. Schiff

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Salzgehalt in der Milchnahrung

In Tabelle 1 sind die Durchschnittswerte von Natrium, Kalium und Chlorid in reifer Frauenmilch [11] und in Kuhmilch [11, 5] einander gegenübergestellt.

Tabelle 1. Natrium-, Kalium- und Chloridgehalt in Frauenmilch und Kuhmilch mg/100 ml

	Frauenmilch (11)	Kuhmilch (11)	Kuhmilch (5)
Natrium	15	47	42 ± 4
Kalium	53	155	155 ± 9
Chlorid	36	90	101 ± 6

Ein Brustkind würde danach bei einer durchschnittlichen Trinkmenge von 800 g in 24 Std am Ende des 2. Lebensmonats 120 mg Natrium, am Ende des 5. Lebensmonats bei einer Trinkmenge von 900 g in 24 Std 135 mg Natrium erhalten. Auf das Kilo Körpergewicht berechnet sind das 25 mg bzw. 20 mg Natrium.

Wird mit Kuhmilch ernährt, so bekommt der Säugling mit einer $^2/_3$-Milchmischung bei denselben Trinkmengen am Ende des 2. Lebensmonats 240 mg Natrium und am Ende des 5. Lebensmonats 270 mg Natrium in 24 Std, also die doppelte Menge an Natrium wie das Brustkind. Auf das Kilo Körpergewicht berechnet sind das 50 bzw. 40 mg Natrium. Diese Überschlagsrechnung gilt unter der Voraussetzung, daß für die Herstellung von Milchmischungen die gebräuchlichen Konsumgetreideprodukte, wie Haferflocken, Mais-Stärkemehl oder feiner Weizengries, verwendet werden. Diese Getreideprodukte enthalten im Durchschnitt 1–5 mg Natrium in 100 g [11].

Die Milchnahrungen werden heute überwiegend mit einem speziell für die Säuglingsernährung hergestellten sog. Trockenschleimpräparat oder einem Mehl-, Grieß- oder Schroterzeugnis zubereitet. Tabelle 2 zeigt, daß sich die einzelnen Präparate im Salzgehalt beträchtlich voneinander unterscheiden. In der Mehrzahl der angeführten Präparate ist der Natrium- und Chloridgehalt größer als in den gebräuchlichen Konsumgetreideprodukten.

Bei Verwendung eines Präparates mit einem Natriumgehalt von 10 mg in 100 g würde sich, bei einer 3%igen Abkochung, der Natriumgehalt in der Milchnahrung um 1% erhöhen. Mit einem Präparat um 100 mg Natrium in 100 g steigt der Natriumgehalt in der Milchnahrung um 10% an. Wird die Milchmischung mit Gerber Haferschleim oder Gerber Kindergrieß angesetzt, so würde das Natriumangebot

Tabelle 2. Milchfreie Kohlenhydratzusätze und milchfreie Zusatznahrungen

in 100 g sind enthalten:		Natrium mg	Kalium mg	Chlorid mg
1. *Präparierte Mehle zur Bereitung von Getreideschleim, sog. Trokkenschleimpräparate* (vorgekochte Mehle; Mehle mit Zusatz von Schleimstoffen)				
Alete	Honigschleim mit Honig, Zucker, Vitaminen	11	67	32
Aponti	Reisschleim	16	232	53
	Dreikornschleim	228	357	398
Gerber	Hafer Schleim mit B-Vitaminen und Mineralsalzen	479	307	345
Milupa	Milana Vollweizen-Flocken	7	365	140
	Hafer-Trockenschleim	4	331	63
	Reis-Flocken	8	93	44
Nestlé	Getreide Vita Schleim mit Vitaminen und Mineralsalzen	404	114	533
2. *Mehl-, Grieß- und Schroterzeugnisse*				
Alete	Kinder-Grieß mit Bienenhonig und Vitaminen	10	174	68
Gerber	Kinder Grieß mit 10% Dextropur und B-Vitaminen angereichert	612	145	458
Humana	Kinder Grieß mit Honig und Vitaminen	4	158	70
Milupa	Miluvit, Honig-Kindergrieß	30	223	69
Nestlé	5 Korn Vita Brei mit 10 Vitaminen	192	58	397
	Fenchel Vita Brei mit Honig und 10 Vitaminen	25	56	97
	Schoko Vita Brei mit Schokopulver, Zucker, Nährzucker und 10 Vitaminen	26	334	84
Pomps	Kindergrieß angereichert mit Vitamin B_1 und B_2	6	121	71
	Vielkorn Brei mit Bienenhonig und Zucker	92	139	192
3. *Getreideflocken*				
Mischungen verschiedener Getreideflocken				
Alete	6-Korn-Flocken mit Vitaminen und Eisen	10	297	56
	Apfel-Brei aus Weizen, Hafer, Reis, mit Bienenhonig und 10 Vitaminen	50	385	56
	Bircher-Brei mit Äpfeln, Bananen, Nüssen, Rosinen und 10 Vitaminen	142	419	43
	Hafer-Brei Hafermehl, Maisstärke, Zwiebackmehl und Vitaminen der B-Gruppe	93	234	171
	Schoko-Nuß-Brei mit Bienenhonig, Schokopulver, Zucker, Haselnüssen, Mandeln, B-Vitaminen	19	536	38
	Reis-Flocken mit Bienenhonig und B-Vitaminen	11	142	29
Aponti	Kinder Köstli mit Orangen, Äpfeln, Grieß, Zwieback, Zucker, Vitaminen und Mineralstoffen	221	184	365
Gerber	7 Korn Brei mit B-Vitaminen, Mineralsalzen und 10% Dextropur	744	431	657
Humana	Apfelbrei mit Honig Äpfel, Weizen, Reis, Mais, Hafer, Honig, Zucker, Vitamine	45	250	93
Milupa	7-Korn-Flocken	158	361	310

Tabelle 3. Milchpräparate für gesunde Säuglinge

in 100 ml trinkfertiger Nahrung sind enthalten:		Natrium mg	Kalium mg	Chlorid mg
1. Nichtadaptierte Milchpräparate				
Milupa	Milumil, flüssig, Süßmilch-Säuglingsnahrung	36	110	75
	Nektarmil, Honig Milch 1	59	111	94
Nestlé	Beba	36	116	84
	Pelargon (15%ig)	32	131	69
2. Teiladaptierte Milchpräparate				
Alete	Aletemil (Pulver)	45	145	60
	Aletemil (flüssig)	38	136	50
	Aletana (Pulver)	49	162	72
	Aletana (flüssig)	41	105	56
Aponti	Schwarzwald Milchnahrung 2	43	133	83
Nestlé	Nidina	29	109	66
Pomps	Pomil	30	110	74
3. Adaptierte Milchpräparate				
Aponti	Schwarzwald Milchnahrung 1	31	99	62
Heyden	Multival Anfangsnahrung 1 (Pulver)	37	111	49
Humana	Humana 1 Anfangsnahrung	53	99	64
	Humana 2 Dauernahrung	38	82	57
Milupa	Aptamil, flüssig	26	88	56
Nestlé	Nan, flüssig	30	86	47
	Nan, Pulver	26	94	49
Wyeth-Pharma	WM Säuglingsnahrung	32	71	42

in der $^2/_3$-Milch 50–60% größer werden. Ein Säugling würde dann am Ende des 2. Lebensmonates mit einer $^2/_3$-Milch statt 240 mg Natrium 360–390 mg in 24 Std und am Ende des 5. Lebensmonates statt 270 mg Natrium 390–420 mg erhalten. Als Kochsalz berechnet würde ein Säugling statt 0,6–0,7 g rund 1,0 g Kochsalz bekommen.

In Tabelle 3 sind der Natrium-, Kalium- und Chloridgehalt einer Anzahl in der Bundesrepublik gebräuchlicher Milchpräparate für den Säugling im 1. Lebenshalbjahr zusammengestellt. Der Natrium- und Chloridgehalt in der Mehrzahl dieser Präparate liegt in Höhe einer $^2/_3$-Milchmischung. Einen geringeren Natrium- und Chloridgehalt haben nur die zur Gruppe der adaptierten Milchnahrungen gehörenden Präparate Aptamil und Nan. In den Präparaten Nektarmil Honigmilch 1 und Schwarzwald Milchnahrung 2 entsprechen der Natrium- und Chloridgehalt in den von uns untersuchten Chargen mehr oder weniger einer Vollmilch. In Aletemil, Aletana und Humana 1 Anfangsnahrung liegt der Natriumgehalt in Höhe einer Vollmilch, der Chloridgehalt entspricht dagegen dem einer $^1/_2$- bzw. $^2/_3$-Milchmischung.

Salzgehalt in Obst-, Obst- und Gemüsesäften und Obstzubereitungen

Durch Zufütterung von Obstsaft aus Apfelsinen (Orangen), Erdbeeren, Himbeeren, Mandarinen, Johannisbeeren (schwarz) oder Zitronen wird das Kochsalzangebot für den Säugling nicht erhöht [11]. Karotten enthalten mit im Durchschnitt 45 mg Natrium in 100 g die 10fache Menge an Natrium wie Obst [11]. Durch Karottensaft wird das Kochsalzangebot aber nur um 5% größer. Die industriell hergestellten Obst- und Karottensäfte unterscheiden sich im Natrium- und Chloridgehalt nicht wesentlich von den Zubereitungen im Haushalt.

Bei den Obstbreien bestehen Unterschiede zwischen den industriellen und den haushaltsüblichen Zubereitungen. In den Obstzubereitungen der Firma Hipp entsprechen der Natrium- und Chloridgehalt den Durchschnittswerten der frischen Früchte. In den Obstzubereitungen der Firmen Alete und Gerber (Deutsche Maizena-Werke) ist der Natriumgehalt mit 20–40 mg in 100 g um das 10fache größer als in frischen Früchten. Bei Verwendung von Obstbreien dieser Firmen für die Ernährung des Säuglings im 1. Lebenshalbjahr wird das Natriumangebot um weniger als 10% erhöht.

Salzgehalt in Gemüsebreien ohne Zusatz von Ei, Fisch oder Fleisch und in feinpürierten Menüs

Karotten und Spinat haben einen durchschnittlichen Natriumgehalt von 45 bzw. 62 mg in 100 g. Im Blumenkohl, Schnittbohnen, Erbsen, Kohlrabi und Kartoffeln liegt der Natriumgehalt zwischen 2 und 20 mg in 100 g [11].

In Tabelle 4 sind der Natrium-, Kalium- und Chloridgehalt einiger gebräuchlicher industriell hergestellter Gemüsekonserven ohne Zusätze von Ei, Fisch oder Fleisch zusammengestellt. Diese Konserven enthalten keine Kartoffeln. Sie sind nach der Deklaration eine vollständige Mahlzeit.

Tabelle 4. Beikost zur Säuglingsernährung

Gemüsebreie als Glaskonserve ohne Zusätze von Ei, Fisch oder Fleisch in 100 g sind enthalten	Natrium mg	Kalium mg	Chlorid mg
Alete			
Karottenzubereitung (6. Woche)	32	204	36
Zartes Gartengemüse (9. Woche)	259	193	395
Gemüse-Allerlei (9. Woche)	87	283	135
Sommergemüse mit Fenchel (9. Woche)	246	152	348
Spinatzubereitung (4. Monat)	160	400	249
Gerber (Deutsche Maizena-Werke)			
Karotten (6. Woche)	97	176	152
Gartengemüse (9. Woche)	107	194	188
Zartes Gemüse (9. Woche)	138	181	257
Spinat (4. Monat)	104	194	164
Hipp			
Frühkarotten (6. Woche)	119	221	192
Salatbrei, aus zartem Kopfsalat mit Banane (6. Woche)	108	91	209
Karotten (9. Woche)	148	180	208
Zartes Gartengemüse (9. Woche)	241	136	345
Gemüse-Allerlei (9. Woche)	136	145	231
Spinat (4. Monat)	139	170	238
Gemüseerzeugnisse in trockener Form in 100 g Pulver sind enthalten:			
Aponti			
Aponti Möhrenflocken (6. Woche)	893	1430	1420
Humana			
Humana Karotten-Tomatenbrei (6. Woche)	72	499	129
Humana Mischgemüsebrei (6. Woche)	100	729	92

Von den aufgeführten Konserven hat nur die Karottenzubereitung der Firma Alete den Natrium-, Kalium- und Chloridgehalt der frischen Ware. Alle anderen Konserven enthalten wesentlich mehr Natrium und Chlorid als die rohen Gemüse. Nach dem Natriumgehalt lassen sich die aufgeführten Konserven in 3 Gruppen teilen: Präparate mit einem Natriumgehalt um 100 mg, Präparate mit einem Natriumgehalt um 150 mg und Präparate mit einem Natriumgehalt um 250 mg in 100 g.

Mit einer Karotten-Kartoffelmahlzeit aus Frischgemüse von 150 g (100 g Karotten und 50 g Kartoffeln) bekommt ein Säugling ca. 40 mg Natrium. Bei Verfütterung von Spinat 60 mg Natrium. Setzt man im 4. Lebensmonat bei einer Trinkmenge von 800 ml $^2/_3$-Milch das Natriumangebot aus der Milchnahrung mit 270 mg pro Tag an (s. o.), so würde der Säugling nach Verfütterung der Gemüse-Mahlzeit insgesamt 310–330 mg Natrium am Tag erhalten. Umgerechnet sind das 0,81 g Kochsalz. 150 g industriell hergestellte Gemüsekonserven enthalten dagegen, wenn wir die Präparate nach ihrem Natriumgehalt in 3 Gruppen teilen (s. o.), 150 mg bzw. 225 mg bzw. 375 mg Natrium. Das Angebot von Natrium am Tag aus Milchnahrung und Gemüsemahlzeit liegt dann bei 420 mg, 495 mg oder 645 mg. Das sind 1,1 g, 1,3 g oder 1,6 g Kochsalz am Tag.

Neben den Gemüsebreien als Glaskonserve werden auch Gemüseerzeugnisse in trockener Form (Tabelle 4) als Beikost angeboten. Bei diesen Präparaten handelt es sich um Gemüsepulver mit Zusatz von Getreideanteilen im Verhältnis von ca. 50:50. 30 g Möhrenflocken bzw. 40 g Gemüsepulver ergeben mit Wasser nach der Deklaration eine tischfertige Mahlzeit von ca. 200 g. In einer solchen Breimahlzeit aus Aponti Möhrenflocken sind 270 mg Natrium, in den Breimahlzeiten aus Humana Gemüsepulvern 30–40 mg Natrium enthalten.

Tabelle 5. Beikost zur Säuglingsernährung

Feinpürierte Menüs als Glaskonserve ab 4.—5. Lebensmonat (Gemüse und/oder Nährmittel mit Zusätzen von Ei oder Fleisch) in 100 g sind enthalten:	Natrium mg	Kalium mg	Chlorid mg
Alete			
Hühnchen, Gemüse, Eiernudeln	183	210	315
Hühnchen in Karotten und Reis	203	101	308
Kalbfleisch in Gemüse, Spaghetti	226	101	366
Leber-Menü aus Karotten, Schweineleber, Frischei-Nudeln	222	237	329
Rindfleisch in Spinat, Kartoffelbrei	181	138	277
Rindfleisch in zarten Erbsen	236	134	306
Karottencreme mit Eigelb	145	167	236
Truthahn in Gemüse und Reis	218	121	327
Gerber (Deutsche Maizena-Werke)			
Kalbfleisch in Gartengemüse	125	82	219
Hühnchen in Karotten	107	87	180
Leber in Karotten	123	112	204
Rindfleisch in zartem Gemüse	158	190	252
Püree mit Kalbfleisch und Eigelb	135	58	189
Rindfleisch in Nudeln	129	50	214
Hühnchen in Reis	101	68	185
Hipp			
Hühnchen in Reis — Gemüsecreme	191	98	341
Rindfleisch in Tomaten-Reis	140	126	254
Leber in Karotten	175	151	293
Leber in Spinat	131	102	249

Setzt man der haushaltsmäßig hergestellten Karotten-Kartoffelmahlzeit von 150 g 20–25 g Kalb-, Rind- oder Hühnerfleisch oder Kalbsleber [11] zu, so erhöht sich der Natriumgehalt der Mahlzeit von 50 mg auf 65–70 mg. Statt Frischfleisch oder Leber kann neuerdings auch pürierte Fleischkonserve der Firma Hipp verwendet werden. Mit 20–25 g Fleisch dieser Konserve wird im Durchschnitt 150 mg Natrium gegenüber 15–20 mg Natrium in entsprechender Menge Frischfleisch [11] gegeben.

In Tabelle 5 sind feinpürierte Menüs (Gemüse und/oder Nährmittel mit Zusätzen von Ei oder Fleisch) für Säuglinge ab dem 5. Lebensmonat aufgeführt. Der Natriumgehalt in diesen Menüs liegt zwischen 100 und 240 mg in 100 g. Je nach Wahl des Präparates erhält ein Säugling mit 150 g Menü zwischen 150 und 360 mg Natrium. Eine Menümahlzeit enthält also praktisch genau so viel Natrium, wie die in Tabelle 4 aufgeführten Gemüsebreie ohne Zusatz von Ei, Fisch oder Fleisch.

Salzgehalt in Milchbreien

Im 6. Lebensmonat wird im allgemeinen anstelle einer $^2/_3$-Milchflasche 200 g Vollmilchbrei (meist 10 g Grieß auf 100 ml Milch) gefüttert. Feiner Weizengrieß enthält im Durchschnitt 1 mg Natrium in 100 g [11]. Der Natriumgehalt im Grieß kann also bei der Berechnung vernachlässigt werden. Bei 45 mg Natrium/100 ml Frischmilch (Tab. 1) erhält der Säugling mit 200 g Vollmilchgrießbrei ca. 90 mg Natrium.

Für die Breizubereitung wird gerne eines der in Tabelle 2 aufgeführten Mehl-, Grieß, Schrot- oder Getreideflockenerzeugnisse benutzt. Der Natriumgehalt dieser Präparate liegt zwischen 4 mg und 744 mg in 100 g. Bei Verwendung eines Präparates mit einem Natriumgehalt bis 50 mg in 100 g erhöht sich der Natriumgehalt in 200 g Vollmilchbrei von 90 mg auf 100 mg, mit Präparaten mit einem Natriumgehalt bis 250 mg in 100 g von 90 mg auf 140 mg und bei Verwendung von Gerber 7 Korn-Brei von 90 mg auf 240 mg Natrium.

Von der diätetischen Lebensmittelindustrie wird auch eine große Zahl milchhaltiger Fertigbreinahrungen für Säuglinge angeboten. Überwiegend handelt es sich um $^1/_2$- und $^2/_3$-Milchbreie. Der Natriumgehalt in 200 g solcher Breie liegt in den meisten Fällen zwischen 50 und 90 mg. In einigen Fällen erreicht der Natriumgehalt 160 mg pro 200 g Breizubereitung.

Diskussion

Für eine normale Entwicklung im 1. Lebenshalbjahr dürfte der Natriumgehalt in der Muttermilch ausreichend sein. Der Bedarf eines gesunden Säuglings an Natrium wäre dann am Ende des 2. Lebensmonates mit durchschnittlich 120 mg, am Ende des 5. Lebensmonates mit 150 mg pro Tag gedeckt. Ein Natriumangebot in dieser Höhe dürfte auch ausreichen, um kurzdauernde Kochsalzverluste, z. B. durch Erbrechen, Durchfall oder mit dem Schweiß auszugleichen.

Bei Ernährung mit Kuhmilchmischungen erhält der Säugling stets mehr Natrium als das Brustkind. Wir [5] fanden bei gesunden Säuglingen zwischen der 8. und 10. Lebenswoche bei Ernährung mit $^1/_2$- und $^2/_3$-Milch mit und ohne Zusatz von 2% Keimöl eine durchschnittliche tägliche Natriumaufnahme von 195 mg (Tab. 6). Über Stuhl, Harn und Haut wurden

Tabelle 6. Natriumbilanz reifgeborener gesunder Säuglinge

	8.-10. Lebenswoche $^1/_2$- u. $^2/_3$-Milch mit/ohne Zusatz von 2% Keimöl Anzahl der Kinder: 23 Anzahl der Bilanztage pro Kind: 21		20.-22. Lebenswoche $^1/_2$- u. $^2/_3$-Milch mit Zusatz von 2% Keimöl u. Beikost Anzahl der Kinder: 7 Anzahl der Bilanztage pro Kind: 21	
	m	s	m	s
Natriumaufnahme (mg/24 Std)	195,0	± 7,3	220,9	± 11,5
Natriumausscheidung (mg/24 Std)				
Stuhl	16,6	± 9,4	48,8	± 35,7
Urin	118,2	± 34,2	125,1	± 35,7
Haut	16,9	± 8,2	16,7	± 8,0
Natriumretention (mg/24 Std)	43,3		30,3	
Natriumretention (% der Aufnahme)	22,2%		13,7%	

m = Mittelwert
s = Standardabweichung

täglich durchschnittlich 152 mg ausgeschieden. Es wurden im Durchschnitt tgl. 43 mg Natrium retiniert. Das sind 22% der Natriumaufnahme. Zwischen der 20. und 22. Lebenswoche beobachteten wir [5] (Tab. 6) bei Ernährung mit $^1/_2$- und $^2/_3$-Milch plus 2% Keimöl und altersgemäßer Beikost eine durchschnittliche tägliche Natriumaufnahme von 220 mg. Hiervon stammten 45 mg aus der haushaltsüblichen Beikost (Karotten, Kartoffeln, Obstmus, Obst- und Gemüsesaft). Über Stuhl, Harn und Haut wurden täglich durchschnittlich 190 mg ausgeschieden. Retiniert wurden täglich 30 mg, das sind 14% der Natriumaufnahme. Eine durchschnittliche tägliche Natriumretention von 1,3–1,8 mÄq, bzw. 30–40 mg, halten auch Gamble [8], sowie McCance und Widdowson [10] auf Grund von Berechnungen für ausreichend, um den Natriumbedarf gesunder Säuglinge im 1. Lebenshalbjahr zu decken. Eine tägliche Natriumaufnahme von 7–8 mÄq, bzw. 160–180 mg, wie Dahl [3] und auch Fomon [6] für künstlich ernährte Säuglinge im 1. Lebenshalbjahr empfehlen, wird bei Ernährung mit Kuhmilchnahrungen nach unseren Untersuchungen immer überschritten.

Ein gesunder Säugling, der mit altersgemäßen Milchnahrungen und haushaltsmäßig hergestellter Beikost ohne Zusatz von Kochsalz ernährt wird, erhält im 1. Lebensvierteljahr pro Tag etwa 200–250 mg Natrium, entsprechend 0,51–0,63 g Kochsalz (Tab. 7).

Im 2. Lebensvierteljahr liegt die tägliche Natriumaufnahme zwischen 300 und 350 mg, entsprechend 0,76–0,88 g Kochsalz. Werden Präparate der diätetischen Lebensmittelindustrie (Milchpräparate, milchfreie Kohlenhydratzusätze, Gemüsebreie, feinpürierte Menüs) verwendet, so ist es möglich, mindestens im 1. Lebensvierteljahr, die angegebenen Natrium- bzw. Kochsalzmengen nicht wesentlich zu überschreiten, wenn die Auswahl der Präparate nur nach ihrem Kochsalzgehalt vorgenommen wird. Wird bei der Auswahl der Präparate der Kochsalzgehalt aber nicht beachtet, so kann die Natrium- bzw. Kochsalzaufnahme bis zu 150% höher liegen (Tab. 7). Ein Säugling am Ende des

Tabelle 7. Natriumaufnahme im 1. Lebenshalbjahr

	Natriumaufnahme mg/Tag	mg/kg × Tag	berechnet als Kochsalz mg/Tag
Ende des 2. Lebensmonats:			
Muttermilch	120	25	300
$^2/_3$-Milch + milchfreie Kohlenhydratzusätze	240— 375	50— 78	605— 945
Milchfertigpräparate	210— 450	45— 93	530—1125
Ende des 4. Lebensmonates:			
Muttermilch u. Gemüse-Kartoffeln (haushaltsmäßig)	190	30	480
Muttermilch u. Gemüsekonserven (industriell)	200— 540	31— 85	505—1360
$^2/_3$-Milch + milchfreie KH-Zusätze u. Gemüse-Kartoffeln (haushaltsmäßig)	310— 510	49— 80	780—1280
$^2/_3$-Milch + milchfreie KH-Zusätze u. Gemüsekonserven (industriell)	320— 812	50— 127	806—2050
Ende des 6. Lebensmonates:			
Muttermilch	120	25	300
$^2/_3$-Milch, Vollmilchbrei + milchfreie KH-Zusätze, Gemüse-Kartoffeln + Fleisch (haushaltsmäßig)	350— 450	48— 62	883—1135
$^2/_3$-Milch, Vollmilchbrei + milchfreie KH-Zusätze, Gemüse-Kartoffeln + industriell)	530—1100	74—153	1333—2780

2. Lebensmonates würde dann pro Tag etwa 1 g, am Ende des 4. Lebensmonates 2 g und am Ende des 6. Lebensmonates etwa 3 g Kochsalz erhalten.

Nach unseren Untersuchungen haben fast alle für die Säuglingsernährung industriell hergestellten Beikostpräparate mehr oder weniger große Zusätze von Natrium bzw. Natriumchlorid. Für die Entwicklung des Säuglings sind Kochsalzzusätze zur Nahrung nicht erforderlich. Erfahrungsgemäß essen Säuglinge Nahrungen mit und ohne Salzzusatz gleich gut. Diese kinderärztliche Erfahrung ist in neuester Zeit von der Arbeitsgruppe um Fomon [1, 7] in vergleichenden Untersuchungen bestätigt worden. Beikost für den Säugling ist ohne Salzzusatz für den Geschmack des Erwachsenen fade. Vermutlich sind die Salzzusätze zur industriell hergestellten Beikost ein Zugeständnis der Hersteller an den Geschmack der Mütter.

Da der Kochsalzzusatz zur Nahrung für den Säugling keinen Nutzen hat, ist zu fragen, ob er gleichgültig oder vielleicht schädlich ist. Unmittelbare Schäden nach regelmäßigem Verzehr industriell hergestellter Beikost mit Salzzusätzen sind u. W. bei Säuglingen bisher nicht beschrieben worden. Dabei ist anzumerken, daß die Natrium- bzw. Kochsalzzusätze in der industriell hergestellten Beikost in den USA z. T. wesentlich größer sind als in den entsprechenden Präparaten auf dem deutschen Markt [1].

Überschüssig mit der Nahrung aufgenommene Salze müssen wieder ausgeschieden werden. Verglichen mit dem Brustkind fallen beim künstlich ernährten Säugling mehr Proteinabbauprodukte und mehr Mineralien zur Ausscheidung an. Salzzulagen zur Nahrung beanspruchen den Wasserhaushalt zusätzlich und können damit die Reserve an „freiem Körperwasser" einengen. Oft diskutiert ist der Zusammenhang zwi-

schen regelmäßigem Genuß von Speisen mit hohem Kochsalzgehalt und der Entwicklung einer Hypertonie. Dahl [4] hat darauf aufmerksam gemacht, daß junge Ratten bereits nach mehrmonatiger Fütterung mit industriell hergestellten Gemüsebreien und feinpürierten Menüs für Säuglinge mit einem Natriumgehalt zwischen 150 und 500 mg pro 100 g Konserve häufiger eine Hypertonie entwickelten und eine höhere Sterberate aufwiesen als die Kontrolltiere. Diese Beobachtungen sind für den Menschen nicht unbestritten geblieben [9]. Nach den bisher vorliegenden Untersuchungen kann nicht entschieden werden, ob die in den industriell hergestellten Fertigpräparaten für Säuglinge enthaltenen Natriummengen für die Entwicklung des Säuglings unschädlich sind. Wir halten es deshalb für zweckmäßiger, Milch- und Beikostnahrungen ohne Zusatz von Kochsalz und anderen Natriumsalzen zu verwenden.

Literatur

1. Anderson, Th. A., Fomon, S. J.: Commercially prepared strained and junior foods for infants. J. Amer. diet. Ass. 58, 520 (1971).
2. Czerny, A., Keller, A.: Des Kindes Ernährung, Ernährungsstörungen und Ernährungstherapie. II. Band, 2. Aufl. Leipzig-Wien: Franz Deuticke 1928.
3. Dahl, L. K.: Salt in processed baby foods. Amer. J. clin. Nutr. 21, 787 (1968).
4. — Heine, M., Leitl, G., Tassinari, L.: Hypertension and death from consumption of processed baby foods. Proc. Soc. exp. Biol. (N. Y.) 133, 1405 (1970).
5. Droese, W., Stolley, H.: Unveröffentlichte Werte.
6. Fomon, S. J.: Infant nutrition. Philadelphia-London: W. B. Saunders Comp. 1967.
7. — Thomas, L. N., Filer, L. J., jr.: Acceptance of unsalted foods by normal infants. J. Pediat. 76, 242 (1970).
8. Gamble, J. L. et al.: Effects of large loads of electrolytes. Pediatrics 7, 305 (1951).
9. Gros, G., Weller, I. M., Hoobler, S. W.: Relationship of sodium and potassium intake to blood pressure. Amer. J. clin. Nutr. 24, 605 (1971).
10. McCance, R. A., Widdowson, E. M.: Mineral metabolism of the foetus and the newborn. Brit. med. Bull. 17, 132 (1961).
11. Souci, S. W., Fachmann, W., Kraut, H.: Die Zusammensetzung der Lebensmittel. Nährwert-Tabellen Band I und II. Stuttgart: Wissenschaftliche Verlagsgesellschaft MBH 1969.

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Salt and Water Metabolism During Weight Reduction

By PETER ELSBACH AND IRVING L. SCHWARTZ

On a metabolic ward, sodium balances were studied in severely obese patients undergoing weight reduction on a formula diet, and in two obese subjects, and two subjects who had never been obese, who received the formula diet in amounts sufficient to maintain weight. Marked, prolonged positive balances were noted in the patients undergoing weight reduction, particularly during moderate salt loading; often these were sufficient to account for failure to lose weight on a 600 Calorie diet. Spirolactone SC 8109 did not significantly influence this salt retention in the two patients to whom it was administered. Similar delays in salt excretion did not occur in the non-obese subjects nor in the obese patients on weight-maintaining diets, although the latter did not adapt to changes in salt intake as rapidly as the non-obese subjects. Divergences in sodium and water balances were also noted in the obese subjects on low-calorie diets during moderate salt-loading, indicating that the obese state may be associated with abnormalities in homeostatic mechanisms regulating the distribution as well as excretion of salt and water.

THIS REPORT PRESENTS observations on weight reduction and salt balance in severely obese patients who participated in a long-term metabolic study under rigidly controlled conditions on a metabolic ward. It was noted that increase in salt intake by patients on a low calorie diet resulted in cessation of weight loss or in actual weight gain. A review of the literature indicated that although frequent allusions to salt and water retention by obese individuals are made, no exact documentation of this phenomenon is recorded. The observations presented below indicate that abnormalities in salt and water metabolism do occur, and may be partially or completely responsible for the remarkable fluctuations in weight and the failure to lose weight sometimes observed in obese patients on a low calorie diet.

METHODS

Severely obese patients (approximately twice ideal weight), who were free of disease of the heart, liver, kidney, and endocrine system by usual clinical and laboratory criteria, were admitted to The Rockefeller Institute Hospital for approximately one year. Because

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of the long period of hospitalization, attempts were made to provide an environment reasonably suited to each individual. An occupational therapy program provided several hours of organized activity every day. During metabolic balance periods the patients were allowed to leave the hospital only under supervision of hospital personnel.

After a short adjustment period the patients were given a formula diet[1] supplemented with vitamins and providing sufficient calories for maintenance of weight. Iodized salt was administered in divided doses following formula feedings, which were usually given 5 times a day at 3-hour intervals. During low salt intake, the patients received 2 to 3 Gm. daily; during high salt intake, 10 Gm. daily. The formula contained small amounts of sodium which were included in the calculation of the balance. Occasional nausea and/or vomiting occurred after salt ingestion, but usually the patients experienced little or no discomfort.

After approximately 3 months of weight maintenance, the daily caloric intake was reduced to 600 Calories, but the composition of the formula and timing of the feedings were unchanged.

Each patient was weighed without clothes, daily, and before the first formula feeding, using a Toledo scale which had an accuracy of ± 5 Gm. Blood pressure readings were taken every day while balance studies were in progress. Daily fluid intake and urine output were measured and recorded. An aliquot of the mixed daily urine collection was taken and stored for determination of Na and K content. Blood samples were obtained several times a week for determination of hematocrit and concentrations of Na and K in plasma. Urine and plasma were diluted to the desired working concentrations for flame photometry, using lithium as an internal standard.

During selected periods of the study, stools were collected in Mason jars and refrigerated. Weekly collections were homogenized after addition of 250 ml. of 7 N sulfuric acid. The homogenate was brought to constant volume with deionized water and an aliquot stored in capped glass bottles. A 20 ml. aliquot was further digested in concentrated nitric acid at 150 C. for 30 minutes, cooled, diluted and filtered prior to addition of lithium for flame photometry.

The balance studies were carried out in 3 groups of individuals:

1. Obese patients on a reducing formula diet after substantial weight reduction;
2. Obese patients on a weight maintaining formula diet prior to weight reduction;
3. Patients of normal weight, who had never been obese, on a weight-maintaining formula diet.

During the balance periods, caloric intake was kept constant and sodium intake varied to suit the requirements of the study.

Accuracy of the Data

The conclusions of this study depend on a comparison of oral ingestion and urinary excretion of sodium. Intake of salt was determined in milliequivalents, by analysis of the formula and by administration of salt supplements in exact amounts. Ingestion of salt tablets by the patients was assured by close supervision by the nursing staff. When, on occasion, vomiting occurred following swallowing of salt tablets, it was assumed that the whole dose was lost.

Fecal, skin, and menstrual losses of sodium were not included in the balance. Table 1 shows that sodium in the feces represented an insignificant portion of the amount ingested, on both a high and low intake of salt. This is in accord with reports in the literature concerning fecal sodium excretion at various levels of salt intake.[2] Balance studies were only carried out during the cool months of the year when cutaneous losses of sodium were insignificant relative to urinary sodium excretion.

In interpreting the results of the balance studies, the differences between intake and output were regarded as significant only when these differences were numerically large relative to the absolute values of intake and output from which they derived. These latter values (sodium intake and urinary excretion) were always greater by several orders of magnitude than uncontrollable variables, such as non-urinary losses of sodium and analytical

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Table 1.—*Average Daily Fecal Sodium Excretion by Two Obese Patients on a Low and a High Sodium Intake**

Intake	55 mEq./day	220 mEq./day
Fecal excretion	.63	1.55
	.85	.88

*Sodium determination on stools collected during 10-day periods on both a low and a high sodium intake.

errors. Moreover, since the balance studies extended over many weeks, random analytical errors would tend to cancel out.

Results

All of the seven obese patients studied and presented in this paper exhibited abnormal salt and water metabolism following weight reduction.

Weight Gain on a 600 Calorie Diet During Chronic Moderate Salt Loading

Patient B. R. weighed 115 Kg. on admission and had lost approximately 30 per cent of her body weight at the start of the first balance period (fig. 1). As shown, sodium was retained on an intake of 35 mEq. per day, while the patient slowly lost weight. However, following an increase in salt ingestion to 172 mEq. per day, her weight increased sharply. After 14 days on the higher salt dose, the patient had gained 6 Kg. and retained more than 1000 mEq. of sodium. During the remainder of the 32 days on the high salt intake, the patient lost all of the weight she had gained, although she still retained half of the salt which she had accumulated during the first 14 days. This amount of salt, approximately 500 mEq. was excreted when her salt intake was reduced to 34 mEq. per day.

Figure 2 shows a balance study started 6 weeks later in the same patient. It is evident that throughout the period of observation weight gain associated with a markedly positive sodium balance was reproduced whenever salt intake was increased. Most of the retained salt was excreted when the salt dose was reduced to 43 mEq. daily.

Remarkable fluctuations in serum sodium, and to a lesser extent in hematocrit, were observed in this patient and also in others. Hypernatremia, with levels of serum sodium as high as 165 mEq./L. was noted repeatedly, but could not be correlated with changes in weight, sodium balance, or with the clinical condition of the patients.

During salt loading the patient complained about headaches, dizziness, difficulty in focusing and occasional nausea. These symptoms were most marked at the peak of weight gain, at which time facial puffiness was apparent, as well as frank pitting edema of feet, ankles, and lower legs, and sometimes of the dorsum of the hands. None of these symptoms or signs was associated with any consistent abnormality of serum sodium concentration. No significant changes in blood pressure occurred at any time during these balance studies. The menstrual cycle seemed to bear no correlation with salt retention on high sodium intake.

One determination of aldosterone excreted in the urine over a 24-hour period

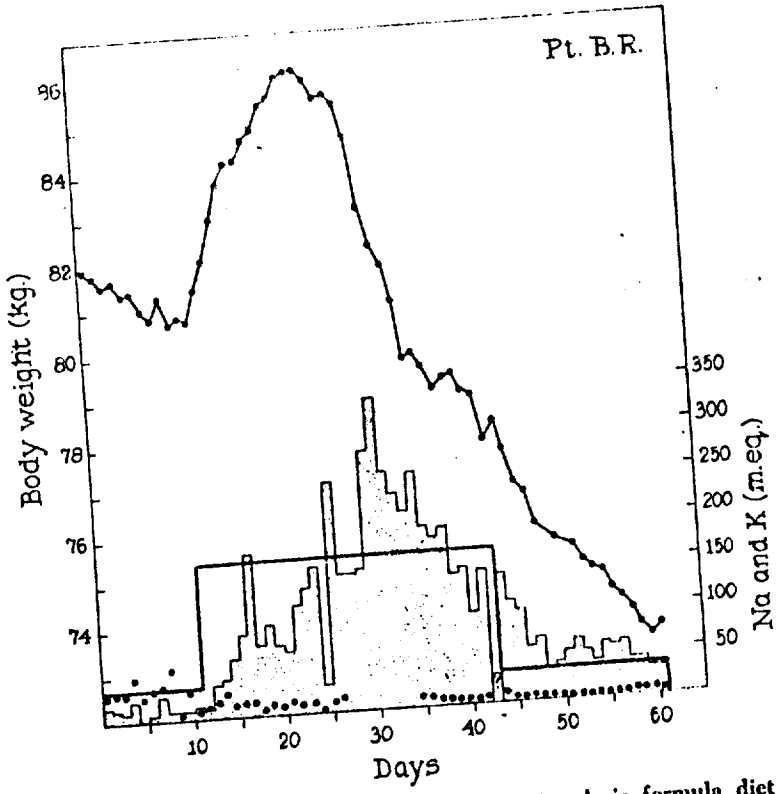

Fig. 1.—Weight gain and salt retention on a 600 calorie formula diet during moderate salt loading (10 Gm. daily). Daily weights in Kg. are shown in the top part of the figure. Daily salt ingestion (heavy line) and daily urinary sodium excretion (thin line, stippled area) in mEq. Sodium excreted is plotted up from the base line. Black dots indicate daily potassium excretion in mEq.

during maximal sodium retention showed a low normal value.* No clear-cut effect of the steroid-17-spirolactone SC 8109 on sodium retention or K excretion during salt loading was observed (fig. 2); however, the weight gain and salt retention following moderate salt loading seemed more marked when no aldosterone antagonist had been given, suggesting that the administration of SC 8109 may have reduced these effects of moderate salt loading to some extent. SC 8109 was given intramuscularly in a dose of 300 to 500 mg. per day, or orally in a dose of 300 to 2500 mg. per day.†

A comparable response to moderate salt loading was exhibited by T. S. (fig. 3). This patient, as B. R., did not begin to excrete retained salt until 14 days after salt loading was started.

It is noteworthy that inulin, urea and creatinine clearances were normal in

*We are indebted to Dr. John Larragh for this determination.

†SC 8109 was generously supplied by Dr. Clarence Gantt of Searle Co., Chicago, Ill.

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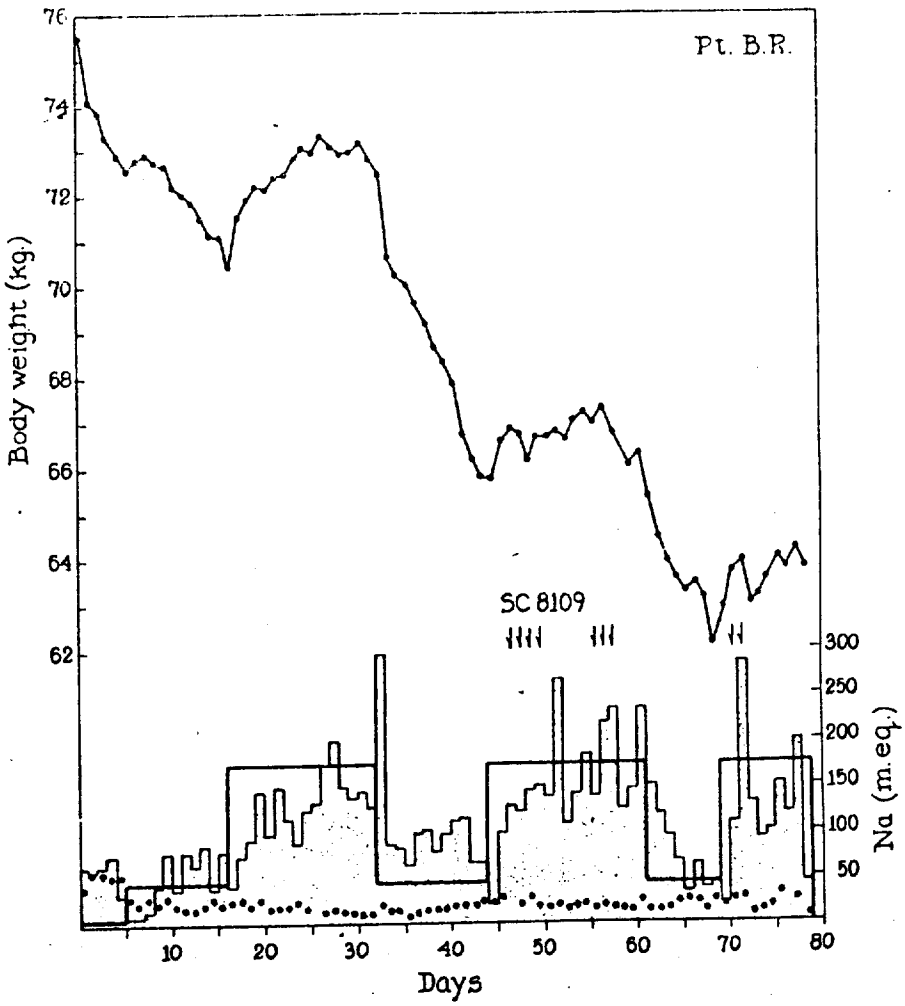

Fig. 2.—Weight gain or failure to lose weight on a 600 Calorie formula diet when salt intake was alternately maintained at levels of 3 and 10 Gm. daily. (Same patient as fig. 1). During the first 5 days of the balance period, the patient received a 600 Calorie "saltfree" diet of normal food.

these patients and that no albuminuria or loss of renal concentrating power was ever demonstrated.

Dissociation Between Sodium and Water Retention on a Low Calorie Diet

Patient S. T. (fig. 4) on a low salt intake and a 600 Calorie diet remained in balance and lost 4 Kg. in 40 days. While still on a low salt dose, the patient stopped losing weight on the 32nd day of the balance period. When the salt intake was increased to 5 Gm. daily, the weight curve showed some fluctuation

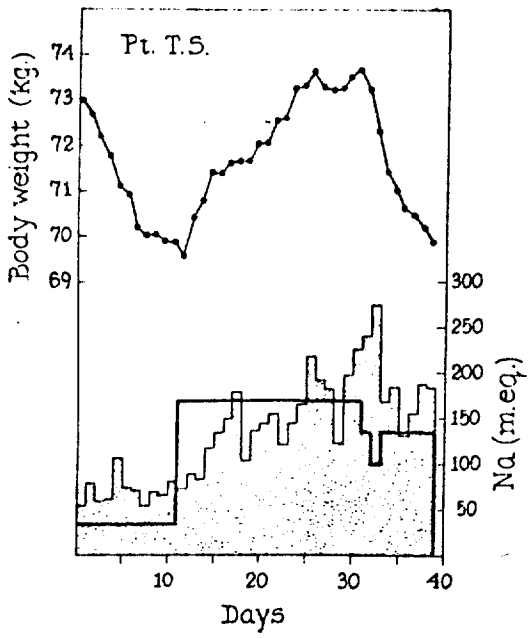

Fig. 3.—Weight gain and salt retention during moderate salt loading while on a 600 Calorie formula diet.

but no significant weight loss occurred during the 35-day period on the higher intake. As shown in the graph, excretion of salt essentially matched intake for a period of 75 days. However, when the salt dose was reduced to 1 Gm. for one day, and then omitted entirely, a massive diuresis and natriuresis followed, as if this patient had retained large amounts of salt as extracellular fluid. The weight loss of 7.0 Kg. associated with this diuresis corresponded to a negative Na balance of more than 1100 mEq. It is of interest that this same patient, during another balance period under apparently similar conditions, showed the response illustrated in figure 5. On 3 Gm. (43 mEq.) of salt this patient did not lose weight and it is clear that she excreted only a fraction of the salt ingested. On the higher salt dose she gained weight, but the weight curve flattened out and reverted downward while the patient continued to retain salt in large quantities. The reduction in salt intake to 128 mEq. per day starting on the 28th day was due to the loss of one dose of salt per day because of nausea and vomiting.

R. G., during the same balance period (fig. 6), displayed a pattern similar to that shown by S. T. in figure 4. R. G. gained weight on a 1000 Calorie formula diet when supplemented with 10 Gm. of salt daily. It is clear, however, from figure 6 that Na excretion did not correspond at all to weight change. For example, on the 17th day of the balance she excreted 470 mEq. of sodium in excess of intake, whereas her weight loss amounted to only 1 Kg. At the end of the 30-day salt-loading period, she was in balance with respect to sodium but she weighed over 6 Kg. more than at the beginning of the period of high salt intake. It was only after reduction of the daily salt intake that

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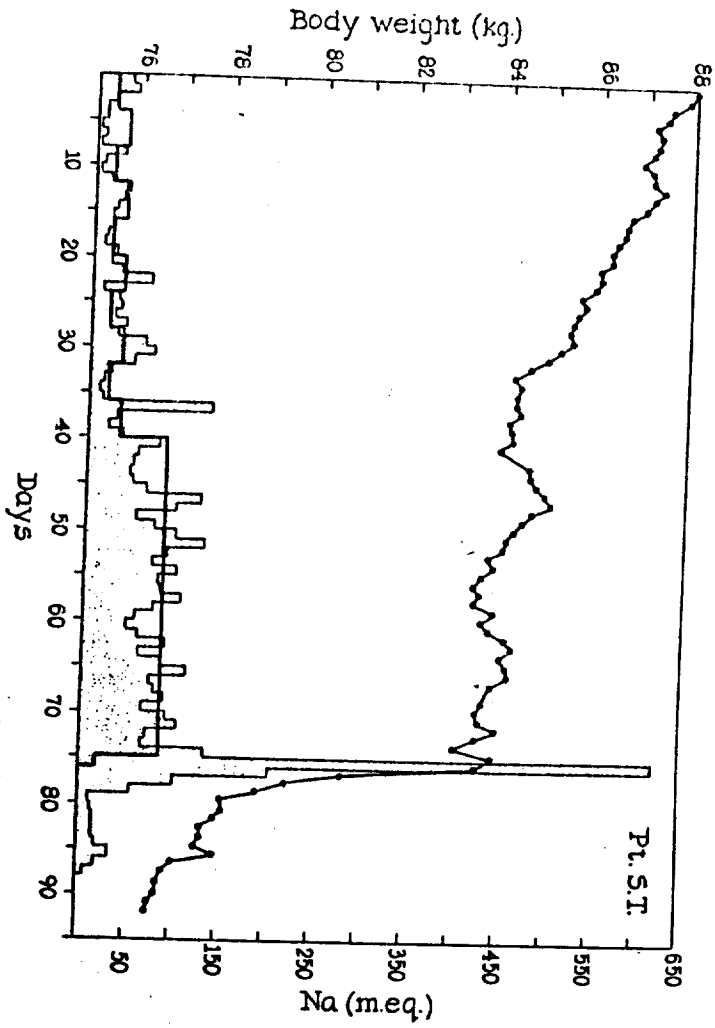

Fig. 4.—Failure to lose weight on a 600 Calorie formula diet in the absence of sodium retention during the period of observation, followed by natriuresis and rapid weight-loss when salt intake was discontinued.

a massive diuresis (6 liters) and a minor natriuresis ensued. Thus Na balance was slightly negative on the 50th day. At this time the caloric intake was increased to 1500 Calories, so that no further follow-up was possible.

Potassium and nitrogen excretion during this balance period was in the same range as the dietary intake, rendering unlikely the possibility of clandestine food ingestion. Moreover, both weight gain and diuresis correlated closely with the manipulation of the salt dose.

Not every patient responded to salt loading with either striking salt retention

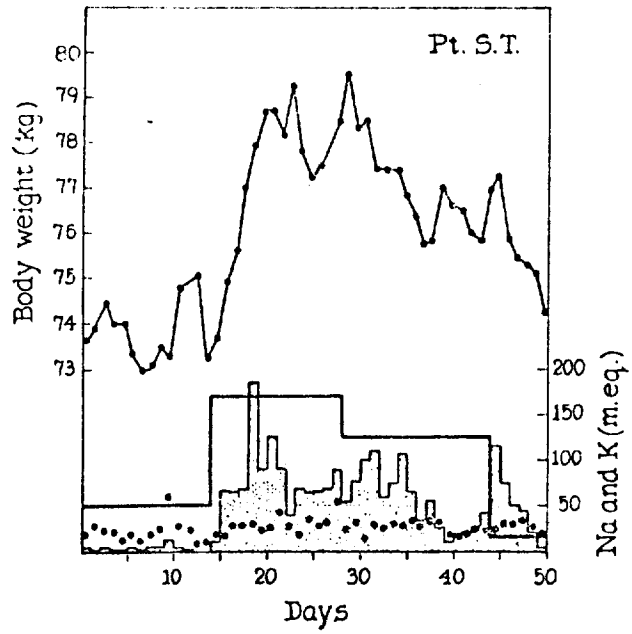

Fig. 5.—Salt retention without corresponding water retention on both a low and a high salt intake while on a 600 Calorie formula diet (same patient as fig. 4).

and/or weight change. For example, S. B. exhibited a clearly lesser alteration of her weight curve (fig. 7) when treated in the same manner as the 4 persons described previously. In fact, at the end of the 32-day period on high salt intake (172 mEq. Na daily), S. B. exhibited a negative balance of 760 mEq., which increased to 1250 mEq. during the subsequent 18 days. This patient was studied in an identical manner and at the same time as patient B. R. (contrast fig. 7 and fig. 1).

Several tests were carried out to determine whether or not the retention of salt and water occurring with change from a low to a high salt intake was due in some manner to long term feeding of an artificial diet or to the possibility that a prolonged low salt intake leads to a conditioning of factors promoting renal salt conservation.

Two non-obese subjects and two obese patients were studied.

When two non-obese patients, J. M. and A. D., who had been on a weight-maintaining formula diet with a daily supplement of 40 mEq. of salt for 4–6 months, were given 172 mEq. of sodium/day, no significant salt or water retention was observed (fig. 8).

Of the two obese patients studied on a weight-maintaining formula, M. V. retained a moderate amount of sodium and showed only minor fluctuations in weight, both on the lower and higher salt intake (fig. 9). However, following 7 months of caloric restriction associated with a weight loss of 25 Kg., this patient showed a striking increase in both water and salt retention in response to elevation of her dietary sodium intake from 34 mEq. day to 172

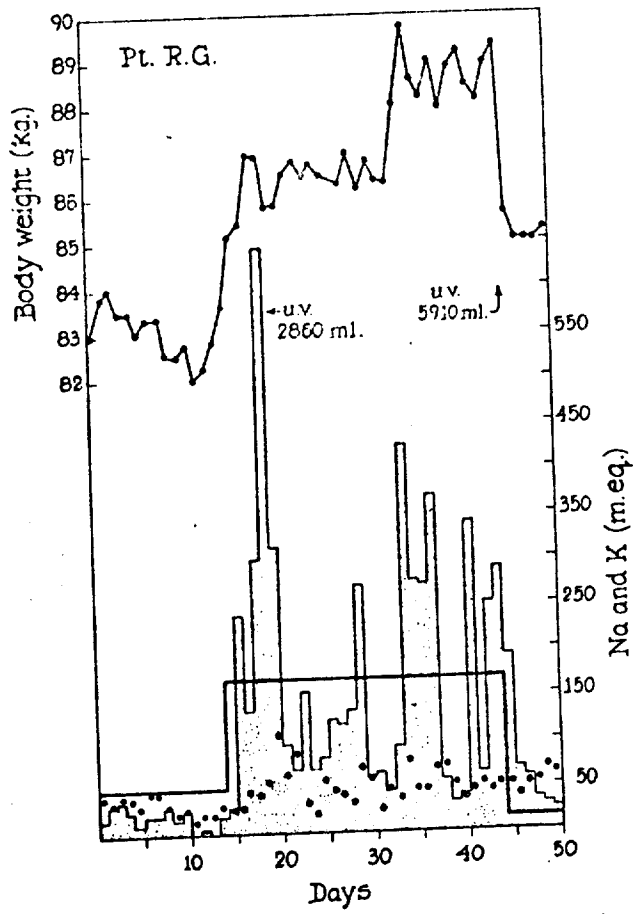

Fig. 6.—Weight gain by a patient on a 1000 Calorie formula diet. Dissociation between water and salt retention (water retention without corresponding salt retention).

mEq./day (fig. 10). This patient's weight curve reached a plateau, but there was no change in her serum sodium concentration while she was retaining salt in large quantities, again suggesting that part of the retained salt was being sequestered in an osmotically inactive state. It is also noteworthy that during the period of caloric restriction (except when the salt loading test was conducted), she received natural food. The other patient, A. E., exhibited retention of water and salt in response to salt loading even prior to the period of caloric restriction (fig. 11), although the enhanced retention following weight reduction (fig. 12) was not as striking as in the case of M. V. (fig. 10).

Discussion

Labbé and Furet first suggested that an abnormality in the excretion of salt and water could occur in association with obesity.[3] These workers recorded

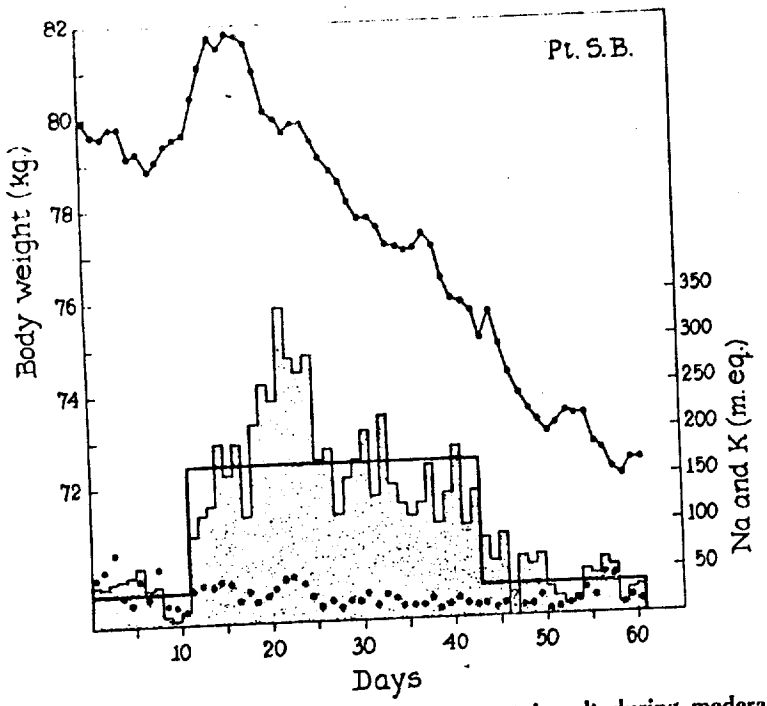

Fig. 7.—Definite but less marked tendency to retain salt during moderate salt loading on a 600 Calorie formula diet (compare with fig. 1).

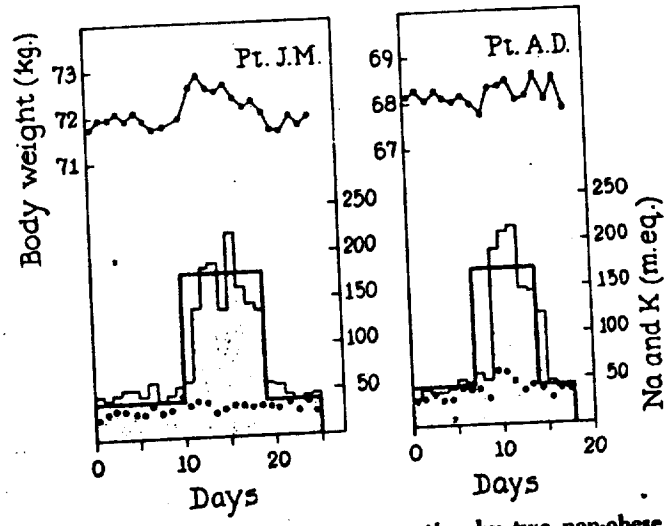

Fig. 8.—Urinary sodium and potassium excretion by two non-obese patients on a weight-maintaining formula diet during moderate salt loading (10 Gm. daily). These two subjects were under study for atherosclerosis. There was no history or present evidence of any of the manifestations of congestive heart failure.*

*We are greatly indebted to Drs. E. H. Ahrens, Jr. and Jules Hirsch for the opportunity to carry out control studies on these non-obese patients.

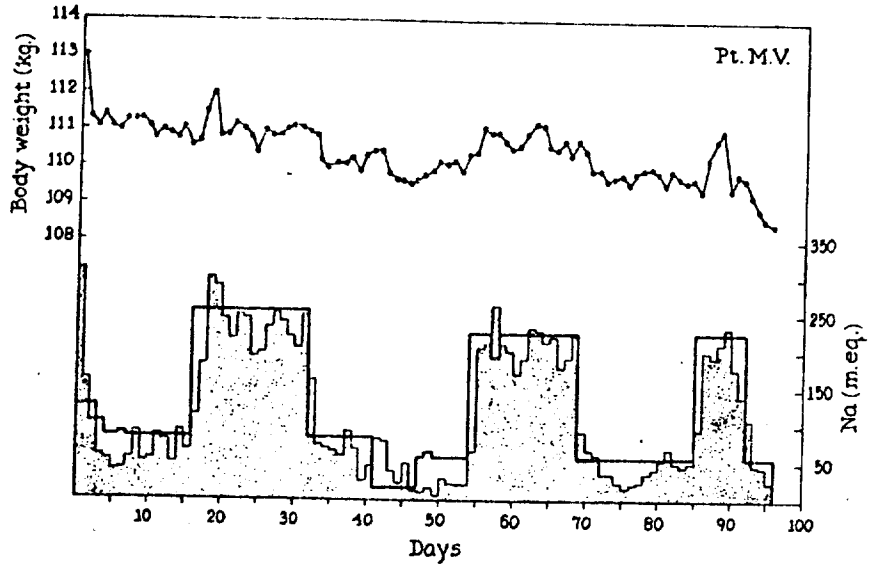

Fig. 9.—Rapid adaptation of sodium excretion during low and moderate salt intake (10 Gm. daily) by an obese patient prior to weight reduction on a weight-maintaining formula diet.

weight and followed the excretion of urinary chloride by normal subjects and by two obese patients on a calorically unrestricted diet with a measured salt intake. One of the obese patients retained 102 Gm. of salt within a period of less than one month; the other retained 42.5 Gm. of salt within 35 days. Although both patients gained weight during the study, the authors point out that there was no close correlation between chloride retention and weight gain and conclude that salt retention could occur without water retention and vice versa. Since this early study there have been suggestions that obese individuals tend to retain salt[4-7] but the authors are not aware of any recorded observations of salt and water balance in obese patients subjected to chronic salt loading during various phases of a weight reduction program.

In contrast to the response of normal subjects, most of the obese patients observed in this study demonstrated a delay in the excretion of sodium in response to moderate chronic salt loading. Usually the retention of salt was associated with isotonic retention of water as indicated by a proportionate gain in weight. However, it was observed that large quantities of sodium could be retained for long periods without sufficient water to produce an equivalent amount of extracellular fluid and without correlated change in serum sodium concentration. Conversely, a patient may fail to lose weight on a daily intake of 600 Calories without demonstrable sodium retention or serum dilution, but, subsequently, on abrupt reduction of salt intake, lose large amounts of sodium and water. In one such instance the excretion of 1100 mEq. of sodium could not be attributed to retention of dietary salt during the preceding 75 days (fig. 4). These observations suggest that some of these patients can accumulate sodium and retain it in an osmotically inactive form for long periods, until an

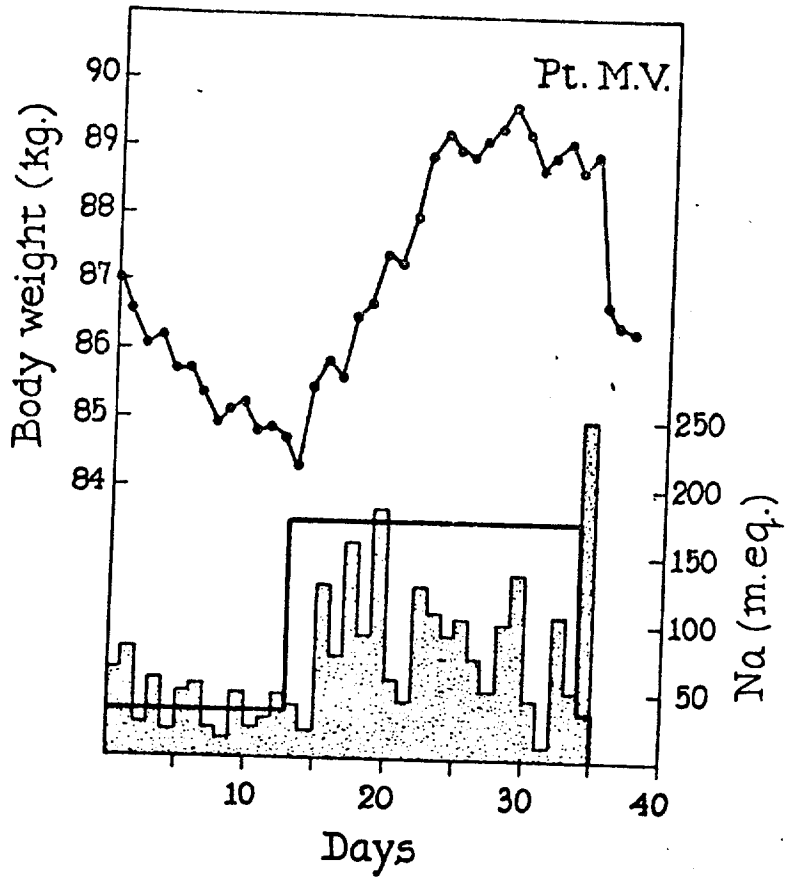

Fig. 10.—Salt retention and weight gain of same patient as in fig. 9 on a 600 Calorie formula diet and a salt intake of 10 Gm. daily, following weight loss of 25 Kg. Compare with response shown by this patient prior to weight reduction (fig. 9).

abrupt change in salt intake serves in some manner to trigger a chain of events that results in an osmotic reactivation of the "bound" sodium. Previously, Elkinton et al.[8] concluded that osmotic activation and inactivation of cell base must occur when their balance studies showed changes in body sodium, potassium and water, which could not be explained if it were assumed that all base was in simple solution. Also, Farber and Soberman[9] and Carroll and Farber[10] have inferred that sodium must be stored in an osmotically inactive state on the basis of their observation that an increased total exchangeable body sodium persisted in "dry cardiacs," i.e., in patients with congestive heart failure who had lost their edema fluid.

The present data show that normal persons on a low-salt formula diet for six months did not manifest significant weight gain when their salt intake was increased and that within 24 hours sodium excretion matched ingestion (fig. 8). Rapid adaptation of sodium excretion was also noted when salt intake was

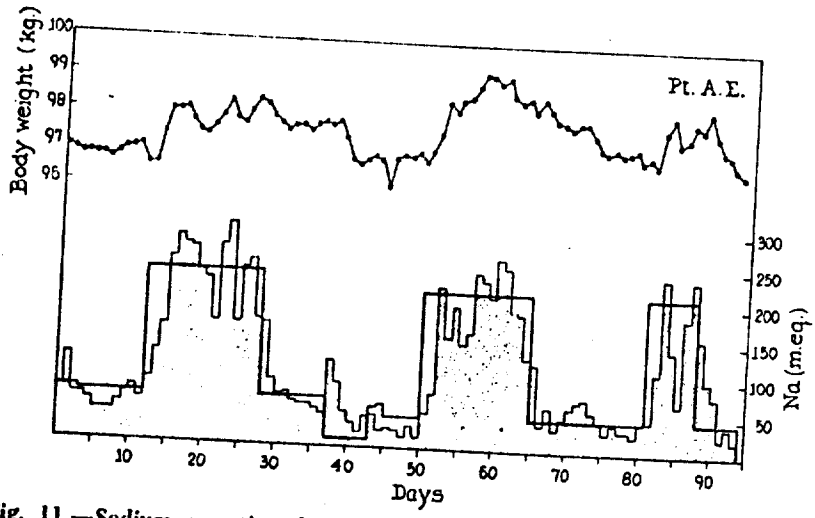

Fig. 11.—Sodium excretion during high and low sodium intake by an obese patient prior to weight reduction on a weight maintaining formula diet.

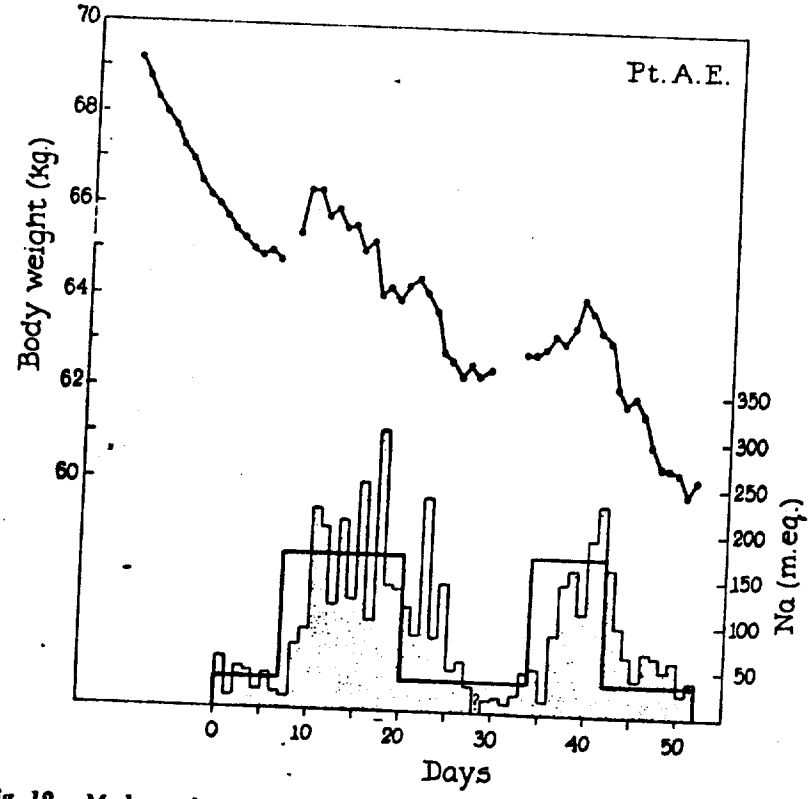

Fig. 12.—Moderate loss of ability to adapt to salt loading (10 Gm. daily) following weight reduction of 27.5 Kg. over a period of 7 months. Compare with response shown by the same patient prior to weight reduction (fig. 11).

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reduced to preloading levels. Two obese patients on a weight-maintaining formula diet also retained little salt under similar conditions (but noticeably more than the non-obese patients). These observations indicate that formula feeding *per se* or salt restriction *per se* does not cause the delayed excretion of sodium in response to moderate salt loading. In addition, it is noteworthy that we have observed repeatedly the retention of sodium by obese patients on a daily intake of as little as 50 mEq. of salt or less.

The retarded natriuretic response to moderate salt loading of patients who have lost weight during drastic caloric restriction raises the question of a possible similarity of the mechanism of this phenomenon and the mechanism of "starvation" edema.[11,12] In the patients described in this study there was no clinical evidence of malnutrition. All patients, after an initial 4–6 weeks of negative nitrogen balance, achieved nitrogen equilibrium.[13] In addition, plasma protein levels and other tests indicated normal liver, kidney and endocrine function. The incidence of infections among the obese patients was no greater than among the rest of the hospital population. Although no evidence of malnutrition was apparent, one cannot but wonder whether the obese patient who has reduced his weight to normal levels differs from the normal individual who had never been or who has no predisposition to become obese, the distinguishing features becoming manifest during semi-starvation.

Little can be said about the pathophysiology of salt retention in severely obese individuals on a reducing diet. The possibility that aldosterone activity was of importance was diminished by the failure of a spirolactone to effect an unequivocal alteration of sodium retention in two patients. That salt conserving steroids may be implicated in related clinical situations is suggested by Leutscher's demonstration of increased urinary excretion of aldosterone by moderately obese young women with periodic edema.[14] Apart from a possible abnormality in the production of mineralocorticoids, it is noteworthy that Bansi[7] has reported on the occurrence of increased antidiuretic activity in the serum of obese patients during water and salt retention.

Whatever its underlying mechanism, it is of practical importance that salt and water retention is often responsible for the failure of obese patients to lose weight on a low calorie diet to which they actually adhere.

REFERENCES

1. Ahrens, E. H., Jr., Dole, V. P., and Blankenhorn, D. H.: The use of orally-fed liquid formulas in metabolic studies. Am. J. Clin. Nutrition 2:336, 1954.
2. Dempsey, E. F., Carroll, E. L., and Albright, F.: A study of factors determining fecal electrolyte excretion. Metabolism 7:108, 1958.
3. Labbé, H., and Furet, L.: Les troubles de l'élimination chlorurée urinaire. Facteurs d'obésité. Presse méd. 809, 1905.
4. Bozenraad, O.: Über den Wassergehalt des menschlichen Fettgewebes unter verschiedenen Bedingungen. Deutsches Arch. klin. Med. 103:120, 1911.
5. Lauter, S.: Über die Beziehungen zwischen Energiehaushalt, Wasserhaushalt und Gewicht bei Fettsucht. Klin. Wchnschr. 5:1696, 1926.
6. Newburgh, L. H.: Obesity. Arch. Int. Med. 70:1033, 1942.
7. Bansi, H. W., and Warninghof, G.: Zur Klinik und Pathogenese der Fet-

tleibigkeit. Medizinische 15:549, 1956.

8. Elkinton, J. R., Winkler, A. W., and Danowski, T. S.: Inactive cell base and the measurement of changes in cell water. Yale J. Biol. & Med. 17: 383, 1944.
9. Farber, S. J., and Soberman, J.: Total body water and total exchangeable sodium in edematous states due to cardiac, renal or hepatic disease. J. Clin. Invest. 35:779, 1956.
10. Carroll, H. J., and Farber, S. J.: In press.
11. Helweg-Larsen, P., Hoffmeyer, H., Kieler, J., Hess Thaysen, E., Hess Thaysen, J., Thygesen, P., and Hertel Wulff, M.: Famine disease in German concentration camps. Complications and sequellae. Acta med. scandinav. Suppl. 274, 1952.
12. McCance, R. A.: Overnutrition and undernutrition. Lancet 685 and 739, 1953.
13. Unpublished observations.
14. Leutscher, J. A., Jr.: Idiopathic edema with increased aldosterone output. Trans. Ass. Amer. Physicians 70:158, 1957.

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The Effect of Drinking Water with a Different Content of Chlorides on the Organism of Experimental Animals

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Source: Gigiena i Sanitariya, 1971, Vol. 36, no. 6, pp. 11-15

Our task was to study the role of an excess supply of sodium chloride with drinking water in the development of experimental hypertension.

Clinical and experimental data of the past years show that electrolytes play an important role in the development of certain cardiovascular diseases (V.V. Parin and F.Z. Meerson; V.M. Bogolyubov; B.N. Brikker, and others). In the secondary peripheral mechanisms of the development of hypertensive disease, an important role is attributed to the conditon of electrolyte metabolism, and in particular, to the metabolism of socium chloride. The pressor effect of mineral cortical products is associated with the retention of sodium chloride in the organism. (Raab; S.M. Shenderov, and others). The above characteristics were used by us in selecting an experimental model of desoxycorticosteroid hypertension (DOC-hypertension). Mineral cortical products contribute as is known, to the retention of sodium chloride in the organism and a rise of arterial pressure.

Experimental studies were carried out by us on 200 rats, which received drinking water with a different content of chlorides and were kept on a standard food ration.

The first series of experiments included six groups of rats, which received during two months, an intramuscular injection of 1 ml of a 0.5% solution of desoxycortiscosterone acetate (DOCA). For drinking, we

gave them solutions of sodium chloride with a Cl content in water of 0.5, 1, 2.5, and 10 g/l, and one group was given Na_2So_4 in a dose of 2.5 g/l. Control animals received tapwater.

We measured the arterial pressure of the animals weekly by means of a blood-free plethismographic method. Once a month, the Na and K content of the blood and urine was determined (by flame photometry), Cl of the blood (by the Bang method), Cl of the urine (according to Mohr), total blood cholesterol (according to Blewer), hemoglobin and erythrocytes (with an erythrohemometer), and 17-ketosteroids of the urine (according to Drekter). In the course of the entire test, the condition, water consumption and weight of the animals were observed.

The results of the first series of tests showed that, against a background of DOC introduction, drinking water with a Cl content of 0.5 and 1 g/l did not cause a rise in blood pressure. As can be seen from Fig. 1, a hypertensive effect under the influence of an excessive supply of sodium chloride and the introduction of DOCA is observed only in rats which received drinking water containing 2.5 and 10 g/l Cl. As compared to the initial background (65 $\pm$ and 2.8 and 68 $\pm$ 2.9 mm), the arterial pressure rose by 16 mm, thus indicating a combined pressor effect of DOCA and of the above concentrations of sodium chloride in drinking water.

Upon further periodical introduction of DOCA, the same rule was observed, with the same degree of expression; apparently, this can be explained by a significant decrease of the general tonus (state) of the animals by the end of the tests. During this period, great disturbances of the water-salt metabolism (lower potassium content of the organism polyuria, weight loss, etc.) were noted in the rats, which in some cases

resulted in their death.

In the determination of electrolytes (Na, K, Cl) in the blood of the animals after 2 months of consumption by them of water with sodium chloride in concentrations of 0.5 - 10 g/l and the introduction of DOCA, no significant changes, as compared to the initial background, were noted.

Later, an increase in the amount of Na and Cl in the blood was recorded, starting with a Cl concentration of 1 g/l and above, and a decrease in the amount of K at Cl concentrations of 2.5 and 10 g/l.

Beginning with the 3rd month of observation, an increased excretion of electrolytes was noted, which continued throughout the entire duration of the test (Table 1).

Our studies showed a certain increase in the total blood cholesterol content of the animals at a Cl concentration of 1 g/l and above during the second month of the test. However, later, the cholesterol content dropped significantly. During analysis of the blood, no significant and reliable changes whatsoever were noted in the content of hemoglobin and erythrocytes, as compared to the initial background, in any of the animal test groups.

An increased urinary excretion of 17-ketosteroids was recorded at the end of the third month when the Cl concentration was equal to 1 g/l and higher. The urinary excretion of 17-ketosteroids increased in proportion to the increase in the concentration of sodium chloride in the water.

The second series of tests was also carried out on six groups of rats, which received in the course of 12 months only salt solutions with the same salt concentrations. Control rats received Moscow tapwater for drinking.

It was found, that the introduction of sodium chloride in concentrations from 0.5 to 10 g/l during two months did not cause any kind of changes in the arterial pressure of the animals (see Fig. 1). When salt solutions were introduced during one year, rats which received a high concentration of sodium chloride in water (10 g/l exhibited a statistically reliable increase of the arterial pressure, from 67.3 $\pm$ 6.3 to 83.7 $\pm$ 1.4 mm). This indicates the significant role of a prolonged action on the organism of an excess amount of sodium chloride.

However, the introduction of DOCA during one month into rats which, during the 11 preceding months, had received for drinking solutions with a NaCl concentration of 2.5 and 10 g/l, was sufficient to cause a hypertensive effect. A statistically reliable rise of the arterial pressure by 22 - 25 mm was found in the animals.

Thus, experimental observations showed that a prolonged consumption of solutions with a high concentration of sodium chloride alone can cause a rise of the arterial pressure.

It should be noted that 20 animals were given, instead of NaCl solutions, solutions containing 2.5 g/l Na_2SO_4; in these rats, no rise of the arterial pressure was observed. This fact indicates that sodium is capable of causing a hypertensive effect only when combined with a chlorine ion.

In the study of electrolyte metabolism in chronic tests on animals which received only NaCl solutions, changes of the same nature were noted as those observed during the simultaneous introduction of salts and DOCA, but these changes occurred later. As in the first series of experiments, an increase of the Na and Cl content and a decrease of the K level in the blood

of animals was noted at a Cl of 1, 2.5 and 10 g/l. The average Na content in the blood during the entire test period was equal to 348 ± 3.3, 354 ± 2.8 and 345 ± 2.5 mg %, resp., at a Cl concentration of 1, 2.5 and 10 g/l, as compared to 329 ± 2.2 mg % in the controls. The average K content in the blood was 15.8 ± 0.35, 15.3 ± 0.24 and 15.4 ± 0.47 mg % (in the controls 17.5 ± 0.37 mg %). An increase in the excretion of Na and K in the urine (Table 2) was noted at Cl concentrations of 2.5 and 10 g/l and above. ($P \leq 0.05 - 0.001$).

The results of the studies indicate a retention of sodium and chlorine and a loss of potassium in the organism.

An increase in the content of chloresterol in the blood was noted by the end of the fourth month in rats, which had received water with Cl in concentrations of 1 g/l and higher ($P \leq 0.05 - 0.02$). Later, the content of cholesterol decreased. The content of erythrocytes and hemoglobin in the blood of animals which consumed water with various concentrations of sodium chloride remained within normal limits during the entire test period. The urinary excretion of 17-ketosteroids increased in comparison with the control group ($P \leq 0.001$).

It is well known that distrubances of the water-salt metabolism characterized by a Na accumulation within the cells, a loss of K by the cells, and an increase of the gradient between the intracellular content, cause an increase of the pressor effect on adrenaline, noradrenaline, and other hypertensive factors. (Aho et al., Oelsner and Skelton). Tests were carried out in which, after long-term (during one year consumption by the animals of drinking water with various concentrations of sodium chloride, their reaction towards adrenalin was determined. Observations were carried

out on 80 rats, which received a subcutaneous injection of 200 micrograms of a 0.1% solution of adrenaline.

For drinking, the various groups of rats received distilled water, water with a chloride content of 0.25, 0.5, 1, 2.5, and 10 g/1 and 2.5 g/1 Na_2SO_4. Moscow tap water served as a control. The blood pressure of the animals was measured every 30 minutes during 3 - 4 hours after the adrenaline loading.

The results of the functional adrenaline test are presented in Fig. 2. As can be seen from this figure, the introduction of adrenaline disclosed a tendency towards a rise of the blood pressure in rats, which consumed for a long time water containing 2.5 and 10 g/1 sodium chloride. In these animals, the blood pressure rose considerably during adrenaline loading. The maximum rise in arterial pressure after such a loading was noted after 1 1/2 - 2 hours. In rats which constantly consumed water with a lower concentration of sodium chloride (0.25, 0.5 and 1 g/1), the introduction of adrenaline did not cause any kind of regular change in blood pressure, as compared with animals of the control group. Thus, an increased hypertensive effect of adrenaline is noted only during chronic (long-term) introduction of large concentrations of sodium chloride in the drinking water (Cl' concentrations of 2.5 and 10 g/1.

A study of the role played by an excess supply of sodium chloride in drinking water in the production of experimental hypertension shows the following: a sodium chloride content in drinking water of up to 1 g/1 (as chlorine ion) contributes to the development of experimental hypertension and does not cause any kind of changes in electrolytic metabolism. At a concentration of sodium chloride in water 10 g/1, a rise in blood pressure is noted.

Prolonged (for one year) consumption by the animals of water with a chloride content at the level of 1 - 2.5 g/l changes in some factors (characteristics) of their water-salt metabolism.

The application of a functional adrenaline loading discloses a tendency to a rise of pressure in rats consuming water with a concentration of chlorides equal to 2.5 g/l.

Solutions containing 2.5 and 10 g/l sodium chloride, when these solutions are introduced simultaneously with DOCA, exert a hypertensive effect at the end of the second month of the test.

The results of the study of the effect of a different content of chlorides in drinking water on the condition of the vascular system confirm the safety of standardizing the content of chlorides in drinking water at a level of 350 mg/l (GOST 2874-54 "Drinking Water") on the basis of an organoleptic hazard symptom.

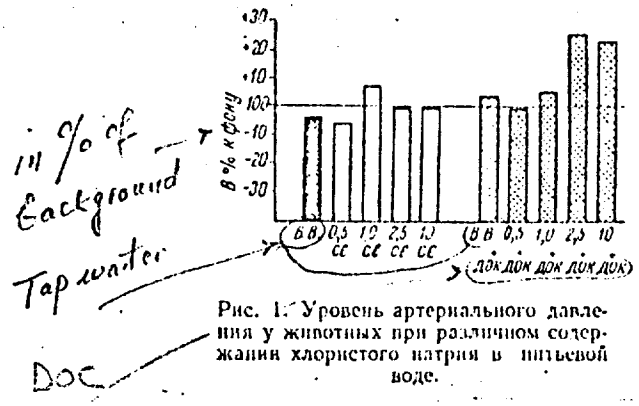

Рис. 1. Уровень артериального давления у животных при различном содержании хлористого натрия в питьевой воде.

Fig. 1 Level of arterial pressure in animals at a different content of sodium chloride in drinking water.

Таблица 1

Содержание Na, Cl и K в моче животных при разных концентрациях хлористого натрия в питьевой воде и введении ДОКА

(1) Исследуемая вода	(2) Выделение с мочой (в мг/сутки)		
	Na	Cl	K
	M±m		
(3) Водопроводная вода+ДОКА	8,4±0,2	72,1±3,5	8,5±0,1
(4) Cl′ в концентрации 0,5 г/л+ДОКА	9,0±0,2	85,9±3,7	8,8±0,1
(5) Cl′ » 1 г/л+ДОКА	15,2±0,8	138,7±6,8	8,7±0,1
(6) Cl′ » 2,5 г/л+ДОКА	16,6±1,2	165,0±10,6	13,2±0,3
(7) Cl′ » 10 г/л+ДОКА	32,9±2,8	235,4±19,0	14,0±0,5

Table 1 Content of Na, Cl and K in the urine of animals at different concentrations of sodium chloride in drinking water and introduction of DOCA.

1. Type of water tested
2. Excreted in urine (mg/24 hours)
3. Tap water + DOCA
4. Cl ion, in a concentration of 0.5 g/l + DOCA
5. Cl ion, in a concentration of 1 g/l + DOCA
6. Cl ion, in a concentration of 2.5 g/l + DOCA
7. Cl ion, in a concentration of 10 g/l + DOCA

Table 2

Таблица 2

Содержание Na, Cl и K в моче у животных при разном содержании хлористого натрия в питьевой воде

(1) Исследуемая вода	Выделение с мочой (в мг/сутки) (2)		
	Na	Cl	K
	M±m		
(3) Водопроводная	8,3±0,2	65,2±1,5	9,0±0,2
(4) Cl′ в концентрации 0,5 г/л	9,0±0,1	64,7±2,0	9,4±0,1
(5) Cl′ » 1 г/л	8,8±0,2	86,4±3,0	9,4±0,2
(6) Cl′ » 2,5 г/л	11,5±0,5	98,7±5,6	10,8±0,3
(7) Cl′ » 10 г/л	11,3±0,4	107,7±5,1	12,7±0,4

Table 2--Content of Na, Cl, and K in the urine of animals at a different content of sodium chloride in drinking water.

1. Type of water tested
2. Excreted in urine (mg/24 hours)
3. Tap water
4. Cl ion, in a concentration of 0.5 g/l
5. Cl ion, in a concentration of 1 g/l
6. Cl ion, in a concentration of 2.5 g/l
7. Cl ion, in a concentration of 10 g/l

Figure 2

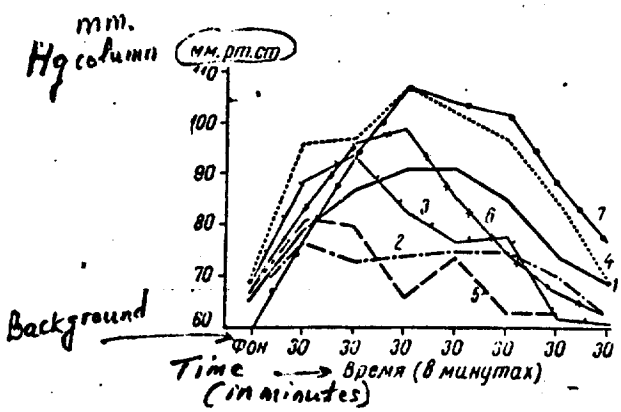

Fig. 2 Level of arterial pressure of experimental animals consuming highly mineralized chloride-containing water, against a background of a functional adrenaline load.

1. Tap water
2. Cl in a concentration of 0.25 g/l
3. Cl in a concentration of 1 g/l
4. Cl in a concentration of 10 g/l
5. Distilled water
6. Cl in a concentration of 0.5 g/l
7. Cl in a concentration of 2.5 g/l

Bibliography

Bogolyubov V. M. Pathogenesis and Clinical Water-electrolyte disorders, Leningrad 1968, p. 295 (in Russian); Brikker V. N. Disturbances of electrolyte metabolism in cardiovascular diseases, Leningrad, 1965, p. 169 (in Russian); Parin V. V. and Meerson F. Z. Outline of clinical physiology of blood circulation, Moscow, 1965, p. 325 (in Russian); Shenderov S. M. In book: Physiology of man and animals. Moscow, 1964., 70 (in Russian); Aho A. S., Linna N. I., Pekkarinck A., Acta pharmocol (Copenhagen), 1961, v. 18, p. 207; Oelsner T., Skelton F. R., Physiol 1961, p. 759; Raab. W. Cardiol, 1939, v. 4, p. 752.

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EFFECT OF DRINKING WATER WITH DIFFERENT CONTENT OF CHLORIDES ON EXPERIMENTAL ANIMALS

The investigations performed on experimental animals showed an excessive intake of sodium chloride to promote the development of a state of hypertension in the body. Sodium chloride content of drinking water exceeding the level of 2.5 g/l produced a distinct hypertensive effect. Drinking water containing chlorides at concentrations within the level of 1 g/l had no effect on the development of experimental hypertonia and caused no changes of water-salt metabolism in animals.

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Gigiena i Sanitariya, 1971, Vol. 36, no. 6, pp. 11-15

ВЛИЯНИЕ ПИТЬЕВОЙ ВОДЫ С РАЗЛИЧНЫМ СОДЕРЖАНИЕМ ХЛОРИДОВ НА ОРГАНИЗМ ЭКСПЕРИМЕНТАЛЬНЫХ ЖИВОТНЫХ

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В нашу задачу входило изучение роли избыточного поступления хлористого натрия с питьевой водой в развитии экспериментальной гипертонии.

Клинические и экспериментальные данные последних лет показывают важное значение электролитов в развитии некоторых сердечно-сосудистых заболеваний (В. В. Парин и Ф. З. Меерсон; В. М. Боголюбов; В. Н. Бриккер, и др.). Большое место во вторичных периферических механизмах развития гипертонической болезни отводится состоянию электролитного обмена и, в частности, обмену хлористого натрия. С задержкой в организме хлористого натрия связывают прессорный эффект минералокортикоидов (Raab; С. М. Шендеров, и др.). Указанные особенности использованы нами при выборе экспериментальной модели дезоксикортикостероидной гипертонии (ДОК-гипертония). Минералокортикоиды способствуют, как известно, задержке хлористого натрия в организме и повышению артериального давления.

Экспериментальные исследования проведены нами на 200 крысах, получавших питьевую воду с различным содержанием хлоридов и находив-

шихся на стандартном пищевом рационе. I серия опытов охватывала 6 групп крыс, которым в течение 2 месяцев внутримышечно вводили 1 *мл* 0,5% раствора дезоксикортикостерона-ацетата (ДОКА). Для питья им давали растворы хлористого натрия с содержанием Cl′ в воде 0,5, 1, 2,5 и 10 *г/л*, а одной группе — Na_2SO_4 в дозе 2,5 *г/л*. Контрольные животные получали водопроводную воду.

У животных еженедельно измеряли артериальное давление бескровным плетизмографическим способом. Раз в месяц в крови и моче определяли Na и K (методом пламенной фотометрии), Cl крови (методом Банга), Cl мочи (по Мору), общий холестерин крови (по Блюру), гемоглобин и эритроциты (эритрогемометром) и 17-кетостероиды мочи (по Дректеру). В течение всего эксперимента вели наблюдения за состоянием, водопотреблением и весом животных.

Результаты I серии опытов показали, что на фоне введения дезоксикортикостерона питьевые воды с содержанием Cl′, составляющим 0,5 и 1 *г/л*, не вызывала повышения кровяного давления. Как видно из рис. 1, гипертензивный эффект под влиянием избыточного поступления хлористого натрия и введения ДОКА отмечается лишь у крыс, которые получали для питья воду, содержавшую Cl′ в воде 2,5 и 10 *г/л*. По сравнению с исходным фоном (65±2,8 и 68±2,9 *мм*) артериальное давление поднялось на 16 *мм*, что свидетельствует о совместном прессорном действии ДОКА и указанных концентраций хлористого натрия в питьевой воде.

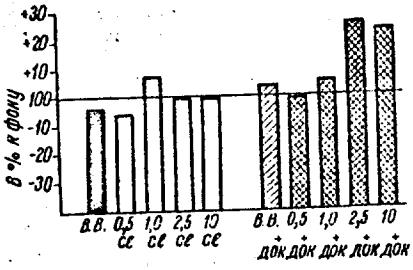

Рис. 1. Уровень артериального давления у животных при различном содержании хлористого натрия в питьевой воде.

При дальнейшем периодическом введении ДОКА выявляется та же закономерность, не менее выраженная, что, по-видимому, можно объяснить значительным понижением общего тонуса животных к концу опыта. У крыс в этот период обнаружены резкие нарушения водно-солевого обмена (обеднение организма калием, полиурия, потери в весе и т. д.), что приводило в ряде случаев к их гибели.

При определении электролитов (Na, K, Cl) в крови у животных после 2-месячного потребления ими воды с хлористым натрием в концентрации 0,5—10 *г/л* и введения ДОКА не выявлено сколько-нибудь существенных изменений по сравнению с исходным фоном.

В дальнейшем регистрируется повышение в крови количества Na и Cl, начиная с концентрации Cl′ 1 *г/л* и выше, и понижение количества K при концентрациях Cl′ 2,5 и 10 *г/л*.

С 3-го месяца наблюдений отмечено усиленное выведение электролитов, которое держалось в течение всего срока эксперимента (табл. 1).

Исследования показали некоторое повышение содержания общего холестерина крови у животных при концентрации Cl′ 1 *г/л* и выше на 2-м месяце опыта. Однако в дальнейшем содержание холестерина значительно понизилось. При анализе крови не обнаружилось каких-либо существенных и достоверных изменений содержания гемоглобина и эритроцитов по сравнению с исходным фоном ни в одной из экспериментальных групп животных.

Повышение выведения с мочой 17-кетостероидов зафиксировано к концу 3 месяца при концентрации Cl′ 1 *г/л* и выше. Выделение с мочой 17-кетостероидов возрастало соответственно повышению концентрации хлористого натрия в воде.

II серия опытов проведена также на 6 группах крыс, получавших в течение 12 месяцев только одни солевые растворы с теми же концентрациями

солей. Контрольным крысам для питья давали московскую водопроводную воду.

Обнаружено, что введение хлористого натрия в концентрации от 0,5 до 10 *г/л* в течение 2 месяцев не вызывает у животных каких-либо изменений артериального давления (см. рис. 1). При введении в течение года солевых растворов у крыс, получавших большую концентрацию NaCl с водой (10 *г/л*), найдено статистически достоверное повышение артериального давления (с 67,3±6,3 до 83,7±1,4 *мм*) что свидетельствует о значении длительного действия на организм избыточного количества хлористого натрия.

Таблица 1

Содержание Na, Cl и K в моче животных при разных концентрациях хлористого натрия в питьевой воде и введении ДОКА

Исследуемая вода	Выделение с мочой (в *мг/сутки*)		
	Na	Cl	K
	M±m		
Водопроводная вода+ДОКА	8,4±0,2	72,1±3,5	8,5±0,1
Cl′ в концентрации 0,5 *г/л*+ДОКА	9,0±0,2	85,9±3,7	8,8±0,1
Cl′ » 1 *г/л*+ДОКА	15,2±0,8	138,7±6,8	8,7±0,1
Cl′ » 2,5 *г/л*+ДОКА	16,6±1,2	165,0±10,6	13,2±0,3
Cl′ » 10 *г/л*+ДОКА	32,9±2,8	235,4±19,0	14,0±0,5

Однако введение ДОКА в течение 1-го месяца крысам, которые в течение 11 предшествующих месяцев получали для питья растворы с концентрацией NaCl 2,5 и 10 *г/л*, оказалось достаточным для гипертензивного эффекта. У животных установлено статистически достоверное повышение артериального давления на 22—25 *мм*.

Таким образом, экспериментальные наблюдения показали, что длительное потребление растворов с высокой концентрацией хлористого натрия само по себе способно вызвать повышение артериального давления.

Следует указать, что 20 животным вместо растворов хлористого натрия давали растворы с содержанием Na_2SO_4, равным 2,5 *г/л*. При этом у крыс не повышалось артериальное давление. Это свидетельствует о том, что натрий только в соединении с ионом хлора способен вызывать гипертензивный эффект.

При изучении электролитного обмена в хронических опытах на животных, получавших одни только растворы NaCl, обнаружены изменения такого же характера, что и при одновременном введении солей и ДОКА, но в более поздние сроки. Как и в I серии опытов, в крови у животных наблюдалось увеличение содержания Na и Cl и понижение уровня K при концентрации Cl′ 1, 2,5 и 10 *г/л*. Среднее содержание Na в крови за весь период исследования составляло соответственно 348±3,3, 354±2,8 и 345±2,5 *мг%* при концентрации Cl′ 1, 2,5 и 10 *г/л* по сравнению с 329±2,2 *мг%* в контроле. Содержание K в крови в среднем составляло 15,8±0,35, 15,3±0,24 и 15,4±0,47 *мг%* (в конт-

Таблица 2

Содержание Na, Cl и K в моче у животных при разном содержании хлористого натрия в питьевой воде

Исследуемая вода	Выделение с мочой (в *мг/сутки*)		
	Na	Cl	K
	M±m		
Водопроводная –	8,3±0,2	65,2±1,5	9,0±0,2
Cl′ в концентрации 0,5 *г/л*	9,0±0,1	64,7±2,0	9,4±0,1
Cl′ » 1 *г/л*	8,8±0,2	86,4±3,0	9,4±0,2
Cl′ » 2,5 *г/л*	11,5±0,5	98,7±5,6	10,8±0,3
Cl′ » 10 *г/л*	11,3±0,4	107,7±5,1	12,7±0,4

роле — 17,5±0,37 *мг*%). Увеличение выведения Na и K с мочой (табл. 2) отмечено при концентрациях Cl′ 2,5 и 10 *г/л* в питьевой воде, а увеличение выведения Cl′ — при концентрациях 1 *г/л* и выше ($P \leqslant 0{,}05$—0,001).

Результаты проведенных исследований указывают на задержку в организме натрия и хлора и потерю организма калия.

Повышение содержания холестерина крови отмечено к концу 4-го месяца у крыс, получавших воду с Cl′ в концентрации 1 *г/л* и выше ($P \leqslant 0{,}05$—0,02). В дальнейшем содержание холестерина понижалась. Содержание эритроцитов и гемоглобина в крови у животных, потребляющих воду с различной концентрацией хлористого натрия, оставалось в пределах нормы в течение всего периода исследований. Выведение с мочой 17-кетостероидов увеличилось по сравнению с контрольной группой животных ($P \leqslant 0{,}001$).

Известно, что нарушения водно-солевого обмена, характеризующиеся накоплением внутри клеток Na, потерей ими K и увеличением градиента между внутриклеточным содержанием, усиливают прессорный эффект на адреналин, норадреналин и другие гипертензивные факторы (Aho и соавт., Oelsner и Skelton). Были поставлены опыты, в которых после хронического (в течение года) потребления животными питьевой воды с различной концентрацией хлористого натрия определялась их реакция на адреналин. Наблюдения проведены на 80 крысах, которым подкожно вводили 200 *мкг* 0,1 % раствора адреналина. Для питья разные группы крыс получали дистиллированную воду, воду с содержанием хлоридов 0,25, 0,5, 1,-2,5 и 10 *г/л* и Na_2SO_4 2,5 *г/л*. В качестве контроля служила московская водопроводная вода. После нагрузки адреналином в течение 3—4 часов каждые 30 мин. у животных измеряли кровяное давление.

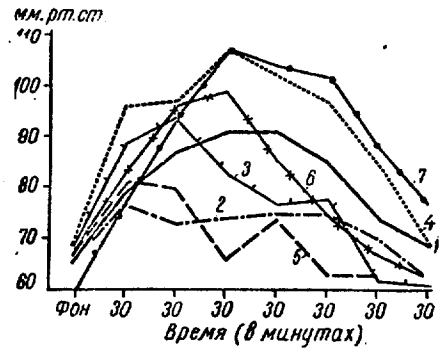

Рис. 2. Уровень артериального давления у экспериментальных животных, потребляющих высокоминерализованные хлоридные воды, на фоне функциональной нагрузки адреналином.

1 — водородная вода; *2* — Cl в концентрации 0,25 *г/л*; *3* — Cl в концентрации 1 *г/л*; *4* — Cl в концентрации 10 *г/л*; *5* — дистиллированная вода; *6* — Cl в концентрации 0,5 *г/л*; *7* — Cl в концентрации 2,5 *г/л*.

Результаты функциональной адреналовой пробы представлены на рис. 2. Как видно из рис. 2, введение адреналина позволило выявить у крыс, длительно потребляющих воду с содержанием хлористого натрия, равным 2,5 и 10 *г/л*, склонность к повышению кровяного давления. У этих животных кровяное давление значительно повысилось при адреналовой нагрузке. Максимальный подъем артериального давления после такой нагрузки отмечен через $1^1/_2$—2 часа. У крыс, постоянно потребляющих воду с меньшими концентрациями хлористого натрия (0,25, 0,5 и 1 *г/л*), введение адреналина не вызывало каких-либо закономерных изменений кровяного давления по сравнению с животными контрольной группы. Таким образом, усиление гипертензивного действия адреналина отмечено лишь при хроническом введении больших концентраций хлористого натрия с питьевой водой (Cl′ с концентрацией 2,5 и 10 *г/л*).

Изучение роли избыточного поступления хлористого натрия с питьевой водой в возникновении экспериментальной гипертонии показало следующее. Содержание хлористого натрия в питьевой воде до 1 *г/л* (по хлориону) способствует развитию экспериментальной гипертонии и не вызывает у животных каких-либо изменений электролитного обмена. При концентрации хлористого натрия в воде, равной 10 *г/л*, отмечается повышение кровяного давления.

Длительное, в течение года, потребление животными воды с содержанием хлоридов на уровне 1—2,5 *г/л* вызывает у них изменения некоторых показателей водно-солевого обмена.

Применение функциональной адреналовой нагрузки выявляет склонность к повышению давления у крыс, потребляющих воду с концентрацией хлоридов, равной 2,5 *г/л*.

Растворы хлористого натрия 2,5 и 10 *г/л* при введении их одновременно с дезоксикортикостероном вызывают гипертензивный эффект в конце 2-го месяца эксперимента.

Результаты изучения влияния различного содержания хлоридов в питьевой воде на состояние сосудистой системы подтверждает безопасность нормирования хлоридов в питьевой воде на уровне 350 *мг/л* (ГОСТ 2874-54 «Питьевая вода») по органолептическому признаку вредности.

ЛИТЕРАТУРА

Боголюбов В. М. Патогенез и клиника водно-электролитных расстройств. Л., 1968, с. 295. — Бриккер В. Н. Нарушение электролитного обмена при сердечно-сосудистых заболеваниях. Л., 1965, с. 169. — Парин В. В., Меерсон Ф. З. Очерки клинической физиологии кровообращения. М.,1965, с. 325. — Шендеров С. М. В кн.: Физиология человека и животных. М., 1964, с. 70. — Aho A. S., Linna N. I., Pekkarinck A., Acta pharmacol. (Kbh.), 1961, v. 18, p. 207. — Oelsner T., Skelton F. R., An. J. Physiol., 1961, p. 759. — Raab W., Am. J. Cardiol., 1939, v. 4, p. 752.

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EFFECT OF DRINKING WATER WITH DIFFERENT CONTENT OF CHLORIDES ON EXPERIMENTAL ANIMALS

V. K. Fadeeva

The investigations performed on experimental animals showed an excessive intake of sodium chloride to promote the development of a state of hypertension in the body. Sodium chloride content of drinking water exceeding the level of 2.5 g/l produced a distinct hypertensive effect. Drinking water containing chlorides at concentrations within the level of 1 g/l had no effect on the development of experimental hypertonia and caused no changes of water-salt metabolism in animals.

Faith, W.L., D.B. Keyes and R.L. Clark. 1965

Industrial Chemicals, 3rd edition

John Wiley & Sons, Inc., N.Y., N.Y.
pp. 635-641; 676-684