VOLUME 2

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SODIUM CHLORIDE, POTASSIUM CHLORIDE

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Edited by the Secretary

The Fatal Dose of Various Substances on Intravenous Injection in the Guinea Pig. S. AMBERG AND H. F. HELMHOLZ. From the Otho S. A. Sprague Memorial Institute, Chicago, Ill.

On intravenous injection at the rate of 1 cc. per minute the fatal dose of a 30 per cent urea solution in guinea pigs of about 250 grams is 4 cc, while the animals recover from a dose of 3.33 cc. The fatal dose of a 1 per cent ammonium chloride solution in guinea pigs of about 230 grams is 5 to 6 cc. that of a 2 per cent solution in animals of 210 to 230 grams is 2 cc.; that of a 3 per cent solution in animals of about 200 grams, 1.5 cc. The fatal dose of some organic ammonium salts as ammonium acetate, ammonium butyrate and ammonium β oxybutyrate in solutions corresponding to a 2 per cent ammonium chloride solution is about the same as that of 2 per cent ammonium chloride solution, that is, the toxicity of ammonium salts tested under the conditions of the experiment is dependent on the ammonium they contained. The fatal dose of a 10 per cent sodium chloride solution in guinea pigs of about 240 grams lies between 6 and 8 cc. On intravenous injection of these strong salts solution the lenses become cloudy and a precipitate is formed. In cases of recovery these manifestations disappear. The fatal dose of a 1 per cent potassium chloride solution in guinea pigs of about 200 grams is 1.5 to 2 cc., that of a 2 per cent solution in animals of 260 grams is about 1 cc. On injections of mixtures containing potassium chloride and sodium chloride, potassium chloride and sodium sulphate, potassium sulphate and sodium chloride, the animals tolerate decidedly more potassium chloride or potassium sulphate than without the addition of these sodium salts. It is possible that at least a part of the detoxifying action of the sodium salts is due either to the depression of ionization by a common ion as in the case of potassium chloride and sodium chloride mixtures, or to more complicated chemical changes taking part in solutions containing for instance potassium chloride and sodium sulphate.

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The Several Factors Involved in the Standardization of Pituitary Extracts. GEORGE B. ROTH. From the Laboratory of Pharmacology, Hygienic Laboratory, U. S. Public Health Service.

The animal employed as a test animal is a prime factor in either the bloodpressure or isolated-uterus method. Dogs are best suited for the bloodpressure method while virgin guinea pigs are best suited for the isolated-uterus method. The isolated-uterus method is applicable to all preparations whereas the bloodpressure method is inadequate. In the isolated-uterus method virgin guinea pigs weighing between 275 and 350 grams should be used. They should be killed without preliminary anesthesia, since the normal contractions are slow in appearing when an anesthetic such as chloroform is used. The examination of six commercial preparations showed no distinct parallelism in activity when examined by the bloodpressure and isolated-uterus methods. The character of the bloodpressure tracing caused by the several preparations varied greatly. A marked difference in activity was found to exist in commercial pituitary extracts when examined by either method.

The Effects of Chelidonin on Surviving Organs. PAUL J. HANZLIK. From the Pharmacological Institute, University of Vienna.

Chelidonin is the alkaloid of *Chelidoneum majus* which belongs to the Papaveraceae. Its empirical formula as given by Schmidt[1] is $C_{20}H_{19}NO_{5}$, and the most soluble salts are the phosphate and sulphate. In 1892, Meyer[2] studied the general or systemic effects of chelidonin and found them to be closely similar to those of morphin with the important difference that chelidonin produces practically no subsequent irritation of the central nervous system. As yet, however, no studies have been made upon surviving visceral organs.

The present study was undertaken with the idea of ascertaining the effect of chelidonin upon surviving organs and those functions in which the presence of smooth muscle plays an important rôle. A preliminary report of the results obtained is here briefly presented.

1. Chelidonin promptly abolishes the spontaneous contractions of the following excised organs: oesophagus, fundus and pylorus of the frog's stomach, intestine of cat and rabbit, and pregnant uterus of guinea pig.

2. Chelidonin removes the effects of pilocarpin, pituitrin, histamin and barium chloride upon surviving organs.

3. The peripheral blood vessels of the frog previously contracted by epinephrin are more rapidly dilated by chelidonin than by Ringer's solution alone. The effects upon the untreated vessels are doubtful.

4. The constriction of the bronchial musculature by histamin in the surviving lungs of the guinea pig is removed by chelidonin. Broncho-

[1] Schmidt, E.: Mittl. aus d. pharmaceut. Inst., Univ. Marburg, 1888, v, 15.

[2] Meyer, H. H.: Arch. exp. Path. u. Pharm., 1892, xxix, 397.

Acta Pædiat Scand 61: 670–676, 1972

RENAL RESPONSE TO AN ORAL SODIUM LOAD IN NEWBORN FULL TERM INFANTS

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In the newborn infant renal function is reported to be restricted with regard to filtration rate (16, 22), concentrating (10), and acidifying capacity (11). Knowledge about the renal control of sodium excretion in the infant kidney is, however, limited.

The present study is an attempt to assay the ability of the newborn full term infant to excrete sodium following a standardized oral load of sodium chloride. As a reference the renal effect of a quantitatively corresponding load to older children has been used (2, 5).

MATERIAL

Thirty-one healthy infants have been studied. The age distribution is shown in Fig. 1. The gestational age ranged between 38–42 weeks according to case history, weight and length and the result of neurological evaluation of the maturity. Twenty-seven of the infants had a normal delivery, 2 were delivered with vacuum extraction and 2 with caesarean section. None of the children had Apgar score below 7 at one minute after birth, all of them had 10 after five minutes. All infants had been fed with breast milk or formula.[1] The studies were carried out in the nursery ward. The body temperature did not change and the babies were disturbed as little as possible during the course of the study. They showed no signs of discomfort. No case of diarrhoea was encountered. One baby who vomited during the study was excluded. Informed maternal consent was given in all cases studied.

This work has been supported by grants from Semper Research Foundation and Karolinska institutet.

[1] Allomin (Semper) sodium content 6.5 mEq/l, or Milkotal (Findus) sodium content 8.7 mEq/l.

EXPERIMENTAL

In order to ensure a good and relatively constant diuresis all studies were carried out during standardized fluid expansion. The infants were fed by stomach tube. During the entire course of study they were given a standardized composition of diluted formula (Allomin, diluted 1 : 3). The diluted formula was calculated so no baby should receive less than 60 kcal/kg body weight and day during the study. The first hour of the study the infants were given the diluted formula in an amount corresponding to 2% of the body weight, followed every 30 min by an amount corresponding to 0.5% of the body weight. Urine was obtained by spontaneous voidings at approximately hourly intervals. A high and relatively stable diuresis was achieved after about 90 min.

The infants were divided into the following three groups:

Group I (20 babies). After the collection of at least one urine sample an oral sodium load, consisting of 95 mEq Na^+ per 1.73 m^2 body surface (0.12 g sodium chloride per kg body weight), was given. The sodium chloride was dissolved in the diluted formula, yielding a 1% saline solution. This modified formula was administered during a 30 min period.

Group II (5 babies). Same as group I, except that 190 mEq Na^+ per 1.73 m^2 was given. The sodium chloride was dissolved as described above, yielding a 2% saline solution.

Group III (6 babies). Same as group I, except that no extra sodium chloride was added to the formula.

After the administration of the sodium chloride load urine was collected for 5–8 hours.

In 5 babies from Group I the glomerular filtration rate was determined by analyzing the disappearance rate of a single intravenous dose of inulin. Following the injection, capillary blood was obtained every 5–10 min for 80 min. Inulin (Laevasar-Gesellschaft), in a 10% solution, was given in an amount of 0.75 ml per kg body weight. The values of GFR were calculated from the formula given by Sapirstein (18):

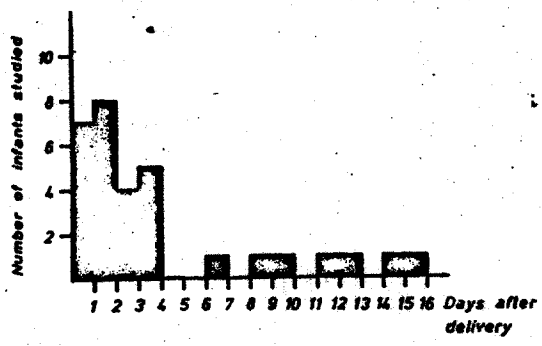

Fig. 1. Age distribution of the infants studied.

$$GFR = I \times \gamma_A \times \gamma_B / A \times \gamma_B + B \cdot \gamma_A$$

(I = the amount of inulin injected)

A typical disappearance curve in a representative infant is shown in Fig. 2.

ANALYTICAL METHODS

The sodium concentration in serum and urine was analyzed by a flame photometer (Eppendorf). Inulin in blood was determined according to Heyrovsky (12). Osmolality in blood and urine was determined cryoscopically with the aid of a Knauer osmometer. Hematocrit in capillary blood samples was estimated in glass capillaries which were centrifuged at 10 000 RPM for 5 min.

RESULTS

Urine sodium excretion

Fig. 3 demonstrates the hourly accumulated sodium excretion in a representative study of

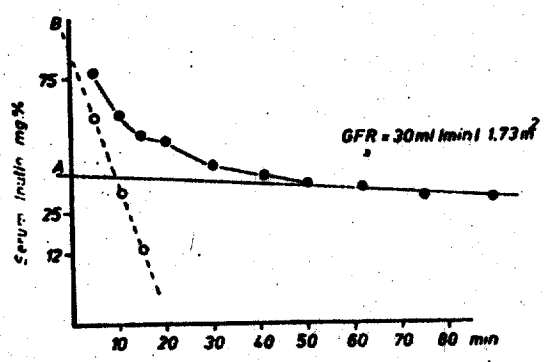

Fig. 2. Plasma disappearance curve of inulin following an intravenous injection of a 10% inulin solution. Filled circles represent actual plasma values, unfilled circles constructed line representing actual disappearance rate from blood compartment. The scale is in log line.

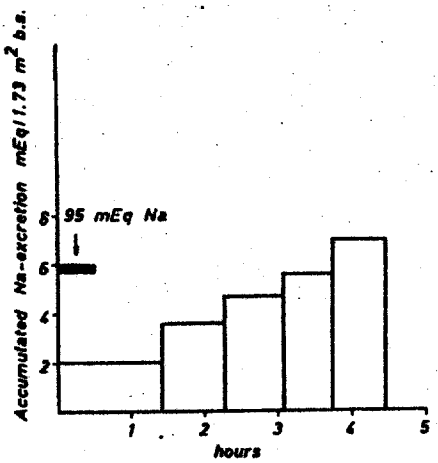

Fig. 3. Accumulated Na^+ excretion in a 6-days-old infant after the administration of a 1% saline solution.

an infant from Group I. The hourly sodium excretion seemed to be fairly constant from 1 to 5 hours after the sodium load, and no peak excretion was observed. It therefore seemed justified to calculate the average hourly sodium excretion from the total excretion between 1 and 5 hours after the load had been given.

The mean values of the hourly sodium excretion following a load with water or saline solution (1 and 2%) are given in Table 1. As a reference, the table also includes the average hourly sodium excretion following a corresponding salt load in children aged 8 to 14 years (5). The urinary sodium excretion was

Table 1. *Sodium excretion mEq per hour per 1.73 m² body surface following an oral load of water or saline chloride solution*

The average hourly urine Na^+ excretion ± one S.D. in infants during high fluid intake with and without an oral salt load. The urinary Na^+ excretion following an oral salt load in older children is given as a reference

Load	High fluid intake only	High fluid intake + 95 mEq Na^+	High fluid intake + 190 mEq Na^+
Infants			
1–15 days	0.53 ± 0.09	1.51 ± 0.28	1.11 ± 0.52
Children			
8–14 years	6.0 ± 2.1	16.0 ± 1.8	25.7 ± 3.5

higher after a salt load than when only water had been given. However, since the sodium excretion did not differ significantly after a load of one or two per cent saline solution, the ability to excrete sodium does not seem to be dose dependent. The natriuretic response to a salt load in infants was significantly lower than it was in older children following the corresponding load. The average hourly urinary excretion of sodium, which was found in infants 1–15 days after birth, is seen in Fig. 4. No increase of the ability to excrete an oral sodium load was noticed. As seen in Fig. 5 there is a significant inverse correlation between the sodium excretion and the hematocrit ($p < 0.001$, correlation coefficient -0.778).

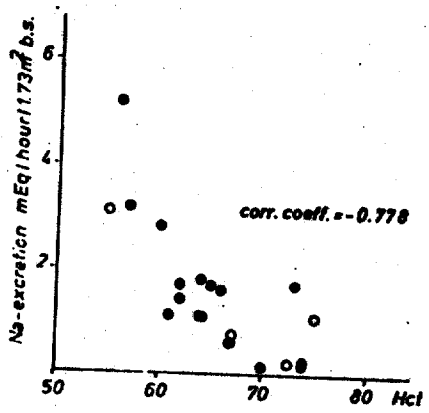

Fig. 5. The relationship between the hematocrit and the average hourly Na^+ excretion following a 1% (filled circles) or a 2% (unfilled circles) saline load. The correlation coefficient, -0.778, was statistically significant.

Glomerular filtration rate

Glomerular filtration rate (GFR) was determined during the course of the oral sodium chloride load in 5 infants. As is seen from Table 2 the values of GFR ranged between 20–34 ml per minute per 1.73 m². No correlation between sodium excretion and glomerular filtration rate was observed.

Urine dilution

Table 2 also includes the average values for diuresis, urine osmolality and sodium clearance during the course of the study.

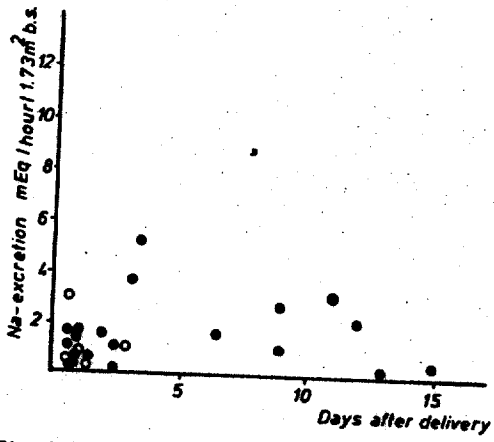

Fig. 4. The average hourly Na^+ excretion following the administration of a 1% (filled circles) or a 2% (unfilled circles) saline load to infants aged 1–15 days.

From the osmolality determination of the hourly sampled urine osmolar clearance (C_{osm}) and free water clearance (C_{H_2O}) can be calculated using the formula:

$$C_{osm} = (U_{osm} \times V)/B_{osm}; C_{H_2O} = V - C_{osm}$$

where V_{osm} and B_{osm} stand for urine and blood osmolality respectively and V stands for the diuresis. Likewise, the sodium clearance can be calculated using the sodium concentration in the hourly sampled urine. The sum of free water clearance and sodium clearance is generally assumed to be an index of sodium delivered to the distal tubule. Free water clearance as such is generally used as an index of distal tubular sodium reabsorption (20, 3). If free water clearance is plotted against the sum of free water clearance and sodium clearance a characteristic relationship is generally found, suggesting that distal tubular sodium reabsorption can be increased as a function of distal tubular sodium availability.

Fig. 6 demonstrates this relationship in the 5 infants included in Table 2 as compared with that found in healthy young adults. It is apparent from the figure that free water clearance in infants is on the upper limit or higher than the free water clearance found in healthy young adults at each level of the sum of free water clearance and sodium clearance. This

Table 2. *Glomerular filtration rate, urinary sodium excretion and urine osmolality in 5 infants following a 1% saline load*

Sex	Age (days)	GFR (ml/min/ 1.73 m²)	Na-excretion (mEq/hour/ 1.73 m²)	Average diuresis[a] (ml/min)	Average urine osmolality[a]	Average Na[a] clearance (ml/min)
Male	3	31	2.8	0.75	61	0.056
Female	9	20	1.1	0.24	70	0.016
Male	11	24	3.2	1.07	43	0.054
Male	13	34	0.3	0.30	55	0.005
Male	15	20	0.5	0.52	47	0.003

[a] Average value from 2–6 urine collection periods.

suggests a more complete reabsorption of sodium in the distal tubule of the infant than in the distal tubule of the adult.

DISCUSSION

The extremely low sodium concentration in human milk, 7 mEq/l, may suggest a reduced ability of the infant kidney to excrete sodium. The finding of an extremely low sodium elimination rate following an acute saline load was therefore not surprising. The finding is also in agreement with the results from a study in piglets on the effect of an oral sodium chloride load (15). The piglets developed edema if they were fed with 0.5% saline solution instead of corresponding amounts of sow's milk. It is well established that the glomerular filtration rate of the newborn infant is low (25–35 ml per minute per 1.73 m² body surface) (16, 22). The infants in the present study had a filtration rate between 20 and 34 ml per minute per 1.73 m² body surface. It has recently been shown in this laboratory that the ability to excrete an oral sodium load is restricted in older children with low filtration rate secondary to renal disease (2). The restriction of the sodium elimination rate is in fact directly related to the glomerular filtration rate. Thus, the sodium elimination rate in those children with glomerular filtration rate between 20–35 ml per minute per 1.73 m² body surface ranges between 6–10 mEq per hour per 1.73 m² body surface. In the infants, however, the sodium elimination rate was 0.1 to 3 mEq per hour per 1.73 m² body surface. Thus, in the infant kidney the reduction of the sodium elimination rate is out of proportion to the reduction of the glomerular filtration rate and must be attributed to a specific inability of the tubules to reject sodium adaptively.

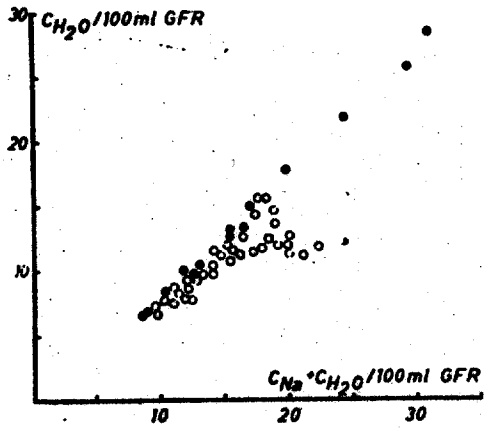

Fig. 6. The relationship between the sum of sodium and free water clearance ("distal tubular Na⁺ delivery") and free water clearance ("distal tubular Na⁺ reabsorption"). Filled circles represent values obtained in 5 infants, unfilled circles values obtained in 14 healthy young adults (3).

Since extracellular volume expansion has a natriuretic effect, which is independent of the glomerular filtration rate (9), the control of tubular sodium reabsorption has been subjected to extensive investigations. During the past years the following factors have all been attributed some importance for the control of tubular sodium reabsorption.

1. Mineral corticoid steroid hormone. This factor can hardly be responsible for the sodium retention observed in infants since aldosteron secretion rate has been shown to be low during the first week of life (21).

2. Physical forces. It is well established that intrarenal physical forces, such as peritubular capillary and interstitial hydrostatic and oncotic pressure influence tubular sodium reabsorption (4, 6, 14). Polyglobulia is a condition in which physical forces have been shown to enhance tubular sodium reabsorption (7, 19). The mechanisms behind this effect is thought to be as follows: high hematocrit results in increased filtration fraction; increased filtration fraction results in increased peritubular capillary oncotic pressure, a situation known to enhance tubular sodium reabsorption. The hematocrit of newborn infants is high. In the present study a significant correlation was found between hematocrit and tubular sodium reabsorption, suggesting that physical forces are at least partially responsible for the enhancement of tubular sodium reabsorption in newborns. It should be pointed out, however, that the effect of the hematocrit on tubular sodium reabsorption in infants is probably less pronounced than in the experimental conditions with polyglobulia, since the plasma oncotic pressure as such is low in infants. In addition the enhancement of distal tubular sodium reabsorption suggested by the analyses of free water clearance cannot be attributed to the effect of physical forces, since so far micropuncture studies have failed to reveal any important effect of this factor on the distal tubule (1).

3. Intrarenal blood flow distribution. It has been claimed that intrarenal blood flow distribution to a certain extent will influence the overall glomerular tubular balance for sodium (17). It is generally agreed that the juxtamedullary nephrons have a higher capacity to reabsorb sodium than the cortical nephrons. If a relatively high proportion of the renal blood flow would perfuse the juxtamedullary nephrons a relatively large proportion of the filtered load of sodium would be reabsorbed. That this mechanism might be responsible for the enhancement of the overall sodium reabsorption in small infants is suggested by a recent study in newborn puppies (13). Using ^{133}Xenon wash out—as well as microsphere technique—it was clearly demonstrated that during the first weeks of life cortical blood flow is low in relationship to total blood flow and will increase markedly after an age of 10–12 weeks.

4. A natriuretic hormone. Presently there are no conclusive evidence of the existence of such a hormone (23).

To sum up: The results from the present study do not reveal the exact mechanism of the relative inability of kidney tubules of newborn infants to reject the reabsorption of sodium adaptively. It is likely that changes in physical forces brought about by the high hematocrit are at least partially responsible for the enhancement of tubular sodium reabsorption. A relative increase in the proportion of renal blood flow perfusing the juxtamedullary nephrons might, however, also contribute to the increased fractional sodium reabsorption during the first weeks of life.

The present study has clearly shown that the infant kidney, as compared to the mature kidney, tends to retain sodium. This suggests a reduced tolerance of the newborn baby to salt administration. The most acutely riskful clinical situation is undoubtedly presented by the seriously acidotic baby to whom repeated doses of sodium bicarbonate is administered. Previous observations have demonstrated that older children can excrete at most 20% of the filtered load in situations of acute salt overloading (unpublished observations in this laboratory). During the first weeks after birth the elimination rate of an acute sodium load was approximately 25% of that found in older children with corresponding glomerular filtration rates. Thus, the newborn infant should, in the situation of a high salt load, only be able to excrete about 5% of the filtered load of sodium. Assuming a glomerular filtration rate

of 20–35 ml per minute per 1.73 m² body surface area at a blood sodium level of about 140 mEq per liter the maximal sodium excretion can be calculated to be about 12 mEq per kg body weight per 24 hours. It may also be pointed out that water is always passively reabsorbed with sodium, at least in the proximal tubule. Thus, enhanced sodium reabsorption is not necessarily associated with increased serum sodium concentration, but may result in an almost iso-osmotic fluid retention of varying degree; most seriously manifested by the development of edema. Another clinical aspect, so far only open to speculation, is the possibility that a moderately positive sodium balance during early infancy might result in future arterial hypertension (8).

SUMMARY

Urinary elimination rate of a standardized oral sodium load has been studied in 31 full-term healthy infants aged 1–15 days.

The elimination rate of sodium was found to be low in newborn infants as compared to what has been observed in older children. The reduction was out of proportion to the restriction of the glomerular filtration rate.

Various possible explanations for the reduced ability of the immature renal tubule to reject sodium adaptively, such as the high hematocrit, and a different distribution of the intrarenal blood flow have been discussed.

The apparent implication of the finding is a low tolerance to sodium in the newborn baby. The maximal tolerated daily dose of sodium has been calculated to be about 12 mEq per kg body weight.

REFERENCES

1. Anagnostopoulos, T., Kinney, M. J. & Windhager, E. E.: Salt and water reabsorption by short loops of Henle during renal vein constriction. *Amer J Physiol, 220:* 1060, 1971.
2. Aperia, A., Berg, U. & Broberger, O.: Control of sodium homeostasis in children with recurrent urinary tract infections and reduced glomerular filtration rates. *Acta Paediat Scand, 60:* 695, 1971.
3. Aperia, A., Broberger, O. & Feychting, H.: Effect of hypotonic mannitol and saline load on diluting capacity in man. *Acta Physiol Scand, 80:* 145, 1970.
4. Aperia, A., Broberger, O. & Söderlund, S.: Relationship between renal artery perfusion pressure and tubular sodium reabsorption. *Amer J Physiol, 220:* 1205, 1971.
5. Berg, U.: Urine elimination of an oral salt and fluid load in healthy children. *Acta Paediat Scand.* Submitted for publication.
6. Brenner, B. M., Falchuk, K. H., Keimowitz, R. I. & Berliner, R. W.: The relationship between peritubular capillary protein concentration and fluid reabsorption by the renal proximal tubule. *J Clin Invest, 48:* 1519, 1969.
7. Burke, T. J., Robinson, R. R. & Clapp, J. R.: Effect of arterial hematocrit on sodium reabsorption by the proximal tubule. *Amer J Physiol, 220:* 1536, 1971.
8. Dahl, L. K., Heine, M. & Tassinari, L.: Effects of chronic excess salt ingestion. Evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J Exp Med, 115:* 1173, 1962.
9. de Wardener, H. E., Mills, I. H., Clapham, W. F. & 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, 1961.
10. Edelmann, C. M., Jr., Barnett, H. L. & Troupkau, V.: Renal concentrating mechanism in newborn infants. Effect of dietary protein and water content, role of urea and responsiveness to antidiuretic hormone. *J Clin Invest, 39:* 1062, 1960.
11. Edelmann, C. M., Soriano, J. R., Boichis, H., Gruskin, A. B. & Acosta, M. I.: Renal bicarbonate reabsorption and hydrogen ion excretion in normal infants. *J Clin Invest, 46:* 1309, 1967.
12. Heyrovsky, A.: A new method for the determination of inulin in plasma and urine. *Clin Chim Acta, 1:* 470, 1956.
13. Jose, P. A., Logan, A. G., Slotkoff, L. M., Lilienfield, L. S., Calcagno, P. L. & Fisner, G. M.: Intrarenal blood flow distribution in canine puppies. *Pediat Res, 5:* 335, 1971.
14. Lewy, J. F. & Windhager, E. E.: Peritubular control of proximal tubular fluid reabsorption in the rat kidney. *Amer J Physiol, 214:* 943, 1968.
15. McCance, R. A. & Widdowson, E. M.: Hypertonic expansion of the extracellular fluids. *Acta Paediat Scand, 46:* 337, 1957.
16. Oh, W., Oh, M. A. & Lind, J.: Renal function and blood volume in newborn infant related to placental transfusion. *Acta Paediat Scand, 56:* 197, 1966.
17. Pomeranz, B. H., Birtch, A. G. & Barger, C.: Neural control of intrarenal blood flow. *Amer J Physiol, 215:* 1067, 1968.
18. Sapirstein, L. A., Vidt, D. G., Mandel, M. J. & Hanusek, G.: Volumes of distribution and clearances of intravenously injected creatinine in the dog. *Amer J Physiol, 181:* 330, 1955.

19. Schrier, R. W. & Earley, L. E.: Effects of hematocrit on renal hemodynamics and sodium excretion in hydropenic and volume expanded dogs. *J Clin Invest, 49:* 1656, 1970.
20. Stein, R. M., Abramson, R. G., Kahn, T. & Levitt, M. F.: Effects of hypotonic saline loading in hydrated dogs: Evidence for a saline-induced limit on distal tubular sodium transport. *J Clin Invest, 46:* 1205, 1967.
21. Weldon, V. V., Kowarski, A. & Migeon, C. J.: Aldosterone secretion rates in normal subjects from infancy to adulthood. *Pediatrics, 39:* 713, 1967.
22. Vesterdal, J. & Tudvad, F.: Studies on the kidney function in premature and full term infants by estimation of inulin and paraamino hippurate clearance. *Acta Paediat Scand, 37:* 429, 1949.
23. Wright, F. S., Brenner, B. M., Bennet, C. M., Keimowitz, R. I., Berlinger, R. W., Schrier, R. W., Verroust, P. J., de Wardener, H. E. & Hollgreve, H.: Failure to demonstrate a hormonal inhibitor of proximal sodium reabsorption. *J Clin Invest, 48:* 1107, 1969.

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tinued his diet for two weeks and had a very severe attack June 14, in which he lost consciousness, and has not felt well since. He is back on the diet. In August he felt well. There had been no attacks. In January 1937 he was feeling well. He had had two brief attacks in spite of a full regimen. He had been taking potassium chloride also.

Case 11.—A man, aged 59, seen Dec. 19, 1935, had had four attacks of tinnitus, vomiting and vertigo during the past year. The last one was December 4. There was nerve deafness. He was given the regimen. Jan. 23, 1936, there had been no attacks but a little dizziness at night. May 26 he had had no attacks and had lost 10 pounds (4.5 Kg.).

Case 12.—A man, aged 49, seen May 7, 1936, complained that two years before he had had deafness and tinnitus of the right ear. Eleven days before examination there was a sudden attack of vertigo to the right with no vomiting. Tinnitus ceased two years before. Lying on the right side would bring on an attack. Nerve deafness was present. The regimen was started. May 21 there had been no severe attacks. He could now lie on the right side. There was some tinnitus of the left ear. June 30 there had been no attacks. He had lost 16 pounds (7.3 Kg.) and felt that pills were hard on the stomach. Ammonium chloride was reduced to three 5 grain (0.3 Gm.) pills with each meal. July 21 he reported one attack of vertigo, after which he returned to the full dose of ammonium chloride. August 26 he felt well. There had been no attacks.

SUMMARY

Severe attacks of vertigo and vomiting in twelve patients suffering from deafness and tinnitus have ceased under the medical treatment described.

412 Beacon Street.

PROTEIN, SALT AND FLUID CONSUMPTION OF 1,000 RESIDENTS OF NEW YORK

9076

BENJAMIN I. ASHE, M.D.
AND
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Various workers in nutrition have stated that a daily protein intake of from 75 to 100 Gm., a daily salt intake of from 2 to 4 Gm.[1] and a fluid intake of from 1,000 to 2,000 cc. are suitable or optimal components of an adequate diet. In the United States particularly it has been believed that most people eat from 75 to 100 Gm. of protein daily.[2] Von Voit[3] suggested 118 Gm. of protein as a desirable daily intake. McLester[4] favors 100 Gm. daily for stamina and vigor. Studies on the twenty-four hour urines which yielded information for the urea, salt and fluid excretion have been made since 1920 on normal individuals who appeared for health examination. These studies provide material for an analysis of the habitual diet of residents of New York City. The study comprises 1,000 such individuals, normal so far as they knew, who came for a "check up" because of their interest in a "health examination," or because a relative had been found to have diabetes or hypertension or for some such reason. From this study were omitted all cases that presented diarrhea, edema or any illness other than a hypertension first made known as a result of this examination. While the subject was on his usual diet there was obtained in each case (1) the volume of urine in twenty-four hours, (2) the sodium chloride excretion, (3) the urea excretion, (4) the hemoglobin and red blood cell count, (5) the blood pressure, (6) the blood urea nitrogen, (7) the blood uric acid, (8) chemical and microscopic analysis of the urine, and in most cases (9) the blood sugar, (10) the blood cholesterol and (11) the blood chlorides.

The data are presented as follows:

1. The protein intake of group A (563 normal individuals), group B (298 individuals who, although they did not know it before this study, showed an elevation of blood pressure over 145 systolic, 90 diastolic in younger people and over 150 systolic, 96 to 100 diastolic in older people), and group C (139 individuals who showed for the first time to their knowledge not only an elevation of blood pressure but also a blood urea nitrogen of from 20 to 40 mg. per hundred cubic centimeters).
2. The salt intake of groups A, B and C.
3. The fluid intake of groups A, B and C.

METHOD

The determination of the amount of protein, salt and fluid intake was based on the nitrogen, sodium chloride and total fluid output in the twenty-four hour urine. This remains the most feasible laboratory method since in normal individuals, especially when a large series is studied, slight positive balances in some subjects are offset by negative balances in others. In previous observations it was found that the urea nitrogen as determined by the hypobromite method was really equivalent to the total urinary nitrogen.[5] Consequently the values obtained by the hypobromite method were regarded as representing total nitrogen and will be so termed in the subsequent discussion.

The sodium chloride in the urine was estimated according to the method of Vollhard.

In the calculations of the protein intake from the urinary nitrogen, allowances were made for nitrogen loss in the feces. Lusk,[6] Mosenthal,[7] Peters and Van Slyke[8] and others have shown that only from 6 to 10 Gm. of protein (from 8 per cent to 10 per cent of the protein eaten) is lost in the stool. Thus the computations based on urinary nitrogen plus 10 per cent for nitrogen lost in the stool furnish a satisfactory formula for estimating protein consumption.

The calculation for protein intake is as follows: Urine urea grams (as obtained by hypobromite method) $\times$ 0.46 (nitrogen comprises 46 per cent of urea) equals urine nitrogen. Urine nitrogen $\times$ 6.25 equals amount of protein represented by the urinary nitrogen (nitrogen is 16 per cent of protein). These two steps may be combined by multiplying the urine urea grams by 2.87 (0.46 $\times$ 6.25 equals 2.87). If to the factor 2.87 is added one tenth (0.28) to represent the one tenth of protein nitrogen of the intake that is lost in the stool, the factor is approximatley 3. Hence the twenty-four hour urine urea figure multiplied by 3 yields the amount of protein represented in the urine and stool. It should be borne in mind that the calculations used in this study have allowed for the protein of the stool.

With regard to estimations of the fluid intake on the basis of urinary output, Soderstrom and Du Bois[9]

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1. McLester, J. S.: Nutrition and Diet in Health and Disease, Philadelphia, W. B. Saunders Company, 1929, pp. 99, 203 and 443.
2. Lusk, Graham: Science of Nutrition, Philadelphia, W. B. Saunders Company, 1928, p. 457.
3. von Voit, Carl: Handbuch der Physiologie, Leipzig, L. Hermann, 6: 519 (pt. 1) 1881.
4. McLester: Nutrition and Diet in Health and Disease, p. 214.
5. Mosenthal, H. O.: Renal Function as Measured by Elimination of Fluids, Salt and Nitrogen, and the Specific Gravity of the Urine, M. Clin. North America 4: 209 (July) 1920.
6. Lusk: Science of Nutrition, p. 53.
7. Mosenthal, H. O.: Observations on the Succus Entericus, J. Exper. Med. 13: 319, 1911.
8. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company 1: 270, 1931.
9. Soderstrom, G. F., and Du Bois, E. F.: The Water Elimination Through Skin and Respiratory Passages, Arch. Int. Med. 19: 931 (May, pt. 2) 1917.

cite the fact that when 1,894 cc. of water is taken the output is 1,750 cc. (a difference of 144 cc.), so that for practical purposes the urinary fluid output may be taken as approximately the fluid intake, particularly when a large series is studied in which positive balances counteract negative balances.

The specimens of urine were collected without difficulty. Ordinary traveling bags were used containing

TABLE 1.—*Protein Intake by One Thousand Subjects*

Amount of Protein, Gm. per Day	Group A: 563 Normal Individuals		Group B: 298 Subjects with Hypertension		Group C: 139 Cases of Hypertension and Nitrogen Retention	
	No. of Cases	Percentage of Group	No. of Cases	Percentage of Group	No. of Cases	Percentage of Group
20 or less	55	9	42	14	...	..
21 to 42	293	52	128	43	87	62
43 to 60	159	28	69	23	35	25
61 to 75	16	2	45	15	13	9
Over 75	40	7	14	4	4	3
Quota of group eating 42 Gm. or less of protein per day	348	61	170	57	87	62
Quota of group eating 60 Gm. or less of protein per day	507	89	239	80	122	87

TABLE 2.—*Protein Eaten by Group A: 563 Normal Individuals (280 Males, 283 Females)*

	Cases	Per Cent	
20 Gm. or less	55	9+	89% ate 60 Gm. or less of protein daily
21 to 42 Gm.	293	52	
43 to 60 Gm.	159	28+	
61 to 75 Gm.	16	2+	
Over 75 Gm.	40	7+	

eight wide-mouthed 20 ounce bottles in which each urine specimen was voided separately. The subjects followed their routine occupations, ate their usual meals and kept a record of their food and fluid intake during the twenty-four hour period of observation.

PROTEIN INTAKE

Table 2 shows that 9 per cent of the subjects in group A ate 20 Gm. of protein daily, or less than half the so-called 45 Gm. "minimal" protein intake; 61 per cent of these normal subjects ate 42 Gm. or less of protein daily; 89 per cent were eating 60 Gm. or less of protein daily. Only forty subjects, 7 per cent of the group, ate 75 Gm. or more of protein daily. These figures for protein consumption must appear surprising when it is shown that half the group ate less than what is felt to be an adequate minimal protein allowance, and only 7 per cent ate the 75 to 100 Gm. that has been advocated as the more fitting daily ration (tables 1 and 2).

Denis and Borgstrum,[10] Brooks [11] and Beard,[12] working with a large number of male medical students who had an ample supply of food and could eat as they pleased, showed that the protein consumed daily was about 71 to 79 Gm. for a person weighing about 70 Kg. Lusk [13] felt that a great number of medical students in New York City do not have an output of over 12 Gm. of nitrogen daily and believed the average protein intake of these students to be about 75 Gm. daily.

10. Denis, W., and Borgstrum, P.: Study of the Effect of Temperature on Protein Intake, J. Biol. Chem. 61: 109 (Aug.) 1924.
11. Brooks, F. P.: Protein Intake of Medical Students, Am. J. Physiol. 89: 403 (July) 1929.
12. Beard, H. H.: Protein Intake of Medical Students, Am. J. Physiol. 82: 577 (Nov.) 1927.
13. Lusk: Science of Nutrition, p. 455.

The data presented in this study coincide with those of Hetler,[14] of Sherman [15] and of Mendel.[16] Hetler,[14] in studies of the women of the Iowa State College in 1927, found that these subjects would take as high as 70 Gm. of protein daily but more often would consume only from 25 to 40 Gm. From studies of the twenty-four hour urine in 1932, Hetler concluded that the average nitrogen excretion was 7.69 Gm., representing 48 Gm. of protein. Sherman [15] reported on 109 experiments indicating an average of 44 Gm. of protein and ranging from 21 to 65 Gm. Mendel [16] in 1932 said:

> In health, the nitrogen content of the kidney output furnishes a reliable index of the nitrogenous (or protein) intake. During the nineties of the last century the estimated protein intake, after due allowance for fecal loss of nitrogen generally approximated an average of at least 100 Gm. (16 Gm. N × 6.25) a day. In the course of the years, these annually secured estimates are showing lower averages. Other teachers have had similar experiences. The newest figures of Hetler on young women in Illinois studied during the years 1928-1930 averaged 7.7 Gm. of nitrogen (equivalent to about 50 Gm. of protein metabolized) daily.

TABLE 3.—*Protein Eaten by Group B: 298 Individuals Who Were Found to Have Hypertension Which They Did Not Suspect (118 Males, 180 Females)*

	Cases	Per Cent	
20 Gm. or less	42	14+	80% ate 60 Gm. or less of protein daily
21 to 42 Gm.	128	43	
43 to 60 Gm.	69	23	
61 to 75 Gm.	45	15	
Over 75 Gm.	14	4+	

TABLE 4.—*Protein Eaten by Group C: 139 Subjects Who Were Found to Have Hypertension and a Blood Urea Nitrogen of 20 to 40 Mg. (82 Males, 57 Females)*

	Cases	Per Cent	
21 to 42 Gm.	87	62	87% ate 60 Gm. or less of protein daily
43 to 60 Gm.	35	25	
61 to 75 Gm.	13	9+	
Over 75 Gm.	4	3—	

TABLE 5.—*Summary of Protein, Salt and Fluid Intake of One Thousand New York City Residents*

	Number of Cases	Percentage
I. Protein intake, Gm. per day		
20 or less	97	10
21 to 42	508	51
43 to 60	263	26
61 to 75	74	7
Over 75	58	6
II. Salt intake, Gm. per day		
4 or less	50	5
4 to 8	416	42
Over 8	534	53
III. Fluid intake, cc. per day		
1,000 or less	258	25
1,000 to 1,500	337	36
Over 1,500	390	39

It has been pointed out by many workers that a low protein ration is not conducive to general health or to mental or physical efficiency. "Nutritional" edema, fatigue, anemia, cloudy swelling of vital organs, lack of resistance to infection, pellagra and other ill effects have been cited.

14. Hetler, R. A.: Protein Intake and Basal Metabolism of College Women, J. Nutrition 5: 69 (Jan.) 1932.
15. Sherman, H. C.; Gillett, L. H., and Osterberg, E.: Protein Requirements of Maintenance in Man, J. Biol. Chem. 41: 97 (Jan.) 1920.
16. Mendel, L. B.: Changing Diet of the American People, J. A. M. A. 99: 117 (July 9) 1932.

It is profitable to compare the extremes among this normal group A as to its use of protein. The "low protein group": (1) females constituted most of this group; (2) low or low-normal blood pressure readings are encountered frequently, yet the "high protein" eater does not have a high blood pressure; (3) underweight occurs frequently; (4) mild secondary anemia is common; (5) the blood urea nitrogen is often found to be from 8 to 10 mg. per hundred cubic centimeters as compared to from 12 to 15 mg., the standard normal.

Table 6.—*Salt Eaten by Group A: 563 Normal Individuals*

	Cases	Per Cent
Less than 4 Gm.	27	5—
4 to 8 Gm.	211	37
Over 8 Gm.	325	57

Table 7.—*Salt Eaten by Group B: 298 Individuals Who Were Found to Have Hypertension Which They Did Not Suspect*

	Cases	Per Cent
Less than 4 Gm.	15	5
4 to 8 Gm.	131	44
Over 8 Gm.	150	50

Table 8.—*Salt Eaten by Group C: 139 Subjects Who Were Found to Have Hypertension and Blood Urea Nitrogen of from 20 to 40 Mg.*

	Cases	Per Cent
Less than 4 Gm.	8	6
4 to 8 Gm.	72	52
Over 8 Gm.	59	42

The "high protein group": (1) males comprised most of this group; (2) the blood pressure is not elevated; (3) these individuals are of average weight, though very rarely overweight is noted; (4) no anemia is demonstrated and rarely a high red blood cell count is seen (e. g., 6,480,000); (5) the blood urea nitrogen figures in a few cases are the upper limit of normal; e. g., from 15 to 18 mg. per hundred cubic centimeters. Yet high protein feeding seems, in the cases comprising this study, to have less effect in keeping the blood urea nitrogen at the high normal figure than has a low water excretion. Thus, while the "low protein eater" tends toward a low blood pressure and a low hemoglobin percentage, the "high protein eater" has no increased blood pressure and his hemoglobin is normal.

Table 3 shows that 57 per cent of the subjects in group B (170 cases) ate 42 Gm. or less of protein daily. This is a close approximation to the 61 per cent of the normal group, who ate a similar low protein ration. Only 4 per cent of these hypertensive persons ate 75 Gm. or more of protein daily. When these 298 subjects of essential hypertension with no impairment of renal function are considered, it is found that these people, if left to their own choice, on their habitual diet since they were unaware of an existing hypertension, were eating practically the same amount of protein as nonhypertensive persons. The same was found to be true of their salt and their fluid intake.

Many of the patients found to have hypertension were seen several times in the course of the years over which this study was made, some of them having followed a low protein diet for periods of from three months to a year. There is no evidence to show that a low protein diet will materially reduce the blood pressure. In the very few instances in which some appreciable drop in blood pressure occurred, protein and total diet restriction had been extreme and had resulted in anemia, undernutrition and general weakness. In several cases low protein intake by patients with hypertension was compensated by an excessive carbohydrate intake to satisfy hunger, which resulted in an increase of weight, thereby handicapping the patient.

Table 4 shows that even the hypertensive person with slight renal insufficiency unknown to the patient does not differ widely from the normal as to the protein content of his diet. The same is true of the salt and fluid intake.

It might be urged that nitrogen retention in these subjects may have caused a low urinary urea excretion so that the protein intake estimations for this group may be low. However, ninety-four out of this group of 139, or 67 per cent of the total, had blood urea nitrogen figures ranging from 20 to 25 mg. per hundred cubic centimeters or but slightly above the average normal; their urinary urea output and, by computation, their protein intake fell within the same range as those of the normal subjects, the hypertensive subjects with normal blood chemical changes and the hypertensive subjects with blood urea nitrogen figures of from 26 to 40 mg. per hundred cubic centimeters.

SALT INTAKE

As shown in table 6, 57 per cent of the subjects in group A ate over 8 Gm. of salt daily; eight individuals ate over 20 Gm. of salt daily, two of these taking as high as 40 Gm. a day (five of these eight subjects were overweight). The "low salt eaters" were chiefly females; the "high salt eaters" were mainly males. Among the "high salt eaters" there was a tendency toward a high normal blood urea nitrogen (from 15 to 18 mg. per hundred cubic centimeters) and a high normal blood uric acid (from 3.5 to 4.5 mg. per hundred cubic centimeters).

Table 9.—*Fluid Taken by Group A: 563 Normal Subjects*

	Cases	Per Cent
Less than 1,000 cc.	152	27
1,000 to 1,500 cc.	185	33
Over 1,500 cc.	226	39

Table 10.—*Fluid Taken by Group B: 298 Cases of Hypertension*

	Cases	Per Cent
Less than 1,000 cc.	71	24
1,000 to 1,500 cc.	107	36
Over 1,500 cc.	120	40

Table 11.—*Fluid Taken by Group C: 139 Cases of Hypertension with Blood Urea Nitrogen Figures of from 20 to 40 Mg.*

	Cases	Per Cent
Less than 1,000 cc.	30	22—
1,000 to 1,500 cc.	65	47
Over 1,500 cc.	44	30+

Six persons of group C ate from 18 to 28 Gm. of salt daily.

FLUID INTAKE

Among the normal individuals, the "high fluid" consumers may be compared with the "low fluid" users. The "high fluid" normal: (1) males make up the major-

ity of this group; (2) the group is equally divided as to obese and thin persons; (3) the blood urea nitrogen is low normal. There is no relation between high fluid and high salt intake. The "low fluid" normal: (1) females comprise most of this group; (2) obesity is often present; (3) there is a fair incidence of the upper limit of normal blood urea nitrogen (from 15 to 18 mg. per hundred cubic centimeters) and of the upper normal limit of blood uric acid (from 3.5 to 4.5 mg.). The relatively high blood urea nitrogen and uric acid in persons who drink but little fluid is interesting in view of the fact that there has been noted a slight increase in the nitrogen elimination if copious amounts of water are taken. This is thought by Hawk[17] to demonstrate the fact that water drinking actually induces increased protein metabolism and is not to be thought of as merely a "washing out" process but rather as a form of "specific dynamic action."

Fluid taken by group B (table 10) is essentially the same as by group A (table 9). It is to be noted that 1,500 cc. or more of fluid was taken by only 30 per cent of the subjects in group C (table 11), as compared to 40 per cent in groups A and B. Whether the slight nitrogen retention of this group is explainable, at least for some of its members by their low fluid intake or whether the two results are merely a coincidence is debatable. It is difficult to see why nitrogen retention of from 20 to 40 mg. per hundred cubic centimeters (in most cases from 20 to 25 mg.) would cause a low fluid output, since urea is an excellent diuretic. It must be noted, as has already been pointed out, that the normal individuals who take little fluid manifested high normal blood urea nitrogen.

SUMMARY AND CONCLUSIONS

1. It was felt from this study of 1,000 cases that the minimal requirement in a study of protein, salt and water consumption is the twenty-four hour urine sample. The patient's statement and even his record of food consumed may be very misleading. Thus we may cite a patient with Bright's disease (not included in this series) who believed himself to be on a low protein diet but who was found to be deriving more than 100 Gm. of protein daily from bread, cereals and cheese.

2. Many people eat less than 45 Gm. of protein daily. Sixty per cent of 1,000 subjects in this study were found to be eating 42 Gm. or less of protein daily (table 5). The evil effects of such a course have been pointed out.

3. The majority of the individuals studied were found to take a low protein (42 Gm. or less), moderately high salt (from 4 to 8 Gm. or more) and moderate fluid (1,000 cc. or more) ration.

4. There is no relation between protein, salt and water intake.

5. While the normal individual eating a low protein diet tends to have a low blood pressure and a low hemoglobin percentage, the normal person eating a high protein diet has no increased blood pressure and his hemoglobin is normal.

6. The high protein diet has less effect in raising the blood urea nitrogen than has a low fluid intake.

7. People with hypertension do not habitually eat more protein or more salt than normal persons. There is no evidence to show that a low protein diet, followed by a hypertensive subject, will materially reduce the blood pressure provided there is no anemia.

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17. Hawk, quoted by McLester,[1] p. 29.

To eat or not to eat salt is now the question. What is the effect of "too little" or "too much"? J. Amer. Dietetic Assoc.

Observations on Dietary Sodium Chloride[1]

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DIETITIANS, PHYSICIANS and, most of all our patients are impaled on the horns of a dilemma (1). We have all seen the benefit of low-salt diets when properly indicated, but we have all been puzzled by those patients who are insidiously or abruptly made worse by salt withdrawal. What is the "correct" amount of salt in the diet? What is the scientific evidence that salt in excess may have bad effects? To these, and the many related questions, we can give only partial answers.

Salt was in wide use by the beginning of recorded history, and history's pages have not been lightened by wars waged, wives and children sold into slavery, decapitation, and other strong measures for control of the traffic in this commodity. In 1584 Jean de Marcomille in Paris could exclaim: "The sacredness and dignity of salt! This mineral is like unto the four elements—earth, air, fire and water, so universal, so necessary to life, it is the fifth element" (2).

Since the beginning of the last century when the element sodium was discovered by Sir Humphry Davy, it has been recognized as an important constituent of the body and an essential element in the diet. Redtenbacher and Schmidt are commonly credited as the first who paid scientific attention to salt in disease states about 1850. But, as Gamble pointed out (3), the honor really lies with a London physician named O'Shaughnesy twenty years earlier. During the ensuing hundred years we have discovered and rediscovered the merit of salt withdrawal in edema, heart failure, and hypertension.

That salt could have deleterious effects has been known since time immemorial. Eating a cupful of salt was a Chinese method of committing suicide. If granular sodium chloride is sprinkled in a turkey run, the sparkling grains attract the poults to eat an excess which will be fatal to them. Sapirstein, Selye, Fukada, and others have observed specific changes due to excessive salt intake. The Minneapolis symposium (4) generously documented the role of salt in hypertension. Dahl and Love observed (5) a positive correlation between the incidence of hypertension and salt-adding habits of 547 adults.

The *optimum* level of sodium chloride in the human diet remains to be defined. The *average* intake of sodium chloride for a normal adult is estimated at 7 to 15 gm. per day (6). A 2500-calorie diet containing 500 gm. nutriment (without water and inert material) might thus contain from 1.4 to 3.0 per cent sodium chloride (Table 1). In an effort to assess the chronic toxicity of sodium chloride we have maintained rats from five weeks of age throughout life at ten levels of added dietary sodium chloride.

TABLE 1 Composition of human diet

NUTRIENT	INTAKE	
	Weight	Food energy
	gm./day	calories/day
Protein	100	400
Fat	100	900
Carbohydrate	300	1200
Total intake	500	2500
Sodium chloride (wt. × 0.028)	14	

The initial experiment started in 1951, the seventh experiment now in progress, and the five intervening experiments have shown remarkably reproducible

[1] This research was supported in part by the Life Insurance Medical Research Fund G-54-24 and the National Heart Institute H-1816. Presented by Dr. Meneely at the 39th Annual Meeting of The American Dietetic Association in Milwaukee, on October 12, 1956.

results. Increased sodium chloride correlated positively with mean water consumption, blood pressure, radio-sodium space, incidence of electrocardiographic abnormalities, serum cholesterol, heart weight, and kidney weight; and correlated negatively with mean stem length, weight, and longevity (7–11).

Materials and Methods

Five hundred and forty-six male and twenty-one female Sprague-Dawley rats five weeks old at the start of the experimental regimen were housed in constant temperature and humidity-controlled quarters with free access to demineralized water and were fed purified diets containing from 0.15 per cent up to 21 per cent sodium chloride. The basic ration, from which these diets were derived (Table 2) contained approximately 0.01 sodium chloride. Seven additional female rats were maintained on a commercial chow diet with added sodium chloride to bring the percentage to 5.6 per cent. The rats were weighed and examined weekly. Systolic blood pressure measurements were made by the cuff method of Kerston *et al.* (12) at monthly intervals in the first experiment and more frequently in the later experiments. Serum cholesterol determinations by the method of Pearson *et al.* (13) were done in young (less than ten months), middle-aged (nineteen to twenty-one months), and old (twenty-three or more months) rats. Measurements of total body sodium and sodium space using radioactive sodium were made in selected male rats at four months and at eight to ten months (14). After nineteen months, electrocardiograms were taken on all surviving rats in Experiment No. 1 using subcutaneous needle electrodes. Autopsies were performed on all rats.

Results

When first placed on the experimental diets, marked polydipsia and polyuria occurred among the rats eating increased sodium chloride. After three days, these rats appeared to be severely dehydrated. Two weeks later they all looked healthy and were gaining weight regularly, although growth was adversely influenced by the amount of added sodium chloride.

EDEMA

The earliest untoward effect of the elevated dietary sodium chloride was evidenced during the third and fourth months. Edema developed in 15 per cent of the rats eating 7 per cent or more of sodium chloride (*Fig.* 1). Sudden weight gains of 40 to 100 gm. occurred. Extracellular water measured with radioactive sodium rose from the control levels of 25 to 57 per cent of body weight. These edematous animals were severely anemic and showed lipemia and hypoproteinemia. At autopsy there were obvious edema and anemia. Otherwise, grossly, all the tissues except the kidneys appeared normal. In relatively bloodless glomerular tufts, there were foam cells containing large amounts of fat-staining material. The tubules exhibited a nephrotic degeneration and contained also much fat-staining material. There were lesser deposits of lipoid in some of the arterioles. This syndrome has an evident resemblance to nephrosis.

The few animals surviving this edematous phase rapidly lost their extra fluid and became extremely emaciated and cachectic. Again, all tissues seemed essentially normal except for a similar but more extensive renal lesion. Many glomerular tufts were obliterated. Tubular degeneration was extreme. No evidence of adrenal failure was found.

The majority of the rats did not develop nephrosis. From the ninth month of the first experiment, the trend of elevated systolic blood pressure in association with increasing dietary sodium chloride became apparent (*Fig.* 2). In later experiments, earlier efforts by adept technicians to train the rats enabled us to obtain reliable, reproducible blood pressures from the second month. In those later experiments the association of hypertension and the sodium chloride content of the diet was evident from the fourth month.

TABLE 2 Basic, low-sodium ration for rat studies

INGREDIENT	PER CENT
Casein, vitamin-test	25.1
Cane sugar	51.8
Shortening, all-vegetable	20.0
Mineral mixture	2.9
Vitamins	0.2

FIG. 1. *Rats on ration provided 8.4 per cent sodium chloride. The edematous rat at right is typical of the 15 per cent of the rats eating 7.0 per cent or more sodium chloride which developed this nephrotic syndrome between two and four months.*

HYPERTENSION

Hypertension was similarly induced in the small series of females, but the mean elevations of blood pressure were somewhat less than in the male. Levels of sodium chloride added to the purified basic ration and to a commercial rat chow produced a similar degree of hypertension (Table 3). Females eating 5.6 and 9.8 per cent sodium chloride mated with normal males and delivered apparently normal young without developing symptoms of eclampsia, although the number of pregnancies, the size of litters, and the survival of the young were unfavorably influenced.

Total serum cholesterol values correlated positively with the dietary percentage of sodium chloride, and as a sequela showed a low order of correlation with the systolic blood pressure.

Electrocardiograms on all survivors at nineteen months showed an incidence of abnormalities paralleling the degree of hypertension amounting to 80 per cent of the rats eating 9.8 per cent sodium chloride. All rats eating 14.0 per cent sodium chloride were dead at fifteen months, those eating 21.0 per cent at nine months. The left ventricular "strain" pattern, similar to many tracings in human hypertension, was present in more than half of the rats eating high levels of sodium chloride. Cardiac hypertrophy correlated well with electrocardiographic abnormalities.

Obesity occurred in a number of the control rats, less frequently in 2.8 and 5.6 per cent groups, but was not seen at the higher sodium chloride levels.

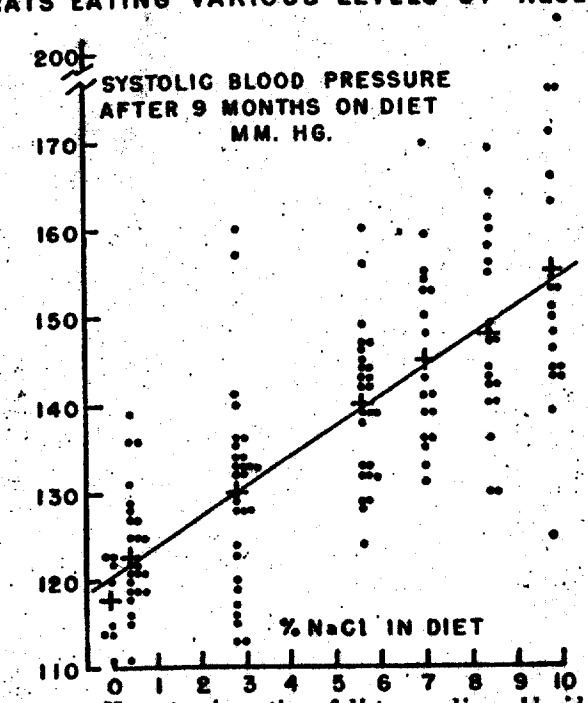

FIG. 2. *Hypertensive action of dietary sodium chloride in male rats.*

Control rats continued to gain weight for significantly longer periods of time than rats consuming 7 per cent or more of sodium chloride. Control rats eating 1.1 and 2.0 per cent sodium chloride had slightly better growth and mortality during youth and reproductive spans than rats on the 0.15 per cent sodium chloride diet. Survival rates showed statistically significant decreases depending on the amount of dietary sodium chloride and the duration of the regimen (*Fig. 3*).

Data on wet organ weights at death, grouped on the basis of three time intervals—less than twelve months, thirteen to nineteen months, and twenty plus months that the rats survived—were expressed as weight of the organ in grams per kilogram body weight. The mean cardiac weight increased with increasing dietary sodium chloride, the greatest cardiac hypertrophy being evident at the 9.8, 14.0, and 21.0 per cent levels. This significant increase was notable even in rats dying in the earlier period. In the survivors to twenty months and beyond, the increased relative cardiac weight was apparent at the 2.8 per cent level. The pattern of renal hypertrophy was similar. The adrenal weight changes were less consistent, although again the rats eating 9.8 per cent plus sodium chloride showed the greatest relative adrenal weight, regardless of the length of time on the experiment. The smallest relative adrenal weights were found in the controls. Animals dying during the first year exhibited increased adrenal weight related to the amount of dietary sodium chloride, those dying after twenty or more months showed a converse relationship.

LIFE SPAN SHORTENED

The pathology of the small percentage of rats eating high levels of sodium chloride having a nephrosis-like syndrome is reported above. Among the remainder, life span was shortened; hypertension developed; there was a disturbance of lipid metabolism; renal and heart failure occurred—all in proportion to the amount of sodium chloride in the diet. The spectrum of hypertensive cardiovascular renal disease seen in the human being was reproduced, ranging from a clinical course resembling that of mild "benign-essential" hypertension with minimal shortening of life span to rapidly progressive and

TABLE 3 Hypertensive action of dietary sodium chloride on female rats after ten months' experiment

RATION	SODIUM CHLORIDE	SYSTOLIC BLOOD PRESSURE	
		Mean	S. D.*
	%		
Purified control	0.15	124.2	2.1
Purified	5.6	139.3	0.4
Purified	9.8	149.8	1.3
Commercial	5.6	140.1	1.2

* Standard deviation.

lethal "malignant" hypertension. Arteriolar lesions occurred in incidence, extent, and degree proportional to the increase in dietary sodium chloride and to the severity of the clinical course. In the more advanced disease, the arterioles were uniformly involved. Stainable lipid formed vacuoles in smooth muscle cells, some of which were necrotic. In many arterioles, the elastic lamina was greatly swollen, frayed, and intensely eosinophilic. The lumina were narrowed or even occluded. Visceral arteriolar lesions were fairly constant among these animals and were almost invariably present in the heart, pancreas, testis, and gastrointestinal tract. In the less severe forms of the disease, the vascular lesion, both renal and general visceral, was less impressive and less constant, usually consisting of medial hypertrophy.

Discussion

It is clearly evident that excessive salt is "rough on rats." What light does this shed on the human problem? Dahl's observations (5) seem to indicate there is a relation between human salt intake and the incidence of hypertension. The evidence assembled in the Minneapolis symposium (4) documents the comment of Braun-Menendez that whatever the experimental method for producing hypertension, increased salt facilitates its obtainment and salt restriction prevents it.

Let me draw your attention to the pattern of survival in our animals. Dismiss the findings with high levels of salt, those of 7 per cent and above. Note that those animals eating from 2.8 to 5.6 per cent salt, the levels most reasonably related to human consumption, did not die at an excessive rate until after the seventeenth month. They had been mildly to moderately hypertensive since perhaps the ninth month, but only this late in life does the disease produced by a lifetime of excessive salt

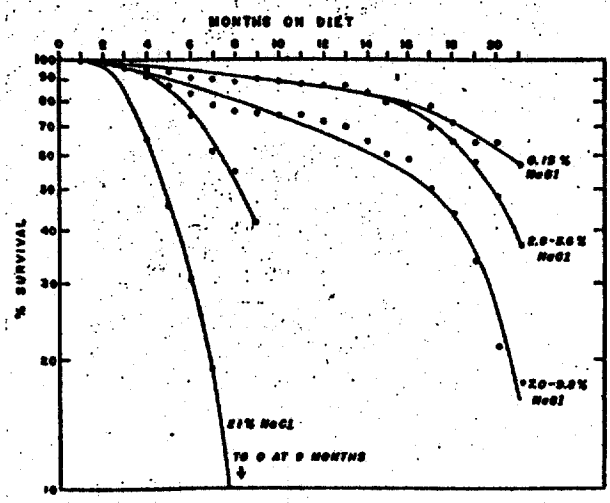

FIG. 3. *Survival curves at twenty-one months, Experiment 1, and at nine months, Experiment 2.*

eating begin to exact its penalty. If ten days for a rat is equivalent to a year for a man, these animals develop their benign (?) hypertension in the third or fourth decade but are in the early fifties before they begin to die more rapidly than their controls. Thus in a rather remarkable way, the human time-course of the disease is reproduced.

"But I eat a lot of salt and my blood pressure is normal," some people can honestly say. Evidently salt is not the whole story of human hypertension; there certainly seems to be an hereditary element. Remember, too, that the salt used in adult life may not reflect the life-time salt-eating habit. A group of elderly rats we obtained from Eli Lilly Company through the courtesy of Dr. Kenneth Kohlstaedt were given the same diets we have described to you and the effects of high salt-eating were not nearly as bad for the older animals as for the young ones in the experiments described above. Other experimental methods for the production of hypertension are more effective in young animals. Perhaps it is our salt-eating habit in youth and early adult life which lays the groundwork for hypertension in middle and older age.

Potassium as an Antidote?

What can we do about it? Can we change a human custom engrained through fifty centuries at least? Perhaps we do not need to do it quite that way. We have thought of a method which may be of some help. If one admixes potassium chloride in ordinary table salt to as high a proportion as 30, even 40 per cent for some people, the taste difference is not detectable. If this were used instead of sodium chloride alone, it would simultaneously diminish the amount of sodium chloride we eat and increase the potassium. Is there any evidence that an increase of potassium chloride in the diet does any good? We think so. In experiments we have just begun to analyze and which we reported in preliminary form at the meeting of the American Physiological Society held recently in Rochester (15), we have found that potassium chloride has a remarkable protective action against excessive sodium chloride intake in rats. The most important observation indicates that life may be greatly prolonged despite high sodium chloride eating; and, at lower levels of excess sodium chloride, the potassium-protected rats outlived the controls!

It is reasonable to suppose that potassium might be of help. Only those animals which have a high potassium intake (the herbivores) take the trouble to go to salt licks for extra sodium chloride. If extra sodium chloride is needed with a high-potassium diet, perhaps an increase in potassium chloride may balance an excess sodium chloride intake. Our evidence seems to support this possibility.

A word of caution is in order, however. We know a great deal about rats, but not nearly as much about

human beings. We do know that excessive potassium intake can be harmful to patients with severe kidney disease. If we are stimulated to experiment with increased potassium feeding, let us do so under controlled conditions with careful laboratory checks for possible harmful effects! Remember that when lithium became commercially available as a substitute for salt, it had a wide vogue before its toxicity was recognized.

References

(1) YOUMANS, J. L.: The horns of a dilemma. Am. J. Med. 111: 133, 1951.

(2) ESKEW, G. L.: Salt, the Fifth Element. Chicago: J. G. Ferguson & Assoc., 1948.

(3) GAMBLE, J. L.: Early history of fluid replacement therapy. Pediatrics 11: 554, 1953.

(4) Hypertension. Symposium held at the Univ. of Minn. on Sept. 18, 19, and 20, 1950, in honor of Elexious T. Bell, Benjamin J. Clawson, and George E. Fahr. (Bell, E. T., ed.) Minneapolis: Univ. of Minn. Press, 1951.

(5) DAHL, L. K., AND LOVE, R. A.: Evidence of relationship between sodium (chloride) intake and human essential hypertension. Arch. Int. Med. 94: 525, 1954.

(6) FOOD AND NUTRITION BD.: Recommended Dietary Allowances, Rev. 1953. Natl. Acad. Sci.—Natl. Research Council Pub. 302, 1953.

(7) MENEELY, G. R., TUCKER, R. G., AND DARBY, W. J.: Chronic sodium chloride toxicity in the albino rat. 1. Growth on a purified diet containing various levels of sodium chloride. J. Nutrition 48: 489, 1952.

(8) MENEELY, G. R., TUCKER, R. G., DARBY, W. J., AND AUERBACH, S. H.: Chronic sodium chloride toxicity in the albino rat. 2. Occurrence of hypertension and a syndrome of edema and renal failure. J. Exper. Med. 98: 71, 1953.

(9) MENEELY, G. R., TUCKER, R. G., DARBY, W. J., AND AUERBACH, S. H.: Chronic sodium chloride toxicity: hypertension, renal and vascular lesions. Ann. Int. Med. 39: 991, 1953.

(10) MENEELY, G. R., TUCKER, R. G., DARBY, W. J., BALL, C. O. T., KORY, R. C., AND AUERBACH, S. H.: Electrocardiographic changes, disturbed lipid metabolism and decreased survival rates in rats chronically eating increased sodium chloride. *In* Proc. South. Soc. Clin. Res. Am. J. Med. 16: 599, 1954.

(11) TUCKER, R. G., DARBY, W. J., AND MENEELY, G. R.: Effect of dietary NaCl on ultimate size, blood pressure and organ weight of rats. Federation Proc. 13: 480, 1954.

(12) KERSTEN, H., BROSENE, W. G., JR., ABLONDI, F., AND SUBBAROW, Y.: A new method for the indirect measurement of blood pressure in the rat. J. Lab. & Clin. Med. 32: 1090, 1947.

(13) PEARSON, S., STERN, S., AND MCGAVACK, T. H.: A rapid procedure for the determination of serum cholesterol. J. Clin. Endocrinol. Metab. 12: 1245, 1952.

(14) KALTREIDER, N. L., MENEELY, G. R., ALLEN, J. R., AND BALE, W. F.: Determination of the volume of the extracellular fluid of the body with radioactive sodium. J. Exper. Med. 74: 569, 1941.

(15) MENEELY, G. R., DARBY, W. J., LEMLEY-STONE, J., TUCKER, R. G., AND BALL, C. O. T.: Survival of rats prolonged by adding potassium chloride to diets containing toxic levels of sodium chloride. *In* Proc. Am. Physiol. Soc. Am. J. Physiol, in press.

Beeson, P.B. and W. McDermott (Eds.) 1971

Cecil-Loeb Textbook of Medicine

W.B. Saunders Co., Philadelphia, Pa.
p. 1058

Arch. f. exp. Path. u. Pharm.
108:39-51, 1924

Studies on the mechanism of sodium chloride poisoning. I. Significance of osmotic processes

by

Behrend Behrens

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It is generally known that sodium chloride, the neutral salt most indifferent even towards body cells, can exercise strong toxic effects not only upon the cell, but upon the entire organism, when it is used as a solid or in concentrated solutions. There are many reports particularly in the earlier literature on poisoning of humans and animals resulting from the ingestion of large quantities of sodium chloride (for literature see Kobert, [1], p. 39); it is even said to have been used in China as a poison for purposes of suicide (see Poulsson, p. 354, [2], p. 39).

The cause of this toxic effect is generally assumed to be the so-called salt effect, by which is meant the osmotic changes suffered by the cells upon contact with concentrated salt solutions. These physical qualities of a salt appear to exist in particularly pure form precisely in the case of sodium chloride, while in other salts they are more indistinct, owing to the specific action of their ions.

Upon intake of NaCl through the mouth such salt effects will

occur primarily on the mucosa of the digestive tract; however, since sodium chloride is readily absorbed and thus the osmotic pressure of the blood increases, these effects are also to be expected in all other tissues of the body.

In textbooks of pharmacology and toxicology the cause of death following the oral intake of sodium chloride in solid form or as concentrated solution is concordantly assumed to be the drying out of vital organs due to osmotic withdrawal of water. On the other hand there is some obscurity and contradiction concerning the site towards which the displacement of water takes place. In the opinion of some, based on the observation of inflammatory irritation of the gastric mucosa and effusions of large quantities of fluid into the gastrointestinal tract, the loss of water by the tissues is caused by the fact that as a result of the inflow of hypotonic fluid from the blood into the lumen of the digestive tract the osmotic pressure of the blood increases and secondarily, again due to osmotic pressure differences, water enters the blood from the tissues. This appears to be the view of Heinz and Haas ([1], p. 40), who have recently carried out experiments on the problem of sodium chloride poisoning; these two authors initially observed a dilution of the blood upon poisoning a chicken with sodium chloride, but towards the end, as they evidently expected, they observed a thickening of the blood.

A more general and more well-founded assumption appears to be the other view, according to which the effect on the tissues

is caused directly by the osmotic pressure which is increased by the absorption of sodium chloride, while no decisive importance is attributed to the changes in the gastrointestinal tract. This assumption is supported above all by experiments according to which NaCl and many other salts, as well as other substances without decided chemical affinities, elicit phenomena similar to the ingestion of sodium chloride when injected intravenously (Münzer[2], Müller[3], Falk[4], Hermanns[5], p. 40).

Some observations, such as the seizures and signs of paralysis frequently described (Hermanns[5], Müller[3], Falk[4], Guttmann[6], Limbourg[7], p. 40), and the sometimes very acute course of NaCl poisoning, raise some doubts as to whether we are justified in regarding the osmotic effect of sodium chloride as the sole explanation for its great toxicity. It is conceivable that by an enormous increase in the Na ions a disturbance comes about in the physiologic cation equilibrium, in other words a "sodium poisoning" plays a part, in the same manner in which we speak of potassium or calcium poisoning when there is a displacement towards one side or the other.

Finally it would seem to be entirely possible that the ingestion of sodium chloride leads to a displacement in the base-acid equilibrium in the organism. Thus it might be thought that in the presence of high sodium chloride concentrations the dissociation of the gastric hydrochloric acid is so strongly repressed that considerable quantities of molecular hydrogen chloride appear. This would create a state such as has been

discussed by Hermann Wieland and Kurtzahn ([1], p. 41) for hydrogen fluoride poisoning: the gastric mucosa which is impermeable to H-ions readily allows the undissociated molecules of hydrogen halide to penetrate; in the tissues or in the blood, where conditions are given, decomposition into ions and thus acid poisoning sets in.

Other possibilities will not be discussed in this report.

A new study of the mechanism of sodium chloride poisoning appears to be urgently desirable because of the obscurities referred to above, particularly since the clarification of this borderline case of the sodium chloride effect might also yield some insight into the physiologic role of this important nutritional and bodily component. In order to place my studies on firm foundations, in this first part of my report I have dealt only with the basic problem of whether death after peroral intake of sodium chloride is the consequence of the presence of the salt in the lumen of the gastrointestinal tract, or is caused by the entry of this substance into the circulation.

Local damage by sodium chloride, in analogy to experiments on individual cells, should be the more severe the higher the concentration of the solution. Upon damage caused by absorption, on the other hand, it might be expected that medium concentrations would be more effective than the highest ones, because at these concentrations the high osmotic pressure presents an obstacle to absorption which is only gradually overcome, by dilution with gastric juice. Thus it was a matter of finding out the degree of

toxicity of sodium chloride, that is to say the lethal dose when solutions of various concentrations are introduced into the stomach, or the period within which one and the same quantity of sodium chloride causes the death of the experimental animal when it is ingested with varying quantities of water.

Method

The selection of animals for such experiments at first presented considerable difficulties. Dogs and cats had to be excluded for such experiments owing to their high price. Also these animals readily tend to evacuate harmful material by vomiting. Rabbits, like guinea pigs, generally have a full stomach and are unsuitable for this reason. On the other hand it was found that the albino mouse was very suitable for such experiments. Aside from its cheapness, the mouse offers further advantages as an experimental animal: after 1 hour the stomach of the mouse is already empty, the mouse cannot vomit and the stomach of fully grown animals is large enough to take up ca. 1.5 cc comfortably. The only difficulty, i.e. that of introducing measured quantities of fluid into the stomach by force, was overcome with unexpected ease. It was found that stomach intubation was fairly easily carried out on the mouse, despite the small dimensions involved. The procedure will be described in detail, since it is suitable for more general use.

Obviously it must be borne in mind that in view of the very delicate tissues of the mouse any rough procedure must be avoided. After a few experiments I found a rigid nickel canula 7 cm long

and 3/4 mm thick to be the only suitable tubing. The end of the canula was closed with a drop of soft solder, rounded off and smoothed. Slightly above the end a small opening was filed laterally into the tube.

When introducing the tube it must be borne in mind that, as can be seen from Fig. 1 ([1], p. 43) the esophagus makes small bends, mainly caused by the peculiar sharp bend in the cervical spinal column characteristic of small mammals, so that the direction of the canula must be changed accordingly when inserting it, as shown half schematically in Fig. 2. The first direction (I) is necessary to advance behind the tongue and reach properly into the esophagus. This can usually be achieved without difficulty. In rare cases it happens that the trachea is compressed and the animal is threatened with suffocation. The second segment of the esophagus can also almost always be penetrated (II). The situation is different with the last part (III). Here it is not always easy to find the point of entry of the esophagus through the diaphragm; it lies somewhat backwards towards the spinal column, giving rise to the 3rd change in direction. The point of entry must be sought out with careful seeking movements. By holding the animal properly it is much easier to to find this point. If the animal is held by the back of the neck (see Fig. 3) between the thumb and index finger of the left hand and the tail is pressed with the fourth finger towards the ball of the thumb, by pulling

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[1] p. 43. The x-ray pictures were kindly furnished by Privy Councilor Matthes.

on the tail it is possible to stretch the spinal column, as a result of which the bend in the throat becomes less pronounced and direction III, particularly in older mice, will diverge less from direction II. As might be expected, there are considerable diversities. An occasionally disturbing feature is the fact that the mice, in making defensive movements with their front paws, clasp the canula. This can be remedied by making the fold of skin by which the animal is held at the nape of the neck large enough to prevent it reaching the canula. At the beginning it sometimes happened, particularly at the point of entry through the diaphragm, that the esophagus was perforated by a clumsy movement. I therefore added a small amount of barium sulfate to the injection solution and checked the dead mice in the autopsies by observing this substance in the stomach or intestines and its absence in the free abdominal cavity. After a certain amount of practice this precautionary measure is no longer necessary. In order to obtain some idea of when to change directions - which obviously varies from one mouse to another - there are marks on my tube at 3.2 and 4.5 cm measured from the tip. The anterior mark indicates roughly when the change from direction II to direction III should occur, while the other mark shows when the tube lies securely in the stomach. This can easily be established by light pressure with the finger on the stomach region.

In giving the injection care should be taken that the contents of the canula should furnish no possibility for inaccuracy. For this reason as a rule the tube was introduced with a closed,

filled syringe, a procedure which is somewhat more difficult than intubation with the canula alone.

Roughly 500 mice were intubated in this manner. In the end less than 1% of the intubations were unsuccessful. The tube can also be introduced repeatedly into the same animal without difficulties and is tolerated without distress. For instance in experimentation on another problem I intubated the same mouse for feeding every day for more than 50 days.

Experiments

Groups of at least 10, if possible identical mice having fasted for 2 hours and weighing 16-19 g received varying quantities of a 2, 3, 4 and 5 molar sodium chloride solution, introduced into their stomach with a tuberculin syringe divided into 1/50 cc. The animals were then observed without giving them access to food or water. Immediately after the injection, as soon as they had recovered from this intervention, which happened within a few seconds, they showed a completely normal behavior. After a certain period distinct somnolence set in and the animals sat crowded together in one corner. At this stage the animals experienced a great feeling of thirst. If they are given the opportunity to drink water, within a short time they drink quantities of more than 1 cc. In the further course of the poisoning the animals became visible more exhausted and were soon no longer capable of moving forward or of turning themselves upright from a dorsal position. The initially accelerated respiration slowed down. At this point seizure-like

states were observed in ca. 20% of cases, similar to those described in other animal species upon administration of sodium chloride. These seizure-like phenomena are the more pronounced the earlier they occur and the less the animal appears to be otherwise affected. Such an animal, which until then had behaved quietly, suddenly began to jump high up, sometimes several centimeters high, stretching out both hind legs simultaneously in a frog-like manner. These springing movements were repeated in rapid succession, until finally the animal came to rest again with a strongly intensified respiration. As a rule this was soon sollowed by a new attack; in particular the slightest touch in the region of the root of the tail almost invariably was promptly followed by an attack. The animals became visibly exhausted from the attacks and as a rule it did not take long until the animals died, apparently of respiratory paralysis. Occasionally opisthotonos was observed. These spasm-like phenomena were probably the most striking features of the entire picture of poisoning and their form was quite characteristic. Similar phenomena have been described by Hermann Wieland ([1], p. 45) and by Pfeiffer ([2], p. 45) upon rapid heating of chilled mice. Mice breathing for a prolonged period in an atmosphere of 30% CO_2 and 70% O_2 also reveal these leaping movements when returned to a normal atmosphere. Whether these spasms are due to a common cause and what the nature of this cause might be will require more thorough investigation.

In some cases, sometimes fairly long before death, I observed a chalky opacity of the eyes. This was not an opacity of the lens, as described in the frog as sodium chloride cataract but of the cornea.

Dissection of the dead animals showed nothing unusual save for pronounced filling of the stomach and intestines with fluid.

The results of the experiments are shown in the next figures. The curves show the precentage of animals that died upon poisoning with a certain dose of sodium chloride of the appropriate concentration; the numbers entered next to individual dots give the number of experiments from which the precentual values are calculated.

It is readily apparent that the 5 m solution is less toxic than the more dilute ones, for while with the 3 m solution there were no more survivors at 105 mg, there were still nearly 10% survivors with the 5 m solution at 120 mg.

The difference is not great, but is nevertheless above the limits of error of the measurements. The lesser toxicity of the more highly concentrated solution could be more distinctly demonstrated by experiments in which the time course of the poisoning with lethal doses of sodium chloride was selected as the standard of toxicity.

Twelve mice of roughly the same weight were selected. Six received 0.36 cc of a 5 m NaCl solution by intubation, the other six twice this amount diluted in the same amount of distilled water, in other words a 2.4 m solution; thus the animals of

both groups each received 105 mg NaCl. The results of this experiment are shown in Table 1.

This experiment clearly demonstrates the stronger effect of the weaker sodium chloride solution: at a time at which the animals treated with the weaker solution had all died, there was only one death among the animals of the group that received the salt in the 5 m solution, and 2 of these animals actually survived the treatment.

It might be objected against these experiments that with fairly massive filling of the stomach by introducing large quantities of fluid, passage into the intestine and therewith absorption takes place more rapidly than when the doses are administered in a higher concentration, in other words occupying a smaller space. It might also be that the reflex closing of the pylorus, which forms an effective protective measure against peroral poisoning in animal species that do not vomit (Hatcher and Weill, [1], p. 48) is made stronger or more prolonged by a higher sodium chloride concentration.

In order to eliminate this objection a series of experiments were made in which a certain quantity of salt, but in varying concentration, was injected directly into the duodenum of mice.

The previously shaved abdomen of fullgrown mice was opened with a longitudinal cut under light ether anesthesia. Next the pylorus was carefully drawn forward under the liver with tweezers and tied off with a thread. The sodium chloride solution was then injected into the duodenum with a fine needle.

After the site of the injection on the intestine had been tied off so as to be sure to avoid any loss of fluid into the abdominal cavity, the abdominal wound was carefully closed with sutures. The entire procedure lasted ca. 10 min. Only those experiments were used in which there was no appreciable bleeding and in which at the latest 3 min after cessation of ether inhalation the animals rose upright from their dorsal position. The results of these experiments are shown in Table 2.

If we compare these results with those of Table 1, we are struck above all by the far quicker effect of the injection into the intestines. This difference certainly cannot be regarded as a consequence of the surgical procedure, since animals that only had their pylorus tied, but received no injection into the intestine, revealed no abnormal behavior and survived the intervention for many hours. But since the time differences are not very great with this more rapid process of poisoning, I repeated the experiment with a small quantity of NaCl (Table 3).

Here the differences are much more distinct: in 10-20% solution, NaCl acts far more rapidly than in 25 (and 5%) solution. It should be noted as a comparison with the experiments reported above that a 25% NaCl solution is approximately 4.3 molar.

Mention should also be made of a few experiments in which NaCl solution (100 mg in 0.3 cc water) was injected into the stomach instead of into the intestines after tying off the pylorus. These animals survived for a very long time and only showed signs of distress after ca. 7 hours, a time at which the control animals, who only had their pylorus tied off, also revealed

signs of distress. Animals that had the same quantity of NaCl introduced into their stomach had died within one hour. The result of these experiments is significant because it eliminates every hypothesis which seeks to link processes in the stomach, whether of osmotic or of some other nature, with death resulting from the ingestion of NaCl.

Behavior of the blood concentration upon oral administration of sodium chloride

It has been established as a clear result of our investigations that in acute NaCl poisoning local osmotic effects are not the essential factor. The fact that NaCl is actually absorbed into the blood is demonstrated by experiments in which the concentration of the blood was followed by a red cell count in the course of peroral NaCl poisoning. It is difficult to obtain sufficient blood for a blood cell count from mice poisoned with NaCl. For this reason the following experiments were carried out on rabbits into whose stomach large quantities of NaCl were introduced with a small amount of water. The cell counts were made according to Bürker's method (320 squares were counted). The experiments are shown in Table 4.

It is apparent from these experiments that in the course of acute lethal NaCl poisoning there was invariably a relative reduction in the number of red cells, in other words a dilution of the blood, or hydremia. I was never able to observe a thickening of the blood as found in the chicken by Heinz and Haas. The appearance of hydremia is distinctly against any sizeable displacement

of water from the blood into the intestinal lumen, since this, if noticeable in the blood at all, would have to be accompanied by a considerable loss of water from the blood, by a rise in erythrocyte values. The change in concentration observed in the blood, however, permits no other conclusion than that larger quantities of NaCl are absorbed into the blood. Whether the loss of water caused thereby is the cause of death is difficult to estimate, since we know neither the degree of tissue dehydration, nor do we know the water content necessary for the functioning of cells vital to life.

Summary

Studies on mice have shown that orally administered NaCl in medium concentrations is more toxic than in higher concentrations. This observation is also valid if the NaCl solution is introduced directly into the duodenum instead of the stomach. If the pylorus is tied off, NaCl introduced into the stomach has a much less toxic effect, if any. In rabbits there is distinct hydremia in the course of NaCl poisoning.

It is concluded from these findings that in acute NaCl poisoning local osmotic disturbances (effusion of fluid into the gastrointestinal tract, gastroenteritic alterations) are of no decisive importance and that the symptoms of poisoning and death after peroral administration of NaCl are linked to the absorption of salt into the blood.

A method of introducing measured quantities of fluid into the the stomach through an esophageal tube is described in detail.

Figures

1. Drawing based on x-ray picture of a mouse fed live with barium sulfate through the esophageal tube. After sacrifice the esophagus was also filled by pressure on the stomach.

2. Half schematic picture based on x-ray.

3. The tube lies in the stomach.

4. Absolute quantity of sodium chloride injected in mg. (abscissa). Ordinate: Number of dead mice %. Lösung = solution.

Tables

1. Key: 1. 5 molar NaCl solution, injected quantity 0.36 cc = 105 mg NaCl. 2. Mouse number. 3. Weight of the mouse in g. 4. Died after... 5. 3 hours and 30 min. 6. Survived some 18 hours.

2. Toxicity of sodium chloride upon injection into the duodenum. Key: See Table 1.

3. Toxicity of sodium chloride after injection into the duodenum. Key: 1. 50 mg NaCl injected in cc water. 2. Death of the animals after - min.

4. Behavior of erythrocyte values after peroral NaCl poisoning. Key: 1. Rabbit number. 2. Body weight in g. 3. Number of erythrocytes before administration of NaCl in Millions per 1 cc. 4. Blood drawn from. 5. Time after administration of NaCl. 6. Hours. 7. Number of erythrocytes after administration of NaCl in millions per 1 cc. 8. Blood drawn from. 9. Remarks. 10. Ear vein. 11. Carotid. 12. Neck Vein. 13. Heart. 14. 8.7 g NaCl in 50 cc water. Animal died the next day.

15. 20 g NaCl in 100 cc water. Death after 2 hours and 32 min. 16. 8.7 g NaCl in 50 cc water. Animal died as a result of an accident. 17. 10 g NaCl in 100 cc water. Death after 3 hours.

Bibliography

For references see footnotes.

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III.

Aus dem Pharmakologischen Institut der Universität Königsberg.

Untersuchungen über den Mechanismus der Kochsalzvergiftung.

I. Mitteilung: Die Bedeutung örtlicher osmotischer Vorgänge.

Von

Behrend Behrens.

(Mit 4 Abbildungen.)

(Eingegangen am 20. III. 1924.)

Es ist allgemein bekannt, daß selbst das Körperzellen gegenüber indifferenteste Neutralsalz, das Kochsalz, nicht nur auf die Zelle, sondern auch auf den ganzen Organismus, wenn es in Substanz oder in konzentrierten Lösungen zur Anwendung kommt, starke giftige Wirkungen entfalten kann. Besonders in der älteren Literatur finden sich ziemlich zahlreiche Berichte über Vergiftungen von Mensch und Tier durch die Aufnahme großer Kochsalzmengen (Literatur bei Kobert[1]); sogar als Selbstmordgift soll es in China eine Rolle spielen (s. Poulsson S. 354[2]).

Als Ursache dieser Giftwirkungen wird allgemein die sogenannte Salzwirkung angenommen, worunter die osmotischen Veränderungen verstanden werden, die Zellen bei der Berührung mit konzentrierten Salzlösungen erleiden. Gerade beim Kochsalz glaubt man diese physikalischen Eigenschaften eines Salzes in besonders reiner Form vor sich zu haben, während diese bei anderen Salzen durch die spezifischen Wirkungen ihrer Ionen mehr oder weniger verwischt werden.

Bei der Aufnahme von NaCl durch den Mund werden solche Salzwirkungen in erster Linie an den Schleimhäuten des Verdauungsapparates auftreten; da Kochsalz aber leicht resorbiert wird

1) Kobert, Lehrbuch der Intoxikationen (2) Bd. 2. S. 169.
2) Poulsson, Lehrbuch der Pharmakologie (3).

und damit der osmotische Druck des Blutes ansteigt, sind sie auch an allen anderen Geweben des Körpers zu erwarten.

In den Lehrbüchern der Pharmakologie und der Toxikologie wird als Todesursache nach peroraler Zufuhr von Kochsalz in Substanz oder konzentrierten Lösungen übereinstimmend die Austrocknung lebenswichtiger Organe durch den osmotischen Wasserentzug angenommen. Dagegen herrschen Unklarheit und Widerspruch über den Ort, nach dem hin die Wasserverschiebung stattfindet. Nach der Meinung der einen, die sich auf die Beobachtung entzündlicher Reizung der Magenschleimhaut und des Ergusses großer Flüssigkeitsmengen in den Magen-Darmkanal gründet, käme der Wasserverlust der Gewebe dadurch zustande, daß durch den Einstrom hypotonischer Flüssigkeit aus dem Blut in das Lumen des Verdauungskanals der osmotische Druck des Blutes ansteigt, und nun sekundär, wiederum auf Grund der osmotischen Druckunterschiede aus den Geweben Wasser ins Blut tritt. Dies scheint die Auffassung von Heinz und Haas[1]) zu sein, die sich jüngst mit der Frage der Kochsalzvergiftung experimentell beschäftigt haben; die beiden Verfasser finden zwar zunächst im Verlaufe der Vergiftung eines Huhnes mit Kochsalz eine Blutverdünnung, gegen Ende aber, wie sie offenbar erwartet haben, eine Eindickung.

Allgemeiner angenommen und besser begründet, scheint aber die andere Auffassung zu sein, nach der die Gewebswirkung unmittelbar durch den infolge von Kochsalzresorption gesteigerten osmotischen Druck bedingt wird, während den Veränderungen im Magen-Darmkanal keine entscheidende Bedeutung zugemessen wird. Für diese Annahme sprechen vor allem Versuche, nach denen NaCl und viele andere Salze, sowie andere Stoffe ohne ausgesprochene chemische Affinitäten ähnliche Erscheinungen hervorrufen, wie die Kochsalzfütterung, wenn sie intravenös eingespritzt werden (Münzer[2]), Müller[3]), Falk[4]), Hermanns[5])).

Manche Beobachtungen, wie die wiederholt beschriebenen Krämpfe und Lähmungserscheinungen (Hermanns[5]), Müller[3]), Falk[4]), Guttmann[6]), Limbourg[7])), dann der mitunter recht akute Verlauf der

1) Münch. med. Wochenschr. 1923, S. 565.
2) Arch. f. exp. Path. u. Pharm. 1898, Bd. 41, S. 74.
3) Dissertation Marburg 1872.
4) Arch. f. path. Anat. u. Phys. Bd. 56, S. 11.
5) Dissertation Marburg 1872.
6) Berl. Klin. Wochenschr. 1865, S. 868.
7) Arch. f. exp. Path. u. Pharm. 1888, Bd. 24, S. 346.

NaCl-Vergiftung lassen Zweifel daran auftauchen, ob wir berechtigt sind, in der osmotischen Wirkung des Kochsalzes eine restlose Erklärung für die große Giftigkeit zu erblicken. Es wäre denkbar, daß durch die enorme Vermehrung der Na-Ionen eine Störung im physiologischen Kationengleichgewicht zustande käme, daß also eine »Natriumvergiftung« mit im Spiele wäre, wie man ja auch von einer Kalium- oder einer Calciumvergiftung spricht, wenn eine Verschiebung nach dieser oder jener Seite erfolgt.

Schließlich erscheint es als durchaus möglich, daß die Zufuhr von Kochsalz zu einer Verschiebung des Basensäuregleichgewichtes im Organismus führt. So wäre daran zu denken, daß bei der Gegenwart hoher Kochsalzkonzentration die Dissoziation der Magensalzsäure so stark zurückgedrängt wird, daß erhebliche Mengen von molekularem Chlorwasserstoff auftreten. Damit wäre ein Zustand geschaffen, wie er für die Fluorwasserstoffvergiftung von Hermann Wieland und Kurtzahn[1]) eingehend erörtert worden ist: Die für H-Ionen undurchgängige Magenschleimhaut läßt die undissoziierten Moleküle des Halogenwasserstoffs leicht durchtreten; im Gewebe oder Blut, wo die Bedingungen gegeben sind, tritt der Zerfall in die Ionen und damit die Säurevergiftung ein.

Andere Möglichkeiten sollen an dieser Stelle nicht erörtert werden.

Eine erneute Untersuchung über den Mechanismus der Kochsalzvergiftung erscheint wegen der angedeuteten Unklarheiten dringend erwünscht, zumal aus einer Klärung dieses Grenzfalles der Kochsalzwirkung auch ein Einblick in die physiologische Bedeutung dieses wichtigen Nahrungs- und Körperbestandteiles erhofft werden konnte. Um meinen Untersuchungen eine sichere Grundlage zu geben, habe ich mich in diesem 1. Teil nur mit der Grundfrage beschäftigt, ob der Tod nach peroraler Zufuhr von Kochsalz eine Folge der Gegenwart des Salzes im Lumen des Magendarmkanals ist oder durch die Aufnahme dieses Stoffes in die Blutbahn bedingt wird.

Eine örtliche Schädigung durch Kochsalz müßte in Analogie der Versuche an Einzelzellen um so schwerer verlaufen, je höher die Konzentration der Lösung ist. Bei einer Schädigung durch Resorption wäre dagegen zu erwarten, daß mittlere Konzentrationen wirksamer sind als die höchsten, weil bei diesen der hohe osmotische Druck ein Resorptionshindernis darstellt, das erst allmählich — durch Verdünnung mit Darmsaft — überwunden wird. Es galt demnach

1) Arch. f. exp. Path. u. Pharm. 1923, Bd. 97, S. 489.

zu prüfen, wie groß die Giftigkeit von Kochsalz, d. h. die tödliche Dosis ist, wenn man Lösungen verschiedener Konzentration in den Magen bringt oder innerhalb welcher Zeit ein und dieselbe Kochsalzmenge den Tod des Versuchstiers herbeiführt, wenn sie zusammen mit wechselnder Wassermenge einverleibt wird.

Methodik.

Die Wahl der Tierart für derartige Versuche machte zunächst große Schwierigkeiten. Hund und Katze mußten für derartige Versuche ihres hohen Preises wegen von vornherein ausscheiden. Auch pflegen diese Tiere leicht Unerwünschtes durch Erbrechen wieder zu entleeren. Kaninchen haben, ebenso wie Meerschweinchen, meist einen vollen Magen und sind schon deshalb ungeeignet. Dagegen zeigte sich, daß die weiße Maus für derartige Versuche sehr gut geeignet ist. Abgesehen von ihrer Billigkeit bietet die Maus als Versuchstier noch weitere Vorteile: nach 1 Stunde ist der Mausemagen bereits leer, die Maus kann nicht erbrechen, und dabei ist der Magen ausgewachsener Tiere groß genug, um etwa 1,5 ccm bequem aufnehmen zu können. Die einzige Schwierigkeit, nämlich die, gemessene Mengen einer Flüssigkeit gewaltsam in den Magen zu bringen, wurde mit unerwartet geringer Mühe überwunden. Es zeigte sich nämlich, daß die Schlundsondierung bei der Maus trotz der kleinen Verhältnisse ziemlich leicht auszuführen ist. Das Verfahren soll eingehend beschrieben werden, da es sicher für eine allgemeinere Anwendung geeignet ist.

Man muß sich natürlich klar sein, daß bei den immerhin recht zarten Geweben einer Maus jedes brüske Vorgehen vermieden werden muß. Als

Abb. 1. Zeichnung nach Röntgenaufnahme einer lebend mit Bariumsulfatsuspension durch die Schlundsonde gefütterten Maus. Nach Tötung wurde der Ösophagus durch Druck auf den Magen ebenfalls gefüllt.

Hohlsonde habe ich nach einigen Versuchen eine starre Nickelkanüle von 7 cm Länge und 3/4 mm Dicke als allein geeignet gefunden. Das Ende

der Kanüle wird durch ein Tröpfchen Weichlot verschlossen, kugelförmig abgerundet und geglättet. Etwas oberhalb des Endes wird seitlich eine kleine Öffnung eingefeilt.

Bei dem Einführen der Sonde ist zu bedenken, daß, wie aus Abb. 1[1]) hervorgeht, der Ösophagus verschiedene, in der Hauptsache durch den den kleinen Säugetieren eigentümlichen Knick der Halswirbelsäule bedingte Biegungen macht, denen entsprechend die Richtung der Kanüle beim Einführen geändert werden muß, wie dies in Abb. 2 halbschematisch dargestellt ist. Die erste Richtung (I) ist nötig, um hinter die Zunge und richtig in den Schlund zu kommen. Es gelingt dies meist ohne Schwierigkeiten. Selten kommt es vor, daß man hierbei die Trachea komprimiert und das Tier zu ersticken droht. Auch die zweite Strecke des Ösophagus zu passieren (Richtung II) gelingt fast immer. Anders ist es mit dem

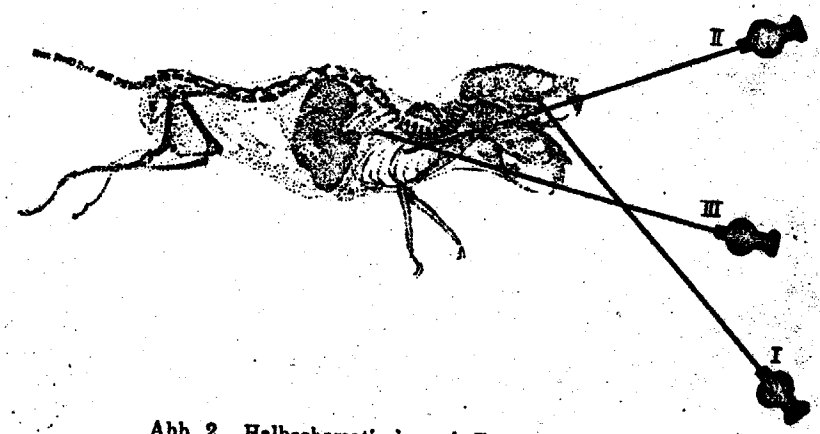

Abb. 2. Halbschematisch nach Röntgenaufnahmen.

letzten Teil (Richtung III); hier ist es nicht immer leicht, die Durchtrittsstelle des Ösophagus durch das Zwerchfell zu finden; sie liegt etwas nach hinten gegen die Wirbelsäule zu, hieraus ergibt sich die 3. Richtungsänderung. Die Durchtrittsstelle muß man mit vorsichtigen, tastenden Bewegungen zu erreichen suchen. Durch richtiges Halten des Tieres kann man sich das Auffinden ganz wesentlich erleichtern. Wenn man (s. Abb. 3), das Tier im Nacken mit Daumen und Zeigefinger der linken Hand faßt und den Schwanz mit dem Ringfinger gegen den Daumenballen drückt, so kann man durch Zug am Schwanz die Wirbelsäule strecken, wodurch der Halsknick kleiner wird, die Richtung III kann dann, besonders bei alten Mäusen, weniger von der 2. Richtung abweichen. Es bestehen, wie auch nicht anders zu erwarten, große Verschiedenheiten. Störend wirkt bisweilen, daß die Mäuse bei Abwehrbewegungen mit den Vorderpfoten die Kanüle umklammern; man hilft sich dagegen, indem man die Hautfalte, mit der

1) Die Anfertigung der Röntgenaufnahmen wurde mir von Herrn Geheimrat Matthes in dankenswerter Weise ermöglicht.

man das Tier im Nacken faßt, so groß nimmt, daß es gehindert ist, bis zur Kanüle zu langen. Im Anfang kam es manchmal, besonders an der

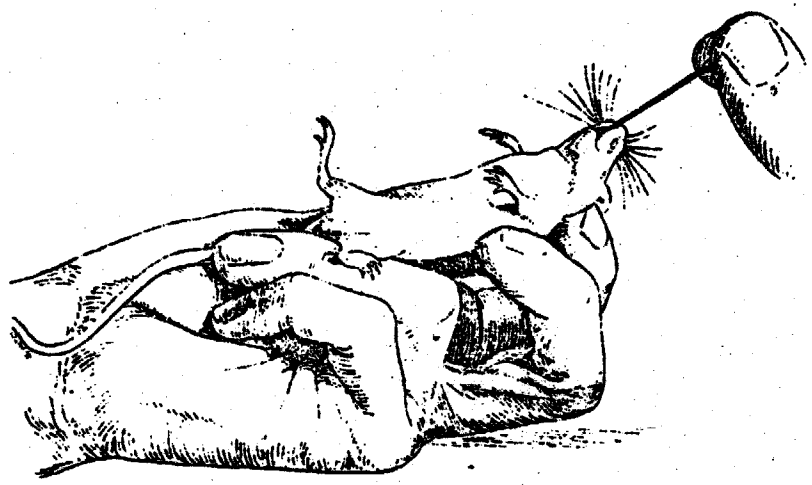

Abb. 3. Die Sonde liegt im Magen.

Durchtrittsstelle durch das Zwerchfell, vor, daß durch eine ungeschickte Bewegung der Ösophagus perforiert wurde. Ich setzte deshalb der zu injizierenden Lösung etwas Bariumsulfat zu und kontrollierte die gestorbenen Mäuse durch Feststellung dieses Stoffes in dem Magen oder Darm und das Fehlen desselben in der freien Bauchhöhle bei der Sektion. Bei einiger Übung ist diese Vorsichtsmaßregel nicht mehr notwendig. Um einen ungefähren Anhalt zu haben, wann die Richtungsänderungen vorzunehmen sind, — es ist natürlich bei den einzelnen Mäusen etwas verschieden —, befinden sich an meiner Sonde, von der Spitze aus gemessen, je eine Marke bei 3,2 und 4,5 cm. Die vordere Marke zeigt an, wann ungefähr Übergang von Richtung II zu Richtung III stattfinden muß, die andere Marke, wann die Sonde sicher im Magen liegt. Dies kann man auch durch einen leichten Druck auf die Magengegend mit dem Finger sehr gut feststellen.

Bei der Injektion ist darauf zu achten, daß der Inhalt der Kanüle keine Ursache zu Ungenauigkeiten gibt; deshalb wurde in der Regel die Sonde mit angeschlossener, gefüllter Spritze eingeführt, ein Verfahren, das etwas schwieriger ist als die Sondierung mit der Kanüle allein.

Auf diese Weise habe ich gegen 500 Mäuse sondiert. Zuletzt ist mir in weniger wie 1% der Fälle die Sondierung nicht geglückt. Auch wiederholtes Einführen der Sonde gelingt ohne Schwierigkeit und wird anstandslos vertragen. Für Versuche zu einer anderen Fragestellung habe ich z. B. bei derselben Maus die Sondierung des Magens mit Fütterung mehr als 50 Tage lang täglich ausgeführt.

Versuche.

Gruppen von mindestens zehn, möglichst gleichen, 2 Stunden nüchternen Mäusen, im Gewicht von 16—19 g, wurden wechselnde Mengen einer 2-, 3-, 4- und 5molaren Kochsalzlösung mit einer Tuberkulinspritze, die in $^1/_{50}$ ccm geteilt war, in den Magen gebracht. Die Tiere wurden dann, ohne daß ihnen Zugang zu Futter und Wasser möglich war, beobachtet. Sie zeigen gleich nach der Injektion, sobald sie sich von dem Eingriff erholt haben, was nach wenigen Sekunden der Fall ist, völlig normales Verhalten. Nach einiger Zeit stellt sich dann ausgesprochene Schläfrigkeit ein, die Tiere sitzen zusammengekauert in einer Ecke. In diesem Stadium ist bei den Tieren ein großes Durstgefühl vorhanden; läßt man den Tieren die Möglichkeit, Wasser zu trinken, so werden in kurzer Zeit von ihnen Mengen über 1 ccm aufgenommen. Im weiteren Verlauf der Vergiftung werden die Tiere zusehends matter, sie sind bald nicht mehr befähigt, sich fortzubewegen oder sich aus der Rückenlage wieder aufzurichten. Die anfangs beschleunigte Atmung wird langsamer. In dieser Zeit können wir nun, etwa in 20% der Fälle, krampfartige Zustände beobachten, ähnlich wie dies bei anderen Tierarten nach Kochsalzgaben beschrieben ist. Diese krampfartigen Erscheinungen sind um so heftiger, je früher sie auftreten, und je weniger das Tier sonst schon mitgenommen erscheint. Plötzlich beginnt ein solches Tier, das sich bis dahin völlig ruhig verhalten hatte, in die Höhe zu springen, mitunter mehrere Zentimeter hoch, indem es froschähnlich beide Hinterbeine gleichzeitig streckt. Diese springenden Bewegungen wiederholen sich in rascher Folge, bis schließlich das Tier mit stark gesteigerter Atmung wieder zur Ruhe kommt. Es dauert jedoch meist nicht lange, und ein neuer Anfall beginnt. Auffällig ist, daß durch den geringsten sensiblen Reiz ein Anfall auszulösen ist; besonders die leiseste Berührung in der Gegend der Schwanzwurzel wird fast immer prompt mit einem Anfall beantwortet. Durch die Anfälle wird das Tier zusehends matter, und es dauert meist nicht lange, bis — anscheinend durch Atemlähmung — der Tod eintritt; mitunter ist dabei das Auftreten eines Opisthotonus zu beobachten. Diese krampfartigen Erscheinungen sind wohl das auffälligste an dem ganzen Vergiftungsbild, ihre Form ist ganz charakteristisch. Gleiche Erscheinungen sind von Hermann Wieland[1]), sowie von Pfeiffer[2]) bei schnellem Erwärmen abgekühlter

1) Arch. f. exp. Path. u. Pharm. 1922, Bd. 92, S. 133.
2) Zeitschr. f. ges. exp. Med. 1922, Bd. 29, S. 75.

Mäuse beschrieben worden. Auch Mäuse, die in einer Atmosphäre von 30% CO_2 und 70% O_2 längere Zeit geatmet haben, zeigen, sobald man sie wieder in eine normale Atmosphäre bringt, diese hüpfenden Bewegungen. Ob diesen Krämpfen eine gemeinsame Ursache zugrunde liegt und welcher Art dieselbe ist, bedürfte einer eingehenden Untersuchung.

In einigen Fällen habe ich, manchmal schon ziemlich lange vor Eintreten des Todes eine kreideartige Trübung der Augen beobachtet. Es handelte sich nicht um eine Linsentrübung, wie sie beim Frosch als Kochsalzstar beschrieben ist, sondern um eine Trübung der Hornhaut.

Die Sektion der gestorbenen Tiere zeigte außer einer starken Füllung des Magens und Darmes mit Flüssigkeit nichts besonderes.

Die Ergebnisse der Versuche zeigt die folgende Darstellung. Die Kurven geben an, wieviel Prozent der Tiere bei der Vergiftung mit einer bestimmten Dosis Kochsalz in der entsprechenden Konzentration eingegangen sind; die den einzelnen Punkten beigefügten Ziffern bedeuten die Anzahl der Versuche, aus der die Prozentwerte berechnet sind.

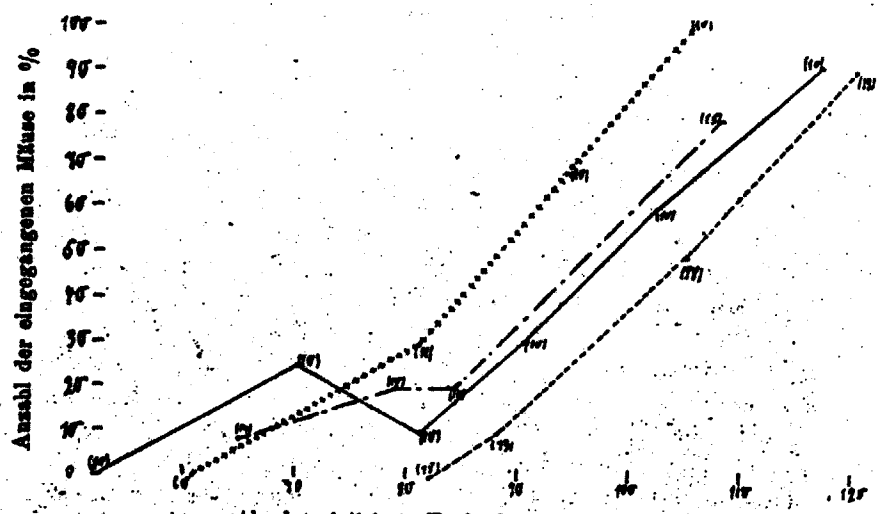

Abb. 4. —— 2 m-Lösung, ++++ 3 m-Lösung, —·—· 4 m-Lösung, ----- 5 m-Lösung.

Es ist ohne weiteres zu erkennen, daß die 5 m-Lösung weniger giftig ist, als die verdünnteren. Während nämlich bei der 3 m-Lösung bei 105 mg keine Überlebenden mehr zu verzeichnen sind, finden wir bei der 5 m-Lösung noch bei 120 mg beinahe 10%.

Der Unterschied ist nicht groß, liegt aber immerhin über der Fehlergrenze der Abmessung. Deutlicher erwiesen wird die Tatsache einer geringeren Giftigkeit der höher konzentrierten Lösung durch Versuche, in denen der zeitliche Verlauf der Vergiftung mit tödlicher Kochsalzgabe als Maßstab der Giftigkeit gewählt wurde.

Es wurden zwölf Mäuse von etwa gleichem Körpergewicht ausgesucht. Sechs erhielten durch die Schlundsonde 0,36 ccm einer 5 m-Kochsalzlösung, die anderen sechs die doppelte Menge derselben mit der gleichen Menge destillierten Wassers verdünnten, also einer 2,5 m-Lösung; die Tiere beider Gruppen demnach je 105 mg Kochsalz. Das Ergebnis dieses Versuches zeigt die folgende Tabelle.

Tabelle 1.

5 m-Kochsalzlösung injizierte Menge 0,36 ccm = 105 mg NaCl			2,5 m-Kochsalzlösung injizierte Menge 0,72 ccm = 105 mg NaCl		
Maus Nr.	Gewicht der Maus in g	Eingegangen nach	Maus Nr.	Gewicht der Maus in g	Eingegangen nach
1a	16,5	3 Stund. 30 Min.	1b	17,0	— Stund. 30 Min.
2a	15,5	9 » 15 »	2b	16,5	— » 40 »
3a	17,0	9 » 15 »	3b	16,5	2 » 40 »
4a	15,0	etwa 18 Stund.	4b	18,5	2 » 50 »
5a	16,0	überlebt	5b	18,0	3 » 40 »
6a	17,0	»	6b	16,0	9 » — »

Dieser Versuch läßt die stärkere Wirksamkeit der schwächeren Kochsalzlösung klar erkennen: Zu einer Zeit, wo die damit behandelten Tiere schon alle eingegangen waren, ist bei der anderen Gruppe, der das Salz in 5 m-Lösung verabfolgt war, erst ein Todesfall zu verzeichnen, und zwei dieser Tiere haben den Eingriff sogar überlebt.

Gegen diese Versuche ließe sich nun der Einwand erheben, daß bei stärkerer Füllung des Magens durch die Einführung größerer Flüssigkeitsmengen der Übertritt in den Darm und damit die Resorption rascher erfolgen, als wenn die Gaben in höherer Konzentration, also in kleineren Raummengen verabreicht werden. Es wäre auch daran zu denken, daß der reflektorische Pylorusverschluß, der bei den Tierarten, die nicht erbrechen, eine wirksame Schutzmaßnahme

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gegen perorale Vergiftung darstellt (Hatcher und Weiß[1]), durch eine höhere Kochsalzkonzentration in stärkerem Maße oder für längere Zeit bewirkt wird.

Um diesen Einwand auszuschalten, wurden Versuchsreihen angestellt, in denen eine bestimmte Kochsalzmenge, aber in wechselnder Konzentration, Mäusen unmittelbar ins Duodenum eingespritzt wurde.

Ausgewachsenen Mäusen wurde in leichter Äthernarkose der vorher rasierte Bauch in der Längsrichtung eröffnet; dann wurde der Pylorus unter der Leber vorsichtig mit einer Pinzette hervorgezogen und mit einem Faden abgebunden. Es wurde nun mit einer feinen Nadel die Kochsalzlösung in das Duodenum injiziert. Nachdem auch die Injektionsstelle am Darm, um ein Austreten von Flüssigkeit in die Bauchhöhle sicher zu vermeiden, abgebunden war, wurde die Bauchwunde mit einer Naht sorgfältig geschlossen. Der ganze Eingriff dauerte etwa 10 Minuten. Es wurden nur die Versuche verwandt, in denen keine wesentlichen Blutungen auftraten und bei denen die Tiere spätestens 3 Minuten nach Beendigung der Äthereinatmung sich aus der Rückenlage aufrichteten. Über die Ergebnisse dieser Versuche unterrichtet die folgende Tabelle.

Tabelle 2.

Giftigkeit von Kochsalz bei Einspritzung ins Duodenum.

5 m-Kochsalzlösung injizierte Menge 0,36 ccm = 105 mg NaCl			2,5 m-Kochsalzlösung injizierte Menge 0,72 ccm = 105 mg NaCl		
Maus Nr.	Gewicht der Maus in g	Eingegangen nach	Maus Nr.	Gewicht der Maus in g	Eingegangen nach
1a	17,8	1 Stund. 28 Min.	1b	19,1	1 Stund. 10 Min.
2a	15,3	1 » 7 »	2b	15,4	— » 47 »
3a	19,3	1 » 4 »	3b	16,6	— » 41 »
4a	16 3	1 » 4 »	4b	17,7	— » 39 »

Wenn wir diese Ergebnisse mit denen der Tabelle 1 vergleichen, so fällt vor allem die viel raschere Wirkung bei der Injektion in den Darm auf. Diesen Unterschied kann man sicher nicht als eine Folge des operativen Eingriffs betrachten, denn Tiere, denen nur der Pylorus unterbunden, aber nichts in den Darm injiziert worden war, zeigen kein abnormes Verhalten und überleben den Eingriff viele Stunden. Da nun bei diesem rascheren Ablauf der Vergiftung die zeitlichen Unterschiede nicht sehr groß sind, habe ich den Versuch mit einer kleineren NaCl-Menge wiederholt (Tabelle 3).

1) The Journ. of Pharmac. and Exp. Therap. 1923, Bd. 22, S. 145.

Tabelle 3.

Giftigkeit des Kochsalzes nach Einspritzung ins Duodenum.

	50 mg NaCl injiziert in ccm Wasser			
	1 (5%)	0,5 (10%)	0,35 (20%)	0 20 (25%)
Tod der Tiere nach Min.	165	50	56	130
	236	78	86	130

Hier sind die Unterschiede sehr viel deutlicher: In 10—20%iger Lösung wirkt Kochsalz viel rascher als in 25 (und 5%iger. Zum Vergleich mit den oben mitgeteilten Versuchen sei bemerkt, daß eine 25%ige Kochsalzlösung etwa 4,3-molar ist.

Anschließend seien noch einige Versuche erwähnt, bei denen Kochsalzlösung (100 mg in 0,3 ccm Wasser) nach Abbinden des Pylorus, statt in den Darm, in den Magen injiziert wurde. Diese Tiere überlebten sehr lange und zeigten erst nach etwa 7 Stunden Störungen, zu einer Zeit, in der Kontrolltiere, denen nur der Pylorus unterbunden war, ebenfalls Veränderungen aufwiesen. Tiere, denen dieselbe Kochsalzmenge in den Darm eingebracht war, waren bereits nach 1 Stunde eingegangen. Das Ergebnis dieser Versuche ist deshalb von Bedeutung, weil damit jede Hypothese hinfällig wird, die Vorgänge im Magen, sei es osmotischer oder anderer Art, mit dem Tod nach Kochsalzzufuhr zu verknüpfen sucht.

Das Verhalten der Blutkonzentration bei peroraler Kochsalzzufuhr.

Als eindeutiges Ergebnis unserer Versuche läßt sich feststellen, daß bei der akuten Kochsalzvergiftung, die lokalen osmotischen Wirkungen nicht das wesentliche sind. Daß Kochsalz in der Tat in das Blut resorbiert wird, zeigen Versuche, in denen die Konzentration des Blutes durch Zählung der roten Blutkörperchen im Verlauf der peroralen Kochsalzvergiftung verfolgt wurde. Von Mäusen, die mit Kochsalz vergiftet worden waren, ist schwer genügend Blut zur Blutkörperzählung zu gewinnen; die folgenden Versuche sind deshalb an Kaninchen angestellt, denen größere Kochsalzmengen mit wenig Wasser in den Magen eingeflößt wurden. Die Zählung der Zellen erfolgte nach der Bürkerschen Methode (es wurden je 320 Quadrate ausgezählt). Die Versuche sind in der folgenden Tabelle zusammengestellt.

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Tabelle 4.

Verhalten der Blutkörperzahl nach peroraler Kochsalzvergiftung.

Kaninchen Nr.	Körpergewicht in g	Erythrocytenzahl vor der Kochsalzzufuhr Millionen in 1 cmm	Blutentnahme aus	Zeit nach der Kochsalzgabe Std.	Min.	Erythrocytenzahl nach Kochsalzzufuhr Millionen in 1 cmm	Blutentnahme aus	Bemerkungen
1	2200	4,9	Ohrvene	5 8	— —	5,1 4,45	Halsvene	8,7 g NaCl in 50 ccm Wasser. Tier am nächsten Tage tod.
4b	3200	4,3	Carotis	2	32	3,95	Herz	20 g NaCl in 100 ccm Wasser. Tod nach 2 Stunden 32 Minuten.
6	2200	4,54	»	5 7	— —	4,65 4,3	Carotis »	8,7 g NaCl in 50 ccm Wasser. Tier starb durch Unfall.
7	1600	4,5	»	3	—	4,3	»	10 g NaCl in 100 ccm Wasser. Tod nach 3 Stunden.

Aus diesen Versuchen geht hervor, daß im Verlauf der akuten tödlichen Kochsalzvergiftung regelmäßig eine relative Verminderung der roten Blutkörperchen, also eine Verdünnung des Blutes, eine Hydrämie eintritt. Eine Eindickung des Blutes, wie sie von Heinz und Haas in einem Versuch am Huhn gefunden worden ist, habe ich nie feststellen können. Das Auftreten der Hydrämie spricht entschieden dagegen, daß eine Wasserverschiebung aus dem Blut in das Darmlumen in wesentlichem Umfang stattfindet, da diese ja, wenn sie sich überhaupt am Blute bemerkbar macht, mit einer Wasserverarmung des Blutes, einem Anstieg der Erythrocytenwerte einhergehen müßte. Die beobachtete Konzentrationsänderung des Blutes läßt im Gegenteil keinen anderen Schluß zu als den, daß größere Kochsalzmengen in das Blut resorbiert wurden. Ob nun der hierdurch bedingte Wasserentzug die Todesursache ist, läßt sich schlecht beurteilen, da wir weder den Grad der Gewebsaustrocknung kennen, noch wissen, welcher Wassergehalt für die Tätigkeit lebenswichtiger Zellen notwendig ist.

Zusammenfassung.

Untersuchungen an Mäusen haben ergeben, daß peroral zugeführtes Kochsalz in mittleren Konzentrationen giftiger ist als in höheren. Diese Feststellung gilt auch dann, wenn man die Kochsalzlösung statt in den Magen, unmittelbar in das Duodenum bringt. Wird der Pylorus unterbunden, so wirkt in den Magen gebrachtes Kochsalz, wenn überhaupt, viel weniger giftig. Bei Kaninchen tritt im Verlauf der Kochsalzvergiftung eine deutliche Hydrämie ein.

Aus diesen Befunden wird geschlossen, daß bei der akuten Kochsalzvergiftung den örtlichen osmotischen Störungen (Flüssigkeitserguß in den Magen-Darmkanal, gastro-enteritischen Veränderungen) keine entscheidende Bedeutung zukommt, sondern daß die Vergiftungserscheinungen und der Tod nach peroraler Kochsalzzufuhr mit der Aufnahme des Salzes ins Blut zusammenhängen.

Ein Verfahren, Mäusen durch die Schlundsonde abgemessene Flüssigkeitsmengen in den Magen zu bringen, wird eingehend beschrieben.

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Best, C.H. and N.B. Taylor. 1966

Physiological Basis of Medical Practice, 8th Edition

Williams and Wilkins Co., Baltimore, Md.
pp. 495; 1351; 1477; 1689-1690

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Arch. int. Pharmacodyn., 1963, 144, No 1-2.

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THE ACUTE ORAL TOXICITY OF SODIUM CHLORIDE

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BOYD and SHANAS (1) have reported that the $LD_{50} \pm$ S.E. of potassium chloride given orally to young, adult, female rats on an empty stomach is 3.02 ± 0.14 gm per kgm body weight. Most of the animals died within 10 hours from respiratory failure, acute gastroenteritis, dehydration, and renal failure. Survivors had anorexia, polydipsia, polyuria, fever, convulsive movements, and gastrointestinal disturbances for 24 hours but rapidly recovered thereafter. To determine to what extent this syndrome was due to potassium ion, an identical study of sodium chloride was made and the results are herein reported.

SPECTOR (2) has reviewed published data upon lethal doses of sodium chloride. He reports doses of from 2.5 to 5.0 gm per kgm to be lethal when given subcutaneously, intraperitoneally, and intravenously to mice, rats, and guinea pigs. These doses were about fourfold those of corresponding reports upon potassium chloride reviewed by SPECTOR (2).

From data reviewed by USSING *et al.* (3), it may be calculated that doses of sodium chloride of the order of 2.9 gm per kgm are lethal when given intravenously as physiological saline to mice, but susceptibility to death increases with increase in the rate of infusion (4). When given by mouth, USSING *et al.* (3) report sodium chloride tolerated in doses which may be variously calculated at about 0.8 gm per kgm in man, 2 gm per kgm in rats, and 2.5 gm per kgm in dogs. The figure for man was derived from experiments in which isotonic saline solution was given by intragastric drip over a period of 21 hours (5).

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From these data, it was decided to try pilot runs at doses of sodium chloride of from 0.8 to 16 gm per kgm given orally as a single dose on an empty stomach. The clinical signs to be anticipated from such doses were estimated from SOLLMANN's review (6) to be a "severe and fatal gastroenteritis", probably accompanied by restlessness, thirst, polyuria, fever, convulsions, coma, and death within 8 hours.

METHOD

The project was performed upon 168 albino rats of a Wistar strain which has been bred in the Animal Quarters of the Department of Pharmacology at Queen's University since 1937. Half of the animals were males of mean $\pm$ standard deviation body weight of 202 $\pm$ 42 gms, and the other half females weighing 167 $\pm$ 27 gms. They were fed Purina fox chow checkers and water ad libitum. For oral dosing, they were placed in metabolism cages, one rat per cage, and given water but no food for 16 hours. Sodium chloride was given by intragastric tube in doses of 0.0, 0.8, 3.0, 3.2, 3.5, 3.8, 4.0, 5.0, 10.0, and 16.0 gm per kgm, with 12 to 44 rats per dose. Each dose was dissolved in distilled water and given in a volume of 20 ml per kgm body weight, except for the two largest doses which were given in larger volumes. Clinical and pathological methods used were those of BOYD and SHANAS (1). The statistical methods employed were those of CROXTON (7) and WAUGH (8).

RESULTS

At doses of 3.0 to 5.0 gm per kgm, regression of dose (Y axis) on percent mortality (Y axis) was linear. The estimating equation $\pm$ its standard error was $Y = (3.05 + 0.0168\ X) \pm 0.30$ for males and $Y = (2.69 \pm 0.0185\ X) \pm 0.30$ for females. The estimated $LD_{50} \pm$ S.E. was 3.89 $\pm$ 0.30 gm per kgm for males and 3.62 $\pm$ 0.30 for females, the difference being statistically insignificant ($P > 0.05$).

Death occurred earlier the larger the administered dose of sodium chloride as shown by results summarized in Figure 1. All animals receiving the two largest doses died within 0.5 to 4.25 hours. There were no deaths in animals receiving the smallest dose. The mean $\pm$ standard deviation hours to death in animals receiving from 3.0 to 5.0 gm per kgm was 9.8 $\pm$ 4.1 for males and 6.9 $\pm$ 3.7 for females with $P = 0.05$ that the two means were identical. Not included in the estimate

of interval for males was 1 animal which died 5 days after receiving a dose of 5.0 gm per kgm. The coefficient of correlation between dose and mean hours to death per dose in males was not significantly different ($P = 0.07$) from that in females as shown in Figure 1.

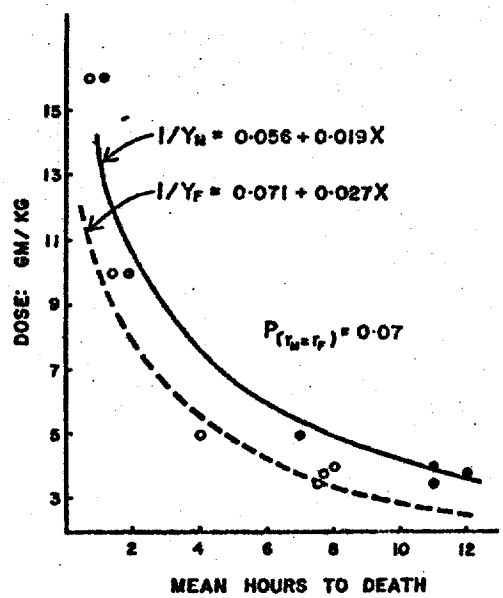

FIG. 1

The regression of dose of sodium chloride on mean hours to death in males (Y_m, solid line, and solid circles) and females (Y_f, interrupted line, and open circles).

The incidence of other clinical signs in males was insignificantly different from that in females. The presence or absence of a series of clinical signs was recorded on each animal during a period of seven hours following drug administration. Over this period, 56 % of animals exhibited convulsive movements, the incidence increasing logarithmically with dose (FIG. 2). The incidence of thirst-drinking immediately after drug administration—averaged 25 % but decreased with increase in dosage, as shown in Figure 2. Other signs were mucous diarrhea in 35 % of animals and flexor rigidity in 15 %. The immediate cause of death was respiratory failure following prostration.

At autopsy on animals which died following drug administration, the most common histopathologic sign was capillary and venous congestion. This was marked in the lamina propria and submucosa of the pyloric stomach, the small bowel, cecum and colon, and in the red pulp of the spleen, the kidneys (especially in the loop region), the meninges, the

cerebrum, and the cerebellum. Congestion was less marked in cardiac stomach, liver, the adrenal glands, testes, ovaries, thymus gland, and heart. No congestion was seen in skeletal muscle, lungs and skin.

Most organs of the body in nonsurviving albino rats had lost significant amounts of water as shown by data summarized in Table I. This, in turn, produced loss of wet weight which was significant in colon, kidneys, liver and testicles. Percentage changes in weight and water in males were insignificantly different from those in females. Hence data upon both males and females were used in compiling the results given in Table I. Values in control rats given no sodium chloride were similar to those reported by BOYD and SHANAS (1).

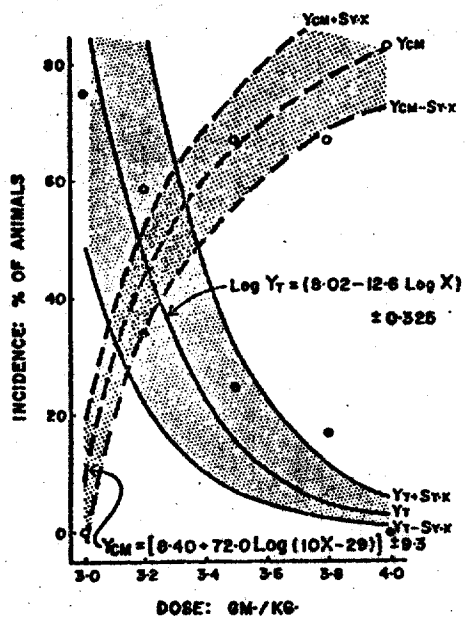

FIG. 2

The regression on dose of the incidence of thirst or immediate drinking (Y_T, solid lines, and solid circles) and convulsive movements, (Y_{CM}, interrupted lines, and open circles) during the first seven hours following oral administration of sodium chloride to albino rats.

Some evidence was obtained which indicated that loss of water in certain organs of rats given the same dose expressed as gm per kg body weight, was correlated with the amount of sodium chloride given per rat. Otherwise expressed, loss of water within a dosage group was greater—in certain organs—the greater the body weight. To test this possibility, weight and water levels were measured in 15 rats given the

TABLE I

Shifts in the wet weight and water levels or organs of albino rats given doses of sodium chloride in the range of the oral median lethal dose (1)

Organ	Nonsurvivors		Survivors at 2 weeks	
	Weight	Water	Weight	Water
Adrenal glands	− 3.2	−20.4*	+ 1.3	− 1.9
Brain	− 5.8*	−31.1*	+ 4.9	− 0.8
G.I. Tract				
a) Cardiac stomach	+ 6.8	−14.4*	+ 5.0	− 8.8*
b) Pyloric stomach	− 6.5	−22.3*	+ 5.7	− 3.7
c) Small bowel	−14.8	− 5.0	− 9.9	−16.9*
d) Cecum	−13.9	− 2.9	+ 8.9	− 4.7
e) Colon	−16.6*	−17.5*	+ 3.9	− 3.7
Heart	− 3.6	−17.2*	− 0.9	+ 1.1
Kidneys	−12.9*	−14.6*	+ 3.0	+ 1.6
Liver	−38.4*	−23.2*	+11.1*	− 1.1
Lungs	− 1.5	− 7.4	+14.4	+ 1.1
Muscle (abd. wall)	− 5.7	−14.8*	+ 3.5	+ 6.4
Ovaries	−11.3	−24.3*	−11.3	+10.1
Skin	+ 8.7	−10.7	+ 3.7	+ 3.5
Spleen	− 7.9	−22.8*	− 3.1	+ 0.5
Testicles	−13.9*	−14.5*	− 5.2	− 0.5
Thymus gland	− 9.0	−15.3*	−12.4	− 1.5
Residual carcass	− 0.4	−15.0*	+ 4.0	− 1.3

(1) Wet weight was measured in gm and water levels in gm per 100 gm dry weight of tissue. Shifts are expressed as a mean percentage change from corresponding controls given no sodium chloride, specifically as $(X_d - X_c)/X_c) \times 100$ where X_d refers to the mean in the drug-treated and X_c in the control animals. Shifts significantly different from control values at $P \leq 0.05$ are indicated by an asterisk.

same dose of sodium chloride, namely 10 gm per kg body weight. All of these animals died within 2 hours except two which died at 3 and 4 hours after the drug. Water levels in brain, kidney and liver were significantly negatively correlated with gms sodium chloride per rat. In most other organs, the corresponding coefficients of correlation were also negative but at $P > 0.05$ that correlation was zero. The regression analysis for brain is shown as an example in Figure 3. Vascular congestion was extremely marked in the meninges and in the grey and white matter of brain (FIG. 4).

The cause of death, therefore, was respiratory failure associated with an acute encephalopathy, accompanied by a fulminating gastroenteritis,

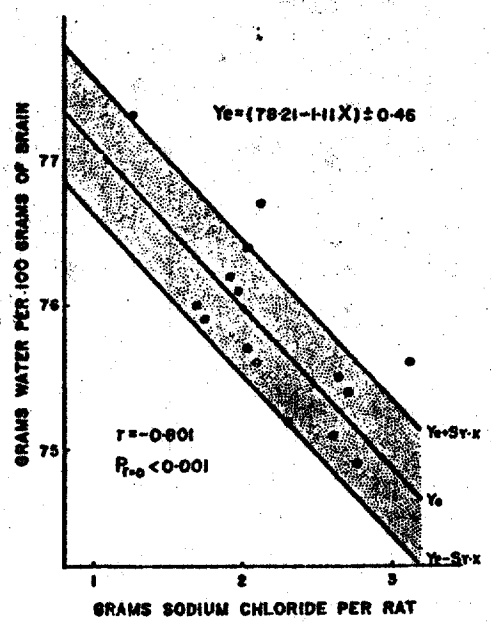

FIG. 3

The regression of brain water levels on grams of sodium chloride given orally to albino rats at a dose of 10 gm per kgm body weight. (Figures on the abscissa also represent body weight expressed in units of tenth kilograms).

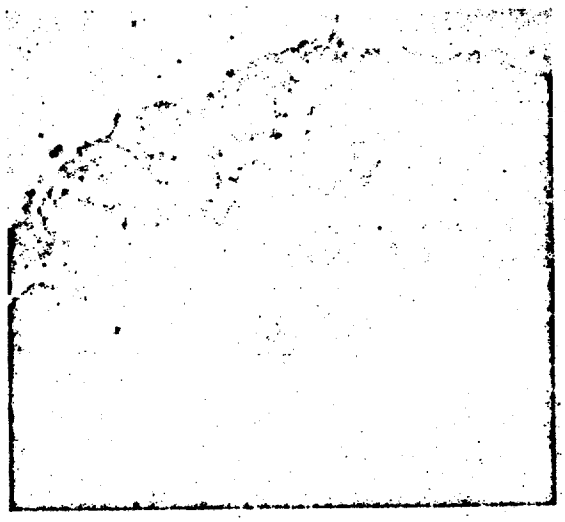

FIG. 4

A microphotograph showing extreme vascular congestion of the meninges and brain in an albino rat which died 2 hours after oral administration of sodium chloride at a dose of 10 gm per kgm body weight.

TABLE II

Shifts in the body weight, food intake, water intake, and colonic temperature of albino rats surviving oral doses of sodium chloride in the range of the LD_{50} (1)

Dose gm per kgm	N	Days after Sodium Chloride 1	2	3
		Body Wt. Gain per 24 Hrs. : Grams (+ or −)		
0.0	44	+ 17.6 ± 8.5	+ 3.7 ± 5.6	+ 2.0 ± 4.8
0.8	12	+ 16.1 ± 4.2	− 0.3 ± 5.5	+ 8.3 ± 5.0
3.0	12	+ 15.0 ± 7.7	+ 1.4 ± 5.4	+ 2.4 ± 3.1
3.2	9	+ 7.0 ± 9.0	+ 8.6 ± 7.5	+ 1.2 ± 4.0
3.5	7	+ 11.7 ± 9.8	− 1.4 ± 7.9	+ 0.7 ± 3.5
3.8	5	+ 4.6 ± 8.8	+ 7.1 ± 6.3	+ 4.2 ± 3.3
4.0	2	+ 4.7 ± 9.1	+ 3.7 ± 3.3	− 0.3 ± 5.5
		Food Intake : gm/kgm/24 hrs		
0.0	44	110 ± 17	101 ± 17	102 ± 13
0.8	12	122 ± 6	101 ± 15	114 ± 14
3.0	12	84 ± 19	106 ± 11	104 ± 14
3.2	9	75 ± 18	90 ± 8	83 ± 22
3.5	7	53 ± 29	98 ± 36	71 ± 23
3.8	5	63 ± 12	91 ± 13	97 ± 10
4.0	2	49 ± 31	81 ± 14	88 ± 6
		Water Intake : ml/kgm/24 hrs		
0.0	44	216 ± 39	165 ± 45	167 ± 29
0.8	12	228 ± 20	142 ± 29	175 ± 48
3.0	12	216 ± 89	177 ± 43	173 ± 35
3.2	9	229 ± 59	193 ± 52	160 ± 45
3.5	7	219 ± 88	150 ± 68	130 ± 67
3.8	5	224 ± 40	163 ± 50	180 ± 8
4.0	2	253 ± 64	196 ± 53	129 ± 28
		Colonic Temperature : °F		
0.0	44	99.3 ± 0.5	99.4 ± 0.9	99.2 ± 0.8
0.8	12	99.0 ± 0.8	99.3 ± 0.6	98.8 ± 0.5
3.0	12	100.2 ± 0.7	99.6 ± 0.6	99.8 ± 0.6
3.2	9	99.5 ± 0.9	99.4 ± 0.9	98.5 ± 1.4
3.5	7	99.7 ± 0.7	100.5 ± 0.5	99.6 ± 0.9
3.8	5	100.5 ± 0.4	100.0 ± 0.7	99.4 ± 0.4
4.0	2	101.5 ± 1.1	100.5 ± 1.1	100.0 ± 1.0

(1) The results are expressed as mean ± standard deviation.

TABLE III

Urinary volume and composition in albino rats surviving oral doses of sodium chloride in the range of the LD_{50} (1)

Dose gm/kgm	N	Days after Sodium Chloride 1	2	3
		Volume : ml kgm/24 hrs		
0.0	44	40 ± 19	36 ± 22	38 ± 24
0.8	12	46 ± 9	41 ± 17	37 ± 16
3.0	12	141 ± 57	48 ± 32	10 ± 9
3.2	9	108 ± 25	54 ± 22	56 ± 32
3.5	7	83 ± 34	29 ± 27	35 ± 30
3.8	5	150 ± 12	35 ± 8	58 ± 17
4.0	2	173 ± 38	53 ± 10	34 ± 12
		Glucose : gm/100 ml		
0.0	44	0.02 ± 0.07	0.08 ± 0.11	0.06 ± 0.10
0.8	12	0.12 ± 0.12	0.17 ± 0.12	0.19 ± 0.17
3.0	12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3.2	9	0.00 ± 0.00	0.02 ± 0.04	0.00 ± 0.00
3.5	7	0.05 ± 0.05	0.10 ± 0.11	0.04 ± 0.06
3.8	5	0.00 ± 0.00	0.07 ± 0.07	0.00 ± 0.00
4.0	2	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.10
		Protein : mgm/100 ml		
0.0	44	80 ± 51	58 ± 36	65 ± 35
0.8	12	19 ± 11	54 ± 17	65 ± 26
3.0	12	14 ± 11	51 ± 36	77 ± 24
3.2	9	13 ± 13	42 ± 17	90 ± 61
3.5	7	14 ± 12	53 ± 36	39 ± 22
3.8	5	5 ± 2	41 ± 24	75 ± 64
4.0	2	3 ± 2	53 ± 25	22 ± 11
		pH		
0.0	44	8.1 ± 0.8	7.7 ± 0.9	7.2 ± 0.9
0.8	12	7.4 ± 0.9	7.6 ± 0.8	7.0 ± 0.9
3.0	12	7.7 ± 0.4	7.9 ± 0.6	7.3 ± 0.5
3.2	9	8.1 ± 0.5	8.2 ± 0.5	7.9 ± 0.2
3.5	7	7.6 ± 0.6	7.6 ± 0.4	7.3 ± 0.7
3.8	5	6.9 ± 0.5	8.6 ± 0.4	8.0 ± 0.3
4.0	2	6.8 ± 0.5	7.5 ± 0.4	7.2 ± 0.5

(1) The results are expressed as mean ± standard deviation.

dehydration, and vascular congestion of many organs, due to oral administration of a single lethal dose of sodium chloride.

Recovery was rapid in animals which survived the lethal action of sodium chloride. At 24 hours, there were no neurological signs and little or no diarrhea. At the same time, as shown in Table II, the controls and animals receiving the lower doses of sodium chloride had recovered body weight lost during the 16 hour period of starvation before drug administration. Survivors of the larger doses of sodium chloride had some anorexia (TABLE II) and polyuria (TABLE III) without polydipsia, so that recovery of body weight was somewhat depressed at 24 hours. Survivors of larger doses also had a fever (TABLE II) and acidosis (TABLE III) at 24 hours. By the end of the third day of convalescence, most of these clinical signs had disappeared (TABLES II and III).

Fifteen survivors were autopsied at 2 weeks after drug administration. There was no gross evidence of residual pathology. The weight and water content of most organs were insignificantly different from values in controls (TABLE I).

DISCUSSION

The separate reporting of data upon male and female albino rats, noted above, indicated that while females were possibly very slightly more susceptible to the lethal action of sodium chloride than were males, in most instances the difference was statistically insignificant. Considering males and females as one group, the $LD_{50} \pm$ S.E. was calculated to be 3.75 ± 0.43 gm of sodium chloride per kgm body weight. The median lethal dose was approximately equivalent to the total body content of sodium chloride (3). This dose was placed in the empty stomach, dissolved in distilled water to a volume of 20 ml per kgm. The drug was administered orally, therefore, as a markedly hypertonic solution.

This solution produced an immediate, violent, inflammatory reaction in the gastrointestinal tract accompanied by anorexia and diarrhea. Columnar epithelium lining the gastric foveoles of pyloric stomach and the intestinal villi were lysed, but the stratified squamous epithelium of cardiac stomach was more resistant and the contact effect appeared to be diluted by the time the solution reached the colon. The capillary and venous congestion of the lamina propria and submucosa was comparable to that seen following oral administration of median lethal doses of benzylpenicillin (9).

The absorption of these doses of sodium chloride into the blood

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stream probably produced an increase in blood volume (3) by drawing water from most tissues of the body (TABLE I). This was accompanied or followed by vascular congestion in most organs with the notable exceptions of lungs, skeletal muscle, and skin. There was a relative polydipsia (decrease in food intake with no decrease in water intake—see TABLES II and III) accompanied by polyuria, a slight aciduria and fever. SELYE (10) reported convulsions, coma and death from intraperitoneal injection into rats of doses of sodium chloride of the order of 2.5 gm per kgm and similar clinical signs were recorded in the project reported herein. Death was due to respiratory failure and occurred earlier the higher the dose of drug, the mean $\pm$ standard deviation hours to death being 8.6 ± 5.3. Recovery of survivors was rapid and apparently complete.

Calculated in terms of millimoles per kgm body weight, the oral $LD_{50} \pm$ S.E. for sodium chloride was 64.1 ± 7.36 and for potassium chloride 40.5 ± 1.88 (1) and the difference was significant at $P < 0.001$. The mean interval to death following sodium chloride was significantly longer than that following potassium chloride. Premortem signs and mean loss of most organ weights were similar in the two toxicity syndromes. Mean loss of weight in spleen, skeletal muscle, pyloric stomach and skin was greater, and in liver less, following potassium chloride. Mean loss of organ water content and vascular congestion of organs at autopsy were significantly greater following sodium chloride.

Survivors from death to both sodium and potassium chloride had a similar decrease in food intake and increase in colonic temperature and urine volume. Polydipsia was more evident in survivors of potassium chloride (1). Absence of appreciable polydipsia in survivors of sodium chloride intoxication may have been associated with stimulation of the zona glomerulosa of the adrenal cortex reported by SELYE (10). The cells of the zona glomerulosa had a swollen, "foamy" appearance, similar to that shown in Figures 3 and 4 of SELYE's (10) paper, but in addition there was congestion of the capillaries and sinusoids. The weight, water content, and appearance of organs were mostly normal in survivors at 2 weeks after administration of both sodium and potassium chloride.

Acute oral sodium chloride intoxication, therefore, differed from the corresponding potassium chloride intoxication in that the LD_{50} was higher, the interval to death longer, dehydration and vascular congestion of organs were more marked, there was less polydipsia, and certain organs had differing changes in weight or appearance.

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Summary

The median lethal dose ± standard error of sodium chloride given orally to young adult albino rats on an empty stomach was 3.75 ± 0.43 gm per kgm body weight. The mean ± standard deviation interval to death was 8.6 ± 5.3 hours. The reaction in males was similar to that in females. The clinical syndrome included convulsive movements, diarrhea, muscular rigidity, prostration and death due to respiratory failure. At autopsy, dehydration and vascular congestion were present in most organs except skeletal muscle, lungs, and skin. Congestion was particularly marked in the meninges and brain. Columnar epithelium lining the gastrointestinal tract was lysed. Survivors had a brief convalescent anorexia, polyuria, fever, and acidosis; appearance, weights, and water contents of organs were essentially normal at autopsy 2 weeks after drug. The cause of death, therefore, was respiratory failure associated with an acute encephalopathy, accompanied by a fulminating gastroenteritis and dehydration and congestion of many organs, due to oral administration of a single lethal dose of sodium chloride.

References

1. — Boyd, E. M. and Shanas, M. N. *Arch. int. Pharmacodyn.*, 1961, *133*, 275.
2. — Spector, W. S. (editor). Handbook of Toxicology, Volume I. W.B. Saunders Company, Philadelphia, 1956, p. 272.
3. — Ussing, H. H., Kruhoffer, P., Thaysen, J. H. and Thorn, N. A. The alkali metalions in biology. Springer-Verlag, Berlin, 1960, p. 231, p. 363, and p. 520.
4. — Winbury, M. M. and Crittenden, P. J. *Proc. Soc. Exper. Biol. & Med.*, 1948, *69*, 220.
5. — Markley, K., Bocanegra, M., Morales, G. and Chiappori, M. *J. Clin. Invest.*, 1957, *36*, 303.
6. — Sollmann, T. A manual of pharmacology, 8th edition. W.B. Saunders Company, Philadelphia, 1957, p. 1038.
7. — Croxton, F. E. Elementary statistics with applications in medicine. Prentice-Hall, Inc., New York, 1953.
8. — Waugh, A. E. Elements of statistical method, 3rd edition. McGraw-Hill Book Company, Inc., New York, 1952.
9. — Boyd, E. M., Broughton, R. J. and James, J. *Arch. int. Pharmacodyn.*, 1960, *123*, 295.
10. — Selye, H. *Acta Neuroveget.*, 1953, *6*, 212.

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CANADA

THE ACUTE ORAL TOXICITY OF POTASSIUM CHLORIDE

BY

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BOYD, BROUGHTON and JAMES (1) reported that the oral median lethal dose of benzylpenicillin was 6.7 ± 0.1 gm per kgm body weight in the albino rat. Benzylpenicillin was given as the potassium salt so that the rats received 0.78 gm of potassium equivalent to 1.49 gm of potassium chloride per kgm body weight along with the LD_{50} of benzylpenicillin. This dose of potassium chloride is considerably above parenteral lethal doses reviewed by SPECTOR (2).

Limited information is available upon the acute toxicity of potassium chloride at the range of the single oral median lethal dose. ULRICH and SHTERNOV (3) gave 1 molar solutions by mouth and reported the average LD_{90} — LD_{95} as 2.43 gm per kgm in rats. STEPHENS (4) reported death rates of 17 % and 84 % after giving 2.5 and 5.0 gm per kgm respectively to rats, presumably on a full stomach. DRESCHER *et al.* (5) found that potassium chloride added to the diet of rats in doses of 20 to 30 gm per kgm per day killed 50 % of the animals in 3 days. The maximal nontoxic dose in man has been estimated at between 0.2 (6,7) and 1 (8) gm per kgm per day.

From these data, it appears that the lethal oral dose of potassium chloride is lower when given as a single administration on an empty stomach than when given mixed with the diet. The cause of death appears to vary between the two methods of administration as well. A single lethal dose given on an empty stomach has been reported to produce convulsions and respiratory failure (3). Given mixed with diet,

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it produced paresthesias, paralyses, and death due to ventricular fibrillation.

In the experiments on benzylpenicillin potassium noted above (1), the drug was given as a single dose to rats on an empty stomach. The toxicity syndrome was somewhat similar to that reported for potassium chloride given in the same manner (3). While this similarity does not necessarily mean that death from benzylpenicillin under these circumstances was due to its potassium content, it was considered advisable to compare the two toxicity syndromes more carefully. The present report is concerned, therefore, with studies upon the acute oral toxicity of potassium chloride in albino rats.

Method

The work was done upon 109 adult female albino rats of a Wistar strain which has been bred in the Animal Quarters of the Department of Pharmacology at Queen's University since 1937. The animals weighed 200 to 300 gm and were fed Purina fox chow checkers and water ad libitum. They were placed in metabolism cages, one rat per cage, and given water but no food for 16 hours before administration of potassium chloride. Potassium chloride was given by stomach tube in doses of 0.0 (controls), 2.1, 2.4, 2.7, 3.3, 3.6 and 3.9 gm per kgm body weight. Each dose was dissolved in distilled water and given in a volume of 20 ml per kgm body weight.

Clinical signs were noted at hourly or other intervals, as indicated, during the acute phase of the toxic reaction and as death appeared imminent. Body weight, food and water intake, urine volume and urinalyses, and colonic temperature were recorded upon survivors at intervals of 24 hours after drug administration until recovery was obvious. Food intake was calculated as gm chow, and water intake and urine volume as ml, per kgm body weight per 24 hours.

Autopsies were performed upon nonsurvivors, a group of 13 survivors at two weeks after drug administration, and upon controls. Gross pathologic observations, wet weight and water levels were noted upon the organs listed below. Blocks of tissue were stained with hematoxylin-phloxine-saffron and examined microscopically.

All data were subjected to t tests of significance of differences between means after the methods of Croxton (9).

Results

Death occurred in approximately half of the rats. By plotting dosage on the ordinate and percent mortality on the abscissa, regression was found to be linear at the doses selected. The regression line ± its standard error was calculated by the method of least squares. The LD50 ± standard error was found to be 3.02 ± 0.14 gm per kgm body weight.

Death occurred in less than 2 hours in 62 % of instances, in 3 to 6 hours in 25 %, and in 7 to 12 hours in 13 %. The mean ± standard deviation interval to death was 3.3 ± 3.2 hrs. Deaths delayed beyond 6 hours occurred only in rats receiving doses of 3.3 gm per kg and over. The most common pre-mortem clinical sign was prostration. Tonic-clonic convulsions occurred in one third of the animals. Death was due to respiratory failure. At autopsy, the parietal cells, mucous neck cells, and surface epithelium of the pyloric stomach were shrunken and there was loss of weight of this organ (Table I). There were also areas of capillary and venous congestion in the lamina propria and submucosa in the pyloric stomach and occasional hemorrhage and necrosis of the surface epithelium. The columnar epithelium covering the intestinal villi was shrunken and the goblet cells dilated. There were areas of early necrosis in the renal tubular epithelium. The heart was dilated with blood and the respiratory alveoli were frequently collapsed. The spleen was contracted. Other organs noted in Tables I and II were normal in histological appearance but most of them had lost weight (Table I) due principally to loss of water (Table II).

The cause of death in these rats was, therefore, respiratory failure with or without convulsions, accompanied by a gastroenteritis, dehydration of most organs, and renal tubular necrosis, due to oral administration of a single lethal dose of potassium chloride.

Survivors recovered quickly from the toxic effects of potassium chloride. At about the time their littermates were dying, one third of the survivors had convulsive movements or brief clonic convulsions. During the remainder of the first day, 23 % of the survivors had diarrheal bowel movements, 18 % were constipated, and 16 % had periods of dyspnea. As illustrated in Figure 1, there occurred in the first 24 hours anorexia, polydipsia, polyuria, and fever, all increasing more or less in proportion to the administered dose of potassium chloride. During the second and third days after drug administration, appetite and water intake, urine volume and colonic temperature, returned to or toward

TABLE I

Shifts in wet weights of organs

Organ	Controls Mean ± St. dev. (gm)	Nonsurvivors % change from controls	Nonsurvivors P	Survivors at 2 weeks: Compared with nonsurvivors % change	Survivors at 2 weeks: Compared with nonsurvivors P	Survivors at 2 weeks: Compared with controls % change	Survivors at 2 weeks: Compared with controls P
Spleen	0.721 ± 0.091	− 23.6	< 0.001	+ 34.6	0.001	+ 2.9	0.7
Abd. muscle	5.13 ± 0.81	− 20.1	0.005	+ 19.3	0.02	− 4.7	0.5
Pyloric stomach	1.00 ± 0.19	− 19.0	0.05	+ 17.3	0.05	− 5.0	0.5
Kidneys	2.02 ± 0.26	16.8	0.005	+ 7.7	0.2	− 10.4	0.05
Lungs	[illegible]	14.7	0.05	+ 20.1	0.05	+ 10.1	0.4
Pancreas	[illegible]	11.0	[illegible]	+ 11.2	[illegible]	[illegible]	[illegible]
Heart	[illegible]	[illegible]	[illegible]	+ 4.8	[illegible]	[illegible]	[illegible]
Colon	[illegible]	[illegible]	[illegible]	+ 4.0	[illegible]	[illegible]	[illegible]
Skin	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Adrenals	[illegible]	[illegible]	[illegible]	8.3	[illegible]	[illegible]	[illegible]
Liver	[illegible]	8	[illegible]	8.1	[illegible]	[illegible]	[illegible]
Testes	[illegible]	6.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Brain	[illegible]	6.1	[illegible]	+ 3.1	0.4	[illegible]	[illegible]
Cecum	0.85 ± 0.29	4.7	0.6	+ 4.9	0.6	0.0	1.0
Residual carcass	131 ± 20	− 3.8	0.6	+ 1.6	0.8	− 2.3	0.7
Cardiac stomach	0.344 ± 0.058	+ 1.7	0.8	+ 1.2	0.9	+ 2.9	0.7
Heart	0.796 ± 0.072	+ 11.3	0.025	− 12.0	0.02	− 2.0	0.6

TABLE II

Shifts in water levels of organs

Organ	Controls	Nonsurvivors		Survivors at 2 weeks			
				Compared with nonsurvivors		Compared with controls	
	Mean ± St. dev. (gm/100 gm dry wt)	% change from controls	P	% change	P	% change	P
Pyloric stomach	349 ± 37	− 22.9	< 0.001	+ 30.9	< 0.001	+ 0.9	0.9
Skin	146 ± 13	− 14.3	< 0.001	+ 12.8	< 0.001	− 3.4	0.3
Cardiac stomach	355 ± 26	− 13.5	0.01	+ 15.6	< 0.001	+ 0.3	0.9
Lungs	241 ± 15	− 11.7	< 0.001	+ 18.0	< 0.001	+ 4.1	0.1
Kidneys	332 ± 22	− 10.5	< 0.001	+ 12.1	< 0.001	+ 0.3	0.9
Colon	449 ± 67	− 10.2	0.05	+ 13.6	0.025	+ 2.0	0.7
Liver	283 ± 13	− 8.8	< 0.001	+ 9.7	0.005	0.0	1.0
Heart	351 ± 8	− 8.3	< 0.001	+ 9.6	< 0.001	+ 0.6	0.6
Cecum	344 ± 44	− 7.6	0.2	+ 12.9	0.025	+ 4.4	0.3
Abd. muscle	224 ± 24	− 5.3	0.3	+ 5.2	0.5	− 0.4	0.9
Ovaries	265 ± 39	− 4.2	0.6	+ 9.1	0.3	+ 4.5	0.7
Residual carcass	201 ± 20	− 4.0	0.4	+ 8.3	0.1	+ 4.0	0.3
Brain	365 ± 11	− 3.8	0.02	+ 4.8	0.005	+ 0.8	0.5
Spleen	316 ± 9	− 1.6	0.4	+ 2.6	0.2	+ 0.9	0.6
Jejunum	415 ± 67	+ 1.0	0.9	+ 10.0	0.2	+ 11.3	0.2
Adrenals	240 ± 31	+ 1.6	0.7	+ 5.7	0.1	+ 7.5	0.1
Ileum	338 ± 65	+ 8.9	0.4	+ 7.4	0.4	+ 16.9	0.05

control levels. The urine of survivors during these three days did not differ significantly from that of controls with respect to the presence or absence of sugar, albumin, occult blood, acetone, or bilirubin.

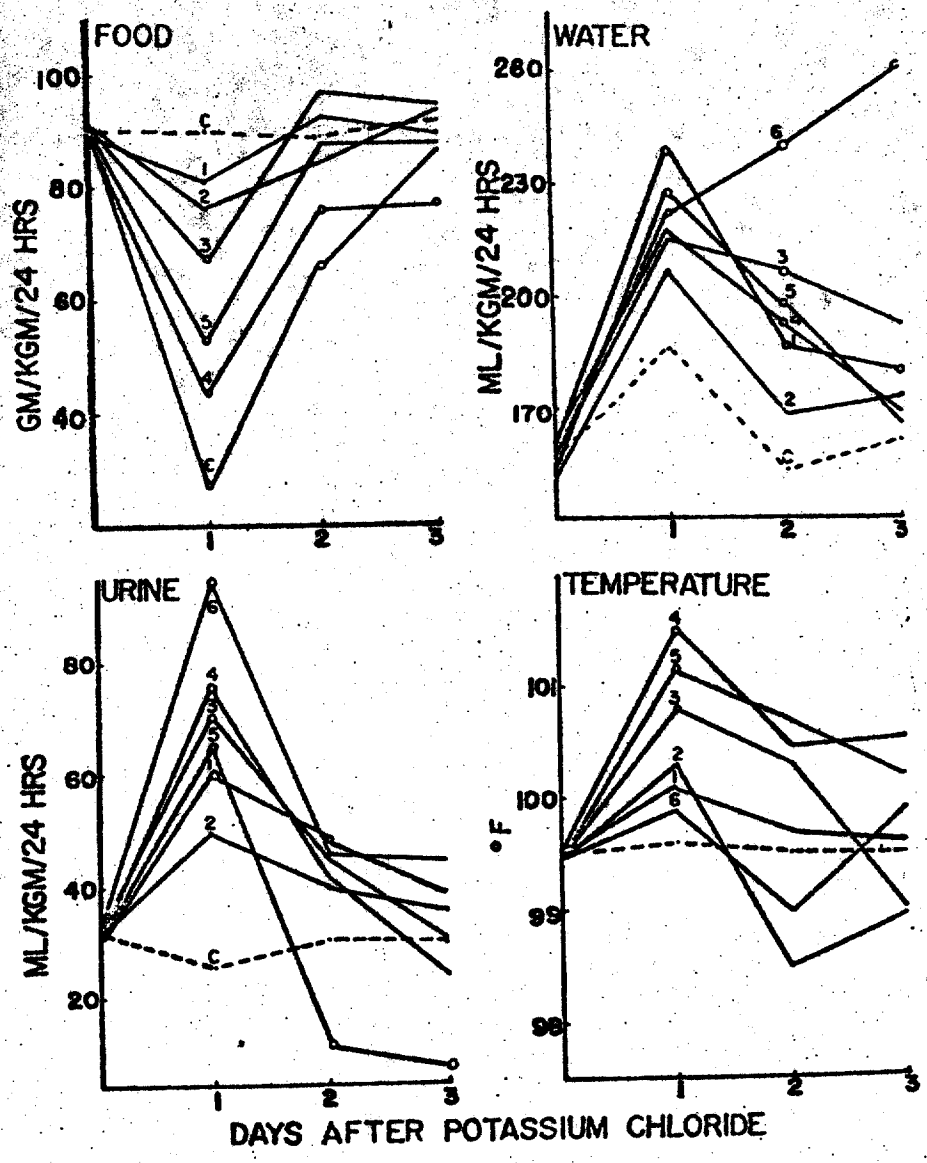

FIG. 1

Shifts in the mean food intake, water intake, urine volume, and colonic temperature of albino rats surviving noted oral doses of potassium chloride; C = controls; 1 = 2.1 gm, 2 = 2.4 gm, 3 = 2.7 gm, 4 = 3.3 gm, 5 = 3.6 gm, and 6 = 3.9 gm per kgm of potassium chloride. Means significantly different from the controls at $P < 0.025$ are indicated by open circles.

A group of survivors was autopsied at 2 weeks after administration of potassium chloride. There was no evidence of residual pathology in these animals. All of the organs except heart had gained weight over that in the nonsurvivors and were insignificantly different in weight from that in the controls (TABLE I). The dilated heart seen in the nonsurvivors had disappeared. The dehydration of organs was no longer present (TABLE II). Survivors were normal by 2 weeks, therefore, insofar as these measurements so indicated.

DISCUSSION

Comparison of these results with those of BOYD, BROUGHTON and JAMES (1) indicates that potassium was not of itself responsible for the death of albino rats from benzylpenicillin potassium but may have contributed in part to the syndrome of toxicity at the range of the oral median lethal dose. Extrapolation of the dose-response regression of mortality from potassium chloride showed that a very small percentage (if any) of rats would die from a dose of potassium chloride (1.49 gm per kgm) containing the amount of potassium in an oral LD_{50} of benzylpenicillin potassium. On the other hand, this amount of potassium could have contributed in part to mortality from free benzylpenicillin. In this connection it is important to note that the LD_{50} of benzylpenicillin given as the ammonium salt (10) is 8.4 ± 0.13 gm per kgm which is significantly higher than that for benzylpenicillin given as the potassium salt (1).

There are some points of similarity between the syndrome of acute toxicity to oral potassium chloride and that to oral benzylpenicillin potassium. In both syndromes, the immediate cause of death is respiratory failure preceded by prostration and often tonic convulsions. In both there occur gastroenteritis, dehydration of most organs, and renal tubular damage.

In other respects, the two syndromes differ. Death occurs earlier from potassium chloride. In the acute toxicity reaction to benzylpenicillin potassium, the gastroenteritis was more marked, there was some hepatic damage, and the lungs, spleen, and adrenals were enlarged and congested.

Survivors to benzylpenicillin potassium recovered more slowly than survivors to potassium chloride. Penicillin survivors had more diarrhea and in addition showed glycosuria, albuminuria, and hematuria. They had no fever and were hyperkinetic. Polydipsia and polyuria appeared

on the second and third days rather than on the first day as in potassium chloride survivors.

There were, therefore, similarities and dissimilarities between the two toxicity syndromes. It may be noted that the points of similarity between the two syndromes, noted above, were also present in the acute toxicity syndrome to oral benzylpenicillin ammonium. From the evidence available, it would appear that the syndrome of toxicity to oral benzylpenicillin potassium was due mostly to the benzylpenicillin part of the molecule.

Summary

The median lethal dose (± standard error) of potassium chloride given orally to adult female rats on an empty stomach was 3.02 ± 0.14 gm per kgm body weight. The animals died in 3.3 ± 3.2 (mean ± standard deviation) hours from respiratory failure, sometimes following convulsions, and accompanied by a gastroenteritis, dehydration of organs, and early renal tubular necrosis. Survivors had anorexia, polydipsia, polyuria, fever, convulsive movements, and gastrointestinal disturbances during the first 24 hours but rapidly returned to normal thereafter.

References

1. — Boyd, E. M., Broughton, R. J. and James, J. *Arch. int. Pharmacodyn.*, 1960, *123*, 295.
2. — Spector, W. S. (editor). *Handbook of Toxicology*, Volume I, W. B. Saunders Company, Philadelphia, 1956, p. 242.
3. — Ulrich, J. L. and Shternov, V. A. *J. Pharmacol. & Exper. Therap.*, 1929, *35*, 1.
4. — Stephens, J. W. *Canad. Med. Assoc. J.*, 1952, *66*, 19.
5. — Drescher, A. N., Talbot, N. B., Meara, P. A., Terry, M. and Crawford, J. D. *J. Clin. Invest.*, 1958, *37*, 1316.
6. — Winkler, A. W., Hoff, H. E. and Smith, P. K. *J. Clin. Invest.*, 1941, *20*, 119.
7. — Keith, N. M., Osterberg, A. E. and Burchell, H. B. *Ann. Intern. Med.*, 1942, *16*, 879.
8. — Ussing, H. H., Kruhoffer, P., Thaysen, J. H. and Thorn, N. A. *The alkali metal ions in biology*. Springer-Verlag, Berlin, 1960, p. 521.

0362

9. — CROXTON, F. E. *Elementary statistics with applications in medicine*, Prentice-Hall, Inc., New York, 1953.

10. — BOYD, E. M., BROUGHTON, R. J. and JAMES, J. *Antibiot. & Chemotherap.*, 1959, *11*, 739.

Symposium on Pediatric Pharmacology

Fluid and Electrolyte Therapy

*Erika Bruck, M.D.**

Maintaining or establishing adequate composition of fluid and electrolytes in patients who suffer from a variety of diseases has become one of the most important functions of modern hospitals. Next to surgery, the need for expert fluid and electrolyte therapy constitutes one of the prime indications for hospitalization of children.

Therapy for disturbed metabolism of fluids and electrolytes is also one of the most frequently misunderstood and most difficult problems for many physicians who are not accustomed to thinking in quantitative terms and who may focus on only one aspect of a complex situation. The proper solution of this problem is even more essential in infants who have less reserves of body water and electrolytes, absolutely and in relation to body weight, than adults have. Newborn or premature infants, in addition, may be deficient in some of the homeostatic functions which protect the adult or the older child against the ravages of abnormal losses or inappropriate therapy. Primary disturbances of the homeostatic mechanisms, as in renal or adrenal insufficiency, also require expert management.

ROUTES OF ADMINISTRATION

The safest and simplest route for administration of fluids is the oral one in all patients in whom adequate absorption from the gastrointestinal tract can be expected.

Feeding through a gastric tube is a substitute for oral intake in patients who are unconscious or unable to swallow. Complete nutrition can be accomplished, including protein, fat, and fat-soluble vitamins. Administration through a gastric tube has the disadvantage that the patient's thirst mechanism is not regulating his fluid intake. Formulas recommended for tube feeding by well-meaning physicians who are concerned with calories and protein often contain insufficient water, and numerous cases of hyperosmolality have been reported in both children and adults receiving this type of feeding.[10, 25, 35]

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Feeding through a tube in the duodenum is rarely indicated. If it is necessary, the solution should be given by slow, continuous drip. Entry of a large single bolus of food in the small bowel can precipitate the so-called "dumping syndrome," which has been observed in patients with gastroenterostomy and is characterized by vascular collapse. The syndrome is thought to result from the presence of a large number of small molecules in the lumen of the gut. High osmolality of the intestinal contents results and causes influx of a large amount of water.[31] Rapid absorption of carbohydrates following administration into the duodenum may cause excessive hyperglycemia followed by prolonged hypoglycemia[45] (Fig. 1).

Isotonic saline or 5 per cent glucose may also be given per rectum as an emergency procedure, if there is no diarrhea.

Intravenous infusion is the preferred form of giving fluids and electrolytes to patients who are unable to tolerate fluids by the gastrointestinal route. With modern equipment* available, at least in the United States, there are no longer any indications for subcutaneous administration of fluid. Fluid given subcutaneously is absorbed very slowly, causes

*"Scalp vein" needles, "intracath," silicone rubber catheters, infusion pumps, and "minidrop" sets

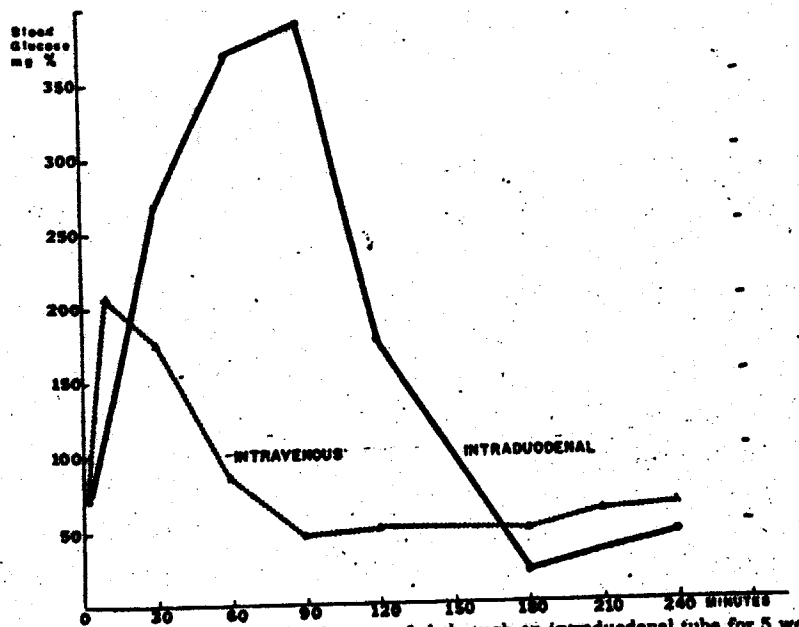

Figure 1. A 10 month old infant was fed through an intraduodenal tube for 5 weeks because of severe rumination. Signs of hypoglycemia occurred; therefore, two glucose tolerance tests were performed with the same dose during the fourth week of this regimen, at an interval of 6 days. The glucose was administered through the intraduodenal tube the first

tissue damage if it is not isotonic, and may lead to cellulitis or abscesses if any microorganisms are introduced. Calcium salts and sodium bicarbonate should not be given subcutaneously even in small amounts (e.g., leaking out of a vein); severe necrosis may result.

When no vein can be found, even by cutdown, as in cases of generalized ekzema, epidermolysis bullosa, or in very small premature infants, fluid may be given by slow infusion into bone marrow (iliac crest in older children, tibia in infants), or by single injection into the peritoneum. Although water, electrolytes, glucose, and even blood are readily absorbed from both of these sites, the danger of infection exists.

In infants in shock, where search for a vein or a cutdown would take too much time, an initial hydrating dose of fluid may, in an emergency, be injected into the superior sagittal sinus through a patent fontanelle. This dose may expand the circulating blood volume sufficiently to permit perfusion of the brain and the kidneys and to allow time for establishing a more stable form of infusion.

Fluid should be given by continuous slow intravenous infusion over a period of 24 hours each day. A continuous protocol, tabular or graphic, should be kept, with hourly records of intake. Deviations in the actual intake from the desired intake in 1 hour have to be compensated for during the following hour to avoid cumulative deficits or excesses.

PURPOSES OF FLUID THERAPY

Fluid therapy may serve to maintain normal body composition when normal food intake is impossible, or to correct acute or chronic disturbances of fluid and electrolyte balance.

Requirement for maintenance therapy arises in unconscious patients or in patients with cerebral or respiratory disturbances where there is danger of aspiration of food, as well as before and after surgery.

Diarrhea, vomiting, diabetic acidosis, and salicylate intoxication are the most common conditions that cause acute imbalance of fluid and electrolytes in children and call for parenteral fluid therapy. Chronic losses from the intestinal tract or via the urine, and renal, adrenal, or pituitary insufficiency present less common but more difficult challenges.

MAINTENANCE THERAPY

Short Term Maintenance

When maintenance problems exist for only 1 to 3 or 4 days, simple solutions providing an adequate amount of water, glucose for minimal caloric requirements, and small amounts of sodium, chloride, potassium, and phosphate to replace basic losses will suffice to permit normal composition of body fluids and normal circulation, renal, and other basic functions, although nitrogen balance will be negative and body fat, glycogen, and protein will be gradually consumed. The requirements for

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maintenance of water and electrolytes have been calculated from published measurements of expenditures in normal children[12] and are listed in Table 1; these are average, not minimal figures. A solution listed in Table 2 as PIS No. 1, or any similar solution which is hypotonic with respect to electrolytes, will provide adequate electrolytes for short term maintenance if the amount given satisfies the water requirements. Normal kidneys and normal adrenal and antidiuretic hormone production will enable the individual to adjust the amounts of fluid and electrolytes retained to his own specific requirements, which may vary as much as 25 per cent from the average, depending on metabolic rate, body and environmental temperature, muscular activity or disease processes. The "homeostatic limits to safe therapy"[67] will not be exceeded if the rate of administration of this solution is approximately 1500 ml. per M.^{2} per 24 hours, or 63 ml. per M.^{2} per hour.

During the first week of life, the requirements of infants are lower than the figures above, and the ability of the kidneys to excrete excess water and electrolytes is underdeveloped. The fluid requirements at this age may be met by giving amounts corresponding to those taken by normal infants at the breast.[12] Suggested amounts for full-term infants are listed in Table 1. Premature infants receive proportionately smaller amounts, i.e., gradually increasing from 35 ml. per kg. to 100 to 120 ml. per kg. per day during the first week. In premature infants, no electrolytes need be given during the first 3 or 4 days; 5 to 10 per cent glucose in water suffices.

Table 1. *Average Daily Requirements of Water and Electrolyte for Maintenance*

INFANT AND CHILD PER M^2 OF BODY SURFACE AREA		NEWBORN INFANT, 3 KG. ABSOLUTE VOLUME	
Water	1500 ml.	*Age*	*ml.*
Sodium	12–30 mEq.	2 days	100
Potassium	15–25 mEq.	3 days	180
Chloride	12–20 mEq.	4 days	240
Glucose	100–150 gm.	5 days	300
		10 days	400

Table 2. *Composition of Solutions Commonly Used for Intravenous Fluids*

	BALANCED HYDRATING SOLUTION	POLYIONIC NO. 1	POLYIONIC NO. 2
Carbohydrate	5%	10%	10%
Sodium	75 mEq./L.	30 mEq./L.	57 mEq./L.
Potassium		15 mEq./L.	25 mEq./L.
Magnesium			6 mEq./L.
Chloride	50 mEq./L.	22 mEq./L.	50 mEq./L.
Lactate	25 mEq./L.	20 mEq./L.	25 mEq./L.
Phosphate		3 mMol./L.	13mMol./L.

Note: Several companies now manufacture solutions with a composition similar to the above.

Long Term Maintenance

If the need for maintenance fluid therapy exceeds 4 to 6 days in a newborn infant or 1 to 2 weeks in an older child, estimation of the requirements as well as the technique of administration becomes more complicated. Hypoproteinemia and edema may appear in infants who suffer from protracted diarrhea, pertussis, encephalitis, head injuries, or other surgical conditions, when they are given protein-free fluids for more than a few days. For long term nutrition, calories, protein, vitamins, and trace metals have to be provided in adequate amounts, in addition to water, sodium, potassium, calcium, magnesium, chloride and phosphate. Essential aminoacids and essential fatty acids have to be included.

Attempts to devise complete artificial nutrition are hampered by the fact that optimal or minimal requirements for several ingredients are not well established even for the normal subject at different ages, much less for the stresses imposed by illness. When the intelligence of the physician has to substitute for the patient's normal instincts of thirst, hunger, and satiety, and for the composition of natural foods, the limitations of man's intelligence may become painfully evident. There is a tendency to provide excessive amounts of protein and perhaps some minerals and often less than the optimal amount of water.

When liquid nutrition for maintenance is given by stomach tube, a liquid diet may be prepared from milk and fruit juices with small amounts of meat, and sources of iron and vitamins. Since the intake of infants normally is in liquid or semi-liquid form, the "normal" food may be mixed and liquefied and given through a stomach tube. Protein intake does not have to exceed the "recommended daily requirements" for the age of the patient.[41] Amounts slightly above the minimum are adequate when there are no abnormal losses. Excessive amounts of protein will result in a large excretion of nitrogen as urea. A high renal load of urea causes osmotic diuresis; therefore, a high protein intake raises water requirements. Some nationally recommended formulas for tube feeding of adults are poorly balanced; most of them contain too much protein and some, in which liver is an ingredient, may contain more vitamin A than can be tolerated in daily doses over extended periods.

Table 3 gives a commercial formula in common use for tube feeding of older children or adults. Even this formula has a rather high protein content (20 per cent of calories) and not enough Vitamin D, thiamine and riboflavin for sick children. In infants, the water requirements are higher in relation to caloric intake than in older individuals; their water intake should be close to 1.5 ml. per calorie.

When the intestinal tract is unable to digest food, e.g., after extensive surgery or peritonitis or with protracted diarrhea, one has to resort to parenteral alimentation. Progress in this field during the last few years has been due mostly to advanced surgical technique.[4, 28, 71] Prevention of infection and of thrombosis are the biggest problems. As prophylaxis, a silicone rubber catheter is inserted into one of the

Table 3. *Formula for Tube Feeding**

Protein	50 gm.†	Vitamin B_6	1.5 mg.
Fat	33 gm.	Vitamin B_{12}	1.5 mg.
Carbohydrate	125 gm.	Niacin equiv.	10 mg.
Minerals (ash)	0.9 per cent	Calcium	1600 mg.
Moisture	79.8 per cent	Phosphorus	1350 mg. = 44 mM.
Vitamin A	12000 USP	Sodium	479 mg. = 21 mEq.
Vitamin D	225 USP	Potassium	1750 mg. = 45 mEq.
Vitamin E	21.1 U	Magnesium	250 mg. = 42 mEq.
Vitamin C	40 mg.	Iron	10 mg.
Thiamine	0.7 mg.	Copper	1.6 mg.
Riboflavin	1.2 mg.	Zinc	6.6 mg.
		Manganese	4.5 mg.

Solute load: 300 to 400 mOsm. per 1000 calories

*"Formula I," Gerber Company

†All per 1000 calories. This formula contains 1 calorie per ml. Ten to 20 per cent extra water should be added for infants and for children who are likely to have high insensible water loss because of fever or hyperventilation.

large central veins so that the infused fluid will immediately be diluted with a large amount of blood. However, the incidence of infection is higher with plastic catheters than with "scalp vein" needles in peripheral veins. Therefore, most experienced clinicians warn that this method should be used only in life-threatening situations when no other means is available.[72] Meticulous asepsis and antisepsis are required.

Considerable controversy still exists among investigators about the optimal composition of an appropriate fluid, although successful growth has been reported in an infant who received 93 per cent of all nutrients by vein for over a year.[23] One problem is the provision of sufficient calories for growth. In oral nutrition, 30 to 40 per cent of the calories are provided by fat. In the United States, as of early 1971, there is no fat preparation licensed for intravenous administration. Fat emulsions (Lipomul, Upjohn) used in the 1950's for this purpose resulted in fever, fat embolism, hemolytic anemia, and hemorrhagic phenomena in many cases[1, 42] and were therefore abandoned. Since 1968, "Intralipid,"* an emulsion of fractionated soy bean oil, has been manufactured in Sweden and is available in Canada. The emulsifying agent in this solution is a compound of egg yolk phosphatides which is said to have less toxic effects than the soy bean phosphatides used in the past.[4] Intralipid may be added to intravenous solutions, providing up to 10 per cent of fat in the solution, or given separately, providing up to 4 gm. of fat (37 calories) per kg. per day. No adverse effects of this procedure were reported at a symposium on Complete Parenteral Alimentation[72] held in Atlantic City in April, 1971, with several hundred participants from the United States and Canada. Hyperlipemic plasma of postprandial blood donors has also been used to provide essential lipids.[28, 71]

*Intralipid: 20 gm. soy bean oil, 1.2 gm. egg phosphatide, 2.5 ml. glycerol, distilled water ad 100 ml. Supplied by A. B. Vitrum, Stockholm, Sweden, and Riker Laboratories, Northridge, California.

The majority of calories are provided by glucose or invert sugar. If the concentration of the solution is raised gradually (i.e., over several hours) from 5 to 10 per cent up to 20 to 25 per cent, hyperglycemia and glycosuria usually do not occur in children. Glucose levels in blood and urine should be monitored during the first 2 to 3 days of the infusions since some very sick infants may not have adequate homeostasis even without such infusions, and the rate of glucose administration may have to be reduced temporarily. The rate of maximum glucose utilization varies between individuals and in the same individual from time to time; no specific upper limit can be recommended.[61] Extreme hyperglycemia and hyperosmolality (blood sugar near 1000 mg. per 100 ml.) has been reported in some infants weighing less than 1200 gm. during the first 3 or 4 days of life when 25 per cent glucose solutions were infused; however, this problem rarely presents itself after the newborn period. There is no need to give complete nutrition during the first few days of life.

At present, two protein hydrolysates available in the United States are suitable for parenteral administration of amino acids: Amigen,* a casein hydrolysate and Aminosol,** a beef fibrin hydrolysate; Neo-Aminosol, a synthetic amino acid mixture, is under investigation. The amino acid composition of these preparations varies from one to another and from human plasma protein; both Amigen and Aminosol contain large amounts of glutamic acid.[64]

The administration of amino acids is best limited to the minimum required for maintenance of cell composition and growth. Since this minimum depends on the supply of the limiting essential amino acids, and also on the condition of the patient, exact figures for the individual patient cannot be categorically given. However, there is probably no need to give more than 4 gm. of protein equivalent per kg. to infants, less for older children. Disease or trauma representing "stress" will result in a negative nitrogen balance, regardless of the intake, but during recovery a higher than average intake may be required to produce a positive balance.

The dangers of giving an excessive amount of amino acids are: (1) Production of large amounts of urea causes osmotic diuresis. (2) Imbalance of plasma amino acid concentration has been reported, although the feared "toxic" effects of the high glutamic acid content, especially of casein hydrolysate, have not been substantiated.[64] (3) The amino acid solutions contain appreciable amounts of ammonia: as much as 20 to 25 mg. per 100 ml. of the mixed infusion has been reported,[66] i.e., more than 500 times as much as normally reaches the posthepatic circulation. Very young or premature infants may be unable to metabolize ammonia as rapidly as it is given,[36] and toxic amounts may reach the brain. (4) Metabolic acidosis occurs in many patients of all ages receiving protein hydrolysates intravenously. This acidosis is thought to result from the large amounts of free amino acids, that is, hydrogen ions, in the solutions.

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Vitamins must never be forgotten when complete alimentation, en- or parenteral, is attempted. The water-soluble vitamins of the B plex are essential for maintenance of carbohydrate metabolism and not stored to any significant extent. Although vitamin C is stored in adrenal cortex in previously well-nourished individuals, these stores be rapidly depleted under the stress of infection, surgery, or severe ydration. The requirements for thiamine, riboflavin, and ascorbic are increased in diseases associated with a negative nitrogen bal- e or other stress.[3] For prolonged artificial alimentation, liberal ounts in the range of normal requirements of all vitamins should be orporated in the regimen (Table 4). The water-soluble vitamins may added to parenteral fluids as well as to intragastric feedings.

Vitamin K or one of its synthetic analogues is required when paren- l fluids are the only source of intake for more than 3 days. Prolonged eding from the sites of venipunctures, and other manifestations of hrombin deficiency have been observed in patients with chronic or te diseases (e.g., meningitis,[7] diarrhea,[37]) when vitamin K was forgot- . Solutions of fat-soluble vitamins are now available for intravenous ninistration.*

CORRECTION OF ACUTE DISTURBANCES OF FLUID AND ELECTROLYTE METABOLISM

Though more dramatic, this task is practically simpler than mainte- nce over long periods. The first aim of therapy in all cases of dehydra- n is rapid restitution of the circulation and of renal function. This aim ccomplished efficiently by rapid intravenous infusion of an amount of id sufficient to expand the circulating volume within 1 hour or less; time may be as short as 10 to 15 minutes. If the infusion takes more n 1 hour, the fluid will diffuse out of the blood vessels into the ex- cellular space and not serve to establish renal circulation. Em- ically, 400 ml. per M.², given within ½ to 1 hour, is almost always suc- ssful.[12] The type of fluid used at this time is not crucial; isotonic saline, lf-isotonic saline with 5 per cent glucose, "balanced hydrating solu-

*For example, Vi-Syneral Injectable, USV Pharmaceutical Company, 10,000 units vi- in A and 1000 units vitamin D in 2 ml.

Table 4. *Recommended Daily Doses of Vitamins During Parenteral Nutrition of Sick Infants and Children*[3]

	MINIMUM	RECOMMENDED MG. PER DAY
Thiamine	1 mg. or 0.5 mg. per 1000 cal.	2
Riboflavin	2 mg. or 0.8 mg. per 1000 cal.	3–4
Niacin	6–9 mg./day (infants — adult)	10–20
Pyridoxine	average, 1.0–1.4 mg./day (infants)	5
Ascorbic acid	30 mg.	100
Vitamin K		1

tion" (see Table 2), Ringer's lactate solution, and even 10 per cent glucose solution[11] have been used successfully for initial hydration. The solution should *not* contain potassium, and its osmolar concentration should not be higher than that of body fluids (cave: concentrated solutions of sodium bicarbonate!).

Diarrhea

Diarrhea is still the most common cause of acutely disturbed fluid and electrolyte balance in infants. Loss of water and electrolytes with the diarrheal stool results in depletion of both extracellular and intracellular fluid and electrolytes. In babies who are sick enough to require hospitalization, the circulating blood volume and, therefore, the renal blood flow are usually diminished, and oliguria or sometimes anuria is present.

The major aim of therapy, after initial repletion of the circulation, is restoration of deficits of water and electrolytes plus supply of these substances for current expenditures, normal and abnormal. Calories in the form of glucose have to be given simultaneously. Although the deficits and current losses differ from one patient to another, it is the great accomplishment of investigators such as Darrow,[18, 21] Gamble,[33] Metcoff,[53] and others to have measured average losses and deficits by balance studies and to have related these deficits to body composition and altered physiologic functions. These investigators established the principle that the normal kidney is able to restore normal body composition if provided with a supply of corresponding amounts of water, sodium, potassium, magnesium, chloride, and phosphate, plus small amounts of lactate or bicarbonate, together with enough glucose to prevent ketosis and to reduce protein catabolism to a minimum.

Numerous regimens for therapy of acute dehydration have been proposed, most of them are based on the figures and principles derived in these investigations. All of them work in the great majority of cases if the fluids contain the above-mentioned elements in "average" amounts calculated from published data. It is neither possible nor necessary to calculate individual requirements from laboratory data or other forms of observation of the patient.

A regimen[12] which has been in successful use at the Children's Hospital of Buffalo for about 20 years requires only calculation of the child's body surface area from standard nomograms and of water requirements based on this figure; using solutions which are now commercially available in the United States, adequate amounts of electrolytes and glucose are automatically supplied (see Table 2). In 20 years, it has rarely been necessary to add specifically calculated amounts of sodium bicarbonate for correction of acidosis or to reduce the amount of sodium and chloride in the fluid in cases of hypertonic dehydration.[11] On the other hand, mistakes are frequently made when physicians try to devise specific solutions for individual patients. The patients are usually critically ill, the house officer is harassed, and excitement, miscalculation, and misinterpretation of laboratory data often result in excessive loads of sodium salts[34] or insufficient amounts of water, chloride, or potassium.

The commercial solutions do not contain calcium since calcium salts

are not stable in solutions containing lactate or bicarbonate. Calcium gluconate, 10 ml. of 10 per cent solution per kg. per day, may be added to the infusion in cases of "post-acidotic syndrome"[28] with hypocalcemia. However, hypocalcemia in infants with diarrhea is now much rarer than it was in the 1940's, perhaps because appreciable amounts of potassium now are regularly contained in any program for parenteral fluid therapy.[30]

The concentration of calcium as well as of potassium in serum should be monitored, particularly in infants under 3 months of age. Both may be low and can be corrected only gradually by supplying the respective ion, since the rate of administration is limited by the slow transfer of the ion from the extracellular space into the cells in the case of potassium, into the bones for calcium. The total amount of potassium in extracellular fluid is 1/40, the amount in plasma is 1/200 of the content of the body; the amount of calcium in plasma is about 1/4000 to 1/2000 of the total amount in the body; therefore deficient bones and cells act as dumps for these ions. The daily requirement far exceeds the amount which is present in the serum or the extracellular space.

Potassium deficit is not corrected in one day by any form of therapy.[21] In careful balance studies, Gamble et al. were impressed with the extremely slow response of the mechanisms which adjust renal excretion of electrolytes to changes in intake. They emphasize "the hazard of over-replacement of deficits of electrolytes in the treatment of diarrheal dehydration."[34] Restoration of deficits is usually completed by oral intake after the acute phase of diarrheal dehydration is over.

Hypernatremia in cases of diarrhea is commonly caused by deficits of more water than sodium, only rarely by an absolute excess of sodium.[10] Gradual correction by polyionic solutions (PIS No. 2) containing total electrolytes in approximately half the osmolar concentration of body fluids and sodium and potassium salts in a ratio of approximately 2:1 has been found satisfactory.[11] Rapid "correction" of osmolality by electrolyte-free fluids has sometimes been associated with convulsions and other pathologic alterations[11, 31, 69] for reasons which are not entirely clear in spite of much research.

Hypotonic dehydration with diarrhea is usually caused by a severe absolute deficit of sodium or potassium or both; the abnormal osmolality in these cases also is corrected with the polyionic solution No. 2. If parenteral fluid therapy is used for only 24 hours or less, restoration of electrolyte deficits, especially potassium, may be inadequate. The oral fluids introduced after the period of starvation (glucose water, tea, ginger ale) often contain little or no electrolyte; therefore hypo-osmolality of body fluids may persist for several days unless electrolytes are given by mouth, e.g., bouillon, fruit juice, milk.

Salt Poisoning

Hyperosmolality caused by excessive loads of salt is more dangerous than hypertonic dehydration with diarrhea. This condition cannot be treated successfully with conventional forms of parenteral fluid therapy. The total volume of body fluids may be excessive rather than deficient. If the patient is conscious and able to swallow, fruit juices or other carbohydrate solutions containing little or no sodium and chloride may be given orally to satisfy thirst and stimulate diuresis. Studies with salt-loaded rabbits[10] have shown that animals that were permitted oral water intake stopped drinking precisely at the right moment and corrected the osmolality of their body fluids without developing convulsions. Unfortunately, infants with salt poisoning are often too sick for oral therapy to be attempted. In severe cases, peritoneal dialysis, using 5 to 8 per cent glucose solution,[32] is probably the safest method of removing excessive loads from the body and restoring normal osmolality gradually. In many cases of "salt poisoning," irreversible damage to the brain has already occurred at the time the diagnosis is made, and permanent sequelae cannot be attributed to faulty therapy.

Salt poisoning may be iatrogenic and result from poorly designed types of therapy for other conditions. In recent years, the preoccupation of some physicians with the dangers of acidosis has resulted in recommendations for infusion of large amounts of sodium bicarbonate in highly hypertonic solution (the concentration of sodium bicarbonate in the commercially sold ampoules is six times the osmolality of body fluids). This form of therapy is often used in newly born or very young infants. Finberg and his associates[29, 46] have repeatedly called attention to the fact that "osmol poisoning" may be produced by such procedures and is associated with dangerous alteration of intracranial pressure, venous pressure, and blood volume. Rapid injection of concentrated solutions of sodium bicarbonate in babies with respiratory distress syndrome resulted in increased fatality in the hospital where this method was first introduced[68] and offered no advantage in a small "controlled" study in another institution.[63]

The experimental studies of Kravath, Finberg, and others, as well as much clinical experience, have convinced this author that spontaneous correction of acidosis by disposal of hydrogen ions via the kidneys and lungs is far more efficient, even in a young infant, than disposition of a sodium load.[11, 34, 54] Also, there are no known permanent sequelae of even severe acidosis (blood pH as low as 6.8).[43] Coma and death associated with severe acidosis are probably caused by the simultaneous lack of oxygen supply to the brain; the cerebral hypoxia results from stagnant circulation in cases of severe dehydration[44] and from insufficient oxygenation of blood in the lungs in cases of pulmonary insufficiency. By contrast, both acute and permanent sequelae of salt poisoning, including death or irreversible cerebral damage, have frequently been observed.[14]

Vomiting and Withdrawal of Upper Gastrointestinal Secretions

The pathophysiology of acute vomiting in children consists mainly of dehydration and starvation; at this stage, most children are acidotic. The principle that the kidney will correct acid-base balance if renal circulation is established and adequate supplies of potassium, chloride, sodium, and water are given, pertains to the treatment of vomiting as well as to diarrhea. Expansion of blood and extracellular volume with water, sodium and chloride and correction of ketosis with glucose will abolish this acidosis rapidly.

Chronic vomiting which is caused by pyloric or duodenal stenosis or

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esia is the most common pathologic cause of metabolic alkalosis in in-
ıts. As H^+ and Cl^- ions are lost, the kidney continues to excrete K^+.
ıce the intake of K under these conditions is usually minimal, severe
›letion of potassium content of cells occurs; the cellular K^+ is replaced
H ions and extracellular alkalosis results. The chronic character of
: condition results in cumulative deficits, particularly of potassium.

The same course of events occurs whenever upper gastrointestinal
id is removed continuously or repeatedly by suction. The amount
noved is often underestimated. particularly in young infants, since the
ume may not *look* very large; and it is not widely known that the con-
ıtration of sodium and chloride in this fluid is similar to their concen-
tion in extracellular fluid[7, 45, 36] and that the potassium excretion in
ne is higher than the loss from the upper gastrointestinal tract. "Only
) ml." of gastrointestinal fluid in 24 hours may, after 3 or 4 days, add
to a considerable proportion of the reserves of an infant weighing 3

The metabolic alkalosis in pyloric stenosis may be extreme; we have
:n pH 7.60, carbon dioxide content as high as 60 mEq. per liter, and
.oride concentration as low as 53 mEq. per liter. The complete repair of
: potassium deficit may require 1 to 2 weeks and, therefore, is usually
: possible before surgery, since such a long delay would aggravate the
.rvation. However, 24 to 48 hours of parenteral administration of
ids containing potassium, chloride, sodium, and glucose before
rgery may be necessary to restore more reasonable electrolyte composi-
n (pH <7.5, carbon dioxide content <35 mEq. per liter, chloride >80
:q. per liter, potassium >3.5 mEq. per liter) which reduces the compli-
tions of surgery. Polyionic solution No. 2 given at the rate of 3000 ml.
r M.² per 24 hours the first day, 2400 ml. per M.² per 24 hours on
bsequent days, accomplishes the correction of metabolic alkalosis of
loric stenosis as successfully as that of the metabolic acidosis of diar-
ea.[12] Since pyloromyotomy effects a complete and rapid correction of
strointestinal function, most infants can take oral feedings promptly
:er the operation; the electrolytes in milk will be used for complete cor-
:tion of body composition.[16]

Iatrogenic production of metabolic alkalosis by removal of upper
strointestinal fluid should be prevented rather than repaired by daily
easurement of the volume and composition of the fluid so removed; a
rresponding amount of fluid should be added to the intravenous regi-
en. The potassium deficit is underestimated by this method since more
tassium is excreted in the urine than with the gastrointestinal fluid.
easurement of urinary excretion of electrolytes is instructive but cum-
rsome. Darrow[19] recommends a solution containing sodium 75 mEq.,
tassium 40 mEq., chloride 115 mEq. per liter for replacement of gas-
c fluids, volume by volume; this solution avoids the excess of water in
S No. 2; the chloride concentration corresponds to that of the gastric
id.

In extensive and meticulous studies of the electrolyte metabolism of
wborn infants during and after surgery, compared with normal in-
nts, Knutrud[45] emphasizes the point that negative balance of sodium

and chloride is due to loss of these ions in aspirated secretions. When there are no extrarenal losses of sodium and water, normal as well as sick infants have a tendency to retain sodium and water during the first week of life; after the third day, urine volume is smaller in babies operated on than in control infants. By contrast, potassium is excreted in the urine, in response to trauma, especially on the 2 days following surgery, even with 0 intake. As in adults, trauma is followed by loss of potassium in the urine, in excess of the amount corresponding to the nitrogen loss. The potassium:sodium ratio in the urine during the first two post-operative days was found as high as 4-8.

Diabetic Acidosis

In diabetic acidosis, the deficits of water, electrolytes and energy are usually higher than in cases of diarrhea or vomiting. The losses occur primarily through the urine; they have often extended and progressively increased over several days or weeks, and they usually continue at least on the first day of corrective therapy. Brodsky et al.[6] emphasized the effect of osmotic diuresis on renal electrolyte loss, even without the severe starvation and acidosis which are elements of acute diabetic failure. In most cases of diabetic acidosis, vomiting contributes critically to the electrolyte loss.

Atchley and Loeb[2] and Butler[13] measured the deficits incurred by previously well-regulated diabetic patients when insulin was withheld for 3 to 4 days. In newly diagnosed cases of diabetes mellitus, the deficits may well be much larger than the amounts measured in these short term experimental studies, but the published figures provide guidelines for therapy. Danowski et al.[17] and Nabarro et al.[55] measured complete electrolyte balances of patients recovering from spontaneously occurring diabetic acidosis for 5 to 12 days. Nabarro pointed out that even after this period, cell composition was not restored and nitrogen balances were still negative. Danowski[15] summarized the published data[2, 13, 17, 55] and calculated that the average deficits, per kg. of body weight, amount to: 76 ml. of water, 0.65 gm. of nitrogen, 8 mEq. of sodium, 6 mEq. of chloride, and 5.5 mEq. of potassium in excess of what would correspond to the nitrogen balance. Butler's data, which were derived from the study of one diabetic patient, form the basis of the repair solution which he proposed and which has been modified only slightly in the polyionic solution No. 2 listed in Table 2.

In the treatment of diabetic acidosis, insulin is, of course, an essential ingredient. Since in the presence of acidosis and "stress," insulin is less effective than in a person with normal body composition,[37] large amounts are needed. A patient with severe diabetic acidosis may receive 2 units per kg. of body weight initially, half of this intravenously. Blood sugar and acetone in urine and serum should be monitored every 2 to 4 hours and additional doses of insulin, 1 or ½ unit per kg., added at these times until acetone disappears.

Insulin alone will not save the patient's life, however, unless circulation and metabolism are corrected by fluid and electrolyte therapy. Patients with diabetic acidosis are frequently in circulatory collapse. The

expansion of blood volume is critical and should be accomplished in no more than one hour. For this purpose, one should give within the first 45 minutes, 400 ml. per M.² of a "hydrating" solution which should contain sodium and chloride, preferably in a proportion resembling the normal proportion of these ions in extracellular fluid; e.g., 2 to 3 parts sodium chloride to 1 part sodium lactate or sodium bicarbonate. During the first hour of therapy, the solution should not contain glucose or potassium, since at this time insulin is not fully effective; at the rapid rate at which this solution is given, potassium ions may raise the serum level dangerously, and 5 per cent or 10 per cent glucose may result in further elevation of blood glucose and, therefore, in increased osmotic diuresis.

Extracellular deficits of water, sodium, and chloride are large but can and should be replaced within 24 hours. It is not necessary to calculate a theoretical requirement of sodium bicarbonate from the "negative base excess" or carbon dioxide content of serum. Darrow[21] and Butler et al.[13] pointed out the fallacy of this concept many years ago. If sodium bicarbonate is administered according to such a calculation, alkalosis almost always results, and the potassium deficiency of the cells is aggravated. The acidosis in diabetes is mainly caused by β-OH-butyric acid and other "ketones" which will be metabolized under the effect of insulin + glucose. Venous blood often has a very low pH because of stagnating circulation. In very severe cases, if the pH of *arterial* blood is below 7.0 and carbon dioxide content below 4 mEq. per liter, bicarbonate may be given in a dose calculated to raise the "base excess" or carbon dioxide content by 5 mEq. per liter and the pH into a range more favorable for insulin action.

Glucose may be withheld as long as blood sugar concentration is above 500 mg. per 100 ml. but is required after the first hour or two of therapy. The amount of insulin given should be adequate to replenish the glycogen stores of the liver which are 0 in untreated diabetic acidosis and to provide energy by metabolism of glucose. The concentration of glucose or invert sugar in the solution is usually 10 per cent since it is impossible to give enough calories with a lower concentration.

Glucose metabolism requires thiamine; since patients in diabetic acidosis and other states of starvation may be deficient in thiamine, riboflavin, and niacin, a preparation of vitamin B complex should be included in the initial therapy.

Intracellular deficits of potassium, phosphate, nitrogen, and magnesium usually require at least 1 to 2 weeks for restoration;[25] final correction of cell composition occurs only after an appropriate diet has been eaten for several days or weeks. However, provision of intracellular ions, especially potassium and phosphate, is very important during the first 24 to 48 hours of parenteral fluid therapy. Phosphorylation of glucose under the influence of insulin and production of high energy phosphates in the depleted cells consume large amounts of inorganic phosphate which is withdrawn from the extracellular fluid. Although stores of inorganic phosphate in the bones may be temporarily accessible to provide phosphate to cells, [P] in serum always falls, sometimes to levels below 1 mg. per 100 ml. Simultaneously, potassium enters the cells from the extracellular space even while urinary losses of potassium continue. [K⁺] in serum may fall to low enough levels to interfere with conduction of impulses in the heart; muscular weakness and depression or disappearance of reflexes are common. No stores of potassium exist in the body; therefore the depleted diabetic patient depends on exogenous potassium for recovery.

Monitoring serum [K⁺] during therapy of diabetic acidosis is at least as important as monitoring glucose or carbon dioxide content in blood. If it is realized that a low concentration of [K⁺] in serum always indicates very severe depletion, the need for large amounts of potassium in the therapy will be evident. The absolute quantity required can only be estimated from published balance studies, not from the serum concentration. The principle of potassium therapy is to give as much as is tolerated without transient elevation of [K⁺] in serum to a dangerous range; this will never be enough to correct the cellular deficit in a few days. The maximal amount of potassium tolerated by intravenous infusion is probably about 6 to 8 mEq. per M.² per hour but varies with the renal function. When the rate of administration of a solution is accelerated, the potassium concentration should not be increased simultaneously without calculating the absolute amount of potassium administered per hour. More potassium can be tolerated by mouth.[17]

Because of the osmotic diuresis which usually continues during the early phases of therapy, the total amount of fluid on the first day of therapy should be at least 4000 ml. per M.² per 24 hours, half of this given during the first 8 hours. The urine volume has to be measured at least every 2 to 4 hours; if it exceeds or approaches the fluid intake, the rate of infusion has to be accelerated, otherwise dehydration will increase instead of decreasing. A program for therapy of diabetic acidosis then may look as illustrated in Table 5 and provide, per M.² within the first 24 hours: water 4150 ml., sodium 244, chloride 208, potassium 80 to 100 and magnesium 22.5 mEq.

Salicylate Intoxication

The aim of fluid therapy in salicylate intoxication is a dual one: promotion of excretion of the salicylate and correction of the dehydration and metabolic acidosis which are present in all severe cases. Oxygen consumption, hyperventilation, and respiratory water loss are extremely high in this condition,[7] while oxidative phosphorylation, i.e., energy production, is blocked, and carbohydrate metabolism is impaired.[73] The severity of acute salicylate intoxication can be estimated from the salicylate concentration in serum in relation to the time elapsed since ingestion of the salicylate, if known, by referring to Done's diagram.[2] This diagram indicates a "half time" of 20 hours. The initial salicylate level can be calculated by extrapolation to 0 time. An initial level of over 100 mg. per 100 ml. serum indicates severe poisoning and a dubious prognosis.

Rehydration and renal correction of acid-base balance can be achieved in most cases by the same regimen which is used in cases of acute dehydration from diarrhea or other causes. But rapid excretion of

Table 5. *Example of a Program for Therapy of Diabetic Acidosis*

TIME	VOLUME (ML./M²)	SOLUTION		INSULIN (UNITS/KG.)
0–45 min.	400	"Hydrating," e.g.:		
		Na	75 mEq./L.	1 i.v.
		Cl	50 mEq./L.	+1 s.c.
		Lactate or bicarbonate	25 mEq./L.	
	ml./M²/hr.			
45 min.–8 hr.	250	PIS No. 2 with added "Solu-B with C" or		1 × 1
		Berocca C, 2 ml.		½ × 2
8–24 hr.	125	PIS No. 2 with added K_2HPO_4, 15–25 mEq./L.		½ × 2

large amounts of salicylate cannot be expected unless the pH of the urine is above that of plasma. The clearance of free salicylate is about 20 to 30 times as high at pH 7.5 as at pH 6,[62] and the formation of salicylurate – which accounts for 70 to 80 per cent of excretion of *low* doses of salicylate – is limited to 0.3 to 0.4 mg. per hr. M.² [49] For this reason, attempts should be made to alkalinize the urine, especially during the first 12 hours of therapy when rapid reduction of high drug levels in body fluids may prevent serious impairment of metabolism.

Alkalinization of urine in the presence of metabolic acidosis is difficult to achieve. The administration of very large amounts of sodium bicarbonate was recommended by Whitten et al.[70] but even these doses do not always ensure urine pH >7 and lead to metabolic alkalosis and hypopotassemia in most cases. Excessive expansion of extracellular fluid with edema may also occur. Acetazolamide, a carbonic anhydrase inhibitor, alkalinizes urine by preventing tubular reabsorption of bicarbonate. If it is given in doses of 5 mg. per kg., the effect lasts approximately 4 hours and results in marked increase of the clearance of salicylate.[62] Two or three doses usually suffice to reduce the salicylate level in blood to a safe range. By the nature of its action, a carbonic anhydrase inhibitor interferes with the normal renal compensation for acidosis. Therefore, it is often necessary to add sodium bicarbonate to the parenteral fluid regimen for patients with severe metabolic acidosis and salicylate poisoning. The amount may be calculated to raise the base deficit (or the plasma bicarbonate) by 6 to 10 mEq. per liter, assuming the volume of distribution to be 50 per cent of body weight. The infused solution should always be hypotonic to body fluids, its [Na^+] should not exceed 100 mEq. per liter since the insensible water loss in salicylate intoxication is very high.[38]

Since acetazolamide also increases diuresis, the volume of infused fluid should be no less than 125 ml. per M.² per hour.

In salicylate intoxication treated with acetazolamide, as well as in diabetic acidosis, obligatory diuresis prevents renal water conservation which operates so effectively in cases of diarrhea or vomiting. In our

experience, *the lack or delay of success in these two conditions can almost always be traced to an unduly slow rate of infusion.*

In cases of extreme salicylate intoxication, particularly if a long time has elapsed since the ingestion and the patient is severely dehydrated, exchange transfusion, peritoneal dialysis, or hemodialysis are sometimes considered as emergency measures. All these methods require a much longer period of preparation than intravenous fluid therapy, and none of them provides as effective removal of the salicylate as the kidney does if it is able to excrete an alkaline urine. Only one fifth of the total amount of salicylate in the body is contained in plasma; the amount recovered in 12 hours in alkaline urine may be 5 to 7 times the amount circulating in plasma.[27] Much of the salicylate in the body is bound to protein, including intracellular proteins, therefore serum albumin added to the fluid used for peritoneal dialysis increases the amount removed by this method.

Burns

Burns constitute another special challenge for parenteral fluid therapy. The deficits occur more suddenly than when electrolytes and water are lost via the intestinal tract or the kidneys; they are of a different nature, and they change rapidly during the first 24 to 48 hours after the burn. The loss of water, sodium, chloride and protein with the exudate is the first and obvious problem. These losses have been measured directly; during the first 24 hours they may amount to approximately 15 ml. of water, 1 mM. of sodium, and 0.03 to 0.1 gm. of nitrogen for every 100 cm.² of burned area.[32, 59] The protein loss calculated from these figures is about 0.7 gm. per 100 cm.²/24 hours on the first day, 0.4 gm. the second, and 0.3 gm. the third. Shock is present in many children who have extensive burns. The clinical signs of shock are associated with reduction of circulating blood volume, renal plasma flow, and glomerular filtration rate. In addition, renal excretion of water and sodium during the first 12 hours after a burn are inhibited, presumably by excess production of both antidiuretic hormone and adrenal hormones in response to the trauma.

The evaluation of deficits and, therefore, of the requirements for replacement therapy in individual cases is difficult. The concentrations of sodium, hematocrit, or plasma protein may give misleading impressions as to requirements. Hematocrit particularly is a poor guide to circulating blood volume. The hematocrit of capillary blood may differ widely from that of the blood in large blood vessels. Blood loss or hemolysis may reduce the number of circulating red blood cells, and plasma proteins leave the circulation, reversibly, via increased capillary permeability.[20] Central venous pressure provides a better measure of adequacy of the circulation.

Accurate weighing of the patient before and after application of dressings and equipment and at frequent intervals thereafter remains the most important guide to correct therapy. Urine volume should be measured, but oliguria during the first 12 hours cannot be used as an indication for giving large amounts of sodium containing fluid. Metcoff et

al.[32] have shown that early oliguria (average 110 ml. of urine per M.^{2} per 12 hours) is caused by unavoidable renal and hormonal changes and that large amounts of saline (as much as 90 gm. sodium chloride per M.^{2} had been recommended in the past) given at this time cause a large positive balance and the edema which had been thought to be an inevitable consequence of burns. They also demonstrated that the hyponatremia, which is sometimes observed on the second day, was caused by shift of sodium into cells and, in addition, by increased antidiuretic hormone production; therefore, it has to be treated by increasing potassium and decreasing water intake, not by giving more sodium salt. Urine volume usually increases considerably on the second day and third day; potassium deficiency develops unless the intake of potassium is high.

Agreement exists that the initial phase of fluid therapy for burns has to include a colloid, which will effect replenishment of the circulating volume and compensate for continuing losses of protein with the exudate. Serum albumin, plasma, or Dextran may be used.

The area of the burn is usually estimated as a percentage of the total body surface according to an empiric table.[30] By multiplying the estimated percentage of burn by the child's surface area, the area of burn can be directly calculated in cm.^{2} The amount of protein to be given may then be determined as 0.7 gm. the first day, 0.4 gm. the second day, and 0.3 gm. the third day for each 100 cm.^{2} of burn area. The volume of replacement fluid *to be added to the usual fluid regimen* is 15 ml. per 24 hours for each 100 cm.^{2} of burn.

In addition to the replacement of losses from the skin, maintenance fluid, of course, has to be provided. The maintenance requirements differ from those of an unconscious but otherwise metabolically normal patient in three respects:[32]

1. A negative nitrogen balance and a disproportionately large negative potassium balance is practically always present.
2. The average insensible water loss is 900 gm. per M.^{2} per day[32] = 37 gm. per M.^{2} per hour and may be as high as 60 to 175 gm. per M.^{2} per hour[40] instead of the basal loss of 20 gm. per M.^{2} per hour which is often assumed; if high protein formulas are used for tube feeding[5] without addition of free water, severe hyperosmolality of body fluids may be produced.[35]
3. Glomerular filtration rate and renal excretion of water and sodium are reduced to about one third of normal during the first 12 hours.

Therefore, a reasonable volume added for maintenance to the replacement of losses could be 50 ml. per M.^{2} per hour during the first 12 hours, 62.5 ml. per M.^{2} per hour the second 12 hours, and 75 ml. per M.^{2} per hour the second day. During the phase of oliguria, this fluid should not contain potassium, but after the first 12 hours, generous amounts of potassium should be provided. PIS No. 2 is an appropriate solution; its concentration of sodium and chloride resembles the concentrations in the exudate, and its potassium content helps to compensate for the large renal losses. Water soluble vitamins are helpful during the recovery from severe trauma and shock. Table 6 is an example of a program calculated according to these principles.

Table 6. *Program for Fluid Therapy in a Burn Patient**

	PROTEIN	FLUID	SOLUTION
1st 12 hours	5.5 gm.	400 ml.	0.45% NaCl in 10% dextrose
2nd 12 hours	5 gm.	500 ml.	PIS No. 2 with added Solu-B with C
2nd Day	6.0 gm.	1100 ml.	PIS No. 2 with added Solu-B with C
3rd Day	4.5 gm.	1100 ml.	PIS No. 2

*30 per cent burn of a child weighing 10.0 kg. Body surface area = 0.5 M^2; burn area 30 × 0.5 × 100 cm.^{2} = 1500 cm.^{2}

The "Evans regimen,"[26] which is still used as a guide to fluid therapy of burns by many surgeons, provides colloid in amounts comparable to those proposed here, though slightly higher. However, the amount of sodium and water suggested by the Evans regimen during the first 12 hours is much higher and exceeds the excretory ability of the kidney at that time.

PROLONGED ELECTROLYTE LOSSES

Gastrointestinal Diseases

Patients suffering prolonged or chronic losses require careful calculation of their fluid requirements because deficits accumulate rapidly. Often such patients have malnutrition and large deficits of electrolytes already before therapy is begun. This is particularly true for patients with protracted diarrhea, ulcerative colitis, or "anorexia nervosa."[24] The deficits in these conditions are only in part caused by the losses from the intestinal tract directly; renal losses of potassium and other electrolytes contribute significantly.

Diuretics and Salt Depletion

When diuretics have been given for several days or weeks, renal excretion of sodium and chloride may have led to severe deficits of these ions and thereby of extracellular fluid. Sometimes the patients also receive a low salt diet, and although they may originally have had edema and therefore an excess of sodium and extracellular fluid, the combination of low salt intake and forced diuresis may result in both hyponatremia and hypovolemia and eventually in circulatory collapse. If glucocorticoids have been given, the body may have been depleted of potassium. The losses are apt to continue during the period of therapy.

In any case of protracted electrolyte disturbance, therefore, volume and electrolyte content of urine and gastrointestinal losses (vomitus or diarrheal stool) as well as the patient's weight should be carefully measured and analyzed. A therapeutic program should be thoughtfully planned with consideration of all the factors that can be derived from the patient's history and from the pertinent scientific literature.

A graphic or tabular record of actual intake and output should be brought up to date at least once daily and compared with the intended in

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take. Any deviation should be compensated within 24 hours. Serum should be analyzed frequently to detect the effects of miscalculations. Next to body weight, carbon dioxide content, chloride, sodium, and potassium provide the most valuable information.[9] Adjustment of intake to the figures obtained by analysis of intestinal secretions and urine can help to correct circulatory collapse and to maintain constant weight and normal composition of body fluids.

RENAL INSUFFICIENCY

In all of the foregoing discussions, it was assumed that normal kidneys and endocrine organs will maintain normal body composition if sufficient materials are provided, and that excesses will be excreted in the urine. The relatively wide margin of safety[67] provided by the functions of these organs narrows down considerably when these functions are impaired and may have the dimension of a razor's edge in cases of anuria.

Impaired renal function may imply oliguria and inability to excrete potassium and nitrogen when glomerular filtration is reduced to less than perhaps 5 per cent of normal; or it may imply polyuria with isosthenuria when concentrating capacity is the main function lost. The latter condition is by far the more common one in cases of chronic renal insufficiency and is tolerated quite well by ambulatory patients for many years as long as their thirst mechanism is functioning and they have access to water. These patients, who may not even be aware of their renal insufficiency, become severely ill if they vomit or become unconscious, or if physicians start restricting their fluid intake, for example, in preparation for surgery. One such patient, who had documented hyposthenuria and polydipsia since childhood, entered the United States Army without knowledge of his condition by either himself or the physician; he collapsed during the first strenuous march in the heat of South Carolina. Such patients should receive large volumes of fluid intravenously; a history of their customary intake may be helpful in estimating the requirement on the first day; thereafter, the patient's weight and the [Na⁺] of his serum are good guides to follow. Thirst reflects fluid requirements faithfully if the patient is able to talk, but does not give reliable information as to electrolyte needs.

Patients with isosthenuria usually are "salt-wasters"—that is, their renal tubules do not reabsorb either sodium or water adequately. Frequently, at this stage of renal insufficiency, the excretion of H ions is impaired also. For these reasons, the initial solution given intravenously should resemble the "balanced hydrating solution," i.e. it should contain sodium in approximately half isotonic solution, and chloride and lactate ions in a ratio of 2:1 or 3:1. "Free" water is necessary for replacement of insensible water loss. The kidneys of patients with advanced renal insufficiency cannot adapt to a schedule which is still customary in some hospitals of alternating one bottle of saline with one bottle of glucose in water. The solutions have to be adapted to the kidneys.

In the treatment of patients suspected of having impaired renal functions, the volume of fluids is often calculated as "insensible loss plus urine output." Although this principle is logical, it frequently leads the physician to prescribe an insufficient amount of fluid and thereby to cause dehydration. He may be unaware that (1) a low urine output may be the effect of a low water intake and dehydration rather than of primary renal disease (in this case his therapy will aggravate the patient's condition); (2) insensible water loss in disease is not well known[8] and is usually underestimated. The lowest basal insensible water loss which has been reliably measured and reported[48] is 20 gm. per M.² per hour in healthy sleeping infants under 1 year of age, 24.7 gm. per M.² per hour over 1 year of age, in an air-conditioned room during a 2-hour period. In Krieger's more recent study,[47] the mean value was 25.9 gm. per M.² per hour or 1.22 gm. per kg. per hour in normal infants, higher in malnourished convalescent infants.

No child remains at basal rest for 24 hours, and muscular activity as well as almost any disease other than hypothyroidism tends to increase the insensible water loss. Sweat is not included in careful studies of insensible water loss, but has to be included in the practical calculation of water requirements. Crude estimation of insensible losses, probably including sweat, of hospitalized sick children[21, 38, 39, 32] has yielded figures averaging 700 to 900 ml. per M.² per 24 hours, but with a range from 300 to 1300 ml. per M.² per 24 hours. The variations with fever, hyperventilation, or crying are so large that it is impossible to rely on a fixed low figure of insensible water loss; respiratory water loss alone may be as high as 20 ml. per M.² per hour in hyperventilation.[7] On the other hand, water of oxidation and water released by catabolism of tissues may contribute significantly to the balance. The contributions from this source are most significant in cases of stress with acute illness, tissue necrosis, or infection. In critically ill adults who had prolonged anuria or severe oliguria, Bluemle et al.[48] observed that as much as 50 per cent of the insensible water loss was offset by water liberated in metabolism.

Therefore, in critical circumstances, a patient with severe oliguria or anuria should be weighed several times a day or, preferably, on a continuously recording balance.[60] He should also be examined clinically for edema or signs of dehydration. After correction of pre-existent underhydration or overhydration, the volume of water intake should be adjusted to keep his weight stable or declining slightly. Oral administration of fluid and carbohydrate is the safest and simplest method for a patient who is conscious and does not vomit. Thirst is a reliable criterion for water intake in this situation; it may be more precise than the balance on which the patient is weighed and certainly more precise than theoretical calculations of insensible water loss. It seems both cruel and unphysiologic to restrict water intake to a thirsty patient because of some preconceived idea of requirements which may or may not be correct for the "average" or "ideal" person, but does not fit the individual case.

Carbohydrate intake should be high; it may be calculated to provide 1000 to 1200 calories per M.² per day. This amount will not prevent protein catabolism completely, but it will reduce breakdown of cell protein and release of potassium from cells to a minimum. Loss of potassium from the cells and elevation of the serum level of potassium is reduced also by correction of acidosis.

Potassium and magnesium should not be given to a patient who has anuria or minimal glomerular filtration rate. Ion exchange resins which are given orally or rectally, often combined with a potassium-free diet, may result in gradual depletion of cell potassium even in the presence of a high potassium level in the serum.

Phosphate also should be omitted from fluids administered to patients with minimal glomerular filtration. The function of phosphate as a buffer in urine which permits the kidney to regulate acid-base balance by excreting H ions does not exist in such patients; therefore, the requirement for phosphate is much lower than in other conditions requiring fluid therapy. The concentration of inorganic phosphate in serum is usually elevated and is thought to stimulate the parathyroid glands; hyperparathyroidism is one of the causes of bone disease which accompanies chronic renal failure. Other electrolytes have to be provided to compensate for losses via the intestinal tract. In the absence of sweating, electrolyte loss from the skin is negligible.

Acidosis of chronic renal insufficiency has to be treated or, better, prevented by alkalinizing salts. Sodium citrate (Shohl's solution), acetate, lactate, or bicarbonate may be used orally; the latter two salts are also available for intravenous use. Except in emergencies, the organic salts are preferable to bicarbonate. The amounts needed have to be determined by trial and error; they depend on the number of H ions produced in metabolism which, in turn, depend on endogenous protein metabolism as well as on the protein and ash content of the diet; for adults the dose varies between 50 and 100 mEq. per day.

Nephrogenic diabetes insipidus is a form of renal disease in which the only missing renal function is reabsorption of water in the collecting tubules in response to antidiuretic hormone. Once the diagnosis has been established, these patients present no serious problem in fluid therapy; their thirst indicates the amount of water needed, which is large; chlorothiazide may effect some reduction of this amount. Like other patients with renal disease, the patients are severely handicapped when they are unconscious or otherwise unable to communicate their thirst, e.g., as young infants, or when they suffer from unrelated acute conditions which limit fluid intake or cause excessive loss, such as diarrhea or vomiting.

The amount of water to be added to the conventional fluid therapy regimen may be estimated by measuring the osmolar load as: urine volume times osmolality of urine, or by calculating it from intake.[74] The usual parenteral fluid regimen[12] is calculated to allow insensible water loss of 750 ml. per M.^{2} per 24 hours plus excretion of urine with an osmolality of 300 mOsm. per liter; a patient with renal diabetes insipidus may excrete a urine with perhaps 75 mOsm. per liter. Therefore, the amount of water needed for urine formation in this patient would be 12 ml. per milliosmol of solute load instead of 3 ml. per milliosmol in the normal person. The urine volume of the patient, even without knowledge of the solute load, is a valuable guide to water requirements and should be measured carefully during treatment.

ENDOCRINE DISTURBANCES

Diabetes Insipidus

Patients with true diabetes insipidus present similar problems when they become ill and require parenteral fluid therapy; however, since they respond to exogenous pitressin, their fluid balance is easier to manage than that of patients with nephrogenic diabetes insipidus.

Inappropriate Antidiuretic Hormone Production

The converse of diabetes insipidus, excessive "inappropriate" production of antidiuretic hormone, presents a more common problem. It occurs either transiently or chronically with various diseases affecting the hypothalamus, such as meningitis or encephalitis, as well as occasionally with pulmonary diseases. The diagnosis is the most important requirement for correct therapy. The clue is hyponatremia associated with hyperosmolality of urine. Not only total osmolality but sodium content of urine may be elevated, since the hypervolemia which is often produced by the excessive water retention inhibits, via the juxtaglomerular apparatus, aldosterone production by the adrenal cortex. Treatment consists of restriction of fluid intake. Depending on extrarenal losses, the total requirement for water may vary from 900 to 1300 ml. per M.^{2} per 24 hours. In most cases, electrolyte intake does not have to be modified. Again, the patient's weight, volume and osmolality of his urine, and [Na^+] of serum should be monitored at least once a day.

Adrenal Insufficiency

Besides the kidney and the pituitary gland, the adrenal cortex is essential for homeostasis of fluid and electrolytes. A patient suffering from salt-losing adrenogenital syndrome or Addison's disease may die in early infancy unless he receives adequate fluid and electrolyte therapy. Conversely, many patients were kept alive in the past by salt therapy alone, even before appropriate hormones were available for treatment. The essential disturbances and common causes of death are cardiac arrest from hyperkalemia and shock and dehydration caused by excessive loads of sodium, and therefore water and chloride, in the urine.

In a critical state of adrenal insufficiency, an intravenous infusion of saline produces dramatic results. The saline solution may be isotonic or half isotonic or intermediate between these concentrations. It may be given orally after the initial shock is corrected, and provided that the patient is not vomiting. Any solution containing potassium, including whole blood, is of course contraindicated in acute adrenal insufficiency. Other electrolytes are not required at this stage, but "balanced hydrating solution" containing 25 mEq. of lactate per liter is as successful as saline solution alone in correcting dehydration and shock.

The acidosis which always accompanies adrenal insufficiency is usually corrected spontaneously as renal circulation and glomerular filtration are restored. However, we have seen a patient who remained acidotic and was hyperventilating vigorously after 8 hours of saline infusion; he had ketoacidosis because glucose had inadvertently been

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omitted from the fluids given to him. Both for this reason, and because patients with adrenal insufficiency may have hypoglycemia, it is recommended that they be treated with fluids containing 5 or 10 per cent glucose.

SUMMARY

Patients with intact renal, adrenal, and pituitary functions are able to maintain or establish normal composition of body fluids if given sufficient amounts of water, electrolytes and glucose. Excess supplies, within "safe homeostatic limits," will be excreted.

The safe homeostatic limits, particularly for sodium, are smaller in newborn infants than after the first month of life.

For patients who are unable to take nutrition by mouth normally, but who have no other disturbances of electrolyte metabolism, fluid therapy serves to compensate for obligatory expenditures. For periods of a few days, water and glucose are the most important ingredients, but since urine always contains electrolytes and sodium and chloride may be lost by sweat, small amounts of sodium, chloride, potassium and phosphate are included in the regimen.

If maintenance therapy is required for more than a few days, amino acids, vitamins, and trace minerals must be provided and caloric expenditures covered, mostly by glucose. Although improved surgical techniques have permitted much progress in the field of complete parenteral nutrition, the safety and adequacy of amino acid solutions available at present has not been established over long periods of time and in all age groups, and satisfactory fat emulsions for parenteral administration are still lacking, at least in the United States.

Acute dehydration caused by gastrointestinal losses can be satisfactorily corrected with solutions devised in the past on the basis of classic research. The regimen consists of establishing circulation by rapid infusion of a potassium-free solution within ½ to 1 hour and following with a continuous slower infusion of a polyionic solution containing glucose. All solutions should be hypotonic to body fluids with respect to electrolytes, since insensible water loss requires electrolyte-free water.

Continuing losses of gastrointestinal fluid, as through a drainage tube, should be replaced daily, in addition to any other therapy. The concentration of sodium and chloride in such secretions may approach the concentration in serum; renal excretion of potassium adds to the deficits.

Diabetic acidosis and salicylate intoxication can also be treated successfully with the same regimen; only slight modifications may be necessary. Because the osmotic diuresis in diabetic acidosis may have been present for many days or weeks and continues during the early phases of therapy, larger amounts of polyionic solution may be required than in other cases of dehydration; for the same reason, the rapidly infused initial hydrating solution should not contain glucose although glucose is required after the first 1 to 2 hours. In salicylate intoxication, excretion

of the salicylate is accelerated if the pH of urine can be raised above 7 by the use of acetazolamide and sodium bicarbonate.

Various programs proposed during the last 20 years are based on the same fundamental balance data. They all work in acute conditions, regardless of the specific deficits or electrolyte composition of the individual patient. "Tailoring" a fluid therapy program to the specific needs of an individual patient is a job for experts; serious mistakes are often made when it is attempted in the excited atmosphere of an emergency by physicians who are not very familiar with the pathophysiology of electrolyte metabolism.

Individual "tailoring" is required for patients whose renal, adrenal or pituitary functions are impaired. Besides expert knowledge of pathophysiology, monitoring of the patient's weight, urine volume, and electrolyte composition of serum and urine is necessary for success in such cases.

An exact and clear record of intake in tabular or graphic form should be kept in all cases in which parenteral fluids are administered. The record has to be brought up to date every hour during acute conditions, or 2 or 3 times a day in more chronic ones. Volume of urine and unusual expenditures, such as gastrointestinal contents from a drainage tube, also should be entered in this record. Deficits or excesses, either of total fluid or of individual ions such as potassium or chloride, can then be corrected immediately by compensating in one period for aberrations during the previous one.

The most common cause for lack of success of a fluid regimen is deviation of the amount actually received by the patient from the intended amount, which may have been ever so carefully calculated.

REFERENCES

1. Alexander, C. S., and Zieve, L.: Fat infusions. Toxic effects and alterations in fasting serum lipids following prolonged use. Arch. Intern. Med., *107*:514–528, 1961.
2. Atchley, D. W., Loeb, R. F., Richards, D. W., Jr., Benedict, E. M., and Driscoll, M. E.: On diabetic acidosis. A detailed study of electrolyte balances following the withdrawal and reestablishment of insulin therapy. J. Clin. Invest., *12*:297, 1933.
3. Beaton, G. H., and McHenry, E. W., eds.: Nutrition. A Comprehensive Treatise. New York and London. Academic Press, 1964, vol. 2, chap. 2, by G. A. Goldsmith.
4. Berg, G., ed.: Advances in Parenteral Nutrition. Symposium of the International Society of Parenteral Nutrition, Prague, Sept. 3-4, 1969. Stuttgart. Georg Thieme, 1970.

4a. Bluemle, L. W., Jr., Potter, H. P., and Elkinton, J. R.: Changes in body composition in acute renal failure. J. Clin. Invest., *35*:1094–1108, 1956.

5. Boles, E. T., and Terry, J. L.: Practical aspects of the management of severely burned children. Amer. J. Surg., *101*:668–676, 1961.
6. Brodsky, W. A., Rapoport. S., and West, C.: The mechanism of glycosuric diuresis in diabetic man. J. Clin. Invest., *29*:1021–1032, 1950.
7. Bruck, E.: Unpublished data.
8. Bruck, E.: Water in expired air: Physiology and measurement. J. Pediat., *60*:869–88[illegible] 1962.
9. Bruck, E.: Laboratory tests in the analysis of states of dehydration. Pediat. Clin. N. Amer. *18*:265–283, 1971.
10. Bruck, E., Abal, G., and Aceto, T., Jr.: Pathogenesis and pathophysiology of hypertonic dehydration with diarrhea. A clinical study of 59 infants with observations of respiratory and renal water metabolism. Amer. J. Dis. Child., *115*:122–144, 1968.
11. Bruck, E., Abal, G., and Aceto, T., Jr.: Therapy of infants with hypertonic dehydration due to diarrhea. A controlled study of clinical, chemical and pathophysiological response[illegible]

two types of therapeutic fluid regimen with evaluation of late sequelae. Amer. J. Dis. Child., *115*:281-301, 1968.
12. Bruck, E., Aceto, T., Jr., and Lowe, C. U.: Intravenous fluid therapy for infants and children. Physiologic principles and a practical regimen with examples of application. Pediatrics, 25:496-516, 1960.
13. Butler, A. M., Talbot, N. B., Burnett, C. H., Stanbury, J. B., and MacLachlan, E. A.: Metabolic studies in diabetic coma. Trans. Assoc. Amer. Physicians, 60:102-109, 1947.
14. Cooke, R. E., and Ottenheimer, E. J.: Clinical and experimental interrelations of sodium and the central nervous system. Advances Pediat., *11*:81-145, 1960.
15. Danowski, T. S.: Diabetes Mellitus with Emphasis on Children and Young Adults. Baltimore, Williams and Wilkins, 1957.
16. Danowski, T. S., Austin, A. C., Gow, R. C., Mateer, F. M., Weigand, F. A., Peters, J. H., and Greenman, L.: Electrolyte and nitrogen balance studies in infants following cessation of vomiting. Pediatrics, 5:57-66, 1950.
17. Danowski, T. S., Peters, J. H., Rathbun, J. C., Quashnock, J. M., and Greenman, L.: Studies in diabetic acidosis and coma, with particular emphasis on the retention of administered potassium. J. Clin. Invest., 28:1-9, 1949.
18. Darrow, D. C.: The retention of electrolyte during recovery from severe dehydration due to diarrhea. J. Pediat., 28:515-540, 1946.
19. Darrow, D. C.: A Guide to Learning Fluid Therapy. Springfield, Illinois, Charles C Thomas, 1964.
20. Darrow, D. C., and Buckman, T. E.: The volume of the blood. II. The volume of the blood and concentration of crystalloids and electrolytes in dehydration and edema. Amer. J. Dis. Child., 36:248-267, 1928.
21. 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, 3:129-156, 1949.
22. Done, A. K.: Salicylate intoxication. Significance of measurements of salicylate in blood in cases of acute ingestion. Pediatrics, 26:800-807, 1960.
23. Dudrick, S. J.: Intravenous feeding as an aid to nutrition in disease. CA, 20:198-211, 1970.
24. Elkinton, J. R., and Huth, E. J.: Body fluid abnormalities in anorexia nervosa and undernutrition. Metabolism, 8:376-403, 1959.
25. Engel, F. L., and Jaeger, C.: Dehydration with hypernatremia, hyperchloremia and azotemia complicating nasogastric tube feeding. Amer. J. Med., 17:196-204, 1954.
26. Evans, E. I., Purnell, O. J., Robinett, P. W., Batchelor, A., and Martin, M.: Fluid and electrolyte requirements in severe burns. Ann. Surg., *135*:804-817, 1952.
27. Feuerstein, R. C., Finberg, L., and Fleishman, E.: The use of acetazoleamide in the therapy of salicylate poisoning. Pediatrics, 25:215-227, 1960.
28. Filler, R. M., Eraklis, A. J., Rubin, V. G., and Das, J. B.: Long-term parenteral nutrition in infants. New Eng. J. Med., *281*:589-594, 1969.
29. Finberg, L.: Dangers to infants caused by changes in osmolal concentration. Pediatrics, 40:1031-1034, 1967.
30. Finberg, L., and Fleishman, E.: Experimental studies of the mechanisms producing hypocalcemia in hypernatremic states. J. Clin. Invest., 36:434, 1957.
31. Finberg, L., and Harrison, H. E.: Hypernatremia in infants. An evaluation of the clinical and biochemical findings accompanying this state. Pediatrics, *16*:1-14, 1955.
32. Finberg, L., Kiley, J., and Luttrell, C. N.: Mass accidental salt poisoning in infancy. A study of a hospital disaster. J.A.M.A., *184*:187-190, 1963.
33. Gamble, J. L.: Deficits in diarrhea. J. Pediat., 30:488-494, 1947.
34. Gamble, J. L., Wallace, W. M., Eliel, L., Holliday, M. A., Cushman, M., Appleton, J., Shenberg, A., and Piotti, J.: Effects of large loads of electrolytes. Pediatrics, 7:305-320, 1951.
35. Gault, M. H., Dixon, M. E., Doyle, M., and Cohen, W. M.: Hypernatremia, azotemia and dehydration due to high-protein tube feeding. Ann. Intern. Med., *68*:778-791, 1968.
36. Gordon, H. H., McNamara, H., and Benjamin, H. R.: The response of young infants to ingestion of ammonium chloride. Pediatrics, 2:290-302, 1948.
37. Guest, G. M., Mackler, B., and Knowles, H. C.: Effects of acidosis on insulin action and on carbohydrate and mineral metabolism. Diabetes, *1*:276-282, 1952.
38. Guest, G. M., Pettit, M. D., Araujo, C., Frid, J., Combes, B., deMenibus, C., and Wittgenstein, E.: Studies of insensible weight loss, clinical and experimental. A.M.A. J. Dis. Child., *96*:578, 1958.
39. Heeley, A. M., and Talbot, N. B.: Insensible water losses per day by hospitalized infants and children. A.M.A. J. Dis. Child., *90*:251-255, 1955.
40. Hogan, G. R., DeVivo, D., Gill, S. R., Master, S., Sotos, J., and Dodge, P.: Development of seizures during rehydration of hypertonic rabbits. J. Pediat., *63*:729, 1963. *Note*: The statement quoted here was made in the verbal presentation of this paper at the 33rd annual meeting of the Society for Pediatric Research, Atlantic City, N.J., May 2, 1963, but it is not contained in the printed abstract or in a later article on this topic in Pediatrics, 43:54-64, 1969.

41. Holt, L. E., Jr.: Nutrition and infant feeding. *In* Barnett, H. L., ed.: Pediatrics. New York, Appleton-Century-Crofts, 14th ed., 1968.
42. Iacono, J. M., Mueller, J. F., and Zellner, D. C.: Changes in plasma and erythrocyte lipids during short-term administration of intravenous fat emulsions and its subfractions. Amer. J. Clin. Nutr., *16*:165-72, 1965.
43. James, L. S.: Physiology of respiration in newborn infants and in the respiratory distress syndrome. Pediatrics, 24:1069-1101, 1959.
44. Kerpel-Fronius, E., Varga, F., and Kun, K.: Anoxia in infantile dehydration. Arch. Dis. Child., 25:156-158, 1950.
45. Knutrud, O.: The water and electrolyte metabolism in the newborn child after major surgery. Oslo, Universitetsforlaget, 1965.
46. Kravath, R. E., Aharon, A. S., Abal, G., and Finberg, L.: Clinically significant physiologic changes from rapidly administered hypertonic solutions: Acute osmol poisoning. Pediatrics, 46:267, 1970.
47. Krieger, I.: The energy metabolism in infants with growth failure due to maternal deprivation, undernutrition, or causes unknown. Pediatrics, 38:63-76, 1966.
48. Levine, S. Z., Kelly, M., and Wilson, J. R.: The insensible perspiration in infancy and childhood. II. Proposed basal standards for infants. Amer. J. Dis. Child., 39:917, 1930.
49. Levy, G., and Yaffe, S. J.: The study of salicylate pharmacokinetics in intoxicated infants and children. Clin. Toxicol., *1*:409-424, 1968.
50. Lund, C. C., and Browder, N. C.: The estimation of areas of burns. Surg. Gynec. Obstet., 79:352-358, 1944.
51. Machella, T. E.: Mechanism of the post-gastrectomy dumping syndrome. Gastroenterology, *14*:237-255, 1950.
52. Metcoff, J., Buchman, H., Jacobson, M., Richter, H., Jr., Bloomenthal, E. D. and Zacharias, M.: Losses and physiologic requirements for water and electrolytes after extensive burns in children. New Eng. J. Med., 265:101-111, 1961.
53. Metcoff, J., Frenk, S., Gordillo, G., Gómez, F., Ramos-Galván, R., Cravioto, J., Janeway, C. A., and Gamble, J. L.: Intracellular composition and homeostatic mechanisms in severe chronic infantile malnutrition. IV. Development and repair of the biochemical lesion. Pediatrics, 20:317-336, 1957.
54. McCance, R. A., and Widdowson, E. M.: Hypertonic expansion of the extracellular fluids. Acta Paediat., 46:337-353, 1957.
55. Nabarro, J. D. N., Spencer, A. G., and Stowers, J. M.: Metabolic studies in severe diabetic ketosis. Quart. J. Med., *21*:225-248, 1952.
56. Peonides, A., Young, W. F., and Swain, V. A. J.: Fluid and electrolyte requirements of newborn infants with intestinal obstruction. Arch. Dis. Child., 38:231-241, 1963.
57. Rapoport, S., and Dodd, K.: Hypoprothrombinemia in infants with diarrhea. Amer. J. Dis. Child., *71*:611-617, 1946.
58. Rapoport, S., Dodd, K., Clark, M., and Sylim, I.: Postacidotic state of infantile diarrhea: Symptoms and chemical data. Postacidotic hypocalcemia and associated decreases in levels of potassium, phosphorus and phosphatase in plasma. Amer. J. Dis. Child., 73:391, 1947.
59. Reiss, E., Pearson, E., Artz, C. P., and Balikov, B.: The metabolic response to burns. J. Clin. Invest., 35:62-77, 1956.
60. Roe, C. F., Kinney, J. M., and Blair, C.: Water and heat exchange in third-degree burns. Surgery, 56:212-220, 1964.
61. Sass-Kortsak, A.: Unpublished communication. April 1971.
62. Schwartz, R., Fellers, F. X., Knapp, J., and Yaffe, S.: The renal response to administration of acetazolamide (Diamox) during salicylate intoxication. Pediatrics, 23:1103, 1959.
63. Sinclair, J. C., Engel, K., and Silverman, W. A.: Early correction of hypoxemia and acidemia in infants of low birth weight: A controlled trial of oxygen breathing, rapid alkali infusion, and assisted ventilation. Pediatrics, 42:565-589, 1968.
64. Stegink, L. D., and Baker, G. L.: Infusion of protein hydrolysates in the newborn infant: Plasma amino acid concentrations. J. Pediat., 78:595-602, 1971.
65. Sullivan, M. B., and Boshell, B. R.: Aetiological factors and therapeutic approach to the dumping syndrome. Brit. Med. J., 5380:414-416, 1964.
66. Sunshine, P.: Unpublished communication. April 1971.
67. Talbot, N. B., Crawford, J. D., and Butler, A. M.: Homeostatic limits to safe parenteral fluid therapy. New Eng. J. Med., 248:1100-1108, 1953.
68. Usher, R.: Comparison of rapid versus gradual correction of acidosis in RDS of prematurity. A sequential study. Pediat. Res., *1*:221, 1967.
69. Weil, W. B., and Wallace, W. M.: Hypertonic dehydration in infancy. Pediatrics, *17*:171-183, 1956.
70. Whitten, C. F., Kesaree, N. M., and Goodwin, J. F.: Managing salicylate poisoning in children. An evaluation of sodium bicarbonate therapy. Amer. J. Dis. Child., *101*:178-194, 1961.
71. Wilmore, D. W., and Dudrick, S. J.: Growth and development of an infant receiving all nutrients exclusively by vein. J.A.M.A., 203:860-864, 1968.

72. Winters, R. W., Cheek, D. B., Holliday, M. A., Swyer, P. R., and Wilmore, D. W.: Intravenous nutrition in pediatrics. The state of the art. Symposium held in conjunction with annual meeting of the Soc. Pediat. Research, Atlantic City, New Jersey, April 1971.
73. Winters, R. W., White, J. S., Hughes, M. C., and Ordway, N. K.: Disturbances of acid-base equilibrium in salicylate intoxication. Pediatrics, 23:260–285, 1959.
74. Ziegler, E. E., and Fomon, S. J.: Fluid intake, renal solute load, and water balance in infancy. J. Pediat., 78:561, 1971.

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Hypertension in patients on maintenance hemodialysis: evaluation of peripheral renin activity and bilateral nephrectomy

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Abstract: Twenty-seven patients with end-stage renal disease and hypertension were studied. Control of hypertension was attempted by low salt diet and ultrafiltration with low sodium dialysate. Peripheral vein renin activity was measured by radioimmunoassay. Twenty-one patients responded favorably to this type of therapy. Renin activity was normal or slightly elevated in most of these patients (Group 1). Of six patients who did not respond to excess salt and water removal by dialysis, four required bilateral nephrectomy for the adequate control of their hypertension. Peripheral vein renin activity was markedly elevated in all these patients (Group 2). On the basis of plasma renin activity, two types of hypertension are recognized in patients with end-stage renal disease; the "salt-dependent" type in the majority of patients and "renin-dependent" type in the minority of patients.

KEY INDEXING TERMS

Hypertension *Renal disease* *Ultrafiltration*

Hypertension has been a common and sometimes a serious complication in patients with end-stage renal disease. It has been suggested[1-6] that increased plasma renin activity may play a role and that hypertension in these patients is due to influences of the renin-angiotensin-pressor system. Other authors[7-15] maintain that the plasma renin activity in end-stage renal disease is variable and bears no definite correlation with the patient's blood pressure level. Several investigators[4,5,11-13,15] report amelioration of the hypertension by a low-sodium dialysis bath and contend that the blood pressure elevation in these patients is a function of the increased body sodium and water. Bilateral nephrectomy has been performed in several centers throughout the world[3,5,14,16-18] in patients in whom the hypertension could not be controlled by drug therapy and its value in controlling hypertension in end-stage renal failure has been questioned.[3,19] The present study was undertaken

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to examine the influences of sodium and water balance and of peripheral vein renin activity (PVR) on arterial blood pressure (BP) in patients undergoing chronic hemodialysis.

Material and Methods

Twenty-seven patients with end-stage kidney disease and hypertension on maintenance hemodialysis were studied. These were initially inpatients until they became stable enough to be maintained as outpatients for in-center dialysis. Pertinent clinical data are presented in Tables I and II. All patients were receiving antihypertensive medications as part of their management prior to initiation of dialysis. All showed hypertensive retinal changes in their fundi which ranged from grade II to grade IV according to the Keith-Wagener-Barker classification.[20] Their urine output ranged from 0 to 800 ml/24 hours, and their creatinine clearance from 0 to 4.9 ml/minute. All patients were anemic, with hematocrits ranging from 17.6 to 25.2 vol %. All patients complained of exertional dyspnea and easy fatigability, but overt congestive heart failure was absent. Digitalis preparations and all antihypertensive medications were discontinued one week prior to initiation of hemodialysis.

At the time of initiation of hemodialysis regimen, the dietary sodium intake was restricted to 500 mg/day (unless there was some source of sodium loss) by carefully regulating the dietary sodium content by computer analysis of the sodium content of the food by the research dietitian of the clinical research center. Fluid intake was regulated according to each patient's daily urine output. Three days each week, 6-hour hemodialyses with the Kolff twin-coil dialyzers were instituted for each patient. The dialysate sodium concentration was adjusted to 130 mEq/L* in an attempt to remove excess salt and water. The between-dialyses weight gain ranged from 2 to 4 lbs, but the excess fluid was removed by ultrafiltration during each dialysis in sufficient amounts to render negative fluid balance until the patients reached their dry weights. Dry weight, as defined by others,[4,15] is that weight at which the patient is free of edema, and attempts at further fluid removal by dialysis result in hypotension.

Blood pressure was recorded in the supine position. The values recorded in Tables I and II represent the mean of all the readings taken during the interdialysis period.

Serum sodium was measured by a flame photometer on samples drawn before and after each dialysis. Three separate determinations of peripheral vein renin activity (PVR) were made on three consecutive days before initiation of the dialysis regimen and repeated when the patients reached their dry weight or when systolic blood pressure was reduced to 100 mm Hg or lower by ultrafiltration. At this level of blood pressure, the stimulation of renin secretion is believed to be maximal.† Plasma renin activity as angiotensin II was measured according to the method of Goeke et al.[21] PVR values in 15 normal subjects ranged between 0.4 and 1.4 ng/ml/hr with a mean normal value of 0.9 ng/ml/hr (Fig. 1).

The paired *t* test was performed to determine the significance of the effect of dialysis on the blood pressure level and changes in the plasma renin activity. Diastolic blood pressure and PVR were correlated in all patients by the value of correlation coefficient (r) and the level of significance determined by the *p* value.

Bilateral nephrectomy was performed in four patients whose diastolic hypertension, associated with high plasma renin, was not controlled by dialysis.

Results

The patients were classified into two groups according to their response to low sodium hemodialysis. Group I consisted of 21 patients (Table I). In these patients, the arterial blood pressure returned to normal upon reaching their dry weight and was stabilized without any antihypertensive medications. This response was achieved after variable periods of dialysis ranging from 9 to 107 days. The mean systolic and

**125 mEq/L sodium concentrate supplied by Cobe Laboratories, Denver, Colorado, plus 5 mEq/L sodium produced by the water softener.*

†The PVR values listed in Tables I and II represent the mean values of these determinations.

TABLE I

RESULTS OF LOW SODIUM HEMODIALYSIS ON HYPERTENSION

No.	Name	Age (yrs) Sex	Primary Disease	Weight (kg) Initial	Weight (kg) Dry	BP (mm Hg)* Before	BP (mm Hg)* After	PVR (ng/ml/hr)* Before	PVR (ng/ml/hr)* After	Fundi K-W-B	EKG	Dialysis Period (Days)	Hct
1	C.H.	34/F	K-W	65	62.5	176/110	130/84	1.8	2.2	III	Ischemic changes	41	20.0
2	D.H.	45/M	CGN	61.8	57.7	190/130	130/94	2.4	2.7	III	IVH	50	19.0
3	C.J.	40/M	CGN	67.7	65.0	180/110	130/70	0.8	1.4	II	LVH voltage	37	20.5
4	M.M.	30/F	CGN	54.5	51.8	170/110	130/80	0.95	1.1	II	NSC	30	21.3
5	D.M.	22/F	CGN	51.8	49.5	190/130	150/90	1.5	2.0	II-III	LVH voltage	88	18.0
6	M.N.	35/F	Phenacetin neprhitis	51.0	45.9	230/130	160/86	2.1	2.9	III	LVH	107	22.0
7	N.W.	59/F	CGN	51.3	49.0	200/105	144/80	1.18	2.05	II	NSC	95	22.5
8	J.W	41/F	CGN	57.7	55.0	184/104	138/78	1.04	1.85	II	NSC	38	23.0
9	F.Y.	47/M	CGN	83.6	76.0	170/104	130/74	0.95	1.12	II	Ischemic changes	28	21.8
10	E.Y.	40/F	SLE	54.5	51.3	190/114	136/86	1.34	2.36	II	NSC	38	19.5
11	D.A.	22/M	CGN	59.0	54.0	190/120	130/80	0.82	1.14	II	NSC	27	17.6
12	G.T.	15/M	CGN	52.3	46.8	160/110	136/90	2.2	3.0	II	NSC	48	18.4
13	T.N.	22/M	CGN	61.4	55.6	160/120	120/90	0.74	1.55	III	LVH	47	18.4

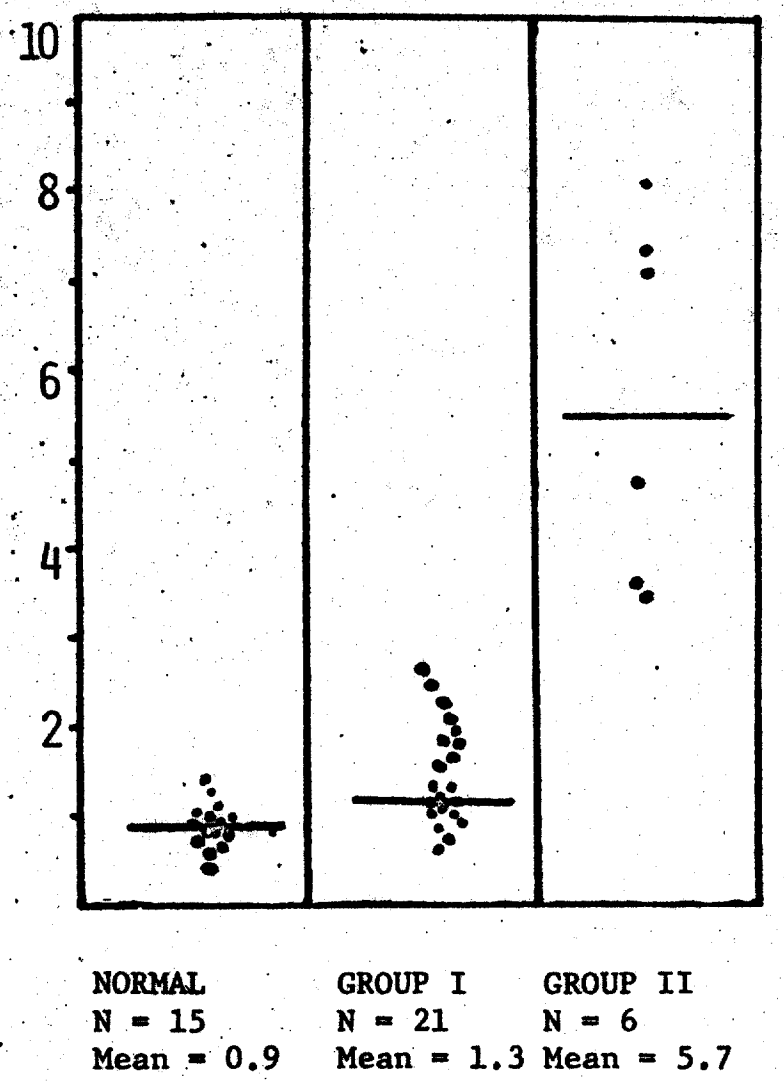

Fig. 1: Renin values in ng/ml/hr in normal controls and hypertensive patients on maintenance hemodialysis.

diastolic blood pressures for this group were 177 mm Hg and 110 mm Hg, respectively, before the initiation of dialysis regimen. On reaching the dry weight, the mean systolic and diastolic blood pressures were 134 mm Hg and 84 mm Hg, respectively. Although significant lowering of blood pressure was obtained by dialysis ($t=3.11$, $0.001<P<0.005$), the changes in body weight did not show any correlation with the changes in diastolic blood pressure ($r=0.03$, $P>0.05$).

Peripheral vein renin was slightly increased in this group after the dry weight was achieved whereas the diastolic blood pressures fell consistently in each patient. No significant correlation was obtained in patients of group I between PVR and diastolic blood pressure ($r=0.262$, $P>0.05$). Figure 2 shows the changes in BP and PVR values after dialysis.

Group II consisted of six patients. The mean blood pressure in this group was 175 mm Hg systolic and 115 mm Hg diastolic before the

TABLE I

RESULTS OF LOW SODIUM HEMODIALYSIS ON HYPERTENSION

No.	Name	Age (yrs) Sex	Primary Disease	Weight (kg) Initial	Weight (kg) Dry	BP (mm Hg)* Before	BP (mm Hg)* After	PVR (ng/ml/hr)* Before	PVR (ng/ml/hr)* After	Fundi K-W-B	EKG	Dialysis Period (Days)	Hct
14	A.H.	46/M	CGN	50.9	49.5	150/100	120/88	0.68	1.2	II	Ischemic changes	40	22.4
15	D.R.	21/M	SLE	60.0	59.0	155/100	136/88	1.15	1.26	II	NSC	9	21.5
16	S.R.	13/F	CGN	34.0	31.9	150/100	118/70	1.04	1.8	II	NSC	17	19.5
17	J.G.	30/F	CGN	51.9	44.5	160/100	170/90	1.3	1.9	II	NSC	18	23.0
18	G.R.	26/M	K-W	55.7	54.0	160/104	130/84	1.6	2.09	III	NSC	23	20.5
19	V.Z.	30/F	CGN	49.0	44.5	180/100	126/74	1.8	1.92	II	NSC	22	21.0
20	K.S.	18/F	CGN	53.6	46.7	170/105	144/80	2.6	3.4	II	NSC	90	17.6
21	R.P.	29/M	CGN	68.2	59.0	168/104	132/80	1.7	2.0	II	LVH	64	22.5

*These values represent the period before and after dry weight was achieved.

PVR = *Peripheral vein renin*
K-W = *Kimmelstiel-Wilson syndrome*
CGN = *Chronic glomerulonephritis*
SLE = *Systemic lupus erythematosus*
KWB = *Keith-Wagener-Barker classification*
LVH = *Left ventricular hypertrophy*
NSC = *Nonspecific changes*

TABLE II

EFFECT OF BILATERAL NEPHRECTOMY ON HYPERTENSION AND RENIN ACTIVITY

No.	Name	Age (yrs) Sex	Primary Disease	Weight (kg) Initial	Weight (kg) Dry	BP mm Hg* Before	BP mm Hg* After	PVR* ng/ml/hr Before	PVR* ng/ml/hr After	Fundi K-W-B	EKG	Period of Dialysis (Days)	BP mm Hg	PVR ng/ml/hr	Renal Histology
1	D.D.	15/F	CGN	68.0	50.0	150/100	224/134	8.2	9.6	III-IV	LVH	300	124/80	0.05	Marked thickening of the vessel wall with endothelial hyperplasia;hyalinized glomeruli.
2	M.C.	47/F	CGN	75.0	64.5	206/136	190/120	4.8	5.6	III	LVH and Strain	47	130/84	0.12	Vascular wall thickening and hyalinized glomeruli.
3	K.B.	17/F	CGN	37.2	35.4	172/108	168/110	7.5	9.0	III	LVH and Strain	21	110/70	0.04	Marked vascular changes and hyalinized glomeruli.
4	M.A.	24/F	CGN	42.5	39.0	192/124	176/104	7.2	8.6	IV	LVH and Strain	19	116/80	0.07	Marked vascular wall thickening due to subintimal hyperplasia simulating "onion-skin" appearance, in contrast to mild glomerular changes.
5	J.C.	48/M	CGN	88.6	78.6	200/120	170/100	3.5	4.9	III	LVH	44	--	--	--
6	M.D.	29/M	CGN	78.0	72.7	180/100	170/100	3.2	4.2	III	LVH	21	--	--	--

These values represent the period before and after dry weight was achieved.

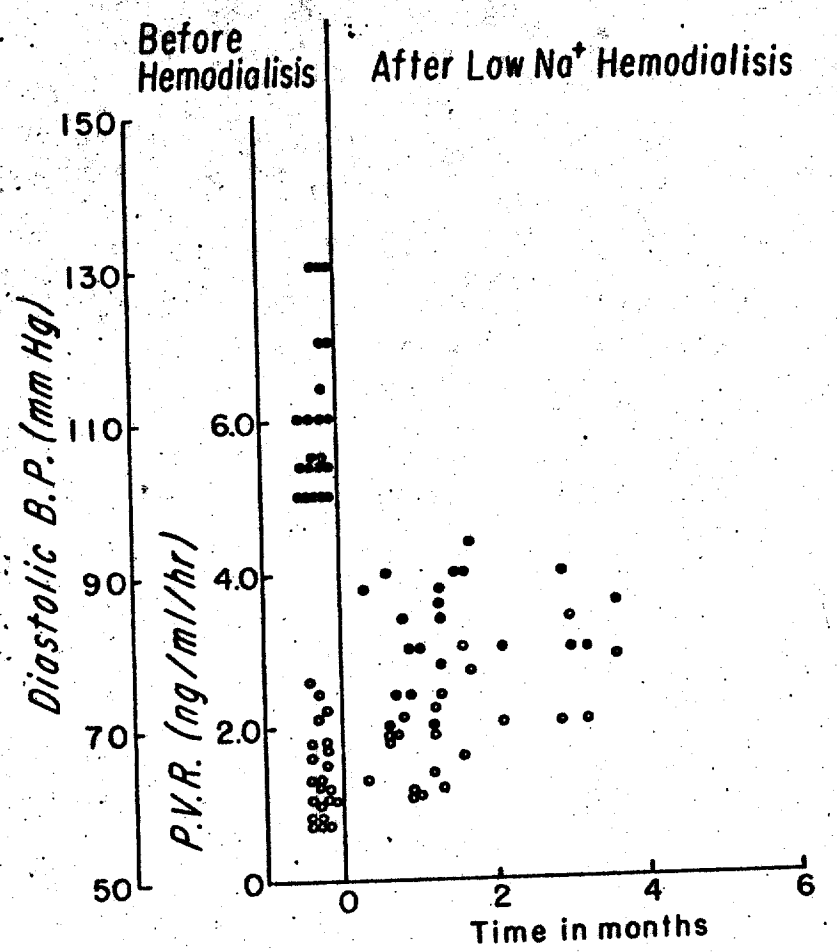

Fig. 2: Effect of dialysis on diastolic BP and PVR values in patients whose hypertension was controlled by dialysis alone. Closed circles = diastolic BP. Open circles = PVR values.

initiation of dialysis regimen. Systolic and diastolic blood pressures were 190 mm Hg and 121 mm Hg, respectively, after the dry weight was achieved. Changes in diastolic blood pressure and body weight by dialysis were not significantly correlated (r=0.184, P>0.05). Diastolic blood pressure in two patients (Nos. 1 and 3, Table 1) was higher after 12 and 3 weeks, respectively, of low sodium dialysis.

The peripheral vein renin on maintenance hemodialysis in group II was significantly higher (mean value=5.7 ng/ml/hr) compared to the renin level (mean value=1.3 ng/ml/hr) in group I (Fig. 1). The renin level in group II increased after the dry weight was reached (Fig. 3). The observed differences between before and after dry weight renin levels were not significant (t=1.23, P>0.05) whereas the renin level in group I before and after dialysis was highly significant (t=2.78, P=0.005). The higher plasma renin concentration in group II was not correlated with the diastolic blood pressure (r=0.554, P>0.05), probably due to the limited number of observations.

Bilateral nephrectomy was performed in four of six patients in group II because of their persistent diastolic hypertension associated with high plasma renin activity which was unresponsive to fluid removal by dialysis and antihypertensive medications. There was a prompt

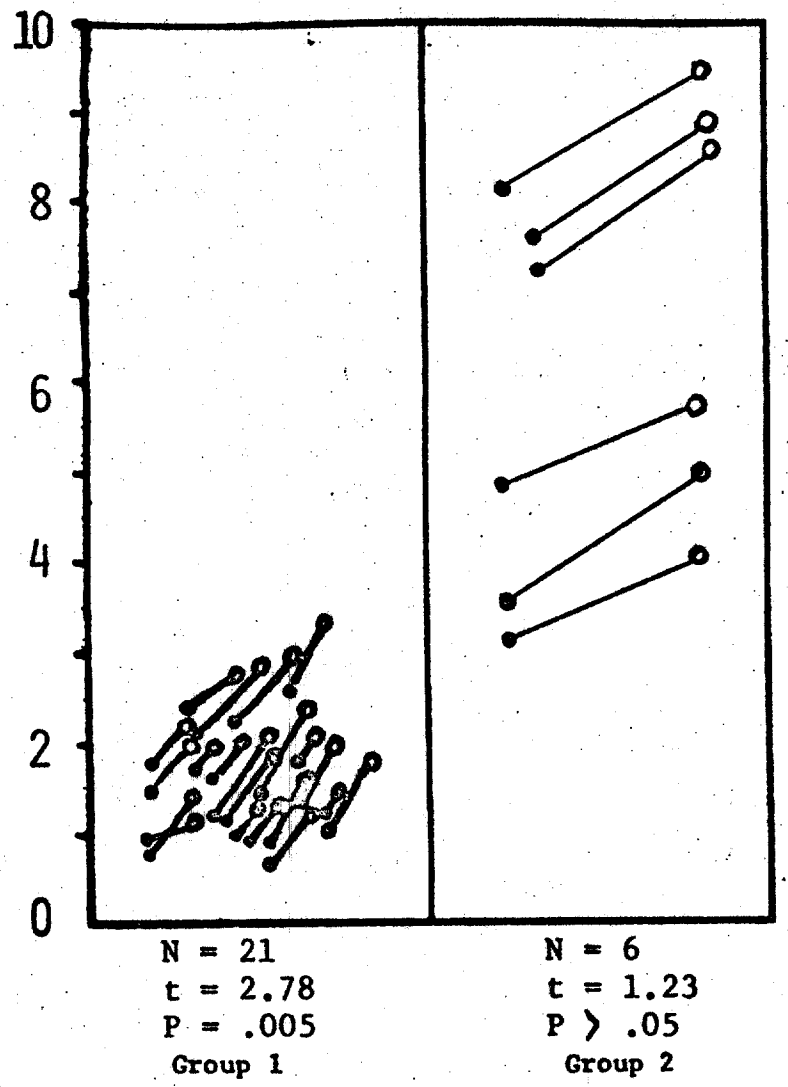

Fig. 3: Changes in PVR values (ng/ml/hr) in patients of Group 1 (left column) and Group 2 (right column) by dialysis. Closed circles = before dry weight. Open circles = after dry weight.

reduction of blood pressure to normal in all four patients and their PVRs to almost undetectable levels within a week after bilateral nephrectomy (Fig. 4). The changes in diastolic BP levels were very significant ($t=3.114$, $0.01<P<0.005$). The histologic findings in the nephrectomized kidneys are listed in Table II. Figures 5 and 6 represent typical vascular changes in the kidneys from patients 1 and 4, respectively (Table II). All four patients remain normotensive, four and six months after bilateral nephrectomy.

Two patients (Nos. 5 and 6, Table II), who were not nephrectomized at the time of this study, continued to be hypertensive and presented difficult problems in titrating their antihypertensive medications due to wide fluctuations in their blood pressures, especially during hemodialysis, with the attended risk of disturbing side effects.

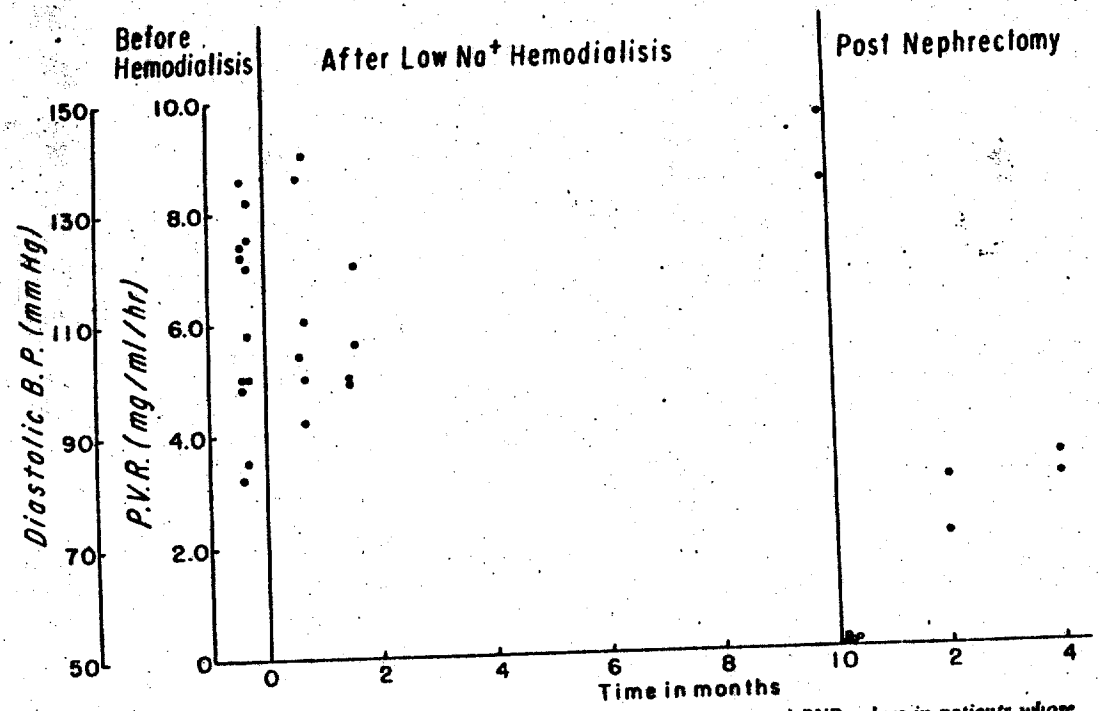

Fig. 4: Effect of dialysis and bilateral nephrectomy in diastolic hypertension and PVR values in patients whose hypertension was not controlled by dialysis alone. Closed circles = diastolic BP. Open circles = PVR values.

Discussion

In our study, 27 hypertensive patients with end-stage renal disease fell into two groups according to their response to low sodium dialysis.

The hypertension in 21 patients (78 per cent) in group I was reduced to normal level by removal of the excess sodium and water (Fig. 4). Hypertension in these patients has been thought to be due to volume expansion secondary to sodium and water retention. Finnerty et al[22] have shown that expansion of the extracellular volume leads to hypertension which is refractory to antihypertensive medications. Reduction of the expanded volume by diuretics renders the patients normotensive or sensitive to antihypertensive medications again. By a similar mechanism, ultrafiltration in patients with end-stage renal disease, with low sodium dialysate, removes the excess of sodium and water and restores BP to normal.

Although in our patients the changes in BP did not correlate with the changes in weight ($r=0.03$, $P>0.05$), we feel that this was masked by the simultaneous gain in lean body mass due to the anabolic effect of increased dietary protein and caloric intake. It correlated well clinically by the uniform disappearance of edema fluid in those patients who presented with it initially, and might have correlated with the total exchangeable sodium (TES). TES was not measured in our patients since it remains a highly disputable finding. Some investigators[4,5,11-14,17] have found good correlation between TES and BP, and have designated it as "salt-dependent hypertension," while others[6] have failed to show such a correlation with the exception of the anephric patients.

Peripheral vein renin levels were normal or slightly elevated in these patients (Fig. 1) and showed no correlation to diastolic BP ($r=0.262$, $P>0.05$). Although low sodium dialysis stimulated renin production in these patients, there was a fall in blood pressure (Fig. 2). This can be explained by the findings of Bianchi et al[23] that sodium depletion stimulates renin secre-

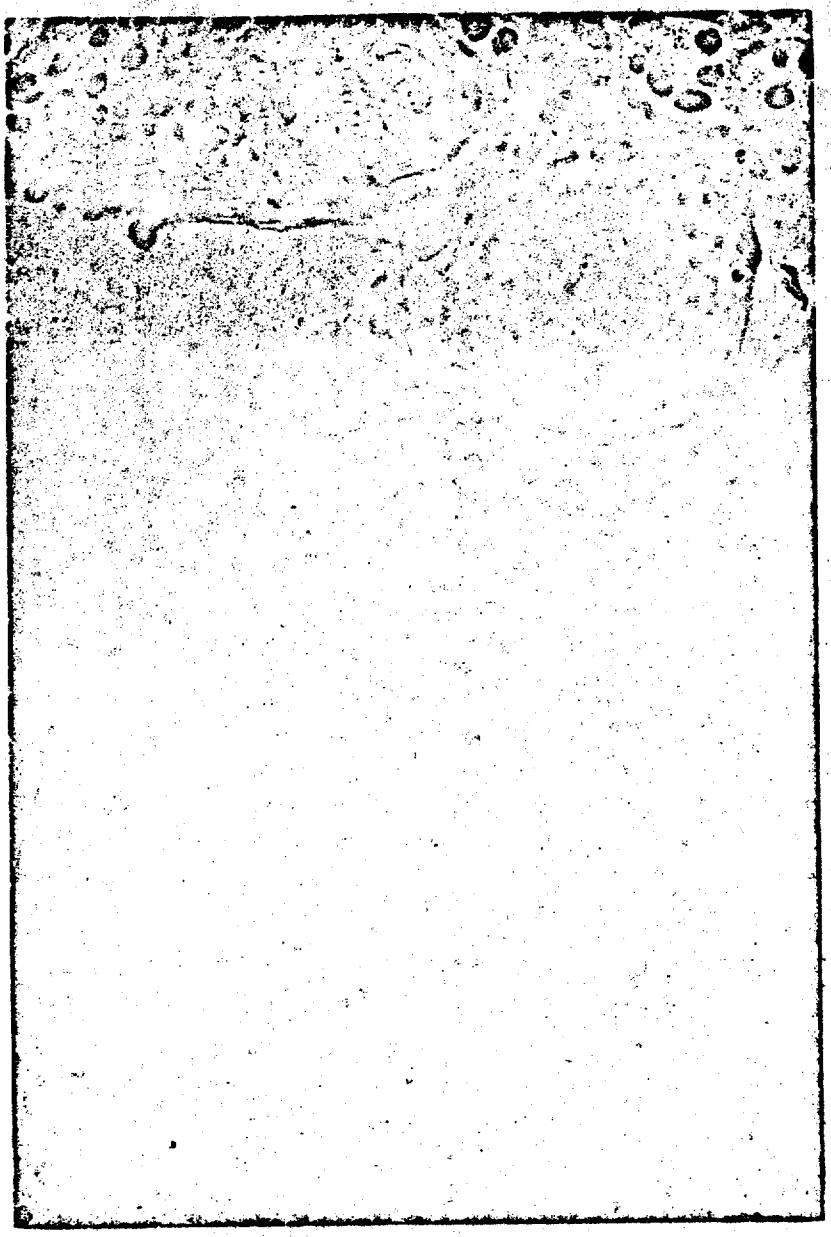

Fig. 5: Table II, Patient No. 1, D.D., 15-year-old white female, showing the thickening of the vessel wall of the kidney with marked narrowing of the lumen. Primary disease, chronic glomerulonephritis. (Hematoxylin and eosin stain, x 300).

tion and at the same time renders the vascular wall less sensitive to renin-angiotensin stimulus. Therefore the need for nephrectomy did not arise. It will be interesting, however, to follow these patients over prolonged periods of time and observe the ultimate response of their BPs to the continuous renin stimulus. Onesti et al[14] and Toussaint et al[18] performed bilateral nephrectomies in patients with normal PVR levels with good results.

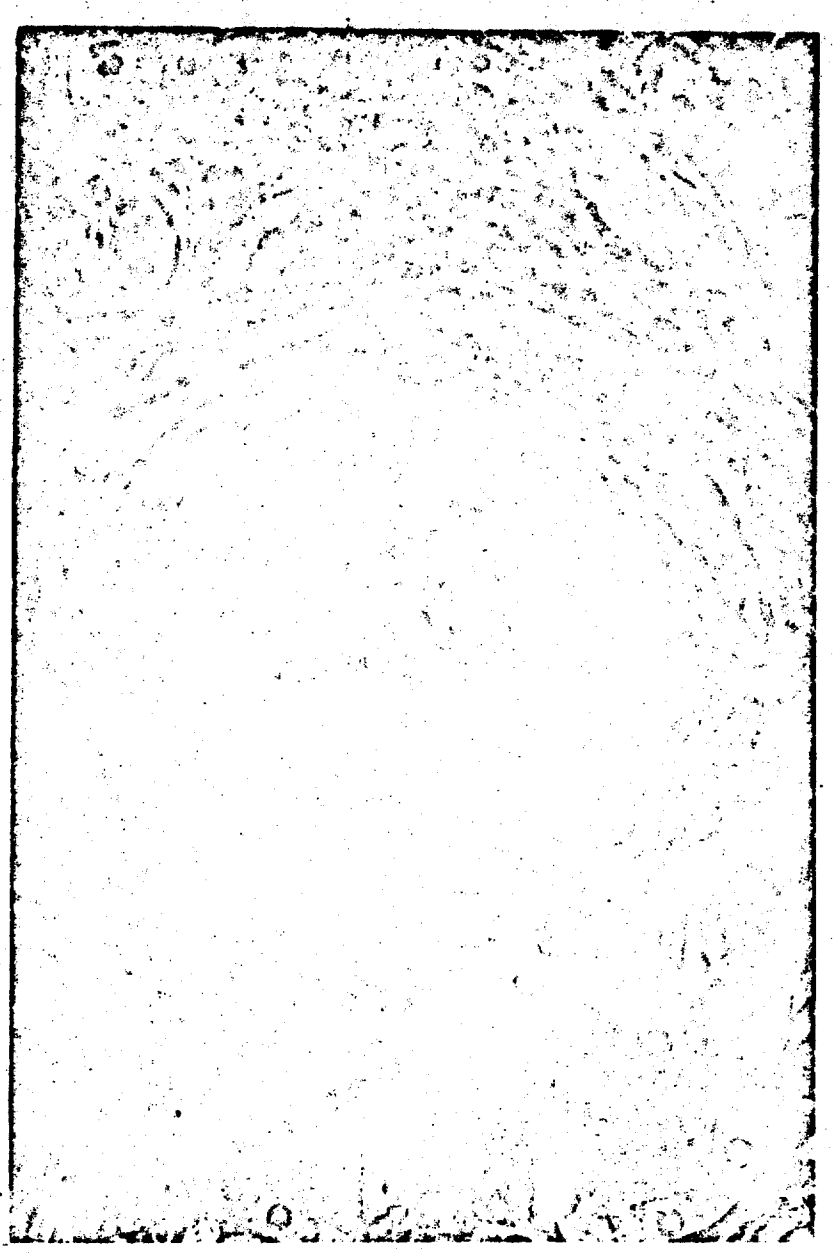

Fig. 6: Table II, Patient No. 4, M.A., 24-year-old white female, showing the marked thickening of the vessel wall of the kidney due to subintimal fibroplasia simulating the "onion-skin" appearance. (Periodic acid Schiff stain, x 100).

Although considerable evidence for control of hypertension by dialysis is now available, there seems little doubt that there is a group of patients in whom hypertension cannot be controlled by the most vigorous salt restriction and ultrafiltration dialysis. This type of hypertension has been designated as "renin-dependent hypertension." These patients, 6 of 27 (22 per cent) in group II, all had higher levels of PVR than the first group (Fig. 1). Some workers[4-6]

have shown a good correlation of diastolic hypertension with the PVR levels in the renin-independent hypertensive patients, while others[14,18] have failed to do so.

Out data did not show any significant correlation between diastolic blood pressure and renin levels of the patients in group II, probably due to the small number of observations. However, the causal relationship of plasma renin to diastolic hypertension was highly significant ($t=3.114$, $0.01<P<0.005$) as was shown by the prompt amelioration of the diastolic hypertension and the simultaneous fall of plasma renin values to almost undetected levels after bilateral nephrectomy.

On the other hand, Kolff et al[3] did not find bilateral nephrectomy always helpful in controlling hypertension and Hampers et al[19] have reported "no response" to bilateral nephrectomy in patients who had normal or slightly increased PVR activity. High plasma renin concentration in hypertensive patients on maintenance hemodialysis, therefore, may have a predictive value with regard to the benefit from bilateral nephrectomy.[4,5,14] Wilkinson et al,[6] however, cast some doubt on the absolute validity of such prediction from their observations of overlap in renin values in the two groups in a small number of patients.

Brown et al[5] maintain that vigorous treatment with low sodium dialysis may aggravate the hypertension in patients with high renin levels intitally and create a vicious circle. This is suggested by the following scheme: high plasma renin→hypertension→further salt and water depletion by dialysis→further rise in plasma renin levels→hypertension. This means that, in spite of the lowering of response of the arterial wall to renin-angiotensin stimulus by sodium depletion, there comes a time when the high renin levels overcome this resistance and result in further vascular spasm and worsening of hypertension. This mechanism might have been responsible for the worsening of diastolic hypertension in two of our patients (Nos. 1 and 3, Table II).

At the present time it is not clear that such an "intractable hypertension" in dialyzed patients is the result of the patients' underlying diseases or of their treatments. Until more evidence is obtained, it seems reasonable to recommend bilateral nephrectomy for intractable hypertension associated with elevated plasma renin levels which cannot be controlled by dialyses alone.

Bilateral nephrectomy proved beneficial in all four patients while those two who were not nephrectomized continue to be hypertensive between dialyses and present problems in drug titration due to wide swings of their blood pressures.

In our experience, bilateral nephrectomy is an effective mode of therapy for intractable hypertension in patients on chronic hemodialysis, and also makes them better candidates for renal homotransplantation.[24]

References

1. Earle DP: Hypertension in parenchymal renal disease. Progr Cardiovasc Dis 8:105-209, 1965.
2. Del Greco F, Simon NM, Goodman S et al: Plasma renin activity in primary and secondary hypertension. Medicine 46:475-490, 1967.
3. Kolff WJ, Nakamoto S, Poutasse EF et al: Effect of bilateral nephrectomy and kidney transplantation on hypertension in man. Circulation 30(Suppl II)23-35, 1964.
4. Vertes V, Cangiano JL, Berman LB et al: Hypertension in end-stage renal disease: mechanisms and treatment. N Engl J Med 280:978-981, 1969.
5. Brown JJ, Curtis, JR, Lever AF et al: Plasma renin concentration and the control of blood pressure in patients on maintenance hemodialysis. Nephron 6:329-349, 1969.
6. Wilkinson R, Scott DF, Uldall PR et al: Plasma renin and exchangeable sodium in the hypertension of chronic renal failure: the effect of bilateral nephrectomy. Q J Med 39:377-396, 1970.
7. Tu WH: Plasma renin activity in acute tubular necrosis and other renal diseases associated with hypertension. Circulation 31:686-695, 1965.
8. Blaufox MD, Birbari AE, Hickler RB et al: Plasma renin activity in homotransplant recipients. N Engl J Med, 275:1165-1168, 1966.
9. Toussaint C, Verniary A: Renin and hypertension. N Engl J Med 276:350-359, 1967.
10. Gutkin M, Levinson GE, King AS et al: Plasma renin activity in end-stage kidney disease. Circulation 49:563-574, 1969.
11. Blumberg A, Hegstrom RM, Nelp WB: Extracellular volume and exchangeable sodium in chronic

hypertensive renal disease. Proc European Dial Trans Assn 1:199-203, 1964.

13. Hegstrom RM, Murray JS, Pendras JP et al: Two years' experience with periodic hemodialysis in the treatment of chronic uremia. Trans Am Soc Artif Intern Organs 8:266-280, 1962.

14. Onesti G, Swartz C, Ramirez O et al: Bilateral nephrectomy for control of hypertension in uremia. Trans Am Soc Artif Intern Organs, 14:361-366, 1968.

15. Thomson GE, Waterhouse K, McDonald HP et al: Hemodialysis for chronic renal failure. Arch Intern Med 120:153-167, 1967.

16. Funk-Brentano JL, Mery JP, Vantelon J et al: Le sodium echangeable des malades atteints d'uremie chronique soumis a des hemodialyses repetees. Ses relation avec le presion arterielle. Proc European Dial Trans Assn 1:203-208, 1964.

17. Seto D, Gritz W, Nakamoto S et al: The effect of bilateral nephrectomy and of sodium and water content on hypertension. Trans Am Soc Artif Intern Organs 9:35-40, 1963.

18. Toussaint CH, Cremer M, Heuse A et al: L'hypertention arterielle maligne incontrolable, indication a la nephrectomie bilaterale dans le mal de Bright au stade ultime. Proc European Dial Trans Assn 3:65-74, 1966.

19. Hampers CL, Skillman JJ, Lyones JH et al: A hemodynamic evaluation of bilateral nephrectomy and hemodialysis in hypertensive man. Circulation 35:272-288, 1967.

20. Keith NM, Wagener HP, Barker NW: Some different types of essential hypertension: their course and prognosis. Am J Med Sci 197:332-343, 1939.

21. Gocke DJ, Gerten J, Sherwood LM et al: Physiological and pathological variations of plasma angiotensin II in man: correlation with renin activity and sodium balance. Circulation (Suppl II):131-146, 1969.

22. Finnerty FA, Davidov M, Mroczek WJ et al: Influence of extracellular fluid volume on response to antihypertensive drugs. Circ Res 38:71-82, 1970.

23. Bianchi G, Brown JJ, Lever AF et al: Changes of plasma renin concentration during pressor infusions of renin in the conscious dog. The influence of dietary sodium intake. Clin Sci 34:303, 1968.

24. Chrysanthakopoulos SG, Kastagir BK, Stevens LE et al: Plasma renin activity in hypertension after renal homotransplantation. Abstracts Am Soc Neph, Washington D.C., 1970, p 15.

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ARTICLES

HYPERTONIC DEHYDRATION (HYPERNATREMIA): THE ROLE OF FEEDINGS HIGH IN SOLUTES

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ACUTE diarrheal disease in infants may lead to loss of water in excess of salt lost with consequent elevation of the osmolarity of the body fluids.[1] Recent reviews[2-5] have described with clarity the biochemical derangements, the clinical manifestations and the therapeutic considerations of this condition.

The number of children presenting with hypertonic dehydration has represented a fairly constant percentage of all admissions for dehydration in each of these series. Skinner and Moll[5] found 16% of 380 patients with sodium in the serum above 150 mEq/l. Weil and Wallace[3] reported 20% of 77 patients with sodium in the serum above 160 mEq/l. In Finberg's series,[4] the children with sodium in the serum above 150 mEq/l represented 25% of the total of 274 cases. The present authors' experience in past years has shown the same type of distribution.

In the winter of 1958, an epidemic of nonspecific, primary gastroenteritis occurred in Minneapolis and St. Paul, Minnesota, and the surrounding suburbs, beginning the latter part of December and spreading in the ensuing months to embrace neighboring communities in the state. It soon became apparent that more than a half of the children admitted to the hospital with gastroenteritis were suffering from a hypertonic type of dehydration.

This marked increase in the incidence of hypertonic dehydration prompted an analysis of the cases of gastroenteritis in infants. Special emphasis was placed on evaluation of the feeding histories of these infants. The purpose of the present report is to point out the role that early treatment of mild to moderate diarrheal disease in the home may play in the increasing frequency of this hazardous complication.

PATIENT MATERIAL AND METHODS

All patients admitted to the University Hospitals and the Minneapolis General Hospitals with a diagnosis of gastroenteritis, from January 1 to March 10, 1958, are included in the study. Concentrations of sodium and potassium in serum, urine and feeding solutions were determined by flame photometry. Chloride determinations were done by a microtitration method using mercuric nitrate with diphenylcarbazone as indicator.[6] Urea nitrogen in the blood was determined by a modification of the method of Peters and Van Slyke.[7] The carbon dioxide combining power was estimated by the method of Segal.[8] Osmolarity of serum and urine was determined with the Fiske osmometer. All stools were cultured for shigella, salmonella and E. coli. Strains of E. coli were typed with specific antisera to serotypes 011-B4, 055-B5, 127-B7 and 126-B8.

RESULTS

Clinical Observations

There were 32 cases (Table I) of gastroenteritis admitted to the hospitals during this period. Seventeen (53%) had concentrations of sodium in the serum above 150 mEq/l or concentrations of chloride in the serum above 125 mEq/l. Eight patients had no electrolyte determinations performed at the time of admission; one of these (Case

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TABLE I

INFORMATION ON ALL CASES OF GASTROENTERITIS OBSERVED FROM JANUARY 1 TO MARCH 10, 1958

Case No.	Initials	Age (mo)	Duration	Chemical Analyses of Blood: Na (mEq/l)	K (mEq/l)	Cl (mEq/l)	HCO_3 (mEq/l)	Urea Nitrogen (mg/100 ml)	Feeding History	Sequelae
				With Hypertonic Dehydration						
1.	R.T.	7	Diarrhea 1 day	—	—	125	6	80	Inadequate history	None
2.	C.B.	7	Diarrhea 5 days	155	—	119	29	—	Lytren®, 2 quarts first 2 days; then solids and whole milk	None
3.	J.B.	1	Diarrhea 2 weeks	—	6.3	141	7	56	Evaporated milk formula, 1:1 dilution from birth; skim milk for 10 days	None
4.	R.N.	7	Diarrhea 8 days	—	6.3	142	13	69	Lytren®, 400 ml for 3 days. Two clyses of 5% dextrose in 0.9% saline, each 250 ml, in week before admission	Spasticity, irritability
5.	G.J.	9	Diarrhea 5 days	174	5.5	146	14	—	Lytren® alternated with skim milk	Irritability for 1 week
6.	E.H.	9	Diarrhea 4 days	165	4.6	126	8	90	Whole milk, 32 oz+3 cans purees first 3 days; whole milk, 2 oz 4th day; whole milk 9 oz +300 ml 5% dextrose and 50 ml 0.9% saline day of admission	Hyperactive irritable
7.	J.L.	13	Diarrhea 6 days	167	4.3	145	8	—	Whole milk, unknown amount on 2nd and 3rd day, skim milk 500 ml on 4th, 5th and 6th day with 1 quart of salt solution containing 1 tbsp salt/qt water on 6th day	None
8.	P.L.	14	Diarrhea 5 days	176	4.8	157	11	46	Skim milk and Lytren® alternated throughout illness	None
9.	D.A.	13	Diarrhea 2 days	172	5.1	129	5	—	Lytren®, unknown amount	Died
10.	C.B.	11	Diarrhea 5 days	160	4.3	131	4	56	Skim milk until day before admission; 3,000 ml Lytren® day prior to admission, 720 ml Lytren® day of admission	Died
11.	E.H.	12	Diarrhea 5 days	164	4.1	133	17	—	Skim milk and Lytren® alternated	None
12.	G.S.	12	Diarrhea 7 days	133	4.6	121	12	—	Whole milk, 5 days	None
13.	K.L.	6	Diarrhea and vomiting 32 hours	151	6	121	11	8	Whole milk	None
14.	D.H.	3/4	Diarrhea 5 days	—	—	172	23	15	Evaporated milk formula, 1:1 dilution from birth; first 5 days of illness, evaporated milk:water formula, 13:7+2 tbsp dextrimaltose. Salt solution* day of admission	Convulsion Died

Case Numbers 26 to 32 had no electrolyte determinations performed.

* Salt solution: 1 quart of boiled water to which was added 2 tsp salt, 1 tsp baking soda, and 3 tbsp sugar.

TABLE I (*Continued*)

Case No.	Initial	Age (mo)	Duration	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)	HCO_3 (mEq/l)	Urea Nitrogen (mg/100 ml)	Feeding History	Sequelae
				Chemical Analyses of Blood						
15.	R.A.	1½	Diarrhea 3 days	244	—	190	—	—	Probably undiluted evaporated milk for 1 week + Lytren® day of admission	None
16.	S.C.	14	Diarrhea 20 hours	173	6.2	129	10	—	Skim milk 1st day. Lytren® in gingerale + Lytren® in skim milk on 2nd day	Convulsions Died
17.	D.W.	13	Diarrhea 7 days	172	3.9	146	—	—	One gallon whole milk daily for 4 days prior to admission; no water	None
				Without Hypertonic Dehydration						
18.	J.F.	4	Diarrhea 3 days Vomiting 3 days	148†	5.9†	121†	7†	—	Water, tea, and skim milk	None
19.	J.K.	7	Diarrhea 2 days	114	5.6	110	20	—	Milk (vomited) 2 days prior to admission, Lytren® (vomited) 1 day prior to admission	None
20.	K.E.	7	Diarrhea 3 weeks	146	—	120	16	—	Skim milk for 10 days	None
21.	K.K.	14	Diarrhea and vomiting 1 day	141	—	111	18	—	Cola syrup, "clear liquids"	None
22.	M.M.	18	Diarrhea and vomiting 2 days	145	—	112	17	—	Tea, skim milk, carbonated beverage	None
23.	J.M.	36	Diarrhea and vomiting 5 days	135	5.3	99	14	—	"Kept nothing down"	None
24.	G.S.	16	Diarrhea 4 days	147	4.5	117	23	—	Half-strength skim milk first 2 days; ¼-strength Lytren® third day; full-strength Lytren® 2 quarts day of admission (half vomited)	None
25.	G.N.	10	Diarrhea 4 days Vomiting 1st day	142†	4.7†	114†	—	—	Eight oz whole milk first 4 days; salt solution* day of admission	None

† Blood withdrawn for analysis on day following admission, after administration of parenteral fluids.

18) had a concentration of sodium of 148 mEq/l in the serum after 12 hours of parenteral fluid therapy. The remaining seven patients had concentrations of sodium and chloride in the serum within the normal range. No correlation between duration of symptoms and the degree of hypertonicity was apparent. No enteric pathogenic organisms were isolated from the stools of the patients in the series. Parenteral infection was not a prominent feature of the outbreak.

There were four deaths in the series (Cases 9, 10, 14, 16). All of the deaths occurred in the hypernatremic group. Case 9 had an elevated concentration of sodium in the serum in another hospital, had been rapidly hydrated with 5% glucose, and was admitted with gross edema and severe neurologic impairment (coma and spasticity). Case 10 was in shock at the time of admission and developed anuria. Cases 14 and 16 both had initial sodium in the serum of over 170 mEq/l. Both infants had convul-

sions upon admission, and death occurred before rehydration could be accomplished. One infant (Case 4) had definite neurologic sequelae (spasticity) at the time of discharge from the hospital.

The 17 infants with hypernatremia all had received, during the period of continuing diarrheal losses, either whole milk without supplemental water, whole milk with supplements of a commercially-prepared, oral, hypotonic electrolyte solution (O.E.S.*) or O.E.S. alone. Three of the infants (Cases 4, 7, 14) received an unusually high salt intake: Case 4 was given 500 ml of isotonic saline solution subcutaneously in addition to O.E.S.; Case 7 ingested a solution containing 1 tablespoon of salt in a quart of water; and Case 14 received a hypertonic oral salt solution. Those infants admitted with normal or low concentrations of sodium in the serum had either vomited all oral feedings or had received half-strength skim milk, weak tea solutions, carbonated beverages or O.E.S. which had been diluted to a fourth the usual recommended dilution.

Osmolarities of the urine of some of the infants are tabulated in Table II. None of the infants had concentrations of solutes in the urine in excess of 700 mOsm/l in the initial urine sample.

TABLE II

SOLUTE CONCENTRATION IN URINE OF INFANTS ADMITTED WITH HYPERNATREMIC DEHYDRATION

	Case No.	Age (mo)	Interval after Admission (hr)	Solute Concentration (mOsm/l)
C.B.	10	14	0–12	415
R.A.	15	1	24–48	657
			48–72	540
			72–96	440
			96–120	524
G.J.	5	9	0–24	350
			24–48	568
J.L.	7	13	0–26	547
			26–48	763

Observations on the Solutions Offered to the Infants During Development of Dehydration

When possible, the actual mixture received by an infant was analyzed. In other cases, the O.E.S. was reconstituted in the laboratory. In addition solutions were made up from the powder, using heaping rather than level measures, because it seemed possible that mothers regarding the powder as "medicine" might err on the side of generosity in preparation of the solution. In addition, attention was given to the other constituent of the final solution—the water supply in the home. The suburban areas surrounding the cities proper are outside the city water supply and rely for water from wells. Because of the high iron and calcium content of this well water, it is customary to "soften" the water by use of an exchange resin charged with sodium chloride. The results of these analyses are tabulated in Table III.

O.E.S. powder when made into a solution according to the recommendations of the manufacturer contains 50 mEq of sodium and 20 mEq of potassium per liter. While there was little variability between lots of powder, wide variations in concentration of the solution could be achieved by differences in mode of preparation. The measure used in the preparation of solutions 1 and 3 (Table III), for instance, had become caked due to the mixture of water with the powder and hence provided less than a full measure. Heaping the powder in the measure increased the sodium concentration by 15 mEq/l. While the sodium content of city water (solutions 4, 7 and 8) was constant and low, the sodium content of well water which had passed through softeners reached a value as high

* Lytren®, Mead Johnson and Company, a powder which, when reconstituted according to the manufacturer's directions, contains per liter: sodium, 50 mEq; potassium, 20 mEq; calcium, 4 mEq; magnesium, 4 mEq; citrate, 30 mEq; sulfate, 4 mEq; chloride, 30 mEq; phosphate, 10 mEq, and lactate, 4 mEq.

TABLE III

DATA ON FEEDINGS

Formula No.	Composition	Sodium (mEq/l)	Potassium (mEq/l)	Protein (gm/l Estimated)
1.	Lytren®, 1 level measure+125 ml distilled water (Case 5)	42.5	19.5	
2.	Lytren®, 1 level measure+125 ml distilled water (Case 19)	47.3	17.7	
3.	Lytren®, 1 heaping measure+125 ml distilled water (same lot and measure as 1)	57	22.8	
4.	Tap water from laboratory	.2	—	—
5.	Water softener A (1 day after regeneration)	14.6	—	—
6.	Water softener B (2 days after regeneration)	6.0	—	—
7.	Water from home of Case 5	.25	—	—
8.	Water from home of Case 10	.2	—	—
9.	Formula from Case 16 (full-strength Lytren®+boiled skim milk, 1:1)	60	40	16.5
10.	Formula from Case 15 (prepared by mother)	64	75	66
11.	Lytren® from Case 15 (prepared by mother)	60.5	20	—
12.	Lytren® from Case 10 (prepared by referring hospital)	43.5	—	—
13.	Cow's milk[12]	25	35	33
14.	Human milk[12]	7	14	12

as 14.6 mEq/l. Boiling 350 ml of water for 5 minutes reduced the volume to 270 ml, so that it is theoretically possible, if water obtained from a softener recently recharged with salt were used, to have had a sodium content as high as 19 mEq/l. The feedings of highest solute content were those in which full-strength O.E.S. was alternated, or mixed 1:1, with boiled, whole or skim milk.

DISCUSSION

An infant with diarrhea is losing water and salt from four sources: lungs, skin, stools and urine. All of these routes lead to loss of water which is hypotonic in electrolytes with respect to plasma, with the exception of urine. The kidney represents the one route where water can be conserved by the production of urine which is hypertonic compared to blood plasma. It is customary to think of the concentrating ability of the kidney in terms of the number of milliosmoles of solute which can be excreted in a given amount of water. The kidneys of the adult, under circumstances of maximal concentration, can achieve a urinary concentration of solutes of 1400 mOsm/l. It has been claimed that in early infancy the kidneys have a maximal concentrating ability of 700 mOsm/l,* which would mean that twice as much water would be required to excrete the same

* This is not firmly established. See reference 13. [Ed.]

amount of solutes (urea, sodium, potassium, chloride and other electrolytes). Pratt *et al.*[10,11] have shown that the concentrating ability of the kidneys in young infants matures quite rapidly, but others have demonstrated that with illness or with dehydration renal function of infants is depressed both as regards glomerular and tubular functions.[12] Analysis of the concentrations of solutes in the first voided urines in some of the patients with hypernatremia reported here suggested a reduction of concentrating ability of the kidneys in that the highest concentrations achieved were less than 700 mOsm/l in infants 12 to 14 months of age. Whether the defective concentration preceded, or resulted from, the dehydration cannot be determined from these data. It is interesting, however, that the solute concentrations in the urine increased during repair in two patients even though more free water was available by this time.

If high-solute feedings are given, the amount of solutes requiring excretion will also increase. In a report of the Committee on Nutrition of the American Academy of Pediatrics[13] it is estimated that, assuming a concentrating ability of 700 mOsm/l, the solutes from unmodified cow's milk require four times as much water for excretion as is the case with human milk. It was further estimated, again assuming a concentrating ability of 700 mOsm/l, that unmodified cow's milk will supply sufficient water for renal and extra-renal losses at an environmental temperature of 23°C, but will not supply sufficient water for such losses at 34°C, due solely to increase in the extra-renal losses of water at higher temperatures. The infant with diarrhea also has increased losses of hypotonic fluid; hyperventilation secondary to fever and acidosis increases insensible loss of water through the lungs, and persistent diarrhea results in increased gastrointestinal losses. As dehydration develops, kidney function may become depressed and renal water losses may increase.

No causative organism could be isolated from any of the infants in this epidemic so that it is impossible to say that the agent causing the gastroenteritis was not also responsible for the unusual incidence of hypertonicity. The occurrence of dehydration without hyperosmolarity of the serum in other infants in the epidemic, ill for the same length of time, may be offered as evidence against such a hypothesis. Since the outbreak occurred in midwinter in a climate not noted for its mildness, high environmental temperatures cannot be implicated in the production of unusually high losses of water through the skin. The low humidity present in this area during the winter months may have greatly augmented loss of water from the lungs.

The most striking difference between the infants having hypernatremia and those without it was the high solute load in the feedings of the infants who ultimately developed hypernatremia. Several factors contributed to this:

1) Continued feeding of whole milk without supplemental water throughout the period of diarrheal losses was frequently encountered. If supplementation of the usual protein-containing food was made, it was usually with O.E.S., a solution which, though hypotonic to plasma, still contains appreciable amounts of electrolytes.

2) A second pattern of feeding was the use of O.E.S. alone. Although a solution such as this is similar in composition to the actual losses observed in diarrheal stools,[3] its use as a substitute for water provides no excess water for other continuing losses (skin and lung) or for the water losses through the kidneys, which may be increased in the face of progressive dehydration and continuing catabolism of protein.

3) Concentrations of sodium in "softened" water, used for reconstitution of both protein and nonprotein formulas, of as high as 19 mEq/l offer another opportunity for introduction of added solute into the diets of young infants.

4) Errors, both on the part of the mother in reconstitution of formulas (Case 15), and of the physicians in prescribing solutions with clearly excessive quantities of salt

(Cases 7, 14), accounted for some of the most severe cases in this series.

5) Finally, the early introduction of unmodified cow's milk, or a 1:1 dilution of evoporated milk as a formula without added carbohydrate, into the diets of very young infants was noted in two cases. One of the two youngest infants (Case 3) had received a 1:1 evaporated-milk formula from birth and with the onset of diarrhea this had been changed to boiled skim milk. Another patient (Case 14) had been given a 1:1 evaporated-milk formula from birth, although prior to admission a food of even higher solute content had been substituted by the parents and a hypertonic salt solution was added by the physician.

CONCLUSIONS

Although the illness in many of the infants in this series was apparently mild initially, the extended duration of symptoms in some, and the appearance of the patients at the time of admission, suggested that parenteral alimentation should have been employed earlier. It is strongly emphasized that the use of a solution containing "electrolytes" should not allow the physician to forget the fact that if losses are exceeding the capacity of replacement by oral means, recourse to parenteral fluids must be made. No routine regimen can substitute for clinical judgment. The availability of solutions theoretically calculated to replace losses may offer a false sense of security to both physicians and parents.

In situations of mild gastroenteritis, the primary need of the young infant, who has higher requirements for water to meet the insensible and renal demands, is for water not obligated to solute. Unmodified cow's milk alone, or supplemented with electrolyte-containing solutions, does not ideally supply this need.

The high mortality rate, which is in agreement with that reported by others in hypertonic dehydration, only emphasizes the gravity of this complication. Even with the most cautious and gradual expansion of body fluid, convulsions were observed in four patients and excessive weight gain in another. One death appeared to be the result of rapid hydration with 5% glucose over a period of 12 hours. Although the use of solute-free solutions would seem to be the logical treatment of hypernatremia, our experience, and that of others,[3] has shown the use of these to result in the development of clinical manifestations resembling "water intoxication," even with elevated concentration of sodium in the serum.

The dangerous effects of giving high-solute feedings to infants enduring increased water losses by "reducing the margin of safety in water balance for any given intake"[10] have been emphasized again and again.[3-5, 10, 13] The experience reported here resulted from inadequate recognition of these potential dangers.

SUMMARY

An epidemic of gastroenteritis during which an unusually high incidence of hypertonic dehydration was encountered in infants is reported.

Analysis of the feedings received by the infants during the initial phase of diarrhea indicates that those who developed hypernatremia had received feedings in which the ratio of solutes to water was higher than that in the estimated continuing losses of fluid through stools, urine, and skin and lungs.

Considerations to be employed in the prevention and treatment of hypernatremia and dehydration during diarrhea are discussed.

The physician must develop his clinical judgment in determining when parenteral rather than oral therapy is required in diarrhea—and not depend on a routine regimen or solution for oral therapy.

REFERENCES

1. Rapoport, S.: Hyperosmolarity and hyperelectrolytemia in pathologic conditions of childhood. Am. J. Dis. Child., 74:682, 1947.
2. Tarail, R., Bass, L. W., and Runco, A. S.: The frequency and nature of hyperto-

nicity of the body fluids in infantile diarrhea. Am. J. Dis. Child., 86:658, 1953.
3. Weil, W. B., and Wallace, W. M.: Hypertonic dehydration in infancy. PEDIATRICS, 17:171, 1956.
4. Finberg, L., and Harrison, H. E.: Hypernatremia in infants: an evaluation of the clinical and biochemical findings accompanying this state. PEDIATRICS, 16:1, 1955.
5. Skinner, A. L., and Moll, F. C.: Hypernatremia accompanying infant diarrhea. Am. J. Dis. Child., 92:562, 1956.
6. Natelson, S.: Routine use of ultramicro methods in the clinical laboratory. Am. J. Clin. Path., 21:1153, 1951.
7. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Vol. II. Baltimore, Williams & Wilkins, 1932, pp. 557-559.
8. Segal, M. A.: A rapid electrotitrametric method for determining CO_2 combining power in plasma and serum. Am. J. Clin. Path., 25:1212, 1955.
9. Hansen, J. D. L., and Smith, C. A.: Effects of withholding fluid in the immediate postnatal period. PEDIATRICS, 12:99, 1953.
10. Pratt, E. L., and Snyderman, S. E.: Renal water requirement of infants fed evaporated milk with and without added carbohydrate. PEDIATRICS, 11:65, 1953.
11. Pratt, E. L., Bienvu, B., and Whyte, M. M.: Concentration of urine solutes by young infants. PEDIATRICS, 1:181, 1948.
12. Calcagno, P. L., and Rubin, M. J.: Effect of dehydration produced by water deprivation diarrhea and vomiting on renal function in infants. PEDIATRICS, 7:328, 1951.
13. Report, Committee on Nutrition, American Academy of Pediatrics: Water requirequirement in relation to osmolar load as it applies to infant feeding. PEDIATRICS, 19:339, 1957.

SUMMARIO IN INTERLINGUA

Dishydratation Hypertonic (Hypernatremia): Le Rolo De Nutrimentos Ric In Solutos

Es reportate un epidemia de gastroenteritis in le qual un inusualmente alte incidentia de dishydratation hypertonic esseva incontrate in infantes.

Le analyse del nutrimentos recipite per le infantes durante le phase initial de diarrhea indica que le patientes de hypernatremia habeva recipite nutrimentos in que le proportion de solutos a aqua esseva plus alte que in le estimate perditas continue de fluido via feces, urina, e pelle e pulmon.

Es discutite considerationes a observar in le prevention e tractamento de hypernatremia e dishydratation durante diarrhea.

Le medico debe basar se super su judicamento clinic in determinar quando un therapia parenteral plus tosto que oral es requirite in diarrhea. Ille non pote sequer de maniera routinari un regime o un solution de therapia oral.

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NUTRITION REVIEWS

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SALT, FAT AND HYPERTENSION: THE JAPANESE EXPERIENCE

While pursuing studies linking salt intake to the development of essential hypertension (L. K. Dahl and R. A. Love, *A. M. A. Arch. Int. Med.* **94**, *525* (*1954*); *J. Am. Med. Assn.* **164**, *397* (*1957*); *New Engl. J. Med.* **258**, *1152*, *1205* (*1958*)), an opportunity was made available to study the adult population of a Japanese farm village in the vicinity of Hiroshima City during February through April 1958. At this time, it was discovered that the clinical picture of hypertension was significantly different from that commonly observed in the United States. On both clinical and laboratory grounds, these Japanese had strikingly little evidence of the usual cardiac complications found so commonly in western adult populations.

Among patients with chronic arterial hypertension who die of effects related to this disease, it has been generally accepted that the chief causes of death are due to the complications of atherosclerosis in the vessels of the heart, brain and kidneys. While it is recognized that there is no necessary relationship between these two diseases, in western society atherosclerosis, particularly of the coronary arteries, is often thought to be a well-nigh inevitable concomitant of chronic hypertension. There is little doubt that in our society hypertension accelerates the progress of established atherosclerosis and it seems likely that the onset of atherosclerosis is materially hastened by the presence of hypertension.

Relative to coronary artery disease for instance, an excellent long-term, continuing study in Framingham, Massachusetts, (T. R. Dawber, F. E. Moore and G. V. Mann, *Am. J. Pub. Health* **47**, *4* (*1957*)) indicated that over a four-year period males aged 45 to 62 with antecedent hypertension developed about three times more clinical coronary artery disease than did males of similar age without hypertension. However, the affected individuals appear to have been on what might be called an atherogenic regimen, *i.e.*, a type of life in which atherosclerosis could be expected to develop in the absence of hypertension.

What is the situation when individuals with hypertension are on a nonatherogenic regimen? Most of the available experimental evidence with animals indicates that on normal diets, atherosclerosis does not develop following prolonged hypertension induced in the rat, dog, rabbit, sheep, goat and probably the chick. By contrast, when changes are effected in lipid metabolism through modifications of diet in the rabbit and chick (in the case of the dog, with the production of hypothyroidism as well), atherosclerosis develops which is clearly intensified and accelerated when the factor of hypertension is added (L. N. Katz and J. Stamler, *Experimental Atherosclerosis*, *p. 224*. *Charles C. Thomas*, *1953*).

The fact that animals with chronic experimental hypertension ordinarily do not develop atherosclerosis suggests the possibility that under certain conditions, human hypertension may be dissociated from this companion disease.

I. Snapper reported that among the Chinese patients at Peking Union Medical College, atherosclerosis was uncommon and when hypertension was present, it was not associated with coronary thrombosis, although heart failure and cerebral hemorrhage were still seen (*Chinese Lessons to Western Medicine*, *p. 171*. *Interscience*, *1941*). It is not clear from his brief discussion just how common either hypertension or the named complications were.

A human situation analogous to that of animals with experimental hypertension may be present in Japan where hypertension is a common and serious disease but coronary artery disease is rare, at least among the non-

professional, laboring majority of the population. In Japan, deaths from hypertension follow primarily cerebrovascular accidents as opposed to western society where deaths are largely due to cardiac complications. For instance, in B. J. Clawson's well-known large autopsy series in the United States (*Hypertension: a Symposium, p. 239. Univ. of Minnesota, 1951*), among those who were classified as dying of causes related to hypertension, 80 per cent died primarily of heart disease and only 14 per cent of cerebral hemorrhage. By contrast, in Japan at least half of the hypertensive deaths in autopsied series are due to cerebrovascular accidents (H. Ueda, *Japan Med. J.* **1740**, *27 (1957)*).

Indeed, these aftermaths of hypertension have been the leading cause of death in Japan since 1951, and one of the leading causes since 1900 (S. Watanabe, *A Brief Report on Public Health Administration in Japan, p. 13. Japanese Ministry of Health and Welfare, 1956*). In 1955, Japanese males of the age group 35 to 80 had two to four times the mortality rate from this cause than American males of similar age, while the mortality from arteriosclerotic heart disease in male Japanese in this age group was from a third to a sixth as common as in their American counterparts (*Annual Epidemiological and Vital Statistics, 1955, pp. 406–411. World Health Organization, 1958*).

It is probable that differences in diagnostic standards account for some of the discrepancies in the incidence of these diseases in the two races, but it is difficult to reconcile the variations on this basis alone. For instance, in one autopsy series of about 10,000 Japanese the incidence of severe coronary atherosclerosis was approximately one tenth that of comparable individuals in the United States (N. Kimura, *Cardiovascular Epidemiology, p. 22. Hoeber-Harper, 1956*).

Furthermore, unpublished observations by American physicians of the Atomic Bomb Casualty Commission in Hiroshima are in accord with the belief that in contrast to hypertension, coronary heart disease is relatively uncommon in the Japanese. Among the 5000 adults studied during the years 1950–55, a diagnosis of arteriosclerotic heart disease was made in 45 patients, infarction of myocardium secondary to arteriosclerotic coronary thrombosis in three, and infarction due to unknown cause in an additional eight patients. In this same series, a diagnosis of hypertensive cardiovascular disease was made in 227 patients, hypertensive vascular disease in 293, and essential vascular hypertension in 430 (Crowley, F. B., Jr., *personal communication. ABCC, Hiroshima*).

Interestingly, while the incidence of cerebrovascular accidents is generally high throughout Japan, there seem to be areas in which it is much higher than others. In general, the highest rates are in the northern areas of the main island of Honshu with progressively lower rates going southward (E. Takahaski *et al.*, *Human Biol.* **29**, *139 (1957)*). This disparity in distribution is not explained by differences in climatic conditions, since the most northerly island of Hokkaido has less than half the rate of the areas noted above in Honshu. The areas in which cerebrovascular accidents are most common are also those in which hypertension is most common.

The primary cause of the cerebrovascular complications, which appear to be so common in the hypertensive Japanese, is less clear. If the lesions are primarily atherosclerotic in origin, this might suggest that the pathogenesis of coronary and cerebral atherosclerosis may be somewhat different.

If the lesions are not atherosclerotic, what are they? No clear-cut answer is available at present, but recent discussions (R. D. Adams, *Cerebral Vascular Diseases, Second Edition, p. 23. Grune & Stratton, 1958*) of hypertensive cerebrovascular disease have reemphasized the evidence for nonatherosclerotic processes such as aneurysm formation from weakened vessels. Certainly the relative mildness of atherosclerosis in the other areas of the body argues for the non-

atherosclerotic origin of some Japanese cerebrovascular accidents. It would also aid in explaining the well-known fact that, while American men have much more mortality from atherosclerotic heart disease than American women, the mortality from cerebrovascular lesions is very nearly equal in the two sexes (*Annual Epidemiological and Vital Statistics, 1955, p. 407. WHO, 1958*).

It is doubtful whether genetic factors are responsible for the striking difference in the incidence of coronary artery disease in Japanese and Westerners, although assessment of this factor is incomplete. It has been reported that the mortality rate from arteriosclerotic heart disease was higher among male Japanese living in Hawaii than among comparable men in Japan. However, Hawaiian Japanese had less disease than Japanese in continental United States, and these, in turn, had less than white Americans of similar age (T. Gordon, *U. S. Pub. Health Reports* **72**, *543 (1957)*). Since in this study most of the Japanese males living in Hawaii and the United States in the susceptible age group, 45 to 64, were immigrants from Japan, it is probable that such individuals remained influenced by cultural (*e.g.* dietary) patterns already established. Moreover, it is well-known that Hawaiian Japanese have retained more of their native customs than have those who migrated farther west.

A study of males of Japanese ancestry who have been exposed to a strictly western environment from birth will be a more critical test, and one might predict that arteriosclerotic heart disease will prove to be the scourge it now is among most other adult American males. Correspondence with cardiologists in Los Angeles and Honolulu suggests that this may be true at present.

There have been numerous western influences in Japan, especially since World War II, and this has included some change in diet, particularly in the large cities among the professional classes. However, the great majority of Japanese still consume the classical Japanese diet, and the current interest in dietary factors which may play a role in the pathogenesis of atherosclerosis and hypertension warrants a passing description of this diet. From the American viewpoint, it might be most simply described as a primarily vegetarian, low fat (largely unsaturated), average protein, high carbohydrate, high salt diet (*Malnutrition and Nutrition Activities in Japan, p. 3. FAO/WHO Nutrition Committee. Japanese Ministry of Health and Welfare. 1956*). The daily fat intake of about 20 to 30 g. is derived almost exclusively from vegetables and fish and accounts for about 10 per cent of the caloric intake. Protein intakes are about 70 g. per day, of which only a quarter comes from animal sources, mostly fish and shellfish. Calories are derived mainly from cereals, of which rice is the principal source.

Salt intake is generally high and appears to decrease from north to south. In two separate studies (T. Fukuda, *J. Med. Soc. of Chiba* **29**, *490 (1954)*; N. Sasaki, *Japanese J. Hygiene* **13**, *11 (1958)*), farmers in northern Honshu were found to eat about 27 g. per day, while those in middle and southern Japan averaged about 17 g. per day. American males eat about 10 g. per day. It is of some interest that the areas in which salt consumption is greatest appear to coincide with those areas where the prevalence of hypertension and cerebrovascular accidents is greatest (E. Takahaski *et al., loc. cit.*).

If high fat and high salt intakes are important etiologic factors in the development of atherosclerosis and hypertension, respectively, it could be predicted *a priori* that the Japanese would be afflicted primarily with hypertension rather than atherosclerosis. This seems to be true.

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Salt and hypertension[1]

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I am pleased to address your group, because it is heavily oriented toward diseases of infancy and childhood. It has long been my belief that the most prevalent cardiovascular diseases, atherosclerosis and hypertension, are generated early in life rather than some 4 or 5 decades later when they are commonly diagnosed. I will consider myself successful if I engage your permanent interest in this concept relative to hypertension, which I have been studying exclusively for more than 2 decades. I will remain unperturbed, however, if you do not accept all the details.

During the 1st decade, our work was focused on the role of sodium in the etiology and pathogenesis of clinical and experimental hypertension. In the 2nd decade, the importance of the genetic substratum was recognized and studied experimentally. In this 3rd decade, we are trying to define the genetic factors responsible for hypertension and to quantify individual genetic vulnerability to this disease. In this review of our work, I will let you judge its overall relevance.

Our work began in 1948 and was based on Kempner's indubitable results with the rice–fruit diet in the treatment of hypertension (1, 2). For reasons that are difficult to fathom, there appeared a great deal of antipathy to Kempner's reports as well as irrational disbelief in the effectiveness of the diet. In a major review of the subject early in 1949, it was stated that "suggestion" had not been ruled out and that "Restriction of salt intake has not been conclusively shown to be independently capable of lowering the blood pressure in hypertensive patients" (3).

We judged Kempner's work only on the basis of his quantitative evidence. We were also aware of several physicians, incapacitated by progressive hypertensive cardiovascular disease who, after being on the regimen for some months, were back practicing medicine. We found it believable that the rice–fruit diet was often useful in the treatment of hypertension, including the "malignant" phase. In so concluding, I have often felt that we became heirs to the antipathies originally directed at Allen and later at Kempner. We decided, nonetheless, to try to detect the dietary factor that made this diet effective. For those of you who are unfamiliar with the diet, let me define it as a low sodium, or a high potassium, or a high carbohydrate, or a low protein, or a low fat, or a high fluid diet. In its pristine form it is made up mostly of rice and fruit including juices, but certainly with no added salt.

In a series of careful metabolic studies carried out over 4 years at the Rockefeller Institute with Drs. Dole and Cotzias, we showed that the blood pressure lowering effect was due primarily to its low sodium content (4–6). Parallel studies at the nearby Goldwater Memorial Hospital in New York City by Watkin et al. (7) and Hatch et al. (8) resulted in similar conclusions.

As a direct outcome of these studies, I began to wonder about the following: if a low salt intake causes a lowering of blood pressure, does a high salt intake cause increased blood pressure? Both the groups mentioned had found that the addition of NaCl to the rice–fruit diet prevented its therapeutic effect, whereas additions of NaCl after the induction of therapy caused reemergence of hypertension. This was not quite the answer to the question that I was asking and which I thought to be original at the time. Later, however, it was learned that several others had had similar thoughts much earlier, namely Ambard and Beaujard in

[1] Supported primarily by the United States Atomic Energy Commission: partial support received from the Public Health Service Grant He 13408-01 and a grant-in-aid from the American Heart Association (AHA 70-772) supported by the Dutchess County (New York) Heart Association.

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1904 (9) and Allen in a series of papers in 1920 and 1922 (10–13) that were summarized in a monograph in 1925 (14). Whereas the paper of Ambard and Beaujard was prophetic, it was based upon remarkably little data. Allen's paper by contrast, was based on long and careful observation. I have since become convinced that Allen was the first to demonstrate clearly the effectiveness of salt restriction in hypertension. He was the first to say that . . . "the morbidity and mortality from renal and vascular diseases would probably be reduced by a general abstinence from salt" (14). He was reluctant to press for such action because of the possibility that ingestion of salt may be based on "an instinct which seems so deep and real." Many heavy salt eaters in this audience would agree. It may be of interest to them then, that in the same discussion, one of Allen's paragraphs concludes with the statement that ". . . races of individuals accustomed to a saltless diet may have no desire but, on the contrary, a distinct dislike for salt."

This dichotomy concerning the need for and harm from added dietary salt continues nearly one-half a century later. In my experience, the chronic heavy salt user resembles the heavy cigarette smoker in that evidence for continued use always seems valid to him, whereas that for stopping is patently fallacious. I might add that my social life is plagued by people who think that I am against their eating salt. I have evolved a standard response which I will pass on to you: I am convinced that adding salt is bad but I do not believe that it is a sin.

It must be remembered that the custom of adding salt to food is ancient. It has sustained a folklore on which I could spend this entire talk. Because some recurring vignettes on man and salt may be as interesting to you as they are to me, you may either refer to earlier papers (15–19) or accept on faith the following:

a) Despite its historical prominence, there were many peoples throughout the world, living in the arctic, desert, jungle, and plain, who went for millenia eating only the salt contained naturally in foods. Primarily herbivorous peoples would average perhaps 2 to 10 mEq Na/day. Primarily carnivorous peoples, even consuming as much as 4,000 kcal/day, would hardly average the 180 mEq/day so easily and commonly exceeded by Americans today.

b) By sharp contrast to processed baby foods, human milk is a low sodium food, averaging about 7 mEq/liter. Yet this food was the major and sometimes exclusive source of nutriments that carried generations of infants well into their 1st years of life.

c) The actual metabolic requirements for sodium are remarkably small. In studies on the period of rapid growth during the 1st year of life, I have calculated (19) that 4 to 8 mEq/day would be a generous allowance. Fomon (20) suggested an advisable intake of 7 to 8 mEq/day. The requirement for healthy adults is similar. For more than 20 years we have been studying active, ambulatory patients maintained chronically on daily sodium intakes averaging 6 to 7 mEq and ranging between 2 and 13 mEq (~ 100 to 750 mg as NaCl). Except for a few individuals with impaired renal function, maintenance of sodium balance on these intakes was never a problem. Contrary to the guesses of many, the problems associated with a low sodium diet are neither medical nor related to palatability, for we have more than 20 years' experience with a highly palatable, restricted fat, low sodium diet. The longest period on this regimen recently came to an end for a patient after more than 15 years. This derelict from the Bowery was referred to us in 1956 from Bellevue Hospital in New York City at the age of 47 after failing to respond to bilateral lumbodorsal sympathectomy for severe hypertension, complicated by two cerebrovascular accidents. During the remaining 15 years of his life, he was active, ambulatory, and went for at least one walk of several miles each day. Throughout this period he was under continuous observation and dietary control on a metabolic ward at Brookhaven. Except for relatively short test periods, his daily sodium intake averaged 5.5 mEq (125 mg). Innumerable studies were carried out on him during this time without evidence that this near-constant low sodium intake affected him other than by lowering his blood pressure to normal.

d) Salt appetite, as it is commonly ex-

ssed, is acquired. One must contrast this the hunger associated with gross salt defi- ency such as in Addison's disease. I am aining to the common variety of appetite, served in any restaurant and most homes. y contrast to salt hunger, which signals salt ed, this appetite readily adapts itself to ac's average dietary level of salt. Some of r patients, for example, felt compelled to lt their food "until it looked like snow" nd recently one volunteered that he salted con and potato chips just to make sure! nd most of these people have readily dapted to our low sodium regimen in a few eeks. I have found four references to primi- ve groups who were presented with salt hen they had previously never added salt to heir food. They uniformly and thoroughly disliked it at first. Still, most individuals in uch groups readily adopted added salt. The Arctic explorer, Stefansson, with whom I corresponded prior to his death, told me that some Eskimos, to whom both salt and sea water were available, preferred unsalted meat nd fish. This was still true in 1968 accord- ng to one Canadian observer (21).

The long treks to salt licks by animals are sometimes cited as evidence for a primordial salt appetite that must have continued in man. But only herbivores make such treks. The omnivores and carnivores may go to licks too, not in search of salt but in search of herbivores! The salt hunger of the herbivores, furthermore, is often due to true salt deficiency; with a few exceptions, all plants are low in sodium. In areas far removed from the sea, the soil can become leached of salt. The plants will, therefore, have extremely low sodium contents, as shown extensively by a group from Melbourne (22, 23).

From each and all of the foregoing, we have concluded that salt appetites are acquired and that the high NaCl intakes that are now so ubiquitous are contrary to physiological needs.

When we first became interested in the subject, we were impressed by the paucity of data about one of the most ancient food additives. We found that nutritionists are remarkably uninterested in salt. If we had been interested, instead, in the protein intake of the Masai, the vitamin A intake of Eskimos, or the rice intake of tea laborers in Assam then nutritionists would have had the data ready for us. Social anthropologists, by contrast, had concerned themselves with salt because it has had long-standing importance in personal and intergroup trading relationships. Early in this work, for example, I called the American Museum of Natural History in New York City for information on salt usage among primitives. I was referred to a man on their staff, now unfortunately nameless, who had just returned from studying a remote tribe in South America. When queried as to whether or not the tribe used salt, he replied, "Oh, my yes. They all carry it around in small bags. It is used for money, never on food. Too valuable, you know." After that, we decided that we had to determine by ourselves the salt intakes in different societies.

We initially evaluated NaCl intake by how Long Islanders used saltshakers at the table: those who never added salt were classified as "low NaCl intake"; those who tasted food before adding NaCl were classified as "averaged intake"; and those who always added NaCl without tasting food, were classified as "high intake." The shortcomings of this classification were obvious to us then. They became even more obvious after we gained sophistication from our genetic experiments. However, this pilot exploration proved eminently serviceable. Over a period of 3 years, the employees at Brookhaven who were examined by Dr. Robert A. Love in the Clinic were queried about their NaCl usage at mealtimes. The results showed a significant difference in the prevalence of hypertension (140/90 mm Hg, or more) between the groups classified as low and high (15, 16). This led to further investigation of the thesis that chronic excess salt ingestion played an etiological role in human hypertension.

Others could not confirm our findings and rejected our concept quickly and vociferously. This was due in part to the fact that not everyone uses a shaker for adding salt. The Japanese, for instance, do not commonly use a saltshaker at the table. Queries about a shaker led some to interpret their NaCl intakes as almost uniformly low when, in fact, they are generally high (24). Furthermore,

the query about the shaker instead of the salt was used by some for estimating the intake of individuals, although we meant it as a primitive epidemiological tool for a specified group of Long Islanders.

This primitive but valuable experiment led us to study different cultures. Instead of questionnaires, we used 24-hr diet and urine collections to estimate NaCl intake. These were first proved valid, provided, and this is an important qualification, that urine collections were complete. Whatever the many problems associated with estimating salt intake, we managed to get evidence suggesting that average intakes in several communities in the world differed. There were marked differences among individuals, however, in the societies where NaCl was freely available. These data are summarized in Table 1.

These data further indicated that if the daily metabolic requirements are generally below 1 g NaCl, most of the subjects studied were consuming amounts well in excess of need.

TABLE 1
Average daily salt intake (based on 24-hr urine excretion) in several societies

Group	Year	Sex	NaCl intake, g/day	
			Avr.	Range
Alaskan Eskimo	1958, 1960	Both	–	1-1[illegible]
Marshall Islander (Pacific Ocean)	1958	Both	7	1.5-1[illegible]
United States (Brookhaven)	1954–1956	Male	10	4-[illegible]
Japan				
Hiroshima (southern Japan)	1958	Male	14	4-[illegible]
Akita (northern Japan)	1954	Both	26	5-[illegible]

Salt and human hypertension

The evidence relating to human hypertension is indirect and derives from three sources: *1*) effects of salt restriction, *2*) effects of salt elimination by diuretics, and *3*) correlations between salt intake and hypertension. I will briefly review the evidence for each in that order.

Effects of salt restriction. The cardinal studies of Allen (10–14) and Kempner (1, 2) have already been mentioned. Between 1945 and 1950, there were a number of careful studies documenting the effectiveness of sodium restriction in hypertension, most of which agreed that about one-third of the patients had an excellent response, one-third had only a modest response, and one-third had little or no response. Even among those patients whose blood pressure does not fall after sodium restriction, however, my impression is that their clinical course was more benign if they were continued on a low NaCl intake, as compared with similar patients who reverted to high salt intakes. This is admittedly difficult to prove in man. In rats, however, there is no question concerning the validity of my position. Among rats from our hypertension-prone strain, the level of NaCl consumed after hypertension develops is critical, whether the disease was induced with added dietary NaCl or unilateral renal artery constriction without added NaCl. High intakes lead almost uniformly to death in a few months. If dietary NaCl is restricted and remains restricted, the course is clearly much more benign even in the animals in which the blood pressure failed to respond. This was evidenced by highly significant extension of life span.

Among our metabolic studies, my favorite was reported in 1958, yet 13 years later, it remains in limbo despite subsequent experience which has only strengthened the original conclusions (25). I need not document the evidence that the incidence of hypertension is significantly greater among the obese than among nonobese individuals. Statements that a decrease in weight results in a decrease in blood pressure are common. We noted, however, that most observers reported falls in blood pressure during the 1st few weeks of caloric restriction, long before significant weight loss had occurred. This timing was precisely what we had observed in numerous hypertensive subjects after restriction of salt unaccompanied by caloric restriction. We therefore postulated that it was not weight loss that lowered blood pressure but the sal

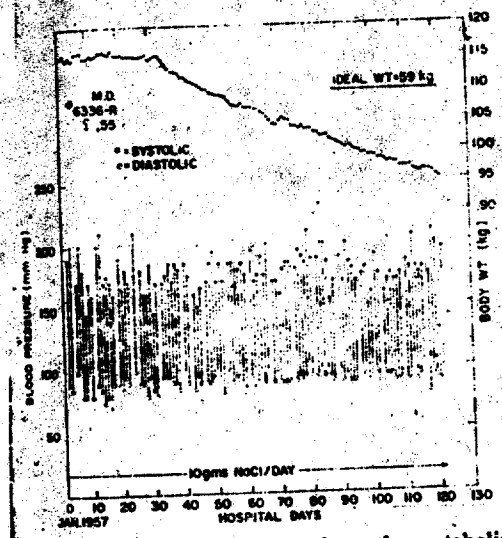

FIG. 1. During the 1st month on the metabolic ward, this obese patient was on a diet sufficient to maintain her admission weight. During the subsequent 3 months of caloric restriction, she lost approximately 20 kg of body wt. A high NaCl intake was maintained throughout the 4 months of hospitalization. No fall in blood pressure occurred.

restriction that must commonly accompany significant caloric restriction. By manipulating caloric and sodium intakes over long periods, we confirmed this postulation. The responses of some representative patients are indicated in Figs. 1 to 3. Under equally well-controlled conditions we have observed this same phenomenon many times since the original report. I am fully aware of the abnormally low blood pressures reported in semi-starvation, but here we are not referring to pathological hypotension. We are talking about diminishing hypertension. In some unpublished studies, we followed the blood pressures of three hypertensive patients maintained on 10 g salt/day but with drastic caloric restriction: not until their weights dropped to approximately 20% below ideal weight and the patients began to appear cadaverous, did blood pressure fall. This seems comparable to the findings reported in semi-starvation under less well-controlled conditions. It is hardly comparable to the usual obese hypertensive person who is reduced toward, or (uncommonly) to, normal. I have no hesitation, therefore, in stating that a fall in blood pressure during a weight-reduction program for obesity is due to restriction of salt, not calories.

Effects of salt elimination by diuretics. Until the thiazide diuretics were introduced in 1957, very few physicians considered that sodium had much to do with hypertension. When these active and relatively safe natriuretics became widely available, their sometimes dramatic effects on blood pressure introduced many physicians for the first time to this possibility. One might ask why it took so long to persuade thoughtful clinicians. I believe the reason for this is that most hypertensive patients whom these men had personally observed had failed to respond to the prescribed sodium restriction. I am further convinced, after using low salt diets for 23 years, that the explanation for this failure is simple: most of their patients had not been on low sodium diets. In my own experience, which has been exclusively by referral, I have never, I repeat never, seen a new hypertensive patient who was, in fact, on a low sodium intake, all protestation to the contrary notwithstanding. I cannot believe this observation is unique to the patients I have observed. One might ask, in turn, why is it so difficult to maintain a low sodium intake?

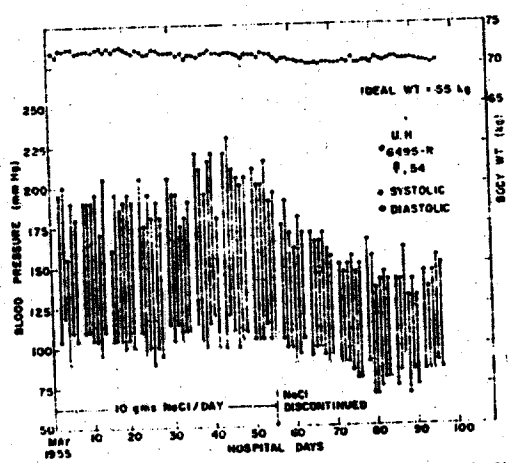

FIG. 2. During the 3 months on a metabolic ward, this obese patient had no weight loss because of a constant high caloric intake. Blood pressure remained constant or perhaps increased toward the end of 55 days on 10 g added NaCl. With no change in caloric intake or weight after the low NaCl regimen began, the blood pressure fell significantly ($P < 0.01$) during the remaining 35 days in the hospital.

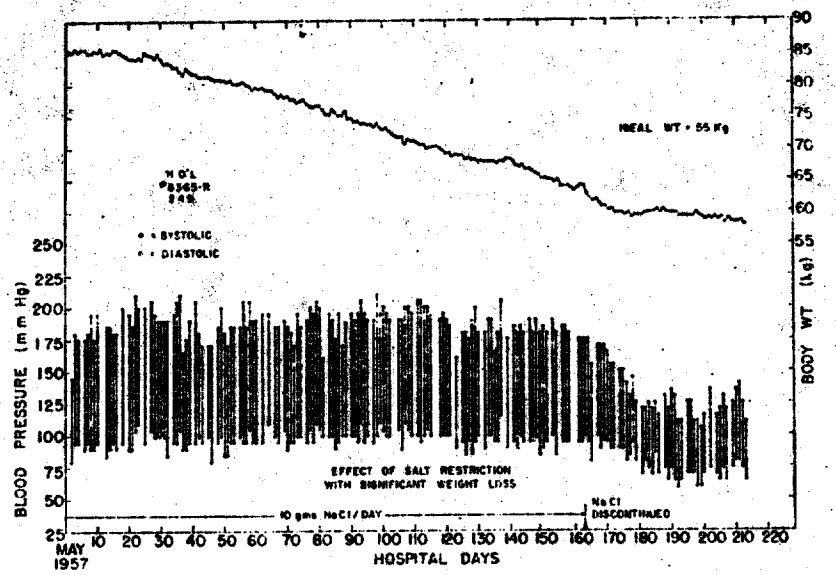

FIG. 3. During the 1st month on the metabolic ward, this obese patient was on NaCl and calories sufficient to maintain her admission weight. After the 1st month, caloric restriction was started but NaCl continued. Over the next 4.5 months, she lost approximately 25 kg body wt with no fall in blood pressure. After 5.5 months of observation, during which time her blood pressure had remained unchanged, the low NaCl regimen was started and, simultaneously, her caloric intake was increased to maintain weight. Only after the NaCl was restricted did blood pressure fall ($P < 0.01$).

There are a number of reasons including at least the following: *1*) To palates accustomed since infancy to saltiness, the absence of salt is at first distressing. Substitution of herbs and seasonings other than salt requires a modicum of intelligence, imagination, and determination. *2*) Most low sodium diets are prescribed haphazardly and unenthusiastically. *3*) Even when prescribed by authoritative sources like the American Heart Association, these diets are so complex as to discourage all but the most persistent.[3] *4*) Most patients are unaware of *a*) the naturally high sodium content of cow's milk, *b*) the added NaCl in cheese, and *c*) the added NaCl in most processed foods.

Correlations between salt intake and human hypertension. There are only a few acute experiments in the literature indicating that large daily intakes of NaCl will increase the blood pressure of nonhypertensive individuals (26–28). Failure to see such acute elevations appears to have been a more common experience (29). It is unrealistic to determine chronic effects of salt by means of acute experiments. Alcoholic cirrhosis does not develop after a binge nor does cancer of the lung occur after a few packs of cigarettes. We sought, therefore, a correlation between chronic average salt intakes and the prevalence of hypertension in various peoples. Even before 1960 (30), we had begun to think of genetic salt interactions and recommended that a family history of hypertension

[3] I would like to suggest the following rule of thumb for attaining a low sodium diet that is both simple and effective: *1*) never add salt to food during preparation or at the table, *2*) avoid milk and milk products, *3*) avoid all processed foods except fruits and juices, and even here examine all labels. By using only this formula, a (calculated) weekly menu with a daily average of 1,565 kcal, 78 g protein, and 408 mg Na (~1 g as NaCl) resulted. Calories may be increased, if desired, by adding sugar, jellies, bread (low Na, 2 mg/slice), fruits and vegetables. Note that nothing is said about the usual proscriptions, e.g., lobster, shrimp, carrots, et cetera. All such foods were included but it was assumed that these would, on the average, not be eaten more than once a week. Like all rules of thumb, this one is not foolproof. Most people can remember these three rules and will end up on a far lower sodium intake than the usual regimen that is soon abandoned because of its complexity.

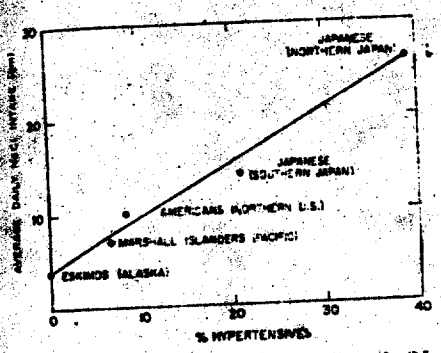

FIG. 4. Correlation of average daily salt (NaCl) intakes with prevalence of hypertension in different geographic areas and among different races. Compare with data in Table 1. This graph suggests a dose-response (30). (Used with permission of the publishers.)

warrants restricted salt intakes (17). Therefore, when we made the group correlations shown in Fig. 4, we indicated that these are not indices that apply to a single individual: genetic variability in man is known to be great. The data in Fig. 4 show increasing group average salt intakes and increasing group prevalence of hypertension, but individual susceptibility is not assessed within each group.

Humphries (31) in a study of 462 Bahamians, concluded that "excessive intake of sodium chloride is an important factor in the causation of hypertension in the Bahamas." Isaacson, Modlin and Jackson (32) measured the 24-hr urinary sodium output of 100 healthy adult Bantu males in South Africa. Using data from other observers on the prevalence of hypertension among male Bantus of all ages, their fit with the line in Fig. 4 was excellent. A number of Japanese observers have found a correlation between the high salt intake in Japan and the prevalence of hypertension and apoplexy in the Japanese (33–35 and others). More recently, Prior et al. [illegible] studied the correlations between blood pressure and sodium output in two groups of Polynesians of "... the same broad ethnic origin" but on separate islands with apparently different average salt intakes. From their data, the group with the higher sodium intake (about 50 mEq/day higher) also had the higher incidence of hypertension. Although there were other differences between these islands, the authors concluded that the data were "... compatible with Dahl's hypothesis that higher salt intake and higher blood pressures are related." The authors were concerned about their failure to "... demonstrate an association between blood pressure and salt intake *within* as well as *between* the populations." (Authors' italics.) This seems an unwarranted requirement except for genetically inbred populations.

In apparent contrast to the foregoing, some studies report absence of correlations between salt intake and hypertension. Phear (40) studied the sodium excretion of 20 men with hypertension and 20 with normal blood pressure. Because the mean sodium excretion of both groups was virtually identical, the author concluded that "... hypertension cannot be related simply to dietary sodium intake." This statement covers maintenance of hypertension once induced. I would, of course, concur.

Miall (41), in restudying a Welsh community, queried 953 residents, fairly equally divided by sex, as to their salt intake habits using our original saltshaker questions (15, 16). No statistically significant correlation appears to have been found from the data given. In a discussion of this problem, Paul (42) opined that there was no correlation between stated salt habits and hypertension among some 2,000 men queried. He reported that frankly hypertensive individuals, given a choice of three test samples of soup with different NaCl concentrations, preferred the two lower concentrations. This is of considerable interest to me, because our genetically hypertension-prone rats behaved similarly. Given a free choice of either saline solution or water these rats ingested less saline than did those from the strain genetically resistant to hypertension (43).

Salt perception is, however, not as simple as may be implied by either Paul's data or ours. This is evident from a report on taste thresholds for salt in hypertensive individuals by Fallis, Lasagna and Tétreault (44). These workers found a highly significant decrease in the ability of hypertensive persons to taste "saltiness" as opposed to appropriate controls. They concluded that the "... data suggest that hypertensives may eat more salt be-

cause they cannot taste it as well as can normotensive individuals." In the Framingham study, Dawber et al. (45) found no correlation between stated salt habits and hypertension or urine sodium and hypertension.

It is clear from the foregoing summary that different experimental protocols have produced different data and have led, therefore, to different conclusions. I would suggest that the following play a role in these divergent results.

a) Asking about NaCl intake in industrial societies is nearly hopeless for obtaining an index of salt intake. Virtually all foods processed for the general market, except fruits and juices, are heavily salted. Unlike cigarettes and alcohol, the consumer cannot be aware of this high intake of salt, because it occurred without his taking a conscious step. It is probable, therefore, that our salt habit questionnaire (15, 16) was useful to us but a disservice to others. As it should be clear by now, unless such questions give an index of actual NaCl intake, they have no relevance whatsoever.

b) In some of the preceding citations, even quantitative estimates of NaCl intake as measured by urine outputs could sometimes not be correlated with hypertension. For this discussion, I am prepared to assume that urine collections were complete even under field conditions in remote lands. I hope, however, that no one expects a linear correlation between individual salt intakes and individual blood pressures on a given day. If I have not yet made this point clear, I will try to do so. Besides daily variations, however, the possibility that "what is one man's meat is another man's poison" seems not to have been considered by these observers. Although these investigators would not expect all two pack-a-day smokers to develop bronchogenic carcinoma, they have difficulties in transferring this concept to salt.

c) Due to the high NaCl intake that is almost uniformly consumed in the United States from early childhood to old age, it is difficult to quantify the etiologic significance of salt in a given individual because this is a factor common to all. Many of us have evolved from a medical past in which diseases had a single primary cause, e.g., infections and parasitic diseases. Acheson (46) has pointed out that these old models may not suffice in the study of the noninfectious, degenerative diseases that now plague mankind. Multifactorial causation has been recognized by some epidemiologists for years. He says, however, that "... because it is uncomfortable and scientifically untidy, we have tended to do lip service to it but to ignore it when we can."

The persistent difficulty in determining the role of dietary lipids for the genesis of atherosclerosis is a parallel case in point. In our society, in which approximately 40 to 50% of calories is derived from fats, it has been impossible to specify the precise role of lipids. Most students of arteriosclerosis agree nonetheless that fats are etiologically important. Atherosclerosis has multifactorial origins. Therefore, although virtually everyone is consuming a high fat diet, some develop atherosclerotic complications early in life, some only late, and some not at all. By studying only our own high fat intake society, one would completely overlook the importance of fats, because they are common to all. Obviously, there must be individual factors that modify, even eliminate, the influence of a high fat intake in individual subjects. I submit that similar considerations must be accorded to NaCl on the basis of the sum of the evidence quoted here.

d) Finally, our animal data (47) suggest that current levels of salt intake in adults can be less critical than early levels in childhood: hypertension-prone rats developed permanent hypertension from brief periods on high NaCl early in life, although maintained thereafter on a low NaCl intake.

Salt and experimental hypertension

In man, one can state only that there is a correlation between salt intake and hypertension. In the rat, however, the cause and effect relationship is clearly demonstrable. The earlier work of others in this field has been reviewed elsewhere (17, 18, 30). Suffice it to say that, between 1940 and 1945, a number of reports had indicated the hypertensinogenic capacity of several steroids when added NaCl was provided, and between 1948-1950, of saline as the sole drinking fluid

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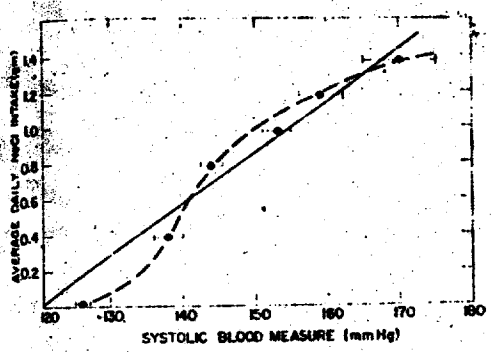

FIG. 5. Correlation between average daily salt (NaCl) intake and mean (±SE) systolic blood pressures of rats after 12 months on various levels of salt intake. Six colonies of rats were maintained on different average NaCl intakes for 1 year. Points may be connected either by a classical sigmoid dose–response curve or a straight line, as in Fig. 4 (48). (Based on data recalculated from Meneely, G. R. et al., *J. Exptl. Med.* 98: 71, 1953. Reprinted by permission of The Rockefeller University Press.)

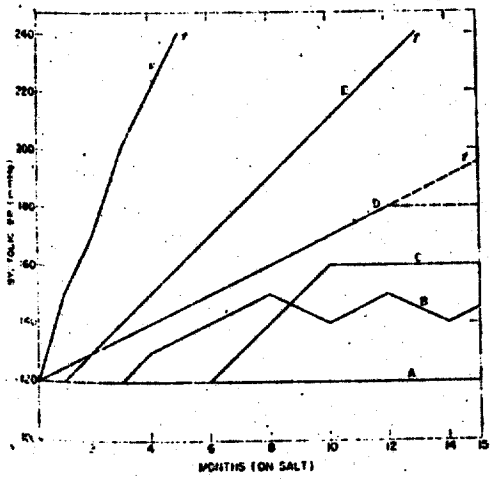

FIG. 6. Schematic representation of systolic blood pressure responses of rats to high salt intakes. Note that some never respond (A), whereas others (F) are dead within a few months with fulminating hypertension (52). (Reprinted by permission of the editors.)

finally in 1953, Meneely and his associates (48) induced hypertension in rats by mixing NaCl with food and allowing ad libitum consumption of food as well as water. As the data in Fig. 5 indicate, they found a classical sigmoidal dose–response curve among the six groups of rats, each on different NaCl intakes. In Fig. 5, the straight line indicates the similarity of the data obtained on rats with those values obtained in man (as shown in Fig. 4).

Our own experience is now based on more than 32,000 rats during the past 20 years. Thousands of these rats developed hypertension from chronic salt feeding (49–51). Among unselected Sprague-Dawley rats, we observed a characteristic response if large numbers of animals were fed salt; this is summarized schematically in Fig. 6 (52). This schema indicates that the response to salt in unselected rats may range from none to gradually increasing pressures including a rapidly malignant phase. Approximately one-quarter of the animals (line A) fail to manifest an elevation in blood pressure even after consuming a very high salt diet over most of their lives. The remaining three-quarters develop elevations of varying degrees, including a small fraction (~ 2 to 3%) that die with fulminating hypertension after only a few months on salt (line F). Such disparate responses, among animals on the same regimen, suggested to me that variations in genetic substrate were modifying the blood pressure response to salt. Therefore, more than 10 years ago, we began exploring this possibility by using the technique of selective inbreeding. In successive generations, we inbred those animals that most rapidly developed significant elevations in blood pressure from salt. Conversely, we also inbred those without elevation in blood pressure from salt ingestion. After three generations of selective inbreeding, it had become obvious that the genetic influence was an important determinant in whether or not the blood pressure became chronically elevated from salt ingestion (53, 54). The data summarized in Fig. 7 were obtained after 5 to 7 generations (55) of such inbreeding. The difference between the two strains was actually greater than shown on this graph. Among the sensitive (S) animals most of the blood pressures were obtained after only 4 weeks on NaCl because, by the time of the second blood pressure measurement at 8 weeks, only 12 of the original 45 sensitive rats were suitably healthy. The remaining 33 were dead or in a terminal state, such that recordings were unreliable. By contrast, the 42 members from the re-

sistant (R) strain were alive and in good health after 10 to 12 months on precisely the same regimen. Similar results continue to obtain after more than 30 generations.

The influence on blood pressure of differences in genetic vulnerability to NaCl is illustrated by the effect of crossing the two strains as shown in Fig. 8 (56). This graph demonstrates conclusively the difficulties in assessing the role of NaCl in genetically mixed populations as commonly occur in man. In the R strain, individual pressures were predictably normal and the opposite was true for the S strain. This was not so, however, for the various crosses. Among the BR (i.e., backcross R = F_1 × R), whereas most animals had normal pressures or were only slightly above the pure R strain, there were a few individuals that developed marked elevations; the reciprocal was true for the

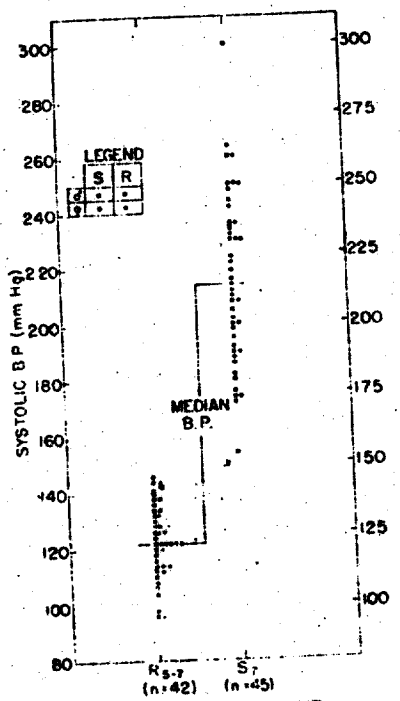

FIG. 7. Genetic influences on BP response to a high salt diet in Sensitive and Resistant strains of rats. S animals after 1 to 2 months on salt; R animals after 10 to 12 months on salt. R 5–7 and S 7 = fifth to seventh generation inbred for resistance or sensitivity to hypertensinogenic effect of salt, respectively. (Reprinted by permission of the publishers, *Postgrad Med. J. 42:* 148, 1966.)

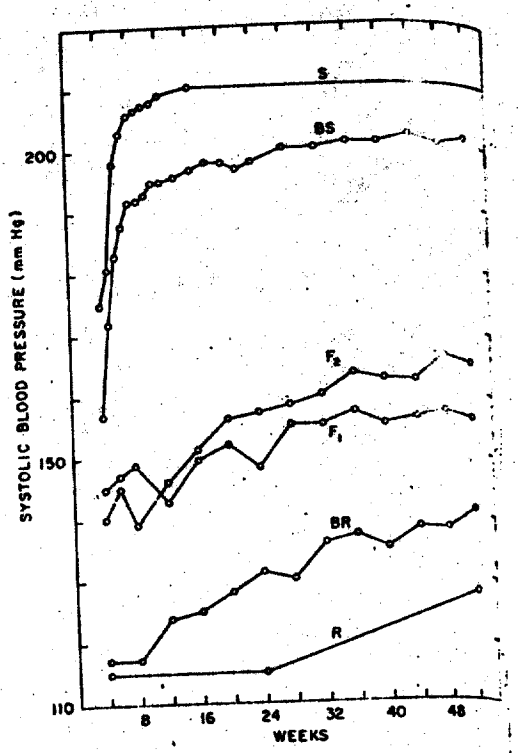

FIG. 8. Average blood pressures of different rat populations on uniform high NaCl (8% in chow) diet during a year of observation. The curves represent average cumulative systolic blood pressure for each cross. Cumulative blood pressures include the last BP when animal was in good health and this value is carried forward whether an animal survived 6 weeks or 12 months on the regimen. Note that no S rat survived beyond the 16th week. F_1 = R × S; F_2 = F_1 × F_1; BS = F_1 × S; BR = F_1 × R. Although R, BR, F_1 and F_2 showed significant differences in BP based on sex (males higher than females), the values have been pooled for the purpose of this graph, which illustrates the definite genetic influence on the reaction to salt intake (56). (Reprinted by permission of The Rockefeller University Press.)

BS (i.e., F_1 × S) cross in which most had high pressures but some remained in the normal range. Among the F_1 (R × S) and F_2 (F_1 × F_1) crosses in which average pressures were roughly midway between those of the parental R and S strains, marked hypertension in some and normal levels in others were observed in members of both crosses. It is evident that despite the foreknowledge of the average daily salt intake from weaning onward, as well as considerable information on

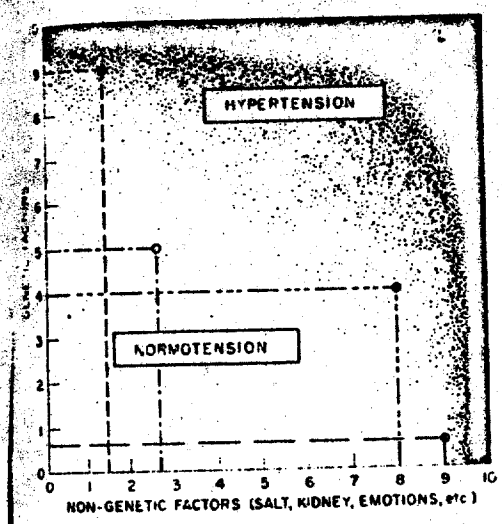

FIG. 9. Diagram to suggest possible relative roles of genetic and nongenetic factors in hypertension. Because outbreeding is customary in man, people with marked genetic predisposition for or against hypertension will be less common than those intermediate between the two extremes. At the same time, the nongenetic influences become more critical in the individual with only modest predisposition as compared with those who have very high innate predisposition for or against hypertension. In the individual with great innate predisposition, protection against the minimal nongenetic influences required to initiate hypertension will be virtually impossible so that hypertension may be inevitable. Just the opposite is true, however, with a person who has great innate resistance to developing hypertension because nongenetic influences will be nearly ineffective. The person with modest genetic predisposition may, or may not, develop hypertension depending upon such things as the character, intensity, and duration of the nongenetic influences (58). (Reprinted by permission of The Rockefeller University Press.)

genetic background, predictions as to the influence of NaCl on blood pressure might differ radically in these rat populations, depending on which individuals or groups were observed. How much more difficult it is to make an assessment in western man in whom both lifetime salt intake and genetic background are unknown.

Discussion

The foregoing allowed a plausible interpretation for the observation that humans with chronic high salt intakes since childhood can remain normotensive. We extended these animal experiments when our peers in this field, using different techniques to induce experimental hypertension, reported failure rates approximately like those we had found for salt. In genetically heterogeneous rats, we thought that variations in genetic susceptibility might be equally responsible for their results. We confirmed this hypothesis by using some of these other techniques: Doca plus NaCl; unilateral renal artery compression without NaCl; adrenal regeneration with NaCl; cortisone without NaCl; and uninephrectomy without NaCl (and with NaCl, unpublished observations) (57–59).

These experiments indicated that the genetic substratum was a critical determinant in whether or not experimental hypertension developed after a number of insults said to play "causal" roles. In this light, we proposed that a similar interaction operates in man (55, 58, 60). We have suggested that hypertension develops from one or more nongenetic factors (e.g., NaCl, kidney disease) acting on the appropriate genetic substrate. Such factors would be more, or less, influential depending on, e.g., the intensity and duration of their action as well as on the genetic predilection for hypertension of the specific individual. If this thesis applies to man, as it clearly does in our two strains of rats, at one extreme there should be individuals with so strong a genetic predisposition as to require only minimal stimuli to develop hypertension; and at the other extreme there should be individuals with so weak a predisposition that they might fail to develop hypertension even after prolonged, intense exposure to the stimuli so effective in others. Because of the outbreeding customary in man, individuals at the two extremes described above will be less common than will be those somewhere in between. At the two extremes, the nongenetic inciting factors would be relatively less important: in the person with great genetic predisposition, protection against all hypertensinogenic stimuli will be practically impossible and therefore hypertension almost inevitably will develop. By contrast, in the person with little or no predisposition, hypertensinogenic stimuli will be so ineffective that hypertension will rarely develop. The

more usual person will be one with only a modest predisposition who may or may not develop hypertension depending upon such things as the nature, severity, and duration of the inciting factor(s). A schema suggests this interaction of genetic and nongenetic factors (Fig. 9). Based on his experience as a quantitative geneticist, Falconer (61) has used similar ideas to develop the concept of liability to ". . . diseases that are not inherited in a simple manner . . ." but which ". . . have nevertheless some hereditary basis." A liability to disease would express ". . . not only the individual's innate tendency to develop . . . the disease, but also the whole combination of external circumstances that makes him more or less likely to develop the disease." The difficulty of the all-or-none character implied in a diagnosis was resolved by assuming a "threshold" on a graded scale of liability. All individuals above this threshold exhibit the disease; all below do not.

There are a number of clinical data compatible with the interpretation illustrated in Fig. 9. It is, for instance, so well established as to require no documentation that first degree relatives of patients with essential hypertension have more hypertension than similar relatives of patients with normal pressures. There is increasing evidence of a similar phenomenon in pyelonephritis; that is to say, in those with pyelonephritis who develop hypertension (and many do not) pyelonephritis probably has served only to unmask a genetic predisposition to hypertension (62–65). This may also be true for patients with chronic nephritis (64). Our hypothesis would predict that it will be found to be generally true in hypertension.

Conclusion

The evidence that salt induces permanent and fatal hypertension is direct, quantitative, and unequivocal in the rat. Because the extensive evidence is circumstantial in man, it is therefore dismissed almost casually by some. If equal evidence had related salt to a similarly fatal but far less common disease, cancer, it would have evoked intense campaigns against it long ago.

Both the emphasis on the negative and the exclusion of the positive are difficult to understand on the assumption that the evidence against excess salt is considered rationally. I have separated acute from chronic effects of salt. I have separated genetic from nongenetic factors and have indicated their interactions. I have also tried to separate the rational from the irrational, and hope, therefore, that this review will serve. However, even Maurice Maeterlinck found it necessary to sustain his enthusiasm. This he did with the following quotation: "At every crossway on the road that leads to the future, each progressive spirit is opposed by a thousand men appointed to guard the past."

References

1. Kempner, W. Treatment of kidney disease and hypertensive vascular disease with rice diet. *N. Carolina Med. J.* 5: 125, 1944.
2. Kempner, W. Treatment of hypertensive vascular disease with rice diet. *Am. J. Med.* 4: 545, 1948.
3. Chapman, C. B., and T. B. Gibbons. The diet and hypertension. *Medicine* 29: 29, 1949.
4. 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.
5. Dole, V. P., L. K. Dahl, G. C. Cotzias, D. D. Dziewiatkowski and C. Harris. Dietary treatment of hypertension. II. Sodium depletion as related to the therapeutic effect. *J. Clin. Invest.* 30: 584, 1951.
6. Dole, V. P., L. K. Dahl, I. L. Schwartz, G. C. Cotzias, J. H. Thaysen and C. Harris. Dietary treatment of hypertension. III. The effect of protein on appetite and weight. *J. Clin. Invest.* 32: 185, 1953.
7. Watkin, D. M., H. F. Froeb, F. T. Hatch and A. B. Gutman. Effects of diet in essential hypertension. I. Baseline study: effects in eighty six cases of prolonged hospitalization on regular hospital diet. II. Results with unmodified Kempner Rice Diet in fifty hospitalized patients. *Am. J. Med.* 9: 428, 1950.
8. Hatch, F. T., A. R. Wertheim, G. H. Eurman, D. M. Watkin, H. F. Froeb and H. A. Epstein. Effects of diet in essential hypertension. III. Alterations in sodium chloride, protein and fat intake. *Am. J. Med.* 17: 499, 1954.
9. Ambard, L., and E. Beaujard. Causes de l'hypertension arterielle. *Arch. Gén. Méd.* 1: 520, 1904.
10. Allen, F. M. Arterial hypertension. *J. Am. Med. Assoc.* 74: 652, 1920.
11. Allen, F. M., J. W. Mitchell and J. W. Sherrill. The treatment of combined diabetes

and nephritis. *J. Am. Med. Assoc.* 75: 444, 1920.

12. ALLEN, F. M. Treatment of arterial hypertension. *Med. Clin. N. Am.* 6: 475, 1922.
13. ALLEN, F. M., AND J. W. SHERRILL. The treatment of arterial hypertension. *J. Metab. Res.* 2: 429, 1922.
14. ALLEN, F. M. *Treatment of Kidney Diseases and High Blood Pressure.* Morristown, New Jersey: The Physiatric Institute, 1925.
15. 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.
16. DAHL, L. K., AND R. A. LOVE. Etiological role of sodium chloride intake in essential hypertension in humans. *J. Am. Med. Assoc.* 164: 397, 1957.
17. DAHL, L. K. Salt intake and salt need. *New Engl. J. Med.* 258: 1152, 1205, 1958.
18. 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.
19. DAHL, L. K. Salt in processed baby foods. *Am. J. Clin. Nutr.* 21: 787, 1968.
20. FOMON, S. J. *Infant Nutrition.* Philadelphia: Saunders, 1967, p. 141.
21. WOODCOCK, G. A northern journal. In: *The Beaver* (Summer). Winnipeg, Canada: Hudson's Bay Company, 1969, p. 13.
22. BLAIR-WEST, J. R., E. BOTT, J. COGHLAN, D. A. DENTON, J. R. GODING, M. WINTOUR AND R. D. WRIGHT. The regulation of electrolyte metabolism of ruminant animals in arid zones. In: *Enviromental Physiology and Psychology in Arid Conditions.* Paris: United Nations Educ., Sci., Cultural Organ. 1963, p. 289.
23. BLAIR-WEST, J. R., J. P. COGHLAN, D. A. DENTON, J. F. NELSON, E. ORCHARD, B. A. SCOGGINS, R. D. WRIGHT, K. MEYERS AND C. L. JUNQUEIRA. Physiological, morphological and behavioral adaptation to a sodium deficient environment by wild native Australian and introduced species of animals. *Nature* 217: 922, 1968.
24. DAHL, L. K. Salt, fat and hypertension: the Japanese experience. *Nutr. Rev.* 18: 97, 1960.
25. DAHL, L. K., L. SILVER AND R. W. CHRISTIE. The role of salt in the fall of blood pressure accompanying reduction in obesity. *New Engl. J. Med.* 258: 1186, 1958.
26. BERCHOFF, R. S., AND A. S. GERACI. The influence of sodium chloride on blood pressure. *Illinois Med. J.* 56: 395, 1929.
27. THOMPSON, W. H., AND I. MCQUARRIE. Effects of various salts on carbohydrate metabolism and blood pressure in diabetic children. *Proc. Soc. Exptl. Biol. Med.* 31: 907, 1933–4.
28. MCDONOUGH, J., AND C. M. WILHELMJ. The effect of excess salt intake on human blood pressure. *Am. J. Digest. Diseases* 21: 180, 1954.
29. GRANT, H., AND F. REISCHSMAN. The effects of the ingestion of large amounts of sodium chloride on the arterial and venous pressures of normal subjects. *Am. Heart J.* 32: 704, 1946.
30. DAHL, L. K. Possible role of salt intake in the development of essential hypertension. In: *Essential Hypertension, an International Symposium,* edited by P. Cottier and K. D. Bock. Berlin: Springer-Verlag, Heidelberger, 1960, p. 53.
31. HUMPHRIES, S. V. A study of hypertension in the Bahamas. *S. African Med. J.* 31: 694, 1957.
32. ISAACSON, L. C., M. MODLIN AND W. P. V. JACKSON. Sodium intake and hypertension. *Lancet* 1: 946, 1963.
33. FUKUDA, T. Investigation on hypertension in farm villages in Akita Prefecture. *J. Chiba Med. Soc.* (Japanese) 29: 490, 1954.
34. SASAKI, N. On the geographic distribution of salt concentration in "miso" and its relation to the death rate from apoplexy in Japan. *Med. Biol.* (Japanese) 38: 187, 1956.
35. SUZUKI, S., T. TEZUKA, S. OSHIMA, T. KUGA, K. YAMAKAWA AND S. NAGAMINE. Nutritional survey on the essential hypertension in Tohoku (east-northern) area. *Japan. J. Nutr.* 17: 159, 1959.
36. SASAKI, N., J. TAKEDA, S. FUKUSHI, T. MITSUHASHI, H. UKAI, E. SAITO, A. ONO AND T. KAWAGISHI. Some problems of salt intake, especially its relation to the apoplexy or hypertension in Japan. *Medicine* (Japanese) 15: 101, 1958.
37. SASAKI, N. High blood pressure and the salt intake of the Japanese. *Jap. Heart J.* 3: 313, 1962.
38. SASAKI, N. The relationship of salt intake to hypertension in the Japanese. *Geriatrics* 19: 735, 1964.
39. PRIOR, I. A. M., J. G. EVANS, H. P. B. HARVEY, F. DAVIDSON AND M. LINDSEY. Sodium intake and blood pressure in two Polynesian populations. *N. Engl. J. Med.* 279: 515, 1968.
40. PHEAR, D. N. Salt intake and hypertension. *Brit. Med. J.* 2: 1453, 1958.
41. MIALL, W. E. Follow-up study of arterial pressure in the population of a Welsh mining valley. *Brit. Med. J.* 2: 1204, 1959.
42. PAUL, O. Discussion of paper by G. R. MENEELY. The experimental epidemiology of sodium chloride toxicity in the rat. In: *The Epidemiology of Hypertension (Proc. Intern. Symp.),* edited by J. Stamler, R. Stamler and T. N. Pullman. Chicago: Grune & Stratton, 1967, p. 248.
43. WOLF, G., L. K. DAHL AND N. E. MILLER. Voluntary sodium chloride intake of two strains of rats with opposite genetic susceptibility to experimental hypertension. *Proc. Soc. Exptl. Biol. Med.* 120: 301, 1965.
44. FALLIS, N., L. LASAGNA AND L. TÉTRAULT. Gustatory thresholds in patients with hypertension. *Nature* 196: 74, 1962.
45. DAWBER, T. R., W. B. KANNEL, A. KAGAN, R. K. DONABEDIAN, P. M. MCNAMARA AND G.

PEARSON. Environmental factors in hypertension. In: *The Epidemiology of Hypertension (Proc. Intern. Symp.)*, edited by J. Stamler, R. Stamler, and T. N. Pullman. Chicago: Grune & Stratton, 1967, p. 255.

46. ACHESON, R. M. Epidemiology of serum uric acid and gout: an example of the complexities of multifactorial causation. *Proc. Roy. Soc. Med.* 63: 193, 1970.
47. 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.
48. 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.
49. DAHL, L. K. Effects of chronic excess salt feeding; elevation of plasma cholesterol in rats and dogs. *J. Exptl. Med.* 112: 635, 1960.
50. DAHL, L. K., AND M. HEINE. Effects of chronic excess salt feeding. Enhanced hypertensogenic effect of sea salt over sodium chloride. *J. Exptl. Med.* 113: 1067, 1961.
51. DAHL, L. K. Effects of chronic excess salt feeding. Induction of self-sustaining hypertension in rats. *J. Exptl. Med.* 114: 231, 1961.
52. DAHL, L. K. Excessive salt intake and hypertension: a dietary and genetic interplay. *Brookhaven Lecture Ser.* No. 12, 1962.
53. 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.
54. DAHL, L. K., M. HEINE AND L. TASSINARI. Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. *Nature* 194: 480, 1962.
55. DAHL, L. K. Genetic influences on experimental hypertension in the rat. In: *First Meeting of International Club on Arterial Hypertension, Paris, July 1965*, edited by P. Milliez and Tcherdakoff. Paris: Gaignault, 1966. L'Expansion Sci. Franc. 1: 268, 1966.
56. KNUDSEN, K. D., L. K. DAHL, K. THOMPSO[illegible] J. IWAI, M. HEINE AND G. LEITL. Effects [illegible] chronic excess salt ingestion: inheritance [illegible] hypertension in the rat. *J. Exptl. Med.* 1[illegible] 976, 1970.
57. DAHL, L. K., M. HEINE AND L. TAS[illegible]RI. E[illegible] fects of chronic excess salt ingestio[illegible] role [illegible] genetic factors in both DOCA-salt [illegible] ren[illegible] hypertension. *J. Exptl. Med.* 118: 605, 1963.
58. DAHL, L. K., M. HEINE AND L. TASS[illegible]RI. E[illegible] fects of chronic excess salt ingestio[illegible] further demonstration that genetic factors influence the development of hypertension: evidence from experimental hypertension due to cor[illegible]one and to adrenal regeneration. *J. Exptl. Med.* 12[illegible] 533, 1965.
59. IWAI, J., K. D. KNUDSEN, L. K. DAHL, M. HEINE AND G. LEITL. Genetic influence on the development of renal hypertension in para[illegible] biotic rats. Evidence for a humoral factor. [illegible] *Exptl. Med.* 129: 507, 1969.
60. DAHL, L. K. Salt and blood pressure. *Lancet* [illegible] 622, 1969.
61. FALCONER, D. S. The inheritance of liability to certain diseases, estimated from the incidence among relatives. *Ann. Hum. Genet. (Londo[illegible]* 29: 51, 1965.
62. CRUZ-COKE, R. A genetic study of blood pressure in chronic pyelonephritis. *Acta Gen[illegible] Statist. Med.* 11: 58, 1961.
63. PLATT, R. Essential hypertension, incidence course, and heredity. *Ann. Internal Med.* 55: [illegible] 1961.
64. HAMILTON, M., G. W. PICKERING, J. A. ROBERTS AND G. S. C. LOWRY. Arterial pressures [illegible] relatives of patients with secondary and malignant hypertension. *Clin. Sci.* 24: 91, 1963.
65. BENGTSSON, U., A. M. HÖGDAHL AND B. HOO[illegible] Chronic non-obstructive pyelonephritis and hypertension: a long-term study. *Quart. J. Med.* 37: 361, 1968.

EFFECTS OF CHRONIC EXCESS SALT INGESTION

Genetic Influence on the Development of Salt Hypertension in Parabiotic Rats: Evidence for a Humoral Factor*

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By selective inbreeding, two strains of rats were evolved with opposite genetic propensities for hypertension from a high NaCl (salt) diet (1, 2). Subsequently it was found that the two strains also showed opposite innate predispositions for developing experimental hypertension from DOCA-salt, unilateral renal artery compression without salt, cortisone, or adrenal regeneration (3, 4). These observations suggest that the basic mechanism of these several "varieties" of hypertension may be the same although the etiological agents differ.

In the present study the technique of parabiosis has been used to explore the hypothesis that a humoral agent was instrumental in the pathogenesis of "salt" hypertension and, by inference, in other "varieties" of experimental hypertension as well. The chronicity of the parabiotic union, with its exchange of extracellular fluids between two animals, could allow subtle humoral influences to modify the blood pressure in a manner that might be inapparent from acute studies. This seemed to be the case, for when two rats, one from each strain, were united in parabiosis, the animal from the strain that normally fails to develop salt hypertensoin rapidly developed chronic hypertension provided that a high NaCl diet was consumed by the pair. This response has been interpreted as being compatible with the transfer of a humoral influence from one parabiotic animal to the other under these conditions. If this is true, such an agent could play a role in evoking the fulminating hypertension characteristically observed in nonparabiotic S animals consuming the same high NaCl diet.

Materials and Methods

The rats used in these studies were derived originally from a Sprague-Dawley strain (1, 2). All animals came from the ninth, or subsequent, generations of selectively inbred animals. The

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strain with the genetic propensity for developing hypertension is called the Sensitive (or S) strain, the other Resistant (or R) strain. Details on the care, feeding, and technique of measuring blood pressure were presented earlier (5–7) and only those pertinent to the current work are included here. All rats had free access to tap water (<1 meq Na/liter) and chows which contained either 0.38% NaCl ("low NaCl") or 8% NaCl ("high NaCl"), but were otherwise identical. Blood pressures (B.P.) and weights were measured under standard conditions at intervals ranging from twice a week to once a month, depending on the observed rate of progression of the hypertension. In instances of hypertension known to be evolving rapidly, B.P. was measured 1–2 times per week but statistical calculations were based on the last measurement made when the animal appeared still in good health; in our experience the most sensitive index of ill health in these rats has been weight loss and we have regarded a weight loss of 10 g as such evidence. Accordingly, we have not included blood pressures associated with a weight change of this magnitude. If the intervals between readings were several weeks or more, we may have missed the peak of the B.P. elevation in S rats on high NaCl since B.P. usually declines during their terminal illness: in such circumstances the reported values are likely to have underestimated the degree of hypertension. We have considered a B.P. of 140 mm Hg or more as indicative of "hypertension" (5–7). Statistical significance for difference between the average blood pressures of groups was assessed by the technique of analysis of variance[1] and P values of <0.05 were accepted as significant. Parabiosis was performed under Pentothal anesthesia according to the technique of Bunster and Meyer (8) on 3-wk-old weanling animals and zero time was considered to begin with this operation. Only female weanling rats from the Sensitive (S) and Resistant (R) strains were used. Females were used arbitrarily because of their availability when this study began, but numerous pairs of males in parabiosis have now been observed to respond like the females reported here. During the 7 days following surgery, all animals were maintained on the low NaCl (0.38%) chow, after which some were changed permanently to the high NaCl (8%) chow. The animals united in parabiosis were identified according to the following combinations: R+R, S+S, and R+S. Some groups were further identified by indicating their NaCl intake, e.g., R+S (8%), or S (0.38%). Rats united in parabiosis were called *parabionts*. In pairs from the same strain, the members were identified as *left*, or as *right*, viewed from above with heads away from observer. A total of 80 pairs of animals in the several combinations shown below were united in parabiosis but death during the first 3 wk after operation left 53 pairs for analysis in this study. Animals not in parabiosis have been called *single*. Four groups of 10 single animals were studied as controls; one animal in the R (8%) group died shortly after the experiment began and has not been included in the final analysis. There were 10 groups of animals divided as follows:

Group No.		*No. in Group*
(1)	Single R on 0.38% NaCl chow	10
(2)	" " " 8% " "	9
(3)	" S " 0.38% " "	10
(4)	" " " 8% " "	10
(5)	R + R (i.e., in parabiosis) on 0.38% NaCl chow	8 pairs
(6)	" " " " " 8% " "	9 "
(7)	S + S " " " " 0.38% " "	9 "
(8)	" " " " " 8% " "	9 "
(9)	R + S " " " " 0.38% " "	9 "
(10)	" " " " " 8% " "	9 "

[1] We wish to thank Keith Thompson for his continued assistance with the statistical analysis in this study.

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OBSERVATIONS

Single Animals (Table I)

The responses were like those we have observed repeatedly, and reported earlier (9) for animals from these two strains on chronic low and high NaCl

TABLE I

*Single Animals**

Strain	R		S		
Diet (% NaCl)	0.38	8	0.38	8	
					Died, *Month*‡
Systolic B.P. of individual animals (mm Hg)	118	104	114	182	2
	120	122	100	202	2
	116	100	122	242	4
	100	146	128	186	3
	118	120	128	194	2
	118	114	120	190	—
	102	120	172	212	2
	98	120	162	164	1
	132	132	150	189	—
	111		149	185	2
No. rats in group	10	9	10	10	
Mean B.P. of group (±S.E.)	113.3 (3.35)	119.8 (4.59)	134.5 (7.21)	194.6 (6.60)	
Median B.P.	117	120	128	190	

Analysis of variance of mean B.P.: S (8%) >** all others; S (0.38%) >** R (0.38%).

* Blood pressures of single female rats at end of study (6 months) or last B.P. before death (see Materials and Methods).

‡ Animal died during month after weaning.

** $P \leq 0.01$, here and in Tables II and III.

diets. Among rats from the R strain, 8% NaCl chow was almost without influence on either B.P. or mortality: at the end of the study all 19 rats were in good health and the mean blood pressures of 113.3 and 119.8 mm Hg for the groups on 0.38 and 8% NaCl, respectively, were not significantly different ($P > 0.05$). However, one R animal on 8% NaCl had a mild elevation in blood pressure on the final measurement, 146 mm Hg. On comparable NaCl intakes, the S rats had significantly higher average B.P. than the R animals ($P < 0.01$). In animals from the S strain on low NaCl chow, 4 of the 10 developed mild hypertension (149–172 mm Hg) but all remained in good health at the end of

the experiment. On high NaCl by contrast, all animals from the S strain rapidly developed severe hypertension and 8 of the 10 were dead before the end of the 4th month. Table I shows that one of these S animals died during the first month with a measured pressure of only 164 mm Hg. We have recorded such blood pressures before in animals with fulminating hypertension that was not recognized in time to institute frequent B.P. measurements: among many similar

TABLE II

*Parabionts from Same Strain**

Strain	R + R				S + S				
Diet (% NaCl)	0.38		8		0.38		8		
	Left	Right	Left	Right	Left	Right	Left	Right	Died‡
									(*months*)
Systolic B.P. of individual parabionts (mm Hg)	122	110	108	122	124	150	198	220	3
	120	110	116	106	140	146	202	210	3
	118	98	112	120	128	144	221	218	3
	124	130§	100	114	124	140	172	211	2
	104	128	122	123	120	132	172	210	2
	120	112	122	106	124	122	184	212	2
	122	124	114	106	150	120	184	170	2
	118	111	102	100	124	120	198	193	3
			110	120	114	120	224	162	3
No. rats in group	8	8	9	9	9	9	9	9	
Mean B.P. of group (±S.E.)	118.5 (2.20)	115.4 (3.87)	111.8 (2.59)	113.0 (2.88)	127.6 (3.63)	132.7 (4.18)	195.0 (6.33	200.7 (7.05)	
Median B.P.	120	112	112	114	124	132	198	210	

Analysis of variance of mean B.P.: In all four parabiont groups, left = right ($P > 0.05$) and S (8%) >** all others.

* Blood pressures of female rats from same strain united in parabiosis.

Results at end of study (6 months) or last B.P. before death (see Materials and Methods).

‡ Pair died during month indicated.

§ Animal died during 3rd month, B.P. of both parabionts at 2½ months.

animals in which B.P. was measured every few days, marked elevations invariably have been found before death.

Animals in Parabiosis

(*1*) *R + R* (*Table II*)

Parabionts in which both members were from the R strain resembled single animals from this same strain in that dietary NaCl appeared to be without significant influence on blood pressure: among these 17 pairs, final pressures among individuals ranged between 98 and 130 mm Hg and blood pressures averaged about 115 mm Hg for the R + R parabiont groups, a figure almost

identical with that for single R animals (Table I). One parabiont on low NaCl died during the 3rd month with normal B.P., 130 mm Hg. (In this instance only, the living member of this pair was separated and was followed to the end of the experiment at which time it was in good health with a B.P. of 114 mm Hg). All other parabionts in these groups appeared healthy after 6 months.

TABLE III
*Parabionts from Opposite Strains**

Strain	R + S			
Diet (% NaCl)	0.38		8	
	R	S	R	S
Systolic B.P. of individual parabionts (mm Hg)	108	122	154	128
	112	118	169	132
	134	130	204	212‡
	110	112	174	170
	124	122	172	184
	102	130	144	157
	122	116	174	128
	110	128	192	150
	110	134	164	204
No. rats in group	9	9	9	9
Mean B.P. of group (±S.E.)	114.7 (3.32)	123.6 (2.47)	171.8 (6.02)	162.8 (10.7)
Median B.P.	110	122	172	157

Analysis of variance of mean B.P.: In both paraboint groups, R = S, but R (8%) or S (8%) >** R (0.38%) or S (0.38%).

* Blood pressures of female rats from both strains united in parabiosis. Results at end of study (6 months).

‡ Died during final week of experiment.

(2) *S + S (Table II)*

Again the results were like those obtained with single animals from this strain on the same NaCl intakes: on low NaCl chow, the average pressures of 127.6 and 132.7 mm Hg in the left and right groups of parabionts were close to the average of 134.5 mm Hg for the single S group: 6 of the 18 *individual* parabionts —there was no pattern by pairs— developed mild hypertension ranging from 140 to 150 mm Hg. All animals seemed in good health when the experiment was terminated. On 8% NaCl chow all of the S parabionts developed hypertension rapidly and none was alive by the end of the 3rd month. The average pressures

of 195 and 200.7 mm Hg for the left and right groups of parabionts were nearly identical with the average of 194.6 mm Hg for the "single S" group on high NaCl; the individual blood pressures of the members in S + S parabiosis rose together, but the values were rarely identical.

(3) *R + S (Table III and Fig. 1)*

On Low NaCl.—

All the individuals in such pairs remained normotensive and in good health when on low NaCl. The average B.P. of the R parabionts was 114.7 mm Hg, a figure very close to that of the other R groups described thus far in this study. The average B.P. of the S parabionts was 123.6 mm Hg, not significantly ($P > 0.05$) different from the other S groups studied here on low NaCl chow. However, the blood pressure response may have differed slightly from that of similarly fed single animals in that none of these S rats developed even mild hypertension (>140 mm Hg). This may be related to the observations now to be described with R + S parabionts on 8% NaCl.

On High NaCl.—

Since the response of the R + S parabionts on high NaCl differed in three significant respects from all previous experience with these animals, it is reported in some detail.

(*A*) *Development of hypertension in R animals* (Table III, Fig. 1): The R animals rapidly developed a rise in blood pressure which generally appeared sooner and reached higher levels than in the S parabionts with which they were united. By the end of the 4th wk, for instance, all but one of the nine R animals had elevated B.P. ranging from 144 to 192 mm Hg (the ninth had 112 mm Hg); in contrast, at this time among their nine S partners, only two had B.P. as high as 142 mm Hg. At the end of the 8th wk, when only one of the R rats had a pressure *below* 150 mm Hg (range 142–212, average 174.3 mm Hg) only two of the S rats were *above* 150 mm Hg (range 82–162, average 136.8 mm Hg). In the period from 10 to 13 wk the average B.P. of the R animals reached a peak of 178 mm Hg, a mean level that their S partners never equalled. Indeed, during the whole period from the 2nd through the 12th wk, the average B.P. of the R parabionts was significantly higher ($P < 0.05$–0.01) than that of their S partners at comparable times. After the 12th wk, the average B.P. among R animals appeared to decline slightly, reaching a low of 154.9 mm Hg about the 16th wk, after which it rose to 171.9 mm Hg in the final week. This is shown in Fig. 1. Caution must be used in interpreting the precise character of the curve for the R animals after the 12th wk, however, since except for the mean B.P. at 16 wk ($P < 0.05$) none of the other means differed significantly from that at 12 wk. In summary, parabiosis of S and R rats on 8% NaCl commonly led to the development of chronic elevations in blood pressure in the R parabionts such as we have

almost never observed in single animals from this strain even after ingesting 8% NaCl for much longer periods.

(*B*) *Amelioration of hypertension in S animals* (Table III, Fig. 1): The rapidly evolving hypertension so characteristic of single S or S + S parabiont animals,

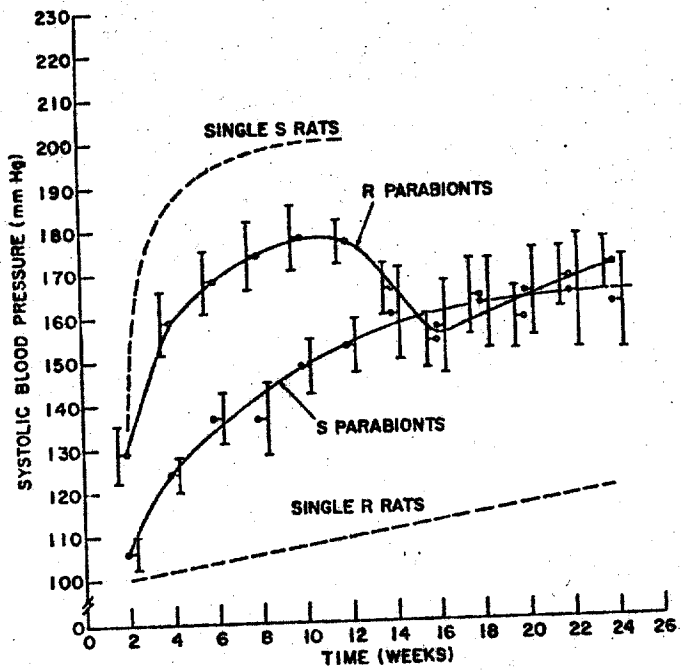

FIG. 1. The symbols with open and closed circles represent mean ±1 standard deviation of B.P. obtained from group 10 (R + S parabionts on 8% NaCl) measured every 2nd wk during the study, and demonstrate the anomalous development of B.P. in this group. The broken lines represent idealized average blood pressures of large numbers of single S and R rats on 8% NaCl chow from both published and unpublished studies in our laboratory. The change in response to NaCl effected by parabiosis between rats, one from each strain, is apparent. From the 2nd through the 12th wk, the average B.P. of the R parabionts was higher ($P < 0.05$) than that of their S partners at corresponding times. After the 12th wk, average B.P. in the two groups was not significantly different ($P > 0.05$). Furthermore, only the average of the R parabionts at 16 wk was significantly different from the average of this R group at 12 wk; for this reason, the curve shown for the R parabionts should be accepted with caution after the 12th wk.

on 8% NaCl chow (Tables I and II) was clearly modified by parabiosis with R animals: both the *rate* of development and the average *level* of hypertension were decreased. The general course of the development of hypertension is shown in Fig. 1. This graph indicates that during the initial 2 months when the R

TABLE IV

*Summary of Final Blood Pressures by Group and Strain**

Group	No. Rats in Group	Mean Systolic B.P. of Group (± S.E.) (mmHg)
	R animals only	
R, Single, 0.38% NaCl	10	113.3 (3.35)
R, " 8% "	9	119.8 (4.59)
R + R 0.38% "		
Left	8	118.5 (2.20)
Right	8	115.4 (3.87)
R + R 8% "		
Left	9	111.8 (2.59)
Right	9	113.0 (2.88)
R [+ S]‡ 0.38% "	9	114.7 (3.32)
R [+ S]‡ 8% "	9	171.8 (6.02)
	S animals only	
S, Single 0.38% NaCl	10	134.5 (7.21)
S, " 8% "	10	194.6 (6.60)
S + S 0.38% "		
Left	9	127.6 (3.63)
Right	9	132.7 (4.18)
S + S 8% "		
Left	9	195.0 (6.33)
Right	9	200.7 (7.05)
[R +]S§ 0.38% "	9	123.6 (2.47)
[R +]S§ 8% "	9	162.8 (10.7)

Statistical Significance of Difference in Mean B.P. among Groups‖

Among all parabionts: right = left.

Among R animals only: All R groups are equal *except* in R + S (8%) where R >** all other R groups.

Among S animals only: All S on 0.38% NaCl are equal; S (8%) = S + S (8%) >** S in R + S (8%) >** all S on 0.38% NaCl.

Among both R and S animals:

S, Single (0.38%)

** > { R, Single (0.38%); R + R (8%) }

¶ > { R + R (0.38%); R + S (0.38%) }

All S on 8% >** all R, *except* in R + S (8%) where R = S; R + S (8%) >** R + S (0.38%)

The most important items to be derived from this summary are (*a*) parabiosis between rats from the same strain (i.e., R *or* S) does not affect B.P. differently from comparable single animals on the same regimen; (*b*) parabiosis between rats from both strains (i.e., R + S) affects B.P. significantly if the animals are on 8% NaCl: those from the Resistant strain have significantly higher ($p < 0.01$) and those from the Sensitive strain significantly lower ($P < 0.01$) B.P. than comparable single rats on 8% NaCl.

* See Tables I–III for primary data.

‖ Definitions: equal or =, $P > 0.05$; **, $P \leq 0.01$; ¶, $P \leq 0.05$.

‡ See data for S animals under "S animals only."

§ See data for R animals under "R animals only."

animals rapidly developed hypertension, their corresponding S parabionts did so only slowly; not until after the 12th wk did the mean blood pressures of the two strains attain roughly equivalent levels ($P < 0.05$) when, between the 14th and 24th wk, S parabionts seemed to reach a plateau at about 160–165 mm Hg. At no time did the average pressure of the group of S parabionts exceed that of their partners significantly although in one pair the S parabiont ended up significantly higher than its R mate (204 vs. 164 mm Hg, respectively). Whereas the final pressures of both the S and S + S groups on 8% NaCl had been found to average about 195–200 mm Hg (Tables I and II), that of the S parabionts in the R + S group was only 162.8 mm Hg ($P < 0.01$). One of the most striking observations was that after 6 months on 8% NaCl three S parabionts remained normotensive with pressures of 128, 132, and 128 mm Hg, respectively: we had not previously observed S rats from this generation of inbred animals that failed to develop significant hypertension after 6 months on 8% NaCl chow.

In summary, parabiotic union with an R animal effected a significant decrease in the average level of hypertension characteristically observed in S rats on 8% NaCl.

(C) *Decreased mortality of S rats on 8% NaCl:* Only one of the nine S rats in the R + S group on 8% NaCl died and this was not until the last week of the study. This is in sharp contrast to the high mortality observed among animals in the single S (Table I) and S + S (Table II) groups, on 8% NaCl: in the former, only 2 out of 10 rats survived 6 months and in the latter, none survived 3 months. In another recent unpublished series, among *40* single S animals maintained after weaning on 8% NaCl, only one sickly animal was alive at the end of the 6th month. Indeed, in our studies involving several thousand weanling S animals on 8% NaCl during the last 3 years, healthy survivors were so uncommon after 3 months that experiments ordinarily were not continued longer except to obtain absolute mortality figures. Therefore, we have considered the increased survival of these S rats in the current studies as markedly divergent from our past experience.

In Table IV, we have summarized the blood pressure responses of the several groups of animals and the statistical comparisons among them.

DISCUSSION

In this study, the influence of parabiosis on experimental hypertension has been explored and, in line with our earlier experience, the genetic substrate has been found to strongly modify the influence of NaCl on the development of hypertension. By appropriate attention to salt intake and to genetic origins of the animals, we could produce pairs both members of which had fulminating hypertension or pairs in which both members had normal blood pressure. These results would have been predicted from our previous experiments; i.e., rats from the Sensitive (S) strain prone to experimental hypertension would develop

severe hypertension on NaCl whereas rats from the Resistant (R) would not. However, when rats, one from each strain, were united in parabiosis and maintained on a high NaCl intake, the genetic influence seemed to be anomalous: hypertension then appeared rapidly in the parabionts from the Resistant strain while their mates from the Sensitive strain had significantly less hypertension than expected.

Hypertension in these R animals was not dependent upon parabiosis *per se* since it was not observed among other groups of R parabionts studied. Neither was this hypertension dependent solely on parabiosis with a member of the S strain, since it was not present among members of the control R + S group maintained on low NaCl chow. Furthermore, hypertension in the R parabiont was not dependent upon the presence of *overt* hypertension in the S partner: elevated pressure usually developed in the R parabiont before becoming manifest in the S partner and in three of these S parabionts, hypertension never appeared. Finally, hypertension in R parabionts was not solely related to dietary NaCl since R + R parabionts on a high NaCl diet manifested no hypertension. Only when an R animal was in parabiosis with a member from the S strain *and* the pair was on a high NaCl intake did hypertension develop in the R partner.

Our observations seem related to those reported by Hall and Hall in 1951 (10) in which DOCA-NaCl was used to induce hypertension in parabionts. In that study, one member, only, of each pair received DOCA implants while both were on added NaCl. Instead of the expected hypertensive disease in DOCA-treated animals, they found that in 15 out of 20 pairs the *untreated* partner suffered while the animals with DOCA implants seemed to be unaffected. Only five pairs showed the expected response: hypertensive disease in the DOCA-treated animal, no disease in the partner. And at autopsy, the parabiosis junction in these five pairs was found to be poor, suggesting the possibility that vascular exchange was less than among the remaining 15 pairs. Subsequently, Hall and Hall (11) reported that without the use of DOCA in about 40% of pairs "spontaneous" hypertension developed in one animal but never in the other. From later studies (12, 13) they concluded that an "unusually" high NaCl intake was not, but that adrenal function was necessary for the development of this hypertension. Since the experimental design of the studies by our two laboratories was different, the results are not wholly comparable; yet the similarities are considerable. In particular, there is a parallelism between their findings with DOCA-NaCl using unselected rats and ours using NaCl and two genetically different strains of rats. In their experiments one has to assume that the hypertensive process started in the animal receiving DOCA. The similarity between the experimental hypertension induced by DOCA-NaCl or NaCl alone (3) allows the inference that in our experiments the hypertension was initiated in the S animals by an interaction with NaCl. Although possible, it is improbable that R animals, genetically resistant to the induction of hypertension by either NaCl or DOCA-NaCl (1–3, 9) would undergo a fundamental reversal by parabiosis: if so, hypertension might have been expected to develop in the R + R

pairs or in the R + S parabionts on low NaCl. This did not occur. It seems more logical to assume that the S animal with the genetic predilection for hypertension was basically responsible for *initiating* the hypertension that developed in the R partners when both were on high NaCl.

It seems clear that some humoral influence can pass the parabiosis junction, thereby modifying the blood pressure of one or both animals. However, except for suggesting that both a high NaCl diet and a genetic propensity for hypertension are required, this humoral influence cannot presently be characterized as to nature, site of origin, or mode of action. Furthermore, if the S rat is assumed to initiate the hypertension that develops in its R partner, it also requires an explanation as to why this should moderate, or even prevent, hypertension in the S rat.

Estimates of the exchange rate between parabiont rats suggest that about 0.5 to 1% of blood volume of each rat is exchanged per minute (14, 15). In unpublished studies from our laboratory, the exchange rate between parabionts similar to those used in these studies was measured with ^{24}Na and was found to support these estimates, namely, 0.1 to 1% of extracellular fluid per minute. The short biological half-lives of the common steriods and vasopressor agents combined with this limited exchange make it difficult to postulate that such agents in concentrations sufficient to induce hypertension would be transferred from the S animals to their R partners. In earlier studies we found that animals from the Resistant strain were less responsive to DOCA (3), cortisone (4), norepinephrine, angiotensin, and possibly vasopressin (16), than were comparable animals from the Sensitive strain. Therefore, it is unlikely that lesser quantities of steroids and vasopressors could be more effective in the R rats than in their S partners. Finally, no vasopressor activity was demonstrable in the blood from rats with either acute or chronic salt hypertension (17). However, for this latter kind of bioassay, pharmacological quantities of pressor agent must exist in the blood and, therefore, one might fail to detect low concentrations that could be effective acting over periods of weeks.

Floyer and Richardson (15) and Floyer (18, as well as by personal communication) have suggested that a higher mean capillary pressure may exist in hypertension. This might be the genetic defect in our S strain of rats, present even before manifest hypertension. Following establishment of joint circulation between parabionts from the two strains, the higher capillary pressure in the S animal could tend to increase the blood volume of the R animal at the expense of the S rat. Venous return and cardiac output would be expected to increase at least transiently, leading to elevation in blood pressure until appropriate homeostatic mechanisms had come into play resulting in return of cardiac output and venous return to normal levels. Potentially, a new "setting" of baroceptors could maintain the pressure at a slightly elevated level and, if the stimulus leading to the initial insult were continuous or repetitive, a chronic and increasing elevation might follow. On the basis of experimental work, Ledingham has proposed a similar mechanism for the inception of hypertension (19, 20).

At this juncture, it is unwarranted to pursue these speculations further. The evidence indicates the existence of a transmittable humoral influence but does not identify it. Studies now under way may aid in characterizing it. While these studies have dealt only with NaCl-induced experimental hypertension, we

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suspect this phenomenon has more general implications for the pathogenesis of hypertension.

SUMMARY AND CONCLUSIONS

Parabiosis has been found to modify the expected blood pressure response of rats from two strains with opposite genetic propensities for experimental hypertension. When a member from one strain was united in parabiosis with a member from the other *and* both were maintained on high NaCl diet, the rat from the strain ordinarily resistant to it rapidly developed hypertension, in contrast to appropriate controls from this strain. The development of hypertension in this resistant animal preceded that in its mate from the strain highly sensitive to hypertension. In the latter, both the level of hypertension and mortality were significantly less than in its control. It seems likely that the hypertension observed is the resistant parabiont was *initiated* in its partner from the sensitive strain. This modification in blood pressures was not observed in the absence of a high NaCl diet.

Parabiosis between animals from the same strain did not alter their response. Thus, as in earlier experiences (1–4) the interaction of a nongenetic factor (NaCl) with the appropriate genetic substrate appeared to be necessary for the development of hypertension.

The findings are interpreted as evidence that a transmittable humoral influence plays an important role in the pathogenesis of rat hypertension. The presence of this agent is genetically determined but, under the conditions of these experiments, it took the added stimulus of dietary NaCl to demonstrate its existence.

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BIBLIOGRAPHY

1. Dahl, L. K., M. Heine, and L. Tassinari 1962. Effects of chronic excess salt ingestion: Evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J. Exptl. Med.* **115**:1173.
2. Dahl, L. K., M. Heine, and L. Tassinari. 1962. Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. Nature, **194**:480.
3. Dahl, L. K., M. Heine, and L. Tassinari, 1963. Effects of chronic excess salt ingestion: Role of genetic factors in both DOCA-salt and renal hypertension. *J. Exptl. Med.* **118**: 605.
4. Dahl, L. K., M. Heine, and L. Tassinari, 1965. Effects of chronic excess salt ingestion: Further demonstration that genetic factors influence the development of hyper-

tension. Evidence from experimental hypertension due to cortisone and to adrenal regeneration. *J. Exptl. Med.* **122:** 533.

5. Dahl, L. K. 1960. Effects of chronic excess salt feeding: Elevation of plasma cholesterol in rats and dogs. *J. Exptl. Med.* **112:** 635.
6. Dahl, L. K., and M. Heine, 1961. Effects of chronic excess salt feeding: Enhanced hypertensogenic effect of sea salt over sodium chloride. *J. Exptl. Med.* **113:** 1067.
7. Dahl, L. K. 1961. Effects of chronic excess salt feeding: Induction of self-sustaining hypertension in rats. *J. Exptl. Med.* **114:** 231.
8. Bunster, E., and R. K. Meyer, 1933. An improved method of parabiosis. *Anat. Record* **57:** 339.
9. Dahl, L. K., and E. Schackow, 1964. Effects of chronic excess salt ingestion: Experimental hypertension in the rat. *Can. Med. Assoc.* **90:** 155.
10. Hall, C. E., and O. Hall, 1951. Hypertensive disease produced by desoxycorticosterone acetate in parabiotic rats. *Am. Med. Assoc. Arch. Pathol.* **51:** 249.
11. Hall, C. E., and O. Hall, 1951. Production and pathogenesis of parabiotic hypertension in the rat. *Am. Med. Assoc. Arch. Pathol.* **51:** 527.
12. Hall, C. E., and O. Hall, 1951. The relationship of sodium intake to the hypertensive hyalinosis syndrome produced in the rat by parabiosis. I. Hypertensive cardiovascular disease. *Texas Repts. Biol. Med.* **9:** 714.
13. Hall, C. E., and O. Hall, 1953. Course of parabiotic hypertension following total adrenalectomy. *Am. J. Physiol.* **173:** 29.
14. Ledingham, J. M. 1951. The nature of the hypertension occurring in the nephrectomized parabiotic rat. *Clin. Sci.* **10:** 423.
15. Floyer, M. A., and P. C. Richardson, 1961. Mechanism of arterial hypertension. Role of capacity and resistance vessels. *Lancet.* 253.
16. Dahl, L. K., M. Heine, and L. Tassinari, 1964. Effects of chronic excess salt ingestion: Vascular reactivity in two strains of rats with opposite genetic susceptibility to experimental hypertension. *Circulation.* **29–30,** (Suppl. 2): 11.
17. Koletsky, S., and W. H. Pritchard, 1964. Failure to demonstrate vasopressor material in salt hypertensive rats. *Am. J. Physiol.* **207:** 152.
18. Floyer, M. A. 1966. The mechanism underlying the response of the hypertensive subject to a saline load. *In* First Meeting of International Club on Arterial Hypertension, Paris, July 1965. Paul Milliez and Philippe Tcherdakoff, editors. L'Expansion Scientifique Francaise. Vol. 1. 440.
19. Ledingham, J. M., and R. D. Cohen, 1963. The role of the heart in the pathogenesis of renal hypertension. Lancet. **2:** 979.
20. Ledingham, J. M., and R. D. Cohen, 1964. Changes in the extracellular fluid volume and cardiac output during the development of experimental renal hypertension. *Can. Med. Assoc. J.* **90:** 292.

Nature

Role of Genetic Factors in Susceptibility to Experimental Hypertension due to Chronic Excess Salt Ingestion

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THERE is considerable evidence indicating a familial trend in human essential hypertension[1]. A familial disease could be due exclusively to common environmental factors, exclusively to common genetic factors, or to an interaction of the two.

We have been investigating the effects of chronic excess salt ingestion in both man and animals and have obtained evidence which supports the thesis that dietary salt plays an aetiological part in human essential hypertension[2]. However, it was observed that some individuals remained normotensive despite the fact that they were chronically consuming large amounts of salt. In salt-feeding experiments with rats, in a given group some never developed hypertension whereas a few developed fulminating hypertension after a short time on the diet. Such wide variations in response to excess salt consumption could be ascribed either to statistical limits of a homogeneous population or to variations in sensitivity to salt based on genetic differences. If the last were true, it should be possible by selective inbreeding to

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Table 1

Strain	Original breeders		S_3		R_3	
Sex	♂	♀	♂	♀	♂	♀
No.	23	24	25	24	29	32
Systolic blood pressure (mm. mercury)						
Mean (± S.D.)	161·6 (± 18·5)	157·6 (± 24·2)	179·3 (± 20·2)	182·3 (± 18·3)	139·5 (± 15·4)	136·1 (± 14·5)
Median	164	161	182	178·5	143	139·5

Effect of L-triiodothyronine-sea salt regimen on systolic blood pressures of Sprague–Dawley rats: response of original breeding stock compared with third generation of animals inbred for sensitivity (S_3) and resistance (R_3) to hypertension from regimen.

By Student's *t* test, mean systolic blood pressure for S_3 > original breeders ($P < 0{\cdot}001$) and R_3 < original breeders ($P < 0{\cdot}001$).

separate strains that differ significantly in their liability to hypertension from excess salt consumption. This communication reports experiments on rats which confirmed this postulate. It seems probable that these observations are pertinent to man as well.

In rats, hypertension from chronic excess salt feeding usually develops slowly[2]. However, we have observed that the administration of L-triiodothyronine to intact salt-fed animals leads to marked hypertension in some animals within one month while animals treated similarly, except fed a low salt control diet, do not develop hypertension. This technique was used to isolate two strains of animals, one of which was resistant while the other was sensitive to the triiodothyronine–high salt regimen. After development of these two strains, salt-feeding without triiodothyronine led to the rapid development of hypertension in the strain known to be sensitive to the triiodothyronine–salt regimen whereas hypertension did not develop in the other resistant strain. The technique was as follows:

Twenty-three male and 24 female unselected 3-week-old Sprague–Dawley weanling rats constituted the initial breeding stock. Each received 50 μgm. triiodothyronine intraperitoneally on each of four successive days and then at about 2-day intervals ten 5 μgm. doses in the next 21 days. All were fed a chow containing 11·6 per cent sea salt containing about 7·3 per cent sodium chloride[3]; systolic blood pressures were measured in a standard fashion[3] 48 hr. after the last injection of triiodothyronine. Males and females with the highest blood pressures were selected for inbreeding as were those with the lowest pressures, and animals with intermediate pressures were discarded. The first generation of animals derived from these matings of parents with high or low blood pressures were called S_1 (sensitive) or R_1 (resistant) respectively; afterwards, no interbreeding between the S and R strains occurred.

The S_1 and R_1 animals were weaned at 3–4 weeks and immediately put on the triiodothyronine–sea salt regimen already described. Animals that responded in the S_1 strain with the highest, in the R_1 strain with the lowest, pressures were selected for further inbreeding and the remaining animals killed: usually brother–sister matings were possible. After two generations of such selective inbreeding there was evidence of a significant increase in the fraction of S_3 animals that responded with high blood pressure and a decrease in the fraction with normal pressures. Just the opposite was true for the R_3 strain. Therefore when the third generation was weaned, half the S_3 and R_3 animals of each sex were put on the triiodothyronine–sea salt regimen, while the other half were treated as described later.

Table 1 is a summary of the blood pressure responses of the S_3 and R_3 animals to the standard triiodothyronine–sea salt regimen. It is compared with the response of the original breeding stock from which the two strains were derived. The difference in response between the S_3 and the R_3 animals was striking and highly significant, statistically. On the basis of this evidence it was concluded that a difference in susceptibility to the triiodothyronine–sea salt regimen had been developed after three generations of selective inbreeding although neither the S_3 nor the R_3 strains were as yet pure.

One hundred and thirty-one siblings of animals from the S_3 and R_3 strains shown in Table 1 received either the control low sodium diet or the same high sodium, sea salt chow used before but no triiodothyronine. Table 2 is a summary of the blood-pressure responses of these animals. The differences in blood-pressure response between the salt-fed S_3 and salt-fed R_3 animals were more striking than was indicated in Table 2: after 6 months of salt feeding none of the 39 R_3 animals had hypertension whereas after only 3 months on the same programme 49 of the 60 S_3 animals were hypertensive and many of them severely so. The 3-month lag was occasioned by differences in times at which litters were delivered as well as because S_3 animals tended to have smaller litters so that a longer time was required to get sufficient numbers of S_3 rats. Therefore, an increase in sensitivity to salt would tend to be minimized in the S_3 strain with differences in response of the two strains thereby being under- rather than over-estimated. When members of the same S_3 strain were maintained on the control low salt diet, hypertension did not develop. Thus, however strong the genetic tendency to hypertension may be in this strain, the addition of a high salt diet seems necessary to unmask it. Among the R_3 animals, the presence or absence of excess dietary salt did not seem to affect the blood-pressure response.

It seems reasonable to expect that similar genetic factors operate in man. If salt plays the important ætiological part that we said it does in human hyper-

Table 2

Strain	S_3				R_3			
Sex	♂		♀		♂		♀	
Diet	11·6 per cent Sea salt chow	Control chow	11·6 per cent Sea salt chow	Control chow	11·6 per cent Sea salt chow	Control chow	11·6 per cent Sea salt chow	Control chow
No.	29	12	31	11	20	10	19	9
Systolic blood pressure (mm. mercury)								
Mean (± S.D.)	150·2 (± 19·4)	125·5 (± 6·5)	150·8 (± 28·4)	112·2 (± 7·3)	119·7 (± 14·3)	120·1 (± 6·5)	103·2 (± 22·5)	106·4 (± 10·7)
Median	150	126	146	114	121·5	119	105	104

Effect of high intake of salt without L-triiodothyronine on systolic blood pressures of Sprague–Dawley rats that were selectively inbred for three generations to develop strains sensitive (S_3) and resistant (R_3) to development of hypertension from salt. Blood pressures of S_3 strain after only 3 months whereas those of R_3 strain were after 6 months on same diet.

By Student's *t* test, the mean systolic blood pressures differed significantly ($P < 0{\cdot}001$) as follows: S_3 salt-fed ♂ > S_3 control-fed ♂; S_3 salt-fed ♀ > S_3 control-fed ♀; S_3 salt-fed ♂ > R_3 salt-fed ♂; S_3 salt-fed ♀ > R_3 salt-fed ♀. By the same test, for comparably fed strains the mean systolic pressures for males exceeded that for females ($P < 0{\cdot}01$).

tension, and given the usual genetically heterogeneous population characteristic of man, it would be illogical to expect all individuals on similar salt intakes to have similar blood pressures. On the contrary, one would expect to find individuals on high average salt intakes who did not develop hypertension as well as individuals on lower average salt intakes who did. It is unlikely that strains of humans can be found with the sensitivity to salt evinced by our rats, although studies of isolated populations might be helpful. In the absence of such genetic homogeneity, it is sufficient to re-emphasize[2b,c] that the available data relating intake of salt to hypertension in man indicate group rather than individual probability of developing hypertension.

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[1] a, Platt, R., *Quart. J. Med.*, 16, 111 (1947). b, Seyhe, P., *Heredity in Essential Hypertension and Nephrosclerosis. A Genetic-Clinical Study of 200 Propositi Suffering from Nephrosclerosis* (NYT Nordisk Forlag, Arnold Busck, Copenhagen, 1948). c, Hamilton, M., Pickering, G. W., Robert, J. A. F., and Sowry, G. S. C., *Clin. Sci.*, 13, 11 (1954).

[2] a, Dahl, L. K., and Love, R. A., *Arch. Int. Med.*, 94, 525 (1954). b, Dahl, L. K., *Nature*, 181, 989 (1958). c, Dahl, L. K., in *Essential Hypertension, An International Symposium*, edit. by Bock, K. D., and Cottier, P. T., 53 (Springer-Verlag, Heidelberg, 1960). d, Dahl, L. K., Smilay, M. G., Silver, L., and Spraragen, S. C., *Nature*, 192, 267 (1961).

[3] Dahl, L. K., and Heine, M., *J. Exp. Med.*, 113, 1067 (1961).

Hypertension and Death from Consumption of Processed Baby Foods by Rats[1] (34700)

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In 1963 we reported (1) that experimental hypertension had been induced in a few female rats fed commercial baby foods. Analyses by ourselves and the U.S. Department of Agriculture (2) indicated that such processed baby foods contained concentrations of salt (NaCl), added by the processors, greatly in excess of the meats and vegetables from which they derived. Viewed in the light of earlier correlations between chronic salt ingestion and hypertension [for review see (3)] this pilot experiment appeared relevant to man: it suggested that a high intake of salt in infancy could enhance the risk of developing hypertension. Since current feeding practices in the U.S. promote the chronic consumption of amounts of NaCl well in excess of the metabolic requirements of infants (4–7), our concern increased when we found that young rats developed permanent, even fatal, hypertension after brief exposures to excessive dietary salt (8). The increasing awareness of delayed injury from chronic exposure to "innocuous" environmental agents stimulated us to confirm and extend our pilot experiment.

Materials and Methods. The protocol of the current study was identical with the original one (1) except that more animals of both sexes were observed for longer times. Forty weanling rats from our hypertension-prone strain (9) were distributed randomly between test and control series. The 25 test rats (14 male, 11 female) were fed only baby foods consisting of processed meats and vegetables purchased on the open market. The 15 controls (7 male, 8 female) were littermates of the test animals and ate our special chow pellets containing 0.4% NaCl (9). Both groups received their respective diets and tap water *ad libitum*. Blood pressures were measured at 2- to 4-week intervals by a standard tail plethysmograph method (8). Our analyses of the baby foods confirmed the earlier findings, namely about 150–500 mg %, as sodium.

Results. The results are summarized in Fig. 1. In brief, these baby foods induced hypertension which was fatal in about half of the rats. By the end of the first month, the average blood pressure in the group eating the baby foods was significantly higher than that of the controls, 130.6 vs. 119.0 mm Hg, respectively, ($p < 0.0002$). The difference increased thereafter ($p < 0.00001$). The maximum difference in means emerged after 6 months at which time the pressures averaged 190.2 versus 138.2 mm Hg for test and control groups, respectively. The slight decline in average pressures in the test group at 7 and 8 months (180.0 and 186.0 mm, respectively), was due to the intercurrent deaths of those with the highest pressures. The experiment was terminated after 8 months because all of the 25 rats on processed baby foods had developed hypertension and about half were dead. The first test animal died between the fourth and fifth months followed by 11 more during the next 2 months; another 2 were seriously ill at the end of the study. In contrast, among their littermates serving as controls, most pressures at 8 months were clustered around 140 mm Hg and the group average was 141.4 mm Hg. One control animal had developed a chronic respiratory infection but the other 14 appeared in good health.

Body weights of the test group were significantly less ($p < 0.05$) during the first 2 and 3 months for both females and males but not

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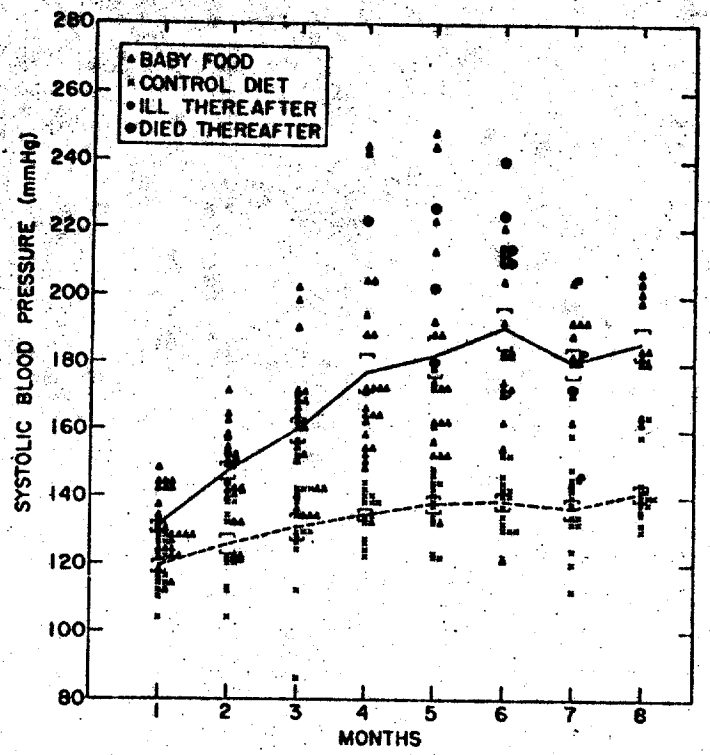

FIG. 1. Effect of processed baby foods on blood pressure of hypertension-prone rats. The mean blood pressures were computed without regard to sex because there were no consistent differences between the responses of the two sexes. Horizontal brackets above and below mean values indicate ± 1 SE. The average pressures of the test group were significantly higher ($p < 0.0002$–0.0001) than those of the control group at every period. Blood pressure shown for animals that died or became ill are the last recorded when animal appeared in good health.

thereafter. This may have been due to a lower average food consumption by the test rats in which case their NaCl intake was correspondingly decreased during this same period.

Discussion. Of necessity, the control diet differed by more than one variable from the experimental one: processed baby foods without added salt were not being manufactured at the time this study was initiated. Even today they are not readily available. We did not make our own baby foods with NaCl as the sole variable, since we wanted to test the baby foods *actually eaten* by American infants. It is conceivable, therefore, that differences in the diet other than the NaCl content were responsible for the serious effects observed in these animals. In similar experiments where both groups received rat chow with NaCl as the only variable, however, the results were equivalent to those reported here. Whatever the explanation, the fact remains that all of the test rats became hypertensive and half of them died. Consumption of processed baby foods caused hypertension and death. This is in sharp contrast to approximately 10,000 rats from this same strain studied during the last 9 years. In our studies on these hypertension-prone rats, we have sometimes observed "spontaneous" development of mild hypertension even on a low salt intake. This was true in the present experiment, for example, in which 2 of the 15 controls had final systolic pressures of 158 and 164 mm Hg, respectively. We do not ordinarily observe our animals after 12 to 15 months of age because such older animals develop murine pneumonia and serve as foci

for respiratory infections in the colony generally. During such a period of observation, however, the mild elevations described seem not to affect health. In any event, we have never observed anything approaching the incidence and severity of the disease displayed by these rats eating baby foods. It seems most probable that the effects were, in fact, due to the high NaCl content of the processed foods but we are now investigating NaCl as the single variable in baby foods prepared by ourselves.

Since the NaCl requirements of the normal infant are met by milk (5, 7, 10) the addition of salt to baby foods is irrelevant to metabolic needs. There is no evidence that the infant is born with a craving for salt. Salt is no longer a necessary preservative for these foods. The initial effect of a chronically high salt intake begun in infancy may be no more than to induce a salt appetite. There is considerable evidence that salt appetite is acquired and that it readily adapts to customary salt intakes however varied (7, 11). This appetite will be satisfied, or enhanced, when the child graduates to processed adult foods which, except for fruits, also generally contain high concentrations of added NaCl (2). Indeed, the addition of NaCl to processed foods is now so ubiquitous that it is difficult to obtain a diet low in salt even when required by prescription. An intake greatly in excess of normal metabolic requirements is thus virtually unavoidable in the western world.

If the primary effect of a chronic high salt intake is merely the induction of a salt appetite, a secondary effect may be the induction of hypertension. The fulminating hypertension displayed by our rats is rarely found before the third or fourth decades of human life but the strong genetic predisposition for hypertension must be equally rare in man. In genetically predisposed persons, our data suggest that a high salt intake, even if confined solely to infancy, might initiate hypertension either then or later. The possibility that evidence of injury from salt might remain covert for years is by no means unique. The appearance of thyroid cancer in adolescence from thymic radiation received in infancy; of blood dyscrasias long after exposure to benzene or radiation; of Parkinson's disease decades after encephalitis; of cancer and emphysema after many years of cigarette smoking provide strong precedents for the possibility that manifestations of injury from salt could be similarly delayed.

As indicated earlier, it is most unlikely that the high salt intake will, in fact, be confined to infancy. It is far more likely that salt will be ingested continually throughout life in large amounts by eating processed foods, supplemented both in the kitchen by the cook and at table by the consumer. We have suggested elsewhere that hypertension may develop thereafter depending on the genetic predilection of the specific individual combined with the intensity and duration of the salt intake (9, 12).

Summary. Some processed baby foods were lethal to hypertension-prone rats. Among 25 rats from a genetically hypertension-prone strain fed solely on such baby foods, all developed significant hypertension (averaging 180–190 mm Hg in the last 3 months of observation), 12 died, and 2 others became seriously ill during the 8 months of study. In contrast, the 15 control rats maintained on a low sodium chow were all alive and their average pressure at 8 months was 141.4 mm Hg. Considerable evidence suggests that the difference in response of test and control groups was due to the high NaCl content added to the processed baby foods. This added NaCl is unnecessary for the health of infants. It may contribute to the later development of hypertension in genetically predisposed individuals.

1. Dahl, L. K., Heine, M., and Tassinari, L., Nature (London) 198, 1204 (1963).

2. Watt, B. K., and Merrill, A. C., eds., "Composition of Foods -- Raw, Processed, Prepared." U.S. Dept. Agr. Handbook, No. 8 U.S. Dept. Agr., Washington, D.C., (1963).

3. Meneely, G. R., and Dahl, L.K., Med. Clin. N. Amer. 45, 271 (1961).

4. Puyau, F. A., and Hampton, L. P., Amer. J. Dis. Child. 111, 370 (1966).

5. Fomon, S. J., "Infant Nutrition," p. 160. Saunders, Philadelphia (1967).

6. Guthrie, H. A., Amer. J. Clin. Nutr. 21, 863 (1968).

7. Dahl, L. K., Amer. J. Clin. Nutr. 21, 787 (1968).

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

9. Dahl, L. K., Heine, M., and Tassinari, L., J. Exp. Med. 122, 533 (1965).

10. Mayer, J., Postgrad. Med. J. 45, 229 (1969).

11. Dahl, L. K., N. Engl. J. Med. 258, 1152, 1205 (1958).

12. Dahl, L. K., Lancet 1, 622 (1969).

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ETIOLOGICAL ROLE OF SODIUM CHLORIDE INTAKE IN ESSENTIAL HYPERTENSION IN HUMANS

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• *The possibility that a high dietary intake of sodium chloride might predispose an individual to hypertension was investigated by classifying 1,346 adults according to their blood pressure and again according to their habitual consumption of table salt. The 105 persons classified as hypertensive were found not to be randomly distributed; there were 61 among the 581 persons with high salt intake, 43 among the 630 with average salt intake, and 1 among the 135 with low salt intake. The departure from randomness of distribution was not explainable as an accident of sampling, and the association between salt intake and hypertension was accepted as significant. Further classification of the subjects as thin, average, overweight, and fat showed that obesity further increased the probability of hypertension in people on high salt intake.*

In 1954 we presented evidence that was interpreted to indicate a relationship between the level of sodium intake and human essential hypertension.[1] The original series of observations on 547 subjects has now been extended to include a total of 1,346 adults. The earlier findings have been confirmed; the data are highly suggestive of a correlation between salt (i. e., sodium chloride) intake and the incidence of hypertension.

The original paper[1b] may be consulted for details of the rationale. The possible role of sodium in the etiology of essential hypertension in humans was suggested by (1) the need for sodium in the production of certain forms of experimental hypertension[2]; (2) the virtual absence of hypertension among primitive races, all of whom appear to have a low-sodium diet[1b]; and (3) the amelioration of hypertension in some persons by restriction of sodium.[3] Given these three observations, the following question was suggested: Did individuals who had an apparently higher salt intake have significantly more hypertension than those with a lower salt intake? If the answer were that high salt intakes were indeed associated more frequently with hypertension, a cause-effect relationship would certainly be implicated, although not necessarily established. While the effects of sodium and chloride could not be separated here, all experimental and clinical evidence indicated that it was the sodium, not the chloride, ion that was important. For this discussion, therefore, the terms sodium chloride and salt may be interpreted to refer only to sodium.

Material and Method

Early in 1953 the present study was begun on every member of the Brookhaven Laboratory staff who reported for annual physical examination to one of us (R. A. L.). Both scientific and nonscientific personnel were included, and no selection by the physician occurred. However, the following selective processes were known to be in operation: 1. Preemployment physical examinations would tend to eliminate individuals with serious diseases of any sort. 2. Because of the laboratory's young age (about 10 years) and the character of the research (the study of the atomic nucleus and isotopes), the mean age of the scientists as well as their associates probably would be lower than the national average. 3. The employment policies at the laboratory limited the age range to from 18 to 65. 4. Females had the frequently exercised prerogative of being examined by a woman physician, so that the total sample had a 5:1 ratio of males to females.

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In our society it seemed likely that the major source of variation in salt intake might derive from the addition of salt at the table by the individual concerned. Of perhaps equal importance, however, was the fact that this addition of salt could be qualitatively analyzed over a lifetime. With this rationale in mind, each person was questioned as to his salt intake and classified into one of three groups, as follows: 1. Low intake—this individual denied adding, or ever having added, salt to his food at the table (the present widespread use of low-sodium diets made it imperative to specify whether the separation from salt shakers had been recent or lifelong). 2. Average intake—this individual added salt to his food, if after tasting, it was insufficiently salty for his palate. 3. High intake—this person routinely added salt to foods customarily salted before tasting them. An individual who would have been classified as a member of the high or average groups by past habits, but who had changed to average or low intake for any reason, was classified as a member of the group with the greater intake to which he had belonged formerly.

As mentioned earlier,[1b] certain defects in this type of classification were obvious: 1. It lacked precision in estimating the intake. 2. No allowance was made for variation in the salt content of foods prior to their arrival at the table due to natural variations in content as well as those diversities due to preparation, preservation, and cooking. 3. No provision was made for differences in perception of saltiness among individuals or even possibly in the same individual at different times. Despite these known limitations, it was believed that with sufficient members in the series some or all of these variables might be canceled out and the conceptual implication of a low versus a high salt intake might be tested.

At the time of the physical examination, record was made of the subject's age, sex, blood pressure, race, and nutritional state. The number of non-Caucasians proved to be so small that race has been disregarded in the calculations. Estimates of nutritional state were based upon the undressed appearance, from which each patient was classified as thin, normal, overweight, or fat. Although this classification was not exact, there appeared to be no need for more detailed anthropometric classification. The blood pressure was measured with a mercury sphygmomanometer while the patient sat quietly in the upright position with the arm resting easily on the examiner's table. Systolic and diastolic blood pressure levels were assumed to be represented by the first and fourth auscultatory phases, as recommended by a committee of the American Heart Association.[4] Hypertension was defined as a level of at least 140 mm. Hg systolic in combination with a pressure of at least 90 mm. Hg diastolic. If the blood pressure was not in this (defined) hypertensive range at the time of the examination but previous incontestable records of such elevation were available, this fact was noted and such individuals were classified as having hypertension. Although not all persons with transient elevations of blood pressure will develop sustained hypertension, available data suggest that among such individuals the disease occurs at least two or three times more often than in persons without such transient elevations.[5] It might be of added significance if even these persons with possible hypertension were found to be absent in the low-intake group.

TABLE 1.—*Uncorrected Data on Salt Intake and Incidence of Hypertension in Humans**

Sodium Chloride Intake	Total Subjects		Subjects with Hypertension	
	No.	% of Total	No.	% of Intake Group
Low	135	10.0	1	0.7
Average	630	46.8	43	6.8
High	581	43.2	61	10.5
Total	1,346	100.0	105	7.8

* The distribution of subjects with hypertension was significantly different among the three groups as tested by the chi-square distribution, with $X^2 = 16.1$ and $p<0.001$.

TABLE 2.—*Sodium Intake, by Age and Sex of Subjects**

Sodium Chloride Intake	Age, Yr.	Males		Females	
		No.	% of Intake Group	No.	% of Intake Group
Low	35.2(±9.2)	101	74.8	34	25.2
Average	34.7(±10.3)	522	82.9	108	17.1
High	34.4(±10.0)	501	86.2	80	13.8
Total	34.6(±10.0)	1,124	83.5	222	16.5

* Sex distribution significantly different among the three groups ($p<0.01$).

Observations

The uncorrected data on 1,346 consecutive patients are shown in table 1. (Data on three subjects were excluded as of no consequence in the present study.[16]) Very briefly, among the 135, 630, and 581 members of the low, average, and high sodium chloride intake groups, there were 1, 43, and 61 persons with hypertension respectively. The group with average salt intake had an incidence of hypertension that did not differ significantly from that predicted by random distribution and therefore is irrelevant to the development of the thesis in this paper. This table indicates that the probability that a distribution of hypertension like that found among these three groups would occur by chance is less than 1 in 1,000 (chi square = 16.1, $p<0.001$). From table 2, it is evident that, while mean ages were the same among the three groups, sex distribution differed significantly, with more females in the low-intake group. Although this difference probably is of no importance at these ages, the data were then analyzed for the 1,124 males, as shown in table 3. The findings remain essentially unchanged from those shown in table 1. Again there was a high degree of statistical significance to the distribution among the three groups ($p<0.001$). When tested against null value, the low-intake group had significantly less ($p<0.01$) hypertension and the high-intake group had significantly more ($p<0.02$) hypertension than would have occurred by chance alone.

The nutritional classifications of the 1,124 male subjects are outlined in table 4. There was no significant difference among the three salt-intake groups; however, the percentage of overweight or fat individuals (46.5%) among the males with hypertension, shown in table 5, exceeded that among the entire male population (26.8%) to a significant degree ($p<0.001$). For statistical analysis the four nutritional groups were combined into two: thin and normal became "non-overweight," and overweight and fat became "overweight." Comparisons then were made among individuals who were classified as having low or high salt intakes and who were overweight or non-overweight. Table 6 summarizes the significant differences found by such comparisons. The following conclusions may be drawn from the data in table 6: 1. Individuals who are not overweight but who are on a high-salt diet will have several times the incidence of hypertension found in similar individuals on a low-salt diet. 2. Among individuals who are on a high-salt diet, those who are overweight will have significantly more hypertension than will those who are not overweight. 3. Those individuals who are both overweight and on a high-salt diet will have a much greater incidence of hypertension than will individuals not overweight and on a low-sodium diet.

TABLE 3.—*Corrected Incidence* of Hypertension in Males Only*

Sodium Chloride Intake	Age, Yr.	Total Subjects		Subjects with Hypertension		
		No.	% of Total	No.	% of All with Hypertension	% of Intake Group
Low	36.8(± 9.4)	101	9.0	1	1	1
Average	36.1(±10.7)	522	46.4	38	39	7.3
High	35.7(±10.2)	501	44.6	58	60	11.6
Total	36.0(±10.4)	1,124	100	97	100	8.6

* For all three groups, $X^2 = 14.8$ and $p<0.001$; for low-intake group, $X^2 = 7.42$ and $p<0.01$; for high-intake group, $X^2 = 5.54$ and $p<0.02$.

Comment

One important question still to be answered is whether the three salt-intake classifications used in this study have any relation to actual salt ingestion. Originally[15] it was thought that the classification might be useful for the low and high groups but that probably the average group would extend over the entire spectrum from low to high. Some preliminary data suggest that this is correct. A series of individuals from all three groups, with and without hypertension, has now been studied by collecting all urines for a minimum period of 7 days (and up to 38), from which an accurate index of sodium intake can be realized.[8] From these data it is pertinent to note that the individuals with a low intake without hypertension had significantly lower urine sodium outputs (and therefore intakes) than the persons with hypertension, whatever the group into which the latter had been classified in this study. Furthermore, the single subject with a low intake who was found to have hypertension had a urine sodium level like that of the other hypertensives, rather than similar to that of the other members of the low-intake group. From these unpublished observations, it is likely that the following conclusions

TABLE 4.—*Nutritional Grouping of 1,124 Males*

Nutritional Groups	Sodium Chloride Intake: Low No.	Low %	Average No.	Average %	High No.	High %	Total No.	Total %	
Thin	16	15.8	70	13.4	75	15.0	161	14.3	73.2
Normal	62	61.4	314	60.2	286	57.0	662	58.9	
Overweight	20	19.8	112	21.4	116	23.2	248	22.1	26.8
Fat	3	3.0	26	5.0	24	4.8	53	4.7	
Total	101	100.0	522	100.0	501	100.0	1,124	100.0	

will emerge: the arbitrary low-average-high classification based on the use of salt at the table, which was devised for exploration of a theory, probably is generally valid. It will, however, be subject to individual exceptions, because of the defects noted earlier in this paper. However, the data suggest that among those individuals who actually have a low-sodium diet, as measured by urine sodium level, hypertension will be significantly less than among those people who have a high-sodium diet, judged in the same fashion. Therefore, the original thesis seems valid; namely, that there is a significant relationship between the level of sodium intake and the development of essential hypertension.

The question will be asked as to whether the enhanced salt intake of individuals predisposed to or with hypertension is caused by, rather than a cause of, the disease. It has been suggested that persons with hypertension have an increased appetite for salt.[7] The present report cannot exclude this possibility. It may be pertinent to note, however, that for eight years one of us (L. K. D.) has used drastic prolonged limitation of sodium (40-125 mg. per day) in the study and treatment of patients in all stages of this illness. This was done under metabolic ward conditions, without evidence of salt-craving, such as might be expected if excessive salt appetite were part of the disease. Since many of these patients had been very heavy users of salt prior to such reduction, this probably is relevant to the present discussion.

TABLE 5.—*Nutritional Grouping of Males with Hypertension*

Nutrition Groups	Sodium Chloride Intake: Low	Average	High	Total No.	Total %	
Thin	...	2	3	5	5.1	53.5
Normal	...	19	28	47	48.1	
Overweight	...	13	20	33	34.1	46.5
Fat	1	4	7	12	12.4	
Total	1	38	58	97	100.0	

It seems fairly well established that essential hypertension occurs more commonly in certain families than in others.[8] If this represents true hereditary rather than common environmental factors, it does not mean that heredity alone is sufficient. In rheumatic fever, for instance, hereditary factors are known to be important[9]; yet, if streptococcic infections are avoided with prophylactic antibacterial therapy, rheumatic fever does not result.[10] We would like to suggest that similarly, in the individual hereditarily predisposed to essential hypertension, the actual etiological agent is environmental; namely, the level of ingestion of salt. In people without such hereditary predisposition, resistance to the effects of ingestion of sodium might be greater, but this resistance could be overcome by a greater ingestion over a longer period.

The failure of many people on a high-salt diet to develop hypertension does not necessarily invalidate the proposed hyothesis: With most disease-inciting agents, exposures of apparently equal degree of different individuals will be followed by development of the disease in some but not in others. Presumably in essential hypertension there are unknown factors that are of importance in modifying the effect of a given level of ingestion of sodium.

It is known that overweight individuals suffer from hyertension more commonly than do non-overweight subjects.[11] Could it be that in eating more food generally, more salt is ingested simultaneously? This is one possible explanation at least.

TABLE 6.—*Statistically Important Difference* in Incidence of Hypertension in Males Only*

Non-overweight subjects: High sodium chloride intake vs. Low sodium chloride intake	$X^2 = 7.2$, $p<0.01$
High sodium chloride subjects: Overweight vs. Non-overweight	$X^2 = 10.8$, $p = 0.001$
Combination resulting in greatest statistical significance: High sodium chloride intake, overweight vs. Low sodium chloride intake, non-overweight	$X^2 = 10.67$, $p<0.001$

* In each pair, the first group has the greater incidence of hypertension. The significance of the difference in incidence of hypertension was estimated by the standard chi-square test.

In view of the foregoing considerations, it is proposed that the level of sodium intake is the primary etiological factor in the development of essential hypertension.

Salt—a dangerous "antidote"

THE TOXIC potential of sodium chloride is not a new discovery. Seafarers have long known of the fatal consequences of drinking sea water. The Chinese have used saturated salt solution as a traditional method of suicide.[1] Salt poisoning has been widely reported in animals and man. Usually, as in the famous Binghamton disaster[2] in which salt was inadvertently substituted for sugar in the preparation of infant formulas, the excessive amounts of sodium chloride have been unintentionally administered. Hypertonic dehydration has been seen rather commonly following treatment of infants with diarrhea using homemade salt solutions. Salt poisoning has also occurred as a result of the intentional administration of hypertonic solutions of sodium chloride. The intra-amniotic injection of hypertonic saline for the induction of abortion[3] can result in symptomatic hypernatremia and permanent damage to the nervous system.

It has come to our attention, through the admission of a seriously intoxicated infant, that concentrated sodium chloride solution has been widely recommended as an emetic agent in the management of poisoning with, or ingestion of, toxic substances. The patient was a 2-year-old boy who had been found playing with a bottle of Darvon from which 4 capsules were thought to be missing. His mother administered a solution of salt water to induce emesis. She gave him four 8 ounce glasses of a solution containing 4 tablespoons of salt in each glass. Two hours later he was admitted in coma, with hyperpyrexia, hyperpnea, cyanosis, hyperreflexia, muscular stiffness, and seizures. The serum sodium was 189 mEq. per liter and the chloride 156 mEq. per liter. The serum osmolality was 407 mOsm. per liter. Blood and urine were tested for Darvon and found to be negative.

With therapy, the concentrations of sodium and chloride in the serum were successfully reduced. However, the patient never recovered cerebral function. He had dilated, fixed pupils and papilledema, and he required a ventilator for the maintenance of respiration. The electroencephalogram showed no activity above base line, and he died on the ninth hospital day.

A level tablespoon of table salt should contain about 350 mEq. of sodium and chloride. The child, therefore, was given a dose approximating 5,600 mEq. of sodium or over 400 mEq. per kilogram. Of course emesis did occur so it is impossible to know how much was retained and absorbed. However, it is clear that this was a lethal dose.

It is frightening to realize how widely disseminated is the advice that this mother followed. Recommendations for the use of saline as an emetic in suspected poisoning are found on labels of common household products, in first aid manuals, and in the pediatric literature. Most of these recommendations are vague or ambiguous in terms of dosage, and they almost never include warnings of the potential hazard of excessive salt ingestion. It may be instructive to look at some of these recommendations:

1. From a manual of medical emergencies intended for the medical assistant or personnel attending to the patient before the arrival of a physician:

"b. Offer several teaspoons of table salt or baking soda in half a glass of water. This is nauseating and will help start vomiting.

c. Repeat above as needed to eliminate evidence of poison in vomitus."[4]

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2. From a government publication for parent education: "To make a child vomit, tickle the back of his throat with your finger or have him drink warm water containing table salt."[5]

3. From a textbook of pediatrics, "Emesis may be induced by administering 15 ml. of syrup of ipecac or strong salt solution. . . ."[6]

Other cases of poisoning and death following saline emetics have been reported.[7, 8] Yet, it is clear that information on the danger of this approach to the management of potential poisoning has not been widely disseminated. Salt has been compared with other agents and with syrup of ipecac as an emetic in cases of poisoning. It has been rated one fourth as effective but equal in safety.[9] In our view, this is inappropriate. However, syrup of ipecac is an effective emetic, and its use has become sufficiently ritualized that accidents are not occurring in its use. Syrup of ipecac, used as recommended, appears to be a safe and reliable emetic in cases of poisoning where emesis is indicated.

Sodium chloride solutions do not appear to meet these criteria. There is additional evidence of sodium chloride toxicity. Syndromes of salt poisoning have been described in animals who have overindulged themselves at salt licks.[10] Calvin and associates[11] reported twins who poisoned themselves similarly on obtaining access to a salt box. Fatal ulcerative tracheobronchitis has been reported following aspiration of salt by a child who pulled the salt canister down from a shelf into his face.[12] Salt belongs on the list of common household products which are potentially hazardous and which should be kept away from children.

Salt may be an occasionally successful emetic. It is a reliable poison. In our view, this approach to emesis is contraindicated. It is clearly a way in which a potential poisoning with a possibly ingested drug can be turned into a lethal intoxication with sodium chloride. It is particularly sad that advice recommending homemade saline emetics is so readily found in pamphlets and other material the parent is likely to turn to first in an emergency. Pediatricians can help to get this advice deleted.

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REFERENCES

1. Johnson, A. S.: Discussion of paper by W. J. Schatz, Med. Rec. Ann. 145: 491, 1937.
2. Finberg, L., Kiley, J., and Luttrell: Mass accidental salt poisoning in infancy, J. A. M. A. 184: 187, 1963.
3. Cameron, J. M., and Dayan, A. D.: Association of brain damage with therapeutic abortion induced by amniotic-fluid replacement: Report of two cases, Brit. Med. J. 1: 1010, 1966.
4. Medical Emergencies, Schering Corporation, 1962, p. 32.
5. Your Child from 1 to 6, United States Department of Health, Education, and Welfare, Children's Bureau Publication No. 30, 1966, p. 92.
6. Nelson, W. E., Vaughan, V. C., and McKay, J. R.: Textbook of pediatrics, ed. 9, Philadelphia, 1969, W. B. Saunders Company, p. 1491.
7. Ward, D. J.: Fatal hypernatremia after a saline emetic, Brit. Med. J. 2: 432, 1963.
8. Schatz, W. J.: Treatment based on physical principles followed by recovery in sodium chloride poisoning, Med. Rec. Ann. 145: 487, 1937.
9. Cashman, T. M., and Shirkey, H. C.: Emergency management of poisoning, Pediat. Clin. N. Amer. 17: 592, 1970.
10. Smith, D. L. T.: Poisoning by sodium salt—a cause of eosinophilic meningoencephalitis in swine, Amer. J. Vet. Res. 69: 852, 1957.
11. Calvin, M. E., Knepper, R., and Robertson, W. O.: Hazard to health—salt poisoning, New Eng. J. Med. 270: 625, 1964.
12. Young, L. G.: Death due to aspiration of table salt, New Zeal. Med. J. 65: 615, 1966.

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Neurophysiologic Disturbances in Hypertonic Dehydration

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Irritability, restlessness, and stupor are the usual neurological signs of hypertonic dehydration in infants and children. Severe degrees of hypertonicity may be associated with irregular twitching, trembling nystagmus, coma, respiratory failure, and occasionally with generalized seizures. All of these findings have been reproduced in experimental animals. The present report is concerned with an analysis of this syndrome in relation to some of the anatomic and physiologic levels of nervous system dysfunction.

Methods and materials: Hypertonicity was induced in 37 adult rabbits of the New Zealand strain by the intravenous infusion of solutions (2,000 mOsm/L) of NaCl, urea, or sucrose. Maximum tonicity was achieved in from one to several hours. Clinical observations were supplemented by polygraphic recordings of electroencephalograms, electromyograms from fore and hind limbs, blood pressure, and respiratory patterns. These were correlated with the plasma osmolality obtained at intervals. In selected animals surgical transection of the mid-dorsal spinal cord and/or femoral and sciatic nerves on one side was accomplished just prior to an experiment.

Results: A predictable series of clinical events occurred during induction of hypertonicity and correlated with the rising tonicity of the plasma. In the NaCl and sucrose infused animals, licking of the lips, restlessness, and startle in response to cutaneous stimulation usually appeared when the osmolality was in the range of 350-375 mOsm/Kg. Nystagmus or rhythmical jerking of the eyes and ataxia and tremulous movements of trunk and limbs appeared next with osmolalities in the 375-400 range; and synchronous and asynchronous jerks, tonic spasms or trembling of the limbs, and reduced responsiveness became increasingly prominent as the osmolality exceeded 400. Death, by respiratory failure, occurred with a mean osmolality of 435. Animals infused with urea developed all of the above signs but at levels of osmolality approximately 100 mOsm/Kg. above the other groups. In a previous report (Sotos, et al., *Pediatrics 26*: 925, 1960) it was demonstrated that comparable degrees of dehydration of the brain were achieved at death in the urea and non-urea groups of animals with these differing osmolalities, suggesting diffusion of some of the urea into the brain during the course of the experiments. In animals administered NaCl rapidly, unresponsiveness and death often obtained in the absence of prominent movement abnormalities.

The electroencephalogram showed no significant alternations until an osmolality of about 400 mOsm/Kg. was reached. As the tonicity increased above this level, there was a gradual loss of normal faster frequencies and the development of bursts of 4-5 and 1-3 per second waves of higher voltage. In an occasional animal a striking reduction in voltage was seen with maximal hypertonicity. A notable lack of paroxysmal electroencephalographic discharges correlating with observed jerking of the body musculature was a striking finding in all animals studied, and sharp waves and spikes were noted at the time of jerking in two animals only. These observations led to the hypothesis that dysfunction of subcortical regions of the nervous system was responsible for much of the motor disorder. In ten of twelve hypertonic animals in which the mid-dorsal spinal cord was transected surgically just prior to the experiment, striking jerks and tonic contractions of muscles identical in appearance to that seen in intact animals were observed and recorded electromyographically below the level of transection. Furthermore, in eight of twelve animals in which sciatic and femoral nerves were severed on one side, recurring jerks and tonic contractions developed in the muscles of that limb. There was quantitatively more activity in muscles cephalad to the transected cord and in the non-denervated hind limb. A total of six animals showing the described motor abnormalities were curarized; all jerking and spasms ceased following this but returned when the curare effect wore off. In one animal undulating muscular fasciculations of the sort observed occasionally in the absence of hypertonicity persisted in the denervated muscle following curarization.

Discussion: The altered responsiveness and EEG changes indicate some disturbance in function of the cerebral hemispheres; the ataxic movement and nystagmus suggest involvement of vestibular-cerebellar mechanisms and death by respiratory failure implicates respiratory centers within the brain stem. The bilaterally synchronous jerks and spasms most probably arise from discharges at various levels of the central nervous system, including the spinal cord; they seem rarely relatable to cerebro-cortical events. At least some of the jerks appear to be of peripheral origin, although precisely where they originate is not clear. They do not arise primarily in the muscle fibers.

From these observations we are led to the general conclusion that hypertonicity of the body fluids in the rabbit has a profound physiologic effect at all levels of the nervous system.

DISCUSSION

CHARLES LUTTHELL (Baltimore, Md.): Dr. Dodge and his collaborators have provided us with a convincing demonstration of an acute metabolic disorder with selective effects at different levels of the nervous system. From their experiments it is clear that acute hypertonicity induces abnormal clinical and electrical activity at cerebral, spinal,

peripheral nerve, and possibly even motor end-plate levels. Muscle jerks and twitchings of the type shown are commonly attributed to cortical disturbances. However, evidence is accumulating that a number of diverse central nervous system diseases or toxic metabolic disorders may affect lower levels of the neuraxis and peripheral nervous system causing myoclonus and muscular twitchings. To this list we may now add acute hypertonic dehydration.

While recognizing the importance of hypertonicity in the interpretation of the results in the present experiments, further elaboration and qualification of possible mechanisms involved must be made. First of all, twitchings, myoclonus, and seizures are all more frequently observed in animals and humans subject to acute cellular desiccation. In the experiments just described the rabbits in a strict sense were not dehydrated during the period of study since their total body water may have been increased. In animals or humans with comparable degrees of hypertonicity developing gradually over a period of days, motor manifestations like those just illustrated are much less prominent. Considered from this viewpoint I would like to suggest that the acutely induced shift of water from the intracellular to the extracellular compartments is the essential ingredient in the widespread disturbance of excitable tissues produced in these rabbits. In the present experiments the physiologically observed differences in animals given solutions of NaCl, sucrose, or urea of the same osmolality further supports this concept. Urea diffuses quickly into cells, NaCl only moderately, and sucrose probably not at all. It follows that cellular damage would be predictably greater in animals receiving hypertonic NaCl or sucrose than those given urea. The results obtained by Dr. Dodge and coworkers appear to confirm this.

In human material these same conclusions are applicable. By way of illustration, of the six infants who died during the recent Binghamton acute salt poisoning incident, all developed tremors and convulsions. In those infants facial twitchings were particularly evident. In contrast, infants and adults who become clinically dehydrated over many days may become lethargic without concurrent twitchings, myoclonus, or seizures.

The physiological approach used by Dr. Dodge and his coworkers is a technique which has long been of value in this type of analysis but is one seldom utilized. In 1894 Turtschaninow became interested in the site of origin of muscular jerks occurring in dogs following administration of 4 to 5% phenol. Since the jerks in those animals were not abolished by brain stem or spinal cord transection though extinguished by section of peripheral nerves, he concluded that the jerking arose from spinal cord. Similar inference can be made from the experiments just described by Dr. Dodge, et al., but additionally they have found persistence of muscular twitchings in a hind limb after sectioning sciatic and femoral nerves.

In addition to the postulated excitatory effect of hypertonicity on the neuromuscular junction, might it not be equally possible that the demonstrated activity arises from the distal stump of the cut peripheral nerves? Rapid desiccation of Schwann cells and surrounding membranes might well deform and stimulate the distal stump of cut nerve producing twitch responses in muscle.

Finally, I would like to ask Dr. Dodge and colleagues if they have: 1) inserted electrodes into spinal cord of animals with mid-dorsal transections to examine the abnormal firing, 2) studied the effects of anaesthetics or procaine blocks of spinal cord and peripheral nerves, and 3) looked for morphological changes in spinal cords and peripheral nerves following acute administration of hypertonic solutions. In closing I wish to congratulate the authors for this careful analysis of an important metabolic disorder.

T. R. Jones (Charlottesville, Va.): I am delighted to have had an opportunity to read the longer version of the paper of Dr. Dodge and his coworkers. In it he has presented evidence which strongly suggests to me that some of the asymmetrical muscle-

twitching that he has observed may be a disturbance within the motor unit or, perhaps more properly, the neuromuscular junction itself.

Using microelectrode recording technique in similar mammalian preparations, Boyd and Martin, Lily, and then Furshpan have demonstrated that changes in osmotic pressure alter the polarization of the nerve terminal. Miniature end-plate potentials are the electrical representatives of quanta, or packets, of acetylcholine which are normally released spontaneously and at random from the nerve terminal. A nerve impulse, or an increase in the concentration of certain ions, will permit a tremendous increase in the frequency of release of the acetylcholine, thus leading to an elevation of the end-plate potential, perhaps to the critical point at which there is initiation of a muscle action potential, and then a muscle contraction. More recently, similar observations have been made in human skeletal muscle by Thesleff, Elmqvist, and myself. On the basis of those studies and these of Dr. Dodge and his coworkers, I would urge Dr. Dodge to continue these fascinating studies on hypertonic dehydration, and to continue his interest in the motor unit as well as the central nervous system.

Dr. Dodge (*closing discussion*): I would certainly agree with Dr. Luttrell that cellular desiccation, as he so nicely puts it, is important. When one considers that the nervous system is made up almost wholly of cells, with little extracellular space, it is not surprising that changes in water content of the nervous system due to alterations in tonicity of the blood will result primarily from cellular hydration or dehyration. In general, our animals lost water from a urinary diuresis during the course of the experiments and were, in fact, dehydrated, but certainly this is not essential for the symptoms of hypertonicity. In our earlier studies in rats with ligated ureters, the entire syndrome developed in the face of an increase in body water, yet the brain was dehydrated as a consequence of hypertonicity of the blood. It is the changes in the nervous system which are important.

The rarity of twitching in man is certainly true. However, I think the Binghamton experience points out that when exogenous solute in the form of saline is given, this can be seen; we have also seen this.

In answer to the specific questions: we have not as yet tried recording potentials from the cord itself. Dr. Konigsmark attempted to record from the peripheral nerve in some preliminary studies in our laboratory, but these studies are as yet incomplete. We have not as yet tried blocking either cord or peripheral nerve with procaine, but this certainly should be done. Our histological studies have been limited to the brain.

I want to thank Dr. Johns for his comments relative to the *in vitro* miniature end-plate potential discharges with hypertonic bathing solutions. We too feel that some of the disturbed function may well be at this level in our animals, but more data must be obtained.

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PATHOLOGY OF ACUTE SALT POISONING IN INFANTS

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On March 9, 1962, 3 infants died unexpectedly in the nursery of the Binghamton General Hospital. The following day 3 more died. In the early morning of Sunday, March 11, it was discovered that a quantity of table salt had inadvertently been added to the sugar used in preparing 1 of the stock formulae and that 5 of the infants who died, and 9 others still living, had received the salted formula between noon of March 7 and the morning of March 11. At an emergency meeting of the pediatric staff at 10:30 A.M. the morning of March 11, the entire hospital was alerted. The staff of the clinical laboratory began at once to perform microchemical determinations of electrolytes on a 24-hr. basis, in order to assist the medical staff in detecting the poisoned infants and in guiding treatment. Peritoneal dialyses following the technic of Miller and Finberg[7] were instituted without delay. On March 12 another infant died in spite of heroic measures to save her life. All 8 of the other treated babies recovered.

Because of the worldwide publicity attending the event, the burden of medicolegal responsibility placed upon the Department of Pathology of the Binghamton General Hospital called for an unusual degree of precision and decisive action. It so happened that a diagnostic microscopic renal lesion was a common denominator of 5 of the 6 infants who died after having been given the salt-laden formula. In the sixth, and last to die, although the renal lesion from salt injury was not well defined, serum sodium levels were known to have attained 250 mEq. per l. prior to death. This was a treated patient. Hence, 6 of the 7 fatalities occurring between March 9 and 12 were established as hypernatremic in etiology and were so reported at a public inquest held at the Broome County Court House on April 26, 1962, by Vincent M. Maddi, M.D., the Broome County Coroner, with jurisdiction in the City of Binghamton.

Although it is known that the salted formula was used in the fatal cases from approximately 41 to 78 hr., the exact amount of salt ingested by each of these infants is not determinable, nor is the median lethal dose for acute poisoning with this agent under different circumstances known with any degree of accuracy. Schatz[8] has estimated the lethal dose to be approximately 3 Gm. per kg., although it is known that less can be lethal in infants and in young lower animals. Smith[10] reports that the lethal dose for swine is 1 Gm. per kg. and that, when thirst develops, water is vital for survival.

Salt is rapidly absorbed in any concentration, whether it is administered by enema, by hypodermoclysis, or by mouth, as demonstrated in a review of prior cases by Schatz.[8] In the case reported by Schatz, a 17-year-old girl had taken a total of approximately 150 Gm. of salt by drinking 4 glasses of salt water (2 heaping teaspoons per glass), 1 every 20 min. "until vomiting was induced" on the advice of a friend, in order to relieve menstrual cramps. Nausea, dyspnea, and vertigo, accompanied by thirst, but no vomiting, occurred after the 4th glass; diarrhea, muscle twitching, fixed contracted pupils, a temperature of 101 F., and unconsciousness rapidly followed. Complete recovery resulted from the intraperitoneal injection of 1500 ml. of distilled water by gravity after gastric lavage with distilled water, followed by 2000 ml. of normal saline solution injected subcutaneously and water administered orally during lucid intervals.

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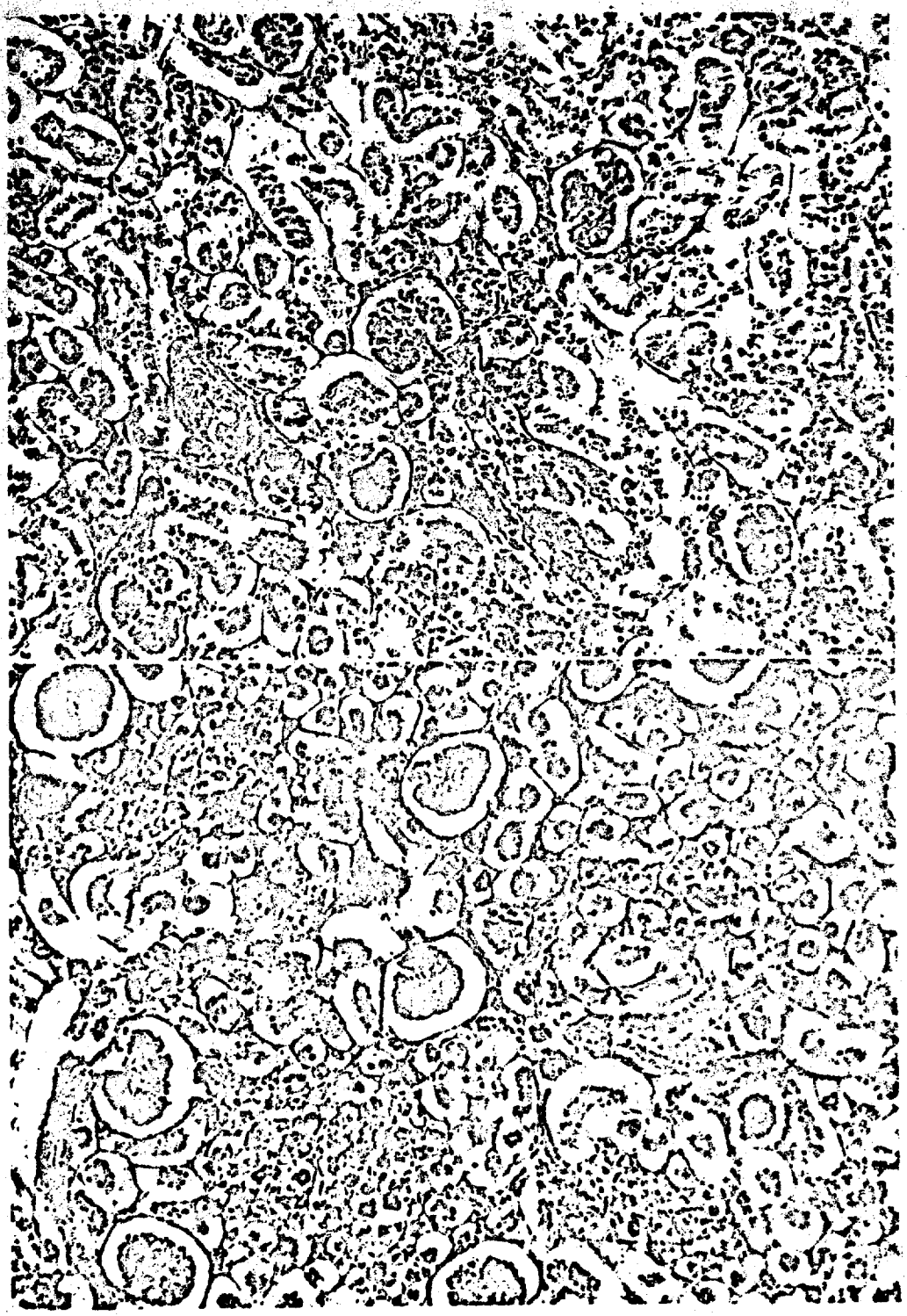

FIG. 1 (upper). Dehydration shrinkage of convoluted tubules in renal cortex of A-58. Identical to lesions of A-57 and A-59. Hematoxylin and phloxin. × 200.

FIG. 2 (lower). Dehydration shrinkage of convoluted tubules in renal cortex of A-57. Identical to lesions of A-58 and A-59. Periodic acid-Schiff. × 200.

TABLE 1

Case Histories and Pathologic Findings

Number	Age at Death	Sex	Estimated Time Fed Formula	Clinical Summary	Gross Pathologic Findings	Histopathologic Findings			
						Kidneys	Central nervous system	Lungs	General
			hr.						
A-57	8 days	M	40	Delivery by cesarean section. Formula every 3 hr. Shallow respirations and generalized convulsions after 24 hr. on formula (March 8). Twitching of eyes. Depression of anterior fontanelle. Extremities limp. Reflexes weak. Subnormal temperature. Spinal tap bloody. Became cyanotic. Convulsions continued to death. Oxygen therapy during illness. Died March 9 at 4:40 a.m.	Subarachnoid and intraventricular hemorrhage. Consolidation, right middle lobe of lung. Urinary bladder contained a few drops of urine.	Diffuse shrinkage of all convoluted tubules in kidneys.	Shrinkage of cerebral cortex with palisading of pyramidal cell layer. Pericapillary hemorrhages in white matter, with occasional lymphocytic cuffing of capillaries. Balloon-like distention of some capillaries in pia-arachnoid and contracted thrombi in others.	Acute suppurative pneumonitis with confluent bronchopneumonia surrounding blood vessels. Acute necrotizing vasculitis of pulmonary artery branches. Foci of intra-alveolar hemorrhage. Remnants of hyaline membranes.	
A-58	32 days	M	55	Removal of encapsulated sympathicogenioma, left adrenal, on 22nd day of life. Condition good until March 8, when vomiting and fever of 103 F. developed. Respirations rapid and noisy on March 9, and convulsions began. Died March 9 at 7:30 p.m.	No evidence of residual adrenal tumor. Massive sagittal sinus thrombosis. Multiple ecchymoses of pia-arachnoid. Brain unusually soft and friable. Urinary bladder empty.	Diffuse shrinkage of all convoluted tubules in kidneys. Collecting tubules packed with amorphous acidophilic casts. Renal infarction and many small vascular thrombi in 1 kidney.	Multiple intracerebral capillary thromboses.	Multiple foci of lobular atelectasis and suppurative pneumonitis.	Capillary thrombosis in perithymic tissue. No evidence of metastatic adrenal tumor.
A-59	3½ days	F	60	Diarrhea with blood and mucus on March 8, followed by respiratory difficulty and sternal tugging. Convulsions developed on March 9. No response to calcium gluconate. Blood sugar 18 mg./100 ml. Eyeballs pointing downward in "setting sun" appearance before death on March 9 at 11:20 p.m.	Subarachnoid and intraventricular hemorrhage. Multiple petechial hemorrhages in lungs. Urinary bladder empty.	Diffuse shrinkage of all convoluted tubules in kidneys.	Multiple intracerebral capillary thromboses.	Partial atelectasis and lobular suppurative pneumonitis. Inhalation of amniotic fluid squames in alveoli).	

A-64	6½ days	F	73	Discharged at age 3½ days from nursery March 7 with supply of salt formula. Readmitted March 8 because she did not take formula well and displayed thirst. Admission temperature 102.4 F. Given clysis of 250 ml. of isotonic saline twice because she continued to refuse formula. Chest film showed miliary atelectasis. Given oxygen by funnel. Became inactive on March 9. Respiration became rapid and noisy a few hours before death on March 10 at 1 p.m.	Body had been embalmed before autopsy. Foci of consolidation and petechial hemorrhages in lungs. Cranial cavity not examined.	Shrinkage of convoluted tubules of kidneys, not generalized, but lobular, in distribution.		Focal suppurative pneumonitis with partial atelectasis in lungs.	
A-66	8 mo.	F	78	Had been in hospital for treatment of fibrocystic disease for several months. An older sibling also had fibrocystic disease. On March 8 coarse breath sounds developed, with fever of 104.2 F. on March 9. WBC 12,050 on March 10. Died on March 10, in spite of oxygen administration, at 6:30 p.m.	Body had been embalmed before autopsy. Lung voluminous and emphysematous. No consolidation. Vessels of pia-arachnoid congested. Urinary bladder contained 15 ml. of urine.	Shrinkage of convoluted tubules of kidneys, not generalized, but lobular, in distribution. Amorphous acidophilic casts in collecting tubules of kidneys (A.F.I.P. observation).	No demonstrable lesions.	Mild pulmonary emphysema with small blebs. Hyaline membrane formation in lungs.	Terminal stage of fibrocystic disease of pancreas.
A-65	4½ days	F	78	Born midnight March 7–8. Spastic movements noted March 10, with tremors of head, rolling of eyes, and labored gasping breathing. Still living when discovery of salted formulae was made. Serum sodium 250 mEq./l. on March 11. 2.5% glucose by clysis on March 11. Blood sugar 140 mg. 100 ml.; hemoglobin 15.9 Gm.; hematocrit 37%; WBC 12,900, on March 11. Serum sodium 194 mEq./l. on March 12. Serum sodium did not fall so rapidly as in other treated cases. Peritoneal dialyses performed 5 times, but respiratory distress increased and x-ray signs of pneumonia appeared before death on March 12 at 11:22 a.m.	Spinal fluid crystal clear. No gross brain lesions. Patchy ecchymoses throughout both lungs, with extensive consolidation. Urinary bladder empty.	No conclusive evidence of shrinkage of convoluted tubules in kidneys. Collecting tubules filled with amorphous acidophilic casts (A.F.I.P. observation).	Multiple intracerebral capillary thromboses and occasional foci of softening in cerebellum.	Confluent suppurative pneumonitis.	

In a discussion of Schatz's report, Johnson[3] pointed out that drinking a pint of saturated salt water is a traditional method of suicide among the Chinese. Johnson also recommended intraperitoneal injection of glucose for treatment.

It should be noted that the sequence of autopsies in the Binghamton cases does not correspond with the sequence of deaths, and that the bodies of 2 of the infants (A-64 and A-66) had been embalmed prior to the completion of arrangements for autopsy.

FINDINGS AND DISCUSSION

Lesions of the central nervous system characteristic of salt poisoning (hypernatremia) are already well described in the literature of human and veterinary medicine. In infants, the recent work of Finberg,[1] Finberg and associates,[2] Luttrell and Finberg,[5] and Luttrell and co-workers[6] has emphasized the frequency of subarachnoid, intracerebral, and intraventricular hemorrhages, as well as sinus thromboses and thromboses of small intracerebral and subependymal vessels, in relative hypernatremia after prolonged diarrhea, as well as in absolute hypernatremia after accidental ingestion of excess sodium chloride.

Findings relative to the central nervous system in the 5 Binghamton cases with available material differ in no significant way from what has already been well described in the literature. In 1 case (A-57), there seemed to be a dehydration of the cerebral cortex, with an accentuated palisading effect of the contracted layer of fetal pyramidal cells. Subarachnoid and intraventricular hemorrhages were noted in 2 cases (A-57 and A-59). Sagittal sinus thrombosis was noted in 1 (A-58). No gross brain lesions were found in 2 (A-65 and A-66). Microscopic lesions consisted of small hemorrhages, mostly in the white matter (A-57), and multiple capillary thromboses, also mostly in the white matter (A-58, A-59, and A-65). An occasional focal area of softening was noted in the cerebellum (A-65). No significant microscopic lesions of the brain were noted in 1 (A-66). In general, vascular stasis was striking, and especially engorgement of vessels of the pia-arachnoid and intracerebral areas, where occasional capillaries were ballooned out so that they appeared to be on the verge of rupture. In other capillaries, thrombi appeared in various stages of development. Further studies on the central nervous system are in progress, and additional findings may subsequently be described by other investigators to whom material has been distributed for research purposes.

In veterinary medicine, the hemorrhagic encephalopathy of hypernatremia is well described in swine and poultry and also in wild life exposed to the sodium chloride used on the highways in winter.[12] Salt poisoning in swine has been extensively studied and described by Smith.[10] In Smith and Jones' text on veterinary pathology,[11] an entire section deals with its occurrence in farm animals.

A renal lesion said to be characteristic of salt injury has been mentioned as occurring after sulfathiazole administration by Luetscher and Blackman,[4] who correlate the lesion with secondary high sodium and chloride levels and with persistent cerebral damage. These authors speak of "atrophied tubules," in the intercalcated segments of the distal convolutions, the spiral portions of the proximal tubules, the collecting cortical tubules, and the ascending loops of Henle, "separated from one another by fairly cellular new-looking scar tissue." The 2 patients in whom this lesion was described were ages 25 and 54, and both had received sulfathiazole. Photomicrographs illustrate the lesion in the convoluted tubules very well.

The kidney lesion in the Binghamton infants is unique and distinctive, and it is demonstrable as a specific effect attributable solely to acute salt poisoning (extrinsic hypernatremia). This microscopic pathology of salt injury may be identified as a diffuse or lobular shrinkage of the proximal and distal convoluted tubules in the cortex, owing to dehydration induced by hyperosmolarity of the extracellular fluid. Periodic acid-Schiff preparations, as well as standard hematoxylin-eosin (phloxin) stains, clearly exhibit a separation of the cell membranes of the tubular complexes from their surrounding basement membranes by what is

FIG. 3 (upper). Dehydration shrinkage of convoluted tubules in renal cortex of A-66. Identical to lesion of A-64. Less striking than in A-57, A-58, and A-59. Hematoxylin and phloxin. × 200.

FIG. 4 (lower). Amorphous acidophilic casts in collecting tubules of kidney of A-58. Similar lesion noted in A-65 and A-66 by observers in the Forensic Pathology Branch, Armed Forces Institute of Pathology (A.F.I.P. No. 62-2901). Hematoxylin and eosin. × 130.

ostensibly hypertonic fluid (Figs. 1 to 3). Weblike processes, probably representing cytoplasmic extensions, are occasionally noted in this halo area. To some extent the halos suggest basal vacuolization to an extreme degree, but little evidence of such a classical pattern exists, and they are hardly comparable to the basal vacuolization seen in other entities, such as carbon tetrachloride nephrosis. There is no convincing evidence of any stages of the intracellular vacuolization generally described in the osmotic nephroses of hypertonic sucrose, glucose, sorbitol, or urea, or of potassium deficiency. The halo formation appears morphologically to be an extracellular phenomenon separating cell membranes from basement membranes. Involvement of the proximal and distal convoluted tubules removes this lesion from the realm of artifact, inasmuch as shrinkage in this area of the nephrons, in the absence of obvious autolysis, is not analogous to that observed in other tubular areas on a generally recognizable artifactual basis in many kidneys. In 1 infant (A-58), focal anemic infarction of a kidney was associated with thrombosis of a small artery. Small vascular thrombi were noted in this kidney when the case was reviewed at the Forensic Pathology Branch of the Armed Forces Institute of Pathology. Renal infarction has been found in salt poisoning in swine (Smith).[10]

Another phenomenon involving the kidney is the presence of amorphous acidophilic casts in the collecting tubules. This was reported by Luttrell and Finberg[5] and is illustrated by an excellent photomicrograph in their paper. In the Binghamton infants, these casts were present to a remarkable degree in A-58 (Fig. 4) and were subsequently noted on review at the Forensic Pathology Branch of the Armed Forces Institute of Pathology in the kidneys of A-65 and A-66.

Acute suppurative pneumonitis to some degree was found microscopically in 5 of the 6 infants involved. In the infant with terminal fibrocystic disease of the pancreas, no pneumonic process was present in the emphysematous lungs. In 2 (A-57 and A-65), there was extensive pneumonitis, with confluent bronchopneumonic areas surrounding vascular channels. In 1 of these (A-57), an acute necrotizing arteritis, possibly analogous to that reported after ingestion of large amounts of sodium chloride in rats by Toussaint and colleagues,[12] was demonstrable (Fig. 5). In 3 others, the polymorphonuclear infiltration involved chiefly atelectatic areas, and it was lobular or microfocal in distribution. No thromboses were found in the pulmonary vessels, although some were observed in the extracapsular tissues of the thymus. Hemorrhagic areas were common in many lobules of lung; sometimes they approached, but did not fully attain, the appearance of infarcts.

The presence of inflammatory reactions in atelectatic areas, especially in those surrounding large vessels, makes it unlikely that the pneumonias originated from inhalation. More probably, the more severe forms at least are related in etiology to hypertonic vascular centers, especially those that follow damage to the walls of blood vessels.

Well defined formation of hyaline membrane was noted in 2 of the infants to whom oxygen had been administered; it is regarded as a not unusual iatrogenic finding in infants who have received such therapy. Inhalation of amniotic fluid as an incidental occurrence was noted in 2.

Just as there is no gross lesion that is pathognomonic of salt poisoning, likewise there is no consistent diagnostic clinical symptom or sign. Lung involvement may give rise to a diagnosis of pneumonia. Muscle twitching and convulsive disorders may arouse suspicion in an epidemic outbreak, but in this epidemic, as in sporadic cases like those reported by Finberg[1] and by Scott and Rotondo,[9] the diagnosis was made only after fortuitous discovery of the accidental administration of salt, whereupon an investigation for an electrolyte disturbance revealed high serum sodium and chloride levels. More often a calcium ion disturbance is suspected.

Recently we have noted an idiopathic renal lesion of salt injury in a 7-month, premature twin that came to autopsy some 6 weeks after the salt poisoning episode

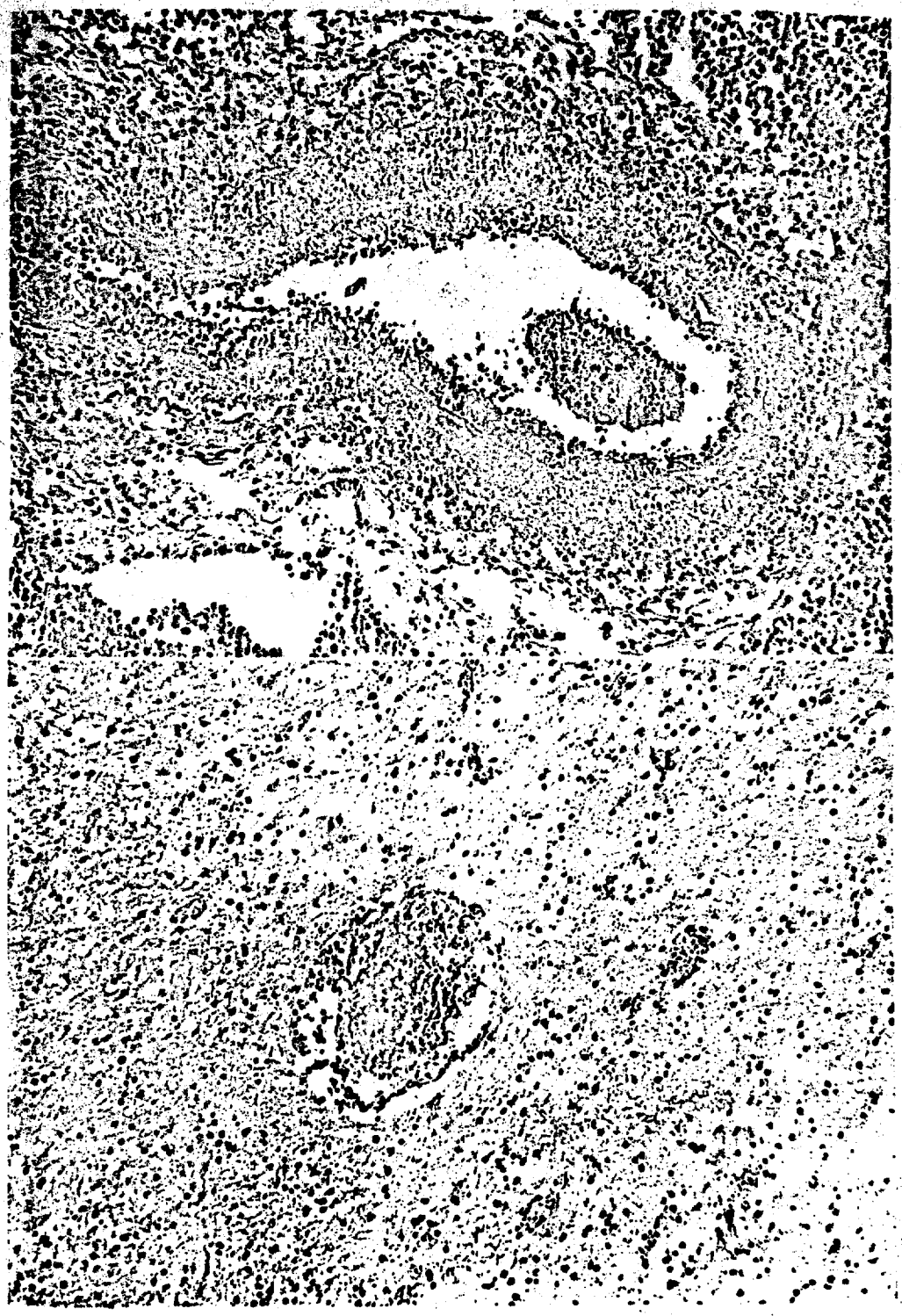

FIG. 5 (upper). Necrotizing arteritis, lung, of A-57. Hematoxylin and phloxin. × 200
FIG. 6 (lower). Partial cuffing of engorged cerebral vessel by lymphocytes in A 57. Eosinophils, which are generally present in this type of infiltrate in swine, have not been noted in human beings. Hematoxylin and phloxin. × 200.

(A-90). This infant, born on April 20, 1962, was the second of twin boys, and he died on April 23. The first twin, born 9 min. earlier, was delivered normally and developed normally. The second was born by breech assist after premature separation of its own separate placenta had occurred. His initial condition was poor; he had labored respirations and was put in an isolette with high humidity and oxygen. Subcostal retractions and tachypnea developed on the third day, and there was poor color and gasping prior to death. At autopsy, the weight of the infant was 1240 Gm. Formation of hyaline membrane (caused by administration of oxygen) was extensive, as was atelectasis (both lungs sank in water). Microscopically the renal lesion of sodium imbalance found was similar to that in Figure 3, but electrolyte studies were not made.

A review of a series of 16 autopsies performed on premature infants at the Binghamton General Hospital during the past 2 years did not reveal a comparable lesion, although equivocal evidence of slight shrinkage of the convoluted tubules seems to be observed occasionally. In another similar review of 18 neonatal deaths of full term infants, occurring up to the age of 3 months, some evidence of shrinkage of convoluted tubules was observed in 2. One of these was a crib death of an infant with a 40-Gm. thymus and partial atelectasis at the age of 8 weeks. The other was a death at the age of 3 weeks, due to Staphylococcus sepsis after exfoliative dermatitis, and the myocardium in this case exhibited formation of microabscess. In neither case was the lesion impressive, however, in either hematoxylin-eosin or periodic acid-Schiff preparations.

Reviews of the autopsy material from the 6 infants involved in the salt-poisoning epidemic and of the additional intercurrent death from hemolytic disease that served as a normal control for the kidney lesion have now been completed in 2 reference laboratories: the Division of Laboratories and Research, New York State Department of Health, Albany, New York, and the Forensic Pathology Branch, Armed Forces Institute of Pathology, Washington, D. C. The protocols and a complete set of wet blocks of tissue have also been sent to the New York State Veterinary College, Cornell University, Ithaca, New York, for comparative pathologic studies in farm animals and wild life. This procedure was deemed advisable because of the worldwide interest in the Binghamton episode.

In general, there has been concurrence with the concepts presented herein, and this policy of review through normal channels of communication in pathology has borne very satisfactory results, as well as notable supplemental findings. Further findings may still be made as these groups continue studies on their own initiative.

The shrinkage of the convoluted tubules in the kidney may be regarded by some as a possible artifact, but inasmuch as it has been a consistent finding in the Binghamton infants and is found in an area of the kidney that is physiologically involved in the resorption of water and chlorides, and as such a pattern has already been noted by Luetscher and Blackman[4] as a specific indication of salt injury in adults with sulfathiazole reactions, it seems reasonable to assume that it is a characteristic lesion. In cases A-57, A-58, and A-59, this lesion was so spectacularly developed in the absence of microscopic evidence of autolysis that artifactual production seemed quite out of the question.

MECHANISM OF SALT POISONING—A WORKING HYPOTHESIS

At first, the pathology of acute salt poisoning seems to involve several seemingly unrelated phenomena. Notable effects are: (1) engorgement of capillaries and vascular channels throughout the body; (2) distention of intracerebral capillaries and those of the pia-arachnoid, with a tendency to overdistention; (3) intravascular thrombosis; (4) suppurative pneumonitis; (5) necrotizing vasculitis; and (6) parenchymatous dehydration, conspicuously demonstrable in the convoluted tubules of the renal cortex.

The basic mechanism of all of these processes seems to be osmolar in nature. The excess of sodium ions occurs largely in the vascular system, where 5 per cent of the body weight consists of water, and in the tissue fluid, where another 15 per cent of the

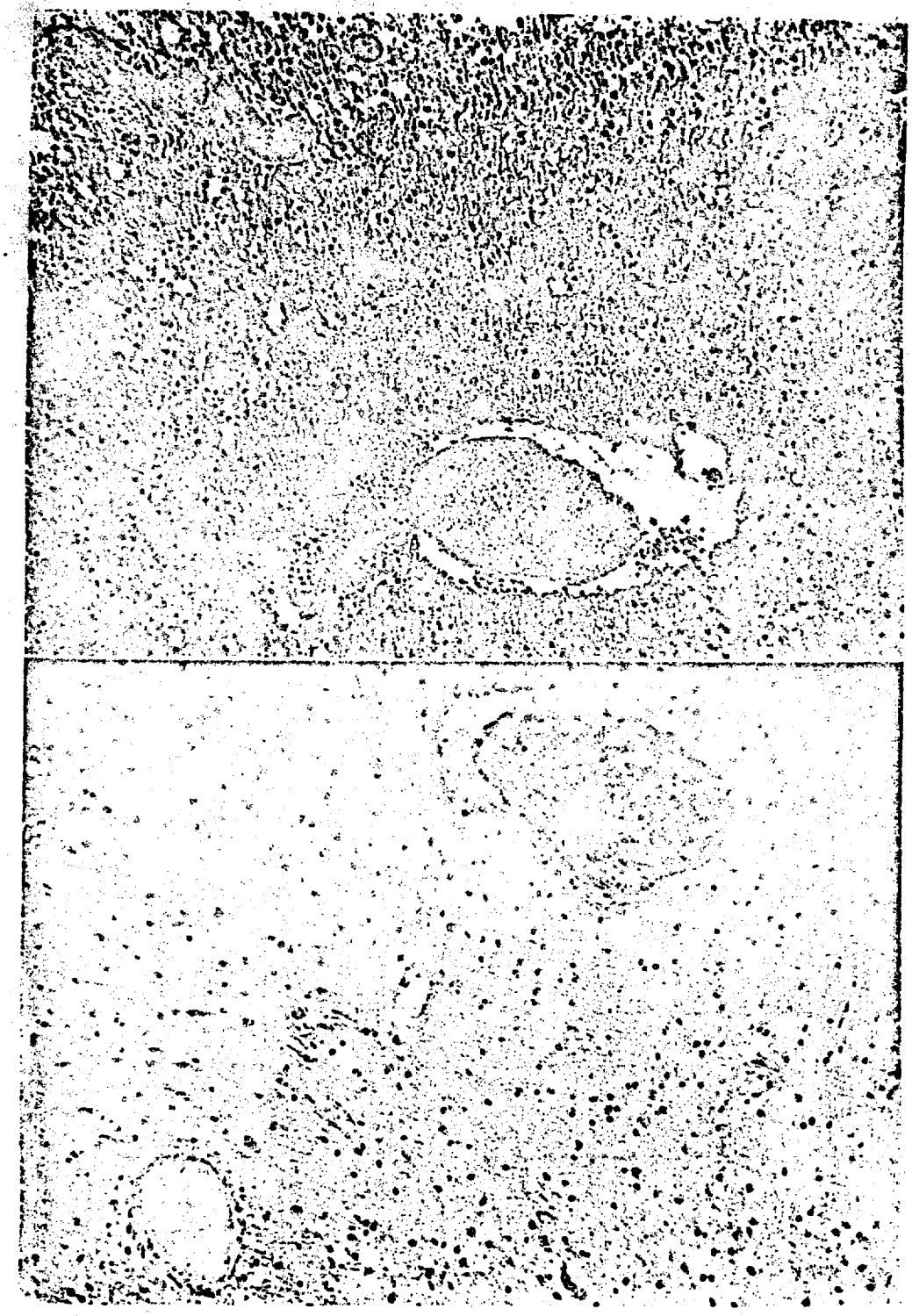

FIG. 7 (upper). Perivascular hemorrhage, brain, in A-57. Hematoxylin and phloxin. × 200
FIG. 8 (lower). Distended capillary and thrombosed capillary, brain, in A-58. Hematoxylin and phloxin. × 200.

body weight is water. When this extracellular 20 per cent of the body weight becomes hypertonic, these areas will draw water by osmosis from the great intracellular water reservoirs that constitute 50 per cent of the body weight. This phenomenon thus would account for the ballooning of capillaries in the central nervous system, especially in the pia-arachnoid, where support is anatomically weak and the surrounding medium is cerebrospinal fluid. Eventual rupture of some of the capillaries could occur at times, with resultant subarachnoid and intraventricular hemorrhage. Thrombosis seems to develop in channels in which vascular stasis occurs in a hypertonic plasma, and it can induce infarctions, as in the kidney and cerebellum, or occlude the lumens in a segmental manner, leading to engorgement proximal to such occlusions. In the central nervous system, thrombosis and engorgement may be seen simultaneously in contiguous vessels.

The pneumonitis, when fully developed to the stage of confluent bronchopneumonia, seems to encircle blood vessels, especially when they are involved in a necrotizing arteritis and is probably a response to the proximity of hypertonic vascular channels in the lung.

The dehydration shrinkage of the convoluted tubules of the nephrons involves an area physiologically concerned with maintenance of water and salt balance in the mammalian organism and is consistent with a morphologic manifestation of parenchymatous dehydration by the action of hypertonic extracellular fluid that contains an excess of sodium ions.

SUMMARY

Pathologic studies of the autopsy material available from 6 infant fatalities that resulted from the ingestion of a formula in which salt was inadvertently substituted for sugar and that occurred at the Binghamton General Hospital in March 1962 have revealed a distinctive microscopic lesion of the kidneys as evidence of salt injury to this organ. This lesion consists of a specific shrinkage of the tubular complexes in the zones of the convoluted tubules of the cortex. Shrunken tubules are surrounded by a clear, slightly webbed zone that separates the tubular cell membranes from their basement membranes. This clear zone is apparently the site of hyperosmolar tissue fluid that has induced a remarkable degree of parenchymatous dehydration.

Damage to the central nervous system is manifested by a high frequency of subarachnoid and intraventricular hemorrhage. Thromboses may involve the sinuses, especially the sagittal sinus. More commonly, however, capillary thromboses are found throughout the brain substance, particularly the white matter, and some have a tendency to develop minute foci of interstitial hemorrhage and focal encephalomalacia. An occasional capillary may manifest lymphocytic cuffing, as in the encephalitides. These lesions of the central nervous system have already been reported, in the literature of both human and veterinary medicine, as characteristic findings in hypernatremia.

A suppurative and hemorrhagic pneumonic process was found in all 5 of the newborn, but was absent in an infant of 8 months that also had a terminal lesion of cystic fibrosis of the pancreas. The suppurative reaction in the lungs occasionally was seen as a broad zone of confluent bronchopneumonia surrounding larger blood vessels and suggestive of a response to stasis in hypertonic vascular foci. In 1 infant, branches of the pulmonary artery manifested a classic necrotizing arteritis. In some lung fields in several cases, the pneumonic process was primarily hemorrhagic; it simulated, but was not typical of, early infarction. No thrombi were demonstrated in the lungs.

SUMMARIO IN INTERLINGUA

Studios pathologic del materiales necroptic disponibile ab 6 infantes morte como resultato del ingestion de un preparato dietari in le qual sal habeva accidentalmente essite substituite pro sucro al Hospital General Binghamton in martio 1962 ha revelate un distinctive lesion microscopic del renes como evidentia del effectos nocive de sal in iste organo. Iste lesion consiste de un specific contraction del complexos tubular in le zonas del convolvite tubulos del cortice

FIG. 9 (upper). "Balloon" type of engorgement of capillary in leptomeninges of A-57. Hematoxylin and phloxin. × 200.

FIG. 10 (lower). Vascular thrombosis, subependymal, brain of A-65. Hematoxylin and phloxin. × 200.

Le contractionate tubulos es circumdate de un levemente membranate zona clar que separa le membranas cellular tubular ab lor membranas basilari. Iste zona clar es apparentemente le sito de liquido tissular hyperosmolari que ha inducite un remarcabile grado de dishydratation parenchymatose.

Lesionage del systema nervose central es manifeste in le alte frequentia de hemorrhagia subarachnoide e intraventricular. In certe casos, le sinos es afficite de thromboses, specialmente le sinus sagittal. Tamen, il es plus commun que thromboses capillari es trovate in omne partes del substantia cerebral, particularmente le substantia blanc. Il occurre que tal thromboses tende a disveloppar minuscule focos de hemorrhagia interstitial e encephalomalacia focal. Occasionalmente un vaso capillari manifesta cincturation lymphocytic del genere incontrate in le encephalitides. Iste lesiones del systema nervose central es jam reportate in le litteratura de medicina tanto human como etiam veterinari como constatation typic de hypernatriemia.

Un suppurative e hemorrhagic processo pneumonic esseva trovate in omne le 5 neonatos sed esseva absente in un infante de 8 menses de etate qui habeva un lesion terminal de fibrosis cystic in le pancreas. Le reaction suppurative in le pulmones esseva vidite occasionalmente como un large zona de confluente bronchopneumonia al intorno de plus grande vasos de sanguine e suggerente un responsa a stase in hypertonic focos vascular. In 1 del infantes, brancas del arteria pulmonari manifestava un classic arteritis necrotisante. In plure casos le processo pneumonic in areas particular esseva primarimente hemorrhagic. Isto stimulava infarcimento sed non esseva typic de infarcimento precoce. Nulle thrombos esseva demonstrate in le plumones.

REFERENCES

1. Finberg, L.: Pathogenesis of lesions in the central nervous system in hypernatremic states. I. Clinical observations of infants. Pediatrics, **23**: 40–45, 1959.
2. Finberg, L., Luttrell, C. N., and Redd, H.: Pathogenesis of lesions in the nervous system in hypernatremic states. II. Experimental studies of gross anatomic changes and alternations of chemical composition of the tissue. Pediatrics, **23**: 46–53, 1959.
3. Johnson, A. S.: Discussion of paper by W. J. Schatz. M. Rec. & Ann., **145**: 491, 1937.
4. Luetscher, J. A., Jr., and Blackman, S. S., Jr.: Severe injury to kidneys and brain following sulfathiazole administration: high serum sodium and chloride levels, and persistent cerebral damage. Ann. Int. Med., **18**: 741–756, 1943.
5. Luttrell, C. N., and Finberg, L.: Hemorrhagic encephalopathy induced by hypernatremia. I. Clinical, Laboratory and Pathological Observations. A. M. A. Arch. Neurol. & Physchiat., **81**: 424–432, 1959.
6. Luttrell, C. N., Finberg, L., and Drawdy, L. P.: Hemorrhagic encephalopathy induced by hypernatremia. II. Experimental observations on hyperosmolarity in cats. A. M. A. Arch. Neurol., **81**: 153–160, 1959.
7. Miller, N. L., and Finberg, L.: Peritoneal dialysis for salt poisoning. Report of a case. New England J. Med., **263**: 1347–1350, 1960.
8. Schatz, W. J.: Treatment based on physical principles followed by recovery in sodium chloride poisoning. M. Rec. & Ann., **145**: 487–491, 1937.
9. Scott, E. P., and Rotondo, C. C.: Salt intoxication: accidental ingestion of a large amount of sodium chloride. Report of a case with autopsy of a 2-year-old patient. Kentucky M. J., **45**: 107–109, 1947.
10. Smith, D. L. H.: Sodium salt poisoning in swine. Thesis for doctorate in philosophy, Cornell University Graduate School, September 1955.
11. Smith, H. A., and Jones, T. C.: Veterinary Pathology. Ed. 2. Philadelphia: Lea & Febiger, 1961, pp. 594–595.
12. Toussaint, C., Wolter, R., and Sibille, P.: Effects de l'ingestion de grandes quantités de chlorure de sodium chez le rat. Compt. rend. Soc. biol., **147**: 1637–1639, 1953.
13. Trainer, D. O., and Karstad, L.: Salt poisoning in Wisconsin wild life. J. Am. Vet. M. A., **136**: 14–17, 1960.

Food and Nutrition Board, National Research Council
National Academy of Sciences. 1968

Recommended Dietary Allowances, 7th ed.

Natl. Res. Counc., Natl. Acad. Sci., Washington, D.C.
pp. 63-64

Food Protection Committee. 1972

Comprehensive GRAS Survey--NAS/NRC 1972 Table 13
Part A--Possible Daily Intakes of NAS Appendix A Substances (Groups I and II), per Food Category and Total Dietary, Based on Food Consumption by Total Sample

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

SAFETY AND SUITABILITY OF SALT

FOR USE IN BABY FOODS

Subcommittee on Safety and Suitability of
MSG and Other Substances in Baby Foods

Food Protection Committee

Food and Nutrition Board

National Research Council

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National Academy of Sciences

September 1970

PREFACE

The National Academy of Sciences was requested by the U.S. Food and Drug Administration to provide advice on the safety and suitability of monosodium glutamate (MSG), salt, and modified starches for use in baby foods. A special advisory committee, under the general direction of the Food Protection Committee, has reviewed and evaluated information concerning salt, and prepared this report under terms of contract CPF 70-10.

Subcommittee on Safety and Suitability of MSG and Other Substances in Baby Foods

Lloyd J. Filer, Jr., Chairman

Pierre M. Dreyfus

Kenneth P. DuBois

Lloyd W. Hazleton

George M. Owen

Virgil B. Robinson

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EVALUATION

We recommend that no more than 0.25 percent salt be added in the commercial preparation of an infant food. This level of salting will result in sodium contents ranging from about 1 to 40 mEq/100 Kcal, depending on the food product. No increases should be made in salting those foods, such as fruits that are now generally acceptable as currently prepared with less than 0.25 percent added salt.

There appears to be no physiologic reason for totally excluding added salt from infant foods, and there is no good physiologic basis for prescribing a precise level of salting. The range of physiologic safety is great, and there is no evidence that a level of salt intake in infancy within this range is associated with later development of any disease state, including hypertension. On the other hand, there is little evidence that added salt is of any benefit to the infant. It may well be that added salt does no more than make infant foods acceptable to mothers.

Addition of moderate amounts of salt to commercially prepared infant foods might, however, serve to control total salt intake of the infant in the sense that totally unsalted food may well be so unacceptable to the mother that she will add salt to her taste, whereas lightly salted foods might be fed without further salting. Obviously, the goal of limiting total salt intake of infants can be achieved by lowering the level of added salt in commercial infant foods only if mothers are somehow persuaded not to add salt themselves.

PATTERNS OF CONSUMPTION

In many areas of the world, infants are breast-fed during the first six to nine months of life and receive little or no solid foods. This was true in the United States 50 years ago; however, there have been extensive changes in feeding patterns with the advent of evaporated milk and later of commercially processed formulas and foods for infants. In reviewing trends in infant feeding, Fomon points out that there have been a dwindling proportion of infants who are breast-fed, increasing numbers fed commercial formulas, and a progressively earlier introduction of solid foods into the diet[1]. Guthrie has demonstrated that infants who begin to ingest solid foods during the first month after birth have consistently higher intakes of sodium at later ages than do infants who begin to consume solids at later ages[2]. It is not known, however, whether there is any relationship between the early introduction of solid foods and the development of a preference for foods with a higher content of salt during childhood or adult life.

Human milk contains 0.5 to 0.6 mEq sodium/100 Kcal, and infants meeting their protein and calorie needs from breast milk obtain about 1 mEq of sodium/kg/day. Most infants in the United States are fed on modified cow's milk formulas, with solid foods being introduced into the diet in the first or second month of life (Guthrie[2]; Puyau and Hampton[3]). By the fourth month, solid foods provide about 20 percent and cow's milk 80 percent of total caloric

intake. The average sodium intake on this type of diet is about 30-35 mEq/day, or about 5 mEq/kg body weight/day (see Table 1). In general, no salt (sodium chloride) is added in the commercial preparation of strained fruits and juices. Most strained meats, egg yolks, "dinners", "breakfasts," and soups contain about 0.4 to 0.6 percent added salt, strained vegetables and desserts from 0 up to 0.4 percent, and dry cereals about 1.0 percent.

A recent survey[4] made to determine the sodium intake of infants reveals that the sodium content of the diet (formula and supplementary infant foods) increases during the first year of life from about 2.2 to about 5.7 mEq/100 Kcal (at the 50th percentile of intake). The total sodium intake increases from about 9 to 60 mEq per day. In contrast, if the child satisfied all his calorie needs from food with the same sodium-calorie relationship as that of human milk, his total sodium intake would increase only from 2.6 to 6.5 mEq per day during this period. In this survey of 364 infants 1 to 12 months of age, eight infants ingested more than 82 mEq of sodium per day. Two of these infants were less than six months of age. The maximum intake recorded was 101 mEq sodium per day. In another survey of 2,160 six-month-old infants selected on a random basis so as to provide a sample compatible with U.S. Vital Statistics, the median daily intake of sodium was 35 mEq with a standard deviation of 15. Thus the upper limit of intake for 97 percent of the sample does not exceed 65 mEq of sodium per day[5].

REQUIREMENTS

Although it is difficult to define the sodium requirement of the infant, it is possible to specify with some confidence the needs for growth (1 to 1.5 mEq/day) and to assess average losses from the skin and stool (1 to 2 mEq/day[6]). Cooke et al. showed that concentrations of sodium in sweat of normal infants (5 to 16 months old) ranged from 1.8 to 7.6 mEq/l, with an average of 4.8[7]. At an environmental temperature of 91° F, calculated volume of sweat was between 45 and 65 ml/kg/day[4]. The maximum sodium a five-month-old infant (7 kg) would lose by this route would be 3.5 mEq/day. Under more temperate conditions the loss would be about 1 mEq/day. Total needs, thus, are about 2-5 mEq/day.

During minor illnesses of the infant, the diet is often substantially changed both in quantity and composition. Depending upon the nature of the illness and magnitude of extrarenal losses of sodium and of water--because of fever, hyperventilation, vomiting, diarrhea, etc.--there will be considerable changes in requirements of both sodium and water.

RENAL SOLUTE EVALUATION

With the current patterns of feeding commonly practiced in the United States, one must be concerned with the capacity of the infant to excrete the sodium load with which he is confronted. The four-month-old infant consuming cow's milk and a combination of commercially prepared

baby foods ingests five times as much sodium as the breast-feeding infant; virtually all the excess is excreted in the urine.

The infant can ordinarily handle an acute load of sodium not exceeding 250 mEq/m^2/day, assuming there is sufficient water available[8]. It would appear that virtually any combination of milk and infant food that would meet the infant's calorie needs could be tolerated from the standpoint of sodium load. The highest levels of sodium intake noted by Fomon et al. and by Puyau and Hampton in their respective studies were between 50 and 60 mEq/day[9,3]. On an acute loading basis, these intakes would amount to no more than 175 mEq/m^2. However, those combinations of foods that impose the highest load of sodium may also impose large loads of other solutes requiring renal excretion[10].

SALT AND HYPERTENSION

The evidence associating salt intake and hypertension in man is inconclusive. There are a number of reports correlating average daily salt intake and frequency of hypertension among different cultures[11,12,13,14]. The study of Prior et al.[14], for example, on two Polynesian populations showed that the one with the higher salt intake (120-140 mEq/day) had a higher range of blood pressure than the one with the lower intake (50-70 mEq/day). Dahl[11] has recorded the incidence of hypertension in adult populations of various cultures in relation to salt intake (Table 2). While these

differences are in some measure suggestive, it is possible that other influences--dietary, cultural, social, and genetic factors--may partially account for the differences.

The effect of salt intake on hypertension within defined populations has been shown to be quite variable. Dahl and Love[15] reported a positive correlation between the salt intake and the prevalence of hypertension among 1,346 laboratory workers who were classified as having low (never adding salt to table food), moderate (adding salt to taste), or high (routinely adding salt irrespective of taste) salt intakes. Other evidence supporting the relationship between salt and hypertension is that of Sasaki[16], who showed a positive correlation ($r = 0.67$) between salt intake and the frequency of stroke among middle-aged Japanese. Dietary fat was the only other nutrient that correlated directly with the incidence of stroke ($r = 0.45$). There was, however, an inverse relation between blood pressure and potassium intake.

On the other hand, Paul[17], in a study of some 2,000 men, found that hypertensives preferred soup with little or no added salt, and reported that replies to the same questions on salting habits as those used by Dahl indicated that hypertensives use less salt than normotensives. Ashe and Mosenthal[18] found that the distribution patterns of salt use (as based on excretion) among 298 patients with hypertension and 563 who were normotensive were similar. Five percent of normotensive patients excreted less than four grams of sodium chloride (70 mEq Na) daily in the urine, 37 percent

excreted four to eight grams (70 to 140 mEq Na), and 57 percent more than eight grams daily. Among those with essential hypertension the percentage distribution at these various levels of sodium excretion was 4, 44, and 50 percent respectively. In the studies of Prior et al.[14] and Dawber et al.[19], no significant correlation was demonstrated between salt intake and blood pressure within the populations selected.

McQuarrie et al.[20] reported development of hypertension in a 13-year-old boy with diabetes who developed a craving for salt and ingested 80 grams (or 1,250 mEq Na) per day. Blood pressure returned to normal when the salt intake was lowered to 5 grams per day. They described another patient who became hypertensive when he received 60 grams (1000 mEq) of salt per day, but became normotensive if 20 grams of potassium chloride was given with the sodium chloride.

It is in this context that the question arises whether nutritional experience with sodium in early infancy has any relationship to later development of hypertension. Observations, primarily in rats, indicate that progressive increases in sodium intake by the young animal are associated with increasing blood pressure. Animals receiving between 0.5 and 7 mEq Na/100 Kcal showed no difference in growth, blood pressure, or longevity. Rats fed sodium at levels of between 10 and 20 mEq/100 Kcal developed moderate hypertension, and those fed at levels exceeding 25 mEq/100 Kcal developed severe hypertension[21].

Dahl et al., by selective inbreeding of rats, developed two strains with opposite genetic susceptibility to development of hypertension[22]. One strain was sensitive to sodium intake and showed progressive increase in levels of blood pressure with levels of intake of sodium exceeding 1.5 mEq/100 Kcal. The other strain was resistant to development of hypertension at any level of intake of sodium. It is of considerable interest that the salt-sensitive strain of rats voluntarily restrict their intake of sodium chloride[23].

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

POTASSIUM-SODIUM RELATIONSHIPS

Both in experimental animals and in man subjected to high sodium intakes, there is evidence that potassium, by an unknown mechanism, exerts a protective or ameliorative effect insofar as hypertension is concerned[21]. Some attention may need to be given to the relationship between sodium and potassium intakes of infants not only in this context but also with respect to acid-base balance.

The ability of the infant to excrete a potassium load (acute) is comparable to his ability to excrete a sodium load, i.e., 250 mEq/m^2/day[8].

In studies cited previously[4,5], the highest intake of potassium by normal infants amounted to no more than 200 mEq/m^2/day, while the lowest intake was 30 mEq/m^2/day, and thus they were well within homeostatic limits.

Regulation of sodium and potassium balance is primarily a function of the distal portion of the renal tubule[24]. Potassium and hydrogen ions within the cells of the distal tubule are exchanged for sodium ions within the tubular lumen, thus the rate at which hydrogen and potassium ions are excreted is dependent upon the amount of sodium available for exchange. When sodium excretion is at a low level, the capacity to secrete hydrogen and potassium ions becomes limited. While the exact mechanism for an active cation transport system within the renal tubule of man is yet to be defined, Charnock et al. have identified an ATPase that requires the combination of both sodium and potassium ions for full activation[25,26]. Thus sodium ion concentration in tubular fluid can become rate limiting. There is no reason to believe, however, that the feeding of unsalted infant foods would result in critically low concentrations of sodium within tubular fluid.

In reviewing growth requirements of various species of mammals for sodium and potassium, Shaw and Phillips[27] concluded that Na:K ratio (w/w) was important and that heavy ingestion of one element raised the requirement of the other. For several species these workers estimated the ratio of dietary Na:K was between 0.5 and 0.25. Based on available

information[4] the range of dietary Na:K ratios of infants receiving formula, cow's milk, and infant foods extends from 2.0 to 0.25. (From Table 3, one could hypothesize that the breast-fed infant consuming one liter of human milk and one jar of fruit juice daily would have an intake of Na:K of about 150:700, approximately 0.22.)

A group of 150 normal infants fed human milk as the major source of calories, but with some calories provided as wet packed cereal or fruits, demonstrated normal growth and serum chemistries during the first four months of life[28]. This group of infants represents that population exposed to a diet relatively low in Na^+ and relatively high in K^+. The opposite divergent situation, i.e., that of high Na^+ intake and low K^+, would rarely occur in a natural setting simply because those foods that ordinarily supply Na also provide K.

CONTROL OF SALT IN THE INFANT DIET

Dahl[29] has stated, "Because it is common practice for mothers to assay baby food by tasting it, the mother's palate must be satisfied. Her palate, nurtured from birth to motherhood on the high NaCl content of most processed foods, would find unsalted vegetables and meat unpalatable." If we accept the proposition that infant foods are seasoned primarily to satisfy the mother's taste, then the addition of limited amounts of salt to processed infant foods may well be recommended, even though there is no good basis for recommending any given level.

In Table 4, the sodium contents of a variety of infant foods as currently marketed, if marketed with no added salt, or if marketed at a level of added salt not to exceed 0.25 percent (0.1 percent added sodium), are compared. Using the data of Fomon et al.[9], it can be calculated that the maximum sodium intake for a four-month-old breast-fed infant given solid foods was 36 mEq/day. If cow's milk replaced breast milk, the sodium intake would be 42 mEq/day. If unsalted infant foods were substituted for salted infant foods, the daily sodium intake would be 10-16 mEq/day. If infant foods with limited salt addition (0.1 percent added sodium) were used, daily sodium intake would be reduced by 17 to 34 percent depending upon the source of milk. The maximum sodium intake for the seven-month-old infants studied by Fomon et al.[9] was 58 mEq/day. If infant foods containing added salt at 0.25 percent were substituted for infant foods as currently salted, a reducting in sodium intake of 33 to 46 percent can be calculated.

In essence, the committee finds no evidence that, insofar as healthy infants are concerned, addition of sodium chloride at current levels to infant foods, in order to increase acceptability of the food (to the mother), is either harmful or beneficial to the infant. There is no valid scientific evidence available in support of the contention that addition of salt to infant foods contributes to development of hypertension or other disease state in adult life, or that salt levels now consumed by infants in the United States

overburden excretory mechanisms. Present salt intakes, however, provide substantially more sodium than is required by the infant. Thus, there is some reason for attempting to limit total intake. It seems likely, however, if commercial infant foods were totally unsalted, that the foods would be salted to the mother's taste--resulting in variable and uncontrolled salt consumption. It seems reasonable, therefore, to recommend that manufacturers add some salt to certain infant foods--particularly those that would most probably be salted by the adult--and that the upper level of added salt in such foods be 0.25 percent. No increases should be made in salting those foods, such as fruits that are now generally acceptable as currently prepared with less than 0.25 percent added salt.

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

TABLE 1

SODIUM CONTENT OF SOME INFANT FOODS

Food	Kilocalories/ 100 ml or 100 g[1]	Sodium		
		mg/100 g	mg/100 Kcal	mEq/100 Kcal (approx.)
Cow's Milk	70	58[2]	83	3.5
Cereal (dry)	308	400[3]	130	6
Meat	84	250[3]	300	15
Vegetables	24	250[3]	1000	44

(1) Calculated from G.H. and E.W. McHenry (Eds.) 1966. Nutrition, Vol. III, Table V. p. 33. Academic Press, New York.

(2) H.J. Heinz Co. 1965. Heinz Handbook of Nutrition, p. 173.

(3) Average data, provided by manufacturers of infant foods.

TABLE 2

CORRELATION OF HYPERTENSION WITH SALT INTAKE

Group	Average Salt Intake gm/day	% with Hypertension B.P. > 140/90
Alaskan Eskimo	4	0
Marshall Islander	7	6.9
United States	10	8.6
Japan (Southern)	14	21
Japan (Northern)	26	39

Adopted from Dahl[8]

TABLE 3

SODIUM AND POTASSIUM CONTENT OF REPRESENTATIVE INFANT FOODS AND FORMULAS

Type of Food	Sodium	Potassium	Na/K Ratio
	mg/jar		
Juices	5	156	0.03
Fruits	37	109	0.34
Meats	212	184	1.15
Vegetables	290	218	1.33
Desserts	177	101	1.75
Cereals	143	74	1.93
High Meat Dinner	388	152	2.56
Dinners	342	101	3.39
	mg/L		
Breast Milk	150	550	0.27
Cow's Milk	410	1810	0.23
Commercial Formulas reconstituted to 20 cal/oz	253	800	0.32

TABLE 4

SODIUM CONTENT OF REPRESENTATIVE INFANT FOODS
Salted at Present Levels, Unsalted, and with 0.25 Percent Added Salt

Variety of Food	Sodium mEq/100 Kcal		
	Salted	Unsalted	0.25% NaCl added
Mixed Cereal with apples and bananas	6.1	0.3	5.2
Peaches*		0.5	
Applesauce*		0.9	
Bananas	3.1	0.4	1.0
Vegetables and Beef	36.0	10.7	18.9
Carrots	52.5	12.2	44.0
Green Beans	35.8	0.8	1.9
Chicken and Vegetables	12.9	1.6	5.3

Calculated from Fomon et al. J. Pediat. 76:242, 1970, and data supplied by H. J. Heinz Co.

*Not salted as generally produced

12. Annotations: Salt and blood-pressure. 1969 (Feb. 8). Lancet 1:295.

13. Isaacson, L.C., M. Madlin, and W.P.U. Jackson. 1963 (April 27). Sodium intake and hypertension. Lancet 1:946.

14. Prior, I.A.M., J.G. Evans, H.P.E. Harvey, F. Davidson, and M. Lindsey. 1968. Sodium intake and blood pressure in two Polynesian populations. N. Engl. J. Med. 279:515.

15. Dahl, L.K., and R.A. Love. 1957. Etiological role of sodium chloride intake in essential hypertension in humans. JAMA 164:397.

16. Sasaki, N. 1962. High blood pressure and the salt intake of the Japanese. Jap. Heart J. 3:313.

17. Paul, Oglesby. In discussing: George Meneely. The experimental epidemiology of sodium chloride toxicity in the rat in The Epidemiology of Hypertension - Proceedings of an International Symposium, p. 248. Jeremiah Stamler, Rose Stamler, Theodore N. Pullman, eds. Grune and Stratton, Chicago. 1967.

18. Ashe, B.I., and H.O. Mosenthal. 1937. Protein, salt and fluid consumption of 1,000 residents of New York. JAMA 108:1160.

19. Dawber, T.R., W.B. Kannel, A. Kagan, R.K. Donabedian, P.M. McNamara, and G. Pearson. Environmental factors in hypertension in The Epidemiology of Hypertension - Proceedings of an International Symposium, p. 255. Jeremiah Stamler, Rose Stamler, Theodore N. Pullman, eds. Grune and Stratton, Chicago, 1967.

20. McQuarrie, I., W.H. Thompson, and J.A. Anderson. 1936. Effects of excessive ingestion of sodium and potassium salts on carbohydrate metabolism and blood pressure in diabetic children. J. Nutr. 11:77.

21. Meneely, G.R., and C.O.T. Ball. 1958. Experimental epidemiology and chronic sodium chloride toxicity and the protective effect of potassium chloride. Amer. J. Med. 25:713.

22. Dahl, Lewis K., Martha M. Heine, Loraine Tassinari. 1962. Effects of chronic excess salt ingestion: Evidence that genetic factors play an important role in susceptibility to experimental hypertension. J. Exp. Med. 115:1173.

23. Wolf, G., L.K. Dahl, and N.E. Miller. 1965. Voluntary sodium chloride intake of two strains of rats with opposite genetic susceptibility to experimental hypertension. Proc. Soc. Exp. Biol. Med. 120:301.

24. Berliner, R.A., T. Kennedy, and J. Orloff. 1954. Factors affecting the transport of potassium and hydrogen ions by the renal tubules. Arch. Int. Pharmadyn. Pi. 97:299.

25. Charnock, J.S., and R.L. Post. 1963. Studies of cation transport: I. The preparation and properties of a cation stimulated adenosine-triphosphate from guinea pig kidney cortex. Aust. J. Exp. Biol. Med. Sci. 41:547.

26. Charnock, J.S., A.S. Rosenthal, and R.L. Post. 1963. Studies of the mechanism of cation transport: II. A phosphorylated intermediate

in the cation stimulated enzymatic hydrolysis of adenosine-triphosphate. Austr. J. Exp. Biol. Med. Sci. 41:675.

27. Shaw, R.L., and Paul M. Phillips. 1953. The potassium and sodium requirements of certain mammals. Lancet 73: 176.

28. Fomon, S.J., R.J. Filer, Jr., L.N. Thomas, and R.R. Rogers. 1970. Growth and serum chemical values of normal breast-fed infants. Acta. Paed. Scand. Suppl. 202.

29. Dahl, Lewis K. 1968. Letter to the editor. Nutr. Rev. 26:125.

Effect of Chronic Salt Depletion on Blood Pressure of Renal Hypertensive Dogs[1]

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THE ROLE of sodium in the development and maintenance of hypertensive states remains obscure. The importance of this problem is emphasized by recent demonstrations that subtle alterations in sodium metabolism occur in association with the development of high blood pressure (1–3). Moreover, low sodium dietotherapy is of undoubted efficacy in many cases of human hypertension (4). Experimental attempts to elucidate the mechanism of the depressor response to low sodium diets have been limited, because renal hypertensive dogs show no fall in blood pressure on such regimens (5, 6). In view of these contradictory observations on the effects of low sodium dietotherapy in man and dog, further studies were undertaken in renal hypertensive animals.

METHODS AND RESULTS

Experiment 1—Therapy of Benign Renal Hypertension[5] With Low Na Diet.[6] Seven dogs with renal hypertension of 4 to 22 months' duration failed to exhibit any fall in blood pressure in response to Lonalac feeding for from 1 to 6 months (table 1).

Experiment 2—Therapy of Benign Renal Hypertension With Low Na Diet Plus Salt Depletion. Five dogs with renal hypertension of ½ to 6 months' duration responded to chronic salt depletion[7] with a significant sustained fall in blood pressure to normotensive levels (table 2). Consistent with the findings in *experiment 1* above (table 1), low Na diet alone failed to elicit a depressor response (*column 4*, table 2). Following an initial peritoneal lavage, which effected a fall in plasma Na level to 130 to 135 mEq/liter (normal 142–150 mEq/liter), blood pressures remained elevated (*column 5*, table 2). However, a second peritoneal lavage, which further lowered plasma Na concentration to 115 to 120 mEq/liter, resulted in a sustained

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[5] Hypertension had been previously produced in these animals by bilateral partial ligation of both renal arteries with linen ligatures (7). All blood pressures (femoral artery) were recorded two to four times weekly on unanesthetized animals, utilizing the Hamilton manometer (8).

[6] Throughout these experiments, the low Na diet used was Mead Johnson's Lonalac. We are grateful to Dr. Warren M. Cox of Mead Johnson & Company, Evansville, Ind., who supplied generous quantities of Lonalac for these experiments.

[7] Salt depletion was accomplished by intraperitoneal injection of 1 liter of 5 per cent glucose in water (9); 3 to 4 hours later this was removed by means of a perforated polyethylene tubing inserted under local anesthesia via an expansile needle (10). Thereafter salt depletion was chronically maintained by exclusive feeding of Lonalac. 168: 500-503

reduction of blood pressure to normotensive levels (*column 6*, table 2). The hyponatremic state was associated with weight loss, lethargy and anorexia; one animal died during this period. When the four surviving dogs were returned to a normal *ad libitum* diet, rapid recovery occurred; blood pressures rose from the normotensive levels attained during Na depletion (*column 7*, table 2).

TABLE 1. EFFECT OF LOW Na DIET ON BLOOD PRESSURE [1] OF RENAL HYPERTENSIVE DOGS

1	2	3	4	5					
DOG NO.	SEX	DURATION OF HYPERTENSION	PRE-LONALAC BLOOD PRESSURE	BLOOD PRESSURES WITH LONALAC DIET					
				1[2]	2	3	4	5	6
		months	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
Z 13....	♀	22	215/120	220/120					
Z 19....	♀	22	190/115	185/115					
Z 77....	♀	6	230/125	235/130					
Z 157...	♀	4	210/125	215/125	170/110	175/115	185/115	190/115	220/100
Z 158...	♂	4	225/140	265/150	255/155	225/150	210/130	225/130	210/110
Z 160...	♂	4	190/115	165/110	180/110	180/130	200/115	200/115	195/110
Z 189...	♀	—[3]	—[3]	220/115					

[1] All blood pressures recorded in this and the following tables represent the average figures for blood pressures taken two to four times weekly during the period indicated. [2] Months on diet. On Lonalac prior to Goldblatt operation.

TABLE 2. EFFECT OF LOW SODIUM DIET AND SODIUM DEPLETION ON BLOOD PRESSURE OF RENAL HYPERTENSIVE DOGS

1	2	3	4	5	6	7
DOG NO.	SEX	PRE-LONALAC BLOOD PRESSURE	PRE-Na DEPLETION BLOOD PRESSURE EFFECT OF LONALAC DIET	BLOOD PRESSURE DURING CHRONIC Na DEPLETION, mm. Hg		BLOOD PRESSURE AFTER RETURN TO NORMAL DIET AND RECOVERY FROM Na DEPLETION
				Plasma Na, 130–135 mEq/l.	Plasma Na, 115–120 mEq/l.	
		mm. Hg	*mm. Hg*			*mm. Hg*
Z 158.....	♂	225/140	230/135	220/120	185/95	210/115
Z 160.....	♂	190/115	195/115		165/100	Deceased
Z 185.....	♀	205/125	—[1]	200/110	155/90	170/105
Z 189.....	♀	—[2]	220/115	210/120	170/100	185/100
Z 202.....	♀	205/130	210/125		155/90	190/115
Duration....		½–6 months	1–6 months	2–3 weeks	2–3 weeks	1–3 months

[1] Lonalac feeding was instituted simultaneously with peritoneal lavage. [2] On Lonalac prior to Goldblatt operation.

Experiment 3—Effect of Low Na Diet Plus Salt Depletion on Normotensive Control Dogs. Three normotensive control dogs fed Lonalac for 1 to 3 months showed no blood pressure alteration (*column 3*, table 3). When Lonalac diet was combined with salt depletion, these normotensive dogs (in contrast to hypertensive animals) failed to develop any blood pressure fall. Pressures remained at control levels even after a second peritoneal lavage, which effected a hyponatremia in the range 115 to 120 mEq/liter, (*column 5*, table 3). Moreover, in contrast to the hypertensive ani-

mals, these dogs exhibited no gross deleterious effects as a result of salt depletion. Rather they appeared to be relatively healthy and none died.[8]

Experiment 4—Attempted Prophylaxis of Benign Renal Hypertension With Low Na Diet. This experiment was done on six dogs, with over-all results that are equivocal and hence are being withheld from publication pending further studies.

Experiment 5—Attempted Prophylaxis of Malignant Renal Hypertension With Low Na Diet. In eight dogs, pretreatment with Lonalac diet for 1 to 2 months before operation failed to influence the course of malignant hypertension produced by almost complete ligation of both renal arteries. Despite continued Lonalac feeding postoperatively, blood pressures rose to hypertensive levels; uremia developed rapidly; vascular pathology typical of malignant hypertension was present uniformly at post-mortem examination.

Discussion

These experiments confirm previous observations (5, 6) that a low Na diet does not lower the blood pressure of renal hypertensive animals. However, they demonstrate that severe and chronic sodium depletion (effected by a combination of low Na diet plus peritoneal lavage) definitely reduces the blood pressure of Goldblatt dogs to normotensive levels. Hence it would appear than an adequate sodium concentration in the extracellular fluid is essential for the maintenance of hypertension in the dog, as well as in man and the rat (4, 13).

TABLE 3. EFFECT OF LOW SODIUM DIET AND SODIUM DEPLETION ON BLOOD PRESSURE OF NORMAL DOGS

1	2	3	4	5	6	7
DOG NO.	SEX	PRE-LONALAC BLOOD PRESSURE	PRE-Na DEPLETION BLOOD PRESSURE EFFECT OF LONALAC DIET	BLOOD PRESSURE DURING CHRONIC Na DEPLETION, mm. Hg		BLOOD PRESSURE AFTER RETURN TO NORMAL DIET AND RECOVERY FROM Na DEPLETION
				Plasma Na, 130-135 mEq/l.	Plasma Na, 115-120 mEq/l.	
		mm. Hg	*mm. Hg*			*mm. Hg*
Z 194	♀	165/100	160/90		155/90	160/85
Z 196	♂	175/90	185/100		200/100	190/95
Z 203	♂	175/95	185/100	185/100	200/100	190/90
Duration		1–2 months	1–3 months	2 weeks	3–6 weeks	1–3 months

The mechanism of the depressor response of hypertensive dogs to salt depletion requires elucidation. Work is proceeding in this laboratory on hemodynamic aspects of the chronic depressor response. Danowski *et al.* (14) reported that acute salt depletion in normal dogs effected an early fall in cardiac output, followed by a reduction in blood pressure. Total peripheral resistance rose in their experiments. These results were related to both plasma hypotonicity and hypovolemia. Whether or not similar changes occur with chronic salt depletion is yet to be determined. In any case, the findings of the present experiments suggest that the chronic depressor response is intimately related to the hypertensive process per se, since salt-depleted normotensive dogs manifest no chronic alterations in blood pressure.

[8] The poorer clinical condition of the hypertensive dogs may be due to pre-existing renal damage, further aggravated by the circulatory and metabolic effects of salt depletion. Random determinations of NPN support this possibility inasmuch as during salt depletion NPN elevations were greater in hypertensive than in normotensive animals. Further, in hypertensive man, low Na diet has been shown to lower GFR and RPF (11, 12).

Summary

Chronic severe sodium depletion with hyponatremia reduced blood pressure of renal hypertensive dogs to normotensive levels. Chronic severe sodium depletion with hyponatremia had no effect on blood pressure of normotensive dogs. As previously observed, low sodium diet alone had no effect on blood pressure of renal hypertensive dogs, nor did pretreatment with this diet influence the course of malignant renal hypertension.

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References

1. Perera, G. A. *Bull. New York Acad. Med.* 26: 75, 1950.
2. Bell, E. T. (editor). *Hypertension* (Symposium). Minneapolis: Univ. Minnesota Press, 1951.
3. Stamler, J., W. Hwang and K. Kuramoto. *Am. J. Physiol.* 165: 328, 1951.
4. Watkin, D. M., H. F. Froeb, F. T. Hatch and A. B. Gutman. *Am. J. Med.* 9: 441, 1950.
5. Grollman, H. *Am. J. Physiol.* 147: 647, 1946.
6. Page, I. H. and L. A. Lewis. *Am. J. Physiol.* 156: 422, 1949.
7. Goldblatt, H., J. Lynch, R. F. Hanzal and W. W. Summerville. *J. Exper. Med.* 59: 347, 1934.
8. Katz, L. N., M. Friedman, S. Rodbard and W. Weinstein. *Am. Heart J.* 17: 334, 1939.
9. Schecter, A. J., M. K. Cary, A. L. Carpentieri and D. C. Darrow. *Am. J. Dis. Child.* 46: 1015, 1933.
10. Fishman, A. P. *J. Lab. & Clin. Med.* 34: 584, 1949.
11. Chasis, H., W. Goldring, E. S. Breed, G. E. Schreiner and A. A. Bolomey. *J. A. M. A.* 142: 711, 1950.
12. Weston, R. E., L. Hellman, D. J. W. Escher, I. S. Edelman, J. Grossman and L. Leiter. *J. Clin. Investigation* 29: 639, 1950.
13. Grollman, A. and T. R. Harrison. *Proc. Soc. Exper. Biol. & Med.* 60: 52, 1945.
14. Elkinton, J. R., T. S. Danowski and A. W. Winkler. *J. Clin. Investigation* 25: 120, 1946.

Effect of Sodium Intake on Hypertensive Vascular Lesions in Rabbits

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Summary

Effects of sodium intake on the development of hypertensive vascular lesions were studied in rabbits. Twenty-one animals (group A) were fed a low-sodium diet (7 mEq of sodium/day) and 21 animals (group B) were fed a high-sodium diet (28 mEq of sodium/day). Hypertension was produced by applying a silver clip (0.9 mm in diameter) on the left renal artery. The right kidney was left intact. Animals were killed 7 days after the surgery and hypertensive vascular lesions were examined on microscopic sections. Blood pressure was measured every day and plasma renin activity was measured on the terminal day. The average rise in blood pressure was significantly greater in group B than in group A ($p<0.05$). The vascular lesions were found in 6 (28.6%) of 21 animals fed on a low-sodium diet (group A) and in 13 (61.9%) of 21 animals fed on a high-sodium diet (group B). The difference in incidence between the 2 groups was statistically significant ($p<0.05$). However, the presence or absence of the vascular lesions did not always depend upon either the level of blood pressure or plasma renin activity.

Additional Indexing Words:
Hypertensive vascular lesions Renal hypertension Renin
Sodium intake

THE development of experimental renal hypertension is critically influenced by the amount of dietary sodium. Restriction of sodium intake prevents and increase in sodium intake augments the development of hypertension.[7),13),16)] There is also experimental evidence to show that the amount of sodium intake influences the development of hypertensive vascular lesions. Koletsky[13)] produced hypertension in rats by ligation of the renal arteries or by the figure of eight ligature of the kidney. Increase in sodium intake served to augment hypertension and hypertensive vascular lesions, whereas restriction of sodium intake had the opposite effect. This observation should be confirmed in other species, because the susceptibility to hypertension and hyper-

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tensive vascular lesions may differ among different species. The rat is a species highly susceptible to them. Both hypertension and hypertensive vascular lesions can be produced in the rat by feeding of a high-sodium diet alone.[14] In the present report, we attempted to study effects of a high- and a low-sodium diet on the development of hypertension and hypertensive vascular lesions in rabbits.

Method

Male rabbits weighing 2.0 to 2.5 Kg were divided into 2 groups. Group A consisted of 21 animals which were fed a low-sodium diet containing 7 mEq of sodium and 26 mEq of potassium per 100 Gm, and group B consisted of 21 animals which were fed a high-sodium diet containing 28 mEq of sodium and 26 mEq of potassium per 100 Gm. Sodium content of the high or low sodium diet was a two-fold or a half respectively of that of the standard diet for the rabbit (CR 1, Japan Clea Ltd) which contains 14 mEq of sodium per 100 Gm. Each animal was given 100 Gm of the pellets per day and water ad libitum. All animals took the total amount of pellets offered to them during the observation period, except for the postoperative one or 2 days. After a 2-week control period hypertension was produced by applying a silver clip of 0.9 mm in internal diameter on the left renal artery. The right kidney was left intact. The operative procedure was performed through a small flank incision under sodium pentobarbital anesthesia (30 mg/Kg, iv.). Blood pressure was measured every morning on the central ear artery by an indirect method.[11] All animals were killed by injecting about 150 mg of sodium pentobarbital 7 days after the surgery. Just before sacrifice about 10 ml of blood were withdrawn with a heparinized syringe for the measurement of plasma renin activity. Plasma renin activity was determined by the method of Pickens et al[18] with a minor modification.[7] Values for renin activity were expressed as nanogram angiotensin II amide (Hypertensin, CIBA) equivalents generated per ml of plasma after incubation for 4 hours. Normal range of plasma renin activity measured by this method was previously described.[8] Plasma renin activity ranged from 1.0 to 9.7 ng/ml with an average of 3.1 ± 2.0 (SD) ng/ml in 20 normal rabbits which were fed a standard diet containing 14 mEq of sodium and 26 mEq of potassium per 100 Gm. After sacrifice the specimen of brain, heart, lung, kidney, aorta, stomach and intestine were fixed in formol saline. Paraffin sections were stained with haematoxyline and eosin, Masson's trichrome and Weigert's elastic tissue stain.

Results

Clipping of the left renal artery was followed by a gradual rise in blood pressure in animals of both groups (Fig. 1). During the postoperative 7 days body weight was reduced by 40.8 ± 31.4 (SE) Gm in group A and by 71.4 ± 21.6 Gm in group B. The average of preoperative blood pressure was about the same in the 2 groups, but the increments were significantly greater after the

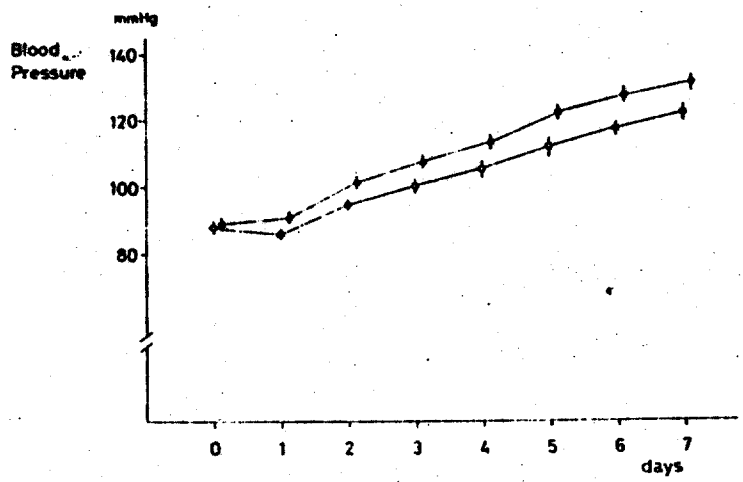

Fig. 1. Blood pressure after clipping of renal artery. Open circles represent animals fed on a low-sodium diet (group A, N=21), and solid circles represent animals fed on a high-sodium diet (group B, N=21). Values are are means ± SE.

fourth day in animals of group B which were fed a high-sodium diet than in those of group A which were fed a low-sodium diet ($p<0.05$).

Table I and Table II show the terminal blood pressure, average of blood pressure during the postoperative 7 days and plasma renin activity on the terminal day. The presence or absence of hypertensive vascular lesions was also indicated in the tables. At autopsy, no gross findings were found in all animals. Hypertensive vascular lesions were examined on microscopic sections. They were defined in the present study as fibrinoid necrosis of the small arteries and arterioles (Fig. 2), cellular proliferation of the intima, or lesions resembling periarteritis (Fig. 3). Necrotic lesions of the glomerular tufts were also included in the vascular lesions (Fig. 4). The most prominent was the fibrinoid necrosis of arterioles which was often found in the submucosal layer of the stomach. The vascular lesions were found in 6 (28.6%) of 21 animals fed on a low-sodium diet (group A) and in 13 (61.9%) of 21 animals fed on a high-sodium diet (group B). There was a significant difference in incidence between the two groups ($p<0.05$). The vascular lesions were found only in the stomach in animals of group A, whereas they were widely distributed among various organs in animals of group B.

Both the terminal blood pressure and the average of blood pressure during the postoperative 7 days tended to be greater in animals with the vascular lesions than in those without the vascular lesion, but there were a wide range of overlaps.

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Table I. Blood Pressure, Plasma Renin Activity (PRA) and Hypertensive Vascular Lesions in Rabbits Fed on Low-Sodium Diet (Group A, N=21)

No,	Blood Pressure (mmHg) Terminal	Average	PRA (ng/ml)	Hypertensive Vascular Lesions
566	123	114	12.5	Present (Stomach)
572	127	96	17.6	Present (Stomoch)
594	143	119	30.1	Present (Stomach)
602	125	103	22.2	Present (Stomach)
632	126	115	6.6	Present (Stomach)
674	125	107	24.0	Present (Stomach)
542	130	114	2.5	Absent
544	90	87	6.4	Absent
546	123	112	1.7	Absent
550	121	108	12.5	Absent
554	130	104	39.5	Absent
574	131	112	2.4	Absent
576	121	108	5.9	Absent
580	140	119	9.3	Absent
604	109	96	1.4	Absent
618	126	104	12.6	Absent
624	123	107	21.3	Absent
630	127	106	30.5	Absent
636	109	94	15.3	Absent
657	109	97	8.8	Absent
703	115	101	8.0	Absent
Means	122.5	105.8	13.9	
S. E.	2.5	1.9	2.3	

Fig. 2. Arteriolar fibrinoid necrosis in submucosa of the stomach. Elastica-Masson's stain. ×400. (No. 633).

Table II. Blood Pressure, Plasma Renin Activity (PRA) and Hypertensive Vascular Lesions in Rabbits Fed on High-Sodium Diet (Group B, N=21)

No.	Blood Pressure (mmHg) Terminal	Average	PRA (ng/ml)	Hypertensive Vascular Lesions
541	123	117	22.2	Present (Stomach)
543	141	118	2.8	Present (Stomach)
545	123	109	5.6	Present (Stomach)
551	143	122	16.2	Present (Stomach, Heart, right Kidney)
571	133	114	6.4	Present (Stomach, right Kidney)
573	132	122	13.6	Present (Stomach, Intestine, Heart)
575	132	108	7.3	Present (Stomach, Intestine)
595	131	114	31.3	Present (Stomach, Intestine)
617	127	117	31.6	Present (Stomach)
621	145	124	22.0	Present (Stomach)
631	146	121	40.0	Present (Stomach, Intestine)
633	151	128	18.8	Present (Stomach, Intestine)
720	125	106	2.0	Present (Stomach)
727	118	110	20.0	Present (Stomach)
553	130	109	22.7	Absent
565	109	97	2.7	Absent
581	129	107	1.5	Absent
601	118	105	44.5	Absent
603	130	106	23.1	Absent
649	142	123	20.0	Absent
699	126	109	18.2	Absent
Means	131.1	113.5	17.7	
S. E.	2.3	1.7	2.7	

Fig. 3. Arterial lesions resembling periarteritis in right ventricle of the heart. Elastica-Masson's stain. ×100. (No. 551).

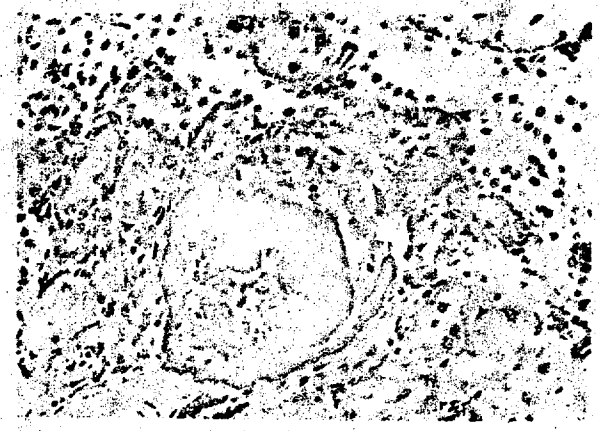

Fig. 4. Glomerular tuft necrosis in the right kidney. Elastica-Masson's stain. ×200. (No. 571).

Plasma renin activity was widely distributed beyond the normal upper limit both in animals of group A and in those of group B. There was no significant difference in plasma renin activity between the 2 groups despite marked difference in the amount of dietary sodium. A wide range of overlaps were also found between animals with the vascular lesions and those without the vascular lesions.

Discussion

The present study revealed a significant role of dietary sodium in the development of renal hypertension and hypertensive vascular lesions. Both the rise of blood pressure and the incidence of hypertensive vascular lesions were significantly greater in animals fed on a high sodium diet than in those fed on a low sodium diet, although sodium content of the high or low sodium diet was not very extreme but was regarded as rather within the physiological range. The present results were essentially similar to those observed in rats by Koletsky.[13] The mechanism responsible for the enhancement of the vascular lesions remains unsolved. The increase in blood pressure did not seem to be the chief factor in enhancing the vascular lesions. The present results indicated that the presence or absence of the vascular lesions did not always depend upon either the level of blood pressure or plasma renin activity.

The vascular lesions observed in animals with renal hypertension correspond to those in patients with malignant hypertension. They have been considered to result from an elevated intravascular pressure.[1,2,9,18] This concept seems to be easy to understand, but there are evidences against it. The vascular lesions can develop regardless of the severity of hypertension in

rabbits as well as in rats.[3),6),10),12)] Hypertension and the vascular lesions often develop simultaneously, but either can develop independently of the other. An increasing attention has been paid to a quite different concept that the clipped kidney can release factors which produce the vascular lesions. One of the vascular permeability factors of renal origin is probably renin.[4)] Administration of crude renin can produce hypertensive vascular lesions in rats.[15)] The vascular lesions caused by renin is also influenced by the amount of dietary sodium.[5)] However, in the present study a certain relation was difficult to find between plasma renin activity and the presence of the vascular lesions. The present results suggest that unidentified factors which are different from renin can be involved in the development of hypertensive vascular lesions. We previously demonstrated that the vascular lesions can be produced by administration of nonpressor fractions of kidney extracts.[17)]

Campbell and Santos-Buch[3)] suggested that factors producing the vascular lesions remained inactive in the presence of an intact contralateral kidney. They produced hypertension in rabbits by wrapping one kidney. The vascular lesions did not develop unless an intact kidney was removed. The observation is not compatible with the results presented here. In the present study hypertension was produced in rabbits by clipping one renal artery and leaving the other kidney intact. This procedure was expected to produce a mild hypertension so that the vascular lesions might develop in a small number of animals. It should be pointed out that the vascular lesions developed as early as 7 days after the surgery even if the opposite kidney was left intact. The difference in results between Campbell and Santos-Buch and the present study may be based on the difference in method to produce hypertension. Wrapping of the kidney is not necessarily the same as the clipping of the renal artery.

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References

1. Allison PR, Blechan N, Brown W, Pickering GW, Robb-Smith AHT, Russell RP: Clin Sci 33: 39, 1967
2. Byrom RB, Dodson LF: J Path Bact 60: 357, 1948
3. Campbell WG, Santos-Buch CA: Am J Path 43: 131, 1963
4. Cuthbert MG, Peart WS: Clin Sci 38: 309, 1970

5. Fernandez LA, Hirano J, Masson GMC: Lab Invest **18**: 8, 1968
6. Flemming HA: J Path Bact **65**: 441, 1953
7. Fujii J, Kurihara H, Yamaguchi H, Ikeda M: Tohoku J Exp Med **97**: 191, 1969
8. Fujii J, Yazaki Y, Kuramochi M, Seki A: Jap Heart J **13**: 339, 1972
9. Hill GS: Circulat Res **27**: 657, 1970
10. Ikeda M, Fujii J, Terasawa F, Hosada S, Kurihara H, Kimata S: Jap Heart J **5**: 466, 1964
11. Kawaguchi H: J Chiba Med Soc **9**: 293, 1931
12. Koletsky S: Arch Path **59**: 312, 1955
13. Koletsky S: Arch Path **63**: 405, 1957
14. Koletsky S: Lab Invest **7**: 377, 1958
15. Masson GMC, Kashii C, Matsunaga M, Page IH: Circulat Res **18**: 219, 1966
16. Miksche LW, Miksche U, Gross F: Circulat Res **27**: 973, 1970
17. Nakao K, Ikeda M, Fujii J, Terasawa F, Kurihara H, Kimata S, Matsushita S, Yamaguchi H: Jap Circulat J **30**: 539, 1966
18. Pickens PT, Bumpus FM, Lloyd AM, Smeby RR, Page IH: Circulat Res **17**: 438, 1965
19. Wilson C, Pickering GW: Clin Sci **3**: 343, 1938

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ecial Article

Infant Feeding Practices—a Predisposing Factor in Hypertension?[1]

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NFANT FEEDING practices in vogue at the turn of the century dictated that the wborn be maintained solely on milk un- 1 year of age. The usual result was a et that failed to promote either a favor- le growth rate or an optimal level of alth. Morbidity and mortality rates were rrespondingly high, and frank deficiency seases were frequently observed. The bsequent trend toward a progressively rlier introduction of solid foods is well cumented (1, 2), and the benefits in rms of growth and development are rec- nized. In the last 2 decades the trend s accelerated until today many infants e being fed solid foods in addition to ilk as early as 2 weeks. Indeed, the ma- ity are receiving a mixed diet con- lerably before 3 months of age (2), the e at which the Committee on Foods and utrition of the Academy of Pediatrics ggests that it is reasonable from a nu- tional and physiological point of view supplement a milk diet with nu- ents, in addition to ascorbic acid and amin D (3).

The proponents maintain that an early ding regime advances the age at which child sleeps through the night, pro- ies a faster and beneficial growth rate; d reduces the incidence and severity of anemia in later childhood. Although there is some evidence that infants in the lower socioeconomic groups—those most susceptible to anemia—may benefit from an iron-enriched formula (4), the hematopoietic effect of solid foods prior to 3 months seems negligible. Experimental evidence to support any of the other theories is lacking.

Although the majority of infants show an amazing ability to adapt to whatever feeding program is prescribed, as more infants are given a mixed diet at an early age more questions are being raised about the possibility of later undesirable effects. Allergists (5) are expressing concern that the allergy-susceptible child will be exposed to a variety of allergens before he has developed adequate defenses against them; nutritionists concerned with obesity cite evidence that patterns of eating predisposing to excessive weight gain may be established in early infancy (6); others reiterate that the child's nutritional needs can be adequately met by a fortified milk diet and that he is physiologically unprepared to swallow solid food or to digest the complex carbohydrates and fats introduced in a mixed diet (7). The fact that such a program is unnecessarily expensive and time consuming for the parent has also been observed (8). More recently, evidence that an elevated sodium intake in early infancy may be a contributing factor to hypertension has suggested a need to

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evaluate the sodium content of infant diets (9, 10). It is becoming apparent that offspring of mothers who increased rather than restricted their sodium intake during pregnancy are more viable (11). However, it is quite conceivable that the young infant with a functionally immature kidney, deprived of the protection afforded by the maternal organism, may be unable to handle the solute load imposed by a high sodium diet. Such a situation is aggravated by a diet in which solid foods with a high renal solute load replace milk, thus increasing the electrolyte levels and at the same time decreasing the total fluid available for their excretion. Likewise the use of undiluted cow's milk under conditions of high extrarenal fluid losses may result in the excessive retention of electrolytes.

Although the relationship between continued high sodium levels in adult diets and hypertension is well established (12) only a few investigators have postulated that high sodium intakes in infancy may predispose to hypertension in later life. The findings of McCance and Widdowson (13), Dahl et al. (9), and Puyau and Hampton (10) have provided evidence that the high level of sodium being ingested by Western infants may be cause for concern. The former investigators found that by adding too much salt to the food of babies they produce an extensive enlargement of the extracellular fluids and some increase in their osmotic pressure.

Dahl et al. (9) fed young hypertension-susceptible rats, from weaning, a diet of meat and vegetables especially processed commercially for young infants, food to which appreciable amounts of sodium chloride are routinely added. After 4 months they found systolic blood pressure elevated to a level associated with drastically reduced life expectancy in five out of seven rats. No increase was noted in rats fed low sodium laboratory chow. These same investigators calculated the sodium content of the diets of 5-month-old infants consuming 1 liter of milk and 100 g of processed meat and vegetables and found it to be comparable to 6.2 mEq/kg per 24 hr, the highest intake recorded for man. This level is found in northern Japan where hypertension is common and severe. Since they found that young rats are more likely to develop high blood pressure on a high sodium diet than are mature animals, Dahl and co-workers postulated that human infants may be more susceptible than adults to the adverse effects of a high sodium intake. The elevation in blood pressure appeared to be self-sustaining even when sodium chloride was reduced.

Puyau and Hampton (10) were motivated to assess the sodium content of infants' diets after observing that a 15-month patient in chronic cardiac failure was consuming 50–60 mEq of sodium daily from a typical diet of homogenized milk and commercial baby foods. In an evaluation of the sodium content of the diet of 1 to 14-month-old infants they found the sodium intake increased from 4.6 to 6.3 mEq/kg per 24 hr, a rate disproportionately higher than that observed for calories. Such an intake is much higher than that of American adults estimated at 1.6 to 3.8 mEq/kg per 24 hr and is very comparable to that which Dahl et al. (9) calculated for the diet of the hypertension-prone Japanese. It approximates intake that Meneely and Dahl (14) showed produced hypertension in rats. While it must be recognized that the infant has an appreciable need for sodium for growth, these values are far in excess of the 2.6 mEq/sodium per 24 hr estimated to meet the needs of a rapidly growing infant. It also exceeds by a wide margin the advisable intake of 8 mEq/day suggested by Fomon (15) to provide a sufficient margin of safety for a wide range of requirements for the expansion of extracellular fluid with growth, the replacement of the obligatory losses of sodium, and the deposition of exchangeable sodium in bone. The estimated require-

TABLE 1

Sodium intake from solid foods by infants fed on solid foods beginning at different ages

Age solid food feeding began, weeks	<3		<5		<7		<9	
Subgroup	U	R	U	R	U	R	U	R
Number of infants	15	20	23	15	7	2	4	3
Age, weeks	*Sodium Content of Solid Foods, mg*							
3	23	48						
	37							
5	53	99	48	39				
	80		44					
7	113	142	65	106	26	9		
	129		81		22			
9	113	152	93	210	40	20	57	61
	135		139		36		59	
11	144	191	120	255	76	36	101	41
	170		171		67		75	
13	213	251	145	272	120	123	106	32
	235		189		122		72	

U = urban, R = rural.
Underlined values represent average values from total of rural and urban groups.

ment of 2.6 mEq/day includes 1.4 mEq/day needed for the increase in extracellular fluid that occurs with growth of 1 oz/day and 1 mEq to compensate for losses through the skin. The advisable intake assumes that 90% of dietary sodium is absorbed.

To investigate the role which the age of introduction of solid foods has on the sodium content of the diets of infants, we examined 1-day food records of the diets of bottle-fed infants. Forty-nine infants from an urban area and 40 infants from a surrounding rural area were studied at 3, 5, 7, 9, 11, and 13 weeks of age. The infants were grouped according to the age at which their diet was first supplemented with solid foods.

The results of this analysis are shown in Table 1. Of the 89 infants studied, 35 were being fed a mixed diet by 3 weeks of age, 73 by 5, 82 by 7, and all by 9 weeks the age at which the Academy of Pediatrics suggests that it may be appropriate to start varying the milk diet for some infants.

For each group, the amount of sodium contributed by solid food increased throughout the 13 weeks of the study. By 13 weeks the infants who had been on a mixed diet from 3 weeks of age were consuming twice as much sodium from solid foods alone as those who started from 5 to 7 weeks and three times as much as those who started at 9 weeks. They were receiving approximately 230 mg. or 10 mEq. sodium per day from solid food alone. Since sodium data were not available for all the modified milks used by the mothers, it was not possible to calculate the contribution of milk to the total sodium intake for the entire group. For those for which data were available the median intakes of sodium from milk fell in the range of 300-350 mg. A few infants received as much as 600 mg. These values do not take into account the sodium content of the water used to reconstitute the formula. White et al. (17) reported that 58% of 2,100 municipalities had water with less

than 20 mg sodium/liter, but that the concentration of sodium ion ranged from 0.4 to 1,900 mg/liter. Therefore, it is conceivable that infants in the high sodium areas could receive an additional 1,000 mg, or 42 mEq, from water. Although such a situation would effect very few infants, the hazards for a hypertension-prone infant should not be overlooked.

For the 13 week old infants who averaged 5½ kg in weight and who received 235 mg sodium from solid foods and 350 mg from liquids for a total of 585 mg, the daily intake amounts to 106 mg, or 1.6 mEq/kg. This is only slightly less than the intake of 5 mEq/kg per 24 hr found by Puyau and Hampton (10) at the same age. Should the infants in our study follow the same pattern of a disproportionate increase in sodium relative to calories with increasing age, their sodium intake would conceivably reach the same high level at a later age. Our data showed a decrease in milk consumption as more solid foods were consumed suggesting a relative as well as absolute increase in the renal solute load. The infants who did not receive solids until 7–9 weeks ingested approximately 160 mg or 1.3 mEq/kg less each day at 13 weeks than the ones who started on solid food at 3 weeks.

For some of the infants who received 400–500 mg of sodium primarily from meat, eggs, and vegetables, the total intake exceeded that found by Puyau and Hampton (10) and exceeds the 6.2 mEq/kg per 24 hr that Dahl et al. (9) found characteristic of hypertensive Japanese adults. It is, however, within the range that Pratt et al. (16) observed could be tolerated without immediate adverse effects under most conditions. Under conditions of high extrarenal fluid loss, relatively low fluid intake, or an impairment in renal concentrating ability such a high renal solute load could exceed the ability of the kidney to concentrate urine (18).

Since a high sodium diet has been shown to induce hypertension that becomes self-sustaining in animals, it is possible that the high sodium content in infants' diets may be a predisposing factor in adult hypertension although the hazards of extrapolating findings from one species to another are obvious. These observations also point to the potential danger of reducing the margin of safety by giving feedings of high solute concentration, especially for the infant whose ability to concentrate urine falls short of 1,000 milliosmols/liter as often observed in the first 2 months of life (19). While most infants apparently adapt to a wide range of intake of sodium with no apparent adverse effects, for those infants for whom it seems desirable to maintain a low solute load, a delayed introduction of solid food is indicated. Fruits, processed for infant feeding, however, have a very low renal solute load.

SUMMARY

Evidence that the sodium content of the diet is markedly elevated when the infant is given foods other than milk in the first few weeks of life may be reason to question the wisdom of such a practice. At the present time evidence is accumulating that diets high in sodium predispose to hypertension in young animals. Data on the sodium in infant's diets indicate that those receiving commercially prepared infant foods are ingesting a level that far exceeds the needs for growth and to compensate for obligatory losses through the skin and kidneys. Data presented here show that the earlier a child is started on the usual sequence of cereal, fruit, eggs, meat, and vegetables in addition to milk, the higher the sodium content of his diet at any age. While there is now no experimental evidence in human infancy to indicate a relationship between dietary sodium levels and hypertension, such studies are indicated. Since the high sodium in infant foods is the result of the addition of so-

sium chloride to make the products more palatable to the mother, consideration might also be given to encouraging manufacturers of infant foods to reduce the amount of salt added. There is no evidence that the infant rejects bland foods.

REFERENCES

1. Butler, A. M., and I. J. Wolman. Trends in the early feeding of supplementary foods to infants. *Quart. Rev. Pediat.* 9: 63, 1954.
2. Guthrie, H. A. Effect of early feeding of solid foods on nutritive intake of infants. *Pediatrics* 38: 879, 1966.
3. Committee on Nutrition. American Academy of Pediatrics. On the feeding of solid food to infants. *Pediatrics* 21: 685, 1958.
4. Adelman, M. B., and B. R. Sered. Utilization of dietary iron by term infants. *Am. J. Diseases Children* 111: 45, 1966.
5. Fruhaler, G. J. Can allergy be prevented? *Southern Med. J.* 58: 836, 1965.
6. Gordon, H. H. A summary of some clinical aspects of obesity. *Pediatrics* 20: 556, 1957.
7. Bakwin, H. Feeding program for infants. *Federation Proc.* 23: 66, 1964.
8. Cochrane, W. A. Overnutrition in prenatal and neonatal life: a problem? *Can. Med. Assoc. J.* 73: 893, 1965.
9. Dahl, L. K., M. Heine and L. Tassinari. High salt content of Western infants' diet: Possible relationship to hypertension in the adult. *Nature* 198: 1204, 1963.
10. Pevay, F. A., and L. P. Hampton. Infant feeding practices. 1966. *Am. J. Diseases Children* 111: 370, 1966.
11. Pike, R. L. Sodium intake during pregnancy. *J. Am. Dietet. Assoc.* 44: 176, 1964.
12. Knudsen, K. N., and L. K. Dahl. Essential hypertension: Inborn error of sodium metabolism? *Postgrad. Med.* 42: 148, 1966.
13. McCance, R. A., and E. M. Widdowson. Physiology of the newborn infant. *Lancet* 2: 585, 1957.
14. Menefee, G. R., and L. K. Dahl. Electrolytes in hypertension: The effects of sodium chloride. *Med. Clin. N. Am.* 45: 271, 1961.
15. Fomon, S. J. *Infant Nutrition.* Philadelphia: Saunders, 1967, p. 141.
16. Pratt, E. L., B. Bienvenu and M. M. White. Concentration of urine solutes by young infants. *Pediatrics* 1: 181, 1948.
17. White, J. M., J. G. Wingo, L. M. Allegood, G. R. Cooper, J. Gutribe, W. Haydaker, T. T. Benack, J. W. Dening and F. B. Taylor. Sodium ion in drinking water. *J. Am. Dietet. Assoc.* 51: 32, 1967.
18. Committee on Nutrition. American Academy of Pediatrics. Water requirement in relation to osmolar load as it applies to infant feeding. *Pediatrics* 19: 339, 1957.
19. Colle, E., E. Ayoub and R. Raile. Hypertonic dehydration (hypernatremia). The role of feedings high in solutes. *Pediatrics* 22: 5, 1958.

Triangle

Salt and Hypertension, Water Hardness and Cardiovascular Death Rate

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The part played by salt in hypertension was suspected earlier than 2300 years B.C. by a Chinese physician, the Yellow Emperor[25], who noted that indulgence in salty foods hardened the pulse and that this predisposed towards apoplexy with aphasia. We must wait over 4000 years more, until 1904, for AMBARD and BEAUJARD[3] to observe the importance of chloride retention in hypertension. Others, in particular ALLEN[2] (1920) and VOLHARD[75] (1935) also studied the role of salt in this condition.

VIERSMA[74] (a student of BORST) employed mercurial diuretics with a low-salt diet to treat hypertension in 1946. KEMPNER[41] (1948) believed that his rice and fruit diet was active on account of its low protein content, but it was the great merit of DOLE, DAHL and COTZIAS et al.[21] to demonstrate in 1951 that the efficacy of the regimen was due to its low sodium content. This clinical work has been pursued until quite recently and has been complemented with numerous experimental data (cf. DAHL[17]). DAHL's hypothesis is that not only is it possible to treat hypertension by restricting the dietary sodium intake, but that essential hypertension is first and foremost due to the combined effects of the ingested salt and genetic factors. This hypothesis was not received enthusiastically by either clinicians or epidemiologists, especially since several conflicting reports were published (MIALL[53], PAUL[62], MALHOTRA[49]). A number of publications of recent years, however, support DAHL's hypothesis.

We must therefore take a close look at the arguments for and against this hypothesis, for if the hypothesis were correct, it would offer us immense possibilities in the realm of preventive medicine.

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Arguments in favour of the importance of salt in high blood pressure

Clinical arguments

Clinical experience has taught us that strict restriction of dietary salt has a favourable influence in the treatment of hypertensive patients. Addition of salt, on the other hand, increases the blood pressure (PERERA et al.[63]). Present-day clinical practice, however, is tending to supplant the salt-free diet by the use of diuretics, and little faith is placed in mild salt restriction. Nevertheless, the small reduction in the daily salt intake of from 12 to 6 g a day produced an average fall in blood pressure of about 1 mm Hg per g of NaCl less ingested. Diet and diuretics produced this effect independently of each other, and it was additive in nature (PARIJS et al.[61]).

Pharmacological arguments

All diuretics without exception, and whatever their effect on potassium excretion, act as hypotensive agents. The variety of chemical structures makes it unlikely that this action should be due to vasodilatation. The only common denominator among these preparations is natriuresis. Various studies (cf. HARRIS et al.[31]) have shown that the reactivity of the arterial wall increases with its sodium content.

Direct epidemiological arguments

Studies of different primitive peoples has shown that populations exist in whom the blood pressure does not increase with age as in our countries, and in whom essential hypertension is virtually unknown (Table 1). The common factor in these populations is the absence of salt in their diet. In contrast, the blood pressure levels of populations using salt, whether they are primitive or developed, do increase with age (Table 2). The population reported by WHITE et al.[76] to use salt and not

Table 1: Populations in which the blood pressure increases little or not at all with age (average of both sexes, in different age groups).

Population	Systolic pressure (mm Hg)			Dietary salt	References
	20 to 29 years	40 to 49 years	60 to 69 years		
Brazil (Carajas)	107	100	109	No salt: use lyes of vegetable ash (K salts)	LOWENSTEIN[48]
New Guinea (Murapins)	126	126	123	0.6 g (24-hour urine)	GOLDRICK et al.[29]
Botswana (!Kung bushmen)	119	116	122	2.0 g (24-hour urine)	TRUSWELL et al.[73]
Cook Islands (Pukapukas)	113	116	125	2.9–4.1 g (24-hour urine)	PRIOR et al.[66]

Table 2: Populations in which blood pressure increases markedly with age (average of both sexes, in different age groups).

Population	Systolic pressure (mm Hg)			Dietary salt	References
	20 to 29 years	40 to 49 years	60 to 69 years		
United States	119	130	149	± 10 g/24 h (DAHL[14])	Nat. Health Survey[58]
Portugal	126	134	155	not measured	DAS NEVES ALMEIDA[18]
Sweden	125	138	159	not measured	CARLSON et al.[8]
Belgium	132	143	163	4 to 20 g (24-hour urine)	JOOSSENS et al.[38]
Cook Islands (Rarotongas)	124	151	165	7.0 to 8.2 g (24-hour urine)	PRIOR et al.[66]
Norway	130	141	167	not measured	BØE et al.[6]
Wales	120	138	169	8.0 g (24-hour urine)	MIALL[53]
Bahama Islands (Negroes)	129	154	176	15 to 30 g (24-hour urine)	MOSER et al.[55]

to exhibit hypertension was not examined for urinary sodium excretion.

Hypertensives have a higher mean taste threshold, as regards salt, than normotensives (FALLIS et al.[26], WOTMAN, LARAGH et al.[77]). This could be due to the greater quantity of salt they consume (JOOSSENS et al.[38]). Chronic and prolonged use of salt affects the taste buds and thus impairs the function of taste. The blood pressure of negro girls was found to be related to sodium excretion in the urine (LANGFORD et al.[45]). In 507 schoolboys a positive correlation was found between the Na/K ratio of morning urine and the diastolic blood pressure (FROMENT et al.[28]). JOOSSENS et al.[38] demonstrated a positive correlation between systolic pressure and 24-hour urinary sodium in 1314 men (Fig. 1) and 713 women aged from 17 to 85 years. They further demonstrated that such a relationship can be found in a small group of persons (39 in this instance), provided that the measurements are repeated sufficiently often. In these 39

persons, more than 8000 blood pressure measurements were made in two months, and 368 24-hour urine samples collected. Employing a similar technique, they recently found that for the majority of persons investigated, the blood pressure increased as the 24-hour urine sodium content, and in inverse ratio to the potassium content. SHAPER et al.[72] observed a rapid rise in blood pressure in young Samburus (used to eating without salt) when given 15 g of salt daily during military service. SASAKI[68] saw the converse phenomenon in Japan: on reducing the salt intake of children taking school meals he found that the blood pressure declined progressively.

Indirect epidemiological arguments

Indirect arguments, in fact, deriving from a study of death rates in various countries, put us on the track of salt and its possible impact on the public health. In 1965 we were investigating the relationship between deaths due to coronary heart disease and lung cancer. Punched cards were prepared with the necessary data, as well as those of other types of mortality such as cerebrovascular accidents (CVA) and stomach cancer (SC). A significant correlation (JOOSSENS[36]) was found between coronary mortality and that of lung cancer, but we were astonished to find a much stronger correlation between the death rates for CVA and SC. Systematic study of this phenomenon (JOOSSENS[37,38,39]) revealed a triple gradient for these two types of mortality, and this triple gradient appeared also to exist for alimentary salt.

Geographical gradient. As a general rule there is a gradient running *from west to east.* The mortality of CVA and SC (cf. DOLL[22]) is relatively low in the United States. It has an intermediate level in England, France, the Netherlands and Belgium, and increases towards Germany and Austria to become very high in Hungary, Czechoslovakia and Finland. The highest death rates are seen in Japan. A *south-north* gradient is also often seen, with mortality generally highest in the north (Japan, Great Britain and Italy), except for the Netherlands. JOOSSENS et al.[38] found a correlation of 0.70 ($p = 0.01$) between the death rates of CVA and SC, for both sexes taken together, on the basis of data from these 12 western countries with well established mortality statistics. Taking the average age-adjusted mortality per thousand inhabitants

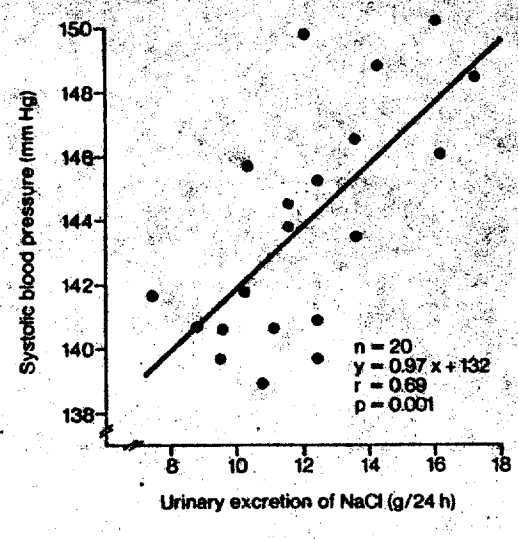

Fig. 1: Relationship between systolic blood pressure and urinary excretion of NaCl. The 1314 men in the group as a whole were first ranked in increasing order of the 24-hour urine NaCl content. Average systolic pressure and urinary NaCl and creatinine excretion were then calculated per subgroup of approximately 66 persons. The quantity of NaCl excreted in 24 hours was calculated with reference to the excretion of creatinine, thus rendering the abscissae independent of any possible errors in the collection of the urine. (After JOOSSENS et al.[38]).

for each country from 1955 to 1957 a regression line with the following formula is found between countries:

$$CVA = 4.17\,SC + 2.47 \quad (n = 12)$$

Historical gradient. Provided that the crude and the age-adjusted death rates are not confused, a marked decrease in the age-adjusted death rate is seen both for CVA and SC, as well as for hypertension, in almost all western countries. In the United States it dates back to about 1925 in respect of CVA (Fig. 2), to at least 1930 for SC (Fig. 3), and to at least 1940 for hypertension (Fig. 4). This decrease is a real one, since it is accompanied by a reduction in total deaths, and continues to obtain for all three causes of death (JOOSSENS and KESTELOOT[39]). The trend set in before any effective treatment of CVA, SC or hypertension was known. The existence of treatment for hypertension has not appreciably affected the slope of the curve in the past decade. JOOSSENS

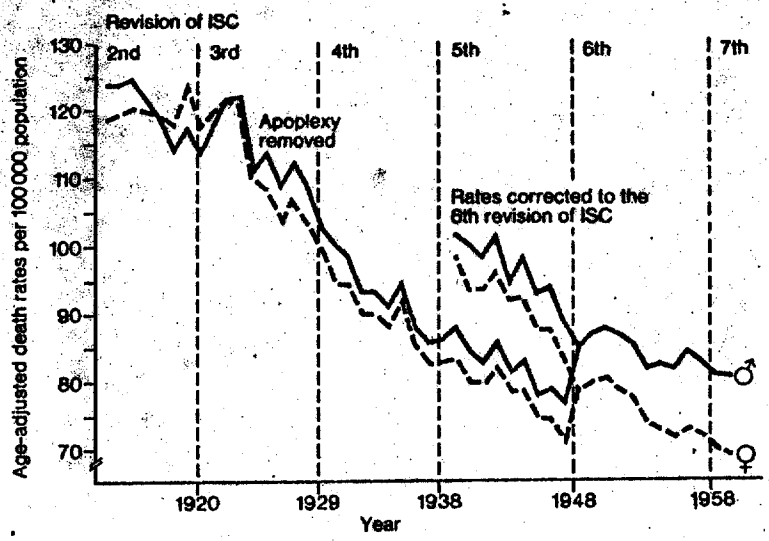

Fig. 2: Age-adjusted death rates for cerebrovascular accidents (both sexes) per 100,000 white inhabitants of the United States from 1914 to 1960, according to the successive revisions of the "International Statistical Classification of Diseases, Injuries and Causes of Death (ISC)". The decline in the death rate has continued after 1960 until the present time. (After ACHESON[1]).

and CLAESSENS[40] set up the formulae of the regression lines of CVA against SC in England and Wales for two periods:

1955 to 1961: CVA = 5.31 SC + 2.18
(r = 0.88) (n = 7)

1962 to 1969: CVA = 5.79 SC + 2.08
(r = 0.84) (n = 8)

There was no significant difference between them. As the treatment of hypertension is continually being improved upon, and since it has no effect on stomach cancer, a better correlation could be expected for the first period than for the second. This was, however, not the case. The same regression line calculated for Japan from 1962 to 1967 yielded

CVA = 5.05 SC + 2.43 (r = 0.91) (n = 6)

Similarly, if the average of the 12 western countries studied is taken for each of the years from 1955 to 1967, we find a relationship between time periods[38]:

CVA = 3.72 SC + 2.63 (r = 0.97) (n = 13)

All these lines are similar, and since their origins are so diverse they cannot be attributed to chance. The common factor between CVA and SC (to all appearances a dietary factor) should be the same for the geographical and historical gradients, should be common to the western countries and Japan, and should have shown a progressive decline with time for more than 40 years. This renders unlikely the participation of pork (MEINSMA[50]) or of smoked and grilled foods (DUNGALL[23]), which are hardly or not at all used in Japan, as well as of rice, consumption of which, while relatively low in the west, is on the increase.

Social gradient. The death rates due to CVA, hypertension (HOWARD and HOLMAN[34]) and SC (CLEMESSEN[10]) show a marked social gradient, all these diseases being more common among the lower social classes. Arterial blood pressure is lower on an average in the upper social classes (CASSEL[9]), as we ourselves have also found, and this is further confirmed by KESTELOOT and VAN HOUTE[42], who examined more than 42,000 members of the Belgian army. No significant difference was found in the Framingham study (DAWBER et al.[20]).

Agreement in habits of salt consumption. The three above-mentioned gradients, geographical, historical and social, are again found for salt, in so far as

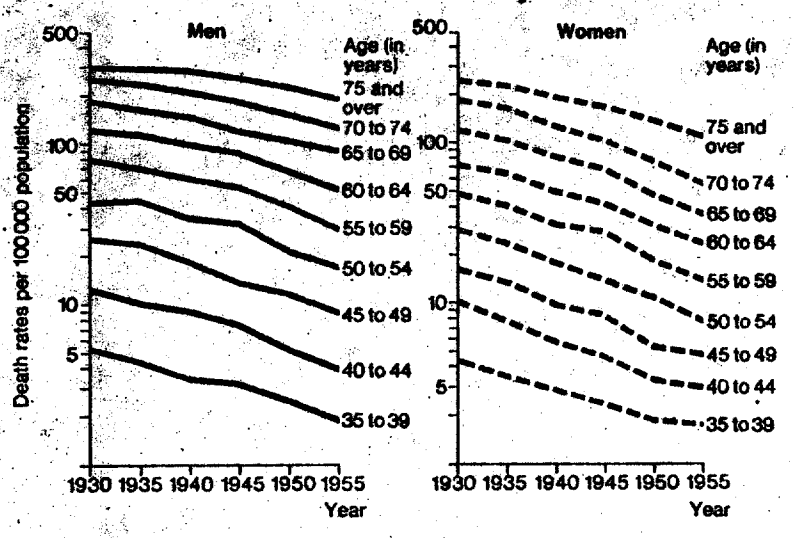

Fig. 3: Specific death rate for stomach cancer in the United States from 1930 to 1955. Here too the downward trend has persisted after 1955. (After HAENSZEL[30]).

data are available. Daily salt consumption was 10 g in the United States (DAHL[14]). SASAKI[68], in 1964, gave values ranging from 18.1 to 27.3 g in the north of Japan and from 14 to 19.9 g in the south. In 1971 KOMACHI et al.[44] found lower figures: 8 to 14 g in Osaki (central Japan) and 20 g in Akita (north Japan).

JOOSSENS et al.[38] found an average of 11.7 g in 1111 men working in an insurance company. Urinary salt excretion was found to be higher in Scotland than in England (DAUNCEY et al.[19]). The historical gradient for salt (progressive decline) is probably due to the increasing use of deep-freezers and refrigerators, making salt unnecessary as a preservative, and to the spectacular decline in the consumption of bread and potatoes. In Belgium a salt consumption of 20 to 30 g can be calculated for about the year 1920, 17 g of which is accounted for by the two last-named foods. Conservation of vegetables, meat and fish in salt has died out almost completely. The existence of a social gradient for salt (accountable to a greater consumption of cheaper foods such as bread, potatoes, sausage meats, etc., by the lower social classes) has been demonstrated in Belgium (JOOSSENS and CLAESSENS[40]).

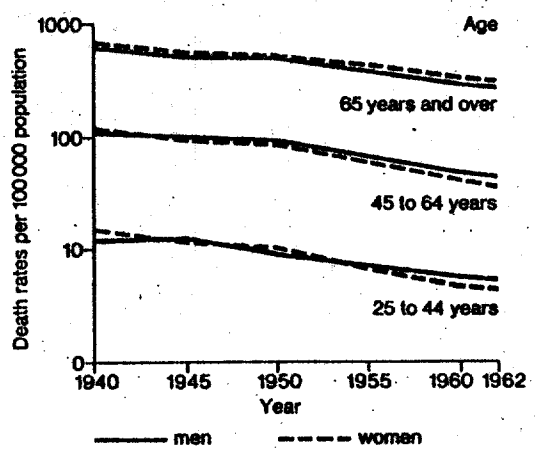

Fig. 4: Specific death rate for hypertension and hypertensive heart disease (both sexes) in the United States from 1940 to 1962. The trend in each sex and age group has been markedly downward since 1962. (Deaths for all years are classified according to the "Sixth Revision of the International Statistical Classification of Diseases, Injuries and Causes of Death" [ISC]). (Origin of data: Cardiovascular Diseases[7]).

It is therefore desirable to organize an international research on the urinary excretion of sodium, to see whether the concept of a triple gradient for salt can be confirmed or not. The results of this enquiry could then be compared with the death rates observed in the participating countries.

Physiopathological mechanisms. The effect of salt might be accounted for by an effect on arterial pressure and thus on deaths due to all causes as well as to CVA or to hypertension. Salt might also favour atrophic gastritis, and thus gastric cancer, both of which are common in Japan (YOSHITOSHI[78]). SATO et al.[69] demonstrated in 1959 the relationship between the incidence of SC in Japan and the quantity of salt in the food. They also induced atrophic gastritis in mice by giving them a salted diet. These findings were confirmed (HIRAYAMA[33]; cf. also World Health[67]).

The osmoreceptors of the duodenum (HUNT[35]) close the pylorus when the food is hypertonic. The latter remains in contact with the gastric mucosa for a long time and can cause its atrophy, just as it promotes atrophy of the taste buds. It is not rare to find elderly persons who are unable to follow a low-salt diet, which they find tasteless. This loss of taste sensitivity, which in younger people is only transitory, may last indefinitely in the elderly.

Experimental arguments

These are conclusive in rats. These animals become hypertensive if their salt intake is increased (MENEELY[52], DAHL[17]). This increase is countered by potassium (MENEELY et al.[51], LOUIS et al.[47]). DAHL et al.[16] deserve credit for having shown that salt is a necessary condition in rats, but is not sufficient in itself. Genetic factors are still required for the development of hypertension. OKAMATO and AOKI[60] bred a strain of rats that always exhibit hypertension, but LOUIS et al.[47] found that this hypertension is aggravated by salt. By treating these animals from the age of 3 months onward it was found possible to maintain the blood pressure at normal levels. If the treatment was stopped after a few months, the pressure was seen to increase gradually to levels that always remained lower than those of control rats (FREIS et al.[27]). It is interesting to note that sea salt, gram for gram of sodium, was more deleterious than purified salt (DAHL and HEINE[15]) in both rats and dogs.

Arguments against the role of salt in arterial hypertension

The evidence here derives above all from observations in man. It is possible to be normotensive, to consume large quantities of salt, and to live to over eighty years of age. The variable action of sodium from one individual to another and the variability in blood pressure and urinary sodium measurements mean that a good correlation with the salt intake is seen only when different countries are compared (correlations "between populations") (Tables 1 and 2). It is much more difficult to determine the presence of this correlation in a given population (correlation "within population"). Spurred on by experimental results, a number of authors have attempted to find such a correlation, but were disappointed to find nothing significant. The explanation for this failure must be sought in the relatively restricted number of tests performed. In the Framingham study[20], the largest to date, only 297 persons were so tested. Our studies have shown a significant correlation between blood pressure and urinary sodium in only one population.

Other theories on the epidemiology of arterial hypertension

Cadmium theory

One theory was suggested by SCHROEDER (cf. PERRY[64]), who incriminates cadmium. There are a number of observations to support this hypothesis. Thus in three out of four studies (333 autopsies) more cadmium was found in the kidneys of hypertensives than in those of normotensives. Further, it is possible to provoke chronic hypertension in rats by giving them water containing 5 mg/l of cadmium. Even accepting that cadmium might explain deaths due to CVA, it is not clear how one should interpret the relationship between CVA and SC. The cadmium theory does not account for the historical gradient of CVA. Rather, the reverse would be expected, since the smoke of 20 cigarettes contains some 30 µg cadmium, absorbed at a rate of 70% (NANDI et al.[57]) and cigarette consumption has risen steeply since 1930.

Stress theory

Another theory is that of stress (cf. HENRY and CASSEL[32]). It is supported mainly by experimen-

tal evidence. The main objections to this hypothesis are the same as for cadmium. To account for the historical trend in the decline of CVA one would have to contend that stress is decreasing with time in our civilization. Any effect it can be expected to have on SC remains to be discovered.

It is possible that cadmium and stress have a part to play in the epidemiology of arterial hypertension, but if so, it is likely to be a secondary one.

Relationship between hardness of water, hypertension and cardiovascular death rate

KOBAYASHI[43] in 1957 was the first to publish a relationship between cardiovascular deaths and the sulphate/carbonate ions of drinking water. SCHROEDER[71] in 1960 found a significant negative correlation between the hardness of the drinking water and deaths due to hypertension and coronary ischaemia. This was confirmed by MORRIS et al. in England, BIÖRCK et al.[5] in Sweden and NERI et al.[59] in Canada. No relationship was found, on the other hand, by LINDEMAN and ASSENZO[46] in Oklahoma, MULCAHY[56] in Ireland and ELWOOD et al.[24] in England, but the groups investigated were small. Since 1960 CRAWFORD et al.[11, 12, 13] have made an intensive study of this question. They showed that infant mortality[13], too, was inversely correlated with water hardness, and that the most important ion in the water appeared to be calcium. In regions in which water was softened[12], they found an increase in mortality, and a diminution when the water was rendered harder. CRAWFORD et al.[11] found more lead in the ribs of persons from soft-water regions at autopsy. ANDERSON et al.[4] in Canada and PETERSON et al.[65] in the United States reported a negative correlation between sudden death and hardness of water. This correlation did not hold for non-sudden death.

To summarize, an inverse relationship between water hardness and cardiovascular deaths (including those due to hypertension) appears to be established. But at the present time it is not possible to state which is the most important factor of the drinking water, although calcium is a strong candidate for this distinction.

The mechanism underlying this relationship is unknown.

Conclusions

Considerable clinical, pharmacological, epidemiological and experimental evidence suggests that dietary salt is not as inoffensive an ingredient of our food as it appears. This emphasizes the urgency of undertaking epidemiological research on an international scale to compare the quantity of salt in the diet not only with blood pressure levels, but above all with mortality due to hypertension, cerebrovascular accidents and stomach cancer. If DAHL's hypothesis were to be confirmed it would be possible to reduce these deaths in a simple and economical manner, by avoiding the addition of salt whenever it is not absolutely necessary for the conservation of food (as in bread, bakery goods, preserves, the preparation of meals, etc.). An important target would be infant foods. For no reason whatsoever, these are made to contain large quantities of salt, while mother's milk, which has carried the human race along throughout its evolution, contains only 0.4 g of salt per litre (four times less than cow's milk).

Research is also necessary to define the possible role of cadmium and stress in hypertension. Finally, it is wiser to abstain from softening the drinking water, except perhaps for non-domestic use.

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References

[1]ACHESON R. M.: Cerebrovascular disease epidemiology. Public Health Monograph Nr. 76. U.S. Depart. of Health Education and Welfare, Washington D.C. 1966, p. 24. – [2]ALLEN F. M.: *J. Amer. med. Ass.* 1920, *74*, 652. – [3]AMIARD L., BEAUJARD E.: *Arch. gén. Méd.* 1904, *1*, 520. – [4]ANDERSON T. W., LE RICHE W. H., MACKAY J. S.: *New Engl. J. Med.* 1969, *280*, 805. – [5]BIÖRCK G., BOSTRÖM H., WIDSTRÖM A.: *Acta med. scand.* 1965, *178*, 239. – [6]BØE J., HUMERFELT S., WEDERVANG F.: *Acta med. scand.* 1957, Suppl. 321. – [7]Cardiovascular Diseases: *Circulation* 1965, *31 & 32*, Suppl. I, II, III. – [8]CARLSON L. A., LINDSTEDT S.: *Acta med.*

scand. 1968, Suppl. 493. – [9]CASSEL J. C.: *Arch. intern. Med.* 1971, *128*, 887. – [10]CLEMESSEN J.: Statistical studies in malignant neoplasms. Munksgaard, Copenhagen 1965, Vol. *I*, p. 138. – [11]CRAWFORD M. D., CRAWFORD T.: *Lancet* 1969, *i*, 699. – [12]CRAWFORD M. D., GARDNER M. J., MORRIS J. N.: *Lancet* 1971, *ii*, 327. – [13]CRAWFORD M. D., GARDNER M. J., SEDGWICK P. A.: *Lancet* 1972, *i*, 988. – [14]DAHL L. K.: *Amer. J. clin. Nutr.* 1958, *6*, 1. – [15]DAHL L. K., HEINE M.: *Amer. J. clin. Nutr.* 1972, *25*, 231. – [16]DAHL L. K., HEINE M., TASSINARI L.: *J. exp. Med.* 1962, *115*, 1173. – [17]DAHL L. K.: *Amer. J. Cardiol.* 1961, *8*, 726. – [18]DAS NEVES ALMEIDA F.: Pression sanguine en rapport avec âge, sexe et profession. *In:* Abstr. VI Congr. Int. Hyg. Med. Prev. Madrid 1971, p. 70. – [19]DAUNCEY M. J., WIDDOWSON E. M.: *Lancet* 1972, *i*, 711. – [20]DAWBER T. R., KANNEL W. B., KAGAN A., DONABEDIAN R. K., MCNAMARA P. M., PEARSON G.: Environmental factors in hypertension. *In:* The epidemiology of hypertension. Eds. J. STAMLER, R. STAMLER, T. N. PULLMAN. Grune and Stratton, New York/London 1967, p. 255. – [21]DOLE V. P., DAHL L. K., COTZIAS G. C., DZIEWIATKOWSKI D. D., HARRIS C.: *J. clin. Invest.* 1951, *30*, 581. – [22]DOLL R.: Prevention of cancer. The Nuffield Provincial Hospitals Trust, London 1967. – [23]DUNGAL N.: *Acta Un. int. Cancr.* 1961, *17*, 365. – [24]ELWOOD P. C., BAINTON D., MOORE F., DAVIES D. F., WAKLEY E. J., LANGMAN M., SWEETNAM P.: *Brit. med. J.* 1971, *II*, 362. – [25]EMPEROR (THE YELLOW): Classical books of internal medicine; written in 2300 B.C.; revised and re-edited during the Ming Dynasty. – [26]FALLIS N., LASAGNA L., TETREAULT L.: *Nature (Lond.)* 1962, *196*, 74. – [27]FREIS E. D., RAGAN D., PILSBURY III H., MATHEWS M.: *Circulat. Res.* 1972, *31*, 1. – [28]FROMENT A., MILON H., VINCENT M., DUPONT J.-CL., DUPONT J.: *Bull. I.N.S.E.R.M.* 1970, *25*, 1237. – [29]GOLDRICK R. B., SINNETT P. F., WHYTE H. M.: An assessment of coronary heart disease and coronary risk factors in a New Guinea Highland population. *In:* Atherosclerosis. Springer, Berlin/Heidelberg/New York 1970, p. 366. – [30]HAENSZEL W.: *Acta Un. int. Cancr.* 1961, *17*, 347. – [31]HARRIS G. S., PALMER W. A.: *Clin. Sci.* 1972, *42*, 301. – [32]HENRY J. P., CASSEL J. C.: *Amer. J. Epid.* 1969, *90*, 171. – [33]HIRAYAMA T.: The epidemiology of cancer of the stomach in Japan with special reference to the role of diet. *In:* Proc. 9th Int. Cancer Congress, Tokyo 1966. UICC Monograph Series, Vol. *10*. Springer, Berlin/Heidelberg/New York 1967, p. 37. – [34]HOWARD J., HOLMAN B. L.: *Milbank mem. Fd Quart.* 1970, *48*, 263. – [35]HUNT J. N.: *Physiol. Rev.* 1959, *39*, 491. – [36]JOOSSENS J. V.: *Verh. vlaam. Akad. Geneesk. Belg.* 1965, *27*, 489. – [37]JOOSSENS J. V.: *Évolut. méd.* 1968, *12*, 381. – [38]JOOSSENS J. V., WILLEMS J., CLAESSENS J., CLAES J., LISSENS W.: Sodium and Hypertension. *In:* Nutrition and cardiovascular diseases. Morgagni Edizioni Scientifiche. Rome 1972[a], p. 91. – [39]JOOSSENS J.V., KESTELOOT H.: Recent trends in cardiovascular mortality. *In:* Nutrition and cardiovascular diseases. Morgagni Edizioni Scientifiche. Rome 1972[b], p. 220. – [40]JOOSSENS J. V., CLAESSENS J.: Epidémiologie de l'hypertension. *In:* Monographie Sandoz sur l'hypertension. Brussels 1972 (in press). – [41]KEMPNER W.: *Amer. J. Med.* 1948, *4*, 545. – [42]KESTELOOT H., VAN HOUTE O.: to be published. – [43]KOBAYASHI J.: *Berichte Ohara Inst. landwirtsch. Biol.* 1957, *11*, 12. – [44]KOMACHI Y., IIDA M., SHIMAMOTO T., CHIKAYAMA Y., TAKAHASHI H., KONISCHI M., TOMINAGA S.: *Jap. Circulat. J.* 1971, *35*, 189. – [45]LANGFORD H. G., WATSON R. L., DOUGLAS B. H.: *Trans. Ass. Amer. Phycns* 1968, *81*, 135. – [46]LINDEMAN R. D., ASSENZO J. R.: *Amer. J. publ. Hlth* 1964, *54*, 1071. – [47]LOUIS W. J., TABEI R., SPECTOR S.: *Lancet* 1971, *ii*, 1283. – [48]LOWENSTEIN F. W.: *Lancet* 1961, *i*, 389. – [49]MALHOTRA S. L.: *Amer. J. clin. Nutr.* 1970, *23*, 1353. – [50]MEINSMA L.: *Voeding* 1964, *25*, 357. – [51]MENEELY G. R., BALL C.O.T.: *Amer. J. Med.* 1958, *25*, 713. – [52]MENEELY G. R.: The experimental epidemiology of sodium chloride toxicity in the rat. *In:* The epidemiology of hypertension. Eds. J. STAMLER, R. STAMLER, T. N. PULLMAN. Grune and Stratton, New York/London 1967, p. 240. – [53]MIALL W. E.: *Brit. med. J.* 1959, *II*, 1204. – [54]MORRIS J. N., CRAWFORD M. D., HEADY J. A.: *Lancet* 1961, *i*, 860. – [55]MOSER M., MORGAN R., HALE M., HOOBLER S. W., REMINGTON R., DODGE H. J., MACAULAY A. I.: *Amer. J. Cardiol.* 1959, *4*, 727. – [56]MULCAHY R.: *J. Irish med. Ass.* 1964, *55*, 17. – [57]NANDI M., JICK H., SLONE D., SHAPIRO S., LEWIS G. P.: *Lancet* 1969, *ii*, 1329. – [58]National Health Survey: Public Health Service Publication No. 1000, Series 11, No. 4. Washington D.C. 1964. – [59]NERI L. C., MANDEL J. S., HEWITT D.: *Lancet* 1972, *i*, 931. – [60]OKAMOTO K., AOKI K.: *Jap. Circulat. J.* 1963, *27*, 282. – [61]PARIJS J., JOOSSENS J. V., VAN DER LINDEN L., VERSTREKEN G., AMERY A.: *Amer. Heart J.* 1973, *85*, 22. – [62]PAUL O.: The natural history of hypertension. *In:* The epidemiology of hypertension. Eds. J. STAMLER, R. STAMLER, T. PULLMAN. Grune and Stratton, New York/London 1967, p. 365. – [63]PERERA G. A., BLOOD D. W.: *J. clin. Invest.* 1947, *26*, 1109. – [64]PERRY H. M., Jr.: *Ann. N.Y. Acad. Sci.* 1972, *199*, 202. – [65]PETERSON D. R., THOMPSON D. J., NAM J.: *Amer. J. Epid.* 1970, *92*, 90. – [66]PRIOR I. A. M., EVANS J. G., HARVEY H. P. B., DAVIDSON F., LINDSEY M.: *New Engl. J. Med.* 1968, *279*, 515. – [67]Santé du Monde: Le Magazine de l'Organisation mondiale de la Santé, avril 1970. – [68]SASAKI N.: *Geriatrics* 1964, *19*, 735. – [69]SASAKI N.: Nippon – Iji – Shinpo *(Jap. Med. J.)* 1970, *2426*, 30. – [70]SATO T., FUKUYAMA T., SUZUKI T., TAKAYANAGI J., MURAKAMI T., SHIOTSUKI N., TANAKA R., TSUJI R.: *Bull. Inst. publ. Hlth (Tokyo)* 1959, *8*, No. 4, 187. – [71]SCHROEDER H. A.: *J. chron. Dis.* 1960, *12*, 586. – [72]SHAPER A. G., LEONARD P. J., JONES K. W., JONES M.: *E. Afr. med. J.* 1969, *46*, 282. – [73]TRUSWELL A. S., KENNELLY B. M., HANSEN J. D. L., LEE R. B.: *Amer. Heart J.* 1972, *84*, 5. – [74]VIERSMA H. J.: De behandeling van hypertensie met zoutloos dieet en uitdrijven van keukenzout. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam 1946. – [75]VOLHARD F.: Elevated blood pressure. *In:* The kidney in health and disease. Ed. H. BERGLUND, G. MEDES. London 1935 (quoted by Viersma). – [76]WHYTE H. M.: *Aust. Ann. Med.* 1958, *7*, 36. – [77]WOLMAN S., MANDEL I. D., THOMPSON R. H., Jr., LARAGH J. H.: *J. chron. Dis.* 1967, *20*, 833. – [78]YOSHITOSHI Y.: Proc. 3rd World Congr. of Gastro-enterology. Tokyo 1966, *I*, 179.

Seminars on Hypertension

Treatment of Hypertensive Vascular Disease with Rice Diet*†

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THE treatment of hypertensive vascular disease with the rice diet[1–5] was suggested by observations made on the protein, fat and carbohydrate metabolism of isolated kidney cells under various pathologic conditions (cell injury and/or changes in pH, sodium bicarbonate concentration, oxygen tension and metabolizable substrate[6–11]).

Until 1944 the consensus was that dietary treatment was useful in kidney disease but of no value in hypertensive vascular disease. "The diet in uncomplicated hypertension requires no essential change from the normal. There is no justification for restriction of protein intake, indeed, such restriction may result in anemia and other evidences of malnutrition. Likewise, in the absence of edema or paroxysmal dyspnea, the restriction of salt is unwarranted; claims that such restriction may lower the blood pressure have not been substantiated. Obesity should be avoided for the same reasons that apply to normal individuals and not because of any demonstrated relationship to hypertensive disease."[12] "No dietary treatment is known which has a specifically favorable effect on essential hypertension."[13]

The rice-fruit-sugar diet is more rigid than any of the fat-poor, salt-poor or protein-poor "hypertension" diets (the therapeutic possibilities and limitations of these will not be discussed here.) The rice diet contains in 2,000 calories not more than 5 Gm. of fat and about 20 Gm. of protein derived from rice and fruit and not more than 200 mg. of chloride and 150 mg. of sodium. A patient takes an average of 250 to 350 Gm. of rice (dry weight) daily; any kind of rice may be used provided no sodium, chloride, milk, etc. has been added during its processing. The rice is boiled or steamed in plain water or fruit juice, without salt, milk or fat. If the sodium concentration of the plain water available is greater than 20 mg. per liter, distilled water should be used. All fruit juices and fruits are allowed, with the exception of nuts, dates, avocados and any dried or canned fruit or fruit derivatives to which substances other than white sugar have been added. Not more than one banana a day should be taken. White sugar and dextrose may be used *ad libitum;* on an average a patient takes about 100 Gm. daily but, if necessary, as much as 500 Gm. daily should be used. Tomato and vegetable juices are not allowed. Usually no water is given and the fluid intake is limited to 700 to 1,000 cc. of fruit juice per day. Supplementary vitamins are added in the following amounts: vitamin A 5,000 units, vitamin D 1,000 units, thiamine chloride 5 mg., riboflavin 5 mg., niacinamide 25 mg., calcium pantothenate 2 mg. No other medication is given unless it is specifically indicated.

During the first period of "regulation" on the diet, the patient should be under constant medical supervision and blood

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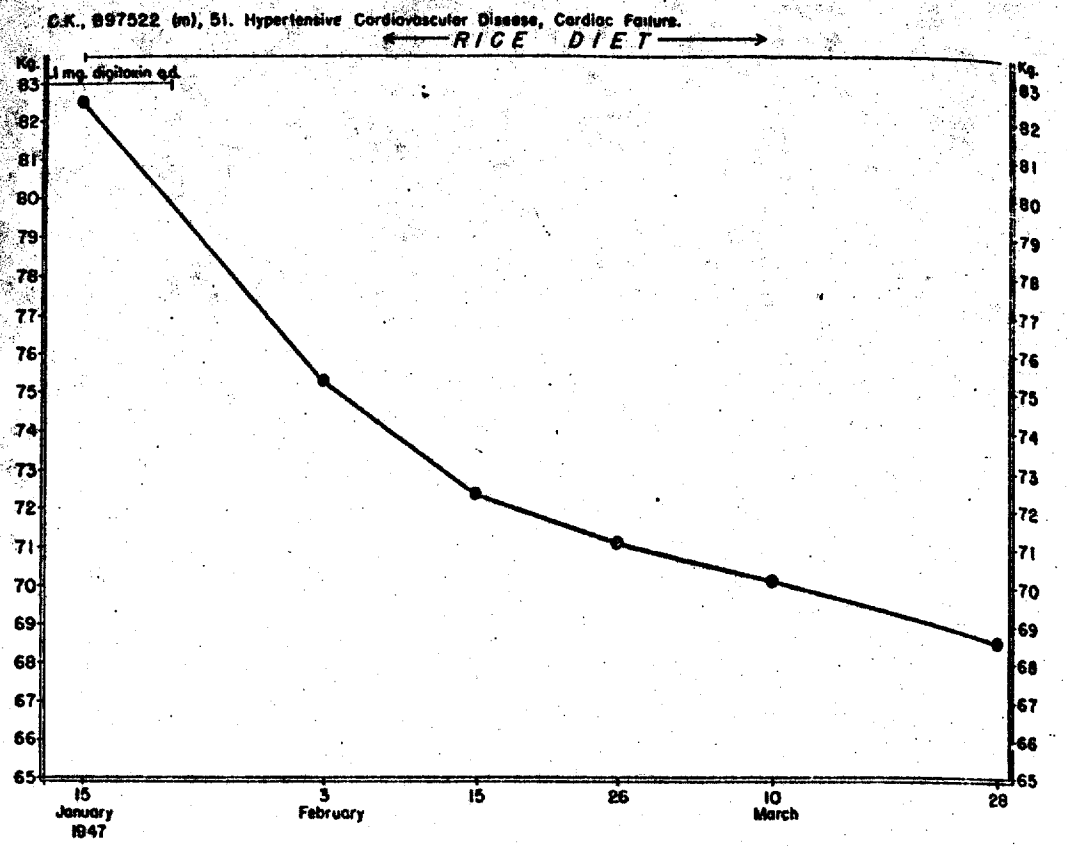


FIG. 1. C. K., male, fifty-one years of age. The patient had hypertensive vascular disease of seven years' duration, auricular fibrillation, cardiac failure of one year's duration, enlargement of liver and spleen and ascites. Previous treatment: digitalis, mercurials, ammonium chloride; codein; low salt, low fat, high protein diet; paracentesis 12 times in past year. January 15 to 21, 1947: Blood pressure, average, 174/97; NPN 44 mg. per 100 cc. blood; venous pressure 380 mm. of saline; total PSP excretion in two hours: 39 per cent. Rice diet started January 18, 1947, was strictly followed. All medication discontinued. On March 17, 1947, NPN 27 mg. per 100 cc. blood March 24 to 30, 1947: Blood pressure, average, 137/82. Ascites and edema unchecked by digitalis, mercurials, ammonium chloride, low salt high protein diet disappeared on rice diet without medication. There was a 14 Kg. weight loss in sixty-eight days.

and urine chemistry should be checked frequently.

Rest in bed, unless the severity of the condition demands it, is neither necessary nor desirable.

It is not unusual for the weight to decrease more or less markedly during the first twenty days. The reason for this weight loss may be that the amount of food given does not cover the caloric requirements; in this case the amount of rice, fruit and sugar must be increased unless reduction of weight is indicated. Another reason may be that the patient does not eat the full amount of his diet during the first period of adjustment. The most frequent cause is the loss of visible or invisible edema; for example, one patient with marked edema lost 63 pounds in the first sixteen days on the diet (no digitalis, mercurials, etc., were given).[5] Figure 1 shows the weight chart of another patient, a fifty-one year old physician, with hypertensive heart disease and auricular fibrillation whose persistent liver enlargement, ascites and edema had not improved in spite of intensive treatment with digitalis, mercurials, ammonium chloride, salt-poor diet and frequent paracenteses.

As a rule the diet should be continued without modification until those conditions

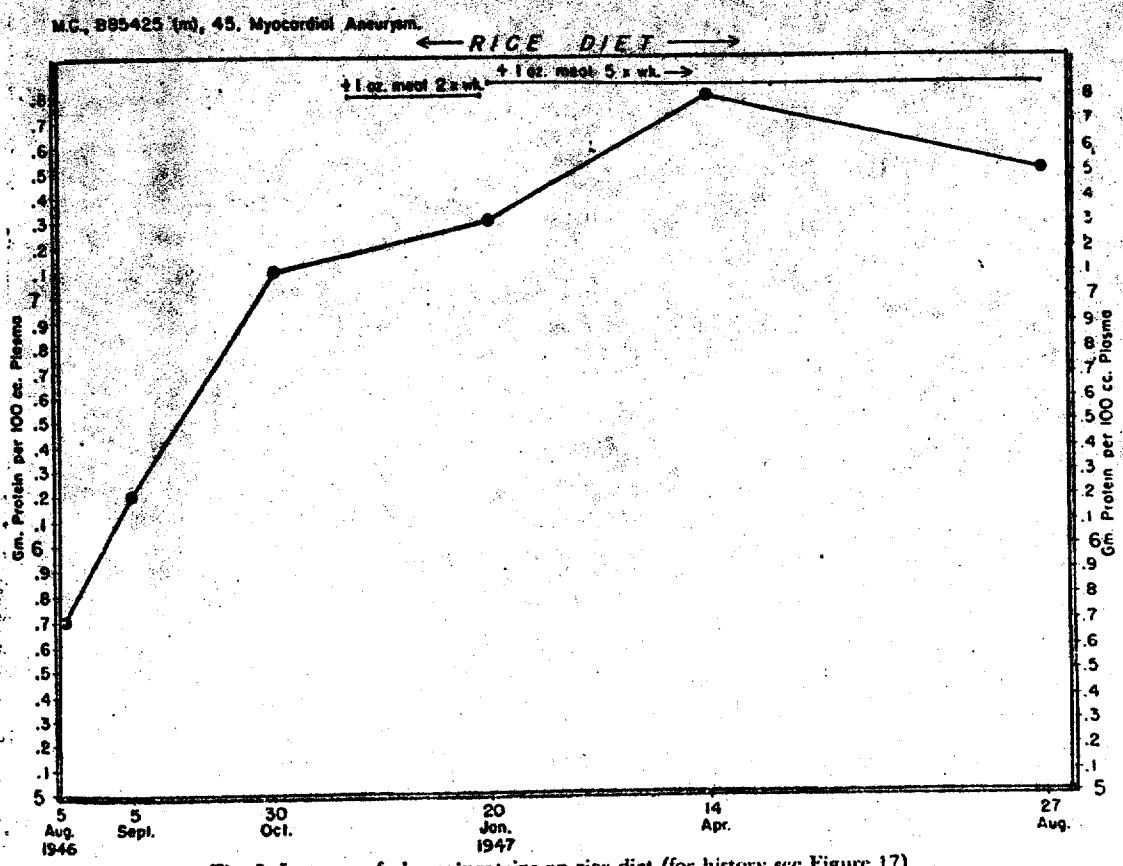


Fig. 2. Increase of plasma proteins on rice diet (for history see Figure 17).

which were the indication for its use have disappeared. Then small amounts of non-leguminous vegetables, potatoes, lean meat or fish (all prepared without salt or fat) may be added. But only so much additional food should be allowed as can be taken without producing undesirable changes in blood pressure, heart size, electrocardiogram, eyegrounds, non-protein nitrogen, etc. When a critical condition of heart, kidney or retina exists, the strict rice diet should be continued indefinitely provided that the equilibrium between intake and loss of those substances which are indispensable for the body is maintained.

CHEMICAL CHANGES PRODUCED BY THE RICE DIET

Nitrogen Metabolism. Because of the protein-sparing effect of carbohydrates, the protein equilibrium is maintained in spite of the low protein content of the rice diet.

A minimum of 50 Gm. of protein (type of protein not specified) has been postulated as the so-called "wear and tear quota" necessary to cover the daily protein requirements. However, since this figure is derived from the total nitrogen excretion of fasting individuals, which is about 7 Gm. in the urine and 0.9 Gm. in the stools, it indicates only the amount of the body protein broken down in fasting ($7.9 \times 6.25 = 49.4$). In patients who have followed the rice diet for two months or more the daily urinary total nitrogen excretion is less than one third of that in fasting. It averages 2.26 Gm.[5] If an allowance of 0.9 Gm. per twenty-four hours is made for the excretion of nitrogen other than that excreted in the urine, the total nitrogen loss in twenty-four hours is about 3.16 Gm. With a daily intake

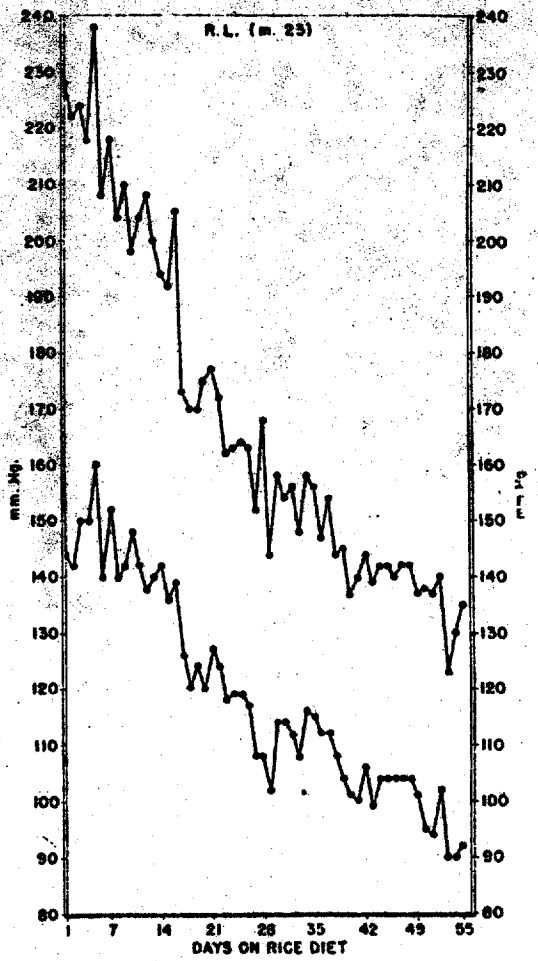


FIG. 3. R. L., male, twenty-three years of age. This patient had hypertensive vascular disease of three years' duration. He was previously treated with a "modified rice diet." EKG T_1 inverted. (Fig. 22.) Total PSP excretion in two hours: 2.5 per cent; NPN 79 mg. per 100 cc. blood; cholesterol 340 mg. per 100 cc. serum. There was advanced retinopathy. (Fig. 30.) Rice diet started December 18, 1945 and strictly followed for three months (8-21 mg. Cl per 100 cc. of urine). March 17, 1946: NPN 60 mg. per 100 cc. of blood; cholesterol 173 mg. per 100 cc. serum.....Decrease in blood pressure started in first week of rice diet.

of $3.16 \times 6.25 = 19.8$ Gm. of protein, these patients are in nitrogen equilibrium.

In fasting the daily urea nitrogen excretion in the urine is about 5.5 Gm. In the urine of patients who have followed the rice diet for two months or more the average daily urea nitrogen excretion is 1.1 Gm.[5]

In fasting the blood non-protein nitrogen and the blood urea nitrogen concentrations

TABLE 1
AVERAGE NPN AND UREA-N OF 261 PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE
(Initial NPN 20 to 45 mg. Per 100 cc. Blood)

No. of Patients	Average Period of Treatment (Days)	NPN Average Before Rice Diet	NPN Average After Rice Diet	Urea-N Average Before Rice Diet	Urea-N Average After Rice Diet
		Mg. Per 100 cc.	Mg. Per 100 cc.	Mg. Per 100 cc.	Mg. Per 100 cc.
NPN and Urea-N Increased					
13	62	31	35	12.5	16.0
NPN Increased, Urea-N Decreased					
10	74	30	32	11.4	7.5
NPN Decreased, Urea-N Increased					
3	83	32	31	8.9	12.8
NPN and Urea-N Decreased					
235	109	34	26	14.4	7.3
Total					
261	106	34	27	14.1	7.8

are higher than normal; on the rice diet they are lower than normal.[5] Table 1 shows the non-protein nitrogen and urea nitrogen in a series of 261 non-uremic patients with hypertensive vascular disease. The non-protein nitrogen before the diet ranged from 20 to 45 mg. per 100 cc. of blood; the average was 34 mg. After the diet it ranged from 18 to 45 mg.; the average was 27 mg. The urea nitrogen before the diet ranged from 4.8 to 30.3 mg. per 100 cc. of blood; the average was 14.1 mg. After the diet it ranged from 1.2 to 30.4 mg.; the average was 7.8 mg.

In starvation, hemoglobin and plasma protein concentrations decrease; on the rice

TABLE II

EFFECT OF HIGH AND LOW PROTEIN DIETS ON URINARY TOTAL NITROGEN AND CREATININE OF NORMAL MAN (FOLIN[14])

	120 Gm. Protein Egg-Milk Diet (3rd Day)	6 Gm. Protein Cream-Starch Diet (7th Day)
Total nitrogen (mg. N per 24 hr.)	16,800	3,600
Creatinine (mg. N per 24 hr.)	580	600

TABLE III

EFFECT OF FASTING ON URINARY CREATININE AND CREATINE OF NORMAL MAN (BENEDICT[15])

	1st Day of Fasting (Weight 59.6 Kg.)	6th Day (Weight 55.9 Kg.)	12th Day (Weight 53.6 Kg.)
Creatinine (mg. N per 24 hr.)	480	390	370
Creatine (mg. N per 24 hr.)	0	130	120
Total creatine bodies (mg. N per 24 hr.)	480	520	490

diet hemoglobin and plasma protein levels are maintained.[5] (Fig. 2.)

The excretion of creatinine plus creatine (total creatine bodies) has been supposed to remain fairly constant in spite of variations in protein intake and nitrogen excretion. (Table II).

The excretion of the total creatine bodies does not decrease in one to twelve days of fasting. The creatine fraction increases. (Table III.)

The excretion of total creatine bodies decreases markedly on the rice diet; the excretion of creatine does not increase. (Table IV.)

The decrease in the excretion of total creatine bodies ranged from 7 to 48 per cent, averaging 29 per cent; the decrease in weight ranged from 0 to 11 per cent, with an average of 6 per cent.

TABLE IV

CREATININE AND CREATINE IN URINE OF TWENTY-TWO PATIENTS (FIFTEEN MEN, SEVEN WOMEN) WITH HYPERTENSIVE VASCULAR DISEASE

	Before Rice Diet	After 35 Days (av.) on Rice Diet
Creatinine (mg. N per 24 hr.)	480	346
Creatine (mg. N per 24 hr.)	40	19
Total creatine bodies (mg. N per 24 hr.)	520	365

TABLE V

TOTAL SERUM CHOLESTEROL OF 284 PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE

Initial Concentration (Mg. Per 100 cc. of Serum)		No. of Patients	Average Period of Treatment (Days)	Mg. Cholesterol Per 100 cc. of Serum (average)		
				Before Treatment	After Treatment	Change
110–218	Increased	18	123	156	180	+24
	Increased to 220 or over	4	93	208	240	+32
	Decreased or constant	61	109	195	157	−38
		83	110	187	165	−22
220–585	Increased or constant	10	146	250	262	−12
	Decreased	59	76	320	253	−67
	Decreased below 220	132	81	273	177	−96
		201	82	286	204	−82
110–585		284	90	257	192	−65

As far as the metabolism of kidney cells is concerned rice protein cannot be indiscriminately replaced by other protein. Proteins differ from each other in regard both to the type and the relative proportion of the various amino acids of which they are composed. They also differ in regard to the rate and degree of assimilation; 30 Gm. of a protein of which 88 per cent is assimilated may be preferable to 50 Gm. of a protein of which only 40 per cent is assimilated.

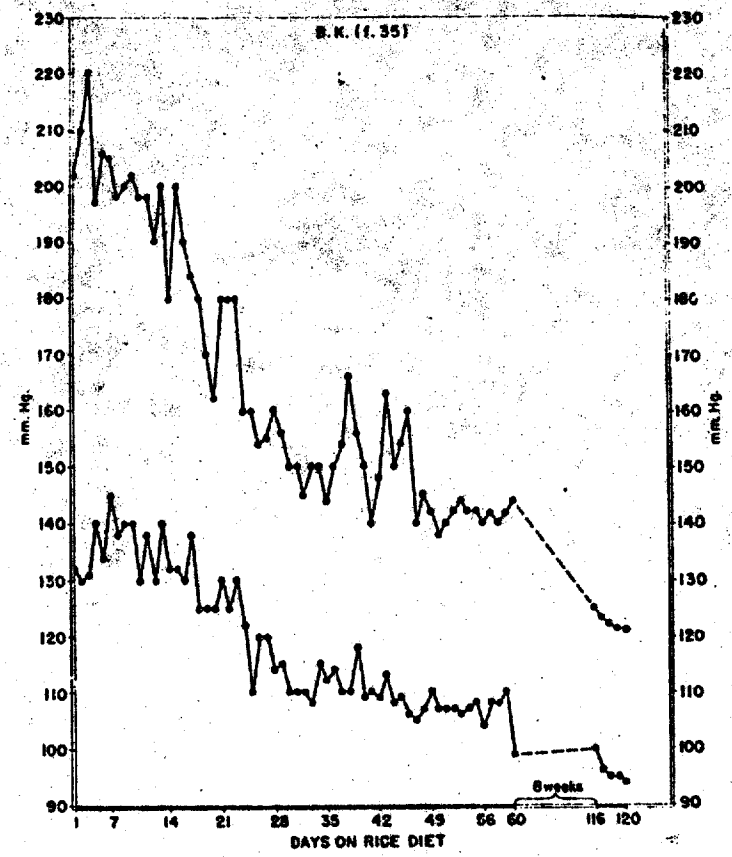


FIG. 4. B. K., female, thirty-five years of age. Patient had hypertensive vascular disease of eleven years' duration beginning during the eighth month of her second pregnancy. Of two brothers with hypertensive vascular disease, one had died at the age of thirty-seven (stroke). The patient had two retinal hemorrhages. Previous treatment: rutin, vitamin K, sedatives. Total PSP excretion in two hours 64 per cent; serum cholesterol 250 mg. per 100 cc. Rice diet was started April 23, 1947, and strictly followed (7–14 mg. Cl per 100 cc. of urine). No medication was given. ———— A decrease in blood pressure began in third week on rice diet.

The factor of assimilation may be important not only because of the amount of protein that can be utilized to meet the body requirements but also because of the amount of the non-utilized protein fraction, the fate and rôle of which have yet to be determined.

Cholesterol. The relation between serum cholesterol and vascular disease (arteriosclerosis, coronary disease, vascular retinopathy, hypertensive vascular disease) has been the subject of extensive study.

Hypercholesterolemia, regardless of its primary cause in a given case, is just as significant a metabolic disturbance as persistent hyperglycemia or hyperuricemia and should probably be considered as serious a disease, as far as potential consequences are concerned, as diabetes mellitus and gout.

Hypercholesterolemia decreases markedly with the rice diet.[1,6,11] Table v shows the effect of the diet on the total serum cholesterol concentration of 284 patients with hypertensive vascular disease. Two hundred one of these patients (i.e., 70 per cent) had hypercholesterolemia (cholesterol concen-

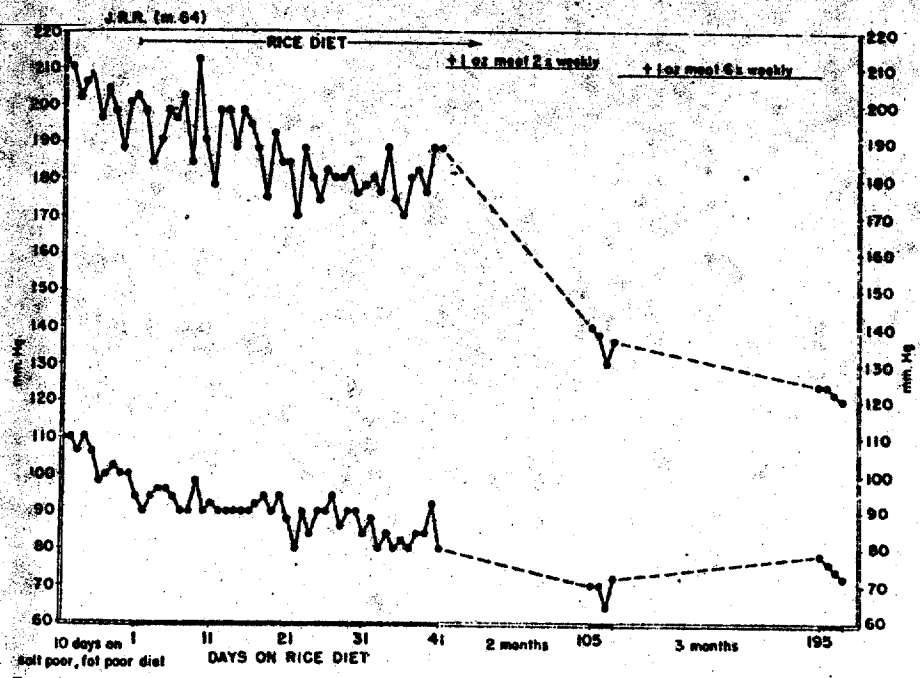


FIG. 5. J. R. R., a male, sixty-four years of age, had hypertensive vascular disease of six years' duration, four retinal hemorrhages and severe headache. He was treated previously with potassium thiocyanate. Total PSP excretion in two hours, 32 per cent. On October 7, 1946: Transverse diameter of heart, 15.2 cm; diameter of great vessels, 10.5 cm.; weight, 62.8 Kg. Rice diet was started October 17, 1946, and strictly followed (4–9 mg. Cl per 100 cc. of urine). No medication was given. The patient was working and was asymptomatic. May 2, 1947: Transverse diameter of heart, 11.9 cm.; diameter of great vessels, 8.6 cm.; weight, 64.4 Kg. No retinal hemorrhages were present. There was reduction in heart size and in size of great vessels. (Fig. 8.) Decrease in blood pressure was definite after 105 days.

tration of at least 220 mg. per 100 cc. serum) at the beginning of the diet.

Four patients whose serum cholesterol concentration was below the upper limits of normal had an increase to a hypercholesterolemic level (average before rice diet 208 mg. per 100 cc. serum, after rice diet 240 mg. per 100 cc. serum). One hundred thirty-two patients who had hypercholesterolemia had a decrease to a normal level (average before treatment 273, after treatment 177 mg. per 100 cc. serum). (Table v.)

Starke[16] examined the concentration of free cholesterol and cholesterol esters in the serum of seventy-nine patients with hypertensive vascular disease who had a total cholesterol concentration of 220 to 463 mg. per 100 cc. of serum at the beginning of the diet. Free cholesterol and cholesterol esters decrease on the rice diet in about the same proportion. (Table VI.)

Chloride, Sodium, Potassium. Therapeutic results with sodium chloride restriction such as those obtained by Allen and Sherrill[17] and by Volhard[18] were explained by Fishberg[13] on the assumption that the unpalatability of the diet led to an inadequate caloric intake and thus to a reduction of the metabolic rate. According to Page[19] the results obtained were due not to salt restriction but to "rest in bed and the psychotherapy of constant attention."

The treatment with the rice diet, which includes rigid sodium and chloride restriction, made it possible to determine the effect of a prolonged minimal intake of sodium and chloride on the concentration of these ions in blood, serum and urine.

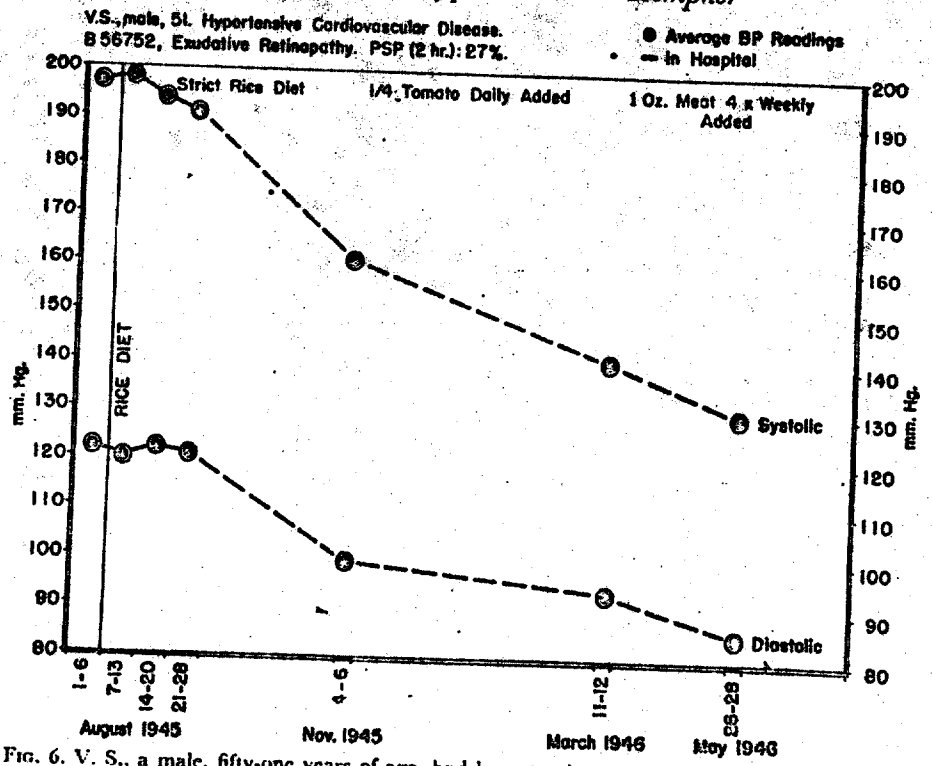


FIG. 6. V. S., a male, fifty-one years of age, had hypertensive vascular disease of eleven years' duration and retinal exudates. Previous treatment consisted of rest, sedatives and a "modified rice diet" for one month. Total PSP excretion in two hours: 21–33 per cent. Rice diet was started August 6, 1945, and strictly followed (5–20 mg. Cl per 100 cc. of urine). No medication was given. Working hours were restricted. There was a gradual decrease of blood pressure after the third month of rice diet.

In a series of 213 patients treated with the rice diet the lowest urine chloride concentration found was 48 mg. Cl per liter with a total urinary excretion of 18 mg. Cl in twenty-four hours in a patient with hypertensive vascular disease who had been on the rice diet for seventy days. The plasma chlorides were 93.1 mEq. (as NaCl: 544 mg. per 100 cc.). The average values of 381 determinations of the plasma chlorides in ninety-one non-uremic patients with hypertensive vascular disease or primary kidney disease were: before rice diet, 97.0 mEq. per 1,000 cc. of plasma; after forty-four days (average) of rice diet, 91.7 mEq. per 1,000 cc. of plasma.[5]

Table VII gives a comparison of the concentrations of chloride, sodium and potassium in the urine of persons on a normal diet and of patients after two months on the rice diet.[11]

The average values of the chloride, sodium and potassium concentrations and their ratios in whole blood, serum and urine in thirty-seven patients with hypertensive vascular disease treated with the rice diet for an average of thirty-six days are shown in Tables VIII and IX.

In thirteen of the thirty-seven patients there was "secondary" renal involvement; in twenty-four patients there was no evidence of renal involvement. The sodium chloride content of the diet of many of these patients had been limited before they were started on the rice diet. None of these patients was in renal failure with sodium chloride leakage.

The following average changes were found: In the urine there was a decrease in the sodium concentration of 99 per cent and in the chloride concentration of 96 per cent and an increase in the potassium concentration of 78 per cent. The sodium to potassium ratio decreased by 99 per cent and the chloride to potassium ratio by 97 per cent. There was a decrease of 79 per cent in the sodium to chloride ratio. All these changes are statistically significant.

TABLE VI
FREE CHOLESTEROL AND CHOLESTEROL ESTERS IN THE SERUM OF 79 PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE

	Before Rice Diet	After 159 Days (Average) on Rice Diet
Free cholesterol (mg. per 100 cc. serum)	80	61
Cholesterol esters (mg. per 100 cc. serum)	205	146
Total cholesterol (mg. per 100 cc. serum)	285	207

TABLE VII
URINE CHLORIDE, SODIUM, POTASSIUM ON "NORMAL" DIET AND ON RICE DIET

	Normal Diet	Rice Diet (after 2 Months)
Chloride (Gm. Cl per 1,000 cc.)	6	0.1
Sodium (Gm. Na per 1,000 cc.)	4	0.01
Potassium (Gm. K per 1,000 cc.)	2	3.0
Gm. Na/Gm. K Ratio	2	0.003

In whole blood there was a statistically significant decrease of 4.3 per cent in the sodium concentration corresponding to an increase in hemoconcentration. There was a statistically significant decrease of 5.6 per cent in the chloride concentration. The sodium to chloride ratio remained constant. There was a statistically insignificant increase of 0.8 per cent in the potassium concentration and a statistically insignificant decrease of 3.4 per cent in the sodium to potassium ratio. The chloride to potassium ratio showed a decrease of 4.7 per cent (T value 2.1; probably statistically significant).

In the serum there was a statistically insignificant decrease of 0.7 per cent in the sodium concentration. Statistically significant changes in the serum were: a decrease of 6.2 per cent in the chloride concentration; an increase of 6.1 per cent in the sodium to chloride ratio; an increase of 11.3 per cent in the potassium concentration; a decrease of 8.6 per cent in the sodium to

TABLE VIII
CHLORIDE, SODIUM AND POTASSIUM CONCENTRATIONS IN WHOLE BLOOD, SERUM AND URINE OF THIRTY-SEVEN PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE BEFORE AND AFTER THIRTY-SIX DAYS (AVERAGE) ON RICE DIET
(Average Values)

	Whole Blood			Serum			Urine		
	Before Rice Diet	After Rice Diet	Change %	Before Rice Diet	After Rice Diet	Change %	Before Rice Diet	After Rice Diet	Change %
	mEq./1,000 cc.			mEq./1,000 cc.			mEq./1,000 cc.		
Chloride	80.2	75.7	−5.6	100.8	94.5	−6.2	86.2	2.50	−96.2
Sodium	82.0	78.2	−4.3	142.8	141.7	−0.7	81.7	0.43	−99.2
Potassium	49.5	49.5	+0.8	4.47	4.86	+11.3	64.4	88.6	+77.8

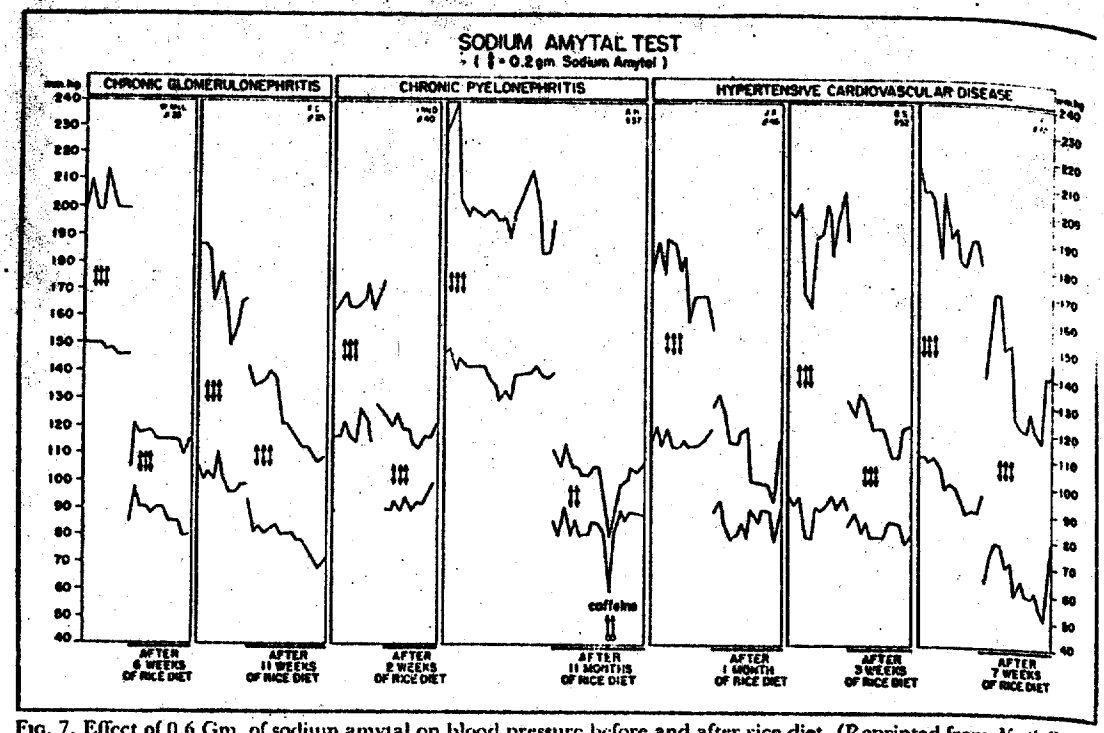


FIG. 7. Effect of 0.6 Gm. of sodium amytal on blood pressure before and after rice diet. (Reprinted from *North Carolina M. J.*, 6: 65, 1945).

TABLE IX

$\frac{\text{SODIUM}}{\text{POTASSIUM}}$, $\frac{\text{CHLORIDE}}{\text{POTASSIUM}}$ AND $\frac{\text{SODIUM}}{\text{CHLORIDE}}$ RATIOS

IN WHOLE BLOOD, SERUM AND URINE OF THIRTY-SEVEN PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE BEFORE AND AFTER THIRTY-SIX DAYS (AVERAGE) ON RICE DIET

(Average Values)

	Whole Blood			Serum			Urine		
	Before Rice Diet	After Rice Diet	Change %	Before Rice Diet	After Rice Diet	Change %	Before Rice Diet	After Rice Diet	Change %
Na/K	1.67	1.61	−3.4	32.7	29.4	−8.6	1.66	0.006	−99.3
Cl/K	1.63	1.55	−4.7	23.1	19.6	−14.0	1.71	0.03	−96.9
Na/Cl	1.02	1.03	+1.6	1.42	1.50	+6.1	0.92	0.18	−79.4

potassium ratio; a decrease of 14.0 per cent in the chloride to potassium ratio.

Sulfate, Phosphate and Ammonia Excretion in Urine. Chloride, sulfate and phosphate account for about 85 per cent of the acid excreted in the urine on a normal diet.

As Tables X and XI show the inorganic sulfate excretion in patients on the rice diet decreases by 80 per cent; the inorganic phosphate excretion decreases by 60 per cent.[20]

Ammonia is formed in the kidney by oxidative deamination of amino acids: blood and tissue acids reaching the kidney

A B

FIG. 8. J. R. R., a male, sixty-four years of age, had hypertensive vascular disease of six years' duration, four retinal hemorrhages and severe headache. He was previously treated with potassium thiocyanate (three years). A, October 7, 1946; Blood pressure 212/110; weight, 62.8 Kg.; total PSP excretion in two hours, 32 per cent. Rice diet was started October 17, 1946, and strictly followed (4–9 mg. Cl. per 100 cc. of urine). No medication was given. B, May 2, 1947; Blood pressure 122/74; weight, 64.4 Kg. No appreciable drop in blood pressure after forty-one days of diet. The patient was working. A definite drop in blood pressure was noted after 105 days of diet. (Fig. 5.) The patient was asymptomatic and retinal hemorrhages had disappeared. There was a reduction in heart size with change in transverse diameter of 28 per cent and a reduction in size of great vessels with change of 22 per cent.

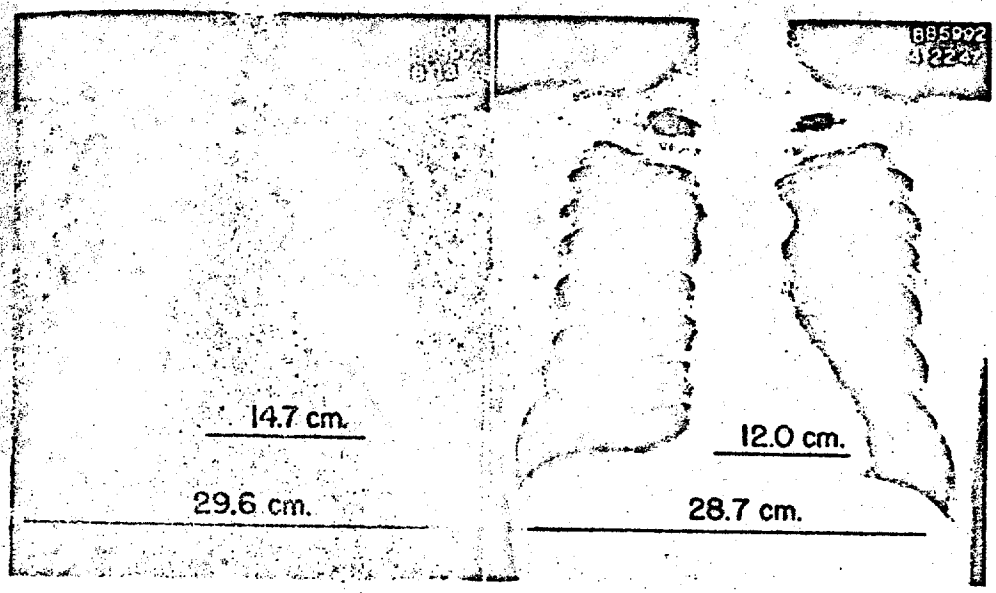
TABLE X
SULFATE EXCRETION IN URINE OF FOURTEEN PATIENTS (TEN MEN, FOUR WOMEN) WITH HYPERTENSIVE VASCULAR DISEASE—NO RENAL FAILURE

	Range		Average		
	Before Rice Diet	After 36 Days (Average) on Rice Diet	Before Rice Diet	After 36 Days (Average) on Rice Diet	Decrease (%)
	(Mg. S in 24 Hr.)		(Mg. S in 24 Hr.)		
Total sulfate	761–471	254–58	592	126	79
Inorganic sulfate	547–362	165–40	452	81	82
Ethereal sulfate	328– 52	115–15	140	45	56

TABLE XI
PHOSPHATE EXCRETION IN URINE OF SEVENTEEN PATIENTS (THIRTEEN MEN, FOUR WOMEN) WITH HYPERTENSIVE VASCULAR DISEASE—NO RENAL FAILURE

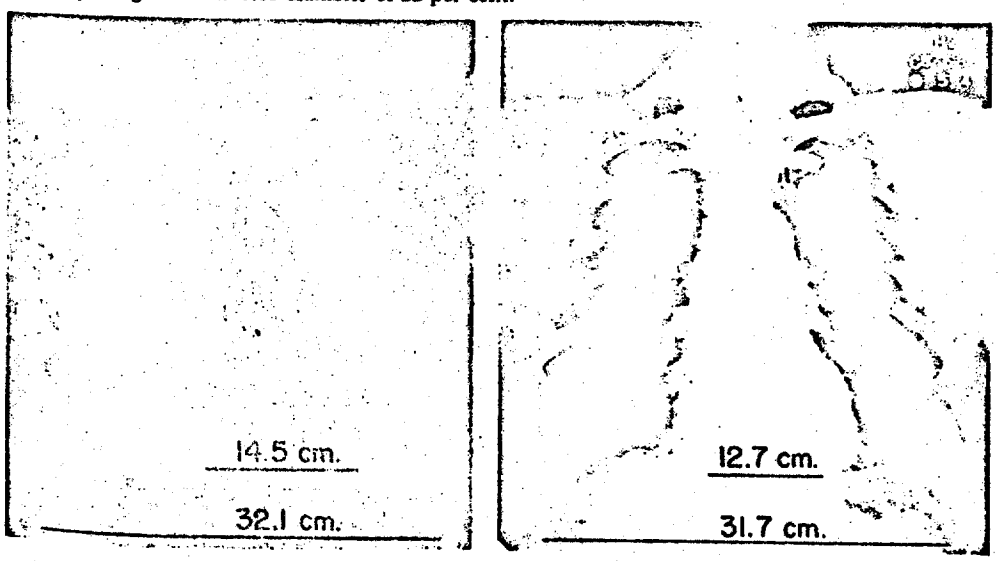
	Range		Average		
	Before Rice Diet	After 34 Days (Average) on Rice Diet	Before Rice Diet	After 34 Days (Average) on Rice Diet	Decrease (%)
	(Mg. P in 24 Hr.)		(Mg. P in 24 Hr.)		
Inorganic phosphate	1055–501	435–170	761	289	62

as salts of fixed base are converted there into ammonium salts and excreted as such in the urine; thus the fixed base in the body is conserved. Under pathologic conditions (e.g., at lowered oxygen concentrations) the rate of deamination of amino acids and of ammonia production in the kidney is decreased.[6,8] The acid must be excreted in the urine as salts of fixed base, the fixed base in blood and tissues decreases and uremic acidosis follows.[9,10] In considering the significance of the figures in Tables X and XI one might speculate about the possibility of forestalling an accumulation of acids in blood and tissue fluids by restricting



A B

FIG. 9. I. S., a male, forty-one years of age, had hypertensive vascular disease of three years' duration, retinal hemorrhages and exudates. Previous treatment consisted of rest and phenobarbital. Total PSP excretion in two hours, 60 per cent. A, August 12 to 13, 1946; Blood pressure 220/150; cholesterol 290 mg. per 100 cc. serum; EKG T_1 diphasic to inverted; weight, 72.5 Kg. Rice diet was started August 17, 1946 and strictly followed for two months (2–7 mg. Cl per 100 cc. of urine); then moderately well followed (35–36 mg. Cl per 100 cc. of urine). No medication was given; the patient was working. B, April 21, 1947; Blood pressure 128/88; cholesterol 155 mg. per 100 cc. of serum; EKG T_1 upright. Retinal hemorrhages and exudates had disappeared; weight 56 Kg.——There was decrease in blood pressure and reduction in heart size with change in transverse diameter of 22 per cent.



A B

FIG. 10. H. H., a male, sixty-three years of age, had hypertensive vascular disease of at least two and one-half years' duration and a stroke 1946. Previous treatment consisted of aminophyllin, rest, sedatives, weight reduction. Total PSP excretion in two hours 56 per cent. A, June 3, 1947; Blood pressure 217/124; weight, 76.3 Kg. Rice diet was started June 7, 1947, and strictly followed for three months (9–23 mg. Cl per 100 cc. of urine.) No medication was given. B, September 7, 1947; Blood pressure 170/98; weight, 70.7 Kg. ——There was a decrease in blood pressure and a reduction in heart size with change in transverse diameter of 14 per cent.

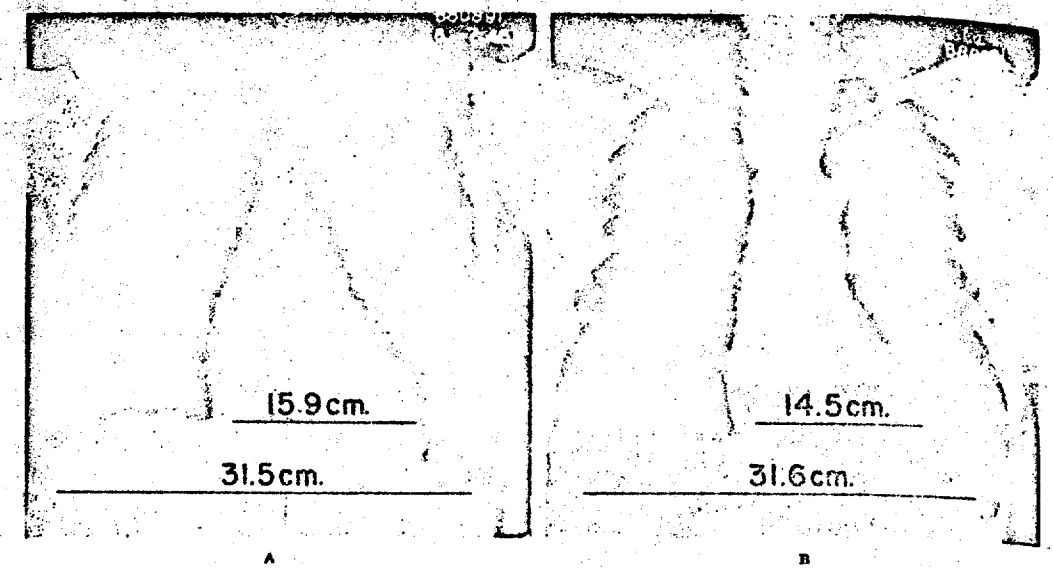


FIG. 11. R. H., a male, fifty-two years of age, had hypertensive vascular disease of three years' duration and pulmonary edema. Was treated with digitalis, mercuhydrin, aminophyllin and morphine. Total PSP excretion in two hours 39 per cent. A, June 8, 1946; Blood pressure 222/130; weight, 78.7 Kg. Rice diet was started June 12, 1946, and strictly followed for four months (11–22 mg. Cl per 100 cc. of urine); then moderately well followed (43–48 mg. Cl per 100 cc. of urine). All medication except digitalis was discontinued at beginning of rice diet. Digitalis was discontinued 7–24–46. B, May 31, 1947; Blood pressure 178/106; weight 71.2 Kg.; the patient was asymptomatic and resumed his practice as surgeon.——There was a decrease in blood pressure and reduction in heart size with change in transverse diameter of 10 per cent.

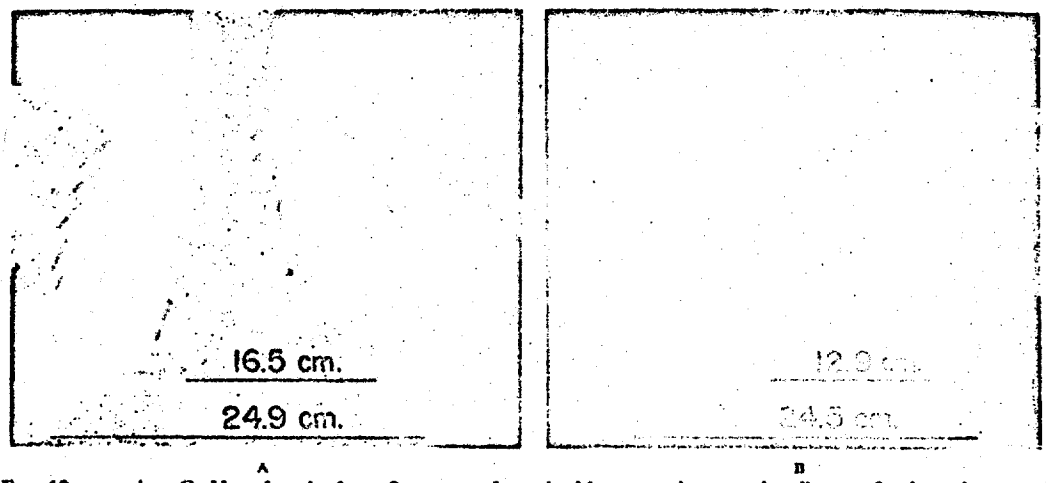
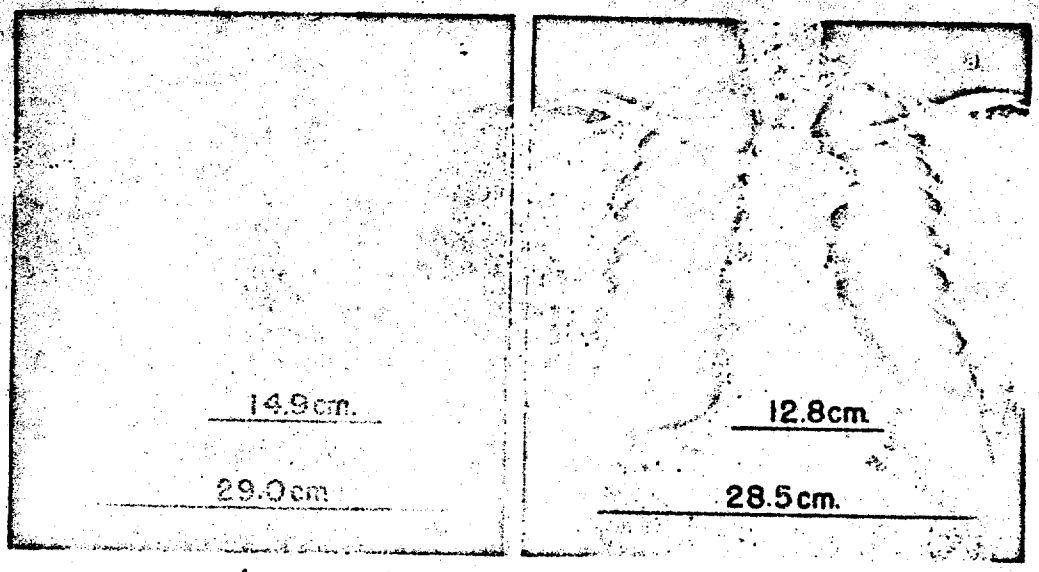
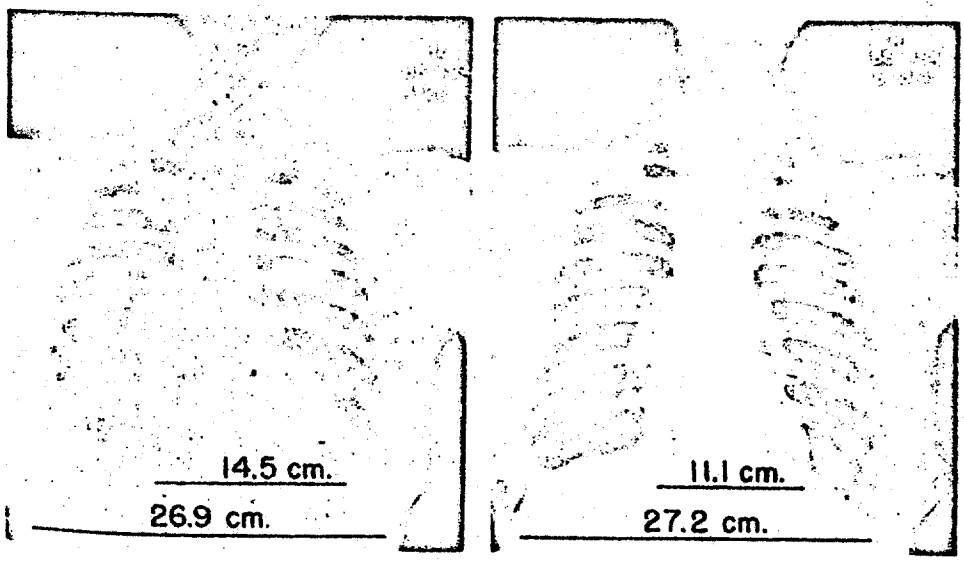


FIG. 12. A and B. G. H., a female, forty-five years of age, had hypertensive vascular disease of at least three years' duration, retinal hemorrhages and exudates. Total PSP excretion in two hours, 52 per cent. A, June 14, 1946; Blood pressure 258/138; EKG T_1 inverted; weight, 64.8 Kg. Rice diet was started June 20, 1946, and strictly followed for four months (4–13 mg. Cl per 100 cc. of urine); then moderately well followed (26–31 mg. Cl per 100 cc. of urine); no medication was given. The patient was active. B, May 28, 1947; Blood pressure 184/98; EKG T_1 upright; weight 59.6 Kg. No retinal hemorrhages or exudates were present.——There was a decrease in blood pressure and reduction in heart size with change in transverse diameter of 28 per cent.



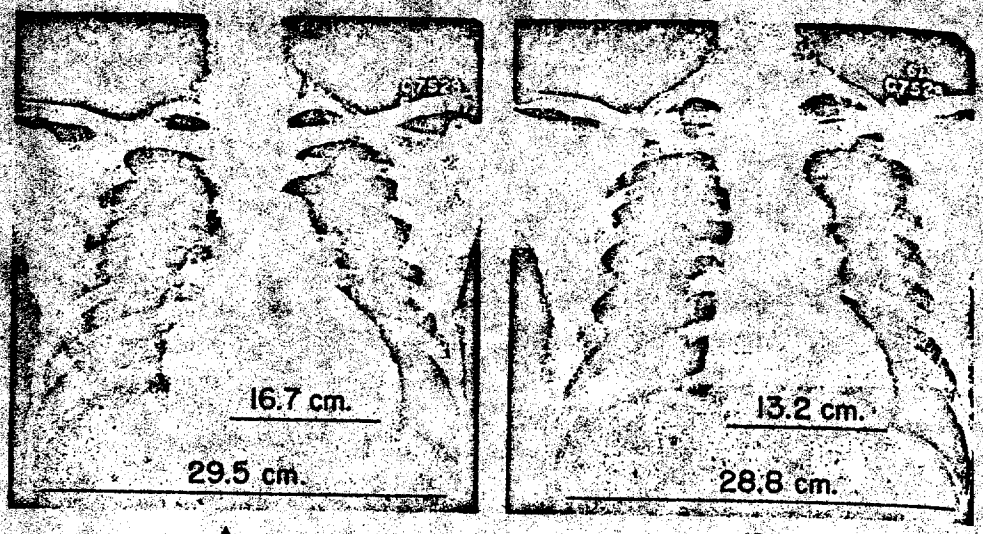
A B

FIG. 13. A and B. O. P., a male, forty-one years of age, had hypertensive vascular disease of five years' duration with severe headache. September 5, 1946; Blood pressure 186/122; EKG T_1 inverted; total PSP excretion in two hours 79 per cent; weight 68.7 Kg. Rice diet was started September 9, 1946; no medication was given. He was asymptomatic and able to do his work. On April 1, 1947; Blood pressure 150/100; EKG T_1 upright; weight, 66.4 Kg.——————There was a decrease in blood pressure and reduction in heart size with change in transverse diameter of 16 per cent.



A B

FIG. 14. A and B. R. Z., a female, fifty-three years of age, had hypertensive vascular disease (of at least five years' duration) and diabetes mellitus. Previous treatment: reduction diet (25 pound weight loss). April 19, 1945; Blood pressure 202/140; weight 53 Kg.; BMR ×45 per cent; total PSP excretion in two hours 62 per cent, sugar, 231 mg. per 100 cc. blood (no insulin). Rice diet was started April 22, 1945; it was well followed through May, 1945, and from January, 1946 to February, 1947 (7–15 mg. Cl per 100 cc. of urine). No digitalis was given. From August, 1945 to December, 1946, 10–30 units of insulin were given daily. May 13, 1947: sugar, 113 mg. per 100 cc. blood (no insulin). May 14, 1947: Blood pressure 224/112; weight, 50 Kg.; BMR −10 per cent.——————There was reduction in heart size with change in transverse diameter of 31 per cent in spite of persistence of high blood pressure.



A B

FIG. 15. A and B, J. P., a male, forty-two years of age, had hypertensive vascular disease of ten years' duration and cardiac failure of six months' duration. Previous treatment: sympathectomy in 1940 and from 1940 to 1946 potassium thiocyanate and phenobarbital. In 1946 pituitary irradiation was given; since 1946, digitalis, mercurials and sedatives (0.6 Gm. sodium amytal daily). May, 1947: Total PSP excretion in two hours, 32 per cent; venous pressure 195 mm. of saline. May 17 to 27, 1947; Blood pressure 220/152; weight 60.3 Kg. Rice diet was started on May 17th and strictly followed for four months. All medication was discontinued on May 17th except digitalis which was discontinued June 9, 1947. September 8, 1947; Blood pressure 214/148; weight 57.1 Kg.——A reduction in heart size occurred with change in transverse diameter of 27 per cent, in spite of persistence of high blood pressure.

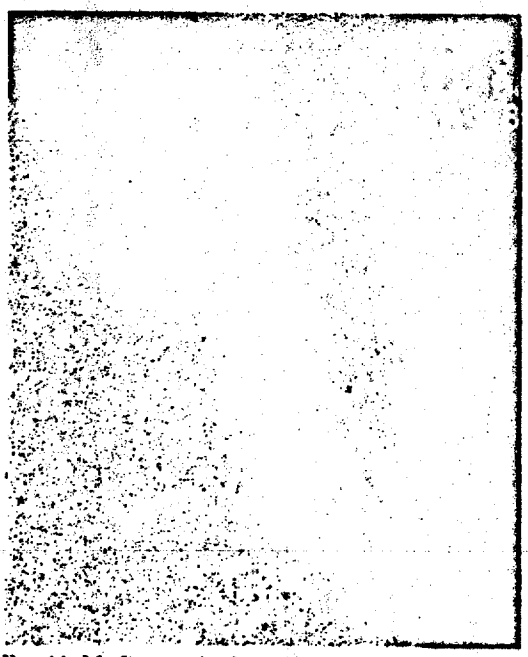


FIG. 16. M. C., a male, forty-five. Planogram of myocardial aneurysm. (See Figure 17.)

now prevailing in other populations such as the Chinese."[23,24]

G. Dick and Schwartz (1947) measured the arterial pressure in dogs in which hypertension had been produced by a nephrosclerosis which followed the intravenous administration of streptococci. At the time when the rice diet was started the hypertension had been maintained for two to four years. Dick and Schwartz found an average decrease of the mean arterial pressure from 181.6 to 138 mm. Hg after eight weeks on the diet. They conclude: "It appears that the Kempner regime is capable of causing significant lowering of the arterial blood pressure of dogs made hypertensive through the induction of nephrosclerosis. The role of weight loss, salt restriction, and nitrogen balance in this result requires further study."[25]

INDICATIONS AND CONTRAINDICATIONS

The apparent simplicity of the rice diet has not infrequently proved a handicap.

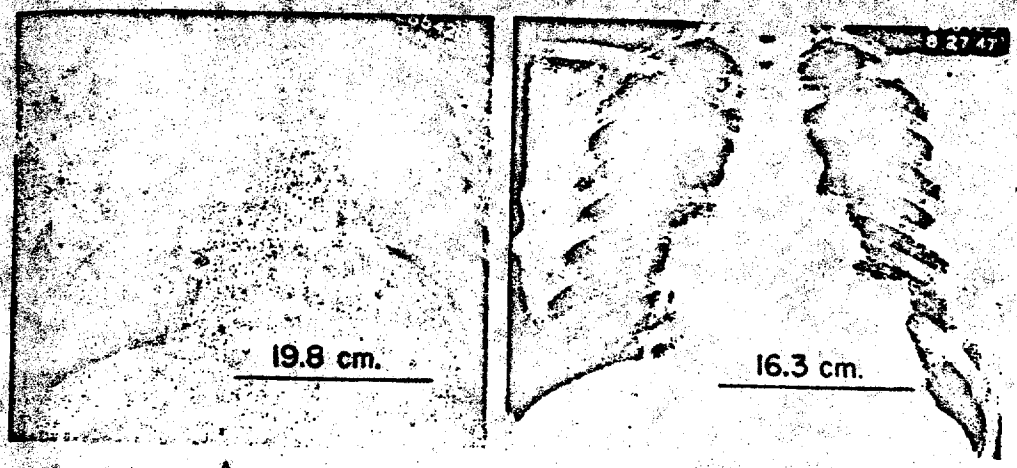


FIG. 17. A and B, M. C., a male, forty-five years of age, had a history of hypertension in 1944, myocardial infarction in 1945, followed by myocardial aneurysm.[26] There was progressive cardiac failure with massive peripheral edema, ascites, liver enlargement, hypoproteinemia (Fig. 2), hypocalcemia, albuminuria, decubitus ulcer and dyspnea. Previous treatment: (four months' hospitalization) low-protein, salt-poor diet, oxygen, digitalis, salyrgan, aminophyllin, ammonium chloride, theominal, coramine, sedatives; i.v. glucose; paracentesis. Rice diet was started August 7, 1946, and strictly followed; paracentesis August 13th; oxygen inhalation. No medication was given except digitalis which was discontinued October 10, 1946. Blood pressure August 6, 1946 was 138/94, August 27, 1947, 118/94.——Advanced myocardial failure unchecked by previous intensive treatment was compensated by rice diet. The patient became asymptomatic and reduction in heart size occurred with change in transverse diameter of 21 per cent.

We have seen patients who had been treated with the diet just because the manometer had shown blood pressure figures above normal and in whom tumors, infections, etc. had been overlooked.

The rice diet is indicated in all serious instances of acute and chronic nephritis;[1–5,11] in heart failure which does not respond to the customary treatment with salt restriction and drugs;[1–5,11,26] in arteriosclerotic and hypertensive vascular disease with cardiac, cerebral, retinal or renal involvement.[1–5,11,26]

The rice diet should be tried in uncomplicated hypertensive vascular disease when a more liberal regimen (fat-poor, salt-poor diets, weight adjustment, restriction of activities, regulation of bowel habits, sedation, etc.) has failed.

The rice diet should be used as a therapeutic test before sympathectomy is considered. If the dietary treatment proves ineffective, it can be discontinued.

In cases complicated by peptic ulcer the rice diet has to be modified. The rice is well tolerated, but raw fruit should be avoided and only cooked, strained fruit should be used. Water or dialyzed milk may be substituted for the fruit juices.

The rice diet is not contraindicated in cases complicated by diabetes mellitus. It may in fact have a special value because of the dangerous rôle played by hypercholesterolemia in this disease.[27] It was expected that in order to maintain the previous blood sugar levels larger amounts of insulin would have to be given. We found instead that in many cases the blood sugar decreased on the rice diet and the insulin dose had to be reduced.

The rice diet is contraindicated unless frequent checks of the patient's blood and urine chemistry are possible. This is of especial importance in patients with renal sodium chloride leakage as the following history may illustrate:

A patient with hypertensive heart disease (Figs. 18 and 19) had been on the diet for seven months. He had followed it very strictly. After three weeks on the diet the serum chloride was 95 and the serum

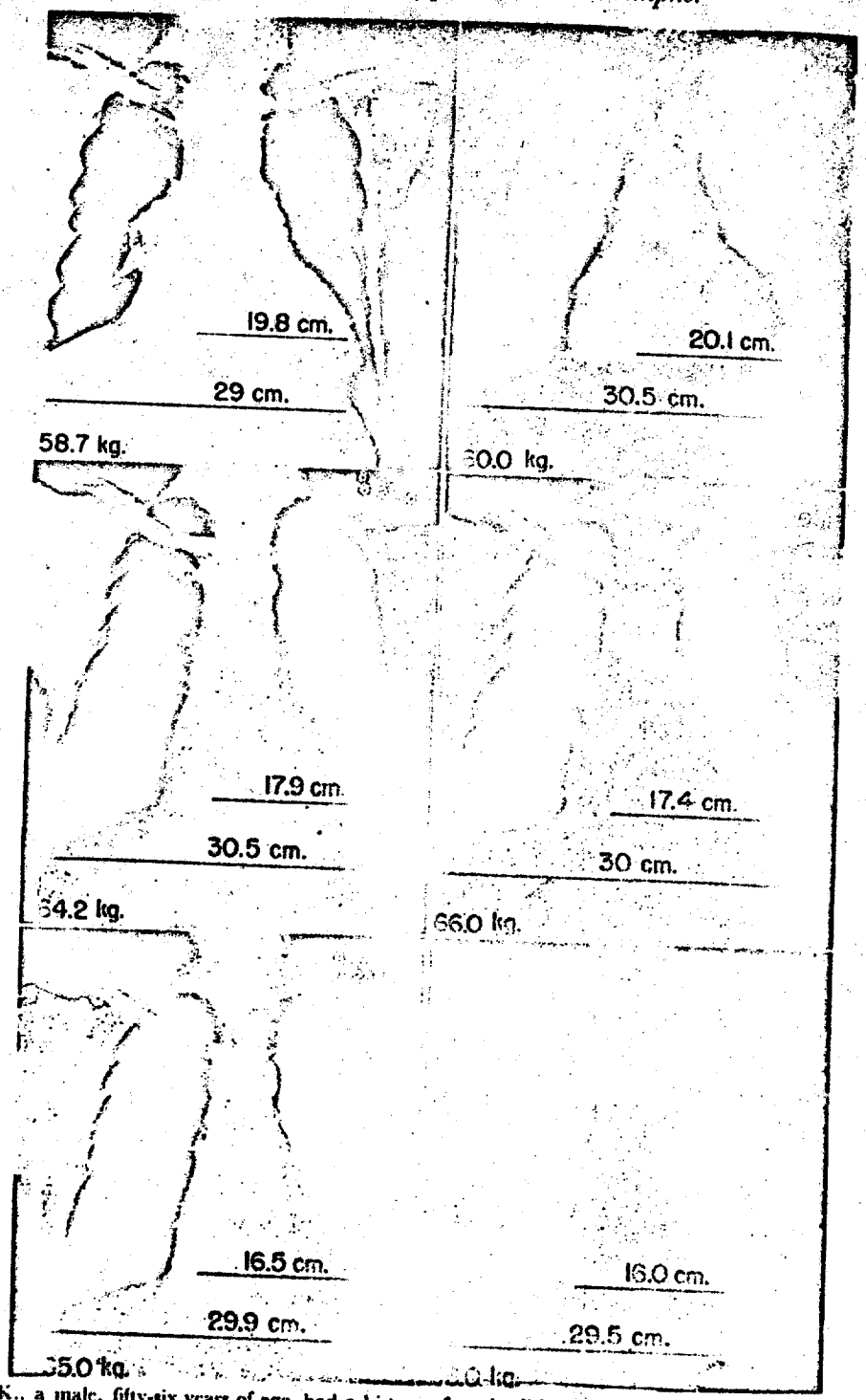


FIG. 18. P. K., a male, fifty-six years of age, had a history of nephrolithiasis, hypertensive vascular disease of more than ten years' duration, nephrectomy (left) in 1940, heart disease of three years' duration; left bundle branch block, dyspnea, edema. Previous treatment: salt-poor diet, digitalis, squill, salyrgan, mercupurin, ammonium chloride, sedatives. Total PSP excretion in two hours, 24 per cent; NPN 45 mg. per 100 cc. of blood; blood pressure 145/90. Rice diet was started April 3, 1946, and strictly followed (1–10 mg. Cl per 100 cc. of urine). All medication discontinued except digitalis. Digitalis was discontinued April 20, 1946. There was a weight gain of 7.3 Kg and a gradual decrease in heart size.

sodium 135 mEq. per liter; after four months, 87 and 138 mEq. respectively. From the fifth month on he had felt well and had been completely asymptomatic. One evening, after some hours work at carpentry, he suddenly became unconscious and remained so for many hours. His hands and feet were extremely cold and, on regaining consciousness, he felt very weak and "faint." The attending internist who was familiar with the treatment made a diagnosis of stroke. However, on the addition of a few vegetables to his diet, all the symptoms disappeared and three days later when the patient was brought to our hospital, examination of the serum revealed a chloride concentration of 69 and a sodium concentration of 125 mEq. per liter.

CLINICAL CHANGES PRODUCED BY THE RICE DIET

A great many patients on the rice diet have experienced relief from headache, giddiness, fatigue, dyspnea and substernal pain. Such subjective improvement has not been accepted as evidence of successful therapy. Only measured results such as decrease in blood pressure, reduction in heart size, loss of edema and reversion of electrocardiogram or eyeground changes have been used to determine the effect of the treatment.

The therapeutic results in eighty patients with acute or chronic primary kidney disease and in 130 patients with hypertensive vascular disease were reported in 1945.[5] By 1946 one hundred patients with primary kidney disease and 222 patients with hypertensive vascular disease had been treated with the rice diet.[11] This paper is limited to the changes obtained in patients with hypertensive vascular disease.

The effect of the diet has been determined in 500 patients most of whom were seriously ill and had failed to respond to other forms of treatment. The diet has been ineffective in 178 of these 500 patients if we include twenty-six patients who were in a critical condition when started on the diet and who died after an average period of thirty-nine days. In 322 of the 500 patients the diet has proved beneficial, i.e., it has produced one or more of the following effects: decrease in "mean" arterial blood pressure of at least 20 mm. Hg; reduction in heart size with change in the transverse diameter of 18 per cent or more; a change in T_1 from completely inverted to upright; disappearance of severe retinopathy.

Blood Pressure. Five hundred patients (207 women, 293 men) with hypertensive vascular disease whose "mean" arterial pressure (sum of systolic and diastolic pressures divided by 2) was 125 mm. Hg or more were treated with the rice diet. The age ranged from nineteen to seventy-three (average, fifty-one) years. Two hundred twenty-nine patients had signs of renal involvement; in 271 no conclusive evidence of renal involvement was found.

The systolic blood pressure levels before treatment ranged from 154 to 264 mm. Hg; the average was 199 mm. The diastolic blood pressure levels ranged from 72 to 172 mm. Hg; the average was 117 mm. Hg.

After they were regulated on the diet under our supervision, most of the patients followed the diet at home, returning at intervals of two to six months for reexamination.

The blood pressure was considered improved if the "mean" arterial pressure had decreased by at least 20 mm. Hg.

The results are summarized in Table xiv. The figures given are averages of the daily readings of three to twenty-four (average, eight) days before and after treatment.

Of the 229 patients in whom the diagnosis of hypertensive vascular disease with "secondary" renal involvement was made, twenty-five died six to ninety-six days (average, thirty-nine days) after the diet was started. Of the 271 patients without evidence of renal involvement, one patient died thirty-six days after the rice diet was started.

Table xv shows the difference in the percentage of improvement when these twenty-six patients who died are not included.

TABLE XIV

EFFECT OF RICE DIET ON BLOOD PRESSURE OF 500 PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE (PERIOD OF DIET 4–898 DAYS)

AVERAGES

No. of Patients	Blood Pressure: Before Rice Diet	Blood Pressure: After Rice Diet	Change in Systolic and Diastolic Pressure	Change in "Mean" Arterial Pressure	Days on Rice Diet
With Renal Involvement					
Hypertension Not Improved					
74	206/121	191/117	−15/−4	−9.5	71
25	226/147	Died	.....	.....	39
Hypertension Improved					
130	207/121	159/98	−48/−23	−35.5	81
Without Evidence of Renal Involvement					
Hypertension Not Improved					
89	186/109	167/102	−19/−7	−13	68
1	248/138	Died	.....	....	36
Hypertension Improved					
181	193/113	147/93	−46/−20	−33	85
Total					
Hypertension Not Improved					
163	195/114	178/109	−17/−5	−11	69
26	227/147	Died	.....	....	39
Hypertension Improved					
311	199/116	152/95	−47/−21	−34	83

TABLE XV

PERCENTAGE OF POSITIVE AND NEGATIVE BLOOD PRESSURE RESULTS (A) INCLUDING AND (B) NOT INCLUDING TWENTY-SIX PATIENTS WHO DIED

A	%	B	%
229 Patients with Renal Involvement		204 Patients with Renal Involvement	
Not improved	44	Not improved	37
Improved	56	Improved	63
271 Patients without Evidence of Renal Involvement		270 Patients without Evidence of Renal Involvement	
Not improved	33	Not improved	33
Improved	67	Improved	67
All 500 Patients		All 474 Patients	
Not improved	38	Not improved	35
Improved	62	Improved	65

TABLE XVI

INFLUENCE OF THE LENGTH OF TREATMENT WITH THE RICE DIET: BLOOD PRESSURE CHANGES IN PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE

	Period of Treatment: 4–34 Days	35–898 Days
	With Renal Involvement	With Renal Involvement
Number of patients	86	143
Not improved	49 = 57%*	50 = 35%†
Improved	37 = 43%	93 = 65%
	Without Evidence of Renal Involvement	Without Evidence of Renal Involvement
Number of patients	109	162
Not improved	47 = 43%	43 = 27%‡
Improved	62 = 57%	119 = 73%
	Total	Total
Number of patients	195	305
Not improved	96 = 49%	93 = 30%
Improved	99 = 51%	212 = 70%

* Including 13 patients who died.
† Including 12 patients who died.
‡ Including 1 patient who died.

TABLE XVII

CHANGES IN DIASTOLIC PRESSURE OF 406 PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE INITIAL DIASTOLIC PRESSURE OF 100–159 MM. HG.

	No. of Patients	Percentage	Average Change
Decrease of 30 mm. Hg or more	52	13	−36
Decrease of 20–29 mm. Hg	101	25	−24
Decrease of 10–19 mm. Hg	158	39	−14
Decrease of 0–9 mm. Hg	77	19	− 5
Increase of 1–22 mm. Hg	18	4	+ 7
Total	406	100	−16.7

Figures 3 to 6 show typical blood pressure curves of patients on the rice diet.

The length of time required for the blood pressure to decrease varies from four days to ten months. The part played by the

TABLE XVIII

EFFECT OF RICE DIET ON HEART SIZE: AVERAGE CHANGES IN TRANSVERSE DIAMETER OF HEART IN 286 PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE

	No. of Patients	Percentage	Period of Rice Diet (Average) Days	Diameter of Chest (Averages)				Transverse Diameter of Heart (Averages)			
				Before Rice Diet	After Rice Diet	Change		Before Rice Diet	After Rice Diet	Change	
				Cm.	Cm.	Cm.	% (Diameter of Chest of Smaller Heart = 100%)	Cm.	Cm.	Cm.	% (Transverse Diameter of Smaller Heart = 100%)
Decrease of 20% or more...	19	6.7	187	29.5	28.9	−0.6	−2.2	15.3	12.3	−3.0	−24.4
Decrease of 10.0–19.9%....	106	37.1	114	29.2	29.1	−0.1	−0.3	14.5	12.7	−1.8	−14.2
Decrease of 0–9.9%.....	146	51.0	112	28.6	28.4	−0.2	−0.7	13.8	13.0	−0.8	−6.2
Increase of 0–8.0%.....	15	5.2	184	27.5	27.8	+0.3	+0.8	13.1	13.5	+0.4	+2.6
Total.....	286	100	122	28.8	28.6	−0.2	−0.7	14.2	12.9	−1.3	−10.1

TABLE XIX

CHANGES IN THE ANGLE OF THE ELECTRICAL AXIS IN 292 PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE AFTER RICE DIET

No. of Patients	Percentage	Angle of Electrical Axis (Degrees)				Period on Rice Diet (Av.) Months
		Range of Change	Before Rice Diet	After Rice Diet	Change (Average)	
1	2	More than −25	−10	−55	−45	2
4		−15 to −25	+19	0	−19	4
175	60	±14	+13	+17	+ 4	6
110	38	+15 to +25	+13	+32	+19	7
2		More than +25	+ 6	+43	+37	8

TABLE XX

CHANGES OF T_1 IN 310 PATIENTS WITH HYPERTENSIVE VASCULAR DISEASE AFTER RICE DIET

No. of Patients	T_1 Before Rice Diet	T_1 After Rice Diet	Period on Rice Diet (Average) Months
Change in Direction to Inverted			
2	diphasic	inverted	3
1	upright	diphasic	6
3	low upright	diphasic	4
1	upright	low upright	2
No Change			
52	inverted	inverted	5
21	diphasic	diphasic	4
5	low upright	low upright	2
136	upright	upright	6
Change in Direction to Upright			
19	low upright	upright	5
4	diphasic	low upright	7
19	diphasic	upright	8
17	inverted	diphasic	7
5	inverted	low upright	7
25	inverted	upright	10

length of time the diet was followed is evident from Figures 3 to 6 and Table XVI. In 125 of the 500 patients (forty with and eighty-five without evidence of renal involvement) the blood pressure figures returned to normal or almost normal values (below 145/95 mm.). The blood pressure of these patients before the rice diet ranged from 222/148 to 158/98, average 181/107 mm.; the average pressure after four to [illegible] days, average ninety-four days, of rice diet was 132/85 mm. Seven patients are

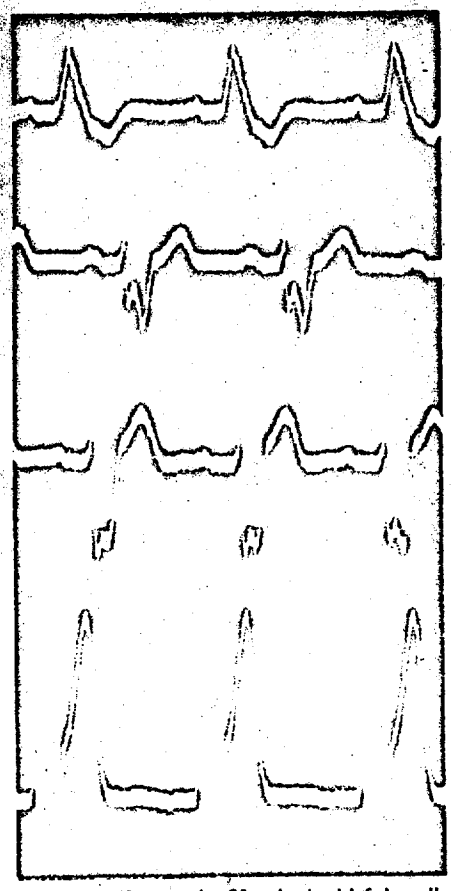


Fig. 19. P. K., a male, fifty-six, had left bundle branch block. The EKG did not change. (See also Fig. 18.)

included in this group whose "mean" arterial pressure dropped less than 20 mm. Hg and who therefore are counted as "not improved" in the previous paragraphs and tables.

The changes in diastolic pressure are analyzed separately in Table XVII.

Of 406 patients whose initial diastolic pressure was 100 mm. Hg or more 388, i.e., 96 per cent, had a decrease of 1 to 62 mm., average 18 mm., whereas only eighteen patients, i.e., 4 per cent, had an increase of 1 to 22, average 7 mm.

It has been assumed that the lowest blood pressure figure obtained after 0.6 Gm. sodium amytal indicates the maximum decrease which could be expected in the individual patient from any form of treatment. Figure 7 shows in three typical charts that the blood pressure values obtained without sodium amytal after rice diet may be far lower than the lowest values reached during the sodium amytal test before the diet.

Heart Size. The assumption that cardiac enlargement in hypertensive vascular disease is desirable in order to overcome the increased peripheral resistance has been a pious self-deception of the physician who had no means of influencing the disease and preventing the progressive cardiac breakdown.

Cardiac enlargement in hypertensive vascular disease has been found to disappear when the patient is given the rice diet. Chest films taken before and after rice diet show decreases in the heart sizes with changes in the transverse diameter up to 30 per cent. Decrease in heart size does not necessarily coincide with decrease in blood pressure. In a number of patients whose blood pressure remained at a constant high level or showed only an insignificant reduction, a considerable decrease in heart size was found. (Figs. 14 and 15.)

Six foot chest films of 286 patients taken before and after one month or more of dietary treatment (no digitalis or other drugs) are available for comparison. Table XVIII combines the averages of the measurements of the transverse diameter of the heart and of the chest diameter grouped according to the extent of change.

Before the rice diet the transverse diameters of the hearts of the 286 patients ranged from 10.2 to 19.4 cm.; the average was 14.2 cm. After the rice diet they ranged from 9.4 to 18.2 cm.; the average was 12.9 cm.

In 15 of the 286 patients (5 per cent) the heart became larger. In these patients the transverse diameter of the heart showed an average increase of 2.6 per cent. The chest diameter (average) increased by 0.8 per cent. The average period on the diet was

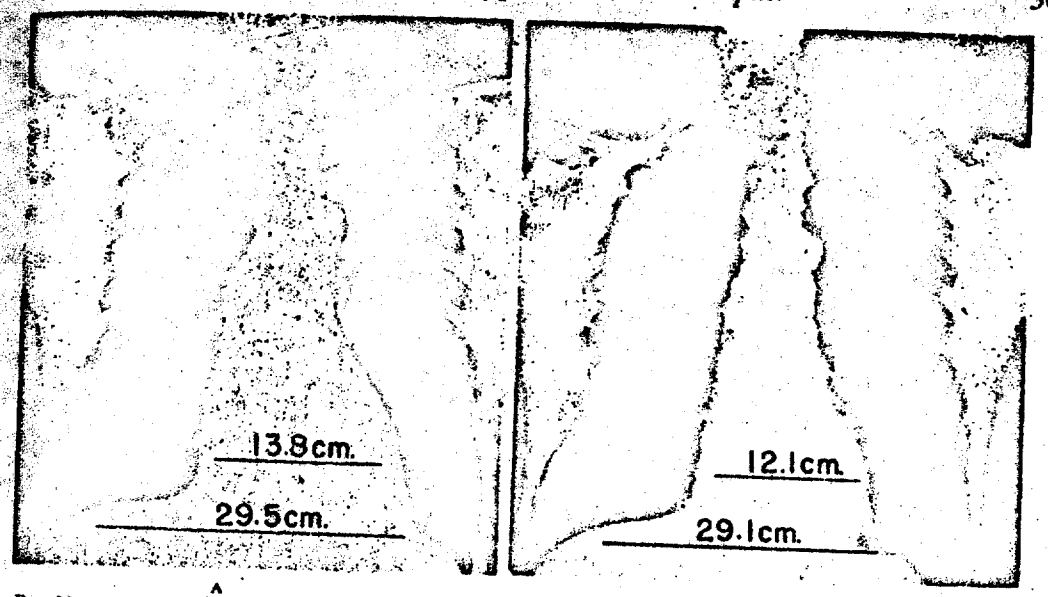


FIG. 20. A and B, C. O. a male, forty-three years of age, had hypertensive vascular disease of ten years' duration, left bundle branch block and dyspnea. Total PSP excretion in two hours, 57 per cent. Rice diet was started May 4, 1946, and strictly followed (10–11 mg. Cl per 100 cc. of urine). No medication was given. The patient became asymptomatic._____There was a decrease in blood pressure and reduction in heart size with change in transverse diameter of 14 per cent. (Fig. 21.)

184 days. The average heart size in this group before treatment was the smallest found.

In 271 of the 286 patients (95 per cent) the heart became smaller with an average change in the transverse diameter of 10.6 per cent. The chest diameter decreased by 0.6 per cent (average). The average period on the diet was 118 days.

Figures 8 to 15, 17, 18, 20 show typical changes in the heart picture produced by the rice diet.

Electrocardiograms. The blood supply to the heart muscle will be inadequate whenever the coronary blood flow is decreased without a simultaneous decrease in the myocardial energy requirements, or whenever the myocardial energy requirements are increased without a simultaneous increase in the blood supply through the coronaries. In either case the effects of the deficiency in oxygen and nutrient substances, with the resulting chemical changes and consequent clinical manifestations, are easily predictable.[7,28] The natural course of these events is recorded by the electrocardiographic findings which indicate advancing myocardial impairment: left axis deviation, T_1 inversion, arrhythmias, conduction defects or myocardial infarction.

An attitude of resignation has prevailed with regard to the abnormal electrocardiogram in hypertensive heart disease. "It is a pertinent feature of records denoting left ventricular strain that the changes are slow in their evolution and more or less permanent once they have appeared."[29] "When once established the T-wave and the RS-T defects described persist and remain unaltered until the death of the patient."[20]

Electrical axis and T_1 waves were studied in the electrocardiograms of 310 patients with hypertensive vascular disease before and after the rice diet. None of these patients received digitalis or any other drug. All electrocardiograms were made with the patient at rest in a recumbent position. The period between the two electrocardiograms compared was one month to thirty-three months, an average of six months. In 18

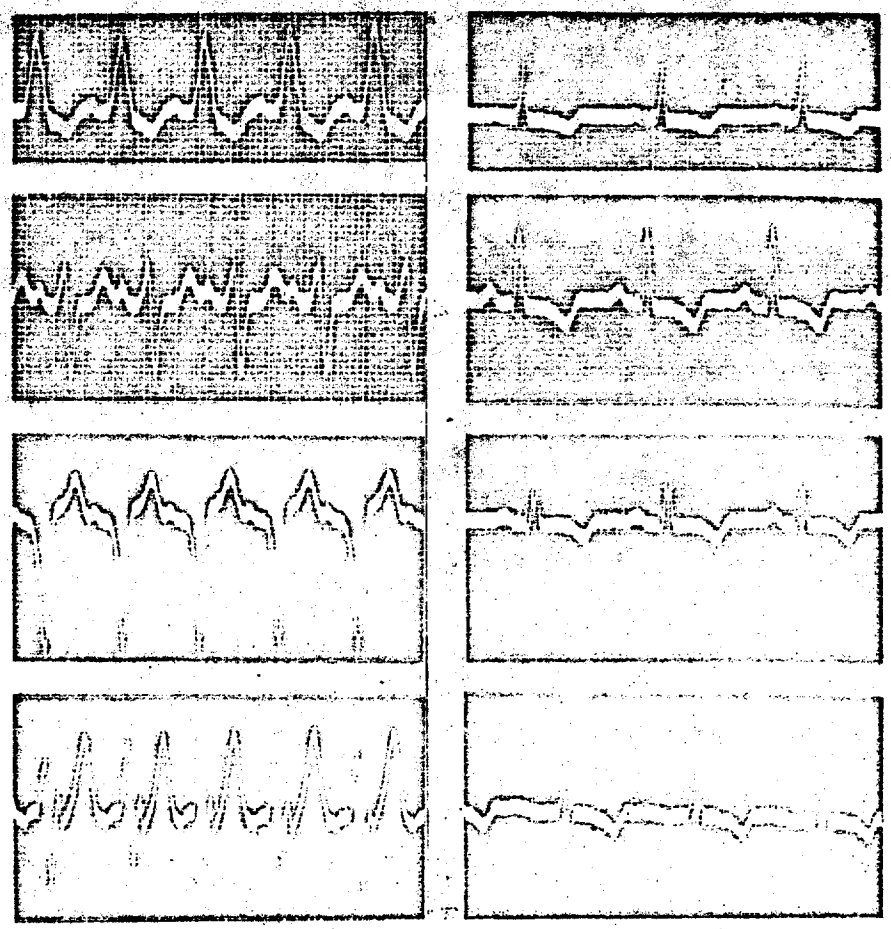


FIG. 21. C. O., a male, forty-three years of age, had hypertensive vascular disease of ten years' duration and dyspnea. April 29 to May 8, 1946: blood pressure, average, 179/126; total PSP excretion in two hours, 57 per cent. Rice diet was started May 4, 1946, and strictly followed (10-11 mg. Cl per 100 cc. of urine). No medication was given. July 24 to July 25, 1946: blood pressure, average, 157/110.——————The patient became asymptomatic and there was disappearance of left bundle branch block. (Fig. 20.)

of the 310 patients the electrical axis could not be evaluated. In the remaining 292 patients the angles of the electrical axis were:

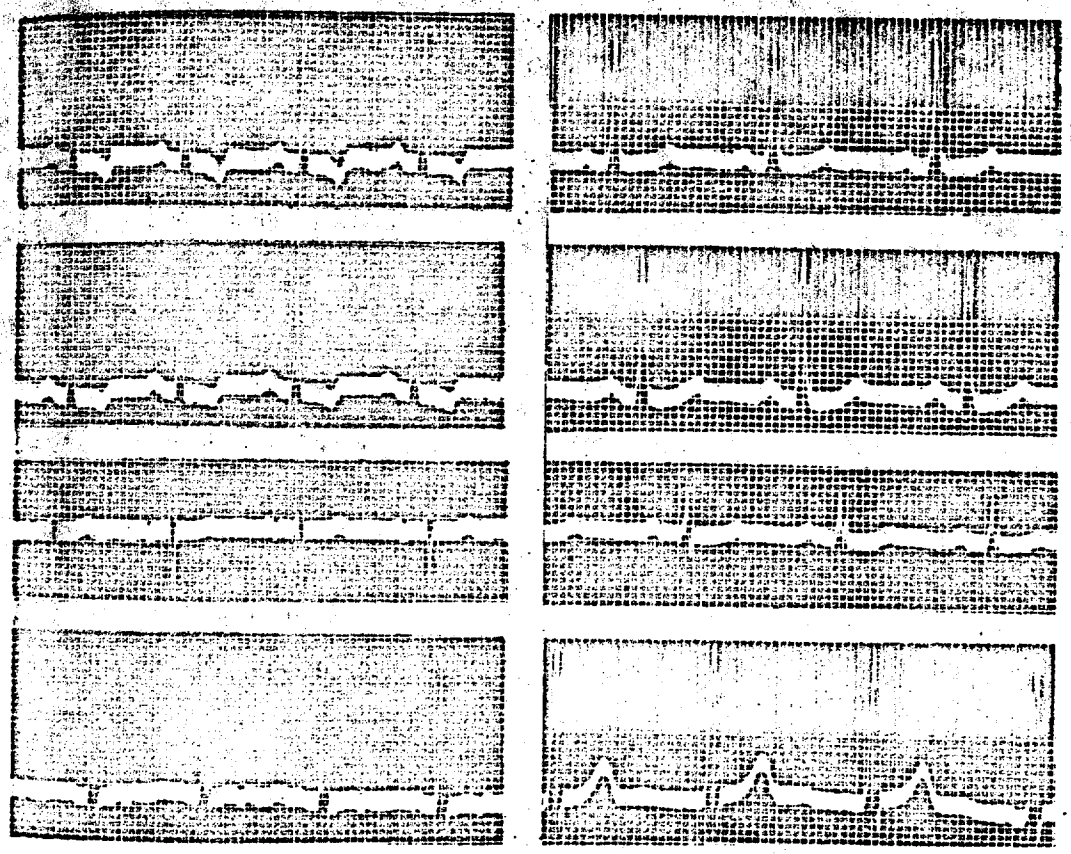
	Before Diet	After Diet
More than +30 degrees	in 89 patients	in 131 patients
0 to +30 degrees	in 97 patients	in 91 patients
Less than 0 degrees	in 106 patients	in 70 patients

The changes in the angle of the electrical axis of these patients are summarized in Table XIX.

Of the 119 patients whose electrical axis changed more than ±14° during the treatment 7, i.e., 6 per cent, showed a decrease; 112, i.e., 94 per cent, showed an increase in the angle of the electrical axis.

The T waves in lead I were evaluated in 310 patients. Before the rice diet was started T_1 was normally upright or low upright in 165, diphasic or inverted in 145 patients. The changes during the treatment are shown in Table XX.

In seven patients there was a change of T_1 in the direction from upright to inverted. In 89 patients there was a change of T_1 in the direction from inverted to upright. In



A B

FIG. 22. A and B, R. L., a male, twenty-three years of age, had hypertensive vascular disease of three years' duration with advanced retinopathy; total PSP excretion in two hours: 2.5 per cent. No digitalis was given. Rice diet was started on December 18, 1945. Blood pressure, average, December, 18 to December 24, 1945: 222/148; January, 15 to January 21, 1946: 153/112. March, 11 to March 22, 1946: 134/94. Inverted T_1 became upright within one month. The lowest blood pressure was reached two months later. (Figs. 3 and 30.)

ninety-nine patients the T_1 waves were completely inverted before treatment. In thirty of these ninety-nine patients T_1 became upright with the diet. In no patient did the reverse occur.

Excluding the patients who at the start of the rice diet already had an inverted T_1 and in whom there could be no further change for the worse according to the grouping of Table xx), the percentage of those changing for the worse during the rice diet was three. Excluding the patients who at the start of the diet already had an upright T_1 and in whom there could be no further improvement according to the grouping of Table xx), the percentage of those changing for the better was fifty-two.

The shortest time in which an inverted T_1 became normally upright was one month. (Fig. 22.) The average was ten months. In the patient whose EKG is shown in Figure 23 it took three years.

Of the 292 patients in whom it was possible to evaluate the changes both in electrical axis and in T_1, eighty-seven patients (30 per cent) had an initial electrical axis above $+10°$ and an upright T_1. Of these eighty-seven patients 7 (8 per cent) showed a change for the worse in that the electrical axis decreased below $+10°$ and or T_1 became diphasic; 80 (92 per cent) did not change with the rice diet.

Of the 292 patients 205 (70 per cent) had an initial electrical axis below $+10°$ and, or

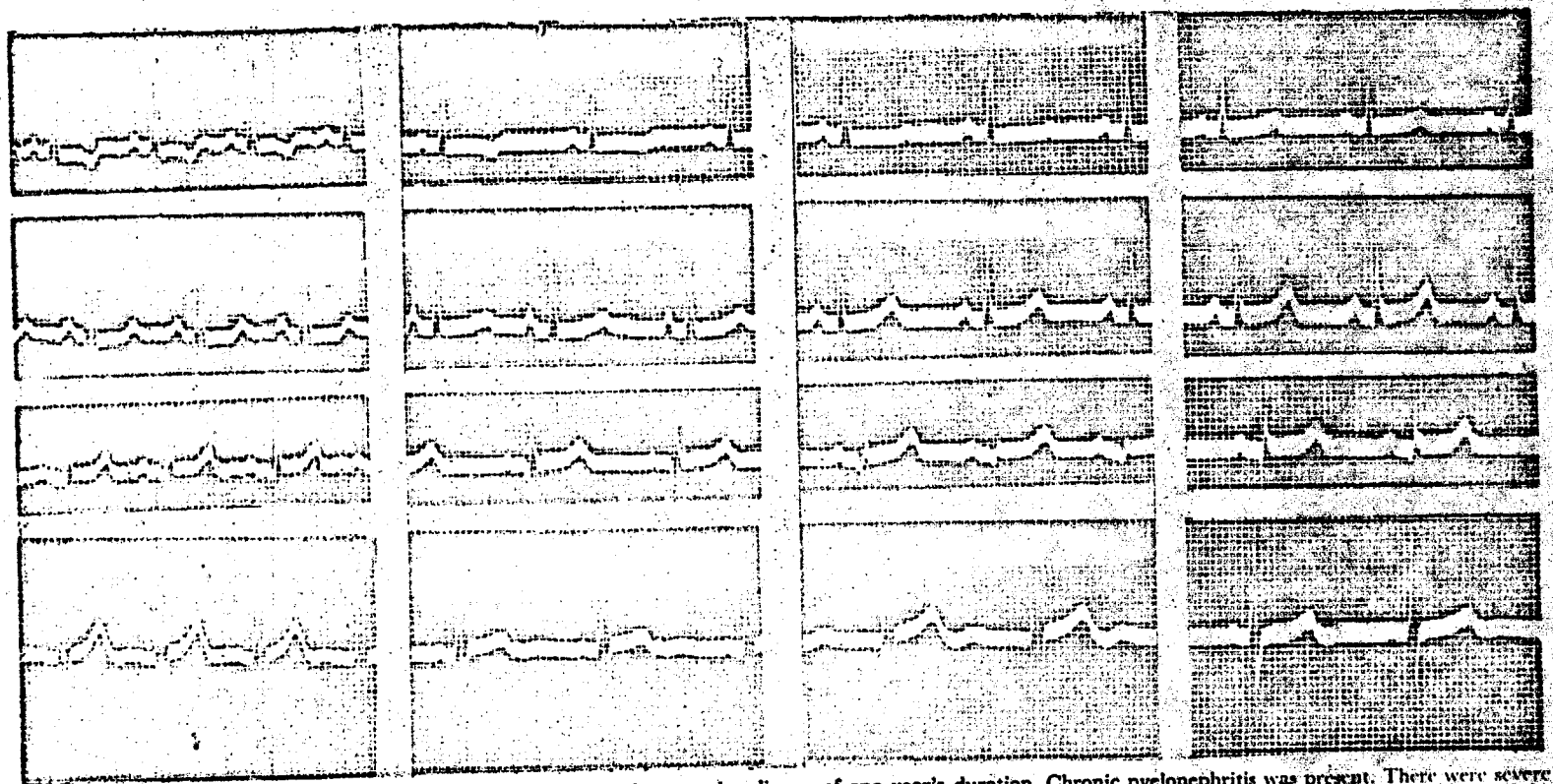
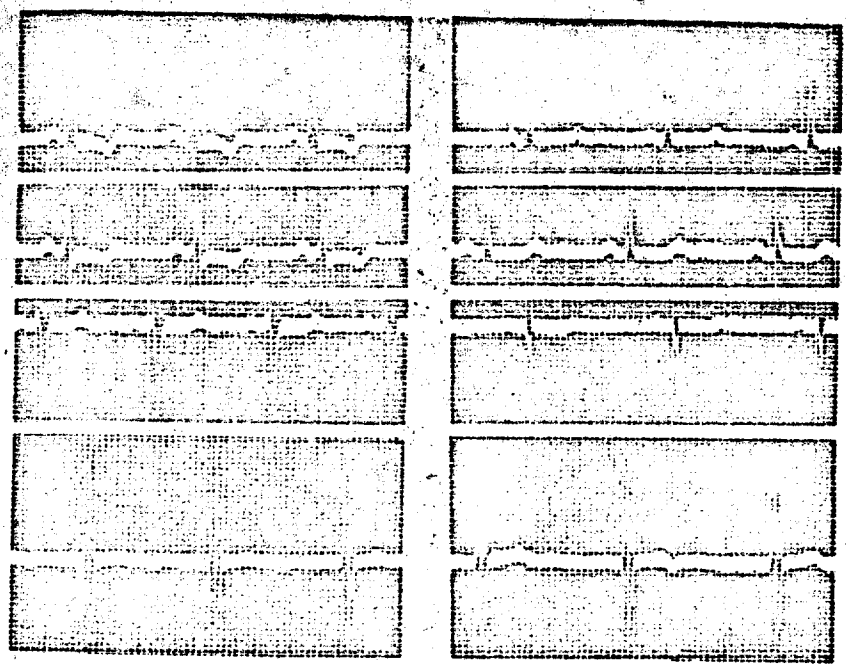


FIG. 23. A. [E. H., female, thirty-six years of age, had hypertensive vascular disease of one year's duration. Chronic pyelonephritis was present. There were severe headaches and retinal hemorrhages and "silver wire arterioles." Previous treatment with salt-poor diet; no digitalis was given. Total PSP excretion in two hours: 9–25 per cent. Rice diet was started April 13, 1943: it was moderately well followed. No medication was given. Patient was asymptomatic and working. Blood pressure averages: April 5 to April 26, 1943: 223/149; March 8 to March 10, 1944: 116/92; Feb. 20 to March 3, 1945: 159/109; May 23 to May 29, 1946: 118/79. Inverted T_1 has become normally upright three years after decrease of blood pressure.



A B

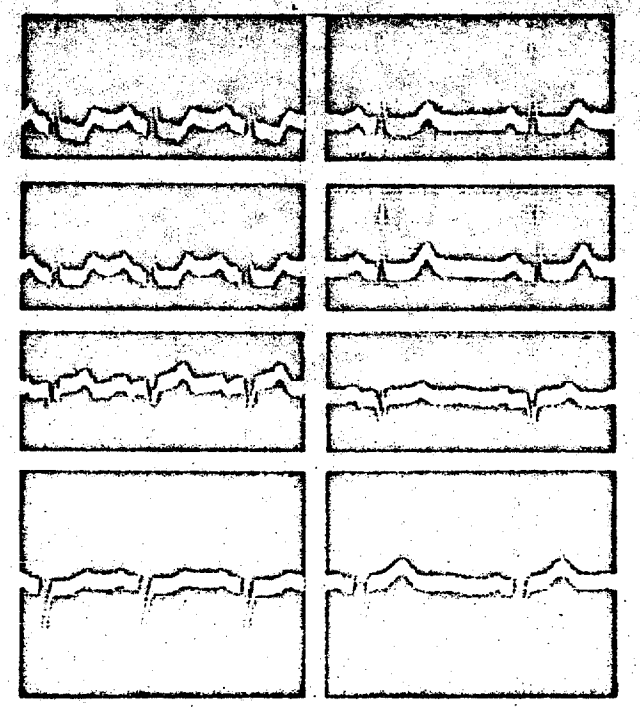
FIG. 24. A and B, C. G., a male, forty-nine years of age, had hypertensive vascular disease of two years' duration with severe headache. Two strokes occurred in 1944. He was treated with sedatives; was given no digitalis; April 6 to April 22, 1945: blood pressure average, was 196/105; total PSP excretion in two hours: 48 per cent. Rice diet was started April 24, 1945: it was followed strictly (5–20 mg. Cl per 100 cc. of urine). October 8 to October 11, 1945: blood pressure average was 136/80.——————There was a decrease in the blood pressure and an increase in the angle of electrical axis. Inverted T_1 became upright.

a diphasic or inverted T_1. Of these 205 patients 119 (58 per cent) remained unchanged; eighty-six patients (42 per cent) showed an increase in the electrical axis to more than +10° and/or a change of T_1 from diphasic or inverted to upright.

Retinopathy. Advanced retinopathy with papilledema, hemorrhages or exudates is a danger signal in hypertensive vascular disease. "Hemorrhages associated with white spots in the retina (hypertensive neuro-retinopathy) are ominous signs. Death commonly follows within a year."[31]

Vascular retinopathy has been found to disappear with the rice diet. The retinal improvement does not necessarily coincide with decrease in blood pressure. Very severe retinopathy has disappeared in patients when the blood pressure remained at a constant high level or showed only an insignificant reduction. (Figs. 27 to 29.)

Papilledema, hemorrhages or exudates, frequently in combination, were present in 140 of the 500 patients. In eighty-eight of these, eyeground photographs taken both before and after the rice diet (one to thirty months) are available for comparison. Papilledema was found in twenty-three of the eighty-eight patients. In seventeen it disappeared completely, in five partially and in one remained unchanged. Hemorrhages were found in fifty-five of the eighty-eight patients. In thirty-nine they disappeared completely, in fifteen partially and in one remained unchanged. Exudates were found in seventy of the eighty-eight patients. In forty-two they disappeared completely, in twenty-three partially and in five remained unchanged. In one of the patients in whom the exudates cleared up partially small hemorrhages occurred after a period of twelve



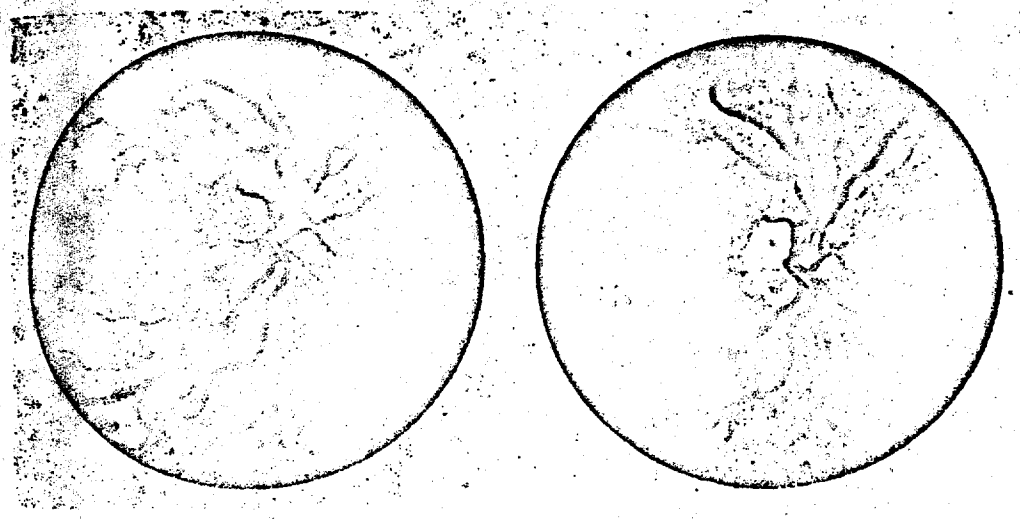
A B

FIG. 25, A and B, F. G., a female, fifty-two years of age, had hypertensive vascular disease of twelve years' duration which began in toxemia of pregnancy. There were severe headaches. She had a stroke in May, 1946. She had previous treatment in a hospital with bedrest and a reduction diet. She had been given sedatives for the last three years. No digitalis was given. August 8, 1946: blood pressure was 238/128; total PSP excretion in two hours: 57 per cent. Rice diet was started August 10, 1946; it was strictly followed for six weeks (5 mg. Cl per 100 cc. of urine). September 20, 1946 to September 26, 1946: blood pressure, average, was 150/100. There was a decrease in blood pressure.——————The diphasic T_1 has become upright.

months on the diet (which had not been strictly followed).

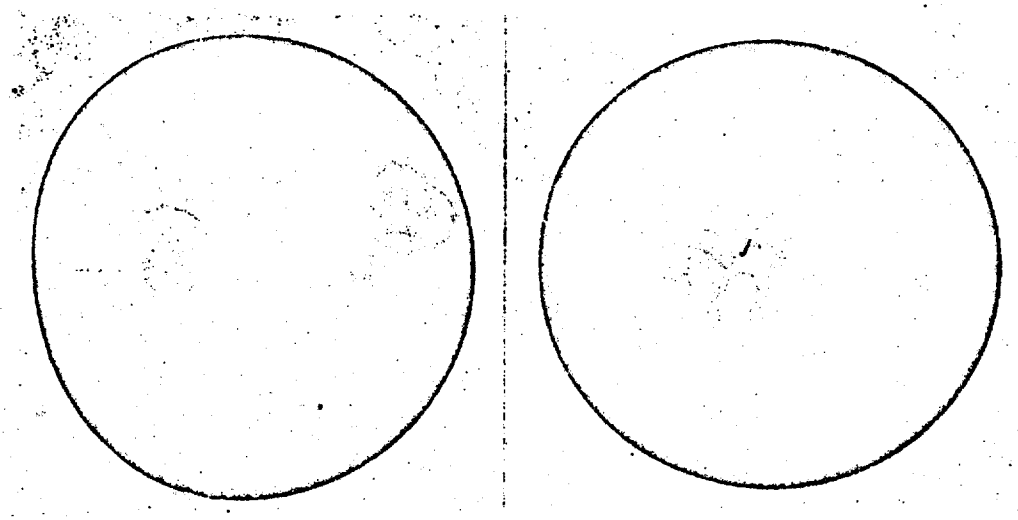
Those patients in whom the retinopathy remained unchanged had been on the diet from one to three and one-half months except for one patient with exudative stippling who was on the rice diet for nineteen months. The patients in whom the retinopathy cleared up only partially had been on the rice diet from one to seventeen months, an average of five months. The period of time in which the retinal changes disappeared completely ranged from two to thirty months, an average of fourteen months.

Figures 26 to 30 show typical eyeground photographs before and after the diet.



A B

FIG. 26. A and B, D. L., a male, fifty-four years of age, had hypertensive vascular disease of at least six months' duration. His previous treatment consisted of low-fat, low-protein diet and sedatives. April 19, 1946 to May 6, 1946: blood pressure average was 221/144; EKG T_1 inverted; total PSP excretion in two hours: 35 per cent. Rice diet was started April 26, 1946; it was strictly followed (5–13 mg. Cl per 100 cc. of urine). March 25 to March 26, 1947: Blood pressure, average, was 177/112; EKG T_1 was upright. September 22 to September 24, 1947: blood pressure, average, was 149/106; EKG T_1 upright.——————There was a disappearance of papilledema and exudates before lowest blood pressure level was reached.



A B

FIG. 27. A and B, W. A., a male, thirty-two years of age, had hypertensive vascular disease of eighteen years' duration. Sympathectomy was performed in the Mayo Clinic in 1945. Since July, 1946, there had been progressive impairment of vision in the left eye. February 19 to February 21, 1947: blood pressure average was 255/158; total PSP excretion in two hours: 40 per cent. Rice diet was started February 21, 1947; it was moderately well followed (12–51 mg. Cl per 100 cc. of urine). September 22 to September 26, 1947 blood pressure, average 230/138.——————There was marked improvement of vision. There was a disappearance of papilledema, almost complete disappearance of hemorrhages and exudates in spite of persistence of high blood pressure.

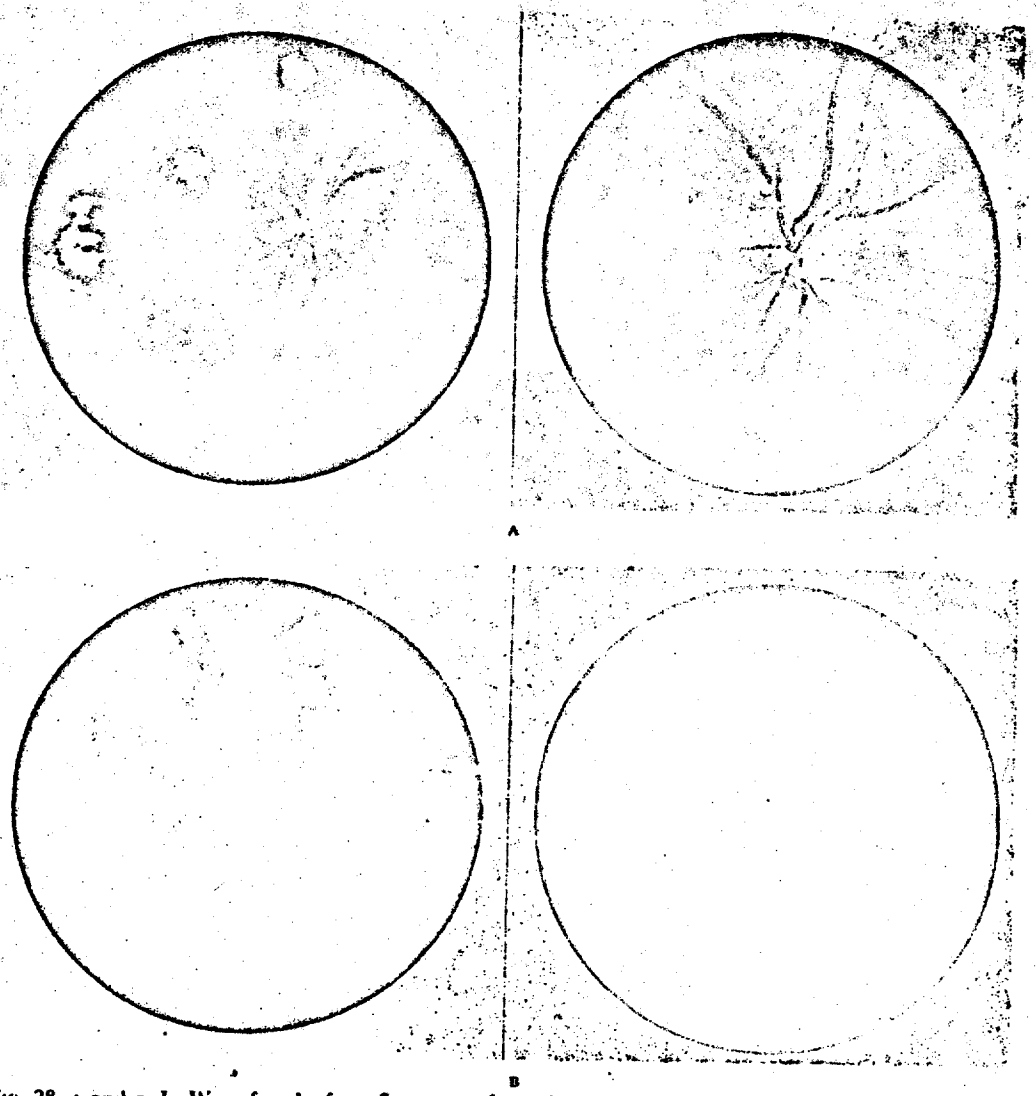
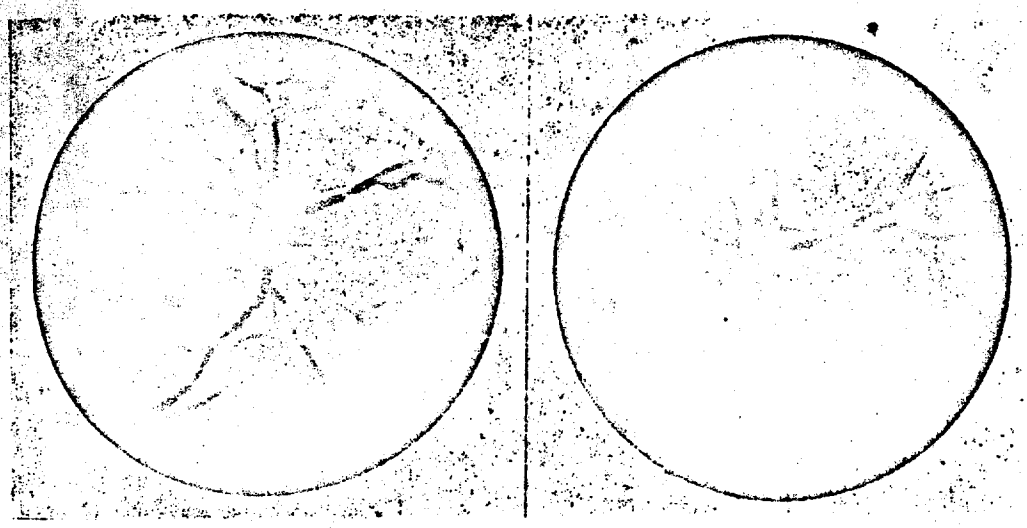
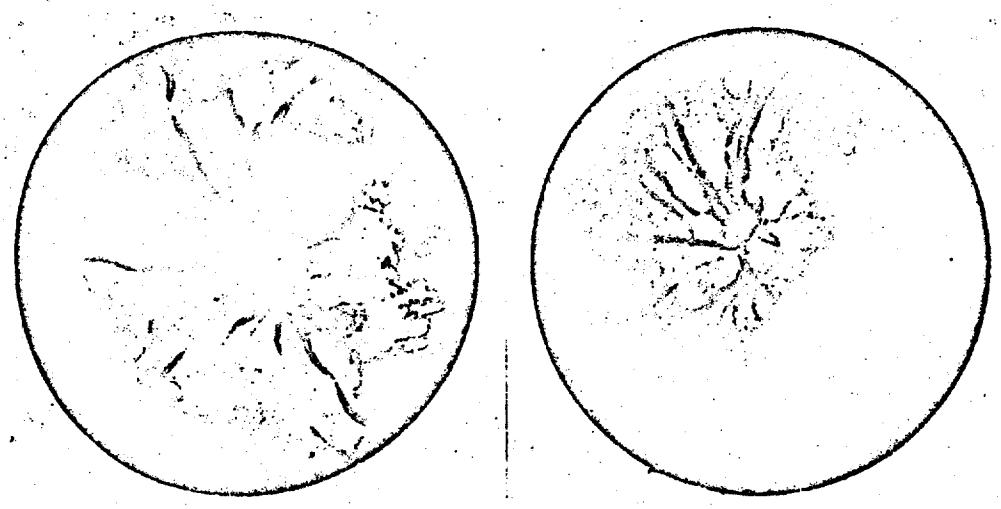


FIG. 28. A and B, L. W., a female, forty-five years of age, had hypertensive vascular disease of at least four months' duration. July 16 to August 5, 1944: blood pressure, average, 225/153; total PSP excretion in two hours, 59 per cent. Rice diet was started July 23, 1944 and strictly followed (4–24 mg. Cl per 100 cc. of urine). August 8 to August 13, 1945: blood pressure, average, 215/138._____There was a disappearance of papilledema, hemorrhages and exudates in spite of persistence of high blood pressure.

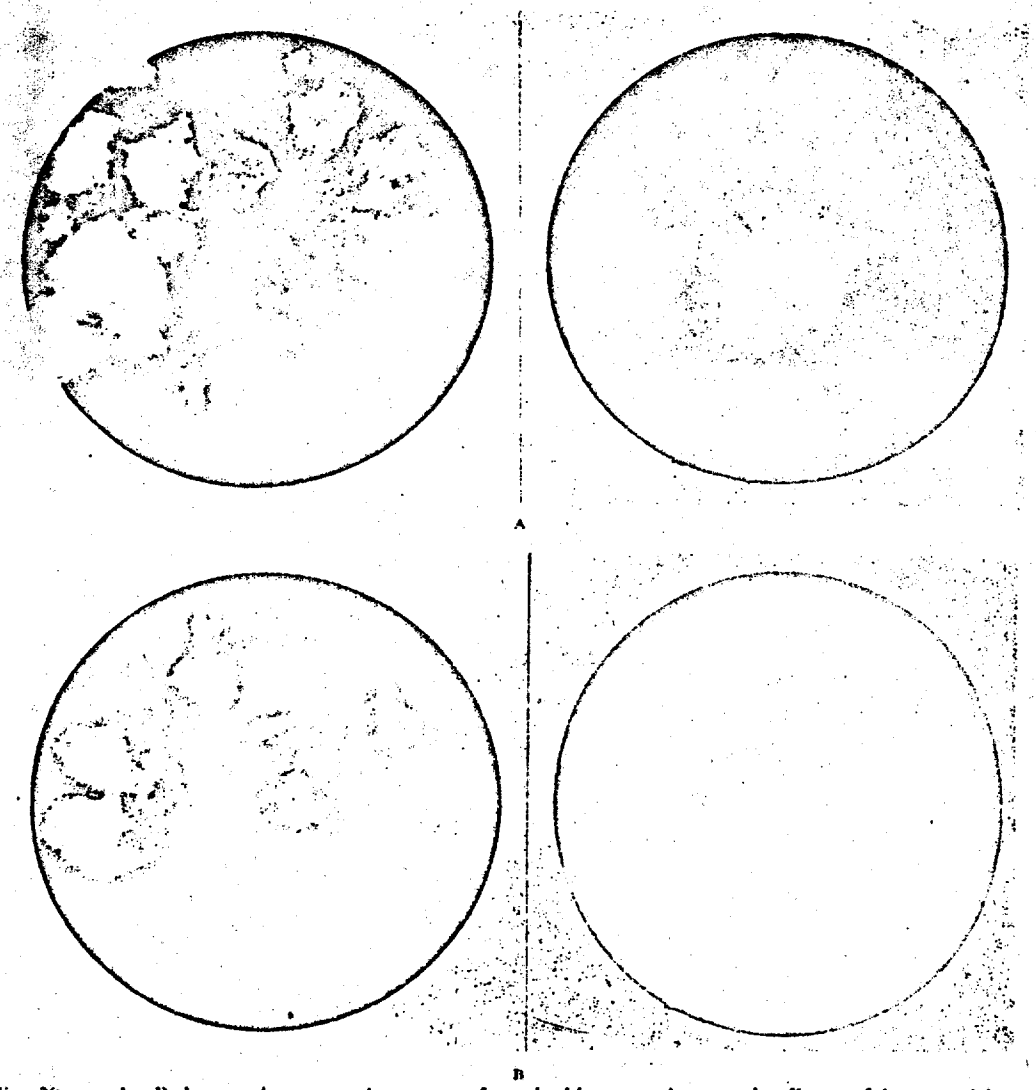


A



B

FIG. 29. A and B, A. McA., a male, thirty-eight years of age, had hypertensive vascular disease of at least one years' duration. He was previously treated with sedatives and low-salt diet. December 8 to 20, 1945: blood pressure, average, 216/132; EKG T_1 inverted; total PSP excretion in two hours, 58 per cent. Rice diet was started, December 13, 1945 but was not strictly followed (28–55 mg. Cl per 100 cc. of urine). May 5 to 8, 1947: blood pressure, average, 208/123; EKG T_1 upright.——Papilledema, hemorrhages, exudates disappeared in spite of persistence of high blood pressure.



A

B

FIG. 30. A and B. R. L., a male, twenty-three years of age, had hypertensive vascular disease of three years' duration. Previous treatment consisted of a "modified rice diet." December 18 to December 24, 1945: blood pressure, average, 222/148; EKG T_1 inverted; total PSP excretion in two hours, 2.5 per cent; NPN 79 mg. per 100 cc. blood; cholesterol 340 mg. per 100 cc. serum. Rice diet was started December 18, 1945 and strictly followed for three months (8–21 mg. Cl per 100 cc. of urine). March 11 to 22, 1946: blood pressure, average 134/94; EKG T_1 upright. After March, diet was poorly followed (192–255 mg. Cl per 100 cc. of urine). August 15 to 21, 1946 and October 2 to 5, 1946: blood pressure, average, 194/133; EKG T_1 upright; NPN 60 mg. per 100 cc. blood; cholesterol 173 mg. per 100 cc. serum. There was a disappearance of papilledema, hemorrhages, exudates and no recurrence of retinopathy although diet was broken and hypertension recurred.

REFERENCES

1. Kempner, W. Treatment of kidney disease and hypertensive vascular disease with rice diet. *North Carolina M. J.*, 5: 125–133, 1944.
2. Kempner, W. Treatment of kidney disease and hypertensive vascular disease with rice diet. II. *North Carolina M. J.*, 5: 273–274, 1944.
3. Kempner, W. Treatment of kidney disease and hypertensive vascular disease with rice diet. *J. A. M. A.*, 125: 48, 1944.
4. Kempner, W. Treatment of kidney disease and hypertensive vascular disease with rice diet. *J. A. M. A.*, 125: 60, 1944.
5. Kempner, W. Treatment of kidney disease and hypertensive vascular disease with rice diet. III. *North Carolina M. J.*, 6: 61–87; 117–161, 1945.
6. Kempner, W. Anoxemia of the kidney as a cause of uremic acidosis: inhibitory effect of low oxygen tension on the deamination of amino acids in kidney tissue. *Am. J. Physiol.*, 123: 117–118, 1938.
7. Lohmann, R. Biologie der Entzuendung. *Ztschr. f. klin. Med.*, 135: 316–346, 1938.
8. Kempner, W. Inhibitory effect of low oxygen tension on the deamination of amino acids in the kidney. *J. Biol. Chem.*, 124: 229–235, 1938.
9. Kempner, W. Verminderter Sauerstoffdruck in der Niere als Ursache der reversiblen uraemischen Acidose. *Klin. Wchnschr.*, 12: 971–973, 1938.
10. Kempner, W. The role of oxygen tension in biological oxidations. Cold Spring Harbor Symposia on Quantitative Biology. 7: 269–289, 1939.
11. Kempner, W. Some effects of the rice diet treatment of kidney disease and hypertension. *Bull. New York Acad. Med.*, 22: 350–370, 1946.
12. Goldring, W. and Chasis, H. Hypertension and Hypertensive Disease. P. 169. New York, 1944. Commonwealth Fund.
13. Fishberg, A. M. Hypertension and Nephritis. 4th ed. Philadelphia, 1939. Lea & Febiger.
14. Folin, O. Laws governing the chemical composition of urine. *Am. J. Physiol.*, 13: 66, 1905.
15. Benedict, F. G. A Study of Prolonged Fasting. Washington, 1915. Carnegie Institution of Washington.
16. Starke, H. Unpublished data.
17. Allen, F. M. and Sherrill, J. W. The treatment of arterial hypertension. *J. Metab. Research*, 2: 429, 1922.
18. Volhard, F. Die Behandlung der Nephrosklerosen, Handbuch der Inneren Medizin. Edited by von Bergmann and Staehelin. 2nd ed., pp. 1753–1782. Berlin, 1931. Julius Springer.
19. Page, I. H. and Corcoran, A. C. Arterial Hypertension. P. 79. Chicago, 1945. Year Book Publishers.
20. Kempner, W., Lesesne, J., Newborg, B. and Whicker, C. The sulfate and phosphate excretion in the urine of patients on the rice diet. (Unpublished.)
21. Grollman, A. and Harrison, T. R. Effect of rigid sodium restriction on blood pressure and survival of hypertensive rats. *Proc. Soc. Exper. Biol. & Med.*, 60: 52, 1945.
22. Selye, H. and Syone, H. Effect of the diet upon the renotropic, nephrosclerotic, cardiotropic and adrenotropic actions of crude anterior pituitary preparations. *Federation Proc.*, 5: 93, 1946.
23. Dock, W. The predilection of atherosclerosis for the coronary arteries. *J. A. M. A.*, 131: 875, 1946.
24. Dock, W. Coronary disease. *J. A. M. A.* 134: 1197, 1947.
25. Dick, G. F. and Schwartz, W. B. Response of experimental hypertension to rice and fruit juice diet. *Proc. Soc. Exper. Biol. & Med.*, 65: 22, 1947.
26. Kempner, W. Treatment of cardiac failure with the rice diet. *North Carolina M. J.*, 8: 128–131, 1947.
27. Rabinowitch, I. M. Experiences with a high carbohydrate-low caloric diet for the treatment of diabetes mellitus. *Canad. M. A. J.*, 23: 489–498, 1930.
28. Kempner, W. and Peschel, E. Stoffwechsel der Entzuendung. *Ztschr. f. klin. Med.*, 114: 439–455, 1930.
29. Kaplan, L. G. and Katz, L. N. The characteristic electrocardiograms in left ventricular strain with and without axis deviation. *Am. J. M. Sc.*, 201: 676–693, 1941.
30. Rykert, H. E. and Hepburn, J. Electrocardiographic abnormalities characteristic of certain cases of arterial hypertension. *Am. Heart J.*, 10: 942, 1935.
31. Cecil, R. L. A Textbook of Medicine. 7th ed., p. 1156. Philadelphia, 1947. W. B. Saunders Co.

Exp. Carcinomas Glandular Stomach Proc. Symp.

[GANN Monograph No. 8-1969]

STUDIES ON FACTORS AFFECTING CHEMICAL CARCINOGENESIS OF MOUSE STOMACH*1

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SYNOPSIS

C3H/HeJ male mice, given by stomach tube 0.5 ml of 0.1% 4-nitroquinoline 1-oxide solution in 10% ethanol solution once a week 10 times or twice a week 8 times starting at 4 weeks of age, were found later with well-established tumors in the stomach. The tumor development was significantly accelerated by feeding the mice 8% low-protein diet (GBI) and by giving 0.5 ml of 10% sodium chloride solution about 5 hours before the 4-nitroquinoline 1-oxide administration and after overnight fasting. In a model experiment of 7 months, all 14 mice had stomach tumors, of which 7 were carcinoma, including one metastasizing adenoacanthoma of the glandular stomach. It was suggested that low-protein diet and strongly salted food might be concerned with high incidence of stomach cancer in Japan especially in rural areas.

INTRODUCTION

Of gastric carcinogenesis, high percent alcoholic drinks, citrus oils, male hormones, etc., have been discussed as promoting factors by various authors. It is remarkable that in Japan, especially in rural districts, people generally take a diet extraordinarily low in protein and extremely high in salt. Average daily per head consumption of salt is said to be as much as 27 g. They love strongly salted vegetable pickles, dried fish, soy-sauce, fermented bean paste, etc. Four years ago it was observed that 0.5 ml of over 3% NaCl solution, given by stomach tube to mice after overnight fasting, caused acute erosions to the stomach mucosa, which were hemorrhagic in the glandular stomach. As the salt concentration was increased, erosions became more extensive.

According to gastrologists, low-protein diet decreases mucus production, leading to less protection of the mucous membrane. Immunopathologists interpret that low-protein diet weakens defensive reactions and reparation processes of tissues. When mice on low-protein (8% casein) diet were used for the pre-

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viously mentioned NaCl experiment, the erosions caused were more severe and slower in healing. When mice were given cow milk in place of drinking water, the particular injuries were considerably slight.

Comparing C3H/HeJ with C57BL/6J and SWR/J, the gastric reactions to a concentrated NaCl solution were strongest in C3H/HeJ, weaker in C57BL/6J, and weakest in SWR/J.

Taking all these points into account, model experiments were undertaken by the use of 4-nitroquinoline 1-oxide as an example of test carcinogenic compound, which has been considered to be quickly inactivated in tissues and not to be so much immunosuppressive as other carcinogenic hydrocarbons.

MATERIALS AND METHODS

C3H/HeJ males, starting at about 4 weeks of age (from 14 to 16 g in body weight) were (a) fed GBI semisynthetic low-protein diet with casein in 8%, (b) after overnight fasting at the 7th day were given 0.5 ml of 10% NaCl solution by stomach tube, and (c) about 5 hr later were administered 0.5 mg/20 g body weight of 4-nitroquinoline 1-oxide in 10% ethanol solution, followed by resumption of feeding further 2 hr later (Fig. 1). Then, (b) and (c) procedures were

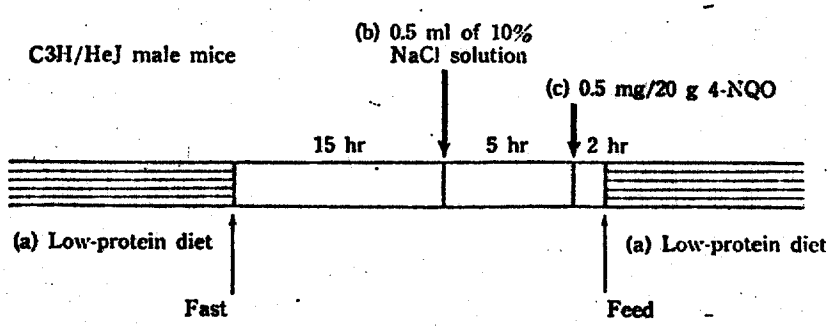

Fig. 1. Scheme of the experiment

repeated once a week 9 times or twice weekly 7 times. Thereafter periodically some animals were sacrificed to study the progress of changes in the stomach. When tumor development was found, all the remaining animals of the particular experiment were sacrificed for autopsy.

For comparison with this regular experiment with (a), (b), and (c) procedures, the control experiment without (a), (b), and (c) procedures, experiments without (c) but with (a) or (b), and experiments with (c) but without (a) or (b) also were studied.

RESULTS AND DISCUSSION

Some series of experiments which yielded significant findings are summarized in Table I. The first series of experiments without (c), or not administered

Table I. Incidence of Gastric Carcinoma in C3H/HeJ Male Mice

Series	Total No. of mice	(a) Low-protein diet	(b) Pretreatment with 10% NaCl	(c) Administration of 0.5 mg/20 g 4-NQO	Days after initial administration	Effective No. of mice	Stomach papilloma	Stomach carcinoma
			(Once a week 10 times)					
1st	5	−	−	−	377	2	0	0
	5	+	−	−	377	5	3	0
			(Twice a week 8 times)					
2nd	15	−	−	+	135	13	12	1
	10	−	+	+	135	9	6	3
	15	+	−	+	132 139	12	11	1
			(Once a week 10 times)					
3rd	10	+	+	−	201	5	0	0
	8	−	+	+	232	8	5	3
	22	+	+	+	215 232	14	7	7*

* One mouse had a massive adenoacanthoma probably originated near the squamo-glandular junction, as shown in Photos 1, 2, and 3.

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4-nitroquinoline 1-oxide, were closed at 377 days of experiment, or at 405 days of age. In the experiment without (a) and (b), or on the normal diet and not NaCl-treated, no abnormal lesions were found, whereas in the experiment with (a) but without (b), or on the low-protein diet but not NaCl-treated, a few small papillomatous lesions were found in the fore-stomach in 3 of 5 mice. Histologically, the lesions showed hyperkaratosis and thickening of the squamous-cell layer without any atypical characteristics. Thus, it was suggested that they were not truly neoplastic, although evidently increased in growth potential.

The second series of experiments with (c) or administered 4-nitroquinoline 1-oxide, were closed at about 135 days. Tumors were found in the fore-stomach of all the animals. Of these, some were carcinoma. This occurred much more frequently in the experiment with (b) or NaCl-treated, compared with the experiments without (b) or not NaCl-treated, and either without or with (a), or either on the normal diet or on the low-protein diet. The salt treatment apparently accelerated the cancer development.

The third series of experiments also with (c) or treated with 4-nitroquinoline 1-oxide, were closed after a longer period of experiment, namely at about 215 days. The percentage of carcinoma cases generally became higher. It was even higher especially in the experiment with (a) and (b), or the low-protein diet and NaCl-treatment. These two procedures apparently showed an aggregate result. Seven of 14 mice produced carcinoma. Furthermore, it was conspicuous that one of them had, besides extensive squamous-cell carcinoma in the fore-stomach, a massive adenoacanthoma, which occupied a large portion of the glandular stomach, infiltrating into the surroundings, penetrating through the stomach wall and out to the peritoneal surface of the serosa, and further peritoneally metastasizing to the liver. Histologically, the tumor tissue was partly adenocarcinomatous and partly squamous-cell carcinomatous (Photos 1, 2, and 3). Evidently, cancer was produced in the glandular stomach in about 7% of animals in such a fairly short period of experiment as about 7 months. Although still low in frequency, yet it was not bad, compared with the reported results by others.

Furthermore, it was conspicuous that squamous-cell carcinoma was produced in a considerably high percentage of animals, for instance, in one-third of animals in the experiment of about 4.5 months. It appeared that the fore-stomach of C3H/HeJ was sensitive, as suspected previously. Accordingly, it was suggested to repeat the experiments with I and/or DBA strains of mice.

Otherwise, it was speculated that the particular carcinogen used, namely, 4-nitroquinoline 1-oxide, by its own nature acted selectively on the fore-stomach. For comparison, 2,7-fluorenylenebisacetamide and aflatoxin are under experimentation.

In any way, the effect of low-protein diet and NaCl-treatment was impressive in promotion of carcinogenesis. The combination of these procedures with the carcinogen administration was a useful modification.

Explanation of Plate

Photo 1. Extensive adenoacanthoma which occupied a large portion of the glandular stomach, probably originated near the squamo-glandular junction. Sacrificed 215 days after initial administration. A case of the third series marked on Table I.

Photo 2. Adenocarcinomatous pattern in the glandular area near the limiting ridge.

Photo 3. Adenoacanthoma, infiltrating through the stomach wall. The same case as Photos 1 and 2.

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Indifferent drugs of great toxicity

by

Victor Kwasniewski

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It has long been customary in pharmacies to store all types of medication, chemicals, drugs and their preparations according to their effect, so as to avoid errors as far as possible. This is done, for instance, by different kinds of labelling: black on white, red on white, white on black. Obviously there are intermediate substances, whose classification in one or the other group is difficult. This applies for instance to volatile oils, which are readily absorbed by the skin and mucosa and with few exceptions may have toxic effects, although only in large or very large doses, and not in the quantities used for therapeutic purposes. Even prolonged inhalation may give rise to headaches and numbeness (flower with a strong scent in the room), and, according to their composition, may give rise to different toxic effects: terpenes paralyze the central nervous system, camphors cause seizures, epileptiform attacks ending in death from respiratory paralysis. Whether in future these should be labelled red on white remains questionable; there are some substances, however, which should certainly be treated separately, since their toxic dose is relatively low. This applies

to wormwood oil, salvia oil and tanacetic oil, as we shall describe in more detail below.

Yet a number of medicinal substances are stored in pharmacies in accordance with current pharmaceutical books which in my view are wrongly classified according to their effect. These are counted among the "indifferent" (=harmless) drugs, while in actual fact they are not, as will be shown in the following brief notes.

Potassium chloride is used both internally for cystitis and pyelitis (formely also for diphtheria) and also for gargling in cases of sore throat. It forms chloric acid in the body, which converts the hemoglobin in the blood into methemoglobin and hematin, which may give rise to thromboses and embolisms. After toxic doses death is caused by cyanosis, since methemoglobin is not capable of releasing any oxygen into the tissues. In adult humans serious symptoms of poisoning develop with 10 g, while 15 to 20 g have a fatal effect.

Potassium nitrate today is seldom used for therapeutic purposes (for instance in Lauder-Brunton mixture, pulvis temperans). Formerly this salt was much in favor as an antiphlogistic agent in acute feverish diseases and was taken in quantities of 0.5 to 1 g. This dose is acceptable, but it has been confused with salts of a similar appearance, such as Glauber's salt or Epsom salt. Taken in quantities of 10 to 30 g it causes severe abdominal pains, profuse bloody vomiting and

diarrhea. This is followed by convulsions, the pulse becomes light and soft and finally there is cardiac arrest, probably as a consequence of the effect of the potassium ion.

Potassium permanganate is still often used as a strong oxidizing agent, for instance in the treatment of wounds. It has a deodorizing and disinfecting effect, and in stronger concentrations a corrosive effect. Formerly it was taken internally (in pills) for stomach ulcers. A 1 to 2% solution already causes strong corrosion of mucous membranes. In the view of some pharmacologists, even its use as a mouth wash is not without risk, since it can lead to slight swelling of the tongue and loss of taste sensitivity lasting for months. Even if poisoning by potassium permanganate is rare, it has nevertheless often been used for suicide. The lethal dose for humans is ca. 10 to 20 g. The picture of poisoning is associated with swelling of the tongue, corrosion of the throat, larynx and esophagus; the cardiac arrest which occurs is presumably the effect of the potassium ion.

Carbon tetrachloride is taken internally (seldom in this country) as an anthelmintic in cases of hookworm in quantities of 2 to 3 cc. Even this therapeutic dose can lead to poisoning and cause liver and kidney damage. In this country its use is more common for technical purposes (spot removal), which should be carried out with caution, if possible with the windows open, since the inhaled vapors can cause nausea as well as numbness accompanied by headache, and in more severe cases

vomiting and convulsions.

Benzene is of little therapeutic importance (it is taken internally in rare cases for intestinal trichinae, or applied externally in the form of Cignolin ointment, as an insecticide, etc.), yet it is very important toxicologically, since even prolonged inhalation of its vapors can lead to loss of consciousness, usually with a fatal outcome; this is preceded by convulsions, euphoria, multiple skin hemorrhages, even fatal uterine hemorrhages. In chronic benzene poisoning the bone marrow is damaged, the number of leukocytes is reduced and anemia develops. Benzene is also absorbed to a considerable degree through the skin.

Pyrogallol is often used in dermatology for psoriasis, lupus, certain fungus diseases, etc. Since it belongs among substances that form methemoglobin, the greatest caution should be exercised in it use (not more than 3 g per day should be rubbed in). Poisoning is manifested by nephritis, jaundice and muscular twitching.

Quinine and its salts, as well as Euquinine: Quinine is a preferred and much used drug. It is prescribed mainly in gynecology to induce labor pains, but also as an antipyretic, in influenza, pneumonia, functional arrhythmias and in malaria. Unfortunately it gives rise to many unpleasant side effects, which may even be observed with therapeutic doses. These are: dizziness, buzzing in the ears, a dimming of consciousness ("quinine intoxication"), all of these disturbances attributed

to paralysis of the central nervous system. Upon more prolonged use (for instance in malaria) hematuria may easily develop (blackwater fever). A particularly unfavorable effect is the sudden development of cardiac weakness, a sign of a toxic effect on the heart. The lethal dose for humans is 10 g.

Naphthalene was formerly taken internally in doses of 0.5 to 3 g per day as an oxyuricide, although it is seldom used today. It was also taken in cases of typhus as an intestinal antiseptic, and also applied externally as a dusting powder for wounds. The greater part of the ingested naphthalene is excreted in the stools and only a small quantity is absorbed by the intestines, whereupon after oxidation into naphthol and naphthoquinone it is transferred via the kidneys into the urine. Here these derivatives cause severe irritation of mucous membranes and give rise of albuminuria. In the literature fatal cases have been described after its ingestion asn an anthelmintic, so that naphthalene can no more be regarded as indifferent than β-naphthol, which has found widespread use in dermatology. In strong concentrations it has a corrosive effect and may even cause necroses in healthy skin. Since it is readily absorbed through the skin, as in the case of naphthalene, upon excretion through the kidneys nephritis frequently develops, with albuminuria and hematuria.

Chrysarobin is probably the most effetive therapeutic agent against psoriasis. It is very readily absorbed by the skin and

its fragments are excreted in the urine, giving rise to renal irritation. Taken internally, even in centigram doses, chrysarobin has a very toxic effect and gives rise to violent gastroenteritis and severe renal damage.

2-Phenylquinoline-4-carboxylic acid (Atophan) is a valuable drug for flushing out uric acid from the body in cases of gout and rheumatism. It also has an antiphlogistic, antipyretic and analgestic effect. The drug may only be taken for a few days (3-5), since otherwise it can easily lead to toxic liver damage (jaundice), and indeed to acute yellow atrophy of the liver (hepatic coma) ending in death.

Salvia oil, in addition to borneol, camphor and cineol, consists up to 50% of very toxic thujone. If we consider that 0.3 g salvia oil is capable of killing a dog weighing 5-6 kg, this should give one pause. Small doses of the oil give rise to epileptiform attacks. The drug most commonly used, which contains 1-2.5% volatile oil, appears to consitute little danger; at any rate there are no detailed data in the literature concerning a possible toxic effect.

Tansy and tanacetic oil containing ca. 1.5% of the drug. This volatile oil consists up to 70% of the toxic anthelmintic β-thujone, which justified use of the drug as an anthelmintic. In therapeutic doses it may already often cause diarrhea and vomiting and severe jaundice has also been observed. Higher dosages cause circulatory collapse. In addition to this use as an anthelmintic it is used in folk medicine to induce abortions

(sometimes with a fatal outcome), and also against migraine, somach cramps, dysmenorrhea, and as an ointment it is popularly used against rheumatism and gout. (The drug Herba tanaceti which is also used medicinally seldom contains more than 0.2 to 0.3% volatile oil; therefore it can remain in its usual classification).

Wormwood oil also contains mostly β-thujone and is popularly used as an anthelmintic, but also to induce abortions. Absinthe beverages are very popular in Romance countries. Because of the danger of causing epileptiform seizures when taken in excess, and so-called "absinthism" upon chronic use, such spirits are forbidden here in Germany and in many other culturally advanced countries.

Areca seed. Areca seeds, in addition to other alkaloids, contain the main alkaloid arecoline in a quantity of 0.4%. They have long been used as an agent against tapeworm. Among Malayan tribes they have been used since ancient times for enjoyment instead of tobaco ("betel chewers"). While arecoline hydrobromide is a poison, the areca seed is still classified as "indifferent", even though poisoning has frequently occurred in humans, with symptoms taking the form of scratching of the throat and violent gastric movements with vomiting. Death is caused by respiratory paralysis. Even 8 to 10 g powdered substance can give rise to a strong toxic effect and it remains questionable whether it is permissible to dispense anthelmintics containing tancy and areca seeds in pharmacies at random to the unsuspecting public, as has often been observed by this author.

Another tapeworm agent is cortex granati, which contains various alkaloids. The most important of these are pelletierine and isopelletierine. For anthelmintic purposes it must be used in fairly high doses (50-60 g as an infusion). An overdosage may easily give rise to toxic phenomena in man: feelings of weakness, dizziness, visual disturbances, symptoms of paralysis and collapse. Death is caused by paralysis of the respiratory center, since pelletierine acts on the central nervous system, like strychnine.

The classification of jalap soap among indifferent substances would also appear to bear careful scrutiny. In high doses resina jalapae, which is contained up to 50% in this substance, causes severe irritation of the intestinal mucosa with intestinal bleeding. While the resin itself must be stored with care, jalap soap, whose effect is intensified by the alkalinity of the added soap, is still labelled with black on white.

It is also pointed out that in homeopathic medicine books thuja mother tincture is entered as an indifferent galenical, a situation which also requires revision, in view of its great toxicity.

This is a small selection of drugs which I most commonly encountered and which in my opinion are wrongly classified. The brief phramacologic and toxicologic descriptions may show distinctly enough that all the drugs enumerated here cannot be designated as indifferent with the best will in the world, but that far rather they may give rise to very unpleasant, often

fatal poisoning. If there is any point at all in the usual pharmaceutical classification of chemicals, drugs and their galenic preparations in indifferent, carefully and very carefully stored substances, then those described above without a doubt belong among those that must be stored with caution, separately.

Summary

The pharmacology and toxicology of a number of drugs is discussed, labelled black on white (as indifferent substances) according to the German Pharmacopeia 6 and its supplement, even though based on their toxic qualities they should be classified among substances to be stored with caution (labelled red on white). In the opinion of this author this includes phenylquinoline-carboxylic acid (Atophan E.W.), benzene, carbon tetrachloride, quinine and its salts and euquinine, chrysarobin, cortex granati, tansy and tanacetic oil, potassium chloride, potassium nitrate, potassium permanganate, naphthalene, β-naphthol, wormwood oil, salvia oil, pyrogallol, jalap soap, areca seeds, and in homeopathic medicine books, thuja.

Bibliography

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„Indifferente" Arzneistoffe mit starker Toxizität

Von Victor Kwasniewski (Königslutter/Braunschweig)

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In der Apotheke ist es seit langer Zeit üblich, alle Arzneistoffe, Chemikalien wie Drogen und ihre Zubereitungen ihrer Wirkung entsprechend aufzubewahren, um Verwechslungen weitgehend auszuschalten. Dies geschieht z. B. durch verschiedenartige Signierung: schwarz auf weiß, rot auf weiß, weiß auf schwarz. Es ist nun selbstverständlich, daß es auch Übergänge gibt, Stoffe, deren Zugehörigkeit zur einen oder anderen Gruppe schwer zu entscheiden ist. Da sind beispielsweise die ätherischen Öle, die leicht von Haut und Schleimhäuten resorbiert werden und mit wenigen Ausnahmen Giftwirkungen zeigen können, jedoch nur in großen bis sehr großen Dosen und nicht bei den therapeutisch gebräuchlichen Mengen. Schon durch längeres Einatmen können sie Kopfschmerzen und Benommenheit erzeugen (stark duftende Blumen im Zimmer) und je nach Zusammensetzung bei Vergiftungen voneinander abweichende Wirkungen zeigen: die Terpene lähmen das Zentralnervensystem, die Kampfer erzeugen Krämpfe, epileptiforme Anfälle mit Exitus durch Respirationslähmung. Ob man diese nun künftig rot auf weiß signieren soll, bleibe dahingestellt; einige müßten jedoch unbedingt zu den Separanden gestellt werden, da ihre toxische Dosis relativ niedrig liegt, so Oleum Absinthii, Oleum Salviae und Oleum Tanaceti, wie weiter unten noch ausführlicher dargelegt werden soll.

Es wird aber in den Apotheken entsprechend den geltenden Arzneibüchern eine Anzahl von Arzneistoffen aufbewahrt, die m. E. ihrer Wirkung entsprechend falsch eingereiht sind; sie werden da nämlich zu den „Indifferenten (= Unschädlichen)" gerechnet, die sie in Wirklichkeit gar nicht sind, wie die folgenden kurzen Notizen zeigen sollen.

Kalium chloricum wird sowohl innerlich bei Cystitis und Pyelitis (früher auch bei Diphtherie) als auch zum Gurgeln bei Angina gebraucht. Es bildet im Körper Chlorsäure, welche das Hämoglobin des Blutes in Methämoglobin und Hämatin überführt, die Thrombosen und Embolien erzeugen können. Der Tod erfolgt nach toxischen Dosen durch Cyanose, da das Methämoglobin keinen Sauerstoff an die Gewebe abzugeben vermag. Beim erwachsenen Menschen treten bei 10 g bereits bedenkliche Vergiftungssymptome auf, während 15 bis 20 g schon tödlich wirken.

Kalium nitricum wird heute therapeutisch nur noch selten gebraucht (z. B. Lauder-Bruntonsche Mischung, Pulvis temperans). Früher war dieses Salz als Antiphlogisticum bei akuten fieberhaften Erkrankungen sehr beliebt und wurde in Mengen von 0,5 bis 1 g genommen. Diese Dosis ist unbedenklich, hingegen sind Verwechslungen mit ähnlich aussehenden Salzen wie Glauber- und Bittersalz vorgekommen. In Mengen von 10 bis 30 g genommen, kommt es zu heftigen Schmerzen im Abdomen, profusem, blutigem Erbrechen sowie Durchfall. Dann folgen Krämpfe, der Puls wird klein und weich und schließlich kommt es zum Herzstillstand, der wahrscheinlich eine Folge der Wirkung des Kaliumions ist.

Kalium permanganicum wird als kräftiges Oxydationsmittel u. a. in der Wundbehandlung noch oft angewandt. Es wirkt desodorierend, desinfizierend und in stärkerer Konzentration ätzend. So wurde es früher auch innerlich bei Magengeschwüren (in Pillen) gegeben. Eine 1- bis 2%ige Lösung verursacht auf Schleimhäuten schon starke Ätzungen. Selbst die Anwendung als Mundwasser ist nach Ansicht einiger Pharmakologen nicht

unbedenklich, da sie schon zu leichten Zungenschwellungen und monatelanger Geschmacksunempfindlichkeit führen kann. Wenn auch Vergiftungen mit Kaliumpermanganat selten sind, wurde es doch schon des öfteren zu Selbstmorden benutzt. Die letale Dosis beim Menschen liegt bei etwa 10 bis 20 g. Zum Bilde der Vergiftung gehören Zungenschwellungen und Verätzungen des Rachens, Kehlkopfes und der Speiseröhre; die auftretende Herzlähmung ist wohl eine Wirkung des Kaliumions.

Carboneum tetrachloratum wird (bei uns selten) als Wurmmittel beim Befall mit Hakenwurm innerlich in Mengen von 2 bis 3 ccm genommen. Jedoch kann schon diese therapeutische Dosis zu Vergiftungen führen und Leber- und Nierenschädigungen machen. Häufiger bei uns ist die technische Anwendung (Fleckenreinigungsmittel), die mit Vorsicht bei möglichst offenem Fenster zu erfolgen hat, da die eingeatmeten Dämpfe neben Benommenheit noch Übelkeit, verbunden mit Kopfschmerzen, in schlimmeren Fällen Erbrechen und Krämpfe erzeugen können.

Benzol hat therapeutisch nur wenig Interesse (innerlich selten bei Darmtrichinen, äußerlich in Form von Cignolinpinselungen, Läusemitteln u. ä.), ist jedoch toxikologisch sehr wichtig, denn bereits längeres Einatmen der Dämpfe führt zu Bewußtlosigkeit mit meist tödlichem Ausgang; voraus gehen Krämpfe, Euphorie, multiple Blutungen in die Haut, sogar tödliche Uterusblutungen. Bei chronischer Benzolvergiftung wird das Knochenmark geschädigt, die Zahl der Leukocyten nimmt ab, es kommt zur Anämie. Benzol wird auch in starkem Maße durch die Haut aufgenommen.

Pyrogallol wird in der Dermatologie häufig gebraucht bei Psoriasis, Lupus, bei gewissen Pilzerkrankungen usw. Da es zu den methämoglobinbildenden Stoffen gehört, ist bei seiner Anwendung größte Vorsicht geboten (nicht mehr als 3 g pro Tag dürfen eingerieben werden). Eine Vergiftung äußert sich in Nephritis mit Hämaturie, Diarrhöen, Frostanfällen, Ikterus und Muskelzuckungen.

Chinin und seine Salze, ferner Euchinin. Chinin ist ein gern und viel gebrauchtes Pharmakon. Besonders in der Gynäkologie zur Anregung der Wehentätigkeit, aber auch als Antipyretikum, bei Grippe, Pneumonie, bei funktionellen Arrhythmien und bei Malaria wird es verordnet. Leider entfaltet es viele üble Nebenwirkungen, die sich oft schon bei therapeutischen Dosen bemerkbar machen, als da sind: Schwindel, Ohrensausen, Trübungen des Bewußtseins („Chininrausch"), alles Störungen, die auf eine Lähmung des Zentralnervensystems zurückgeführt werden. Bei längerem Gebrauche (z. B. bei Malaria) kommt es leicht zur Hämaturie (Schwarzwasserfieber). Besonders übel ist die plötzlich auftretende Herzschwäche, ein Zeichen der Giftwirkung auf das Herz. Die Dosis letalis für den Menschen liegt bei 10 g.

Naphthalin wurde früher innerlich in Dosen von 0,5 bis 3 g täglich als Oxyurenmittel gegeben, heute wird es jedoch nur selten gebraucht. Auch bei Typhus wurde es als Darmantiseptikum sowie äußerlich als Wundstreupulver angewandt. Der größte Teil des eingenommenen Naphthalins geht mit dem Kote ab und nur eine kleine Menge wird vom Darme resorbiert, wobei es nach seiner Oxydation zu Naphthol und Naphthochinon durch die Nieren in den Harn übergeht. Hier verursachen diese Derivate schlimme Reizungen der Schleimhäute und sind Anlaß zu Albuminurie. Auch sind in der Literatur Todesfälle nach Einnahme als Wurmmittel beschrieben, so daß man das Naphthalin wohl ebensowenig als indifferent

bezeichnen kann wie das β-Naphthol, das in der Dermatologie weite Verbreitung gefunden hat. In starker Konzentration ätzt es und bewirkt sogar bei gesunder Haut Nekrose. Da es leicht durch die Haut aufgenommen wird, kommt es bei der Ausscheidung durch die Nieren wie beim Naphthalin oft zu Nephritiden mit Albuminurie und Hämaturie.

Chrysarobin stellt therapeutisch das wohl wirksamste Mittel bei Psoriasis dar. Es wird sehr leicht von der Haut resorbiert und seine Spaltstücke werden durch den Harn ausgeschieden, wobei sie Nierenreizungen erzeugen. Innerlich wirkt Chrysarobin schon in Zentigrammdosen sehr giftig und ist Anlaß zu heftigen Gastroenteritiden und schweren Nierenschädigungen.

Acidum phenylchinolincarbonicum (Atophan E. W.) ist ein wertvolles Arzneimittel zur Harnsäureausschwemmung aus dem Körper bei harnsaurer Gicht, Rheumatismus; auch wirkt es entzündungshemmend, antipyretisch und analgetisch. Die Medikation darf nur wenige Tage (drei bis fünf) fortgesetzt werden, da es sonst leicht zu toxischen Leberschädigungen (Ikterus), sogar zur tödlich endenden akuten gelben Leberatrophie (Coma hepaticum) kommen kann.

Oleum Salviae besteht neben Borneol, Kampfer und Cineol zu 50 % aus dem sehr giftigen Thujon. Wenn man sich vor Augen hält, daß 0,3 g Salbeiöl einen 5 bis 6 kg schweren Hund zu töten vermögen, so sollte diese Tatsache zu denken geben. Kleine Dosen des Öles rufen epileptiforme Anfälle hervor. Die meistens gebrauchte Droge, die 1 bis 2,5 % ätherisches Öl enthält, scheint wenig gefährlich zu sein; jedenfalls sind in der Literatur keine näheren Angaben über eine evtl. Giftwirkung zu finden.

Flores Tanaceti und das der Droge zu ca. 1,5 % enthaltene Oleum Tanceti. Dieses ätherische Öl besteht zu 70 % aus dem giftigen und wurmwidrigen β-Thujon, das eine Verwendung der Droge zu Wurmmitteln rechtfertigt. Bei therapeutischen Dosen treten bereits oft Durchfall und Erbrechen auf; auch schwerer Ikterus wurde beobachtet. Größere Dosen bewirken Kreislaufkollaps. Neben dem Gebrauche als Wurmmittel steht die volkstümliche Anwendung als Abortivum (mit manchmal letaler Vergiftung); auch gegen Migräne, Magenkrämpfe, Dysmenorrhoe und zu Einreibungen bei Rheuma und Gicht wird die Droge vom Volke benutzt. (Die ebenfalls offizinelle Droge Herba Tanaceti enthält selten mehr als 0,2 bis 0,3 % ätherisches Öl; es kann daher in diesem Falle bei der bisherigen Einstufung bleiben.)

Oleum Absinthii enthält gleichfalls vor allem β-Thujon und wird vom Volke als Wurmmittel, aber auch zu Abtreibungen gebraucht. In den romanischen Ländern sind Absinthliköre sehr beliebt; wegen ihrer Gefahr, bei Mißbrauch leicht epileptiforme Krämpfe und bei chronischem Gebrauch den sog. „Absinthismus" zu erzeugen, sind derartige Spirituosen bei uns in Deutschland sowie in vielen anderen Kulturstaaten verboten.

Semen Arecae. Die Arekasamen enthalten neben verschiedenen anderen Alkaloiden das Hauptalkaloid Arekolin zu 0,4 % und stellen ein altes Bandwurmmittel dar. Bei den malaischen Völkern werden sie von alters her als Genußmittel an Stelle von Tabak verwandt („Betelkauer"). Während das Arekolinhydrobromid zu den Giften gehört, ist die Droge Semen Arecae immer noch als „Indifferente" eingereiht, obwohl des öfteren Vergiftungen beim Menschen vorgekommen sind, deren Symptome Kratzen im Halse, heftige Magenbewegungen mit Erbrechen sind. Der Tod erfolgt durch Atmungslähmung. Schon 8 bis 10 g gepulverte Droge können eine starke

Giftwirkung entfalten, und es sei dahingestellt, ob es angängig ist, daß Wurmspezialitäten mit Flor. Tanacet. und Sem. Arecae in Drogerien kritiklos an das ahnungslose Publikum abgegeben werden, wie Verfasser das des öfteren beobachten konnte.

Ein anderes Bandwurmmittel ist Cortex Granati, die verschiedene Alkaloide enthält. Die wichtigsten davon sind das Pelletierin und Isopelletierin. Die Droge muß zur Wurmabtreibung ziemlich hoch dosiert werden (50 bis 60 g als Dekokt). Eine Überdosierung zeitigt beim Menschen leicht Vergiftungserscheinungen: Schwächegefühl, Schwindel, Sehstörungen, Lähmungs- und Kollapserscheinungen. Der Tod tritt durch Lähmung des Atemzentrums ein, da das Pelletierin zentral erregend wirkt, ähnlich wie Strychnin.

Einer ernsten Nachprüfung wert scheint auch die Einreihung von Sapo jalapinus unter die indifferenten Stoffe zu sein. Resina Jalapae, das zu 50% in dieser Mischung enthalten ist, erzeugt bei hohen Gaben starke Darmschleimhautreizungen mit Darmblutungen. Während das Harz selbst vorsichtig aufbewahrt werden muß, ist Sapo jalapinus, deren Wirkung durch die Alkalität des Seifenzusatzes verstärkt wird, immer noch schwarz auf weiß signiert.

Ferner sei noch darauf hingewiesen, daß im Homöopathischen Arzneibuche Thuja-Urtinktur als indifferentes Galenicum aufgeführt ist, ein Zustand, der hinsichtlich der großen Giftigkeit gleichfalls einer Revision bedarf.

Dieses ist eine kleine Auslese der mir am meisten aufgefallenen und m. E. falsch eingeordneten Pharmaka. Die kurzen pharmakologischen und toxikologischen Erläuterungen dürften wohl deutlich genug zeigen, daß alle hier aufgeführten Arzneistoffe mit bestem Willen nicht als „indifferent" bezeichnet werden können, daß sie vielmehr sehr unangenehme, oft tödlich endende Vergiftungen hervorrufen können. Wenn die ganze in der Apotheke übliche Einteilung der Chemikalien, Drogen und ihrer galenischen Zubereitungen in indifferente, vorsichtig und sehr vorsichtig aufzubewahrende Stoffe überhaupt einen Sinn hat, so gehören die oben im einzelnen beschriebenen unbedingt in die Reihe der vorsichtig aufzubewahrenden Arzneistoffe, der Separanden.

Zusammenfassung

Es wird die Pharmakologie und Toxikologie einer Anzahl von Arzneistoffen besprochen, die nach dem DAB. 6 und dem Ergänzungsbuch zum DAB. 6 schwarz auf weiß signiert sind (als indifferente Stoffe), während sie ihrer toxischen Wirkung nach in der Reihe der vorsichtig aufzubewahrenden Pharmaka (rot auf weiß signiert) stehen müßten. Hierzu gehören nach Ansicht des Verfassers Acidum phenylchinolincarbonicum (Atophan E. W.), Benzol, Carboneum tetrachloratum, Chinin und seine Salze sowie Euchinin, Chrysarobin, Cortex Granati, Flores und Oleum Tanaceti, Kalium chloricum, Kalium nitricum, Kalium permanganicum, Naphthalin, β-Naphthol, Oleum Absinthii, Oleum Salviae, Pyrogallol, Sapo jalapinus, Semen Arecae sowie im Homöopathischen Arzneibuche Thuja.

Schrifttum

1) Poulsson, Lehrbuch der Pharmakologie (Leipzig 1934).
2) Braun, Pharmakologie des DAB. 6 und des Erg.-B. 6 (Stuttgart 1949).
3) v. Czetsch-Lindenwald, Pflanzliche Arzneizubereitungen (Stuttgart 1943).
4) Jaretzky, Lehrbuch der Pharmakognosie (Braunschweig 1949).
5) Deutsches Arzneibuch, 6. Ausgabe (Berlin 1926).
6) Ergänzungsbuch zum DAB. 6 (Berlin 1941).
7) Homöopathisches Arzneibuch (Leipzig 1934).

Bull. Sciences Pharmacol. 39(4): 217-227, 1932

Antagonism of Camphor and Potassium Chloride

When the variations of the cardiac rhythm were followed in the heart *in situ* by the method of suspension, it was found that the intramuscular injections of camphorated oil and some soluble derivatives of camphor produced no apparent effect on the normal heart of the dog. This was not the case when the injections were given to animals preliminarily poisoned with an ionized potassium salt, the toxicity of which was pointed out by Claude Bernard and Grandjean and studied in detail by Busquet and Pachon (1) after injection of large doses by the intravenous route. We proposed to apply this antagonism to the comparative study of some derivatives of camphor. We then ran up against a certain number of difficulties because from the beginning of our experiments, we noted on the one hand, that small amounts of potassium injected by the intravenous route caused only a very slightly durable depressive effect so that the antagonistic effect of the camphor could be estimated, and on the other hand, large doses of potassium irremediably caused death no matter what the amount of camphor later injected.

We then operated in different ways, and we injected potassium chloride to dogs having first of all received varying doses of camphorated oil or aqueous solutions of some derivatives of camphor. Under these conditions we observed a notable diminution of the depressive cardiac effects when we injected small or average doses of KCl and a considerable slowing down of the ventricular vibrations from massive doses of KCl.

Further in guinea pigs having initially received camphor, we observed a percentage of survival greatly exceeding that obtained in animals to which the same amount of KCl was injected alone.

Thus, camphor seems to exert an antagonistic effect in relation to cardiac poisoning caused by potassium chloride.

Before formulating hypotheses suggested to us by these facts, let us examine briefly from data in the literature, on the one hand, what are the effects of camphor on the cardiac muscle and on the other hand, what is the mechanism for the poisoning of the heart by potassium chloride.

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(1) Busquet and Pachon, C.R. Ac. Sc., 1907, 144, p. 1065; J. Physiol. Pathol. generale, 1909, 2, 242.

I. Cardiac Action of Camphor - The cardiac action has been known in therapeutics for a long time, but in spite of numerous experiments carried out with this medication, the mechanism of its action has not yet been completely elucidated.

We will rapidly summarize the experiments performed with this purpose, and we will distinguish three orders of facts to look for: on the one hand, the effect of camphor on the isolated heart of warm-blooded and cold-blooded animals; on the other hand, the effect on hearts subjected to electrical stimulation; and finally the effects on hearts subjected to the action of various substances.

1-Effect of camphor on the isolated heart of warm-blooded and cold-blooded animals. It is known that in the isolated heart of the frog, camphor in quite large dilutions and in addition varying between very narrow limits, can improve the amplitude and the rhythm of the movements of the heart (1). When these concentrations are exceeded, camphor acts as a cardic depressant (2). It is undoubtedly because of a similar action on the heart of the cat that Seligman (1)* obtained apparently contradictory results. Finally, Frolich and Pollak (2)*, then Bouisset (loc. cit.) found analogous results, the former on the heart of the rat, and the latter on the hearts of the cat and the rabbit.

2-Effect of camphor on the heart subjected to electrical stimulation. Gottlieb[3] showed that in the heart of the dog in situ or the heart of the cat in situ, camphor injected intravenously suppresses the fibrillations that a current of fixed frequency normally produces in these animals. Camphor also suppresses the wave-motions caused by an electric current on the heart of the frog, according to Frolich and Grossman[4] and Boruttau[5]. This last author further observed that the action of cardiac repair may be observed by perfusing the heart of a frog injured by electrical stimulation with a liquid rich in

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(1)Frolich and Pick, Arch. Exp. Pathol. Pharm., 1918, 84, p. 250, Joachimoglu. Arch. Exp. Path. Pharm. 1917, 80, p. 259.

(2)Alexander-Lewin, Arch. Exp. Path. Pharm. 1890, 27, p. 226; Bouisset J. de Phys. et de Path., 1926, 24, p. 255; Tamura, Kittara and Ishidatr., Proc. Imp. Ac., 1930, 6, p. 175. These last authors pointed out a late cardiotonic effect which would be due to the product of the transformation of comphor which they have isolated and of which the cardiotonic effects are immedicate. Handovsky (arch. exp. Path. Pharm. 1923, 99, p. 115) had earlier pointed out similar facts without isolating the substance.

potassium and deficient in calcium. Potassium in small amounts would thus have under these conditions an effect similar to that of camphor.

3-Effect of camphor on the heart subjected to a certain number of medications.—Boehm[6] and after him, Chiodo[7], showed that the heart of a frog damaged by the perfusion of chloral hydrate regained its amplitude and original rhythm if it is perfused with solutions of camphor in weak concentrations. Frolich and Grossman[8] showed that in the frog camphor suppressed the cardiac effects of digitalis; Frolich and Pollack (loc. cit.) in experimenting on the heart of rats poisoned with chloroform, phosphorus strophanthine or yohimbine, showed the antidotal effect of camphor which in every case consisted of a diminution in excitability.

In summary, camphor, by increasing heterotropic stimulation seems to protect the injured heart not only from electric currents, but also from any cardiac poison.

II. Cardiac Effect of the Potassium Ion - If small amounts of ionizable salts of potassium added to a perfusion liquid are capable of restoring the isolated frog heart injured by Faradaic stimulation, on the other hand, large amounts of potassium produce in the heart of warm-blooded and cold-blooded animals, a definitve arrest.

Busquet and Pachon[9] showed that diastolic arrest of the heart due to potassium is clearly differentiated from cardiac arrest caused by pneumogastric excitation. From their experiments they concluded that "potassium caused cardiac arrest by directly paralyzing the cardiac muscle fibers, rendering them unable to contract."

III. Antagonism of Camphor and Potassium - We have just seen how potassium acts on the heart by poisoning the cardiac muscle fibers, and that in a general way, camphor exerts in regard to certain poisons an antagonistic action, without the mechanism of this action being clear. We thought in relation to the camphor-potassium antagonism, that the camphor perhaps exerts a protective effect on the cardiac fibers either because it prevents the ultimate fixation of potassium or because it makes this fixation more lax and enables the more facile elimination of the potassium ion.

Wanting to exactly define this hypothesis, we administered dosages of potassium in the plasma and the heart of dogs having received or not received camphor and poisoned by the same quantity of KCl, but the numbers obtained were not sufficiently consistent so that we could make a statement.

We will thus restrain outselves to pointing out this new antagonism of camphor and potassium, without it being possible for us to indicate the actual mechanism.

In the experimental description which follows, we have collected experiments done on guinea pigs and dogs.

In every case, we have preliminarily determined the toxic amounts of KCl on control animals, then examined the effect of the same amounts on animals having first received camphor.

I. Experiments on the guinea pig. - We performed two series of experiments on this animal: (1) we fixed the amount of KCl capable of causing death in 70 to 80% of the animals in about an hour; (2) we injected obviously equal or higher doses of KCl to guinea pigs having first received by the hypodermic route soluble derivatives of camphor; we were able to verify that for the most part, the animals resisted poisoning caused by amounts of KCl generally fatal for normal animals. The various experiments which we did are described below.

1. Determination of the toxic dose of KCl in the guinea pig. In our experiments, we used a 16% solution of KCl, and we gave each guinea pig an intramuscular injection. The signs of poisoning due to potassium most often appeared quite late, 10 to 15 minutes before death; respiration was then panting, the heartbeat became arhythmic, the animal lay down on its side with paralysis of the anterior quarters. From time to time, violent and uncoordinated movements appeared. Finally after some deep respiration, followed by respiration whose amplitude progressively diminished, death occurred.

The experimental results which are summarized in table I showed us that the amount of 0.95 g/kg of animal of KCl caused death in about 78% of the guinea pigs in the experiment in a time of about 60 minutes.

TABLEAU I.

N° D'ORDRE	1 POIDS des animaux (gr.)	2 TEMPS qui s'écoule entre l'injection et la mort (minutes)
1	520	58
2	800	64
3	580	58
4	700	48
5	510	45
6	620	59
7	700	Survie. 3
8	660	86
9	700.	Survie. 3

1. Weight of the animals (g)
2. Time between injection and death (minutes)
3. Survival

2. Antagonistic effect of soluble derivatives of camphor in regard to poisoning caused by KCl. -a) In a first series of experiments, we injected animals with an aggregate amount of a soluble derivative of camphor, camphosulfonate soda or diethylamine camphocarbonate, corresponding to 0.10 g of camphor[1], then an amount of 0.95 g KCl per kilogram of animal; we found that under these conditions, 75% of the animals survived. These results are found in Table II.

TABLEAU II.

N° D'ORDRE	1 POIDS des animaux (gr.)	2 TEMPS qui s'écoule entre l'injection et la mort (minutes)
1	750	Survie. 3
2	450	Survie. 3
3	520	Survie. 3
4	560	Mort en 65 4
5	550	Survie. 3
6	560	Mort en 65 4
7	500	Survie. 3
8	500	Survie. 3

1. Nous sommes heureux de remercier ici les firmes DAUSSE et ROBERT et CARRIÈRE qui ont bien voulu nous préparer l'une du camphosulfonate de soude, l'autre du camphocarbonate de diethylamine titré à 10 % en camphre. Nous avons d'ailleurs obtenu des résultats analogues avec des solutions de camphocarbonate de soude.

1. Weight of the animals (g)
2. Time between injection and death (minutes)
3. Survived
4. Dead in 65 (minutes)

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(1) We are pleased to thank the firms of Dausse and Robert and Carriere which prepared for us the camphosulfonate soda and the other, the diethylamine camphocarbonate having a 10% concentration of camphor. In addition we obtained analogous results with solutions of camphocarbonate.

b) In a second series of experiments, we injected the guinea pig with the same amount of camphor, or 0.10 g, then 1 g KCl per kilogram; under these conditions, the control animals and the animals having received first of all one of the soluble derivatives of camphor, all died within a period of time varying from 50 to 70 minutes.

c) Finally, in a third series of experiments, we first injected guinea pigs with a double dose of camphor (o.20 g), then 1 g of KCl per kilogram. Under these conditions, the animals survived in a proportion of 2 out of 3. On the other hand, the animals having received only KCl, in amounts of 1 g per kilogram all died within 60 to 65 minutes.

II. Experiments on the dog.- We perfomred two series of experiments on this animal. The first had for its purpose the determination of the toxic dose of KCl in the dog either by injecting small doses repeated in intervals from 2 to 5 minutes or by injecting large doses at the onset.

In a second series of experiments, we injected amounts analogous to those received by animals having first received soluble derivatives of camphor intravenously. We found that under these conditions the dog resisted poisoning from KCl, and at the very least when potassium chloride injected once did not exceed the minimum lethal dose (3 cg per kilogram), it was necessary in order to obtain death to use total quantities of KCl much higher than the toxic dose previously determined. On the other hand, if at the outset, the amount of 3, 4, or 5 cg/kg was injected, the animal died even if it later received significant quantities of camphor.

1. Determination of the toxic dose of KCl in the dog. -a) Effect of small repeated doses of KCl: We have first of all studied in the dog, the toxic effect of KCl injected in small repeated doses by the intravenous route and followed the course of the cardiac poisoning by recording the movements of the ventricle and the auricle of the chloralosed dog in which the thorax has been opened and upon whom artificial respiration is practiced. In these experiments, we used a 4% solution of KCl.

The first injections of 0.5 or 1 cg of KCl per kilogram had only a slight effect on the amplitude and the frequency of the cardiac rhythm. However, when the animal had already received 7 to 9 cg of KCl, each new injection caused a decrease in amplitude, a decrease especially marked for the auricle, and a notable slowing of the cardiac rhythm. Little by little these phenomena worsened and death occurred a few seconds after the last injection from diastolic

arrest of the heart, followed by fibrillation.

As can be seen in experiments 1, 2, 3, 4, 5 summarized in table III, under these conditions, death can occur when the animal has received between 6 and 26.5 cg of KCl per kilogram (the injections of 0.5 cg and 1 cg of KCl per kilogram being given in intervals from 2 to 5 minutes).

TABLEAU III.

N° D'ORDRE	1 POIDS des chiens (K°)	2 DOSE DE ClK injectée en une fois (centigr. par K°)	3 QUANTITÉ TOTALE de ClK déterminant la mort (centigr. par K°)
1 . . .	♂ 8,300	6 injections de 1 centigr. »	6
2 . . .	♂ 16,500	3 — de 1 centigr. » 4 — de 2 centigr. »	11
3 . . .	♂ 20 »	6 — de 0 centigr. 5 5 — de 1 centigr. » 2 — de 2 centigr. »	12
4 . . .	♂ 5,150	9 — de 1 centigr. » 8 — de 2 centigr. »	25
5 . . .	♀ 7 »	9 — de 0 centigr. 50 20 — de 1 centigr. » 2 — de 2 centigr. »	26,5

1. Weight of the dogs (kg)
2. Amount of KCl injected at one time (cg/kg)
3. Total quantity of KCl causing death (cg/kg)

Furthermore, if at the outset, amounts of 2 cg of KCl per kilogram are injected, whatever is the later conditon of the heart, we always observed at the same time as a significant diminution of auricular and ventricular amplitude, a more marked auricular diminution, a slowing of the beat with in some cases, diastolic arrest. The heart which was very dilated extends beyond the pericardium on which it rests. In some cases (57.50% of the experiments) death occurred from the first injection of 2 cg of KCl per kilogram, from auricular, ventricular fibrillation. But when the animal resisted this first injection, the heartbeat regained its speed, cardiac amplitude increased and also rapidly exceeded the initial amplitude; the injections following most often produced the same phenomena but not as severe. After this period, the phenomena worsened and death occurred, most often when the animal had received 2 to 10 cg of KCl per kg (exp. 6, 7,,8, 9, 10, 11); as exceptions, the most resistant animals (exp. 12 and 13) could receive 18 to 20 cg of KCl per kilogram from doses of 2 cg/kg.

We gathered the results of the experiments done on 8 dogs in Table IV.

TABLEAU IV.

N° D'ORDRE	1 POIDS des chiens (Kg)	2 DOSE DE ClK injectée en une fois (centigr. par Kg)	3 QUANTITÉ de ClK déterminant la mort (centigr. par Kg)
6. . .	♂ 18,900	1 injection de 2 centigr.	2
7. . .	♂ 13,900	1 — de 2 centigr.	2
8. . .	♀ 17,000	1 — de 2 centigr.	2
9. . .	♀ 7,300	1 — de 2 centigr.	3
10. . .	♀ 7,200	2 — de 2 centigr. 1 — de 4 centigr.	8
11. . .	♂ 10,000	3 — de 2 centigr. 1 — de 4 centigr.	10
12 . .	♀ 12,000	9 — de 2 centigr.	18
13. . .	♂ 9,200	10 — de 2 centigr.	20

1. Weight of the dogs (kg)
2. Amount of KCl injected at one time (cg/kg)
3. Quantity of KCl causing death (cg/kg)

b) Effect of large amounts of KCl injected at one time. The amounts of 3 cg, 3.5 cg and 4 cg per kilogram of animals showed themselves to be always fatal (fig. 1) in the dog when they were injected at once and caused death in 100/100 of the injected animals (10) experiments).

2. Antagonistic effect of the soluble derivatives of camphor in regard to poisoning caused by KCl. Our experiments were done as follows. We injected intravenously repeated doses of some soluble derivatives of camphor in the amounts of 0.10 g, 0.20 g, 0.30 g and 0.60 g of camphor at one time. The camphor thus injected did not act on the normal heart of the dog, and we noted no appreciable change in cardiac rhythm and amplitude. After the injections of camphor, we gave injections of KCl, Each injection of KCl acted again on the cardiac rhythm and amplitude by slowing the former down and by diminishing the latter, but these two effects are clearly less significant in the animal first treated with camphor than in the normal animals.

We can compare (fig. 2 and 3) the effect on the auricle and the ventricle of one dose of 2 cg of KCl per kilogram in two dogs of which one is normal and the other (fig. 3) first received a dose of 0.6 g of camphor. In the normal dog, 2 cg/kg of KCl caused cardiac arrest which lasted 7 to 8 seconds. By contrast, in the dog having first received 0.60 g of camphor the auricular and ventricular movements were greatly slowed down, but not completely inhibited.

FIG. 1.

Fig. 1--Effect of KCl on the heart *in situ* Dog ♂, 10 kg. From top to bottom, auricular and ventricular tracings, time in seconds. At X, intravenous injection of 4 cg of KCl per kg. Definitive diastolic cardiac arrest.

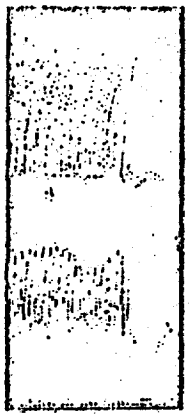

FIG. 2.

Fig. 2--Dog ♀, 12 kg. From top to bottom, auricular and ventricular tracings, time in seconds. At X, intravenous injections of 2 cg of KCl per kg. Diastolic cardiac arrest for 5 to 8 seconds.

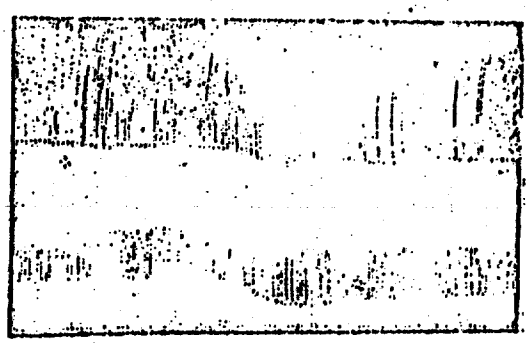

Fig. 3--Dog ♀, 20 kg. From top to bottom, auricular and ventricular tracings, time in seconds. At X, intravenous injection of 0.6 g of camphor and 2 cg of KCl per kilogram. Slowing of cardiac rhythm, decrease in auricular and ventricular amplitude. Tendency towards diastolic arrest.

a) Effect of medium repeated doses of KCl in dogs having first received camphor. In this series of experiments, we always injected at once to dogs having received camphor, amounts of KCl at least equal to 2 cg/kg of animal. If we exclude two experiments (19 and 20) in which the animals died after having received 2 to 4 cg per kilogram of KCl, all the others, in other words, 5 out of 7, resisted large doses of KCl, 19 to 31 cg/kg (experiments 14, 15, 16, 17, 18). Of course, it was necessary to give injections of 0.10 g, 0.20 g, 0.30 g of camphor between the injections of KCl so that the total amount of camphor injected varied from 2 to 6 g. We have seen above that under the same conditons, the animals not having received camphor succumbed in the proportion of 6 out of 8 when the amounts of KCl injected varied from 2 to 10 cg.

We give in Table V a summary of the experiments which we have performed. We have added to this table to the columns indicating the weight of the dog, the number of injections of KCl and the total quantity of KCl injected; a fourth column indicating the amounts of camphor received by the animals in the experiment.

TABLEAU V.

1 N° d'ordre et poids des chiens Kg	2 DOSES DE ClK injectées en une fois (centigr. par Kg)			3 QUANTITÉ totale de ClK déterminant la mort (centigr. par Kg)	4 DOSE totale de camphre injectée
14, ♀ 15,000	8 injections	de 2 centigr.		19	2 gr. 2
	1	--	de 3 centigr.		
15, ♂ 5	7	—	de 2 centigr.	23	1 gr. 7
	2	—	de 3 centigr.		
	4	--	de 1 centigr.		
16, ♀ 9,000	14	—	de 2 centigr.	28	»
17, ♂ 10,000	13	—	de 2 centigr.	28	6 gr. 5
	3	—	de 1 centigr.		
18, ♂ 5,500	15	—	de 2 centigr.	30	1 gr.
19, ♂ 17,000	2	—	de 2 centigr.	4	0 gr. 5
20, ♂ 8,300	1	—	de 2 centigr.	2	0 gr. 9

1. Number and weight of the dogs (kg)
2. Amounts of KCl injected at one time (cg/kg)
3. Total quantity of KCl causing death (cg/kg)
4. Total amount of camphor injected

b) Effect of lethal doses of KCl injected into dogs having first received camphor. We will distinguish two series of experiments, those in which the amount of KCl injected is 3 cg/kg, and those in which this amount is 3.5 or 4 cg/kg. In the latter case and no matter what the dose of camphor initially administered to the animal, the amounts of 3.5 cg and 4 cg/kg showed themselves to be fatal in 100% of the cases. On the other hand, when a dose

of 3 cg/kg of KCl was administered to dogs having first received camphor, death was observed in 20% of the cases. Very generally, the animal resisted this amount of KCl and could even resist doses varying from 6 to 20 cg per kilogram. These various experiments are summarized in Table VI.

TABLEAU VI.

1 N° D'ORDRE et poids des chiens	2 DOSES DE C.K. injectées en une fois (centigr. par K°)	3 QUANTITÉ totale de KCl déterminant la mort (centigr. par K°)	4 DOSE totale de camphre injectée
21, ♂ 15,800	2 injections de 3 centigr. 1 — de 4 centigr.	10	0 gr. 50
22, ♂ 11,100	2 — de 3 centigr.	6	0 gr. 80
23, ♂ 9,500	2 — de 3 centigr. 1 — de 4 centigr.	10	0 gr. 90
24, ♂ 5,500	7 — de 3 centigr.	21	1 gr. 40
25, ♀ 9,000	1 — de 3 centigr.	3	0 gr. 20

1. Number and weights of the dogs
2. Amounts of KCl injected at one time (cg/kg)
3. Total quantity of KCl causing death (cg/kg)
4. Total amount of camphor injected

Conclusions

1. The salts of soda and of organic bases of camphosulfonic and camphocarbonic acid exert an antagonistic effect in regard to cardiac poisoning caused by potassium chloride in the guinea pig and the dog. This antagonistic effect changes neither the meaning nor the means of potassium poisoning; it simply diminishes the intensity of the cardiac phenomena and considerably retards ventricular fibrillation.

2. In the guinea pig, the amounts of 0.95 g or 1 g per kilogram by intramuscular injection caused respectively the death of 75 to 100% of the animals injected; the same doses in animals having received intramuscular injections of the salts of camphosulfonic and camphocarbonic acids caused death in only 25 to 60% of the animals experimented on.

3. In the dog, while doses of 2 cg/kg of KCl may be repeated as often as four to five times, amounts of 3, 3.5, and 4 cg of KCl per kilogram given all at once, kill the animal. In the dog having first received intravenous injections of the salts of camphocarbonic and camphosulfonic acid, intravenous injections of 2 cg/kg KCl can usually be repeated 13, 14 and even 15 times and intravenous injections of 3 cg/kg KCl, two three and even seven times.

4. This antagonistic effect of camphor or its derivatives in relation

to KCl does not exceed a certain limit; it is no longer evident when the animal having received camphor is injected all at once with very high doses of KCl (1.20 g/kg intramuscularly in the guinea pig; 3.5 to 4 cg/kg of KCl intravenously in the dog).

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Antagonisme du camphre et du chlorure de potassium.

Lorsqu'on suit les variations du rythme cardiaque sur le cœur *in situ* par la méthode de suspension, on constate que les injections intramusculaires d'huile camphrée et d'un certain nombre de dérivés solubles du camphre ne produisent aucun effet apparent sur le cœur normal de chien. Il n'en est plus de même lorsque les injections sont faites chez un animal intoxiqué préalablement avec un sel de potassium ionisé dont la toxicité a été signalée par Claude Bernard et Grandjean et étudiée en détail par Busquet et Pachon [1], après injection de fortes doses par voie intraveineuse. Nous nous sommes proposé d'appliquer cet antagonisme à l'étude comparée de quelques dérivés du camphre. Nous nous sommes alors heurtés à un certain nombre de difficultés, car dès le début de nos essais nous avons remarqué, d'une part, que de petites doses de potassium injectées par voie intraveineuse provoquent un effet dépressif trop peu durable pour que l'on puisse juger

1. Busquet et Pachon. *C. R. Ac. Sc.*, 1907, 144, p. 1063; *Journ. de Physiol. et de Pathol. générale*, 1909, 2, 242.

de l'action antagoniste du camphre et que, d'autre part, de fortes doses de potassium provoquent irrémédiablement la mort, quelle que soit la dose de camphre injectée ultérieurement.

Nous avons alors opéré différemment et nous avons injecté le chlorure de potassium à des chiens ayant préalablement reçu des doses variables d'huile camphrée ou de solutions aqueuses de quelques dérivés du camphre. Dans ces conditions, nous avons observé une diminution notable des effets dépressifs cardiaques lorsque nous injections de petites doses ou des doses moyennes de ClK, et un retard considérable de la trémulation ventriculaire lors de l'injection de doses massives de ClK.

De plus, chez les cobayes ayant reçu préalablement du camphre, nous avons observé un pourcentage de survie dépassant de beaucoup celui obtenu chez des animaux auxquels la même dose de ClK est injectée seule.

Ainsi le camphre semble exercer une action antagoniste vis-à-vis de l'intoxication cardiaque causée par le chlorure de potassium.

Avant de formuler les hypothèses que nous ont suggérées ces faits, examinons très brièvement, d'après les données de la littérature, quels sont, d'une part, les effets du camphre sur le muscle cardiaque et, d'autre part, le mécanisme de l'intoxication du cœur par le chlorure de potassium.

I. Action cardiaque du camphre. — L'action cardiaque est connue depuis de longues années en thérapeutique, mais malgré de nombreuses expériences effectuées avec ce médicament le mécanisme de son action n'est pas encore complètement élucidé.

Nous résumerons rapidement les essais effectués dans ce but, et nous distinguerons trois ordres de faits, à savoir : l'action du camphre, d'une part, sur le cœur isolé des homéothermes et des holéothermes; d'autre part, sur des cœurs soumis aux excitations électriques; enfin, sur des cœurs soumis à l'action de diverses substances.

1° *Action du camphre sur le cœur isolé des holéothermes et des homéothermes.* — On sait que, sur le cœur isolé de grenouille, le camphre, à des dilutions assez grandes et variant d'ailleurs dans des limites très étroites, peut améliorer l'amplitude et le rythme des mouvements du cœur [1]. Dès que ces concentrations sont dépassées, le camphre agit comme dépresseur cardiaque [2]. C'est sans doute par suite d'une action

1. Frölich et Pick. *Arch. exp. Pathol. Pharm.*, 1918, 84, p. 250; Joachimoglu *Arch. exp. Path. Pharm.*, 1917, 80, p. 259.

2. Alexander-Lewin. *Arch. exp. Path. Pharm.*, 1890, 27, p. 226; Boulisset. *Journ. de Phys. et de Path.*, 1926, 24, p. 235; Tanura, Kittara et Ishidate. *Proc. Imp. Ac.*, 1930, 6, p. 175. Ces derniers auteurs signalent une action cardiotonique tardive qui serait due au produit de transformation du camphre qu'ils ont isolé et dont les effets cardiotoniques sont immédiats. Hanovsky (*Arch. exp. Path. Pharm.*, 1923, 99, p. 115) avait signalé antérieurement des faits analogues sans isoler de substance.

analogue sur le cœur de chat que Seligmann [1] a obtenu des résultats apparemment contradictoires. Enfin Frölich et Pollak [2], puis Bouisset (*loc. cit.*), d'autre part, signalent des résultats analogues, les premiers sur le cœur de rat, le second sur des cœurs de chat et de lapin.

2° *Action du camphre sur le cœur soumis à des excitations électriques.* — Gottlieb [3] a montré que sur le cœur de chien *in situ* ou sur le cœur de chat *in situ* le camphre injecté par voie intraveineuse supprime les fibrillations qu'un courant de fréquence déterminée produit normalement chez cet animal. Le camphre supprime également les mouvements ondulatoires provoqués par le courant électrique sur le cœur de grenouille par Frölich et Grossmann [4], et par Boruttau [5]. Ce dernier auteur observe en outre que des effets de restauration cardiaque peuvent s'observer en perfusant un cœur de grenouille lésé par des excitations électriques avec un liquide riche en potassium et pauvre en calcium. Le potassium à faibles doses aurait donc, dans ces conditions, une action analogue à celle du camphre.

3° *Action du camphre sur le cœur soumis à l'action d'un certain nombre de médicaments.* — Boehm [6] et, après lui, Chiodo [7] ont montré qu'un cœur de grenouille lésé par perfusion d'hydrate de chloral reprend son amplitude et son rythme primitifs si on le perfuse avec des solutions de camphre de faibles concentrations. Frölich et Grossmann [8] ont montré sur la grenouille que le camphre supprime les effets cardiaques des digitaliques; Frölich et Pollack (*loc. cit.*), en expérimentant sur le cœur de rats intoxiqués par du chloroforme, du phosphore, de la strophanthine ou de l'yohimbine, ont mis en évidence l'action antidotique du camphre qui consisterait dans tous les cas en une diminution de l'excitabilité.

En résumé, le camphre en augmentant l'excitation hétérotrope semble protéger le cœur lésé non seulement par le courant électrique mais encore par un poison cardiaque quelconque.

II. Action cardiaque de l'ion potassium. — Si de faibles doses de sels ionisables de potassium ajoutées au liquide de perfusion sont capables de ranimer un cœur isolé de grenouille lésé par des excitations faradiques, par contre de fortes doses de potassium produisent sur le cœur isolé des holéothermes et des homéothermes un arrêt définitif.

Busquet et Pachon [9] ont montré que l'arrêt diastolique du cœur du

1. Seligmann. *Arch. exp. Path. Pharm.*, 1905, 62, p. 333.
2. Frölich et Pollak. *Arch. exp. Path. Pharm.*, 1920, 86, p. 104.
3. Gottlieb. *Zeits. f. exp. Path.*, 1906, 2, p. 784.
4. Frölich et Grossmann. *Arch. exp. Path. Pharm.*, 1921, 89, p. 1.
5. Boruttau. *Zeits. f. exp. Path.*, 1919, 20, p. 44.
6. Boehm. *Arch. exp. Path. Pharm.*, 1905, 52, p. 348.
7. Chiodo. *Arch. int. Physiol.*, 1928, 20, p. 223.
8. Frölich et Grossmann. *Arch. exp. Path. Pharm.*, 1918, 82, p. 177.
9. Busquet et Pachon. *C. R. Soc. Biol.*, 1907, 72, p. 785.

au potassium se différencie nettement de l'arrêt du cœur produit par excitation du pneumogastrique. De leurs expériences, ils concluent que « le potassium produit l'arrêt du cœur en paralysant directement la fibre musculaire cardiaque impuissante dès lors d'elle-même à se contracter ».

III. Antagonisme du camphre et du potassium. — Nous venons de voir que le potassium agit sur le cœur en intoxiquant la fibre musculaire cardiaque, et que d'une façon générale le camphre exerce vis-à-vis de certains poisons une action antagoniste sans que le mécanisme de cette action soit élucidé. Nous avons pensé que, pour ce qui concerne l'antagonisme camphre-potassium, le camphre exerce peut-être une action protectrice de la fibre cardiaque, soit parce qu'elle empêche la fixation ultérieure de potassium, soit parce qu'elle rend plus lâche cette fixation et permet l'élimination plus facile de l'ion potassium.

Voulant préciser cette hypothèse, nous avons effectué des dosages de potassium dans le plasma et dans le cœur de chiens ayant ou non reçu du camphre et intoxiqués par la même quantité de ClK, mais les chiffres obtenus n'ont pas été suffisamment concordants pour que nous puissions en faire état.

Nous nous bornerons donc à signaler ce nouvel antagonisme du camphre et du potassium sans qu'il nous soit possible d'en indiquer le véritable mécanisme.

***

Dans l'exposé expérimental qui suit, nous avons rassemblé les expériences effectuées sur le cobaye et sur le chien.

Nous avons, dans tous les cas, déterminé préalablement les doses toxiques de ClK sur des animaux témoins puis examiné l'action des mêmes doses sur des animaux ayant préalablement reçu du camphre.

I. Expériences chez le cobaye. — Nous avons effectué sur cet animal deux séries d'expériences: 1° nous avons fixé la dose de ClK capable de déterminer la mort de 70 à 80 °/₀ des animaux en une heure environ; 2° nous avons injecté des doses sensiblement égales ou supérieures de ClK à des cobayes ayant reçu préalablement par voie hypodermique des dérivés solubles du camphre; nous avons pu constater que ces animaux résistaient, pour la plupart, à l'intoxication provoquée par des doses de ClK généralement mortelles pour des animaux normaux. Les diverses expériences que nous avons réalisées sont développées ci-dessous.

1° *Détermination de la dose toxique de ClK chez le cobaye.* — Nous avons, dans nos expériences, utilisé une solution de ClK à 16 °/₀ et nous avons pratiqué chez le cobaye une injection intramusculaire. Les signes de l'intoxication due au potassium apparaissent le plus souvent assez tardivement, dix à quinze minutes avant la mort; la respiration est alors haletante, le cœur devient arythmique, l'animal se couche sur le flanc avec une paralysie du train antérieur. De temps en temps, apparaissent

des mouvements violents et incoordonnés. Enfin, après quelques respirations profondes, suivies d'une série de respirations dont l'amplitude diminue progressivement, survient la mort.

Les résultats expérimentaux qui sont résumés dans le tableau I nous ont montré que la dose de 0 gr. 95 de ClK par kilogramme d'animal provoque la mort d'environ 78 °/₀ des cobayes mis en expérience, dans un temps voisin de soixante minutes.

Tableau I.

Nos d'ordre	Poids des animaux (gr.)	Temps qui s'écoule entre l'injection et la mort (minutes)
1	520	52
2	300	64
3	380	53
4	760	48
5	310	45
6	620	59
7	700	Survie.
8	660	86
9	700	Survie.

2° *Action antagoniste des dérivés solubles du camphre vis-à-vis de l'intoxication provoquée par ClK.* — *a*) Dans une première série d'expériences nous avons injecté aux animaux une dose globale d'un dérivé soluble du camphre, camphosulfonate de soude ou camphocarbonate de diéthylamine, correspondant à 0 gr. 10 de camphre [1], puis une dose de 0 gr. 95 de ClK par kilogramme d'animal; nous avons constaté que, dans ces conditions, 75 °/₀ des animaux ont survécu. On trouvera ces résultats consignés dans le tableau II.

Tableau II.

Nos d'ordre	Poids des animaux (gr.)	Temps qui s'écoule entre l'injection et la mort (minutes)
1	750	Survie.
2	450	Survie.
3	520	Survie.
4	560	Mort en 65
5	550	Survie.
6	560	Mort en 65
7	500	Survie.
8	500	Survie.

1. Nous sommes heureux de remercier ici les firmes Daubsz et Robert et Carrière qui ont bien voulu nous préparer l'une du camphosulfonate de soude, l'autre du camphocarbonate de diéthylamine titré à 10 °/₀ en camphre. Nous avons d'ailleurs obtenu des résultats analogues avec des solutions de camphocarbonate de soude.

b) Dans une deuxième série d'expériences, nous avons injecté au cobaye la même dose de camphre, soit 0 gr. 10, puis 1 gr. de ClK par kilogramme; dans ces conditions les animaux témoins et les animaux, ayant reçu préalablement un des dérivés solubles du camphre, sont tous morts dans un laps de temps variant de cinquante à soixante-dix minutes.

c) Enfin, dans une troisième série d'expériences, nous avons injecté préalablement au cobaye une dose double de camphre (0 gr. 20), puis 1 gr. de ClK par kilogramme. Dans ces conditions, les animaux ont survécu dans la proportion de 2 sur 3. Au contraire, les animaux n'ayant reçu que le ClK, à raison de 1 gr. par kilogramme, sont tous morts dans un délai variant de soixante à soixante-cinq minutes.

II. Expériences chez le chien. — Nous avons effectué sur cet animal deux séries d'expériences. Les premières ont eu pour but de fixer la dose toxique de ClK chez le chien, soit qu'on injecte de petites doses répétées par intervalles de deux à cinq minutes, soit qu'on injecte d'emblée de fortes doses.

Dans une seconde série d'expériences nous avons injecté des doses analogues à des animaux ayant reçu préalablement des dérivés solubles du camphre par voie intraveineuse. Nous avons constaté que le chien résiste dans ces conditions à l'intoxication due au ClK et que, tout au moins, quand le chlorure de potassium injecté en une fois ne dépasse pas la dose minima mortelle (3 centigr. par kilogramme), il faut, pour obtenir la mort, utiliser des quantités totales de ClK très supérieures à la dose toxique préalablement déterminée. Par contre, si on injecte d'emblée la dose de 3 centigr. 5 ou de 4 centigr. par kilogramme, l'animal meurt même s'il a reçu antérieurement des quantités considérables de camphre.

1° *Détermination de la dose toxique de ClK chez le chien.* — a) *Action des petites doses répétées de ClK* : Nous avons étudié préalablement chez le chien l'action toxique de ClK injecté par petites doses répétées par voie intraveineuse et suivi la marche de l'intoxication cardiaque en enregistrant les mouvements du ventricule et de l'oreillette du chien chloralosé dont on a ouvert le thorax et chez lequel on pratique la respiration artificielle. Nous avons utilisé dans ces essais une solution de ClK à 4 °/₀.

Les premières injections de 0 centigr. 5 ou de 1 centigr. de ClK par kilogramme n'ont qu'une action assez faible sur l'amplitude et la fréquence du rythme cardiaque. Cependant lorsque l'animal a déjà reçu 7 à 9 centigr. de ClK, chaque nouvelle injection provoque une diminution de l'amplitude, diminution surtout marquée pour l'oreillette et un ralentissement notable du rythme cardiaque. Peu à peu ces phénomènes s'exagèrent et la mort survient quelques secondes après la dernière injection par arrêt diastolique du cœur suivi de fibrillation.

Comme on le voit, dans les expériences 1, 2, 3, 4, 5 résumées dans le tableau III, dans ces conditions la mort peut survenir lorsque l'animal a reçu entre 6 et 26 centigr. 5 de ClK par kilogramme (les injections de 0 centigr. 5 et de 1 centigr. de ClK par kilogramme étant effectuées de deux à cinq minutes d'intervalle).

Dans une première colonne, nous avons indiqué le poids des animaux mis en expérience, dans une deuxième colonne le nombre d'injections de ClK, dans une troisième colonne la quantité totale de ClK (centigr. par kilogramme reçu par l'animal).

Tableau III.

N°s d'ordre	Poids des chiens (K°s)	Dose de ClK injectée en une fois (centigr. par K°)	Quantité totale de ClK déterminant la mort (centigr. par K°)
1 . . .	♂ 8,300	6 injections de 1 centigr. »	6
2 . . .	♂ 16,500	3 — de 1 centigr. » 4 — de 2 centigr. »	11
3 . . .	♂ 20 »	6 — de 0 centigr. 5 5 — de 1 centigr. » 2 — de 2 centigr. »	12
4 . . .	♂ 5,150	9 — de 1 centigr. » 8 — de 2 centigr. »	25
5 . . .	♀ 7 »	9 — de 0 centigr. 50 20 — de 1 centigr. » 2 — de 2 centigr. »	26,5

D'autre part, si l'on injecte d'emblée des doses de 2 centigr. de ClK par kilogramme, quel que soit l'état antérieur du cœur, on observe toujours, en même temps qu'une diminution importante de l'amplitude auriculaire et ventriculaire, une diminution plus marquée du côté auriculaire, un ralentissement du rythme avec, dans certains cas, un arrêt diastolique. Le cœur qui est très dilaté déborde le péricarde sur lequel il repose. Dans certains cas (57,30 °/₀ des expériences) la mort survient dès la première injection de 2 centigr. de ClK par kilogramme, par fibrillation auriculaire ventriculaire. Mais lorsque l'animal résiste à cette première injection, le rythme cardiaque reprend sa vitesse, l'amplitude cardiaque s'amplifie et dépasse même rapidement l'amplitude initiale; les injections suivantes produisent le plus souvent les mêmes phénomènes mais atténués. Après cette période, les phénomènes s'exagèrent et la mort survient, le plus souvent lorsque l'animal a reçu de 2 à 10 centigr. de ClK par kilogramme (exp. 6, 7, 8, 9, 10, 11); exceptionnellement des animaux plus résistants (exp. 12 et 13) peuvent recevoir 18 à 20 centigr. de ClK par kilogramme par doses de 2 centigr. par kilogramme.

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Nous avons réuni dans le tableau IV les expériences effectuées sur 8 chiens.

TABLEAU IV.

N° D'ORDRE	POIDS des chiens (K°)	DOSE DE ClK injectée en une fois (centigr. par K°)	QUANTITÉ de ClK déterminant la mort (centigr. par K°)
—	—	—	—
6. . .	♂ 18,900	1 injection de 2 centigr.	2
7. . .	♂ 13,900	1 — de 2 centigr.	2
8. . .	♀ 17,000	1 — de 2 centigr.	2
9. . .	♀ 7,300	1 — de 2 centigr.	8
10. . .	♀ 7,200	2 — de 2 centigr. 1 — de 4 centigr.	8
11. . .	♂ 10,000	3 — de 2 centigr. 1 — de 4 centigr.	10
12 . .	♀ 12,000	9 — de 2 centigr.	18
13. . .	♂ 9,200	10 — de 2 centigr.	20

b) *Action des fortes doses de ClK injectées en une fois.* — Les doses de 3 centigr., de 3 centigr. 5 et de 4 centigr. par kilogramme d'animal se sont toujours montrées mortelles (fig. 1) chez le chien, lorsqu'elles étaient injectées d'emblée, et ont provoqué la mort de 100/100 des animaux injectés (10 exp.).

2° *Action antagoniste des dérivés solubles du camphre vis-à-vis de l'intoxication provoquée par ClK.* — Nos expériences ont été effectuées de la façon suivante. Nous avons injecté par voie intraveineuse des doses répétées des dérivés solubles du camphre à raison de 0 gr. 10, 0 gr. 20, 0 gr. 30 et 0 gr. 60 de camphre en une fois. Le camphre ainsi injecté n'agit pas sur le cœur normal du chien et nous n'avons observé aucune modification appréciable du rythme cardiaque et de son amplitude. Après les injections de camphre, nous avons pratiqué des injections de ClK. Chaque injection de ClK agit encore sur le rythme et l'amplitude cardiaque en ralentissant celui-là et en diminuant celle-ci, mais ces deux actions sont nettement moins importantes chez l'animal traité préalablement par le camphre que chez l'animal normal.

On peut comparer (fig. 2 et 3) l'effet sur l'oreillette et le ventricule d'une dose de 2 centigr. de ClK par kilogramme chez deux chiens dont l'un est normal et dont l'autre (fig. 3) a reçu préalablement une dose de 0 gr. 6 de camphre. Chez le chien normal, 2 centigr. par kilogramme de ClK provoquent un arrêt cardiaque qui se prolonge sept à huit secondes. Au contraire chez le chien ayant reçu préalablement 0 gr. 60 de camphre les mouvements auriculaires et ventriculaires sont extrêmement ralentis, mais non complètement inhibés.

a) *Action des doses moyennes répétées de ClK chez les chiens ayant reçu préalablement du camphre.* — Nous avons toujours injecté d'emblée, dans cette série d'expériences, à des chiens ayant reçu du camphre des

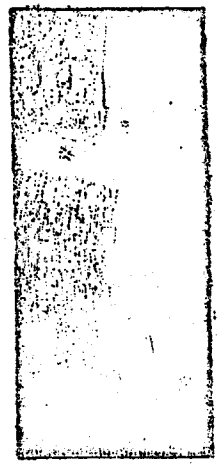

FIG. 1.

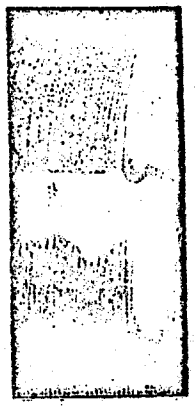

FIG. 2.

FIG. 1. — Action de ClK sur le cœur *in situ*.
Chien ♂ 10 K°. De haut en bas, tracés auriculaire et ventriculaire, temps en secondes.
En X, injection intraveineuse de 4 centigr. de ClK par kilogramme. Arrêt cardiaque diastolique définitif.

FIG. 2. — Chien ♀ 12 K°. De haut en bas, tracés auriculaire et ventriculaire, temps en secondes.
En X, injection intraveineuse de 2 centigr. de ClK par kilogramme. Arrêt cardiaque diastolique pendant cinq à huit secondes.

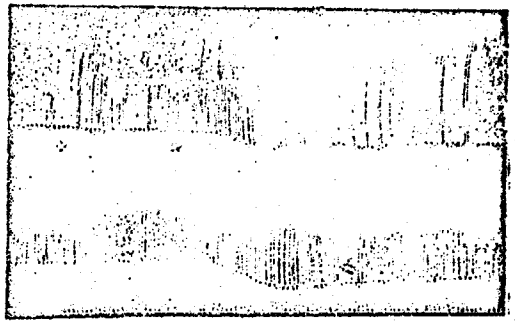

FIG. 3. — Chien ♀ 20 K°. De haut en bas, tracés auriculaire et ventriculaire, temps en secondes.
En X, injection intraveineuse de 0 gr. 6 de camphre et de 2 centigr. de ClK par kilogramme. Ralentissement du rythme cardiaque, diminution de l'amplitude auriculaire et ventriculaire. Tendance à l'arrêt diastolique.

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doses de ClK au moins égales à 2 centigr. par kilogramme d'animal. Si nous exceptons deux expériences (19 et 20) dans lesquelles les animaux sont morts après avoir reçu 2 à 4 centigr. par kilogramme de ClK, tous les autres, c'est-à-dire 5 sur 7, ont résisté à de fortes doses de ClK, 19 à 31 centigr. par kilogramme (exp. 14, 15, 16, 17, 18). Bien entendu il a été nécessaire de pratiquer entre les injections de ClK des injections de 0 gr. 10, 0 gr. 20, 0 gr. 30 de camphre, de telle sorte que la dose de camphre totale injectée a varié de 2 à 6 gr. Nous avons vu ci-dessus que dans les mêmes conditions les animaux n'ayant pas reçu de camphre succombent dans la proportion de 6 sur 8 lorsque les doses de ClK injectées varient de 2 à 10 centigr.

Nous donnons dans le tableau V le résumé des expériences que nous avons effectuées. Nous avons ajouté dans ce tableau aux colonnes indiquant le poids du chien le nombre des injections de ClK et la quantité totale de ClK injectée, une quatrième colonne indiquant les doses de camphre reçues par les animaux mis en expérience.

TABLEAU V.

N°s D'ORDRE et poids des chiens K°s	DOSES DE ClK injectées en une fois (centigr. par K°)	QUANTITÉ totale de ClK déterminant la mort (centigr. par K°)	DOSE totale de camphre injectée
14, ♀ 13,600	8 injections de 2 centigr. 1 — de 3 centigr.	19	2 gr. 2
15, ♂ 5 »	7 — de 2 centigr. 2 — de 3 centigr. 1 — de 4 centigr.	25	1 gr. 7
16, ♀ 9,300	14 — de 2 centigr.	28	»
17, ♂ 10,000	13 — de 2 centigr. 3 — de 1 centigr.	28	6 gr. 5
18, ♂ 5,500	15 — de 2 centigr.	30	1 gr.
19, ♂ 17,500	2 — de 2 centigr.	4	0 gr. 9
20, ♂ 8,300	1 — de 2 centigr.	2	0 gr. 9

b) *Action des doses mortelles de ClK injectées chez des chiens ayant préalablement reçu du camphre.* — Nous distinguerons deux séries d'expériences, celles dans lesquelles la dose de ClK injectée est de 3 centigr. par kilogramme, et celles dans lesquelles cette dose est de 3,5 ou 4 centigr. par kilogramme. Dans ce dernier cas et quelle que soit la dose de camphre administrée préalablement à l'animal, les doses de 3 centigr. 5 et de 4 centigr. par kilogramme se sont montrées mortelles dans 100 °/₀ des cas. Au contraire, lorsqu'on a administré à des chiens ayant reçu préalablement du camphre une dose de 3 centigr. par kilogramme de ClK, on observe la mort dans 20 °/₀ des cas. Le plus généralement l'animal résiste à cette dose de ClK et peut même résister à des doses variant de 6 centigr. à 20 centigr. par kilogramme. Ces diverses expériences sont résumées dans le tableau VI.

TABLEAU VI.

N°s D'ORDRE et poids des chiens	DOSES DE ClK injectées en une fois (centigr. par K°)	QUANTITÉ totale de ClK déterminant la mort (centigr. par K°)	DOSE totale de camphre injectée
21, ♂ 15,800	2 injections de 3 centigr. 1 — de 4 centigr.	10	0 gr. 50
22, ♂ 11,400	2 — de 3 centigr.	6	0 gr. 80
23, ♂ 9,350	2 — de 3 centigr. 1 — de 4 centigr.	10	0 gr. 90
24, ♂ 5,500	7 — de 3 centigr.	21	1 gr. 40
25, ♀ 9,600	1 — de 3 centigr.	3	0 gr. 20

CONCLUSIONS. — 1° Les sels de soude et de base organique des acides camphosulfonique et camphocarbonique exercent une action antagoniste vis-à-vis de l'intoxication cardiaque provoquée par le chlorure de potassium chez le cobaye et le chien. Cette action antagoniste ne change ni le sens ni les modalités de l'intoxication potássique; elle diminue simplement l'intensité des phénomènes cardiaques et retarde considérablement la fibrillation ventriculaire.

2° Chez le cobaye les doses de 0 gr. 05, de 1 gr. de ClK par kilogramme provoquent respectivement par injection intramusculaire la mort de 75 à 100 °/₀ des animaux injectés; les mêmes doses chez des animaux ayant reçu des injections intramusculaires de sels des acides camphosulfonique et camphocarbonique ne provoquent plus la mort que de 25 à 60 °/₀ des animaux expérimentés.

3° Chez le chien, tandis que les doses de 2 centigr. de ClK par kilogramme peuvent être répétées le plus souvent quatre à cinq fois, celles de 3 centigr., 3 centigr. 5 et de 4 centigr. de ClK par kilogramme tuent d'emblée l'animal. Sur le chien ayant reçu préalablement des injections intraveineuses de sels d'acide camphocarbonique et camphosulfonique, on peut le plus souvent répéter les injections intraveineuses de 2 centigr. de ClK par kilogramme, treize, quatorze et même quinze fois, et les injections intraveineuses de 3 centigr. de ClK par kilogramme deux, trois et même sept fois.

4° Cette action antagoniste du camphre ou de ses dérivés vis-à-vis de ClK ne dépasse pas une certaine limite; elle ne se manifeste plus lorsqu'on injecte d'emblée aux animaux qui ont reçu du camphre des doses trop élevées de ClK (1 gr. 20 par kilogramme par voie intramusculaire chez le cobaye; 3 centigr. 5 à 4 centigr. par kilogramme de ClK par voie intraveineuse chez le chien).

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MINERAL METABOLISM OF THE FOETUS AND NEW-BORN

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1 Development and composition
2 Mineral metabolism of the foetus
3 Mineral metabolism of the new-born animal
References

1. Development and Composition

For the whole of its life, before birth and afterwards, the animal body has within it a variety of different inorganic elements, most of which are vital to its existence. Some ten years ago the mineral metabolism of the foetus and new-born was discussed from the incremental point of view (Widdowson, 1950; McCance & Widdowson, 1951; Widdowson & Spray, 1951). This still remains the fundamental approach, and there is no need to modify anything that was written then, but since that time further analyses have been made, and our knowledge at present is set out from rather a new angle in Table I. The great general principle brought out by a study of this table is that development both before and after birth is not just a matter of an increase in size. It also involves a change in composition. Thus, if a foetus weighing 175 g. were to increase 20 times in size it would weigh 3.5 kg., but it would not have the composition of a full-term baby; and were the full-term baby to multiply itself 20 times it would weigh as much as a 70-kg. man, but it would not have the same composition. Owing to the steady fall throughout development in the amount of water and extracellular fluids in the body, mere increase in size both before and after birth would give a product containing too much water and too many chloride ions. Against this, normal development involves the deposition of more nitrogen, potassium and magnesium than would be called for by increase in size alone, and this is because of the increase in cell substance in all parts of the body. The outstanding example of this relationship is the great increase in the deposition of calcium, and to a lesser extent of phosphorus, over and above the amounts called for by size alone. This is due to the maturation and with it the calcification of the bones—tissues which hardly exist in early foetal life, and can have little or no function until after birth. As the bones become calcified, magnesium and sodium are also incorporated into their structure and this explains why the amount of sodium per kg. does not fall so much as the amount of chloride, and why the magnesium rises more than the potassium.

Owing to the increase of structural cellular material throughout foetal life at the expense of watery extracellular fluid, the composition of foetal tissues may differ materially from that of adults. This is well illustrated by Table II (Dickerson & Widdowson, 1960), which shows the composition of skeletal muscle in immature foetuses, new-born infants and adults, of two different species. In each of them the solids—potassium, magnesium and phosphorus—in the muscles of the foetus were well below those in the muscles of the adult, and for the reasons just given. The high concentrations of calcium, however, were probably intracellular and could not be explained in the same way. They were attributed to the high proportion of nuclei in the foetal muscle cell and the high concentrations of calcium therein. Other examples of the effect of growth and development on structure have been given by Dickerson (1960) and Widdowson & Dickerson (1960).

2. Mineral Metabolism of the Foetus

Ion transport, sodium pumps and potassium–hydrogen exchange mechanisms have so dominated physiological thinking in the last few years that it is difficult to carry one's mind back to the days when so much was regulated, or thought to be regulated, by diffusion. It goes without saying that the

TABLE I. Changes Taking Place in Development, Set Off Against Those Due to a Simple Increase in Size

	Water (kg.)	Total N (g.)	Na (m-equiv.)	K (m-equiv.)	Cl (m-equiv.)	Ca (g.)	Mg (g.)	P (g.)	Fe (mg.)	Cu (mg.)	Zn (mg.)
A foetus weighing 175 g. contains	0.154	2.45	18.2	7.4	13.7	0.60	0.024	0.42	9.25	0.575	3.50
This foetus × 20 would contain	3.08	49	364	148	274	12.0	0.48	8.4	185	11.5	70
A baby weighing 3.5 kg. contains	2.4	66	243	150	160	28.2	0.76	16.2	262	13.7	53
This baby × 20 would contain	48	1,320	4,860	3,000	3,200	564	15.2	324	5,240	274	1,060
A man weighing 70 kg. contains	42	2,000	5,150	4,050	2,940	1,320	27.4	740	4,350	100	1,640

TABLE II. Effect of Development on the Composition of Skeletal Muscle of Man and the Pig

(Values expressed per kg. fresh muscle)

	Water (g.)	Total N (g.)	Na (m-equiv.)	K (m-equiv.)	Cl (m-equiv.)	Ca (m-equiv.)	Mg (m-equiv.)	P (m-mole)
Man:								
Foetus, 20 weeks	887	14.8	90.6	57.6	65.6	7.1	10.5	40.0
New-born	804	20.7	60.1	57.7	42.6	4.3	14.8	47.0
Adult	790	30.8	36.3	92.2	22.1	2.6	16.7	58.8
Pig:								
Foetus, 46 days	916	8.5	106	46.7	72.7	7.3	9.0	30.7
New-born	823	15.4	54.3	72.5	36.8	2.9	15.3	56.8
Adult	735	31.1	24.2	92.5	21.1	2.3	23.2	68.0

principles of active transport which control the mineral composition of an adult's cells are likely to apply also to those of the foetus. The sources of energy, however, may not necessarily be the same. It has recently been shown, for instance (Whittam, 1960; Capek, 1961), that the foetal kidney can utilize the energy derived from anaerobic metabolism to maintain the mineral integrity of its cells, whereas that of the adult cannot. This is entirely in keeping with the accepted ability of foetal and new-born animals to survive much longer periods of oxygen deficiency than their parents.

Many investigators have compared the concentrations of sodium, potassium, calcium, and magnesium in foetal and maternal plasma, and these are highly suggestive both as regards ion transport and the mineral metabolism of the foetus. There is general agreement (see Smith, 1959) that foetal plasma usually contains more calcium and magnesium and certainly more phosphorus than maternal plasma. This has been demonstrated in pigs, cows and rabbits as well as in man; and a further, and highly significant, fact has been added, namely that in cattle (Sato, 1933) and in man (Hallman & Salmi, 1953; Salmi, 1954) the concentration of calcium in the umbilical vein exceeds that in the umbilical artery. It may be inferred that some of this calcium reaches the umbilical vein by active transport through the placenta, because the concentrations of calcium in the maternal sera have been found to be below those in the umbilical vessels, and the placenta, if not over-mature and becoming pathologically calcified, contains negligible amounts (Widdowson & Spray, 1951).

The concentrations of potassium have also been found to be higher in foetal than in maternal plasma (Widdowson & McCance, 1956; Westin, Kaiser, Lind, Nyberg & Teger-Nilsson, 1959), and reasoning similar to the above suggests that the maintenance of these concentrations must be the result of active transport. By the time blood has been withdrawn, however, the foetus is generally already suffering from an oxygen deficiency, and it has been suggested (James, personal communication, 1958) that the high serum potassium concentrations found in the foetus, and sometimes in the premature and full-term baby, have been due to anoxaemia, since dangerously high concentrations of potassium have been found in the sera of hypoxic human infants suffering from respiratory distress. This criticism cannot be made of the high concentrations of potassium found in the sera of normal new-born pigs. Salmi (1954), moreover, was alive to this danger in all her work on calcium and showed that, although experimental hypoxia did raise the concentration of calcium in the serum, her conclusions were not upset by it. She showed furthermore that the concentrations of magnesium, frequently found to be higher in foetal than in maternal serum, were not raised by low oxygen tensions.

The work carried out with radioactive isotopes gives results which are in agreement with the above general hypothesis (Shirley, Jeter, Feaster, McCall, Outler & Davis, 1954; Feaster, Hansard, Outler & Davis, 1956). Thyroxine is not transferred across the placenta, but the concentration of inorganic iodine may be five times higher in the foetal than it is in the maternal plasma towards the end of pregnancy in rabbits (Logothetopoulos & Scott, 1955; Hall & Myant, 1956; Friis & Hall, 1957). Fuchs & Fuchs (1957a, 1957b) showed that injected phosphorus passed much more rapidly into the foetus against a concentration gradient than it did in the reverse direction, and Wilde, Cowie & Flexner (1946) had previously concluded that all the phosphorus reaching the foetus was trapped there by the growth of soft tissues and bone. There is also evidence from other directions (see section 3) that the supply of phosphorus may govern the rate of growth both of the foetus and the newly born. As regards water (Plentl, 1958) and most other elements, this is certainly not so, for Wasserman, Comar, Nold & Lengemann (1957) found that nearly twice as much calcium was transferred to the foetus than the foetus required for growth. All the iron actively transferred to the foetal rabbit has also been shown to remain there, but in this case the supply is greater than the immediate demands of growth and most of the iron is deposited in an inorganic state (Bothwell, Pribilla, Mebust & Finch, 1958).

The mineral structure of foetal membranes and fluids has been examined by Alexander, Nixon, Widdas & Wohlzogen (1958), Davies (1958), Economou-Mavrou & McCance (1958), McCance & Stanier (1960), McCance & Widdowson (1960) and Stanier (1960). There would appear to be a rapid build-up of calcium in the pig's chorio-allantoic membrane between the 20th and 46th day of gestation, with subsequent decline to much lower concentrations as the rate at which calcium is deposited in the foetal bones goes up. In the light of the evidence just quoted it is reasonable to suppose that this calcium does not reach the inside of the membrane solely by diffusion, but at present there is no proof of this.

All are in agreement that the allantoic fluid contains less sodium and chloride than the maternal plasma, and is frequently hypotonic in consequence. This is particularly so in the pig, and McCance & Widdowson (1960) added further facts about this fluid, namely, that by the 46th day of gestation (gestation period, 120 days) it has a pH of about 6, largely

owing to carbonic acid, and that it contains ammonia. The allantoic fluid is often regarded as the product of the foetal kidney, but in the sheep it frequently contains less sodium and chloride than urine collected soon afterwards (Alexander *et al.* 1958), and at the 46th day of gestation the mesonephros of the foetal pig seems to produce a fluid containing as much chloride as the plasma (Stanier, 1960). Crawford & McCance (1960), however, have found an active sodium transport mechanism in the pig's chorio-allantoic membrane, which transfers sodium actively from the fluid to the endometrial surface. The process is self-regulated in that it is inhibited by a fall in pH, particularly if this is caused by carbonic acid, and by the presence of some substance not yet identified in the allantoic fluid itself. If some such system as this operates in the placentas of other species it may help us to unravel some of the knotty problems of mineral metabolism before birth.

3. Mineral Metabolism of the New-Born Animal

The human baby, unlike the new-born of other species, is not provided with a free supply of milk for the first 48 hours of its life. This makes it rather a special case for this period, but thereafter the principles underlying its mineral metabolism closely resemble those which control the metabolism of other species.

All new-born animals have a great capacity for growth, and this dominates the whole of their metabolism. Growth and the composition of the diet are in large measure responsible for the maintenance of a normal internal environment, for the kidney of some new-born animals is so immature that it can keep the proportions of water, sodium, and potassium within normal limits only if growth is unrestricted and its requirements are being met by a perfect diet.

Smith, Yudkin, Young, Minkowski & Cushman (1949), Hansen & Smith (1953) and McCance & Widdowson (1954, 1955) have studied the metabolism of premature and full-term human infants during the first days of life, and McCance & Widdowson (1956) have supplemented this by an experimental study of fasting new-born pigs. Some loss of weight is inevitable at this time, but it can be reduced by the administration of water, and still more by water and glucose. The loss of weight is due to the expenditure of glycogen, fat and tissue protein to maintain life, and the products of metabolism are either excreted or retained. The ratio of nitrogen to potassium in the urine is, however, only half that of adults subjected to comparable degrees of food and water deficiencies. The breakdown of tissue protein is exceedingly small at this time of life (McCance & Strangeways, 1954), for the balance between anabolism and catabolism is weighted more strongly in favour of the former than it is in adults. More potassium, however, is excreted during starvation than is called for by the breakdown of tissue protein during the first two days of life, and much more potassium is retained as soon as the infant begins to receive adequate amounts of breast milk. The movements of potassium may reflect movements of glycogen. The excretion of sodium and chloride by the normal infant is not large in view of the amount of extracellular fluid in the body, but it may be considerable if the infant is oedematous and becoming less so. The volume of urine depends upon whether additional water is being administered or not, and is regulated, if no water is being given, by the amount of osmolar material being eliminated by the kidney. The latter is not affected by additional water (Hansen & Smith, 1953).

It has already been suggested that the rate of growth of the foetus may depend upon the amount of phosphorus reaching it, and the human infant normally excretes practically no phosphorus during the first 24 hours of its life. In spite of being given no food, it begins to excrete phosphates during the next 24 hours in response to a rise in the concentration of inorganic phosphate in the serum. This is due to the breakdown of glycogen and tissue protein, necessitated by the absence of food, for by the 7th day, when the intake of phosphorus from breast milk has risen to about 25 mg./kg./24 hr., the serum phosphorus has fallen to its level at birth, and phosphates have disappeared from the urine. All aspects of this syndrome can be reproduced in new-born pigs (Widdowson & McCance, 1959).

Many instances might be given of the dominating influence of food and growth on the electrolyte stability of the new-born animal (McCance, 1959). It has been shown, for instance, that the new-born puppy cannot tolerate the amount of water in a normal supply of milk unless it is accompanied by the milk solids. No cell growth can take place, the kidney is too immature to excrete the water, which is in consequence retained, and the concentrations of electrolytes in the serum and cells are forced down to pathological levels (McCance & Widdowson, 1958a). The converse of this has been demonstrated in infants and in piglets by adding salt to the milk.

Owing to the very large intake of fluid by the new-born animal, quite a small percentage of salt in the milk can lead to the ingestion of more salt per unit of body-weight than an adult would normally take. Given the corresponding amount of water, however, an adult would excrete both the water and the salt without a breakdown in the stability of the body fluids. Not so the human infant. Figure 1 shows the effect of making up the milk for three premature babies so that it contained 0.7% of sodium chloride. In each one the normal gain in weight from day to day altered to a more rapid one, which was in fact pathological. In a short time the babies became oedematous and sick, but on putting them back on to their proper intake of water and salt their weights fell and they began to gain weight again at their previous and more satisfactory rate (McCance & Widdowson, 1957). Similar

FIG. 1. Weight Charts of Three Infants Before, During and After the Period of Salt Administration

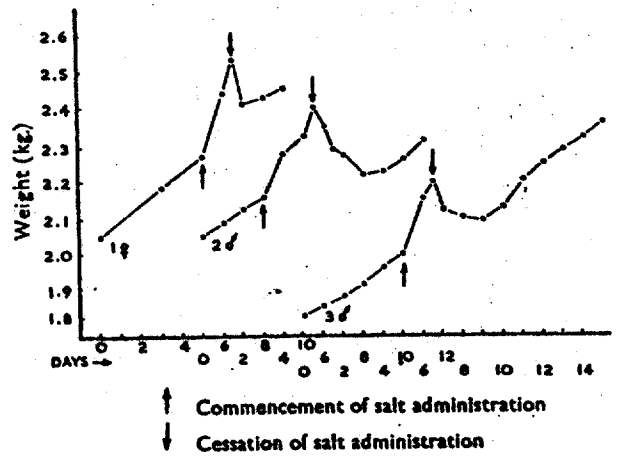

TABLE III. Effects of Giving New-Born Piglets Water or an Equal Volume of Sodium Chloride Solution (0.9 g.% (w/v))

	Water alone	Water+salt
Change of weight in 40 hr. (g./kg. birth-weight)	−53	+96
Percentage of water in bodies	82.7	87.4
Na balance (m-equiv./kg./24 hr.)	−0.3	+35.5
Serum Na (m-equiv./l.)	138	147

results have been obtained by Martínek, Stanichová & Janovský (1961).

Table III shows exactly the same effect in piglets to which no food was given, but only water or salted water. The gain of weight in piglets given the solution of salt without food was quite clearly pathological. Table III, however, shows other features. Firstly, although the amount of water retained in the body was large, it was not large enough to prevent a rise in the concentration of sodium in the serum. This also happened in the babies, and in both species the administration of the salt led to hypertonic expansion of the extracellular fluids.

Our knowledge about mineral metabolism in the suckling period has largely been derived from metabolism experiments but, owing to the technical difficulties involved, very few have been carried out on animals, and most of the information we possess has been derived from studies of the relatively slow-growing human infant, conducted, moreover, after the strictly new-born period. The initial stages of extra-uterine growth remained largely neglected until Widdowson, Slater, Harrison & Sutton (1960) and Slater (1961) set themselves the task of filling in this gap, and in particular of comparing the retentions and mineral balances of infants on breast milk with those on a preparation of cow's milk. These authors studied the infants during the 6th, 7th and 8th days of their lives, and some of their results are shown in Table IV. As might have been expected from a knowledge of the composition of breast milk and cow's milk, the babies on cow's milk were being given considerably more potassium, calcium, strontium, magnesium and phosphorus than the others were getting from their breast milk, and the table shows that they were absorbing and retaining considerably more calcium, strontium, magnesium and phosphorus and nearly twice as much potassium. This was not unexpected, and raises the problem previously voiced by others (Beem and Smith, see McCance & Widdowson, 1958b) as to the fate of the additional potassium retained, for babies fed on these and similar preparations of cow's milk do not retain nitrogen in proportion to potassium, and grow no faster than breast-fed infants.

TABLE IV. Mineral Balances of Infants 6–8 Days Old on Breast Milk and Cow's Milk

(Values expressed per kg. body-wt./24 hr.)

	Na (m-equiv.)	K (m-equiv.)	Ca (mg.)	Mg (mg.)	Sr (μg.)	P (mg.)
Intake:						
Breast milk	2.4	2.3	36	3.5	8	20
Cow's milk	2.5	4.6	135	11	72	103
Net absorption:						
Breast milk	2.2	1.9	20	2.2	−6	18
Cow's milk	2.2	4.0	82	7.0	53	86
Urinary excretion:						
Breast milk	1.7	0.7	4.4	0.6	14	0.5
Cow's milk	2.3	2.0	2.4	0.3	2	35
Retention:						
Breast milk	0.5	1.2	15	1.6	−20	18
Cow's milk	−0.1	2.0	79	6.7	51	51

The additional calcium can find a lodgement only in the bones, and the potassium must go to the soft tissues. From an experimental approach in pigs, McCance & Widdowson (1958b) concluded that the muscles were probably the most important site, but the chemical changes at cell level are quite unknown. There is more evidence about the calcium, strontium and magnesium. It is generally agreed that over the growth period as a whole the skeleton becomes more calcified, but there is evidence that in fast-growing animals, such as kittens (Burns & Henderson, 1936; Weidmann & Rogers, 1958; Slater and Widdowson, unpublished), the calcification of the skeleton does not keep pace with its increase in size over the period of intensely rapid growth during suckling; even in human infants the percentage of calcium in the body may be no higher at six months than it is at birth (Stearns, 1939). There is, therefore, in all probability a place for the additional calcium, phosphorus, magnesium and strontium in the partially calcified osteoid tissues. This process of additional calcification in the early phases of extra-uterine growth is clearly self-limited and must be expected to lead to relatively less mineral deposition later when growth slows down.

A further point of some importance is brought out by Table IV. In spite of the large amounts of calcium, magnesium, and particularly of strontium, absorbed and retained by the babies having cow's milk, less of each of these elements was excreted in the urine. This would be quite in order if the amounts of phosphorus available in breast milk were limiting the deposition of these divalent elements in bone. To prove this, babies fed on breast milk were given a supplement of neutral sodium and potassium phosphates, with the results shown in Table V. These speak for themselves.

The behaviour of strontium, vis-à-vis calcium, has still to be explained. It will be noted that the breast-fed babies were excreting more strontium in the faeces than their food contained, and seven times as much in the urine as the infants on

TABLE V. Effect of Supplementing Breast Milk with Inorganic Phosphate on the Urinary Excretion of Calcium, Magnesium, Strontium and Phosphorus in the New-Born Period

(Values expressed per kg. body-wt./24 hr.)

	Ca (mg.)	Mg (mg.)	Sr (μg.)	P (mg.)
Breast milk unsupplemented	4.4	0.6	14	0.5
Breast milk supplemented with phosphate	2.1	0.2	2.9	20
Cow's milk	2.4	0.3	2.0	35

cow's milk. It is known from the work of Chen & Neuman (1955), Comar, Whitney & Lengemann (1955), Comar (1956) and Wasserman *et al.* (1957) that calcium and strontium compete for the biological transport mechanisms hitherto investigated, and that calcium is preferentially absorbed from the gut, deposited in the bones of the mother and transferred to the foetus. It is also known that there are probably considerable species differences in all this. With phosphorus limiting the rate of mineralization in breast-fed infants, and from what is known about the rapid turnover of minerals, even in an apparently static tissue such as bone, it is easy to see why the babies on breast milk should have been in such large negative strontium balance, although it is not yet possible to fill in all the details.

"... thou knowest not ... how the bones do grow in the womb of her that is with child ..." (*Ecclesiastes*, **11**, 5).

REFERENCES

Alexander, D. P., Nixon, D. A., Widdas, W. F. & Wohlzogen, F. X. (1958) *J. Physiol.* **140**, 1

Bothwell, T. H., Pribilla, W. F., Mebust, W. & Finch, C. A. (1958) *Amer. J. Physiol.* **193**, 615

Burns, C. M. & Henderson, N. (1936) *Biochem. J.* **30**, 1207

Capek, K. (1961) In: Hahn, P., ed. *The development of homeostasis, with special reference to factors of the environment. Proceedings of a Symposium of the Czechoslovak Academy of Sciences, Prague.*, Czechoslovak Academy of Sciences, Prague (In press)

Chen, P. S., jr & Neuman, W. F. (1955) *Amer. J. Physiol.* **180**, 632

Comar, C. L. (1956) *Ann. N.Y. Acad. Sci.* **64**, 281

Comar, C. L., Whitney, J. B. & Lengemann, F. W. (1955) *Proc. Soc. exp. Biol., N.Y.* **88**, 232

Crawford, J. D. & McCance, R. A. (1960) *J. Physiol.* **151**, 458

Davies, J. (1958) In: Villee, C. A., ed. *Gestation. Transactions of the Fourth Conference, March 5-7, 1957, Princeton, N.J.*, p. 11. Josiah Macy, Jr Foundation, New York

Dickerson, J. W. T. (1960) *Biochem. J.* **75**, 33

Dickerson, J. W. T. & Widdowson, E. M. (1960) *Biochem. J.* **74**, 247

Economou-Mavrou, C. & McCance, R. A. (1958) *Biochem. J.* **68**, 573

Feaster, J. P., Hansard, S. L., Outler, J. C. & Davis, G. K. (1956) *J. Nutr.* **58**, 399

Friis, T. & Hall, T. (1957) *Acta endocr., Copenhagen*, **24**, 411

Fuchs, F. & Fuchs, A. R. (1957a) *Acta physiol. scand.* **38**, 379

Fuchs, F. & Fuchs, A. R. (1957b) *Acta physiol. scand.* **38**, 391

Hall, P. F. & Myant, N. B. (1956) *J. Physiol.* **133**, 181

Hallman, N. & Salmi, I. (1953) *Acta paediat., Stockh.* **42**, 126

Hansen, J. D. L. & Smith, C. A. (1953) *Pediatrics, Springfield*, **12**, 99

Logothetopoulos, J. & Scott, R. F. (1955) *Nature, Lond.* **175**, 775

McCance, R. A. (1959) *Arch. Dis. Childh.* **34**, 361; 459

McCance, R. A. & Stanier, M. W. (1960) *J. Physiol.* **151**, 479

McCance, R. A. & Strangeways, W. M. B. (1954) *Brit. J. Nutr.* **8**, 21

McCance, R. A. & Widdowson, E. M. (1951) *Brit. med. Bull.* **7**, 297

McCance, R. A. & Widdowson, E. M. (1954) *Arch. Dis. Childh.* **29**, 488

McCance, R. A. & Widdowson, E. M. (1955) *Arch. Dis. Childh.* **30**, 405

McCance, R. A. & Widdowson, E. M. (1956) *J. Physiol.* **133**, 373

McCance, R. A. & Widdowson, E. M. (1957) *Acta paediat., Stockh.* **46**, 337

McCance, R. A. & Widdowson, E. M. (1958a) *J. Physiol.* **141**, 81

McCance, R. A. & Widdowson, E. M. (1958b) *J. Physiol.* **141**, 88

McCance, R. A. & Widdowson, E. M. (1960) *J. Physiol.* **151**, 484

Martinek, J., Stanichová, J. & Janovský, M. (1961) In: Hahn, P., ed. *The development of homeostasis, with special reference to factors of the environment. Proceedings of a Symposium of the Czechoslovak Academy of Sciences, Prague.* Czechoslovak Academy of Sciences, Prague (In press)

Plenti, A. A. (1958) In: Villee, C. A., ed. *Gestation. Transactions of the Fourth Conference, March 5-7, 1957, Princeton, N.J.*, p. 71. Josiah Macy, Jr Foundation, New York

Salmi, I. (1954) *Ann. paediat. Fenn.* **1**, Suppl. No. 2

Sato, Y. (1933) *Acta Soc. path. jap.* **23**, 257

Shirley, R. L., Jeter, M. A., Feaster, J. P., McCall, J. T., Outler, J. C. & Davis, G. K. (1954) *J. Nutr.* **54**, 59

Slater, J. E. (1961) *Brit. J. Nutr.* **15**, 83

Smith, C. A. (1959) *The physiology of the newborn infant*, 3rd ed. Thomas, Springfield, Ill.; Blackwell Scientific Publications, Oxford

Smith, C. A., Yudkin, S., Young, W., Minkowski, A. & Cushman, M. (1949) *Pediatrics, Springfield*, **3**, 34

Stanier, M. W. (1960) *J. Physiol.* **151**, 472

Stearns, G. (1939) *Physiol. Rev.* **19**, 415

Wasserman, R. H., Comar, C. L., Nold, M. M. & Lengemann, F. W. (1957) *Amer. J. Physiol.* **189**, 91

Weidmann, S. M. & Rogers, H. J. (1958) *Biochem. J.* **69**, 338

Westin, B., Kaiser, I. H., Lind, J., Nyberg, R. & Teger-Nilsson, A. C. (1959) *Acta paediat., Stockh.* **48**, 609

Whittam, R. (1960) *J. Physiol.* **153**, 358

Widdowson, E. M. (1950) *Nature, Lond.* **166**, 626

Widdowson, E. M. & Dickerson, J. W. T. (1960) *Biochem. J.* **77**, 30

Widdowson, E. M. & McCance, R. A. (1956) *Clin. Sci.* **15**, 361

Widdowson, E. M. & McCance, R. A. (1959) *Acta paediat., Stockh.* **48**, 383

Widdowson, E. M., Slater, J. E., Harrison, G. E. & Sutton, A. (1960) *Lancet* **2**, 941

Widdowson, E. M. & Spray, C. M. (1951) *Arch. Dis. Childh.* **26**, 205

Wilde, W. S., Cowie, D. B. & Flexner, L. B. (1946) *Amer. J. Physiol.* **147**, 360

FOLLOW-UP STUDY OF ARTERIAL PRESSURE IN THE POPULATION OF A WELSH MINING VALLEY

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During three months of the winter of 1953–4 arterial blood-pressure measurements were made in a random sample of the general population of the Rhondda Fach, one of the Welsh coal-mining valleys (Miall and Oldham, 1955). Subsequently a similar survey was carried out in the agricultural population of the Vale of Glamorgan (Miall and Oldham, 1958). Both surveys were designed to give measurements which would form the basis for longitudinal studies.

Four years after the first survey in the Rhondda Fach, during the same three months of the winter of 1957–8, measurements were repeated under exactly comparable conditions for the survivors of the original population. The findings confirmed the influence of parity and occupation on arterial pressure found previously, and a fuller analysis of the results will be published when the first follow-up survey of the agricultural population has been carried out. In this paper other findings from this first follow-up study are presented and discussed in relation to changes in cardiovascular mortality reported in the most recent publications of the Registrar-General.

Methods

Population Sample.—The original objective was a study of genetic factors in arterial pressure, and for this 250 families were studied, the propositi being selected by a random method from the general population. All members of the families studied then formed the population for the follow-up; the material available is shown in Table I. Repeat measurements were obtained for 1,114 (99.6%) of the 1,119 subjects still alive and resident within 25 miles of the valley.

Technique.—Casual blood-pressure measurements were made by the same observer using the identical technique previously described. Medical, obstetrical, and occupational histories were taken, and measurements were made of height, weight, arm girth, and arm skinfold. All new measurements were made without reference to the previous records. Dahl and Love (1954, 1957) have claimed that hypertension is commoner in those who customarily take additional salt with their food than in those who do not; questions about salt intake were therefore asked in this survey also.

Analysis.—In surveys of this kind, where casual arterial pressure measurements are recorded, it would be surprising if the results found did not reflect the well-known variability of pressure recorded in this way. Fluctuation in pressure or observational error will result in those with "falsely" high initial pressures tending to have lower subsequent readings, and those with "falsely" low initial pressures having higher subsequent readings. Changes in pressure and initial pressure are also not statistically independent pieces of information, so that in comparing the observed change in pressure of two groups selected because they have high and low average initial pressures it is unsafe to assume that the number of falsely high or low readings is the same in each group and wrong to expect that the pressure change should be the same in both groups if no disturbing factor is operating. In the following analyses, however, it has been assumed that the "falsely" high or low readings are randomly distributed, and that the correlation between the mean *inter-survey pressure* (taken as the mean of the two survey readings) and the change of pressure is small. The tables show pressure change related to this mean pressure for the various groups studied. A more rigorous analysis will be published when further follow-up material is available.

Relationship Between Pressure and Age at the Two Surveys

As these data were collected in exactly comparable ways during each survey no systematic difference between the mean values in five-year age-groups in 1954 and 1958 was expected. For females this was so (Fig. 1), but for males there is a consistent suggestion of a secular change—a change occurring in subjects of the same age as time passes. For each age group between 15 and 55 both systolic and diastolic mean pressures were higher in 1958 than they were in 1954. Though the mean differences are small and may have little meaning medically, they are highly significant statistically, and it is difficult to imagine how an artifact would be likely to produce a difference of this kind in males but not in females. Furthermore, when the data are subdivided into those for random samples and those

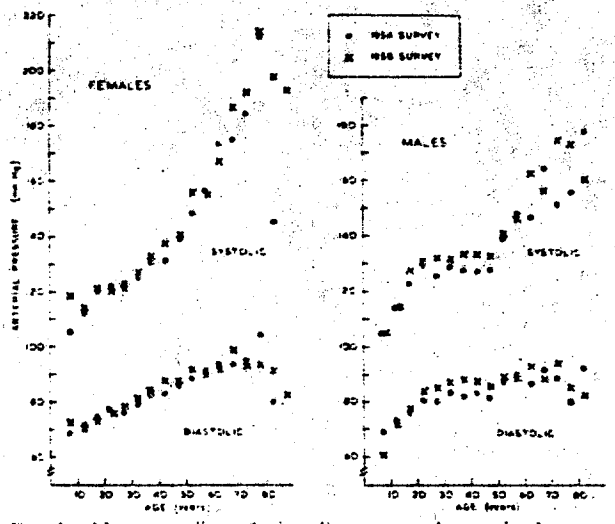

FIG. 1.—Mean systolic and diastolic pressures by age in the two surveys.

for relatives, identical results are found—there is no suggestion of a secular trend in either group of females, but similar changes in both groups of males.

The difference between the findings at two surveys is not due to differences in the populations; though 11% of the original male population had died or left the area, removing them from the 1954 results does not materially influence the difference found between the two surveys.

Those age groups in males which appear to show this secular trend are examined in greater detail later. The trend cannot be explained by changes in arm girth: the mean values differed between the surveys by less than ¼-in. (6 mm.) for each 5-year age-group. As arm girth is highly correlated with weight ($r=0.9$) it seems improbable that weight changes could explain the findings either; but, unfortunately, weight was not recorded for all subjects in the original survey.

Influence of Occupation on Arterial Pressure

It was found in the analysis of the data collected in our first surveys in the Rhondda Fach and the Vale of Glamorgan that arterial pressure is significantly higher in men previously employed mainly in light occupations.

TABLE I.—*Follow-up of Male and Female Populations, Rhondda Fach, 1954–8*

	Males				Females				Total			
	Random Sample		1st Degree Relatives		Random Sample		1st Degree Relatives		Random Sample		1st Degree Relatives	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Followed up	120	88	404	89	103	90	487	93	223	89·2	891	91·[illegible]
Left area	9	7	28	6	6	5	23	4	15	6·0	51	5·[illegible]
Died	5	4	22	5	5	4	9	2	10	4·0	31	3·[illegible]
Refused follow-up	2	1	1	—	0	—	2	—	2	0·8	3	0·[illegible]
Original population	136	100	455	100	114	100	521	100	250	100	976	10[illegible]

The differences in the mean systolic and diastolic pressures as at age 45 between men in light occupations and those in heavy occupations were 10 mm. and 8 mm. Hg respectively. Men in heavy jobs have larger arms, so that applying a correction for arm girth enhances the difference between the groups.

Though these findings were in apparent conflict with those of Brown *et al.* (1957), and Humerfelt and Wedervang (1957), who both found lower pressures in professional groups, they were strongly supported by the recent *Occupational Mortality Supplement* of the Registrar-General (1958). Standardized mortality ratios for hypertension, vascular lesions of the central nervous system, and coronary disease were greatly increased in the light occupation groups (Table II). A more detailed analysis of the effect of occupation on rate of change of pressure will be possible after the follow-up of the rural population. The Rhondda Fach population is less satisfactory for this type of study as there is a deficiency of men in light occupations, particularly in the older age groups.

The findings from this first follow-up are interesting but difficult to interpret. Men whose predominant past occupation was classified as light or moderately light had higher mean inter-survey pressures than those in heavy jobs (Table III) except between the ages of 35 and 45, where the effect of family size (see later) predominates and causes little difference between the occupational groups.

Between the two surveys, however, the increase in both systolic and diastolic pressure is greatest in those previously employed in heavy occupations. Changes in pressure analysed by inter-survey occupation rather than predominant past occupation also showed a greater increase in the four-year interval for those engaged in heavy work.

TABLE II.—*Standardized Mortality Ratios for Males Aged 20–64 by Socio-economic Groups, 1949–53*

	Hypertension (440–447)	Coronary Disease (420–422)	Vascular Lesions of C.N.S. (330–334)
1. Farmers	70	62	76
2. Agricultural workers	60	55	73
3. Higher administrative, etc.	123	147	124
4. Other administrative, etc.	109	116	104
5. Shopkeepers	118	123	117
6. Clerical workers	117	132	118
7. Shop assistants	93	96	86
8. Personal services	114	105	108
9. Foremen	90	99	88
10. Skilled workers	102	102	101
11. Semi-skilled	87	84	89
12. Unskilled	102	89	102

TABLE III.—*Mean Inter-survey Systolic and Diastolic Pressures, and Mean Change of Pressure 1954–58 for Males Aged 15–65 at the Time of the First Survey, Whose Predominant Past Occupation was Light or Heavy Before the 1954 Survey*

Age in 1954	Occupation	No.	Mean Inter-survey Systolic Pressure	Mean Inter-survey Diastolic Pressure	Mean Systolic Change 1954–58	Mean Diastolic Change 1954–58
15–	Light	35	130·6	81·7	+2·7	+2·5
	Heavy	49	126·7	79·8	+6·1	+6·1
25–	Light	19	135·0	87·5	+3·4	+3·6
	Heavy	73	128·9	84·3	+5·0	+5·9
35–	Light	12	126·7	83·0	0	+1·7
	Heavy	74	130·2	85·2	+6·4	+4·0
45–	Light	17	142·7	88·9	+3·9	+2·2
	Heavy	64	136·1	84·8	+8·9	+2·7
55–	Light	9	160·6	94·2	+9·5	+2·2
	Heavy	44	152·5	88·4	+14·4	+3·4

It is difficult to reconcile these findings with the fact that those in heavy occupations previously had lower pressures except in terms of a secular trend which is now influencing the heavy occupation group. It is possible that the considerable social changes which are occurring in the Welsh mining valleys are influencing those in heavy manual work more than those in light jobs, but it would seem unlikely that any recent change in the nature of the work itself has occurred which could have any direct effect of this kind.

Association Between Family Size and Arterial Pressure

We have previously shown that the pressures of married men and women show an inverse correlation with the size of their families—the larger the family the lower the pressure. Similar but less marked trends were reported for women by Humerfelt and Wedervang (1957) in their analysis of the Bergen survey.

In our data this trend was really clear-cut only in the "child-bearing" age-groups, which were taken as 15–45 for females and 15–50 for males (Miall and Oldham, 1958), though, as can be seen in Table IV, in the Rhondda Fach childless men and women have fairly consistently higher pressures than have those up to age 75 with children, where differences in survival probably become important.

TABLE IV.—*Systolic and Diastolic Mean Pressures by Age for Rhondda Fach Females and Males With and Without Children (Based on Data from 1954 and 1958 Surveys)*

Age	Children	Females No.	Females Systolic	Females Diastolic	Males No.	Males Systolic	Males Diastolic
15–	Without	169	121·4	75·7	164	127·8	79·4
	With	32	116·9	72·4	17	124·0	76·9
25–	Without	42	129·2	81·8	69	132·4	85·6
	With	167	123·4	78·1	120	127·7	82·9
35–	Without	23	134·5	82·3	40	135·4	88·0
	With	205	132·5	84·6	155	129·2	84·3
45–	Without	12	142·1	87·9	19	138·8	86·5
	With	150	145·1	87·9	147	134·5	85·7
55–	Without	13	169·8	96·4	14	155·0	91·1
	With	127	161·3	91·0	109	149·7	89·1
65–	Without	10	188·5	97·5	14	163·6	93·2
	With	63	182·8	94·5	60	161·8	90·2
75+	Without	4	180·0	83·8	1	147·5	52·5
	With	20	206·0	97·5	27	164·0	83·6

The data available from these two surveys enable us to see to what extent this results from selection or from having a child. The mean pressures at the time of the first survey were somewhat lower for those women who subsequently had children than for those who did not (Table V). The difference between the two groups for mean inter-survey pressure was more marked. In several of the age-groups the mean pressure on the second occasion had actually fallen in the women who had had children, whereas this did not occur in any age-group for those who had not.

The first survey pressures were also slightly lower in the men who subsequently increased their family size than in those not doing so, and though there was not the same tendency as in women for pressures to fall, they rose less in that group than in those who had no children in the interval (Table VI).

The lower initial pressures of those subsequently having children did not appear to be explained by differences in "parity," but in our previous analysis we found unmarried men and women had higher pressures than those married without children, and this fact must contribute to the initial differences between the groups.

TABLE V.—*Mean Systolic and Diastolic Pressures in 1954, Mean Inter-survey Systolic and Diastolic Pressures, and Mean Change of Pressure 1954–8 for Females Aged 10–45 at Time of First Survey Who Have and Have Not Increased Their Families Between the Surveys*

Age in 1954	Increase in Family Size, 1954–8	No.	Mean Systolic Pressure, 1954	Mean Diastolic Pressure, 1954	Mean Inter-survey Systolic Pressure	Mean Inter-survey Diastolic Pressure	Mean Systolic Change, 1954–8	Mean Diastolic Change, 1954–8
10–	No	55	112·6	72·2	117·0	73·7	+ 8·7	+ 2·9
	Yes	2	110·0	65·0	111·3	65·0	+ 2·5	0
15–	No	35	120·2	73·9	120·2	74·2	0	+ 0·6
	Yes	10	118·0	75·0	117·3	73·8	1·5	2·5
20–	No	28	121·8	78·2	122·6	78·2	+ 1·6	0
	Yes	22	121·6	74·1	119·7	75·4	− 3·9	+ 2·5
25–	No	25	123·5	76·9	125·3	78·3	+ 3·6	+ 2·8
	Yes	18	121·1	74·4	123·3	78·5	+ 4·4	+ 8·1
30–	No	51	125·6	79·8	128·5	82·0	+ 5·8	+ 4·3
	Yes	12	125·0	78·3	126·7	80·4	+ 3·3	+ 4·2
35–	No	52	128·5	81·5	132·0	83·7	+ 7·0	+ 4·4
	Yes	4	152·5	103·8	149·4	99·4	− 6·3	8·8
40–	No	48	131·1	83·1	135·1	85·4	+ 7·9	+ 4·6
	Yes	2	125·0	77·5	118·8	73·8	12·5	7·5

TABLE VI.—*Mean Systolic and Diastolic Pressures in 1954, Mean Inter-survey Systolic and Diastolic Pressures, and Mean Change of Pressure 1954–8 for Males Aged 15–50 at Time of First Survey Who Have and Have Not Increased Their Families Between the Surveys*

Age in 1954	Increase in Family Size, 1954–8	No.	Mean Systolic Pressure, 1954	Mean Diastolic Pressure, 1954	Mean Inter-survey Systolic Pressure	Mean Inter-survey Diastolic Pressure	Mean Systolic Change, 1954–8	Mean Diastolic Change, 1954–8
15–	No	40	122·5	76·4	126·8	79·6	+ 8·5	+ 6·3
	Yes	5	122·5	73·5	121·0	74·0	− 3·0	+ 1·0
20–	No	26	132·5	84·4	135·9	86·3	+ 6·7	+ 3·8
	Yes	13	124·8	75·2	126·2	75·6	+ 2·7	+ 0·8
25–	No	21	128·0	83·0	131·6	86·4	+ 7·1	+ 6·7
	Yes	18	123·3	77·2	126·1	80·4	+ 5·6	+ 6·4
30–	No	38	127·9	82·4	129·9	84·9	+ 4·0	+ 5·0
	Yes	15	132·2	86·2	133·6	88·2	+ 2·7	+ 4·0
35–	No	40	127·3	83·8	130·5	85·8	+ 6·3	+ 3·9
	Yes	7	124·6	78·9	127·5	82·5	+ 5·8	+ 7·1
40–	No	37	127·1	83·2	129·8	84·6	+ 5·3	+ 2·8
	Yes	2	127·5	80·0	127·5	81·3	0	+ 2·5
45–	No	42	128·0	81·1	132·6	84·3	+ 9·2	+ 6·2
	Yes	2	125·0	82·5	131·3	83·8	+ 12·5	+ 2·5

Weight changes are again unlikely to explain the blood-pressure association with family size, for in our data and in the Board of Trade (1957) survey of women's measurements and sizes women with children are somewhat heavier than those without.

Though selection may play some part in causing the difference in pressure between those who did and did not subsequently have children, it appears to be less important than some change which occurs as a result of having a child, which in women can cause an actual fall in pressure. The difference in pressure increments between those who had children and those not doing so are small in a four-year interval, but the cumulative effects on pressure of an active or inactive reproductive life, whether they are direct physiological effects or an indirect effect associated with standards of living or energy expenditure, would be considerable, as judged from Tables V and VI.

It is not unreasonable to expect that the process, whatever its mechanism, would account entirely for the 20 mm. and 9 mm. Hg differences which were found previously in systolic and diastolic pressures between childless women and women with six children at age 45–50. For males the greater rise in pressure for those not having children is also large enough to explain the considerable differences between men with no children and those with large families found previously, without introducing hypotheses involving medical selection to account for the findings.

Influence of Salt on Arterial Pressure

That there are abnormalities of electrolyte metabolism and excretion in hypertension is now well established. High blood-pressure can be reduced by salt starvation (Kempner, 1948) and induced by a very large salt-intake in man (Perera and Blood, 1947) and in animals (Meneely *et al.*, 1953). Retention of sodium is a well-known finding in Cushing's disease, where hypertension is characteristic. Addison's disease manifests the opposite findings. Some workers—for example, Holley *et al.* (1951)—have found high serum sodium values in hypertensive subjects; others—for example, Cottier *et al.* (1958)—found no difference in sodium levels between normotensive and moderately and severely hypertensive subjects, though mildly hypertensive subjects in their study showed consistently a slightly high value. Though the evidence suggests that hypertensive humans and animals have higher sodium values in the body, hypertensives reject sodium after a saltload to a greater extent than do normal subjects (Farnsworth and Barker, 1943; Farnsworth, 1946; Cottier *et al.*, 1958).

The relationship between blood-pressure levels and normal salt intake is less well established. Dahl and Love, 1954, 1957) presented evidence that those who take additional salt with their food at table have higher pressures than those who do not. They questioned both men and women about their salt habits but confined their analysis to men, in whom they found that the prevalence of hypertension (140/90 and over) was increased in those taking salt in this way, and much increased in those who were both overweight and added extra salt.

Similar questions to those used by Dahl and Love were asked in this follow-up study, and on the response to these questions each subject was classified into one of three groups: (1) "high salt intake"—for those habitually adding extra salt to a cooked meal, without previously tasing it; (2) "medium salt intake"—for those adding extra salt to such a meal only occasionally, and after previously tasting it; and (3) "low salt intake"—for those normally not taking additional salt with such a meal.

It cannot be assumed that the questions asked necessarily detect subjects with different salt-intakes, especially in women. It is possible that those women who regularly add salt at the table are those who salt the food insufficiently in its preparation. The three questions asked do, however, produce a clear-cut response and classify the population into well-defined groups, and there is an interesting sex-difference in the response to the questions (Table VII). A smaller

TABLE VII.—*Response to Standard Questions on Salt Intake Habits. Males and Females.* 1958

	Salt Intake Group—All Ages						
	High		Medium		Low		Total
	No.	%	No.	%	No.	%	No.
Males	228	48·9	133	28·5	105	22·5	446
Females	183	36·1	145	28·6	179	35·3	507

percentage of women than men are in the high salt-intake group (36.1% against 48.9%), and a larger proportion are in the low salt-intake group (35.3% against 22.5%). Dahl and Love also found a similar significant difference in the sex distribution. These sex differences in salt habits at table may be occupational and due to loss of salt from sweating in men, or may merely indicate that women, rightly or wrongly, believe that they have added sufficient salt in the kitchen.

The mean pressures of the high intake and low intake groups are shown in Fig. 2. There is no suggestion of any systematic difference between the two groups of males. Among the females there is a consistent difference between the two groups, but, surprisingly, it is in the opposite direction from that expected from the findings of Dahl and Love for males—women who habitually add salt show lower systolic and diastolic pressures than those who do not. All subjects advised to omit salt on medical grounds have been excluded from this comparison. Not only had those women who habitually take extra salt lower pressures in 1958 than those not taking salt, but their pressures had increased less in the previous four years, and, except in old age, they were lighter in weight (Table VIII). Furthermore, their pulse pressures were less in most age-groups over age 35, and had increased less rapidly in the preceding four years (Table IX). These findings appear to merit further investigation.

Separate analyses showed no correlation between parity and salt intake in women, nor between the energy expenditure of occupation and salt intake in men.

These results only suggest that the findings of Dahl and Love for American men do not necessarily apply in other populations where salt habits may be different. Phear (1958) also found in a small series of hospital patients a greater proportion of high salt-takers in his normotensive than in his hypertensive subjects. The questions presumably correlate with salt appetite, but we need an indication of how this is related to the sodium content of the body, and this is not a practicable investigation to tackle by using epidemiological techniques. If salt retention is accompanied by a smaller appetite for salt, these findings accord with the general body of evidence relating sodium metabolism and pressure.

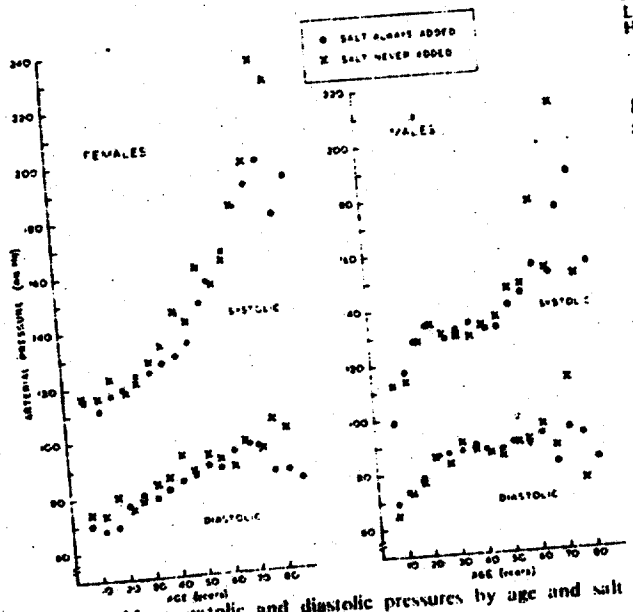

FIG. 2.—Mean systolic and diastolic pressures by age and salt habits in the 1958 survey.

TABLE VIII.—*Mean Systolic and Diastolic Pressures* (1958), *Changes in Pressure* (1954–8) *and Weights* (1958). *Females Aged* 15–90, *by Salt Intake*

Age in 1958	Salt Intake Group	No.	Mean Systolic Pressure, 1958	Mean Diastolic Pressure, 1958	Change in Systolic Pressure, 1954–8	Change in Diastolic Pressure, 1954–8	Weight 1958 lb.	Weight 1958 kg.
15–	Low	23	119·9	77·3	+ 3·0	+1·5	125·6	57·0
	High	37	117·5	72·9	− 2·0	0·1	121·3	55·0
25–	Low	37	124·9	81·0	+ 0·2	+3·8	132·0	59·9
	High	38	123·8	79·4	+ 5·4	+4·4	130·6	59·2
35–	Low	44	140·1	89·4	+ 9·5	+6·0	143·7	65·2
	High	37	128·7	83·0	+ 3·1	+4·0	138·2	62·7
45–	Low	29	149·4	90·3	+13·3	+5·2	150·2	68·1
	High	28	138·6	87·2	+ 7·2	+4·0	143·0	64·9
55–	Low	25	158·7	90·1	+11·6	+2·2	160·1	72·6
	High	27	162·2	91·4	+ 7·0	+0·7	151·0	68·5
65–	Low	16	187·8	94·2	+21·3	+3·8	146·2	66·3
	High	9	185·8	95·8	+11·7	+6·1	168·1	76·3
75+	Low	5	230·5	102·5	+ 2·0	−7·0	120·5	54·7
	High	7	189·7	85·3	+ 7·1	−2·9	144·6	65·6

TABLE IX.—*Mean Pulse-pressures* (1958) *and Changes in Pulse-pressure* (1954–8). *Females Aged* 15–90, *by Salt Intake*

Age in 1958	Salt Intake Group	No.	Pulse-pressure, 1958	Change in Pulse-pressure 1954–8
15–	Low	23	47·0	+ 1·5
	High	37	44·6	− 1·9
25–	Low	37	43·8	− 3·9
	High	38	44·5	+ 0·6
35–	Low	44	50·7	+ 3·5
	High	37	45·6	− 0·8
45–	Low	29	59·1	+ 8·1
	High	28	51·4	+ 3·2
55–	Low	25	68·6	+ 9·4
	High	27	70·7	+ 6·3
65–	Low	16	93·7	+17·5
	High	9	90·0	+ 5·6
75+	Low	5	128·0	+ 9·0
	High	7	104·3	+10·0

TABLE X.—*Mean Values for* 24-*hour Urinary Excretion of Sodium by Systolic Pressure and Salt Intake in Post-menopausal Women*

Salt Intake Group	Systolic Pressure Over 200 mm. Hg: No. of Specimens	Systolic Pressure Over 200 mm. Hg: Na Output in 24 Hours in Milli-equivalents: Mean	Systolic Pressure Over 200 mm. Hg: Na Output in 24 Hours in Milli-equivalents: S.D.	Systolic Pressure Under 150 mm. Hg: No. of Specimens	Systolic Pressure Under 150 mm. Hg: Na Output in 24 Hours in Milli-equivalents: Mean	Systolic Pressure Under 150 mm. Hg: Na Output in 24 Hours in Milli-equivalents: S.D.
Low ..	26	123·1	42·60	61	130·7	48·72
High ..	23	122·1	36·49	43	146·3	48·31

We have determined the sodium output in 24-hour specimens of urine for four groups of post-menopausal women in the sample; all women over age 50 in the high intake and low intake groups with systolic pressures over 200 mm. or under 150 mm. Hg were asked to provide 24-hour urine specimens daily for a week. The completeness of the specimens was checked by measurement of urinary creatinine. The results, based in each case on the highest creatinine values (provided they varied by less than 25%, which we have found to be normal variability on known 24-hour specimens), are shown in Table X.

The hypertensive women in this sample excreted less sodium than those with low pressure, whether in the high or the low salt-intake group, but the differences are not significant at the 5% level. There was no difference in sodium output between hypertensive women who did and did not add extra salt to their

food, but a rather suggestive difference ($0.20 > P > 0.10$) between the two groups of normotensive women.

Relationship Between the Survey Findings and Mortality Rates

Though it would be rash to attach much significance to the secular change in arterial pressure which seems to be occurring amongst middle-aged males in this survey unless it is confirmed in subsequent studies, it is nevertheless possible that the higher pressures found in the second survey are a manifestation of the process which is causing the secular trend in male mortality from cardiovascular disease.

The total number of deaths attributed to cardiovascular diseases is increasing in both sexes in Britain. Some of the apparent increase is due to changes in the age structure of the population; changes in the habits of those responsible for certifying and classifying deaths may also have resulted in changes in the reported rates for specific causes of death. Deaths of hypertensives are apt to be classified under four main headings: (1) hypertensive diseases (*International Classification*, 440–447); (2) arteriosclerotic and degenerative heart disease (420–422); (3) vascular lesions affecting the central nervous system (330–334); and (4) nephritis and nephrosis (590–594).

The last group is small and can be ignored. By pooling the other three we can overcome changes in certification and classification and get an indication of any secular change which is occurring in those diseases most directly affected by arterial pressure.

Between 1950 and 1956 the overall mortality rates for males from these three groups have shown a very definite increase, which is confined to the middle-aged; there is a clear upward trend in each five-year age-group from 25 to 60 (Table XI). In females there is no such increase; indeed, the death rates for the combined group are tending to fall in age-groups over 45 (Table XII).

Examination of the separate groups shows that the increase in the male mortality rates is entirely due to the arteriosclerotic and degenerative heart disease group. The rates for cerebrovascular lesions show no change and there is a slight fall in those attributed to hypertension in males between ages 45 and 65. It is not clear what kind of patients would be certified as dying of hypertension, but certainly the majority of hypertensives who die would not be so classified, but would occur in one of these other groups.

The secular trend in mortality in middle-aged males is therefore largely due to coronary artery disease, the attack rate of which is significantly higher in hypertensives (Dawber *et al.*, 1957; Chapman *et al.*, 1957).

The changes in male mortality from coronary artery disease are certainly occurring in the same age-groups as we have found to show a secular change in pressure. In our survey it is the heavy-occupation groups which are contributing most to this rise, and it is perhaps significant that the *Occupational Mortality Supplement* of the Registrar-General (1958) shows a flattening of the downward mortality gradient from social class I to social class V between 1930–2 and 1949–53 (Table XIII). Furthermore, there is other evidence suggestive of a marked secular trend for coronary disease. The mortality ratios for 10-year age-groups for 1949–53 show, in males aged 25–34, a clear reversal of the normal downward gradient by social class seen in older age-groups (Table XIII). The heavy-occupation groups are apparently starting to show higher mortality than those engaged in lighter work; our survey findings may be reflecting this trend.

Further confirmation of the relationship between hypertension, physical activity, and ischaemic heart disease has been provided by Morris and Crawford (1958). In their analysis of a national necropsy survey they found that hypertension, based on clinical and pathological findings, was less common and occurred 10 to 15 years later in men previously employed in heavy occupations than in others; they also found that within each occupational group, graded according to physical activity, myocardial scarring was commoner in those with evidence of hypertension. When the social classes were broken down into groups categorized by physical activity and graded as light, active, and heavy, these occupational groups showed no social class trend in ischaemic heart disease, but the relationship between myocardial fibrosis and physical activity was then evident within single social class groups.

Mortality by marital status is not given in any detail in the Registrar-General's reports. An analysis of deaths in the United States for the years 1949–51 (U.S.

TABLE XI.—*Death Rates (Per Million of Living Population) from Arteriosclerotic and Degenerative Heart Disease (420–422), Hypertensive Diseases (440–447) and Vascular Lesions Affecting the Central Nervous System (330–334). Males. England and Wales*, 1950, 1952, 1954, *and* 1956.

Age	1950	1952	1954	1956
15–	18	20	20	18
20–	40	45	45	42
25–	51	66	74	73
30–	120	126	159	147
35–	275	316	330	373
40–	658	696	748	783
45–	1,570	1,576	1,646	1,727
50–	2,205	3,349	3,468	3,456
55–	5,909	6,127	6,284	6,512
60–	11,307	11,106	11,237	11,274
65–	19,273	19,508	19,584	19,466
70–	33,168	32,986	32,721	33,013
75–	54,823	56,217	55,215	56,039
80–	89,994	95,105	89,589	89,720
85+	139,721	155,714	136,932	138,262

TABLE XII.—*Death Rates (Per Million of Living Population) from Arteriosclerotic and Degenerative Heart Disease (420–422), Hypertensive Diseases (440–447), and Vascular Lesions Affecting the Central Nervous System (330–334). Females. England and Wales.* 1950, 1952, 1954, *and* 1956.

Age	1950	1952	1954	1956
15–	10	14	14	12
20–	28	28	25	25
25–	35	51	44	36
30–	84	74	92	83
35–	163	155	179	143
40–	290	315	317	297
45–	762	760	681	669
50–	1,586	1,646	1,466	1,347
55–	3,058	3,030	2,847	2,708
60–	6,376	5,979	5,796	5,631
65–	12,528	11,993	11,625	11,290
70–	24,533	22,791	20,368	21,999
75–	45,468	42,508	41,327	41,163
80–	77,827	75,774	71,802	72,482
85+	128,062	138,754	124,770	128,486

TABLE XIII.—*Standardized Mortality Ratios* (20–64) *for Coronary Disease by Social Class*, 1930–32 *and* 1949–53, *and Mortality Ratios for Coronary Disease by Social Class* (1949–53). *Males (Registrar-General*, 1958)

Social Class	S.M.R.s (20–64)		Mortality Ratios			
	1930–32	1949–53	25–34	35–44	45–54	55–64
I ..	237	147	81	108	144	154
II ..	147	110	86	104	110	111
III ..	96	105	97	100	102	108
IV ..	67	79	100	84	80	78
V ..	67	89	128	108	93	86

Public Health Service, 1956) shows much higher rates for cardiovascular deaths at all ages for the single, widowed, and divorced males than for the married. The differences are even more pronounced for the non-white population. Among females the widowed and divorced show a similar excess mortality from these causes; single coloured women also show a very marked excess. Single white women, both in the United States and in Britain, show no excess cardiovascular mortality.

This favourable mortality experience of the married is not confined to the cardiovascular group of diseases, however; similar results are found for such diverse conditions as pneumonia, peptic ulcer, and cirrhosis of the liver. There are many mechanisms which may account for such findings, and it is impossible to determine to what extent they are due to a beneficial influence on health of married life; to the deprivations and worries of the single, widowed, and divorced; or to the health selection which occurs at marriage.

We have, fortunately, too few deaths in this follow-up study to be able to relate our blood-pressure readings to mortality rates or expectation of life, and too few widowed and divorced to warrant their separate analysis until further material is available.

Conclusions and Summary

The results of the first follow-up survey of arterial pressure in the population of one of the Welsh mining valleys are reported. The original measurements were made in 1954 on a random sample of this population and all first-degree relatives living within 25 miles. Repeat readings were made four years later, at the same time of year, in 1958. The recordings were all taken by one observer, under identical circumstances, without reference to previous readings.

The results suggest the possibility that arterial pressure, when full allowance is made for age, may be increasing in middle-aged males. This trend is occurring to a greater extent in men previously engaged in heavy jobs, though they still have lower pressures than those previously in light occupations. These findings are discussed in terms of the secular trend in mortality from cardiovascular diseases reported by the Registrar-General.

Blood-pressure was again found to correlate with family size, and increases in pressure were less in those subjects who had children during the four-year interval. The differences in the rate of increase of pressure with age between those having children and those not having them appeared sufficient to explain the marked difference found previously between childless men and women and those with large families.

In women, those adding salt at table to cooked meals, who presumably have a larger salt appetite, were found to have lower blood-pressures and smaller pulse-pressures in 1958, to have gained less in blood-pressure and pulse-pressure during the previous four years, and to be lighter than women who do not take additional salt in this way. No comparable differences were found in the men.

I should like to record my thanks to the people of the Rhondda Fach, who have again so willingly co-operated in this survey; to my colleagues at the Pneumoconiosis Research Unit, in particular Dr. J. C. Gilson, the Director, and Dr. A. L. Cochrane and Mr. P. D. Oldham for valuable discussion and advice; to Dr. T. G. Morris for the analyses of sodium output; to Miss G. Jones and all members of the field survey team who helped with the collection and analysis of the material, and especially Miss L. Roberts, who provided secretarial help, and Mr. F. Moore, who assisted with all the field work.

References

Board of Trade (1957). *Women's Measurements and Sizes*. H.M.S.O., London.
Brown, R. G., McKeown, T., and Whitfield, A. G. W. (1957). *Canad. J. Biochem.*, 35, 897.
Chapman, J. M., Goerke, L. S., Dixon, W., Loveland, D. B., and Phillips, E. (1957). *Amer. J. publ. Hlth.*, 47, Suppl., April, p. 33.
Cottier, P. T., Weller, J. M., and Hoobler, S. W. (1958). *Circulation*, 17, 750.
Dahl, L. K., and Love, R. A. (1954). *A.M.A. Arch. intern. Med.*, 94, 525.
—— —— (1957). *J. Amer. med. Ass.*, 164, 397.
Dawber, T. R., Moore, F. E., and Mann, G. V. (1957). *Amer. J. publ. Hlth.* 47, Suppl., April, p. 4.
Farnsworth, E. B. (1946). *J. clin. Invest.*, 25, 897.
—— and Barker, M. H. (1943). *Proc. Soc. exp. Biol. (N.Y.)*, 52, 74.
Holley, H. L., Elliott, H. C., and Holland, C. M. (1951). Ibid., 77, 561.
Humerfelt, S., and Wedervang, F. (1957). *Acta med. scand.*, 159, 489.
Kempner, W. (1948). *Amer. J. Med.*, 4, 545.
Mencely, G. R., Tucker, R. G., Darby, W. J., and Auerbach, S. H. (1953). *Ann. intern. Med.*, 39, 991.
Miall, W. E., and Oldham, P. D. (1955). *Clin. Sci.*, 14, 459.
—— —— (1958). Ibid., 17, 409.
Morris, J. N., and Crawford, M. D. (1958). *Brit. med. J.*, 2, 1485.
Perera, G. A., and Blood, D. W. (1947). *J. clin. Invest.*, 26, 1109.
Phear, D. N. (1958). *Brit. med. J.*, 2, 1453.
Registrar-General (1958). *Decennial Supplement, England and Wales, 1951. Occupational Mortality*, Part II. H.M.S.O., London.
U.S. Public Health Service (1956). *Vital Statistics, Special Reports*, 39, 303.

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SUMMARY

Because of the general belief that chickens are very readily poisoned by common table salt, and because of the common occurrence of salt in wastes and by-product feeds for poultry, it was considered important to determine definitely whether the use of such wastes and feeds is dangerous to health or retards growth. This experiment was planned, therefore, in order to determine (1) the maximum percentage of salt that may be fed to growing chickens without harmful results, and (2) the maximum single dose of salt that a chicken can tolerate, or the smallest dose that would cause death.

In all, 75 chickens from three breeds were used. Fifty chickens were given a basal ration made up of yellow corn, bran, dried buttermilk, steamed bone meal, and ground limestone, containing percentages of salt of 1, 2, 4, and 8. Twenty-five other chickens were used in determining the maximum single dose of salt that an adult chicken can tolerate.

It was found that chickens could be raised from 9 to 21 weeks of age on rations containing as high as 8 percent of salt with no apparently detrimental effects. While it took some time for the chickens to become accustomed to such a salty ration, they soon learned to eat it in amounts sufficient to promote a rate of growth approximately the same as that of chickens fed the check ration.

When the salt was mixed in the feed, a daily intake of 6 to 8 grams of salt per bird appeared to have no harmful effect on the birds that were 9 weeks old or older.

Salt put directly into the crop in two equal doses amounting to 12 to 16 grams daily was quickly fatal in the case of birds weighing 2 to 4 pounds each.

Salt given in solution twice daily proved to be more toxic than equal amounts consumed in the feed.

The minimum lethal single dose of salt for birds weighing from 3 to 5 pounds was found to be close to 4 grams per kilogram of body weight.

THE TOXICITY OF SALT FOR CHICKENS

By H. H. Mitchell, L. E. Card, and G. G. Carman[1]

It is a general belief among poultrymen that chickens are very readily poisoned by common table salt, and many instances have been reported in which chickens were killed by eating salt meat or fish, salty kitchen wastes, or brine left from the curing of meat or from the freezing of ice cream. It has been recommended by some writers that the proportion of salt in a mash for chickens be no higher than 5 to 10 ounces to 100 pounds of mash when the mash mixture comprizes about half the total ration, and that no salt be given to young stock until they are two months old. However, only a few controlled experiments concerned with a determination of the minimum lethal dose of salt for chickens have been reported in the literature.

In 1892 Collier[2] reported the results of feeding varying amounts of salt to mature hens. No harmful results were noticed in these experiments involving six hens until the intake of salt reached 0.063 ounce per head per day. An intake of 0.042 ounce per head per day was not accompanied by any noticeable symptoms. In 1909 Suffran[3] attempted to determine the minimum toxic dose of common salt for chickens. From experiments on 5 chickens, he concluded that a dose of 4 grams per kilogram of body weight is sufficient to produce death, tho one chicken in the experiment resisted this dose successfully. Salt was administered in solution and was injected into the crop after the meal. The symptoms noticed were inability to stand, intense thirst, pronounced muscular weakness, and convulsive movements just before death. A viscous discharge from the beak was also noted. Post-mortem examination revealed lesions in many organs, but particularly hemorrhages and a severe congestion in the gastro-intestinal tract.

The attention of Edwards[4] was directed toward the problem by a case in which several ducks, pigs, and chickens were killed by being fed the sweepings from the floor of a bakery, afterward found to contain about 22 percent of salt. The symptoms noted in the chickens were very similar to those above reported. An experiment was then

[1]H. H. Mitchell, Chief in Animal Nutrition; L. E. Card, Chief in Poultry Husbandry; G. G. Carman, formerly Assistant in Animal Nutrition.

[2]Collier, Peter. N. Y. Agr. Exp. Sta. (Geneva), Bul. 39, N. S. 1892.

[3]Suffran, F. Rev. Gen. Med. Vet. 13, No. 156, 698-705. 1909. Exp. Sta. Rec. 23, 793. 1910.

[4]Edwards, J. T. Jour. Compar. Path. and Ther. 31, 40-43. 1918.

performed upon three pigeons. Solutions containing 0.625, 1.25, and 2.50 grams of salt per kilogram of body weight, respectively, were injected into the crops of the birds. No ill effects from these doses were noted, except a temporary depression of the two birds given the largest doses. Five days later the same birds received doses amounting to 2.50, 3.33, and 4.50 grams per kilogram of body weight. The bird receiving the highest dose died in 18 hours, the bird receiving the next highest dose died in 23 hours, while the one receiving the smallest dose showed no symptoms.

Other experiments concerned with the symptoms of salt poisoning have been performed,[1] but in these experiments no attempt was made to determine the smallest toxic dose of salt. They need not, therefore, be reviewed here.

The evidence bearing on the toxicity of salt for chickens is meager and somewhat contradictory. Because of the common occurrence of salt in domestic and industrial wastes and by-product feeds that are occasionally or regularly fed to poultry, it is of importance to determine definitely whether the use of these wastes and feeds is a constant source of danger to the poultry flock. The experiments reported in this bulletin were undertaken, therefore, with two objects in view: first, to determine the effect of varying percentages of salt in the ration on the rate of growth of chickens; and second, to determine the smallest dose that would cause death.

OUTLINE OF EXPERIMENT

Five lots of 10 chicks each at the poultry farm and five lots of 5 chicks each at the nutrition laboratory were fed a well-balanced basal ration containing varying amounts of salt. Lot 1 at both the poultry plant and the nutrition laboratory received 1 percent of salt in their ration; Lot 2, 2 percent; Lot 3, 4 percent; Lot 4, 8 percent; and Lot 5, 1 percent with free access to rock salt. The lots fed at the poultry farm were kept in shelters open to the south and had the run of lots free from vegetation (Fig. 1). They were group-fed and were weighed individually each week. Feed records were kept on all lots.

The chickens grown in the laboratory were confined in individual pens having a floor space of 4 square feet. They were fed individually and were weighed weekly.

All lots had free access to feed at all times, and the chickens fed individually at the nutrition laboratory were also given as much feed as they would consume. It was hoped that all chickens in the experiment could be selected from the same breed of birds at the same age, but unfortunately this ideal could not be realized. Each lot kept at

[1] Heinz and Haas. München. Med. Wchnschr. 70, 565-66. 1923.

the poultry farm contained 5 White Wyandotte chickens and 5 White Leghorn chickens. The chickens grown at the nutrition laboratory were all Rhode Island Reds. The initial age of all chickens was 8 weeks. In the distribution of the birds among the several lots, the number of cockerels and pullets in each lot was made the same.

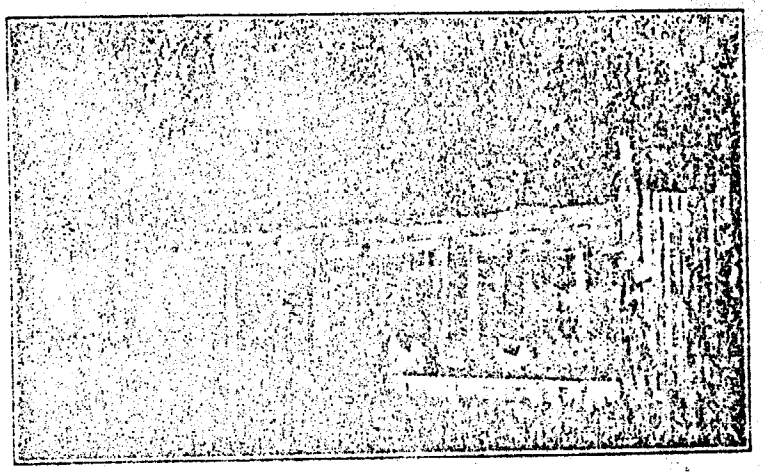

FIG. 1.—EXPERIMENTAL FEEDING PENS AT THE POULTRY PLANT USED FOR THE VARIOUS LOTS

Chicks fed at the poultry farm were kept in shelters open to the south and had the run of the lots, which were free from vegetation.

The feeding experiment at the poultry farm was continued for 13 weeks and that at the nutrition laboratory for 12 weeks. At the end of this time the birds were given salt either in capsules or in solution, in order to determine the minimum amount they could tolerate in repeated doses and the minimum single toxic dose.

EXPERIMENTAL RESULTS

The rations used at the poultry farm and at the nutrition laboratory are given in Table 1. The lots at the farm and at the laboratory receiving equal percentages of salt will be referred to by the same numbers. Lot 5 differed from Lot 1 in having access to salt at all times besides receiving 1 percent in its feed. It was found, however, that these chickens did not consume any of the salt offered to them, or if any was consumed it was so small an amount as to be obscured by the changes in moisture content of the salt offered. The salt intake of Lots 1 and 5 was therefore the same.

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For the first week of the experiment all lots were fed the ration containing 1 percent salt. On June 26, 1925, the lots were put upon the

TABLE 1.—BASAL RATION AND VARIATIONS IN SALT CONTENT FOR VARIOUS LOTS OF CHICKENS

Feed	Lots 1 and 5	Lot 2	Lot 3	Lot 4
Corn	60	59	57	53
Wheat bran	15	15	15	15
Dried buttermilk	15	15	15	15
Steamed bone meal	5	5	5	5
Calcium carbonate ($CaCO_3$)	4	4	4	4
Common salt (NaCl)	1	2	4	8

Ninety-eight parts of the above mixture was in each case mixed with 2 parts of cod-liver oil.

different experimental rations and kept under observation for 12 weeks in the case of the birds at the poultry plant, and for 11 weeks in the case of the birds in the nutrition laboratory.

EFFECT OF SALT ON GROWTH

The rate of growth of the White Wyandotte chickens from June 19 is shown graphically in Fig. 2, the growth of the White Leghorns in Fig. 3, and the growth of the Rhode Island Red chickens in Fig. 4. Among the lots kept at the poultry farm, the growth of Lots 1, 2, 3, and 5 was very similar. Lot 4, receiving 8 percent of salt in its ration, lagged somewhat behind the other lots in its rate of growth, altho this is mainly due to a retardation of growth in the initial weeks of the experiment because of the slow adjustment of the chickens to the extremely salty ration. When the feed intake approximated that of the other groups, the rate of growth was about the same as that of the chickens getting smaller percentages of salt.

There was some mortality among the lots kept at the poultry farm, but the cause of death seemed to be unrelated to the method of feeding. In Lots 1, 2, and 3 there were no deaths, in Lot 4 one White Wyandotte chicken died one week after experimental feeding started, and in Lot 5 two White Leghorn chickens died 11 and 32 days, respectively, after experimental feeding started. No abnormal symptoms referable to the salt intake were observed in any of the groups. Group photographs of Lots 1 and 4 getting, respectively, 1 and 8 percent of salt in their feed, are shown in Figs. 5 and 6. No differences in the condition of the two groups of birds can be seen from a comparison of these photographs. Fig. 7 shows two of the individual birds in Lot 4, one a White Leghorn and the other a White Wyandotte,[1]

[1]Most of the White Wyandottes used in this experiment, including the one shown in Fig. 4, had single combs.

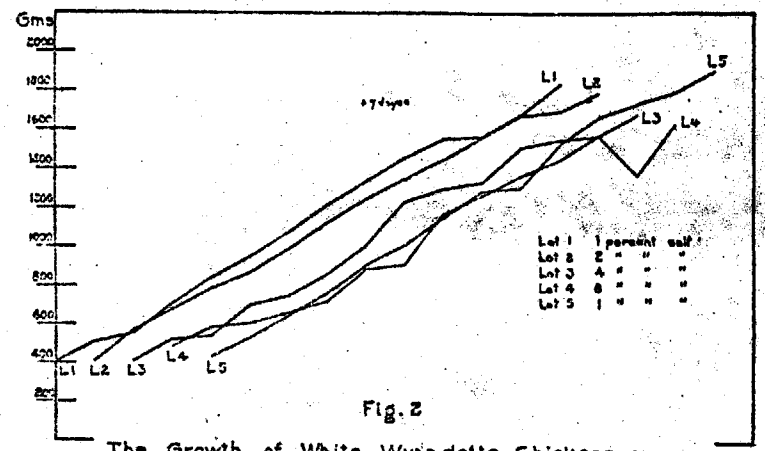

Fig. 2
The Growth of White Wyandotte Chickens on Rations Differing Only in Their Content of Salt

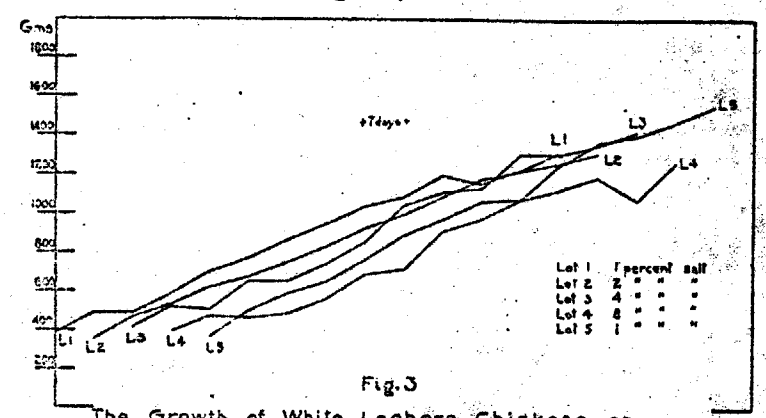

Fig. 3
The Growth of White Leghorn Chickens on Rations Differing Only in Their Content of Salt

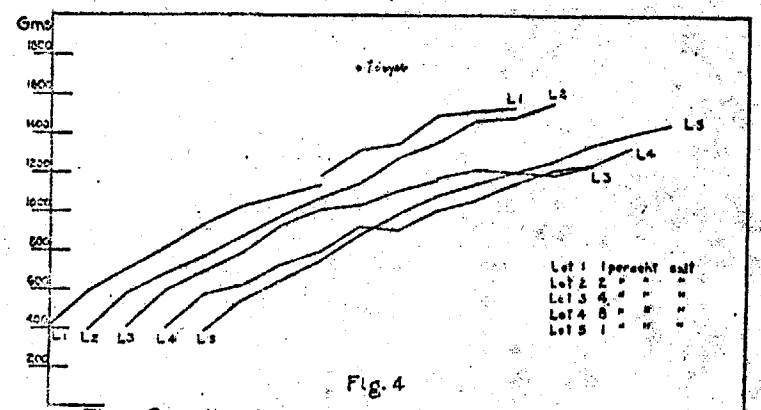

Fig. 4
The Growth of Rhode Island Red Chickens on Rations Differing Only in Their Content of Salt

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FIG. 5.—LOT 1 AFTER THIRTEEN WEEKS ON A RATION CONTAINING 1 PERCENT OF SALT

No difference could be observed in the condition of the birds in Lot 1, which received 1 percent of salt, and Lot 4, which received 8 percent (see Fig. 6).

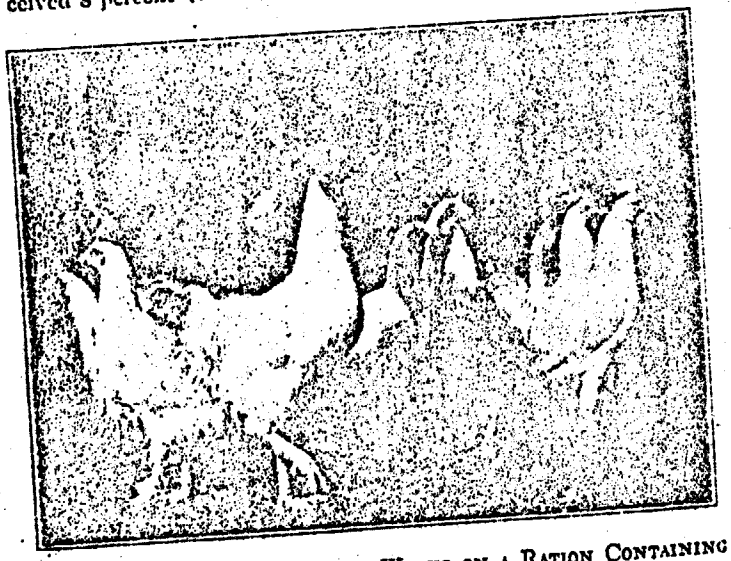

FIG. 6.—LOT 4 AFTER THIRTEEN WEEKS ON A RATION CONTAINING 8 PERCENT OF SALT

The chickens in this lot lagged behind those in the other lots in rate of growth, owing chiefly to retardation of growth in the first weeks of the experiment before they became adjusted to the extremely salty ration. When their feed intake approximated that of the other groups, the rate of growth was about the same as for the chickens receiving less salt.

taken at the end of the feeding test. The chickens in Lot 4, particularly, drank and excreted large amounts of water, this fact accounting for their untidy appearance.

The relative growth observed among the five lots of Rhode Island Red chickens kept at the laboratory was very similar to that of the birds kept at the poultry farm. The growth in Lot 4 again was somewhat slower than that in the lots getting smaller percentages of salt, but after the birds became accustomed to the highly salty ration, the growth in Lot 4 was quite as rapid as that in the other lots; in fact it

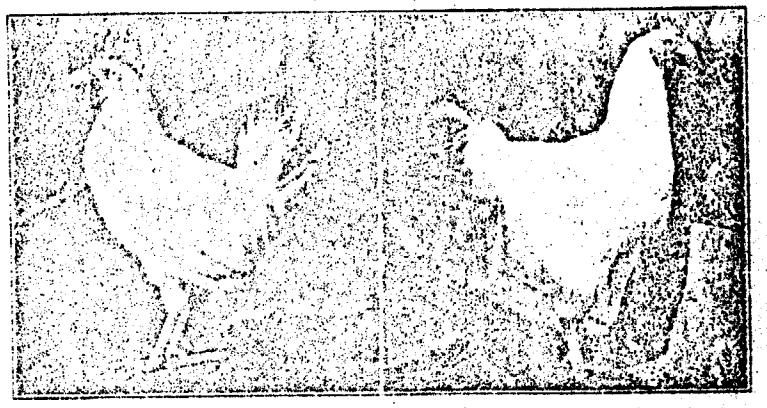

FIG. 7.—INDIVIDUAL BIRDS FROM LOT 4 FED A RATION CONTAINING 8 PERCENT OF SALT

Even this extremely salty ration did not prove to be poisonous to chickens that were eight weeks old when the experiment was started. It was observed, however, that the chickens in this lot drank and excreted large amounts of water.

was somewhat better than that observed in Lot 3, which received 4 percent of salt.

The mortality in these lots also seemed to bear no relation to the method of feeding. One chicken in Lot 1 died after 6 weeks of feeding, and 2 chickens in Lot 3 after 7 weeks of feeding. Continued growth in the lots kept in the nutrition laboratory was prevented by the appearance of leg weakness among many of the birds. Whether this was due to the small percentage of cod-liver oil in the rations or to the confinement of the birds in such small pens cannot be determined.[1] It was entirely unrelated to the salt content of the diet. The feeding experiment in the laboratory was discontinued one week sooner

[1] In an attempt to determine whether the leg weakness observed was due to rickets the tibias and fibulas from each of 7 affected chickens and 5 unaffected chickens were dissected out and analyzed for ash and calcium. The bones were ground, dried, and extracted with ether before analysis. The bones from 3 of the affected birds were normal in appearance and had a normal content of ash

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than that at the poultry farm, because of the prevalence of leg weakness, which interfered seriously with the feeding of many of the birds. The severity of this symptom is illustrated by the three chickens shown in Fig. 8.

The average gains and average feed records of the White Wyandotte and White Leghorn chickens fed at the poultry farm are given

FIG. 8.—BIRDS RAISED IN INDIVIDUAL FEEDING PENS IN THE NUTRITION LABORATORY

Leg weakness developed in many of the birds in the laboratory lots irrespective of the amount of salt in the rations. This condition may have been brought on by the small percentage of cod-liver oil in the rations, or by the confinement of the birds in such small pens.

in Tables 2 and 3. The average daily gains of Lots 1 and 2 receiving 1 and 2 percent of salt in the feed, respectively, were not significantly different for either breed. In Lot 3 the average daily gain of the White Leghorns was slightly higher than the gains for the same breed in Lots 1 and 2. The White Wyandottes in Lot 3, however, gained on the average rather distinctly less per day than the birds of the same breed in Lots 1 and 2. In view of the great variability among the individual gains of Lots 1 and 2, it is probable that the lower average gain in Lot 3 bears no relation to the larger intake of salt. The average daily gain of Lot 4, both with the White Wyandottes and White Leghorns, was slightly less than the average gains of the other groups, probably due to the longer time required by the chickens in this lot to become accustomed to their ration. Lot 5, receiving the same ration as Lot 1, gained on the average at a distinctly better rate, this being true of both the White Wyandotte and White Leghorn chickens that survived thruout the feeding experiment.

and calcium. The bones from 2 of the affected birds were bent, but otherwise normal in appearance, and showed a content of ash and calcium somewhat below that of most of the bones from the unaffected birds. The bones from 2 of the affected birds were bent, soft at the ends, and possessed a distinctly subnormal percentage of ash and calcium and a subnormal percentage of calcium in the ash. The bones from the unaffected birds were perfectly normal in appearance and contained an average of 58.95 percent ash and 22.35 percent calcium on the fat- and moisture-free basis, and an average of 37.91 percent calcium in the ash.

TABLE 2.—AVERAGE WEIGHTS AND GAINS OF THE WHITE WYANDOTTE AND WHITE LEGHORN CHICKENS DURING A TEST OF 84 DAYS
(All weights in grams)

	White Wyandottes						White Leghorns					
Chick No.	1	2	3	4	5	Average	1	2	3	4	5	Average
Lot 1—1 percent of salt												
Initial weight	500	490	560	490	520	512	450	550	560	410	500	488
Final weight	2 030	1 030	1 840	1 690	1 970	1 830	1 060	1 450	1 880	1 150	1 500	1 314
Gain	1 530	1 150	1 280	1 200	1 450	1 318	610	900	880	740	1 000	826
Average daily gain	18.2	13.5	15.2	14.3	17.3	15.70	7.3	10.7	10.5	8.8	11.9	9.84
Lot 2—2 percent of salt												
Initial weight	430	610	570	660	590	572	490	510	490	520	420	468
Final weight	1 670	1 430	1 590	2 120	2 120	1 786	1 070	1 470	1 600	1 220	1 220	1 316
Gain	1 240	820	1 020	1 460	1 530	1 214	670	960	1 110	700	800	848
Average daily gain	14.8	9.8	12.1	17.4	18.2	14.46	8.0	11.4	13.2	8.3	9.5	10.08
Lot 3—4 percent of salt												
Initial weight	420	560	450	640	550	524	460	590	570	520	480	524
Final weight	1 610	1 550	1 610	1 780	1 750	1 690	1 240	1 640	1 600	1 060	1 470	1 420
Gain	1 190	1 000	1 160	1 140	1 200	1 156	780	1 050	1 120	540	990	896
Average daily gain	14.2	13.0	13.8	13.6	14.3	13.78	9.3	12.5	13.3	6.4	11.8	10.66
Lot 4—8 percent of salt												
Initial weight	530	.....[1]	610	530	680	338	500	430	550	380	480	474
Final weight	1 300	.....	1 700	1 530	1 960	1 630	1 260	1 230	1 330	1 100	1 400	1 264
Gain	770	.....	1 090	1 000	1 310	1 042	760	800	750	720	920	790
Average daily gain	9.2	.....	13.0	11.9	15.6	12.42	9.0	9.5	8.9	8.6	11.0	9.40
Lot 5—1 percent of salt												
Initial weight	510	460	470	610	620	534	580	470	450	.....[2]	500[3]	.....
Final weight	1 560	2 020	1 970	2 210	1 750	1 902	1 850	1 690	1 220	.....	560	.....
Gain	1 050	1 560	1 500	1 600	1 130	1 368	1 270	1 220	770	.....	60	.....
Average daily gain	12.5	18.6	17.9	18.9	13.5	16.28	15.1	13.3	9.2	.....	2.1	12.53[4]

[1]This bird died after 1 week on experiment, and is not considered in this connection. [2]This bird died after 11 days on experiment. [3]This bird died after 28 days on experiment. [4]Not including No. 5.

The average daily consumption of feed in the lots kept at the poultry farm was practically the same for Lots 1, 2, 3, and 5, and slightly less for Lot 4. The average amounts of feed required per gram of gain were 6.67 grams for Lot 1, 6.72 grams for Lot 2, 6.80 grams for Lot 3, 7.01 grams for Lot 4, and 6.08 grams for Lot 5. These figures are closely related, of course, to the average daily gains of the lots—the greater the average daily gain, the smaller the average amount of feed required per gram of gain.

The feed and weight records for the Rhode Island Red chickens were obtained for each individual bird. These records with the group

TABLE 3.—FEED RECORDS FOR THE WHITE WYANDOTTE AND WHITE LEGHORN LOTS
Each lot contained 5 birds of each breed.

	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
	grams	*grams*	*grams*	*grams*	*grams*
Total feed	71 552	69 328	69 765	57 445	61 314
Average daily feed	85.18	82.52	83.05	75.29	85.75
Average daily gain	12.76	12.27	12.21	10.74	14.10
Feed per gram gain	6.67	6.72	6.80	7.01	6.08

averages are given in Table 4. The average daily gains of the five lots were, respectively, 11.64, 12.72, 9.60, 9.87, and 11.74 grams. The average daily feed intakes for the five lots were 79.6, 81.8, 69.8, 76.2, and 76.0 grams. The average economy of gains as expressed in the amount of feed required per gram of gain was approximately the same for Lots 1, 2, and 5, namely, 6.85, 6.50, and 6.66 grams, respectively. The average economy of gains in Lots 3 and 4 was distinctly less, namely, 7.99 and 7.80 grams of feed per gram of gain, tho some of the individuals in these lots were as economical in the feed cost of gains as individuals in the other lots.

It may be concluded from the results of these feeding experiments that the rate and economy of gains in growing chickens is distinctly retarded by 8 percent of salt in the diet, tho this effect is largely due to a slow adjustment of the appetite of the chickens to such a salty ration. It cannot definitely be concluded whether 4 percent of salt in the diet retards the growth of chickens. Some evidence favoring the belief that growth was retarded was obtained with White Wyandottes and Rhode Island Red chickens, tho some of the individual gains obtained on the 4-percent salt ration were as high as or higher than the gains secured on the ration containing only 1 percent of salt. The Rhode Island Red chickens receiving 4 percent of salt showed such great variation among the individual birds that the averages cannot be considered representative of the group.

TABLE 4.—AVERAGE WEIGHTS, GAINS, AND FEED CONSUMPTION OF THE RHODE ISLAND RED CHICKENS
(All weights in grams)

Lot 1—1 percent of salt						
Chick No.	1	2	3	4	5	Average
Days on experiment	77	42	77	77	77	.....
Average—						
Initial weight	562	537	792	574	491	591
Final weight	1 360	952	1 850	1 500	1 430	.....
Total gain	798	415	1 058	926	939	.....
Daily gain	10.36	9.88	13.74	12.03	12.19	11.64
Total feed	5 873	2 821	7 098	5 753	6 755	.....
Average daily feed	76.3	67.2	92.2	74.5	87.7	79.6
Feed per gram of gain	7.37	6.80	6.71	6.19	7.19	6.85
Lot 2—2 percent of salt						
Chick No.	1	2	3	4	5	Average
Days on experiment	77	77	77	77	77	.....
Average—						
Initial weight	610	545	575	449	725	581
Final weight	1 850	1 560	1 450	1 300	1 640	1 560
Total gain	1 240	1 015	875	851	915	979
Daily gain	16.12	13.18	11.36	11.05	11.88	12.72
Total feed	6 972	6 727	6 594	5 551	5 603	.....
Average daily feed	90.5	87.4	85.6	72.1	73.5	81.8
Feed per gram of gain	5.61	6.63	7.54	6.52	6.19	6.50

TABLE 4.—*Continued*

Lot 3—4 percent of salt						
Chick No.	1	2	3	4	5	Average
Days on experiment	70	49	77	49	77	.....
Average—						
Initial weight	758	605	512	565	551	598
Final weight	1 230	1 036	880	1 255	1 600	.....
Total gain	472	431	368	690	1 049	.....
Daily gain	6.74	8.80	4.78	14.08	13.62	9.60
Total feed	5 285	2 653	3 794	4 039	6 748	.....
Average daily feed	75.5	54.1	49.3	82.4	87.6	69.8
Feed per gram of gain	11.20	6.15	10.31	5.85	6.43	7.99

Lot 4—8 percent of salt						
Chick No.	1	2	3	4	5	Average
Days on experiment	77	77	77	77	77	.....
Average—						
Initial weight	546	569	683	587	505	578
Final weight	1 190	1 320	1 590	1 400	1 190	1 338
Total gain	644	751	907	813	685	760
Daily gain	8.36	9.75	11.78	10.56	8.90	9.87
Total feed	6 048	6 020	6 482	5 572	5 201	.....
Average daily feed	78.5	78.2	84.2	72.4	67.5	76.2
Feed per gram of gain	9.39	8.02	7.15	6.86	7.58	7.80

TABLE 4.—*Concluded*

Lot 5—1 percent of salt						
Chick No.	1	2	3	4	5	Average
Days on experiment	77	77	77	77	77	.....
Average—						
Initial weight	512	608	496	590	534	548
Final weight	1 590	1 380	1 245	1 300	1 745	1 452
Total gain	1 078	772	749	710	1 211	904
Daily gain	14.00	10.03	9.73	9.22	15.73	11.74
Total feed	6 629	6 083	4 970	5 306	6 279	.....
Average daily feed	86.1	79.0	64.5	68.9	81.5	76.0
Feed per gram of gain	6.15	7.88	6.63	7.47	5.18	6.66

Information concerning the daily salt intake per bird and per kilogram of body weight is contained in Table 5 for the White Wyandotte and White Leghorn chickens, and in Table 6 for the Rhode Island Red chickens, the individual data being given in the latter case. The average daily intake of salt for all groups ranges from 0.72 gram to 6.32 grams per kilogram of body weight. The average daily intake of salt

TABLE 5.—AVERAGE SALT INTAKE PER DAY AND PER KILOGRAM OF BODY WEIGHT FOR THE WHITE WYANDOTTE AND WHITE LEGHORN CHICKENS

Each lot contained 5 birds of each breed.

	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
	grams	*grams*	*grams*	*grams*	*grams*
Average weight	1 044	1 064	1 045	955	1 131
Average daily feed	85.18	82.52	83.05	75.29	85.75
Average daily salt intake	0.852	1.650	3.322	6.023	0.857
Salt intake per kilogram of body weight per day	0.816	1.551	3.179	6.306	0.758

per bird for the lots getting 8 percent in their ration was 6.32 grams for the Rhode Island Red chickens and 6.31 grams for the White Wyandottes and White Leghorns. The largest intake of salt per kilogram of body weight per day among the individual Rhode Island Red birds of Lot 4 was 6.87 grams for bird No. 1.

The figures just considered refer to the average daily intake of salt for the entire period of feeding. For the individual weeks of the experiment the intake of salt was in general higher in the early weeks. For example, in Lot 4 at the poultry farm, consisting of the White Wyandotte and White Leghorn chickens, the average intake of salt per kilogram of body weight per day for the first 4 weeks of the experiment was, in order, 5.66, 8.37, 8.75, and 6.85 grams.

The daily intake of salt per kilogram of body weight for the individual Rhode Island Red chickens in Lot 4 for all weeks of the experiment will be found in Table 7. It is evident that some of the individual birds in this group tolerated 8 to 9 grams of salt per kilogram of body weight per day without developing any pathological symptoms or showing a markedly diminished rate of growth.

MINIMUM LETHAL DOSE OF SALT

At the conclusion of the feeding experiment the birds were given varying doses of salt, either in the solid form in capsules, or in a 20-percent solution, in order to determine the maximum amount of salt that could be tolerated both in repeated doses and in single doses. In the first tests undertaken in this connection, the birds were given two doses a day at approximately 8:30 a. m. and 4:00 p. m. In these tests the doses were not adjusted to the size of the bird, but in the last test the doses were graduated according to the body weight.

The first test, which was made upon Rhode Island Red chickens from the laboratory lots, was concerned with the determination of the maximum dosage of salt that could be tolerated by the birds when given repeatedly at the rate of two doses a day. In the first day of the test two doses of 2, 4, 6, 8, and 10 grams were given groups of 3 or more birds taken from Lots 1 to 5. The salt was given by pipette in a 20-percent solution. During the test the birds had no access to feed, but water was kept before them continually except when the water containers became exhausted at night. All birds getting doses of 6, 8, and 10 grams were dead either on the afternoon of the first day or by morning of the second day. None of the birds getting 2- and 4-gram doses died during the first day. The birds surviving on the morning of the second day were therefore divided into two lots and given, respectively, 4- and 6-gram doses. Of those getting 6-gram doses, 5 survived two doses only, and 2 survived four. Of the birds getting 4-gram doses, 2 survived four doses only, 1 survived six doses, and the other bird, which was the lightest in the lot, tolerated two 4-gram doses daily for about a month with no apparent detriment. The results of the test are summarized in Table 8. The body weights given in this table were the final weights of the birds in the feeding experiment, which terminated exactly one week before this test was started.

The symptoms of the birds that died were very similar to those that have been reported in the literature. Intense thirst accompanied the treatment in all cases, and death was preceded by a period of apparent stupor in which the bird stood or squatted with closed eyes and hanging head. No pronounced convulsions were noted preceding death.

Thru the courtesy of the Laboratory of Animal Pathology and Hygiene, 9 of the birds that succumbed in this test were given post-mortem examination by Dr. E. A. Tunnicliff. The mucous membrane of the digestive tube was in all cases found to be irritated, as evidenced by hyperemia, petechia, or ecchymosis of the blood vessels. Congestion of the liver was a constant finding.

It is evident from this test that 8 grams of salt per day could not be tolerated continuously by a majority of chickens. Doses larger than 8 grams per day were quickly fatal.

In continuing the experiments in the individual dosing of birds with salt, the question arose as to whether the consumption of feed had anything to do with the survival period of the birds. The second test was therefore concerned with determining whether a difference in susceptibility to salt poisoning exists between birds in a fasting and a fed condition. Three groups of 6 birds each taken from Lots 1 to 5 at the poultry farm were given, respectively, twice daily, 4-, 5-, and 6-gram doses of salt. In each of the three groups 3 of the birds were

2 2 9 4

TABLE 6.—AVERAGE SALT INTAKE PER DAY AND PER KILOGRAM OF BODY WEIGHT FOR THE RHODE ISLAND RED CHICKENS
(All weights in grams)

Lot 1—1 percent of salt						
Chick No.	1	2	3	4	5	Average
Average weight	1 105	804	1 375	1 065	981	.....
Average daily feed	76.3	67.2	92.2	74.5	87.7	79.6
Average daily salt intake	0.76	0.67	0.92	0.74	0.88	0.80
Salt intake per kilogram body weight per day	0.69	0.84	0.67	0.70	0.89	0.76

Lot 2—2 percent of salt						
Chick No.	1	2	3	4	5	Average
Average weight	1 253	1 097	1 032	913	1 232	.....
Average daily feed	90.5	87.4	85.6	72.1	73.5	81.8
Average daily salt intake	1.81	1.75	1.71	1.44	1.47	1.64
Salt intake per kilogram body weight per day	1.44	1.59	1.66	1.58	1.19	1.49

Lot 3—4 percent of salt						
Chick No.	1	2	3	4	5	Average
Average weight	1 163	895	793	935	1 094	.....
Average daily feed	75.5	54.1	49.3	82.4	87.6	69.8
Average daily salt intake	3.02	2.16	1.97	3.30	3.50	2.79
Salt intake per kilogram body weight per day	2.60	2.42	2.49	3.52	3.20	2.85

TABLE 6.—*Concluded*

Lot 4—8 percent of salt						
Chick No.	1	2	3	4	5	Average
Average weight	914	941	1 143	974	864	.....
Average daily feed	78.5	78.2	84.2	72.4	67.5	76.2
Average daily salt intake	6.28	6.26	6.74	5.79	5.40	6.09
Salt intake per kilogram body weight per day	6.87	6.65	5.89	5.95	6.25	6.32

Lot 5—1 percent of salt						
Chick No.	1	2	3	4	5	Average
Average weight	1 076	1 040	921	1 124	1 152	.....
Average daily feed	86.1	79.0	64.5	63.9	81.5	76.0
Average daily salt intake	0.86	0.79	0.64	0.69	0.81	0.76
Salt intake per kilogram body weight per day	0.80	0.76	0.70	0.61	0.71	0.72

TABLE 7.—AVERAGE SALT INTAKE PER DAY AND PER KILOGRAM OF LIVE WEIGHT BY THE RHODE ISLAND RED CHICKENS IN LOT 4 GETTING A RATION CONTAINING 8 PERCENT OF SALT

(All weights in grams)

Week	Salt per day						Salt per day per kilogram weight					
	1	2	3	4	5	Average	1	2	3	4	5	Average
1	4.56	5.28	5.12	4.64	4.32	4.78	7.79	9.25	7.06	7.81	8.23	8.02
2	4.72	5.36	5.28	4.56	4.64	4.91	6.97	8.43	6.49	6.91	7.64	7.29
3	4.88	5.84	5.60	4.72	4.88	5.18	6.38	7.74	6.21	6.65	6.67	6.73
4	6.16	6.40	6.64	5.76	5.68	6.13	7.25	7.47	6.49	7.32	7.09	7.12
5	6.00	6.64	6.64	6.56	6.16	6.40	6.81	7.43	6.14	7.45	7.22	7.01
6	6.88	6.72	6.88	6.88	5.68	6.61	7.45	7.24	6.19	7.11	6.45	6.89
7	6.72	5.60	6.64	5.68	4.56	5.84	6.69	5.70	5.52	5.22	4.95	5.62
8	3.00	6.32	7.44	6.16	5.52	6.69	7.60	6.04	5.70	5.28	5.71	6.07
9	6.32	6.32	7.44	5.60	5.12	6.16	5.83	5.47	5.27	4.49	4.97	5.21
10	8.00	7.44	8.40	6.24	6.00	7.22	7.20	6.02	5.73	4.93	5.54	5.88
11	6.88	6.88	8.00	6.88	6.88	7.10	5.92	5.33	5.21	5.22	5.99	5.53

fasted during the test while the 3 remaining birds were given access to feed. The results of this test are summarized in Table 9.

The results do not show any marked differences in the survival period, whether the birds were fasting or had access to feed. A slight difference favoring the fasting birds may be noted, but it is questionable whether the difference is significant in view of the different behavior among birds given the same treatment. All doses proved to be rather quickly fatal, except in the case of one bird receiving 8 grams of salt daily, which survived for 10 days. The salt in this test was fed in the solid form and was administered in capsules.

A third test, the results of which are summarized in Table 10, was concerned with the toxicity of 3-, 4-, and 5-gram doses of salt given twice daily. While the results indicate an increased mortality with increasing dosage, it is evident that even the smallest dose could not be tolerated by these birds for any great length of time, altho the birds in Lot 4 consumed an average of over 6 grams of salt daily thruout the feeding experiment.

TABLE 8.—MINIMUM LETHAL REPEATED DOSE OF SALT FOR RHODE ISLAND RED CHICKENS

(Doses given twice daily, approximately 8:30 a. m. and 4:00 p. m.)

Bird No.	Lot	Body weight[1]	Size of dose	Number of doses taken before death
		grams	*grams*	
2	5	1 380	4	4
1	5	1 500	4[2]	4
3	4	1 590	4	6
2	4	1 320	4	indefinite[4]
4	1	1 500	6	2
3	2	1 450	6	2
1	1	1 360	6[2]	2
1	2	1 850	6	2
2	2	1 560	6[3]	2
3	3	880	6[3]	4
1	4	1 190	6[3]	4
5	1	1 430	8	2
4	2	1 300	8	2
4	4	1 400	8	2
4	5	1 300	8	2
5	2	1 640	10	2
5	4	1 190	10	2
5	5	1 745	10	2

[1]These weights were taken 7 days before the first dosage of salt.
[2]Received 2 doses of 2 grams of salt the day before.
[3]Received 2 doses of 4 grams of salt the day before.
[4]This chicken was given 4 grams of salt twice daily, for almost a month, at the end of which time it was in good condition.

TABLE 9.—SUSCEPTIBILITY TO SALT POISONING OF BIRDS IN FASTING AND FED CONDITIONS

Breed	Body weight[1]	Number of doses given before death					
		4-gram doses		5-gram doses		6-gram doses	
		Fasting	Fed	Fasting	Fed	Fasting	Fed
	grams						
Wyandotte	1 500	2	...	...	...	...	...
Leghorn	1 220	4	...	...	...	...	...
Wyandotte	2 210	20	...	...	...	...	...
Wyandotte	2 020	...	2	...	...	...	...
Leghorn	1 590	...	4	...	...	...	...
Leghorn	1 850	...	5	...	...	...	...
Wyandotte	1 970	...	...	2	...	...	...
Wyandotte	1 970	...	...	3	...	...	...
Leghorn	1 500	...	...	4	...	...	...
Leghorn	1 090	...	...	...	2	...	...
Wyandotte	1 690	...	...	...	2	...	...
Leghorn	1 150	...	...	...	2	...	...
Leghorn	1 450	...	...	...	...	2	...
Wyandotte	2 030	...	...	...	...	3	...
Leghorn	1 500	...	...	...	...	4	...
Leghorn	1 380	...	...	...	...	...	2
Wyandotte	1 840	...	...	...	...	...	2
Leghorn	1 620	...	...	...	...	...	4
Leghorn							
Wyandotte							
Wyandotte							

[1]These weights were taken 3 days previous to the first dosage of salt.

In the tests so far reported, no attention was paid to the body weights of the birds, tho a study of the survival periods does not indicate that the heavier birds were markedly more resistant to salt than lighter birds given the same treatment. In the next two tests, however, the dosage was given in proportion to the live weight of the birds. In the first of these tests, three groups of 4 birds each were given, respectively, 2, 4, and 6 grams of salt per kilogram of body weight, the salt being administered in a 20-percent solution. Only one dose was given in this case, the attempt being to find the minimum lethal single dose of salt. All of the birds getting the 6-gram dose per

TABLE 10.—MINIMUM LETHAL REPEATED DOSE OF SALT FOR WHITE WYANDOTTE AND WHITE LEGHORN CHICKENS

(Doses given twice daily, approximately 8:30 a. m. and 4:00 p. m.)

Breed	Lot	Bird No.	Body weight[1]	Size of dose	Number of doses taken before death
			grams	*grams*	
Leghorn	2	2	1 470	3	3
Leghorn	4	3	1 330	3	3
Wyandotte	4	1	1 300	3	11
Leghorn	3	2	1 640	3	12
Wyandotte	4	5	1 990	3	12
Wyandotte	2	5	2 120	3	12
Leghorn	3	5	1 470	4	2
Wyandotte	4	3	1 700	4	2
Leghorn	4	2	1 230	4	2
Leghorn	2	3	1 600	4	3
Wyandotte	3	1	1 610	4	8
Leghorn	4	5	1 400	4	10
Wyandotte	2	1	1 670	4	10
Leghorn	2	5	1 220	5	2
Wyandotte	3	3	1 670	5	2
Leghorn	4	2	1 230	5	2
Wyandotte	2	4	2 120	5	3
Leghorn	3	3	1 690	5	3
Wyandotte	3	4	1 780	5	11

[1]These were the final weights of the birds in the feeding experiment which ended 7 days before the test reported upon in this table.

kilogram of body weight and two of those getting the 4-gram dose were dead within 24 hours. All other birds survived the treatment. The test indicated that the minimum single lethal dose was close to 4 grams per kilogram of body weight.

In an attempt to determine the minimum lethal dosage more definitely, 6 birds from the White Wyandotte and White Leghorn groups were given, first, a single dose of 3 grams of salt per kilogram of body weight; no deaths occurred in the following 72 hours. At the end of this time they were given a second dose of 4 grams of salt per kilo-

gram of body weight, and at the end of 72 hours all birds were still alive. A third dose of 5 grams per kilogram of body weight was then administered, and within the following 24 hours 5 of the 6 birds had died with symptoms typical of salt poisoning. The remaining bird was unaffected. This test indicates rather clearly that 4 to 5 grams of salt per kilogram of body weight is close to the minimum lethal single dose of salt when administered in solution. There is no reason to suppose that these results would have been different if salt in solid form had been given, provided free access to water had been permitted, as was done in all the tests here reported. However, the preceding test indicates that 4 grams of salt per kilogram of body weight in a single dose may be fatal to some birds.

CONCLUSIONS

Chickens may be raised from 9 to 21 weeks of age on rations containing as high as 8 percent of salt with no apparent detrimental effects on their condition. Furthermore, after the birds have become accustomed to such salty rations, they will consume them in such amounts as to promote a rate of growth approximating that of birds receiving much smaller percentages of salt in their feed.

A daily intake of salt *in the feed* of 6 to 8 grams per bird appears to exert no harmful effect in birds of 9 weeks or more in age.

For birds weighing from 2 to 4 pounds each, 12 to 16 grams of salt daily administered directly in the crop in two equal doses is quickly fatal. Eight grams of salt daily, given in two doses, can ordinarily be tolerated for as long as 5 days, and occasionally indefinitely. However, even on this dosage death within 24 hours may occur in some cases. Six grams per day given in two portions is generally tolerated for 5 to 6 days, tho in a small proportion of cases death results much sooner.

Salt administered in solution twice daily cannot be tolerated so well as equal amounts of salt ingested with the feed. However, the consumption of feed after the administration of toxic doses of salt by injection into the crop, does not alleviate the symptoms produced nor prolong the life of the bird to an appreciable extent.

The minimum lethal single dose of salt for birds weighing from 3 to 5 pounds is close to 4 grams per kilogram of body weight. Most of the birds so treated survive, tho a few may succumb within 24 hours. Birds given 3 grams of salt per kilogram of body weight survive the treatment.

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POST-GRADUATE MEDICAL SCHOOL AND HOSPITAL

THE TREATMENT OF HIGH BLOOD-PRESSURE

A BLOOD-PRESSURE that is higher than normal may occur as a symptom of nephritis, certain cerebral disturbances, nervous excitement, aortic insufficiency, and of other conditions. If all such symptomatic hypertensions are excluded there remains a far greater number whose supernormal blood-pressure cannot be explained on clinical grounds. These cases have been variously named essential hypertension, hypertensive cardiovascular disease, hyperpiesis, etc. Objection has been made to these terms because they do not adequately describe the disease. This criticism is perfectly correct; in answer, however, it may be stated that these names are honestly conceived, because at the present time we know nothing more than the fact that hypertension may exist without known cause and without accompanying symptomatology or pathology of any kind. It is the treatment of this malady which we wish to take up this morning. Every one does not agree with all of the above statements, especially in relation to our lack of knowledge in regard to the causation of essential hypertension. It is very necessary that we should have as clear an understanding as possible concerning the causation and mode of production of this condition, for it is only upon this basis that we can agree upon the proper therapy for it. Hence these questions should be carefully considered at this time.

Some Facts Regarding the Relation of the Kidneys to Essential Hypertension.—In 1836 Richard Bright observed that cardiac hypertrophy and dilatation were often present in nephritis. Such changes in the heart have been and are still

considered to indicate that an increased blood-pressure existed during life. This conclusion is correct in the majority of instances. There are some cases of marked cardiac hypertrophy and dilatation that occur in adults and occasionally in children without apparent cause, the so-called idiopathic cardiac hypertrophies, that may for the moment be disregarded.

If we study the relationship of hypertension to nephritis from another angle, that is, during life, instead of at the autopsy table, we find that when the blood-pressure is raised above the normal a nephritis of some sort or degree is almost certainly present. Coupling these two corroborative facts as developed by the clinician, on the one hand, and the pathologist, on the other, it is evident that kidney disease and hypertension are closely related to one another. Thus far our reasoning has been perfectly sound. If in the joy of acquired knowledge we now recklessly assume that every instance of hypertension is secondary to kidney involvement, we are taking unwarranted liberties with the facts at our command. However, this is precisely what has been done, and has led to the belief that an increased blood-pressure is an inevitable sign of Bright's disease; this idea was firmly adhered to until about fifteen years ago; today it is still unduly honored, but not to the same extent.

It is worth while to pause for a moment and take stock of our precise knowledge, clinical and experimental, of the relation of the kidney to blood-pressure. The pivotal idea has always been that a diminished excretory activity of the kidney results in efforts intended to restore normal renal function. Among these changes an increase of arterial pressure is considered to be one of the most important. A great deal of evidence both for and against this theory has been developed.

In animal experiments it has been demonstrated that a reduction of kidney substance results in an increase of blood-pressure (Janeway, Pässler, and Heineke). A total removal of both kidneys, however, does not bring this about. This curious fact has its counterpart in clinical medicine. Prostatic hypertrophy with its attendant, mechanical interference with

urinary elimination, often results in a rise of arterial pressure (O'Conor, Monakow, and Mayer). The blood-pressure has a tendency to return to a normal level when the urinary flow is again properly established by a retention catheter or operative relief. Complete anuria, on the other hand, leaves the blood-pressure comparatively unchanged. This is well illustrated when a fatal termination is brought on by complete suppression of urine in bichlorid of mercury poisoning when the blood-pressure remains unchanged; there are occasional exceptions to this rule; because of their rarity such cases have been treasured; Janeway and Volhard have reported examples of this sort in which there was a very moderate increase in arterial pressure. It is evident that there is a distinct relation between urinary flow and hypertension, but that there is not a mathematic increase in the latter as the former progressively diminishes. There must be another element in the situation that has not been satisfactorily determined. We can approach the problem a little more intelligently if we are able to grasp the vascular mechanism that is responsible for hypertension.

The Part Played by the Vascular System in Producing Hypertension.—There are two factors concerned in maintaining the arterial blood under pressure. These are the volume of blood injected into the arterial reservoir with each heart-beat and the tonicity of the arteries, both large and small. The character of the heart-beat may be considered as the main element determining the systolic pressure, the tonicity of the artrieoles controls the diastolic tension.

When the heart is not beating, that is, during diastole, the degree of arterial pressure depends principally on the tonicity of the arterial wall. It is clear that in a rigid system of pipes with a pump causing an inflow of fluid at one end and an outlet provided at the other an increase of pressure of the contained fluid occurs only when the pump exerts its force; if this ceases the inelastic walls of the system allow the contained fluid to remain at a zero pressure. This state of affairs is approached in conditions of arteriosclerosis, when the blood-vessels become hard, lose their elasticity, contractility, and

consequently their tonicity. The diastolic pressure in such cases must fall below the normal level and the systolic rise, since the blood propelled by the heart meets an unyielding resistance. In former years, before the diastolic blood-pressure was as much studied as it is today, "hardening" of the arteries was considered to be one of the main causes of hypertension. Today we know that very extensive sclerosis of the arteries may exist without very much change in the blood-pressure. This is a matter of clinical observation. These cases are the ones in which the sclerotic process in the blood-vessels has been a primary one. There is another group of patients in which hypertension and thickened arteries occur simultaneously; this is far the more frequent type. In this instance the strain imposed by the increased blood-pressure has brought on the degenerative changes in the arteries. If rigidity of the blood-vessels—arteriosclerosis—lowers the diastolic arterial pressure and raises the systolic but slightly, where are we to seek for the cause of hypertension?

An increased blood-pressure is not a constant phenomenon, as is shown in Figs. 222–224. In these charts it is evident that the degree of variation that occurs is fairly large. This variability of arterial tension furnishes a clue as to the nature of high blood-pressure. There can only be one factor responsible for it under the circumstances, and that is an augmented muscular action on the part of the heart and the blood-vessels. When the tonicity of the arteries is increased the diastolic pressure must rise, for the blood is then held under greater tension than usual throughout the cardiac cycle. It follows, of course, that the systolic pressure increases as well, for the heart forces the blood into a reservoir which presents a greater resistance that must be overcome if the circulation is to be maintained. It has frequently been assumed that such a tonic state of the arterial musculature is the sole factor responsible for hypertension; if this were so the diastolic and systolic pressures should vary in constant proportion. This is not the case. The following data taken from a number of patients may furnish evidence to substantiate this statement (Table 1).

TABLE 1

SHOWING THE VARIOUS RELATIONS THAT MAY EXIST BETWEEN SYSTOLIC AND DIASTOLIC BLOOD-PRESSURES

Blood-pressure.		Remarks.
Systolic.	Diastolic.	
230	92	Group No. 1: Examples of comparatively low diastolic pressure and markedly increased systolic pressure.
184	90	
220	98	
180	95	
225	94	
200	96	
234	134	Group No. 2: Examples of simultaneous increase of systolic and diastolic blood-pressure.
232	132	
200	130	
245	140	
280	136	
230	135	
170	120	Group No. 3: Examples of increased diastolic pressure with a comparatively slight rise in systolic tension; these cases are comparatively rare.
177	120	
161	120	
190	130	
170	110	

From Table 1 it is apparent that the systolic pressure usually rises more in hypertensive disease than the diastolic, and when the increased blood-pressure diminishes the systolic drops much more than the diastolic (Fig. 222). These facts make it evident that the force of the individual heart-beat is likely to vary independently of the state of arterial tone.

The variations in diastolic pressure indicate the degree of tonicity in the arteries. This is not necessarily increased in hypertension, which may affect only the systolic pressure as shown in Group No. 1, Table 1. On the other hand, in the rather uncommon cases in which the diastolic pressure is disproportionately raised as compared to the systolic tension it is evident that the function of the arteries is mainly disturbed, and that of the heart scarcely at all.

We may conclude, therefore, that two factors are concerned in the production of hypertension, the increased force of the heart-beat and the pathologic degree of tonicity in the arteries, and that to a great degree these elements act independently of one another.

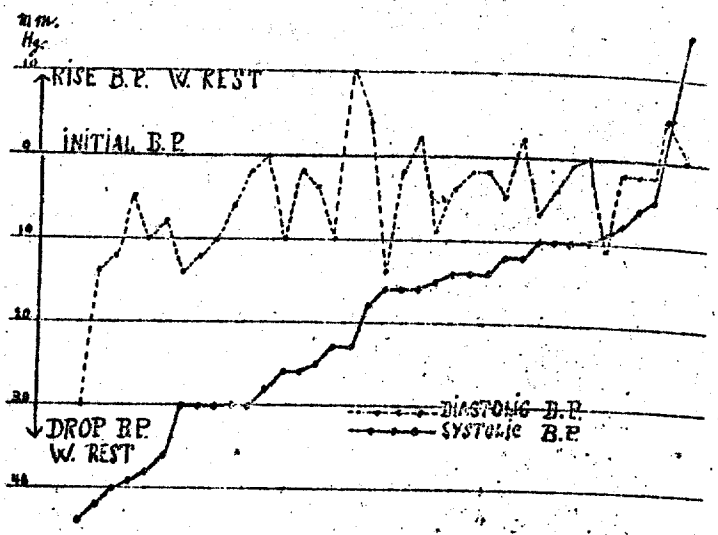

Fig. 222.—Variation of systolic and diastolic blood-pressures from the initial pressure on lying down (short periods of rest) in hypertensive patients (systolic blood-pressure of 160 or higher). The horizontal line marked 0 represents the initial pressure. Every vertical pair of dots represents the greatest drop recorded in each case. The distance below the line indicates the amount of drop obtained. The systolic readings of the various cases are connected by a solid line, the diastolic, by a broken line. The observations are charted in the order of degree of drop in the systolic pressure.

The variations are decidedly less in the diastolic than in the systolic pressure. The drop in diastolic pressure, except in the first case, is so slight that it does not appear to be of great clinical significance; this is in marked contrast to the changes that occur in the systolic pressure, which often shows a very great fall on resting.

The Cause of the Increased Heart Action and Tonicity of the Arterioles in Hypertension.—It is well established that nervous influences cause the blood-pressure to rise and fall in a most bizarre way. O'Hare and Boas have called attention

to this fact recently. In Figs. 222–224 are given the results obtained by short and more prolonged periods of rest. It is perfectly evident that within a brief space of time, fifteen minutes to one hour, relaxation brings about a drop in blood-pressure (Figs. 222, 223); if the stay in bed is prolonged over a period

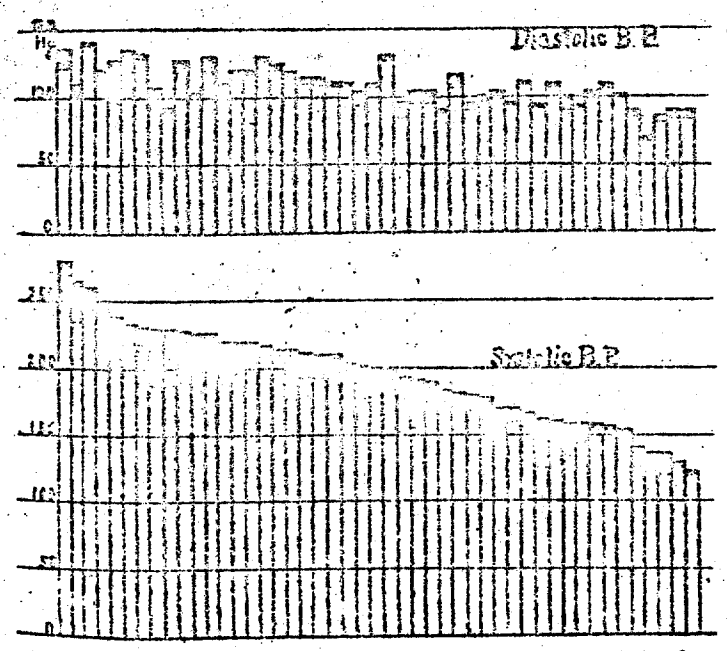

Fig. 223.—Drop in blood-pressure obtained with short periods of rest. The lower columns represent the systolic blood-pressure; the superimposed upper column, the diastolic blood-pressure in the same patient. Each set of columns corresponds to a different case. The entire column represents the initial blood-pressure; the dark portion of the column indicates the drop in blood-pressure which occurred.

of several days the diminution in blood-pressure is much more marked and the normal level is approached in many instances (Fig. 224). The drop in tension affects both the systolic and diastolic pressures, though the drop in diastolic pressure is not as constant nor as great (Fig. 222). Such observations make it certain that the release from overactive nervous impulses

affects both the action of the heart and arterioles favorably, and inasmuch as the systolic pressure undergoes the greater improvement it is only fair to surmise that the heart is more readily influenced than the arterioles. All these facts are of great importance in directing the treatment of these cases, and will be taken up a little later.

At this point I wish to accentuate one fact, which may be gleaned particularly from the marked fluctuations in the blood-

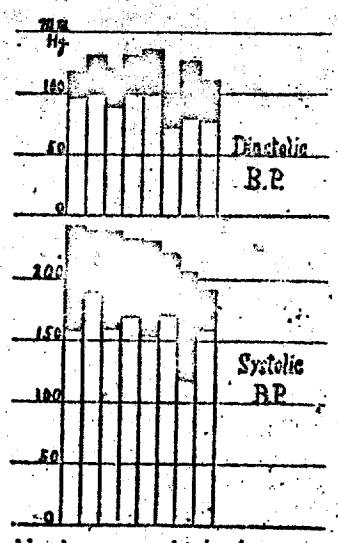

Fig. 224.—Drop in blood-pressure obtained over a period of several days' rest in bed. The columns and their dark areas have the same significance as those in Fig. 223. The diminution of blood-pressure is much greater with prolonged rest than it is in shorter periods of quiet, as a comparison with Fig. 223 shows.

pressure shown in Fig. 224: Hypertension is apparently not a compensatory factor that cannot be dispensed with; the patients shown in Fig. 224 were as comfortable and as symptomless when the blood-pressure was low as when it was high. Hence the idea, prevalent some years ago, that an increased arterial tension should not be interfered with because it was a compensatory exaggeration of function necessary to maintain the well-being of the individual does not appear to be correct, and

we are at liberty to lower the blood-pressure as near to normal as possible without causing any harm.

Why Should Hypertension Be Reduced?—If an increased blood-pressure causes no symptoms, why should attempts be made to lessen it? A rise in arterial tension in itself does no harm, but if it continues indefinitely it results in certain changes that are of vital importance to the patient. These are:

1. Cardiac hypertrophy and dilatation, and finally myocardial insufficiency.
2. Cerebral arteriosclerosis, entailing changes in the brain, especially hemorrhage.
3. Arteriosclerosis of the renal vessels, resulting in primary contracted or arteriosclerotic kidney.

The three sets of changes mentioned above are not the only ones that commonly occur, but they are the usual three vital processes that are the sequels to an increased blood-pressure. This must be recognized, for if the hypertension cannot be controlled, then prophylactic treatment must be instituted to forestall as far as possible the development of symptoms from its almost inevitable complications.

It is of distinct help in the regulation of the treatment of essential hypertension to know which of the three organs—heart, brain, or kidney—is most likely to be involved. Thanks to the statistics of Theodore Janeway and others, which I believe anyone who practices internal medicine will be able to affirm from his clinical experience, myocardial insufficiency is the usual cause of death in these patients, next in frequency is the occurrence of cerebral complications, and last and by far the least common is uremia induced by deficient activity of the kidney. The actual percentages of the causes of death in Janeway's series were as follows:

Cardiac:	Cardiac insufficiency	35.0	46.7 per cent.
	Angina pectoris	7.3	
	Edema of lungs	4.4	
Uremic:	Convulsions, coma, or gradual uremia		22.6 per cent.
Cerebral:	Apoplexy or its results		14.6 per cent.

(It is freely acknowledged today that many of the cases that have been diagnosed as uremia are, in reality, due to the changes accompanying cerebral arteriosclerosis. A study of renal function by more modern methods has revealed this fact. It is probable, therefore, that the above figures should show a higher percentage of deaths due to apoplexy and its results and a lower mortality from uremia.)

It is interesting to note how completely this more modern conception of hypertension differs from the older views in regard to it. Formally an increased blood-pressure was supposed to be secondary to the "small red kidney," now we are certain that the primary contracted kidney develops as the result of a hypertension, and that in most cases a fatal termination does not depend upon the renal involvement, but upon the heart.

THE CONTROL OF HYPERTENSION

has been attempted in many ways. None of them have been completely successful; some of them rest upon hearsay and tradition rather than upon clinical observation. Something may be accomplished along these lines in certain cases. However, they cannot be treated as a group, but must be studied individually, as the subsequent discussion will attempt to show.

The Effect of Protein in the Diet.—For many years protein foods were considered to be at the root of the "blood-pressure evil." The dietary prescription of meat once a day or less and as few eggs as possible has been advocated until the meatless diet has become a popular slogan. There is no doubt about the fact that many of our citizens through an overindulgence in starchy foods are becoming obese and favoring the development of "high blood-pressure" and "hardening of the arteries" by the very diet that is supposed to prevent these maladies.

The feeding of protein, as meats or otherwise, will not tend to increase the blood-pressure of a hypertensive individual. The following case (Table 2) taken from a series of such observations[1] will demonstrate this:

[1] Mosenthal, H. O., Amer. Jour. Med. Sci., 1920, clx, 808.

TABLE 2

Blood-pressure observations in a case of hypertension. (Male, aged sixty-seven years. Secondary contracted kidney; at times a trace of albumin and a few hyaline and granular casts; a phthalein excretion of 25 per cent. in two hours and ten minutes; urea nitrogen, 34 mg. per 100 c.c. of blood; moderate arteriosclerosis.) There is no notable change in the level of the arterial pressure on a high or a restricted protein intake, while the total number of calories is maintained at a constant level.

	Blood-pressure.		Diet.		
Day.	Systolic.	Diastolic.	Protein, gm.	Total calories.	Character.
1	190	120	91 (28)[1]	2344	Normal diet.
2	175	105	86 (19)	2498	
3	195	115	80 (28)	2501	
4	190	110	125 (69)	2065	High protein.
5	178	112	125 (74)	2065	
6	194	120	125 (75)	2065	
7	183	125	125 (76)	2065	
8	188	115	125 (76)	2065	
9	187	119	125 (69)	2065	
10	190	116	125 (79)	2065	
11	170	103	125 (64)	2065	
12	175	105	125 (80)	2065	
13	195	108	125 (78)	2065	
14	182	104	125 (68)	2065	
15	185	113	125 (76)	2065	
16	187	107	125 (80)	2065	
17	175	115	125 (72)	2065	
18	194	109	50 (18)	2065	Restricted protein.
19	181	109	50 (16)	2065	
20	178	100	50 (29)	2065	
21	173	103	50 (18)	2065	
22	209	114	50 (29)	2065	
23	178	105	50 (16)	2065	
24	203	115	50 (11)	2065	
25	188	117	50 (16)	2065	
26	173	106	50 (16)	2065	
27	183	110	50 (16)	2065	
28	182	110	50 (16)	2065	
29	182	110	50 (17)	2065	
30	190	115	50 (14)	2065	
31	206	125	50 (17)	2065	
32	190	125	50 (14)	2065	
33	210	120	50 (20)	2065	
34	195	115	50 (19)	2065	
35	201	115	50 (31)	2065	
36	185	115	50 (21)	2065	
37	205	110	50 (13)	2065	
38	205	105	50 (29)	2065	
39	202	112	50 (18)	2065	

[1] Figures in parentheses are the quantity of protein from meat or fish.

There is another side to this problem of the relation of blood-pressure to protein food. If the protein constituent of the diet is either curtailed or given in excess over a long period, certain changes are brought about in the body's make-up that entail a lowering or raising of the blood-pressure. Benedict and his collaborators have shown that undernutrition brings about a secondary anemia, diminished vitality, and a lowered arterial tension; it must be borne in mind that the patient pays the price of lessened efficiency if this therapeutic goal is to be reached by these means. The physician must be the judge of how far these two factors may be balanced against each other in each case, and regulate the protein content of the food accordingly.

An increased number of red blood-cells may be accompanied by a rise in blood-pressure. This is true of some cases of polycythemia, but not of all. Why it should occur in some of these patients and not in others has not been determined. However, in certain instances a reduction of the protein food may serve to diminish the red blood-cells and at the same time to lower the blood-pressure. This is a matter that has not been studied sufficiently in clinical medicine, but it may possibly yield favorable results if it is tried out.

The former idea that the retention of the end-products of protein digestion act as irritants that stimulate the heart and the arteries to increased activity and raise the blood-pressure cannot be considered to be correct. Many of these cases do have a high blood-pressure, but its cause must be sought in another direction. Dietetic therapy in these instances demands a lowered protein intake, but while ordering it we must be clear in our mind that we are doing it to relieve the kidney of its burden and not to diminish the hypertension. A case with a high blood chemistry and a normal blood-pressure is the following:

D. K. Age twenty. Blood urea nitrogen 86.2 mg. per 100 c.c., blood uric acid 8.3 mg. per 100 c.c., and blood creatinin 5 mg. per 100 c.c.; the urine contained a considerable amount of albumin and a few red blood-cells; the case was evidently a secondary

contracted kidney of marked degree; blood-pressure readings taken at various times were 132/80, 132/85, 136/85, 142/80, 148/80, 164/83; the two last were the highest readings obtained of a considerable number that are not charted; some of these observations were made during office visits when the blood-pressure would naturally show a tendency to be at its highest level because of the nervous tension involved.

In regard to the use of proteins in essential hypertension it may be concluded that enough protein food should be allowed to maintain the body in an efficient condition, and that there is nothing to be gained by the routine restriction of meat, fish, or eggs. A deficient renal function in these cases may demand a curtailment of proteins for other reasons.

The Effect of Carbohydrate in the Diet.—The starches have always been considered the most innocuous of foods. Their final digestive products are carbon dioxid and water, which put no strain on any of the eliminative functions. This is all perfectly correct and deserves consideration in the dietetic therapy of many diseases. Today the amateur doctor advocates a high starch diet for himself and his friends to prevent high blood-pressure among a number of other ills. The one drawback to this mode of living is that it is prone to engender obesity in the individual who pursues this cult.

Many persons may become obese and exhibit no increase in their arterial tension; others are not so fortunate. If the combination of obesity and increased blood-pressure does exist, a restriction of the starchy foods may bring relief. In one patient the blood-pressure dropped from 146/88 to 112/72, while the weight diminished by 25 pounds. Dr. W. W. Herrick tells me that by reduction of weight through regulation of the starchy element in the diet he was able to lower the systolic pressure from above 200 to normal in an obese woman.

In overweight individuals a control of the starchy food may, therefore, be of distinct importance in regulating the blood-pressure.

The Effect of Fats in the Diet.—What has been said in regard to the relation of starches to obesity and hypertension may be

repeated for the fatty foods. The fats in themselves appear to have no other specific effect upon the level of the blood-pressure.

The Influence of Sodium Chlorid upon Blood-pressure.—In 1904 Ambard and Beaujard expressed the belief that salt retention resulted in an increased blood-pressure. Such an inefficient excretion of salt was supposed to be the result of nephritis and renal insufficiency. Various Frenchmen, especially Ambard, confirmed the above theory. Even in the published cases of these authors there is a considerable number that do not exhibit hypertension; furthermore, the very obvious fact has been pointed out that it is generally appreciated that those types of nephritis characterized by salt retention have as their outstanding features albuminuria, edema, and no increase in

TABLE 3

Cases demonstrating the lack of relationship between blood-pressure and concentration of sodium chlorid in the blood. These were consecutive cases of hypertension. There are fewer cases exhibiting a sodium chlorid concentration greater than 500 mg. per 100 c.c. than a lower level.

Case.	Sodium chlorid, mg. per 100 c.c. whole blood.	Blood-pressure, mm. mercury.
1	627	226/90
2	588	184/110
3	557	208/94
4	538	224/120
5	519	180/76
6	500	214/136
7	500	200/82
8	500	212/130
9	494	224/120
10	494	224/106
11	488	206/126
12	482	156/100
13	478	216/132
14	478	210/114
15	475	210/116
16	462	246/120
17	450	240/145
18	429	204/128
19	429	170/110
20	429	224/136

blood-pressure. Looking at the matter from the point of view of a kidney complication it would not seem that inability on the part of the kidney to excrete salt was productive of a rise in blood-pressure.

The sodium chlorid in the blood may be high for a number of reasons. If salt is a factor in bringing about hypertension it may be that its increased concentration in the blood is the factor that is responsible for a rise in arterial pressure. This matter is easily determined because clinical material collected over even a very short period shows very definitely that no very close relation exists between blood-pressure and the concentration of salt in the blood. (See Table 3.) If we regard this matter from another angle and note the blood-pressures in cases that exhibit an unusual amount of sodium chlorid in the blood (Table 4) it is very evident that an increased amount

TABLE 4

Consecutive cases exhibiting an increased concentration of salt in the blood. There is no direct relationship between the sodium chlorid and the arterial tension. About one-half the cases exhibit a normal blood-pressure in the presence of a raised blood chlorid concentration.

Case.	Sodium chlorid, mg. per 100 c.c. whole blood.	Blood-pressure, mm. mercury.
1	676	105/52
2	627	226/90
3	625	98/80
4	588	140/84
5	588	184/110
6	562	150/86
7	557	208/194
8	550	130/70
9	550	104/78
10	544	148/90
11	538	224/120
12	538	108/82
13	532	108/74
14	530	148/86
15	529	120/66
16	519	180/76
17	513	170/86
18	511	136/70

of sodium chlorid does not necessarily entail a rise in blood-pressure. O'Hare's experience in regard to the blood chlorids is very similar to that given above. It is as follows:

"An interesting sidelight is thrown on the subject by a series of 45 or 46 cases of hypertension. These have been arranged according to the height of the maximum blood chlorid. About 20 cases or a little under one-half showed normal blood chlorids. These normal cases were largely cases of vascular hypertension, whereas those cases showing high blood chlorids were, on the whole, chronic nephritics, with retention of nitrogen bodies as well as salt. Curiously enough, those cases that showed the normal chlorids averaged the higher blood-pressure."

Increasing or diminishing the salt in the diet in hypertension is, according to Allen, prone to be followed by a corresponding rise or fall in blood-pressure. McLester and Strouse have failed to obtain the same. Dr. James Short and I, working in this clinic, have not succeeded in affecting arterial tension by the administration of 10 grams of sodium chlorid. The detailed description of these observations I wish to reserve for another time. The disastrous effect which this amount of sodium chlorid is supposed to have in cases of hypertension we have witnessed only once. This was a patient who had a marked renal insufficiency. In cases of essential hypertension unaccompanied by renal or other complications it is perfectly safe to administer salt as desired.

From the above it appears that sodium chlorid does not affect the blood-pressure, and that no object is attained by putting burdensome restrictions in this regard upon patients suffering with essential hypertension. That nephritis of any type may be accompanied by insufficient chlorid elimination must be borne in mind; the salt intake must be adjusted to the ability of the kidney to handle the situation, and no attention need be paid to height of the arterial pressure.

The Effect of Fluid Intake Upon Blood-pressure.—No consideration of the effect of diet on blood-pressure could approximate thoroughness unless this subject were considered. Recently

Miller and Williams reported the finding that there was a marked increase in arterial tension when huge amounts of fluid were drunk. This observation is extremely interesting; its clinical application, however, is another matter. When only such quantities of liquid are ingested that come within the range of clinical probability there is no rise of note in the blood-pressure; a large number of researches bear witness to this statement. Furthermore, the intravenous administration of any of the solutions commonly used up to 1000 c.c. and more apparently does not affect the blood-pressure. Most of us have watched patients during blood transfusion and failed to find any change in arterial tension as the result of this measure. It may be concluded, therefore, that it is not worth while to limit the quantity of fluids in the diet with a view to controlling blood-pressure.

Control of Blood-pressure By Relaxation.—The previous methods given cannot be said to be productive of anything that may be considered to be a constant or reliable result in reducing blood-pressure. The only procedure that has been at all satisfactory in my hands as yielding a drop in blood-pressure that may be regarded as a definite effect of therapeutic interference has been nervous relaxation obtained by longer or shorter periods of rest.

Relief from nervous strain may reduce arterial tension. It is difficult to demonstrate this because we have no absolute way of measuring the amount of worry and anxiety a patient may be harboring at any one time. In addition, there is the other side of the problem to be considered, namely, the receptivity of the individual; responsibilities that make one man grow restless, nervous and unequal to his task, only serve in another to spur him on to greater activities without ruffling him in the least. It cannot be said without reservation that removal of nervous strain will lower blood-pressure because the matter is almost impossible of absolute proof under usual conditions. However, O'Hare has recently shown that conversing about worrisome topics will raise arterial pressure to an amazing degree; there are many impressions that this is correct; Dr.

James Short, recently working in our wards, was able to confirm O'Hare's results.

One incident may serve to show how undue anxiety will increase the blood-pressure. An elderly gentleman, whose life was very methodical and free from almost any unusual interference, was in the habit of visiting me about once a month and have his blood-pressure determined. Over a period of several years it fluctuated between 170 and 190. At one time it reached 240, to return to its accustomed level a few weeks later and remain there. On investigation it developed that the patient's brother had been the subject of an exploratory laparotomy for what the attending surgeon had diagnosed as a carcinoma; the tumor proved to be a benign one and the recovery of the brother seemed assured. With the favorable turn of events the blood-pressure of our patient dropped.

Occurrences of this sort indicate how vital it is to regulate the business and family cares of hypertensive patients. The difficulty of doing this with any degree of success is fully appreciated and demands an unusual amount of tact, co-operation, and forebearance on the part of all concerned.

Rest over shorter or longer periods can be studied more satisfactorily than the intangible effect of nervous influences. O'Hare and Boas have recently done this, and have come to the conclusion, as have others, that relaxation in the recumbent position will diminish arterial tension. A series of cases is presented (Figs. 222, 223) which demonstrates the same point.

There are several facts that should be noted if the full significance of these observations is to be taken advantage of. Figure 222 shows how great the drop in blood-pressure may be. A diminution of 40 mm. or more of mercury in the systolic pressure of 3 of the cases is proof of this. On the other hand, the effect upon the diastolic tension is decidedly less marked and ranges within comparatively narrow limits regardless of the fluctuations that occur in the systolic readings. From what was said as to the mode of the production of blood-pressure it becomes apparent that such short periods of rest serve to diminish the overactivity of the heart much more than they

relax the hypertonic condition of the arteries. As Boas has shown, the drop in blood-pressure obtained on lying down for a short time may persist for a considerable period after the upright position is resumed. Both these effects—the drop in pressure and its duration—are valuable aids in relieving the heart of overstrain, which, as we have seen, is the main sequela of hypertension which we have to contend with if we desire to carry out prophylactic therapeutics.

Figure 223, which pictures most of the cases given in Fig. 222 and includes some others (those with a systolic pressure below 160), serves to demonstrate some of the limitations which short periods of rest have. Striking as we have noted the lessening of the arterial tension to be when measured in millimeters of mercury, it becomes very much less imposing when viewed from another angle. If we regard Fig. 223 for a moment, it becomes very apparent that arterial tension in seeking a lower level does not eliminate the entire pathologic pressure that is present. In other words, we have not succeeded in overcoming the strain upon the heart entirely; our success has only been a partial one, and if we value the results obtained by looking at Fig. 223 we are really bitterly disappointed to see what a very small fraction of the abnormal degree of tension has been done away with by our short period of rest. The enthusiasm engendered by a study of the considerable change in the blood-pressure in Fig. 222 is somewhat dampened. It becomes clear that this mode of treatment affords only partial relief from the cardiac and arterial strain, and nothing more; it is, however, extremely valuable, because it is one of the very few measures that accomplishes even this.

The results of periods of prolonged rest (several days at least) are given in Fig. 224. It is very evident that in these cases much more remarkable results are achieved than in those recorded in Figs. 222, 223, in which the recumbent position was assumed for an hour or less. The blood-pressure approximated the normal in these instances. It becomes perfectly evident that if it is urgent to relieve the heart and blood-vessels from overstrain, prolonged periods of rest are much more

effective than the shorter ones. Which method of treatment is to be resorted to depends upon the condition of the heart, the degree of hypertension, and the response of the individual patient to the various forms of treatment.

It must be distinctly understood that the hypertension is not as successfully set aside in all cases as Fig. 224 would indicate. Comparing the blood-pressure observations taken day by day of 2 patients who are now in the ward you will note how in one the arterial tension was done away with, while in the other no impression was made upon it by prolonged rest.

TABLE 5

ILLUSTRATING HOW THE BLOOD-PRESSURE WILL DROP WITH REST IN SOME PATIENTS, WHILE IN OTHERS IT DOES NOT

Day of rest.	Blood-pressure.	
	Case 1.	Case 2.
1	238/134	205/126
2	210/118	185/110
3	218/130	190/100
4	228/138	175/100
5	214/130	165/100
10	.....	120/80

It is, therefore, very necessary to follow the blood-pressure by daily observations to determine whether or not the desired effect of relieving the circulatory strain is accomplished.

The Use of Drugs to Control Blood-pressure.—The various vasodilators have had their day and have failed to furnish us with a means of permanently reducing hypertension. This is the consensus of opinion at the present time, and I do not believe that these drugs are being used extensively today. Any number of medications may be used to correct symptomatic disturbances, such as constipation, etc., but none of them has a specific therapeutic value. What is true of the vasodilators also applies to the endocrins. It may be that the future holds some medication of value in store for us, but this is a matter to be deter-

mined. Chloral hydrate is one drug that has not been used frequently enough. The former idea that it is a cardiac depressant seems to be losing ground; patients, even with doses large enough to promote sleep, do not exhibit any symptoms referable to the heart. Chloral may act as a vasodilator; it certainly ameliorates nervous tension and promotes quiet. On this score it is a very valuable medication in cases of hypertension.

SUMMARY

Much has been left unsaid that might have been included in this hour. However, if certain facts stand out it is because in the practical handling of high blood-pressure a knowledge concerning them is of the utmost importance. It is worth remembering that essential hypertension is a disease that occurs commonly and is in its early stages not associated with kidney involvement; that as the increased blood-pressure persists certain secondary changes occur, these in their order of vital importance to the patient are: cardiac hypertrophy, dilatation and failure, apoplexy, and nephritis and uremia; that one of the most important elements in the treatment of hypertension is the prevention of these sequelæ; that aside from symptomatic therapy there is in the majority of instances no specific effect to be expected from any form of treatment; that relief from nervous strain and rest for shorter or longer periods offers the best means of reducing the blood-pressure and relieving the strain upon the heart and arteries, for a time at least; that diet as a whole or its various constituents, proteins, fats, carbohydrates, fluids, or salt, have no direct effect upon the degree of arterial tension; indirectly the regulation of the various dietary factors so as to reduce weight, diminish the number of red blood-cells or bring about undernourishment may lower the blood-pressure; that a careful general survey of the patient will indicate what other meaures should be taken in regards to the teeth, gastro-intestinal canal, genito-urinary tract, etc., which in some instances may be productive of far-reaching results. The above represents the facts concerning the treatment of essential hypertension as we possess them today; there

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is much in the routine therapy of this disease that is traditional and that demands investigation before it can be successfully or conscientiously applied.

BIBLIOGRAPHY

Allen, F. M.: Jour. Amer. Med. Assoc., 1920, lxxiv, 652.
Ambard and Beaujard: Arch. gén. de méd., 1904, i, 520.
Benedict, F. C., Miles, W. R., Roth, P., Smith, M. H.: Human Vitality and Efficiency Under Prolonged Restricted Diet, Carnegie Institution of Washington, Publication No. 280, 1919.
Boas, E.: Med. Clin. No. Am., 1920, iv, 257.
Janeway, T. C.: Arch. Int. Med., 1913, xii, 755; Am. Jour. Med. Sci., 1913, cxlv, 625.
McLester, J. S.: Jour. Am. Med. Assoc., 1921, lxxvii, 88.
Miller, J. L., and Williams, J. L.: Am. Jour. Med. Sci., 1921, clxi, 327.
Monakow, F., and Mayer, F.: Deutsch. Arch. f. klin. Med., 1918, cxxviii, 20.
O'Conor, V. J.: Arch. of Surgery, 1920, i, 359.
O'Hare, J. P.: Proceedings American Society for Clinical Investigation, 1921, p. 6; Am. Jour. Med. Sci., 1920, clix, 373.
Pässler, H.: Deutsch. Arch. f. klin. Med., 1906, lxxxvii, 569.
Pässler and Heineke: Verhandl. d. deutsch. path. Gesellsch., 1905, ix, 99.
Strouse, S.: Med. Clin. No. Am., 1921, v, 229.

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Teratogenic effects of sodium chloride in mice

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Hundreds of chemical substances have been reported to be teratogenic in experimental animals (reviews of KALTER and WARKANY [1959], KARNOFSKY [1965], DAGG [1966] and NISHIMURA [1968]). Some nutrients, constituents of the body as well as drugs commonly used in man are included amongst these "teratogens". However, the doses used in these experiments are relatively large. It might be suggested that any chemical substance would have adverse effects on embryos if a sufficiently large dose of it could be administered safely to a pregnant animal and transmitted to the embryos. As a trial, we administered to mice a high dose of sodium chloride, an indispensable ingredient in body fluids, with the hope of determining its teratogenicity.

Material and method

Virgin female mice of Japanese *dd* strain produced in the Mice Center of Kyoto University were left with males overnight. Those with vaginal plugs after mating were regarded as 0 day pregnant. At 10 or 11 days of gestation, the presumably pregnant mice were treated once with either of two dose levels (2500 mg/kg and 1900 mg/kg). Sodium chloride prepared as a 25% aqueous solution was injected subcutaneously into the nape. The animals were maintained on pellets (Japan Clea Co., Tokyo) and fresh water *ad libitum* until day 18 of gestation when they were sacrificed. The implantation sites and maceration of the fetuses were inspected *in situ* and all living fetuses were removed, weighed, and examined for external malformations.

The control animals were pricked once with a needle at the nape at 10 or 11 days of gestation.

Results and discussion

The maternal animals in the experimental groups did not show any obvious symptoms and lost no weight after the injection. The effects on the embryos are summarized in table 1.

Embryocidal, growth suppressing, and teratogenic effects were observed in the experimental groups. There was strong evidence of a dosage effect. The decrease in food consumption which might have occurred for a short period after the injection cannot be ruled out as the cause of such effects. However, this is unlikely because it has been shown (NISHIKAWA [1963]) that maternal fasting for 24 hours at day 10 or 11 of gestation in *dd* mice produces a low incidence of such defects as clubfoot and deviation of the toe but no severe appendicular defects of the type observed in our experiments (fig. 1). So, it is likely that the high concentration of sodium chloride in the maternal body had a teratogenic action. HOLTFRETER [1933] reported

Table I. Embryocidal and teratogenic effects of 25 % sodium chloride solution on mouse offspring (subcutaneous injection)

Treatment			Condition of offspring				
Day of gestation	Dose (mg/kg)	No. of mothers	Total implants	Dead or resorbed (%)	Body weight of survivors (g)	Survivors malformed (%)	Type and No. of malformations***
10	2500	10	77	48*1	1.25*2	18*1	1Ad. 2Cf. 1Dd, 1Pd. 1St. 2Sl
	1900	10	75	8	1.33	12*1	8Cf. 1Dd
	Control**	10	73	5	1.36	0	–
11	2500	10	68	37*1	1.23*2	16*1	5Cf. 1Mw
	1900	10	76	45*1	1.30	2	1Cf
	Control**	10	73	4	1.31	0	–

*1 Significantly different from the control ($P < 0.01$). *2 Significantly different from the control.

** The nape was pricked once with a needle on the respective day.

*** Ad = adactyly, Cf = clubfoot, Dd = deviation of digit(s), Mw = maldirected wrist joint, Pd = polydactyly (toe), St = shortness of foot, Sl = shortness of forelimb.

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Fig. 1. Left micromelic forelimb of a fetus at 18 days induced by sodium chloride injection (2500 mg/kg). (The cheek is cut for examination of the oral cavity.)

that when the embryos of an amphibian were exposed to the medium of sodium chloride solution instead of water, malformations or cessation of development were induced with increasing grade in proportion to the concentration. In view of this finding it seems plausible that the increased osmotic pressure of blood in embryos is linked with the teratogenic effect of the large amount of sodium chloride.

Aside from the exact causal mechanism of the teratogenicity, our data present a clear-cut example that a certain ordinary ingredient of the body can be teratogenic under appropriate conditions of dosage etc. In conclusion, it was indicated that when positive results in teratogenic tests of chemical compounds are obtained in experimental animals, the question whether or not comparable conditions, especially in terms of the quantity of the compound or its metabolites in embryos could occur in man, should be considered in judging the predictable effect with respect to human teratogenicity.

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Summary

A single subcutaneous injection of sodium chloride into pregnant mice of Japanese dd strain at the level of 2500 mg/kg at 10 or 11 days of gestation produced embryocidal,

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growth-suppressing and teratogenic effects in the fetuses. Similar treatment at the level of 1900 mg/kg at 10 days of gestation caused teratogenic effects and at 11 days of gestation embryocidal effects. Gross malformations consisted of shortness of the forelimb and of the foot, malformed wrist and ankle joint and various digital defects. It is speculated that the increased osmotic pressure of embryonic blood is linked with such deleterious effects on the fetuses.

Résumé

Une injection sous-cutanée de chlorure de sodium à 25 % chez des souris portantes (race japonaise *dd*), à une dose correspondant à 2500 mg/kg, au 10e ou 11e jour de la gestation, détermine des effets très graves sur les embryons: destruction, arrêt du développement ou malformations. Si l'on réduit la dose à 1900 mg/kg au 10e jour de la gestation, on produit des effets tératogènes; la même dose, appliquée au 11e jour, détruit les embryons. Les malformations rencontrées au cours de ces expériences comportent: raccourcissement de la jambe et du pied, malformations des articulations du poignet et de la cheville, anomalies digitales. On suppose que l'augmentation de la pression osmotique du sang embryonnaire serait associée à ces effets délétères sur les embryons.

Zusammenfassung

Wird einer trächtigen Maus des japanischen dd-Stammes eine einzige subkutane Injektion von Natriumchlorid in einer Dosis von 2500 mg/kg am 10. oder 11. Trächtigkeitstag verabreicht, so treten keimtötende, wachstumshemmende oder teratogenetische Effekte bei den Feten auf. Eine entsprechende Behandlung mit einer Dosis von 1900 mg/kg am 10. Trächtigkeitstag führt zu keimtötenden Schädigungen. Die schweren Mißbildungen bestehen in einer Verkürzung der vorderen Extremität und des Fußes, in mißgebildeten Fußwurzel- und Kniegelenken und in verschiedenen Defekten an den Zehen. Es wird angenommen, daß der erhöhte osmotische Druck des embryonalen Blutes für diese schädlichen Wirkungen auf den Fetus verantwortlich ist.

References

DAGG, C. P.: Teratogenesis; in GREEN, E. L. Biology of the laboratory mouse, pp. 309–328 (McGraw-Hill, New York/Toronto/Sydney/London 1966).

HOLTFRETER, J.: Die totale Exogastrulation, eine Selbstablösung des Ektoderms vom Entomesoderm. Roux' Arch. EntwMech. *129*: 669–793 (1933).

KALTER, H. and WARKANY, J.: Experimental production of congenital malformations in mammals by metabolic procedure. Physiol. Rev. *39*: 69–115 (1959).

KARNOFSKY, D. A.: Drugs as teratogens in animals and man. Ann. Rev. Pharmacol. *5*: 447–472 (1965).

NISHIKAWA, M.: Influence of maternal fasting for a short period upon the development of mouse embryos [Japanese]. Acta anat. nippon. *38*: 181–184 (1963).

NISHIMURA, H.: Experimental teratology. Chemical teratogens; in U. MURAKAMI, M. SUZUKI and K. BABA Prenatal medicine – Research and practice on congenital anomalies (Japanese), pp. 250–276 (Igaku Shoin, Tokyo 1968).

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Development of a Strain of Spontaneously Hypertensive Rats*

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Experimental hypertension can be induced in animals by several methods; renal[1)2)3)4)], renoprival[5)], adrenal regeneration[6)], DCA[7)], salt[8)9)10)] or neurogenic[11)] provocation. Researches on such induced hypertension have been conducted most thoroughly; however, there has been very little experimental work on spontaneous hypertension in animals. The latter type of hypertension, which may be considered analogous to essential hypertension in man, has been studied with respect to inherited hypertension in rats by Smirk et al.[12)13)14)15)], on spontaneously hypertensive rabbits by Alexander et al.[16)17)18)] and Rosenfeld et al.[19)], on spontaneous hypertension in dogs by Wakerlin et al.[19)20)21)] on experimental congenital hypertensive rats by Okamoto et al.[22)] and on teratogenic induction of hypertension in offsprings from McCollum-Evans strain rats by Grollman et al.[23)]. Only Smirk et al[12)] and Alexander et al.[16)17)], among these workers, have succeeded in isolating a strain of animals susceptible to spontaneous occurrence of hypertension.

We recently mated a male rat of the Wistar strain showing spontaneous hypertension with a female rat of the same strain with a blood pressure slightly above the average to obtain F_1, from which a pair with spontaneous hypertension was selected and mated again. In this way, the succeeding generations were obtained, starting with F_n, in which approximately 100 per cent occurrence of spontaneous hypertension was observed.

Materials

The Wistar strain rat was used in this study. The rats from the Wistar Laboratory came to the Veterinary Phsiology Laboratory, Agricultural Department, Tokyo University (Director: Prof. Osawa) in 1938, hence to the Science Department, Hokkaido University (Director: Prof. Makino), in 1944, and to the Animal Center Laboratory, Kyoto University Faculty of Medicine in 1951. The animals have been maintained ever since by inbreeding.

Sixty-eight weanling rats supplied by the Animal Center were housed in the Department of Pathology under normal conditions. The control group consisted of sixty-six animals. One male rat that had shown persistent high blood pressure (150 to 175 mmHg) since 7 weeks after birth (P_1) was mated with a female rat with a blood pressure slightly above the average (from 130 to 14[illegible] mmHg) (P_2). Four matings were made with P_1 and P_2 always after blood pressures of above 15[illegible] mmHg in P_1 and pressures of 130 to 135 mmHg in P_2 had persisted for more than one month, and a total of 36 F_1 rats were obtained. Of these F_1 animals, pairs of rats with hypertension (blood pressures exceeding 150 mmHg persisting over a month), were selected and mated by brother-sister combinations to obtain F_2 rats. Pairs of F_2 rats with persistent hypertension were again mated to produce F_3. The process was repeated to the 6th generation of F rats. A few rats of F_2 and F were obtained by cross breeding (see Fig. 1). The inbred group consists of animals obtained by brother-sister matings and the cross-bred group of those born from non-litter mates.

Systolic blood pressures above 150 mmHg in rats were considered hypertension (H) and those below 149 mmHg as normotension (N), in compliance with the generally accepted criteria for

* Outlines of this study were reported at 50th annual meeting of the Japanese Pathological Society in 1961, the 11th Kinki Regional Meeting of the Japanese Circulation Society in 1961, the symposium at the 35th annual meeting of the Japan Endocrinological Society in 1962, and the 51st annual meeting of the Japanese Pathological Society in 1962.

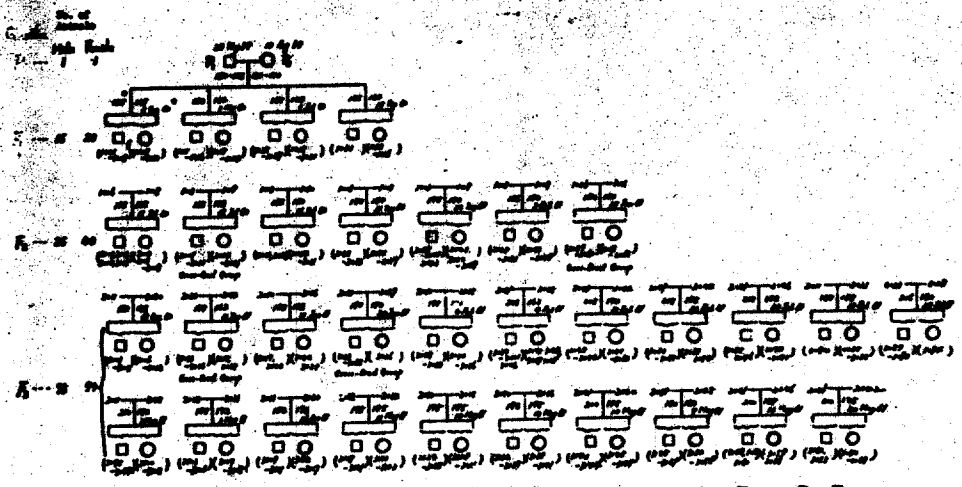


Fig. 1 (a). Pedigree of a Strain of Spontaneously Hypertensive Rats ($P \sim F_3$).
Note: □ Male, ○ Female, * Systolic Blood Pressures of Mated Animals, in mmHg.
† Born, § (Case No.)

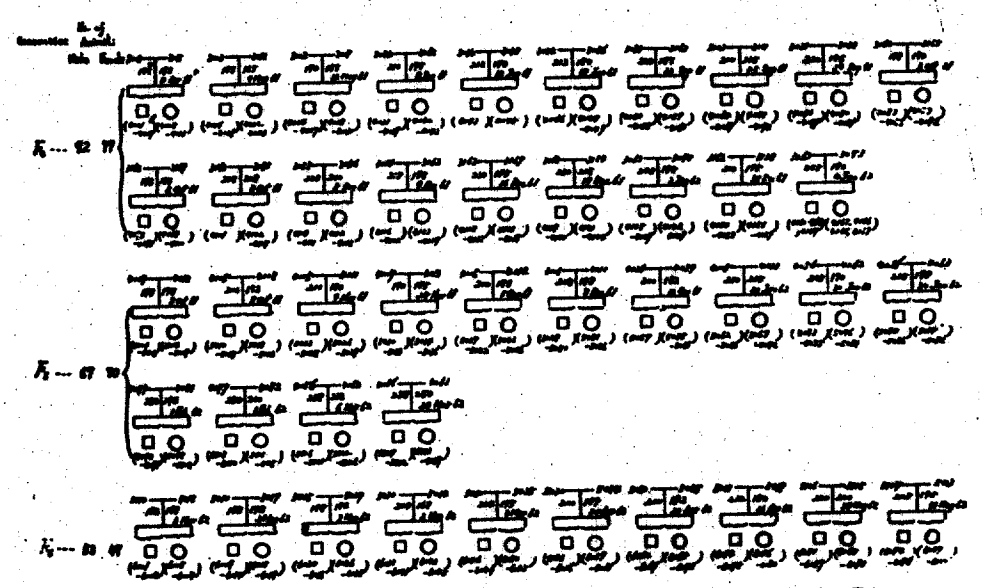


Fig. 1 (b). Pedigree of a Strain of Spontaneously Hypertensive Rats ($F_4 \sim F_6$).
Note: □ Male, ○ Female, * Systolic Blood Pressures of Mated Animals, in mmHg.
† Born, § (Case No.)

hypertension[3)4)13)15)16)18)17)22)23)] and normotension.

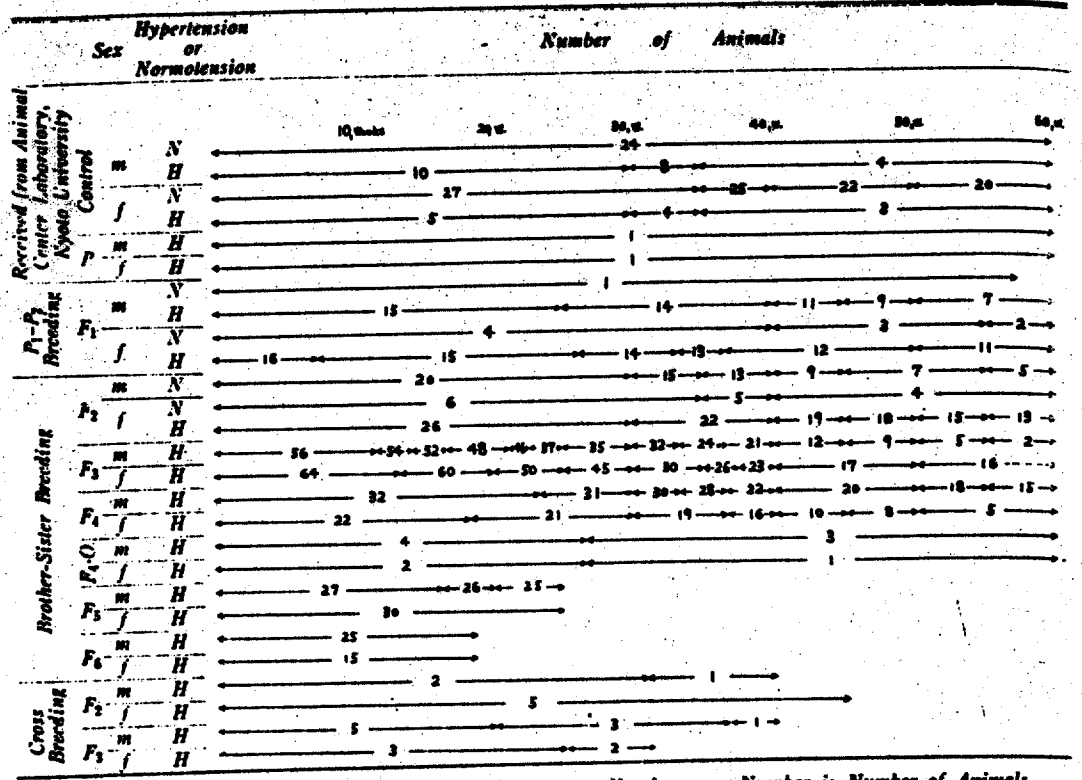
A total of 448 rats were used in this study, consisting of 66 controls, 2P's, 36F_1's, 59F_2's, 128 F_3's, 60F_4's, 57F_5's and 40F_6's. In Table I are shown the number of animals subjected to chronological observation as itemized by weekly age.

Methods

The weights and blood pressures of all rats were measured once weekly, beginning at 4 weeks of age.

For blood pressure measurements, the tail-water-plethysmographic method of Williams et al.[24], Byrom et al.[25], Sobin[26] and Umesawa[27], with

TABLE I NUMBER OF CONTROL AND SPONTANEOUSLY HYPERTENSIVE RATS



a slight modification as follows, was employed. A transparent acrylic plastic tube was used to make the plethysmograph chamber, in the upper part of which was drilled an exhaust outlet (to be closed after discharging the air). The plethysmograph chamber, the cuff and the rat holder were made to be fixed on separate trough-shaped holders provided with springs. The rat to be tested was warmed for 10 minutes in a thermostated box kept at 38°C, then its tail was placed in the plastic chamber through the cuff in a thermostat box kept at 37°C as usual[20]. A cuff of 2 cm width was applied. The plethysmograph chamber was filled with warm water of 37°C, and the blood pressure was measured without anesthesia. The average of four stable measured values (within range of deviation of 5 mmHg) was recorded.

Statistical analysis of the data in each experimental group were performed, using "t" test for the 99 per cent confidence limit ($p < 0.01$).

The animals were kept in a room with a well regulated temperature. The diet consisted of Manitoba wheat mixed with cooked dried sardine, and seasoned with a little salt (to contain 0.3 per cent dietary sodium), and green vegetables. The 6 rats of F_4-O group were fed Oriental's stock chow (NMF, containing 0.276 per cent sodium). All rats were given tap water to drink.

RESULTS

The body weights of F rats and the controls are shown in Table II. Male rats were usually heavier than the females of the same age. The controls were slightly heavier than the F rats, but the difference was not significant.

Blood pressure

(a) *Average systolic blood pressure*

Blood pressures were measured once weekly in all rats from 4 weeks through 60 weeks of age. Many cases of spontaneous hypertension (blood pressure exceeding 150 mmHg persisting over 4 weeks) were observed. The

TABLE II MEAN BODY WEIGHTS OF CONTROL AND SPONTANEOUSLY HYPERTENSIVE RATS ACCORDING TO AGE

	Sex	No. of Animals Init.†	Fin.§	Age (Weeks) 5	10	15	20	25	30
Control	m*	34	34	88±9	231±38	284±51	324±54	363±49	379±51
	f♀	32	32	80±7	166±45	185±42	220±33	240±31	245±28
F_1	m	16	15	85±5	208±52	250±36	266±46	283±42	300±38
	f	20	18	78±5	133±52	167±42	192±65	205±82	205±72
F_2	m	20	20	73±5	211±34	274±41	302±40	311±52	337±33
	f	32	32	72±5	165±32	197±25	210±28	219±30	232±25
F_3	m	56	35	92±2	238±25	296±25	318±28	346±35	387±31
	f	64	45	85±2	171±20	200±17	221±15	223±20	225±20
F_4	m	32	31	69±2	195±20	267±23	298±30	341±30	351±32
	f	22	21	68±2	158±15	186±18	206±22	214±24	222±23
F_5	m	27	25	87±8	191±45	255±25	275±22	290±29	300±22
	f	30	30	79±8	152±42	188±20	203±22	226±25	226±30
F_6	m	25	25	78±4	188±18	252±20			
	f	15	15	70±3	158±10	182±12			

Body Weight (g)

Note: * *Male*, ♀ *Female*, † *Inital*, § *Final*, ± *Standard Deviation*

average systolic blood pressures of each group; male and female P, F and control rats with spontaneous hypertension (H) and those remaining normotension (N) are presented in Tables III and IV, and Figs. 2 and 3.

Blood pressures in the group of N control rats showed a slight rise from 5 to 10 weeks of age, but then became stationary, remaining at the level of 131±9.5 to 136±7.8 mmHg in males and 130±9.9 to 135±7.2 mmHg in females in subsequent weeks. In the H control animals, the blood pressure steadily rose with age.

In normotensive F rats, blood pressures at 10 weeks of age and later ranged from 122 to 148 mmHg in the male and 123 to 137 mmHg in the female. In the F_1 the values were 128 to 140 mmHg in the female F_2, which were a little higher than in N control rats, the differences were not statistically significant. However all male F_1 animals eventually became hypertensive subsequent to 10 weeks of age.

In hypertensive F rats, the blood pressures showed a steady rise with age. For example, in male F_1 rats, the blood pressures rose from 136±13.4 mmHg at 5 weeks of age, to 15[illegible]±17.5 mmHg in 15 weeks, 197±16.5 mmHg in 25 weeks, 200±14.8 mmHg in 35 weeks, 205±16.5 mmHg in 45 weeks and 206±16.2 mmHg in 55 weeks. This tendency toward increasing blood pressures with age was

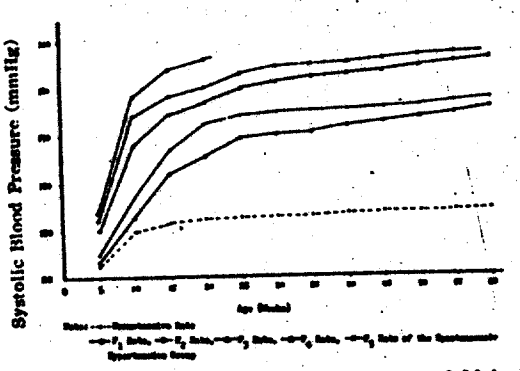


Fig. 2. Average Systolic Blood Pressures of Male Normotensive Rats of the Control Group and of Male Hypertensive Rats of the Spontaneously Hypertensive Group (Brother-Sister Breeding).

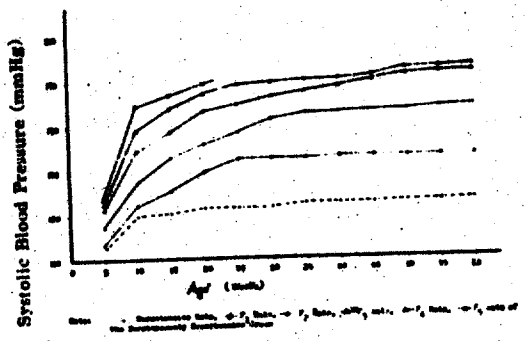


Fig. 3. Average Systolic Blood Pressures of Female Normotensive Rats of the Control Group and of Female Hypertensive Rats of the Spontaneously Hypertensive Group (Brother-Sister Breeding).

TABLE III AVERAGE SYSTOLIC BLOOD PRESSURES AMONG MALE CONTROLS AND

		No. of Animals Init.†	Fin.§	Age (Weeks) 5	7	9	11	13	15	17	19	21
Control	N*	24	24	114 ±12.1	128 ±8.5	131 ±10.2	131 ±9.5	133 ±5.7	133 ±8.0	133 ±8.0	135 ±7.5	134 ±6.6
	H:	10	4	115 ±5.8	131 ±9.3	130 ±13.3	141 ±10.0	142 ±13.3	142 ±16.9	153 ±11.8	148 ±7.0	152 ±11.6
P_1	H	1	1	125	148	155	160	166	175	158	165	155
F_1	N	1	0	108	112	118	122	130	128	128	132	142
	H	15	7	115 ±3.7	124 ±8.8	132 ±7.9	139 ±6.7	149 ±10.2	156 ±12.0	154 ±13.0	153 ±11.6	158 ±1[illegible]
F_2	H	20	5	119 ±7.5	123 ±12.3	147 ±12.3	152 ±15.1	163 ±11.2	164 ±15.0	165 ±12.4	174 ±14.5	175 ±1[illegible]
F_3	H	56	2	133 ±13.7	156 ±13.6	165 ±12.1	172 ±13.4	176 ±14.7	180 ±14.7	181 ±16.9	184 ±15.9	184 ±17.[illegible]
F_4	H	32	15	136 ±13.4	163 ±9.3	174 ±13.2	181 ±17.8	182 ±15.8	188 ±17.5	188 ±15.4	191 ±16.6	194 ±17.5
F_4-O	H	4	3	143 ±4.0	160 ±6.2	168 ±8.8	178 ±7.5	180 ±6.0	182 ±15.4	188 ±8.5	185 ±7.7	195 ±8.5
F_5	H	27	25	136 ±12.9	174 ±15.6	183 ±14.2	190 ±18.6	194 ±15.0	198 ±17.9	197 ±19.5	196 ±18.5	202 ±19.5
F_6	H	25	25	135 ±10.5	151 ±7.7	178 ±8.8	180 ±8.5	185 ±10.6	184 ±10.5	186 ±20.7	188 ±13.9	

Note: * *Normotensive Group,* : *Hypertensive Group,* † *Initial,* § *Final,* ± *Standard Deviation*

TABLE IV AVERAGE SYSTOLIC BLOOD PRESSURES AMONG FEMALE CONTROLS AND

		No. of Animals Init.†	Fin.§	Age (Weeks) 5	7	9	11	13	15	17	19	21
Control	N*	27	20	115 ±11.2	128 ±12.1	130 ±9.5	130 ±7.0	133 ±10.6	131 ±6.8	133 ±9.2	130 ±9.9	133 ±6.0
	H:	5	3	120 ±7.4	122 ±5.3	132 ±2.1	138 ±12.5	140 ±8.0	142 ±5.6	145 ±6.1	151 ±5.0	147 ±3.4
P_2	N	1	1	122	130	130	132	138	135	132	135	132
F_1	N	4	2	111 ±3.0	120 ±1.2	128 ±2.1	129 ±6.1	128 ±13.6	123 ±9.6	124 ±9.2	126 ±13.2	129 ±14.0
	H	16	11	115 ±2.7	122 ±7.2	128 ±9.0	130 ±4.7	135 ±14.6	140 ±11.5	142 ±13.2	148 ±18.0	147 ±11.0
F_2	N	6	4	110 ±4.9	124 ±12.5	131 ±15.1	135 ±13.1	136 ±12.6	133 ±11.9	128 ±9.8	137 ±9.4	137 ±7.7
	H	26	13	125 ±12.2	124 ±11.8	138 ±11.8	146 ±13.0	150 ±13.1	156 ±13.9	158 ±14.4	160 ±15.9	162 ±13.5
F_3	H	64	16	133 ±12.4	151 ±14.2	160 ±10.0	164 ±11.0	168 ±11.1	168 ±12.5	174 ±13.4	176 ±15.6	177 ±16.0
F_4	H	22	5	133 ±12.1	161 ±11.8	170 ±17.6	173 ±14.0	176 ±18.7	178 ±19.6	179 ±14.5	183 ±18.9	185 ±13.6
F_4-O	H	2	1	133	155	169	168	172	174	180	181	181
F_5	H	30	30	135 ±9.1	171 ±14.0	175 ±15.5	177 ±14.2	176 ±13.5	184 ±20.2	189 ±20.5	184 ±19.5	196 ±21.5
F_6	H	15	15	135 ±10.5	149 ±6.5	168 ±8.8	172 ±6.5	175 ±8.5	177 ±4.5	176 ±16.0	176 ±10.2	

Note: * *Normotensive Group,* : *Hypertensive Group,* † *Initial,* § *Final,* ± *Standard Deviation*

AMONG MALE SPONTANEOUSLY HYPERTENSIVE RATS, BROTHER-SISTER BREEDING

Age (Weeks)												
23	25	27	29	31	33	35	37	39	45	50	55	60
134 ±8.2	135 ±7.9	135 ±7.8	138 ±6.4	132 ±6.5	134 ±7.8	136 ±7.8	133 ±7.2	135 ±8.5	135 ±8.2	136 ±7.4	136 ±6.8	136 ±7.2
154 ±12.0	160 ±9.4	160 ±8.7	162 ±9.8	163 ±10.0	161 ±8.9	158 ±9.2	106 ±9.4	165 ±8.5	165 ±6.5	166 ±5.5	166 ±6.8	168 ±8.5
168	158	152	165	165	165	166	158	165	164	160	165	165
125	138	139	128	140	135	134	148	145	146	142	144	
165 ±13.3	169 ±12.6	164 ±13.0	170 ±11.7	170 ±12.5	171 ±13.0	168 ±12.6	169 ±13.0	175 ±13.4	177 ±16.2	178 ±15.8	175 ±16.4	182 ±18.5
174 ±16.0	179 ±14.0	176 ±16.1	178 ±15.2	175 ±13.8	183 ±13.5	179 ±14.7	178 ±13.5	177 ±14.0	178 ±15.5	182 ±14.5	185 ±15.8	183 ±16.0
187 ±18.2	192 ±18.0	195 ±18.2	190 ±16.2	192 ±20.9	194 ±21.2	195 ±17.6	194 ±18.9	196 ±17.2	198 ±23.2	202 ±26.4	202 ±19.6	205
194 ±15.6	197 ±16.5	199 ±14.0	197 ±13.4	197 ±13.7	198 ±14.8	200 ±14.8	200 ±14.8	200 ±14.8	205 ±16.5	204 ±15.5	206 ±16.2	206 ±17.0
195 ±6.5	193 ±10.8											
203 ±19.0	206 ±18.5											

Blood Pressure (mmHg)

AMONG FEMALE SPONTANEOUSLY HYPERTENSIVE RATS, BROTHER-SISTER BREEDING

Age (Weeks)												
23	25	27	29	31	33	35	37	39	45	50	55	60
133 ±7.3	133 ±7.0	131 ±7.2	133 ±6.3	134 ±7.2	132 ±7.4	135 ±3.0	134 ±7.2	134 ±7.6	134 ±7.5	135 ±6.8	135 ±7.0	135 ±7.2
158 ±3.4	152 ±5.3	151 ±4.2	157 ±6.4	152 ±6.2	158 ±6.8	169 ±6.8	170 ±5.6	175 ±7.2	172 ±7.2	174 ±7.5	173 ±8.0	174 ±8.2
134	134	134	125	128	134	134	130	130	128	130	132	132
123 ±14.0	131 ±11.8	137 ±10.1	137 ±9.4	128 ±10.0	124 ±8.8	125 ±9.2	125 ±9.0	134 ±9.6	130 ±10.5	132 ±12.2	134 ±15.2	136
148 ±13.3	156 ±14.1	154 ±9.5	157 ±10.8	155 ±9.8	152 ±10.0	154 ±13.2	154 ±13.0	151 ±11.0	155 ±10.5	156 ±11.2	156 ±11.4	155 ±12.8
135 ±7.[illegible]	136 ±3.7	136 ±5.2	138 ±13.1	132 ±7.5	128 ±9.2	130 ±9.5	136 ±8.5	133 ±8.5	140 ±9.4	140 ±8.8	140 ±8.5	138 ±8.6
165 ±11.7	167 ±15.6	172 ±15.0	172 ±12.5	173 ±12.9	174 ±13.5	175 ±12.9	175 ±13.5	176 ±13.0	176 ±15.4	176 ±15.5	178 ±15.2	178 ±16.0
177 ±14.5	179 ±17.4	182 ±17.6	184 ±17.0	184 ±15.7	186 ±18.7	185 ±22.4	185 ±21.4	186 ±21.2	190 ±18.1	194 ±27.9	192 ±26.1	192 ±26.1
16[illegible] ±[illegible]	189 ±17.4	190 ±17.3	188 ±17.2	185 ±13.3	186 ±12.3	185 ±15.6	186 ±15.4	186 ±16.2	192 ±16.8	195 ±15.8	190 ±18.5	195 ±17.5
17[illegible]	187	195										
19[illegible]	193 ±20.5											

Blood Pressure (mmHg)

also seen in all other hypertensive F groups, and it was particularly marked up to 25 weeks of age. The blood pressure levels in these animals began to be significantly higher than in the N controls after 20 weeks of age in female F_1, after 15 weeks in male F_1 and male and female F_2, and after only 10 weeks in F_3, F_4, F_5, F_6 rats.

Blood pressures in male hypertensive F rats 25 weeks of age were 169±12.6 in F_1, 179±14.0 in F_2, 192±18.0 in F_3, 197±16.5 in F_4 and 206±18.5 mmHg in F_5, showing step-wise increases in successive generations. The tendency toward increasing blood pressures with step-wise rises in successive generations occurred also at other weekly-ages in both male and female rats. Comparisons of the blood pressures of consecutive generations, of F_1 to F_2, of F_2 to F_3, etc., showed that the differences were significant in only a few cases at 10 weeks of age and later (at the level of $0.01<P<0.05$ or $P<0.01$). However, the differences were always significant ($P<0.01$), when data from one generation were compared with those from the third generation, as F_1 to F_3, F_2 to F_4 and F_3 to F_5.

In N controls, sex differences in blood pressure were not significant, although male blood pressures were slightly higher. In hypertensive F rats, the blood pressures of male rats after 11 weeks of age were higher by 10.6 mmHg on the average (range from 2 to 19 mmHg) than those of females rats of the same generation and age.

The offsprings from the male F_2 2041 and the female F_2 2042 "β-line" revealed higher blood pressures than those from male F_2 2020 and female F_2 2020 "α-line", as observed in the respective progenitors. There was a significant difference in blood pressure after 9 weeks of age in male F_4, 22 weeks of age in female F_4; 11 weeks of age in male F_5, and 13 weeks of age in female F_5. The blood pressure level of the "α-line" gradually rose from generation to generation, from F_4 through F_6.

The blood pressures of F_4·O rats fed Oriental's stock chow, showed the same blood pressure levels as in the F_4 group fed our mixed diet (Tab. III and IV).

The blood pressure level of the cross-bred group was not different from the blood pressures of the brother-sister inbred group (Tab. VI).

TABLE V AVERAGE SYSTOLIC BLOOD PRESSURES OF THE α-LINE (OFFSPRINGS OF 2011** AND 2020**) AND OF THE β-LINE (OFFSPRINGS OF 2041** AND 2042**) IN SPONTANEOUSLY HYPERTENSIVE RATS

	Sex	Line	No. of Animals † Init.	No. of Animals § Fin.	Age (Weeks) 5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35
F_1	♂	α	21	20	130 ±9.9	164 ±10.4	167 ±6.9	173 ±8.5	175 ±9.2	178 ±10.1	182 ±12.4	183 ±11.8	184 ±11.5	186 ±11.5	192 ±12.3	193 ±13.0	193 ±12.6	194 ±13.0	199 ±14.0	201 ±15.3
		β	8	6	132 ±12.4	160 ±14.7	194 ±15.7	206 ±6.9	205 ±8.5	212 ±14.6	210 ±11.6	213 ±19.0	220 ±28.3	216 ±20.4	218 ±15.8	214 ±14.1	216 ±17.3	208 ±10.8	206 ±14.6	204 ±18.0
	♀	α	12	10	127 ±8.7	152 ±7.9	167 ±11.5	170 ±9.3	173 ±8.5	174 ±7.5	174 ±8.6	173 ±8.2	176 ±9.0	176 ±9.4	178 ±12.4	178 ±8.5	177 ±8.0	178 ±11.8	180 ±14.3	181 ±14.4
		β	9	8	129 ±8.3	157 ±14.1	175 ±17.1	178 ±17.1	184 ±18.8	187 ±18.0	188 ±17.0	193 ±16.5	195 ±16.5	197 ±16.5	204 ±17.5	204 ±22.1	202 ±20.5	198 ±15.2	200 ±16.0	202 ±16.2
F_2	♂	α	14	14	131 ±5.5	164 ±9.8	177 ±11.0	179 ±10.8	184 ±9.9	183 ±7.1	185 ±10.6	187 ±13.3	192 ±11.0	190 ±11.6	193 ±12.0	192 ±12.2				
		β	13	13	132 ±11.1	182 ±11.9	188 ±12.9	202 ±8.3	204 ±11.8	214 ±15.4	218 ±17.3	205 ±10.4	216 ±14.8	220 ±22.5	225 ±20.5					
	♀	α	14	14	133 ±8.2	164 ±11.6	168 ±10.9	170 ±9.4	174 ±8.2	176 ±9.0	175 ±8.7	175 ±9.8	178 ±11.6	176 ±12.7	178 ±13.0	175 ±12.9				
		β	16	16	137 ±9.7	176 ±12.6	185 ±9.8	186 ±11.1	188 ±12.2	195 ±15.2	198 ±16.3	193 ±17.4	206 ±20.5	209 ±18.8	212 ±20.2					

Blood Pressure (mmHg)

Note: * *Male.* ♀ *Female.* † *Initial.* § *Final.* ** *Case No.* ± *Standard Deviation*

(b) *Incidence of spontaneous occurrence of hypertension*

The spontaneous occurrence of hypertension was not rare in the Wister strain rats used in this study. The incidences are shown in Table VII. At 25 weeks of age, the incidence was 26.4% in male and 15.6% in female controls; 93.5% in male and 36.8% in female F_1; 75.7% in female F_2, and 100 per cent in male F_2 and male and female F_3 to F_6 rats. The incidence increased to 100 per cent at 25 weeks of age in male F_2, 15 weeks in male and female F_3, 10 weeks in male F_4 and 15 weeks in female F_4, and 10 weeks in all F_5, F_6 rats. It can be said therefore, that the incidence of spontaneous hypertension increased and occurred at younger ages from generation to generation. The incidence of

TABLE VI AVERAGE SYSTOLIC BLOOD PRESSURES OF SPONTANEOUSLY HYPERTENSIVE RATS, CROSS-BRED GROUP

Generation	Sex	No. of Animals †Init.	□Fin.	Age (Weeks) 5	10	15	20	25	30	35	40	45	
F_2 H*	m♂	2	0	125	148	167	175	172	175	182	178		Blood pressure (mmHg)
	f♀	5	5	125 ±11.1	148 ±12.0	160 ±11.6	168 ±15.2	168 ±13.2	179 ±14.0	180 ±13.5	195 ±11.5	194 ±15.2	
F_3 H	m	5	0	140 ±10.1	165 ±8.0	168 ±9.0	178 ±11.5	183 ±5.4	182 ±6.4	186 ±3.0	185		
	f	3	0	135 ±2.5	160 ±11.0	162 ±5.2	180 ±15.5	171 ±15.5	175				

Note: * *Hypertensive Group,* ♂ *Male,* ♀ *Female,* † *Initial,* □ *Final,* ± *Standard Deviation*

TABLE VII INCIDENCE OF HYPERTENSION AND DISTRIBUTION OF SYSTOLIC BLOOD PRESSURE AT SEVERAL AGES IN CONTROL AND SPONTANEOUSLY HYPERTENSIVE RATS

Blood Pressure Group (mmHg)	Male, Age (Weeks) 5 No. %	10 No. %	15 No. %	20 No. %	25 No. %	30 No. %	Female, Age (Weeks) 5 No. %	10 No. %	15 No. %	20 No. %	25 No. %	30 No. %
Control less than 149	34 100	33 97.1	29 85.3	28 82.3	25 73.6	25 73.6	32 100	31 96.9	31 96.9	29 90.6	27 84.4	27 84.4
150~199	0	1 2.9	5 14.7	6 17.7	9 26.4	9 26.4	0	1 3.1	1 3.1	3 9.4	5 15.6	5 16.6
above 200	0	0	0	0	0	0	0	0	0	0	0	0
Totals	34	34	34	34	34	34	32	32	32	32	32	32
F_1 less than 149	16 100	16 100	16 100	4 25.0	1 6.5	1 6.5	20 100	19 100	14 73.7	13 68.4	12 63.2	7 38.9
150~199	0	0	0	12 75.0	14 93.5	14 93.5	0	0	5 26.3	6 31.6	7 36.8	11 61.1
above 200	0	0	0	0	0	0	0	0	0	0	0	0
Totals	16	16	16	16	15	15	20	19	19	19	19	18
F_2 less than 149	22 100	13 59.1	4 18.2	1 4.5	0	0	34 91.9	27 73.0	21 56.8	13 35.1	9 24.3	6 16.2
150~199	0	9 40.9	17 } 81.8	20 } 95.5	20 } 100	18 } 100	3 8.1	10 27.0	16 43.2	23 } 64.9	27 } 75.7	29 } 83.8
above 200	0	0	1	1	2	4	0	0	0	1	1	2
Totals	22	22	22	22	22	22	37	37	37	37	37	37
F_3 less than 149	53 86.9	4 6.6	0	0	0	0	57 85.1	8 12.0	0	0	0	0
150~199	8 1.31	56 } 93.4	51 } 100	44 } 100	26 } 100	21 } 100	10 14.9	59 88.0	61 } 100	53 } 100	41 } 100	35 } 100
above 200	0	1	6	9	14	17	0	0	1	4	8	8
Totals	61	61	57	53	40	38	67	67	62	57	49	43
F_4 less than 149	28 87.5	0	0	0	0	0	21 95.5	2 9.0	0	0	0	0
150~199	4 12.5	28 } 100	23 } 100	22 } 100	18 } 100	16 } 100	1 4.5	19 } 91.0	19 } 100	16 } 100	14 } 100	14 } 100
above 200	0	4	9	10	13	15	0	1	3	5	7	7
Totals	32	32	32	32	31	31	22	22	22	21	21	21
F_5 less than 149	23 82.2	0	0	0	0		28 93.3	0	0	0	0	
150~199	5 17.8	22 } 100	17 } 100	15 } 100	11 } 100		2 6.7	27 } 100	23 } 100	21 } 100	19 } 100	
above 200	0	5	10	11	14		0	3	7	9	11	
Totals	28	27	27	26	25		30	30	30	30	30	
F_6 less than 149	24 96.0	0	0				12 80.0	0	0			
150~199	1 4.0	25 100	25 100				3 20.0	15 100	15 100			
above 200	0	0	0				0	0	0			
Totals	25	25	25				15	15	15			

Note: * *F_6 rats consist of a-line only*

hypertension in F_2 rose from 0% at 5 weeks of age to 40.9% at 10 weeks, 81.8% at 15 weeks, 95.5% at 20 weeks and 100% at 25 weeks and later. This steady rise in the incidence of hypertension with age was also observed in other H groups.

The frequency of severe hypertension with blood pressures exceeding 200 mmHg also increased with age and with successive generations, registering 56% (14/25) in male and 37% (11/30) in female F_3, at 25 weeks of age. As all F_6 rats belonged to the α-line, the incidence in F_6 rats can not be studied in comparison with that in earlier generations, which were comprised of rats of both α- and β-lines.

Discussion

A mating of a spontaneously hypertensive male rat (hypertension persisting over a month) and a female rat with blood pressure slightly above the average was used to obtain a hypertensive Wistar rat strain. Upon repeated selective breeding, we have succeeded in causing development of spontaneous hypertension in all animals, F_3 through F_6 offsprings, of 10 to 15 weeks of age. It was confirmed that the spontaneously hypertensive rats showed increasing blood pressures with age, and hypertension developed at earlier ages in the later generations. Moreover, the severity of hypertension increased from generation to generation. Among the F_3 rats, the average blood pressure value at 25 weeks of age was 206 mmHg in male and 193 mmHg in female rats, and severe hypertension with blood pressures exceeding 200 mmHg developed in 25 of 55 rats. Thus, we have obtained a strain in which the incidence of spontaneous hypertension is 100 per cent. We shall refer hereafter to these rats as "spontaneously hypertensive rats, (SHR)".

In 1958, Smirk and Hall[12] obtained a hypertensive strain by breeding rats with blood pressures above the average from the stock colony at Otago (of Wistar strain). The incidence of hypertension (blood pressure with exceeding 140 mmHg) was 30 per cent by inbreeding in the Otago rats, and 40 per cent by selective cross breeding. Smirk et al. designated this effect as inherited hypertension in rats. In 1960, they[13] reported that 50 per cent of the male rats had blood pressures exceeding 150 mmHg, and that the average blood pressure was 147.2 mmHg. In 1954, Alexander et al.[16] obtained a hypertensive strain by inbreeding of rabbits with high blood pressures in the New Zealand white, Dutch and Californian rabbits and obtained an incidence of hypertension of 73 per cent. They called these animals spontaneously hypertensive rabbits, and in 1956[17], reported that the incidence of spontaneous hypertension (exceeding 150 mmHg) was 86 per cent in the males and 53 per cent in the females and that the average blood pressure was 169 mmHg in the male and 152 mmHg in female rabbits. Blood pressure levels and incidences of hypertension of our strain were significantly higher compared with those of inherited hypertension in rats and spontaneously hypertensive rabbits. The reasons for the differences in data are not clear, but we may infer that the following factors are of significance here; (i) Okamoto et al.[20][22][24] have made it clear in their studies on experimental diabetes mellitus that when the disease had persisted for a sufficiently long period in the parents, a diabetogenic factor for the offspring is produced which gradually grows more potent with generation. Tanaka[21] and Taguchi[22] have determined that the minimum period required for manifestation of the diabetogenic factor as 24 days in female and 26 days in male animals prior to mating. Taking such data into consideration, we have always selected and mated rats in which hypertension had persisted for over a month (almost over 2 months). (ii) Whereas we measured blood pressures without anesthesia, Smirk et al.[12] measured blood pressure under light ether anesthesia. The use of anesthesia might have affected the blood pressure values, for, as seen in the blood pressure of controls, their recorded values (124 mmHg in male and 116 mmHg in female rats)[12] were somewhat lower than in our normotensives. (iii) The incidence of spontaneous hypertension in our stock colony of control animals 25 weeks of age was 26 per cent in the male and 16 per cent in female rats, while among rabbits used by Alexander et al.[16] this value was only 2.7 per cent (ex-

ceeding 160mmHg). It may be inferred that the original incidence of hypertension in the stock colony was higher in our Wister strain rats than in their rabbits. (iv) We have shown the average systolic blood pressures of normotensive and hypertensive groups separately, but Smirk et al.[12] and Alexander et al.[16][17] did not make separate calculations, showing only the total average of normotensive and hypertensive animals.

Medoff et al.[33] reported that the incidence of hypertension (exceeding 160mmHg) in Wistar strain rats was 12.2 per cent at 110 to 140 days of age, 24.1 per cent at 450 to 500 days and 55.6 per cent at 825 to 910 days. These data, as well as our data of the control group, seem to suggest that the incidence of spontaneous hypertension is rather high in Wistar strain rats.

As detailed above, the experimental animals showed a trend to increasing blood pressures with age, and consequently, the incidence of spontaneous hypertension also rose in older rats. Similar increases in blood pressures with age and the higher incidence of hypertension with advanced age was confirmed by Alexander et al.[16][17] in spontaneously hypertensive rabbits, by Medoff et al.[33] in control rats of the Wistar strain and by Pickering[4] in human essential hypertension.

According to Alexander et al.[17], higher blood pressures and earlier occurrence of hypertension were seen in populations of rabbits of later generations than among the earlier generation, as we have also observed in our spontaneously hypertensive rats. Such results seem to have been obtained by selective mating and by the fact that hypertensive predisposition is produced and grows stronger in later generations of offsprings from hypertensive ancestors, as can be inferred from the results obtained by Okamoto et al.[20][22][29] cited above.

It was found that in β-line rats born of parents with particularly high blood pressures the blood pressure was significantly higher than in α-line rats of the same generation and age. This suggests that blood pressure levels in the parents regulate the grade of hypertension in the offsprings.

Smirk et al.[12] found that selective cross breeding has produced higher pressures than inbreeding. This is perhaps due to the fact that in cross breeding, pairs with particularly high blood pressures were selected, while such a selection was not made in inbreeding. In the present study, selective mating had been always adhered to, both in brother-sister breeding or in cross breeding, so that the blood pressures of the cross breeding group did not differ from those obtained by inbreeding.

In our study, male rats had higher blood pressures than females, whether normo- or hypertensive. This agrees with the data reported by Smirk et al.[12] (average blood pressures 135.8 mmHg in the male and 129.2 mmHg in the female), by Alexander et al. (average blood pressues 150mmHg in the male and 145 mmHg in the female among F_2 to F_4 rabbits of 3 to 4 months of age[16], and 156 mmHg in male and 149 mmHg in female among F_3 to F_6 rabbits of the same age[17]). Medoff et al.[33] have reported, however, that no sex differences were noted in blood pressures among control Wistar rats.

Most of our experimental animals were fed our mixed diet, but a small number were fed with stock chow. The blood pressures of F_4 with stock chow did not differ from F_4 animals on mixed diets. This shows that the spontaneous occurrence of hypertension was not attributable to the diet employed.

In conclusion, we wish to point out that since spontaneous hypertension in our strain of rats occurred without any treatment to induce hypertension, it is very similar in characteristics to human essential hypertension, but it is still premature to identify definitively the two varieties of hypertension.

Summary

A male rat with spontaneously high systolic blood pressures of 150 to 175 mmHg persisting for more than one month and a female rat with blood pressures slighty above the average, 130 to 140 mmHg, were selected from among 68 Wistar strain rats in normal condition and mated to obtain F_1 rats. Of these F_1 rats, males and females with hypertension (blood pressure exceeding 150 mmHg) persisting for more than a month (mostly over

2 months) were mated to produce F_2 rats. The procedure was repeated to obtain F_3, F_4, F_5 and F_6 rats totaliag 380 animals. The weights and blood pressures (by the tail-water-plethysmographic method) were measured once weekly biginning at 4 weeks of age, and the results can be summarized as follows:

1. In body weight, the F rats showed little difference from the normal controls.

2. The blood pressures of F rats rose with age and from generation to generation, increasing significantly above those of normotensive controls of the same age after 20 weeks of age among female F_1, after 15 weeks among male F_1 and also male and female F_2, and after 10 weeks among all F_3 to F_6 rats. E. g., the average systolic blood pressure of F_5 at 25 weeks of age was 206±18.5 mmHg in the male and 193±20.5 mmHg in female rats. The blood pressures of normotensive controls remained at 131 to 136 mmHg in the male and 130 to 135 mmHg in female rats after 10 weeks of age.

3. Many F rats showed spontaneous hypertension. The incidence of the spontaneous occurrence of hypertension increased, and the development of hypertension occurred at younger ages from generation to generation. All of the F_3 to F_6 rats developed spontaneous hypertension within 15 weeks of age. Severe hypertension with blood pressures exceeding 200 mmHg began to observed among F_2. The incidence of such severe hypertension increased with each generation, so that among male animals it inceased from only 9 % in F_2 to 35% in F_3, 42% in F_4, and 56 % in F_5, and in female animals from 3% in F_2, 16% in F_3, 33% in F_4, and 37% in F_5. The authors have named this Wistar strain of rats with spontaneous occurrence of hypertension as "spontaneously hypertensive rats (Okamoto-Aoki)".

4. The blood pressures of β-line rats from parents with very high blood pressures were significantly higher than among α-line rats from parents with moderately high blood pressures at the same age.

5. There was no difference in the blood pressures of offsprings resulting from inbreeding and those from cross breeding. The male blood pressure averaged about 10.6 mmHg above the female value in spontaneous hypertension, and hypertension developed at lower ages in the male.

6. It is a question for future study whether the spontaneous hypertension induced in rats in this study is comparable in characteristics with essential hypertension in man.

Epitome

Repeating the process of mating Wistar strain rats with spontaneous hypertension (blood pressure exceeding 150mmHg persisting over a month), F_1 to F_6 rats were obtained. 1) Many of the F rats developed spontaneous hypertension. In the F_3 to F_6 rats, 100 per cent of the animals developed spontaneously hypertensive within 15 weeks of age. The authors have named these rats as "spontaneously hypertensive rats". Severe hypertension (blood pressure exceeding 200 mmHg) occurred in 56 per cent of male and 37 per cent of female F_5 rats. 2) The average systolic blood pressure at 25 weeks of age was 206 mmHg in male 193 mmHg in female F_5 rats. The blood pressure level in spontaneous hypertension was higher by 10.6 mmHg on the average in male than in female rats. The blood pressure rose from generation to generation from F_1 through F_6. The hypertensive rats showed increasing blood pressures with age, but this was not the case among normotensive controls. In normotensive controls, blood pressures remained at 131 to 136 mmHg in male and 130 to 135mmHg in female rats after 10 weeks of age. 3) The question of whether the spontaneous hypertension in our F rats is comparable with human essential hypertension is left for future study.

REFERENCES

1) Goldblatt. H.: Effect on blood pressure of moderate constriction of both main renal arteries: The benign phase of experimental renal hypertension. The renal origin of hypertension, 18-26, Charles C. Thomas Co., Springfield, Illinois, 1948.
2) Loomis. D.: Hypertension and necrotizing arteritis in the rat following renal infarction. *Arch. Path.*, 41, 231-268, 1946.
3) Page, I. H.: The production of persistent arterial hypertension by cellophane perinephritis. *J. Am. Med. Ass.*, 113, 2046-2048, 1939.
4) Koletsky, S. and Goodsitt, A. M.: Natural history and pathogenesis of renal ablation hypertension. *Arch. Path.*, 69, 654-662, 1960.
5) Grollman, A., Muirhead, E. E. and Vanatta, J.: Role of the kidney in pathogenesis of hypertension as determined by a study of the effects of bilateral nephrectomy and other experimental procedures on the blood pressure of the dog. *Am. J. Physiol.*, 157, 21-30, 1949.
6) Skelton, F. R.: Adrenal regeneration and adrenal regeneration hypertension. *Physiol. Rev.*, 39, 162-181, 1959.
7) Sturtevant, F. M.: The biology of metacorticoid

hypertension. *Ann. Inter. Med.*, **49**, 1281–1293, 1958.

8) Dahl, L. K.: Effects of chronic excess salt feeding. Induction of self-sustaining hypertension in rats. *J. Exp. Med.*, **114**, 23–1236, 1961.

9) Meneely, G. R., Tucker, R. G., Darby, W. J. and Auerbach, S. H.: Chronic sodium chloride toxicity in the albino rat. II. Occurrence of hypertension and of a syndrome of edema and renal failure. *J. Exp. Med.*, **98**, 71–79, 1953.

10) Tobian, L.: Interrelationship of electrolytes, juxtaglomerular cells and hypertension. *Physiol. Rev.*, **40**, 280–322, 1960.

11) Rosenfeld, S., Thomas, H. V. and Drury, D. R.: Effects of renal denervation on cerebral hypertension in the rabbit. *Am. J. Physiol.*, **178**, 392–398, 1954.

12) Smirk, F. H. and Hall, W. H.: Inherited hypertension in rats. *Nature*, **182**, 727–728, 1958.

13) Phelan, E. L. and Smirk, F. H.: Cardiac hypertrophy in genetically hypertensive rats. *J. Path. Bact.*, **80**, 445–448, 1960.

14) Laverty, R. and Smirk, F. H.: Observations on the pathogenesis of spontaneous inherited hypertension and constricted renal-artery hypertension in rats. *Circulat. Res.*, **9**, 455–464, 1961.

15) Phelan, E. L., Eryetisher, I and Smirk, F. H.: Observations on the responses of rats with spontaneous hypertension and control rats to pressor drugs and to hexamethonium. *Circulat. Res.*, **10**, 817–824, 1962.

16) Alexander, N., Hinshaw, L. B. and Drury, D. R.: Development of a strain of spontaneously hypertensive rabbits. *Proc. Soc. Exp. Biol. Med.*, **86**, 855–858, 1954.

17) Alexander, N., Hinshaw, L. B. and Drury, D. R.: Further observations on development of a colony of spontaneously hypertensive rabbits. *Proc. Soc. Exp. Biol. Med.*, **92**, 249–253, 1956.

18) Alexander, N., Tibbs, W. T. and Drury, D. R.: Cold-pressor responsed in families of rabbits with spontaneous hypertension. *Proc. Soc. Exp. Biol. Med.*, **95**, 356–359, 1957.

19) Katz, J. I., Skom, J. H. and Wakerlin, G. E.: Pathogenesis of spontaneous and pyelonephritic hypertension in the dog. *Circulat. Res.*, **5**, 137–143, 1957.

20) Wakerlin, G. E.: A summary of the laboratory observations on humoral agents as a cause of hypertension. Hypertension. The first Hahnemann symposium on hypertensive disease. 201–21[illegible]. W. B. Saunders Co., Philadelphia. 1959.

21) S[illegible], H. E., Graham, L., Brennan, B. B. and W[illegible]in, G. E.: Renin concentration of normotensive and hypertensive dog kidney. Its relation to serum antirenin titer. Circulat. Res., **10**, 696–70[illegible]. 1962.

22) Okamoto, K., Okuda, T. and Tanabe, K.: Studies on offsprings from rats experimentally made hypertensive for several successive generations. *Acta Sch. Med. Univ. Kioto* in press.

23) Grollman, A. and Grollman, E. F.: The teratogenic induction of hypertention. *J. Clin. Invest.*, **41**, 710–714, 1962.

24) Williams, J. R., Harrison, T. R. and Grollman, A.: A simple method for determining the systolic blood pressure of the unanesthetized rat. *J. Clin. Invest.*, **18**, 373–376, 1939.

25) Byrom, F. B. and Wilson, C.: A plethysmographic method for measuring systolic blood pressure in the intact rat. *J. Physiol.*, **93**, 301–304, 1938.

26) Sobin, S. S.: Accuracy of indirect determinations of blood pressure in the rat: Relation to temperature of plethysmograph and width of cuff. *Am. J. Physiol.*, **146**, 179–186, 1946.

27) Umesawa, H.: A indirect method for measuring blood pressure of the unanesthetized rat. *Tokyo Jikeikai Med. J.*, **66**, 193–199, 1952 in Japanese.

28) Okamoto, K.: Studies on rabbits from ancestors diabetic for several successive generations especially on spontaneous occurrence of diabetes in F_4 and F_5 rabbits. Experimental pathology of diabetes mellitus Report II. *Tohoku J. Exp. Med.*, **61**. Suppl. III. 62–94. 1955.

29) Okamoto, K.: Development of diabetic disposition and of spontaneous diabetes mellitus in the offsprings of diabetic animals. *Tonyobyo*. **3**. 33–47, 1960 in Japanese.

30) Okamoto, K., Midorikawa, O., Mayama, S., Tashima, E., Sato, T. and Yamamoto, Y.: On Wistar rats descended from ancestors diabetic for successive generations, especially, on their Langerhans' islets and their spontaneous diabetization. *Acta Sch. Med. Univ. Kioto*. **36**. 245–272, 1960.

31) Tanaka, T.: Experimental studies on the relation between the duration of diabetes in the male Wistar rat and the disturbance of development in Langerhans' islet in their offsprings. *Folia Endocrinol. Jap.*, **36**. 1350–1370, 1960 in Japanese.

32) Taguchi, T.: Experimental studies on the relation between the duration of diabetes in the Wistar rat and the disturbance of development in Langerhans' islets in their offsprings. *Folia Endocrinol. Jap.*, **37**. 529–554. 1961 in Japanese.

33) Medoff, H. S. and Bongiovanni, A. M.: Age, sex and specis variations on blood pressure in normal rats. *Am. J. Physiol.*, **143**. 297–299. 1945.

34) Pickering, G. W.: Inheritance of high blood pressure. Essential hypertension. An international symposium. 30–38. Springer-Verlag Co., Berlin. 1960.

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THE TOXICITY OF POTASSIUM CHLORIDE AND OF EXTRACTS OF *PSALLIOTA XANTHODERMA* FOR MICE AND RABBITS

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The sporophores of the edible mushroom *Psalliota xanthoderma*, which have been shown (Atkinson, 1946) to exert a powerful antibacterial action, have yielded dried aqueous extracts which contain a high concentration of potassium chloride. Accordingly, in view of the pronounced physiological action (Miller and Darrow, 1940) of the potassium ion in the animal body, it was considered worthwhile to investigate the toxicity of dried aqueous extracts of *Psalliota xanthoderma*.

The experimental animals used were mice and rabbits and the response of these animals to parenteral injection of *Psalliota xanthoderma* extract was compared with that observed when potassium chloride was injected.

It has been shown in this paper that the toxic effect of the mushroom extract upon these animals can be attributed to its potassium content.

EXPERIMENTAL.

MATERIALS AND METHODS.

The potassium chloride used was British Drug Houses AR quality and was dissolved in distilled water to give a 20 p.c. solution. In the case of the lower doses, where the volume would have been inconveniently small for injection, the 20 p.c. solution was diluted to 10 p.c.

The *Psalliota xanthoderma* solutions used were prepared from various dried extracts by dissolving in distilled water. The dried extracts had been prepared by mincing the sporophores, grinding the minced material with water, expressing the juice, filtering free of cellular debris and foreign material and finally drying from the frozen state.

Potassium was estimated in the *Psalliota xanthoderma* extracts and in the specimens of serum from the experimental animals by the sodium-cobalti-nitrite method of Kramer and Tisdall (1921).

The Toxicity of Potassium Chloride.

Mice: Intraperitoneal Injection.

Twenty-five mice, each weighing approximately 20 gm., were randomized into five equal groups and were given intraperitoneal injections of potassium chloride. The doses given to

each group formed a graded series from 0·715 gm./kg. body weight to 2·74 gm./kg. in 40 p.c. increments and the results obtained were subjected to statistical analysis to determine the LD 50. The method used was the method of probits as described by Fisher and Yates (1938).

The results and protocol are shown in Table I.

TABLE I.

Toxicity for mice of potassium chloride: intraperitoneal injection.

Mouse No.	Weight gm.	Dose level gm./kg.	Vol. injected ml.	Death time mins.
1	20·0	0·715	*0·14	S
2	21·0		0·15	S
3	19·5		0·14	S
4	20·0		0·14	25½
5	20·0		0·14	S
6	20·0	1·0	0·10	24
7	23·0		0·115	S
8	19·5		0·097	22
9	20·0		0·10	S
10	22·5		0·113	1
11	22·0	1·4	0·154	S
12	22·0		0·154	S
13	18·0		0·126	S
14	19·0		0·133	63
15	21·0		0·147	S
16	20·5	1·96	0·20	S
17	20·0		0·196	11
18	21·0		0·21	S
19	18·0		0·176	S
20	16·0		0·157	7
21	19·0	2·74	0·26	7
22	21·0		0·29	113
23	21·0		0·29	10
24	21·0		0·29	6
25	16·5		0·25	4
26	22·5	2·74	*0·63	S
27	21·0		0·58	5½
28	22·0		0·60	6
29	20·5		0·56	4
30	24·0		0·66	5½
31	24·5		0·67	6
32	23·0		0·63	4½
33	22·5		0·62	4
34	24·0		0·66	207

* 10 p.c. aqueous solution used. S survived.

The mice which died after the injection showed the signs and symptoms of potassium intoxication. Immediately after injection they showed a disinclination to move about and they remained quiet and huddled together. General muscular weakness then became apparent. The rear legs were particularly affected and the mice were unable to use them. When they were disturbed, the mice moved with great difficulty, dragging the paralysed rear limbs behind them. Dyspnoea and convulsions preceded death, which usually occurred soon after the onset of the convulsions. At post mortem the heart was seen to be in diastole.

The LD 50 of potassium chloride for mice injected intraperitoneally was calculated by the method of probits as 1·3 gm./kg. body weight.

Rabbits: Intravenous Injection.

Six rabbits received injections of potassium chloride into an ear vein. Three rabbits received no injection of potassium chloride and were used as normal controls to establish the normal serum potassium level. Two were killed by air embolism, 25 ml. of air being injected into an ear vein, while the third was killed by concussion.

Samples of heart blood were obtained with a needle and syringe by direct puncture, after exposing the heart by opening the thoracic cavity within three minutes of death. The serum was separated by immediate centrifugation, great care being taken to avoid haemolysis, and was kept in the cold room at 4° C. until the potassium content could be estimated. Table 2 shows the results and protocol.

TABLE 2.

Toxicity for rabbits of potassium chloride: intravenous injection.

Rabbit No.	Weight kg.	Dose level mg./kg.	Vol. injected ml.	Death time mins.	Serum potassium mg./100 ml.	Mean
1	2·71	40	1·1	S	—	
2	2·80	56·6	1·58	S	—	
3	2·71	80	2·17	1½	92	
4	2·56	80	1·89	1½	100	117 ± 29·5
5	2·48	80	1·98	1½	167	
6	2·31	80	1·85	1½	109	
Controls						
1	2·92	Nil	Nil	killed AE	31	
2	2·86	Nil	Nil	killed AE	35	30 ± 4·5
3	2·50	Nil	Nil	killed C	24	

S Survived. — Not estimated. Killed AE: Killed by air embolism. Killed C: Killed by concussion.

Deaths occurred in from 1¼–1½ minutes after the completion of the injection; convulsions preceded death in two out of the four rabbits and profound dyspnoea was observed in all cases.

The two rabbits which received 40 mg./kg. and 56·6 mg./kg. were both dyspnoeic and were disinclined to move at 1–1½ minutes after injection but they gradually recovered and were completely recovered within four hours.

Toxicity of Extracts of Psalliota xanthoderma.

Mice: Intraperitoneal Injection.

Three separate batches of dried aqueous extract of *Psalliota xanthoderma* were taken and after analysing to determine their potassium content were dissolved in distilled water to give a dose level, expressed as potassium chloride, of 2·74 gm./kg. body weight.

Twenty-nine mice were used in this experiment and the results and protocol are shown in Table 3.

The signs and symptoms observed in the mice injected at the dose levels of *Psalliota xanthoderma* extract corresponding to 2·7 gm./kg. potassium chloride were exactly the same as those observed in the mice which were injected with potassium chloride alone at the same level.

TABLE 3.

Toxicity for mice of solution of dried extract of Psalliota xanthoderma: *intraperitoneal injection.*

Mushroom batch No.	Mouse No.	Weight gm.	Vol. injected ml.	Dose level gm./kg. Psalliota extract	Dose level gm./kg. expressed as KCl	Death time mins.
2 B Dry material contained 16·6 p.c. KCl	1	21·5	0·56			4
	2	19·0	0·49			5
	3	21·0	0·55			11
	4	21·0	0·55			8
	5	23·0	0·60			30
	6	20·0	0·52			6½
	7	18·5	0·48			5½
	8	19·5	0·50	18·11	3·00	3½
	9	20·0	0·52			6
	10	22·0	0·57			51
	11	16·5	0·43			5½
	12	23·0	0·60			6
	13	23·5	0·61			6½
	14	21·0	0·55			5
	15	19·0	0·49			7
21 I Dry material contained 17·2 p.c. KCl	1	20·0	0·49			250
	2	20·0	0·49			6
	3	22·0	0·54			5½
	4	20·0	0·49			65
	5	20·0	0·49	15·95	2·74	5
	6	20·0	0·49			43
	7	20·5	0·50			75
	8	19·0	0·47			3½
	9	18·0	0·44			75
24 G Dry material contained 13·3 p.c. KCl	1	20·0	0·63			123
	2	18·5	0·59			196
	3	21·0	0·67	20·06	2·75	5½
	4	15·0	0·48			6½
	5	18·0	0·57			5

Rabbits: Intravenous Injection.

Four rabbits were injected intravenously with an aqueous solution of a dried extract of *Psalliota xanthoderma.* Table 4 shows the protocol and results obtained.

TABLE 4.

Toxicity for rabbits of solution of dried extract of Psalliota xanthoderma: *intravenous injection.*

Rabbit No.	Weight kg.	Vol. injected ml.	Dose level mg./kg. Psalliota extract	Dose level mg./kg. expressed as KCl	Death time mins.	Serum Potassium mg./100 ml.	Mean
1	2·30	2·23	400	50	S	—	
2	2·66	3·00	566	71	1½	116	
3	2·30	3·16	566	71	1½	116	111 ± 6·4
4	1·86	2·13	566	71	1½	100	

S Survived. — Not estimated.

Deaths occurred in from 1½–1¾ minutes after injection of 71 mg./kg. expressed as potassium chloride; convulsions and profound dyspnoea were observed in the three rabbits from the moment of injection to the time of death. The rabbit which received *Psalliota xantho-*

dense extract at a dose level corresponding to 50 mg./kg. potassium chloride, became very quiet and dyspnoeic at 1½ minutes, but was completely recovered within four hours. The similarity of the signs and symptoms with those observed after injection of potassium chloride alone, indicate that the toxicity of *Psalliota xanthoderma* extracts for rabbits after intravenous injection can be attributed to the potassium chloride content.

DISCUSSION.

The action of the potassium ion upon heart muscle is to reduce its contractility and favour its relaxation. This effect is antagonized by the calcium ion but, if the potassium ion is in excess, more and more of the cardiac cycle is occupied by diastole and the heart ultimately comes to rest in the completely relaxed state—a condition known as potassium inhibition (Best and Taylor, 1945). In the experiments reported in this paper the heart stopped in diastole in all cases in both the potassium chloride and the *Psalliota xanthoderma* groups.

Miller and Darrow (1940) record a series of experiments on the toxicity of potassium chloride injected intraperitoneally into adult male rats. They found the normal serum potassium level of 13 rats to be 16 mg./100 ml. with a standard deviation of 0·9 mg. and stated that, in their experience, death occurred or was about to occur when the serum potassium level rose to 80 mg./100 ml.

In the present paper the figures obtained for the serum potassium level in rabbits have been 30 mg./100 ml. with a standard deviation of 4·5 mg. for normal rabbits, 117 mg./100 ml. with a standard deviation of 29·5 mg. for rabbits injected with potassium chloride and 111 mg./100 ml. with a standard deviation of 6·4 mg. for rabbits injected with an extract of *Psalliota xanthoderma*. The toxicity of *Psalliota xanthoderma* extracts at the concentration injected into rabbits by the intravenous route is thus due to the potassium chloride which they contain.

Miller and Darrow (1940) further state, that if death does not occur in rats receiving intraperitoneal injections of potassium chloride within 50–66 minutes of injection, then recovery usually occurs. In the present paper, of the 13 mice which died after receiving intraperitoneal injections of potassium chloride, 2 (15·4 p.c.) survived for longer than 60 minutes, while of the 29 mice which died after receiving *Psalliota xanthoderma* injections, 6 (20·7 p.c.) survived for longer than 60 minutes. The observed toxic effects of *Psalliota xanthoderma* extracts, injected intraperitoneally into mice at a dose level corresponding to 2·7 gm./kg. of potassium chloride, appear to be the same as that observed when potassium chloride alone is injected at the same dose level.

The possibility of a slower acting toxic substance cannot be excluded however, because the rapid killing effect of the potassium ion would mask it.

SUMMARY.

The parenteral injection of potassium chloride into mice and rabbits produces the typical picture of potassium inhibition of heart action.

Psalliota xanthoderma extracts show the same action due to their content of potassium chloride.

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REFERENCES.

Atkinson, N. (1946): Austral. J. exp. Biol., 24, p. 169.

Best, C. H. and Taylor, N. B. (1945): The Physiological Basis of Medical Practice, 4th Edition, Williams and Wilkins, Baltimore.

Fisher, R. A. and Yates, F. (1938): Statistical Tables for Biological, Agricultural and Medical Research, Oliver and Boyd, London.

Kramer, B. and Tisdall, F. F. (1921): J. biol. Chem., 46, p. 339.

Miller, H. C. and Darrow, D. C. (1940): Amer. J. Physiol., 130, p. 747.

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MALABSORPTION OF VITAMIN B_{12} DURING TREATMENT WITH SLOW-RELEASE POTASSIUM CHLORIDE

PRELIMINARY REPORT

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Megaloblastic anaemia in a patient with congestive heart failure and without any known reason for vitamin B_{12} malabsorption aroused our interest in the possible relationship between the slow-release potassium preparations and the absorption of vitamin B_{12}. This is a preliminary report of the low Schilling test values observed during treatment with slow-release potassium chloride.

MATERIAL

The series consisted of 30 hospitalized patients given potassium supplementation. The preparation used was Kalidiuron® (Orion Ltd., Helsinki, Finland) containing [illegible] mg of potassium chloride in a slow-release tablet. The Schilling tests (5) were performed using 0.5 μg of $Co^{57}B_{12}$ (Institutt for Atomenergi, Kjeller, Norway) as a oral dose. Patients with pernicious anaemia or other known reasons for vitamin B_{12} malabsorption were excluded.

RESULTS

In 11 of the 30 patients studied the Schilling test value was lower during potassium treatment than without it. The test values for these patients are presented in Table I. The impairment of B_{12} absorption during potassium treatment was striking in some instances, the Schilling test value being on average 4.8% compared with 11.4% for the same patients without treatment. In the remaining 19 patients the test values were normal with and without potassium, the mean values being 15.4% and 15.5%.

DISCUSSION

The tendency to hypopotassaemia during treatment with diuretics has been the main reason for the need for long-term potassium supplementation. To relieve the hypochloraemic alkalosis occurring at the same time, potassium chloride as a slow-release preparation has been recommended (3) and widely used. On the other hand, absorption of vitamin B_{12} occurs in the distal ileum (2). The intrinsic factor effect in the uptake of B_{12} by ileal mucosa is optimal at pH 6.6, being minimal or absent at pH 5.5, and absent below that level (1). Thus it seems reasonable to think that in some patients the slow-release potassium chloride tablets are able to cause an acidification of the contents of the ileum sufficient to disturb B_{12} absorption. Using radiotelemetric equipment (4) we have obtained measurements of pH in the intestines of some patients, and the first observations support this view. If the pH recorded during potassium chloride treatment was below 6, an impairment of the B_{12} absorption was seen in the

Table I. *Eleven patients who had low Schilling test values during treatment with slow-release potassium*

Pat. no.	Schilling test value, %	
	During potassium	Without potassium
1	1.0	5.5
2	3.0	6.7
3	3.0	12.0
4	3.5	18.0
5	5.0	8.0
6	5.0	18.0
7	5.5	8.5
8	6.8	10.0
9	7.6	10.0
10	8.0	14.0
11	8.3	15.0
Mean	4.8	11.4

Schilling test. No effect on B_{12} absorption occurred in patients with ileal pH above 6 in spite of the potassium treatment. A more extensive study of this type of vitamin B_{12} malabsorption is indicated, including the possibility of developing a B_{12} deficiency.

As long-term potassium supplementation is a very common problem in heart patients, the side effects of such treatment must be carefully evaluated.

REFERENCES

1. Carmel, R., Rosenberg, A. H., Lau, K.-S., Streiff, R. R. & Herbert, V.: Gastroenterology 56: 548, 1969.
2. Drapanas, T., Williams, J. S., McDonald, J. C., Heyden, W., Bow, T. & Spencer, R. P.: J. Amer. med. Ass. 184: 337, 1963.
3. Editorial. Brit. med. J. 2: 191, 1967.
4. Nöller, H. G.: Fortschr. Med. 80: 351, 1960.
5. Schilling, R. F.: J. Lab. clin. Med. 42: 860, 1953.

Study of the Toxic Properties of Potassium Chloride for Small Cattle (Sheep and Goats)

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Source: Izvest. Akad. Nauk Armyan. SSR, Biol. i Sel'skokhoz. Nauki 8, No. 7, 59-62 (1955).

Reports in the literature by A. M. Vil'ner (2), A. G. Zaitseva (3), O. E. Maksimyuk (4), E. Frener (5) and V. Z. Chernyak et al. (6) indicate the occurrence of cases of poisoning among farm animals, following a severe clinical course with a fatal outcome, as a result of careless and negligent handling and poor storage of mineral fertilizers, such as ammonium nitrate and superphosphate.

This study is concerned with the problem of the toxicity of potassium chloride for animals in view of its extensive use as a mineral fertilizer in pasture fields and the possible poisoning of farm animals. The toxicity of potassium chloride and the development of a method for determining its presence in tissues and organs were first investigated in tests performed on 9 rabbits.

As a result of these preliminary tests it was found that: 1) potassium chloride, given in a single dose of 3 g/kg, has an acute lethal effect and causes the death of the rabbit within 45 minutes to 1.5 hour; 2) a single dose of 2 g/kg leads to the death of the rabbit within 7 - 36 hours; 3) a dose of 1 g/kg does not kill the animal, but certain poisoning symptoms are noted.

To determine the content of potassium chloride in tissues of the organism, the reaction with acid sodium tartrate was tested, used in analy-

tical chemistry (1).

From the dead rabbit, small pieces of parenchymatous organs and muscles, the urine and the contents of the gastrointestinal tract were collected. Samples of parenchymatous organs and muscle, weighing 10-15 g, were cut up with scissors into small pieces and were ground in a porcelain mortar, 10 ml water was then added, the contents of the mortar were mixed and then filtered through a paper filter. The content of the stomach and of the large intestine was collected in an amount of 20-25 g each, while the entire content of the duodenum and small intestine was tested, in view of its small amount. These samples were also diluted with water and filtered. The filtrate was tested for the presence of potassium chloride by the above-mentioned reaction. The urine was tested without being processed. A postive reaction was indicated by the appearance of a white crystalline deposit.

Among all tested samples, potassium chloride was found to be present in the contents of the gastro-intestinal tract.

After definite results were obtained with rabbits, tests were carried out on 3 groups of 15 head of small cattle. All 3 groups were given the same feed, except for the 3rd group, which did not receive any table salt in its feed ration. The test animals were subjected to a clinical study for 3 days, and also during the entire course of the test, and studies of the blood and urine were also performed. The tests were carried out in the morning, prior to feeding.

The 1st group of animals consisted of 6 sheep and 2 goats. On these animals, the minimum single lethal dose of potassium chloride (tested in doses of 2, 3 and 4g/kg) was determined for small cattle under normal feed

frequent defecation. During the first 3-5 minutes after KCl administration, vomiting movements and expectoration of food were noted. In addition, tetanic spasms of the muscles of extremities, a backward tilting of the head, at times moaning and bleating, pupil dilatation and accelerated pulse and breathing were also observed.

Autopsy of the dead goats disclosed the presence of a flaccid (flabby) heart muscle, swollen kidneys, and strongly congested capillaries. A strong hyperemia (congestion) of the rumen and reticulum mucosae was noted, as well as a slight hyperemia of the abomasum and duodenum mucosae.

For control purposes, one goat, which was also kept for 1.5 months on a salt-free diet, was given 4 g/kg of table salt; as a result of this treatment, the goat did not die, and no noticeable deviations from the norm or any clinical symptoms were observed, except for thirst and frequent urination.

The 3rd group of animals included 4 sheep, on which KCl doses of 1 and 2 g/kg administered daily for 10 days were tested. These sheep were maintained on a salt-free diet for 3 months prior to the test. Each dose was tested on 2 sheep. The results of this study showed that KCl doses of 1 and 2 g/kg did not cause the death of the animals. During the first 3 - 4 hours, the clinical symptoms included a depression of the general state of the organism, a refusal to eat, and an accelerated pulse and breathing. The blood picture disclosed the presence of leucocytosis and a lowered alkali reserve.

From each dead animal small pieces of parenchymatous organs and muscles, and the urine and contents of the gastro-intestinal tract were tested for the presence of KCl by the method described above. As a control, similar

ration conditions.

The results of this study showed that the clinical symptoms noted during the first few hours after administration of doses of 2 and 3 g/kg included a strong depression of the general condition of the organism, a refusal to eat, a polyuria and an acceleration of the pulse and breathing; the temperature remained almost within the normal range. After 4-6 hours, the above symptoms disappeared and the animals returned to normal.

A dose of 4 g/ kg was tested on 2 sheep and 2 goats, as a result of which both sheep died within 40 minutes to 1 hour and 25 minutes, with the following symptoms: spasms, staggering walk, accelerated breathing, heart throbbing, salivation, temporary vomiting movements and expectoration of food, flatulence, pupil dilatation (mycriasis), a comatose state, and death.

The goats which had received the same dose did not die. However, they also displayed the same clinical symptoms noted above during the first day after administration of potassium chloride. Later, no noticeable deviations from the norm were observed. Upon autopsy of the dead animals, no macroscopic changes in parenchymatous organs were noted. In the gastro-intestinal tract, a hyperemia of the abomasum and duodenum mucosae was noted, and in some spots also of the mucosa of the small intestine.

The <u>2nd group of animals</u> included 2 goats, on which the minimum single lethal dose of 4 g/kg KCl was tested under salt deficiency (starvation) conditions. These goats, prior to the test, were kept for 1.5 months on a ration free of table salt. The results showed that a dose of 4 g/kg leads to the death of the goats within 32 minutes to 1 hour and 25 minutes. The following clinical symptoms were noted: a considerable depression of the general condition of the organism, refusal to eat and drink, polyuria and

material taken from a non-poisoned sheep was tested. As a result of this study, the presence of KCl was found in the contents of the abomasum, omasum, reticulum, rumen, duodenum and small intestine of animals which died from lethal doses of KCl.

Our tests allow us to draw the following conclusions.

1. Potassium chloride, given in a single dose of 4 g/kg, in case of a normal food ration, has an acute lethal effect on sheep and is non-lethal for goats.

2. A dose of 2 and 3 g/kg does not cause the death of the animals, but in this case a strong depression of the general condition of the organism is noted, as well as refusal to eat, polyuria and an accelerated pulse and breathing.

3. A single dose of 4 g/kg KCl has an acute lethal effect on goats under salt deficiency conditions.

4. Potassium chloride, when given daily for 10 days in a dose of 1 and 2 g/kg does not cause the death of sheep under salt deficiency conditions, although some poisoning symptoms are noted.

5. The presence of potassium chloride in the contents of the gastro-intestinal tract of small cattle (sheep and goats) can be determined by means of the acid sodium tartrate reaction.

6. In the multiple (repeated) administration of KCl to small cattle (sheep and goats), the most characteristic deviations in the blood include leucocytosis and a lowering of the alkali reserve.

BIBLIOGRAPHY

1. V. N. Alekseev. Qualitative Analysis Course, Goskhimizdat, 1941 (in Russian).

2. A. M. Vil'ner. Food poisoning in farm animals. 1952 (in Russian).

3. A. G. Zaitseva. Poisoning of domestic animals by insecticides and fungicides, and the prevention of such poisoning, in the journal "Veterinariya", 8, 1948.

4. O. E. Maksimyuk. Poisoning of farm animals by toxic mineral products, in the journal "Veterinariya". 5, 1950.

5. E. Frener. Handbood of pharmacology for veterinarians, 1937 (in Russian).

6. V. Z. Chernyak et al. Foundations of legal-veterinarian tests, 1951 (in Russian).

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ИЗВЕСТИЯ АКАДЕМИИ НАУК АРМЯНСКОЙ ССР
Բիոլ. և գյուղատնտ. գիտություններ VIII, № 7, 1955 Биол. и сельхоз. науки

ТОКСИКОЛОГИЯ

М. С. Топчян и К. Г. Аверьянова

К вопросу изучения токсических свойств хлористого калия для мелкого рогатого скота

В литературе имеется указание А. М. Вильнер [2], А. Г. Зайцева [3], О. Е. Максимюк [4], Е. Френер [5] и В. З. Черняк с соавторами [6] о том, что, вследствие неосторожного и небрежного обращения с минеральными удобрениями — аммиачной селитрой и суперфосфатом—и неудовлетворительного их хранения, наблюдаются случаи отравления среди сельскохозяйственных животных, протекающие с тяжелой клиникой вплоть до смертельного исхода.

В данной работе изучался вопрос токсичности хлористого калия для животных ввиду широкого его применения в качестве минерального удобрения полей и возможности отравления им сельскохозяйственных животных. Токсичность хлористого калия и разработка метода определения его наличия в тканях и органах предварительно были изучены в опытах, поставленных на 9 кроликах.

В результате опытов на кроликах было установлено: 1) хлористый калий в однократной дозе в 3 г на кг веса остросмертелен и вызывает гибель кролика в течение от 40 мин. до 1,5 часа 2) доза в 2 г на кг веса при однократном введении вызывает гибель кролика в течение от 7 до 36 часов; 3) доза в 1 г на кг веса гибели кролика не вызывает, но отмечается наличие некоторых симптомов отравления.

Для определения содержания хлористого калия в тканях организма испытывалась реакция с применением кислой винно-натриевой соли, практикуемая в аналитической химии [1].

От павшего кролика брались кусочки паренхиматозных органов, мышца, моча и содержимое желудочно-кишечного тракта. Пробы из паренхиматозных органов и мышц весом в 10—15 г измельчались ножницами на мелкие кусочки, растирались в фарфоровой ступке, затем прибавлялось по 10 мл воды, все размешивалось в ступке и фильтровалось через бумажный фильтр. Содержимое желудка и толстого отдела кишечника бралось в количестве по 20—25 г, а содержимое 12-перстной кишки и тонкого отдела кишечника, ввиду малого количества его, исследовалось полностью. Пробы эти также разбавлялись водой и фильтровались. При помощи вышеуказанной реакции фильтрат испытывался на наличие хлористого калия. Моча

исследовалась без обработки. На положительную реакцию указывало появление белого кристаллического осадка.

Из всех испытывавшихся проб наличие хлористого калия было установлено на содержимом желудочно-кишечного тракта.

Получив определенные результаты на кроликах, опыты были проведены в трех группах, состоящих из 15 голов мелкого рогатого скота. Кормление для всех групп животных было одинаковое, за исключением III группы, у которой из рациона была исключена поваренная соль. В течение 3 дней, а также и на протяжении всего опыта, за подопытными животными велось клиническое наблюдение, и проводились исследования крови и мочи. Опыты ставились утром до кормления.

I группа животных состояла из 6 овец и 2 коз. На этих животных устанавливалась минимальная однократная смертельная доза хлористого калия для мелкого рогатого скота в условиях нормального рациона. Были испытаны дозы препарата в 2, 3 и 4 г на кг живого веса.

Результаты исследования показали, что дозы в 2 и 3 г на кг веса клинически в первые часы после дачи вызывали сильное угнетение общего состояния организма, отказ от корма, полнурию, учащение пульса и дыхания, температура оставалась почти в пределах нормы. Через 4—6 часов указанные признаки проходили, и животные приходили в норму.

Доза в 4 г на кг веса была испытана на 2 овцах и 2 козах, в результате чего обе овцы пали в течение от 40 мин. до 1 ч. 25 мин. при следующих явлениях: судороги, шаткая походка, учащенное дыхание, сердцебиение, слюнотечение, временами рвотные движения и выбрасывание пищевой массы, вздутие, расширение зрачков, коматозное состояние и смерть.

Получившие ту же дозу козы не пали. Однако и у них, после дачи хлористого калия, в первый день констатировались все вышеуказанные клинические признаки. В дальнейшем видимых отклонений от нормы не наблюдалось. При вскрытии трупов макроскопических изменений в паренхиматозных органах не было установлено. В желудочно-кишечном тракте отмечалось: гиперемия слизистой сычуга, 12-перстной кишки и местами слизистой оболочки тонкого отдела кишечника.

II группа животных состояла из 2 коз. На этих козах испытывалась минимальная однократная смертельная доза хлористого калия в 4 г на кг веса при условии солевого голодания. Указанные козы до опыта, в течение 1,5 месяца, находились на рационе, лишенном поваренной соли. Результаты показали, что доза в 4 г на кг веса вызывает гибель козы в течение от 32 мин. до 1 ч. 25 мин. Клинически было отмечено: значительное угнетение общего состояния организма, отказ от корма и воды, полиурия и частая дефекация. В первые 3—5 минут после дачи наблюдались рвотные движения и вы-

брасывание пищевой массы. Кроме того, констатировались тетанические судороги мышц конечностей, забрасывание головы назад, временами стоны и блеяние, расширение зрачков и учащение пульса и дыхания.

При вскрытии павших коз макроскопически было установлено: дряблая сердечная мышца, набухшие почки, сильно налитые капилляры. Слизистая рубца и сетки в сильной степени гиперемирована, сетка гиперемирована и слизистая сычуга и 12-перстной кишки.

В целях контроля одной козе, также в течение 1,5 месяца выдерживавшейся на рационе, лишенном поваренной соли, задавали поваренную соль из расчета 4 г на кг живого веса, в результате чего коза не пала, и заметных отклонений от нормы и со стороны клиники не отмечалось, за исключением жажды к воде и частого мочеиспускания.

III группа животных состояла из 4 овец. На этих овцах испытывались дозы хлористого калия в 1 и 2 г на кг веса при ежедневном введении в течение 10 дней. В течение 3 месяцев до опыта эти овцы находились на рационе, лишенном соли. Каждая указанная доза испытывалась на 2 овцах. Результаты исследований показали, что дозы как в 1, так и в 2 г на кг веса гибели животного не вызывали. Клинически, в течение первых 3—4 часов, наблюдалось: угнетение общего состояния организма, отказ от корма, учащение пульса и дыхания. В картине крови отмечались лейкоцитоз и понижение щелочного резерва.

От каждого трупа исследовались кусочки паренхиматозных органов, мышца, моча и содержимое желудочно-кишечного тракта на присутствие хлористого калия по вышеописанной методике. В качестве контроля исследовался аналогичный материал, взятый от неотравленной овцы. В результате исследования наличие хлористого калия было установлено в содержимом сычуга, книжки, сетки и рубца, 12-перстной кишки и тонком отделе кишечника трупов животных, павших от смертельных доз хлористого калия.

Наши опыты позволяют сделать следующие выводы:

1. Хлористый калий в однократной дозе в 4 г на кг веса, при нормальном рационе, остросмертелен для овец и несмертелен для коз.

2. Доза в 2 и 3 г на кг веса не вызывает гибели мелкого рогатого скота, но при этом отмечается сильное угнетение общего состояния организма, отказ от корма, полиурия, учащение пульса и дыхания.

3. Однократная дача хлористого калия в дозе 4 г на кг веса в условиях солевого голодания для коз остросмертельна.

4. Хлористый калий в дозе 1 и 2 г на кг веса, при ежедневном введении в течение 10 дней, в условиях солевого голодания не вызывает смерти овец, но наблюдаются некоторые симптомы отравления.

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5. Наличие хлористого калия в содержимом желудочно-кишечного тракта мелкого рогатого скота может быть определено при помощи реакции с применением кислой винно-натриевой соли.

6. Наиболее характерными отклонениями в крови мелкого рогатого скота при многократном введении ему хлористого калия следует считать лейкоцитоз и понижение щелочного резерва.

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ЛИТЕРАТУРА

1. *Алексеев В. Н.* Курс качественного анализа. Госхимиздат, 1941.
2. *Вильнер А. М.* Кормовые отравления сельскохозяйственных животных, 1952.
3. *Зайцева А. Г.* Отравления домашних животных инсектофунгисидами и профилактика этих отравлений. Журн. „Ветеринария", 8, 1948.
4. *Максимюк О. Е.* Об отравлениях с.-х. животных минеральными ядами. Журн. „Ветеринария", 5, 1950.
5. *Френер Е.* Руководство по фармакологии для ветеринарных врачей. 1937.
6. *Черняк В. З.* и др. Основы судебно-ветеринарной экспертизы, 1951.

Մ. Ս. Թոփչյան, Կ. Գ. Ավերյանովա

ՄԱՆՐ ԵՂՋԵՐԱՎՈՐ ԱՆԱՍՈՒՆՆԵՐԻ ՆԿԱՏՄԱՄԲ ԿԱԼԻՈՒՄ ՔԼՈՐԻԴԻ ԹՈՒՆԱՎՈՐՄԱՆ ՀԱՏԿՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅԱՆ ՄԱՍԻՆ

ԱՄՓՈՓՈՒՄ

Կատարված փորձերի տվյալները մեզ հիմք են տալիս անելու հետևյալ եզրակացությունը.

1. Ոչխարների կերակրման նորմալ պայմաններում 1 կգ կենդանի քաշին միանվագ 4 գ կալիում քլորիդ տալու դեպքում արագ մահ է առաջ բերում։

Նույն գոգան այծերի համար մահացու չէ։

2. 1 կգ կենդանի քաշին տրված 2—3 գ գոգան ոչխարների մահ չի առաջացնում, սակայն նկատվում է ընդհանուր թմրություն, կերից հրաժարում, երակազարկի և շնչառության արագացում։

3. Օրգանիզմում ազոտի պակասության դեպքում, 1 կգ կենդանի քաշին միանվագ տված 4 գ կալիում քլորիդը այծերի արագ մահ է առաջ բերում։

Օրգանիզմում ազոտի պակասության դեպքում, 10 օրվա ընթացքում 1 կգ կենդանի քաշին օրական 1—2 գ տրված կալիում քլորիդը ոչխարների մեջ մահ չի առաջացնում, սակայն նկատվում են թունավորման որոշ նշաններ։

THE COMPARATIVE ACTION OF HYPERTONIC SOLUTIONS OF THE CHLORATES AND CHLORIDES OF POTASSIUM, SODIUM, CALCIUM AND MAGNESIUM

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In a previous publication it was shown that in acute poisoning in animals from large doses of potassium, sodium, calcium and magnesium chlorates, the cations of these salts revealed a marked intensity of action and they were to be regarded as the toxic constituents rather than the common anion ClO_3' as is generally supposed. Some doubt may be expressed in respect to the Na-ion in sodium chlorate when it was given in very large doses, for osmotic "salt action" may have played a part in the death of animals. It was also shown that the common anion ClO_3' of the chlorates is not uniformly active in the formation of methemoglobin in the blood in vivo. This substance was formed by only two of the four chlorates. It was never formed after administering the salts of the alkaline earth metals calcium and magnesium, seldom in the case of potassium chlorate, but unfailingly in a definite range of doses by sodium chlorate. Since the different cations of these chlorates are toxic a comparison is now made with the cation constituents of the chlorides.

Joseph and Meltzer (1907) (1909) determined the degree of toxicity of magnesium, calcium, postassium and sodium chloride in dogs. Solutions of each of these salts were injected intraarterially or intravenously until death ensued which was indicated by a complete stopping of the heart. They found that the toxic constituents were the cations and the degree of toxicity is in accordance with a scale in which $MgCl_2 > CaCl_2 > KCl > NaCl$. Osmotic "salt action," even in the case of sodium chloride was be-

lieved to play a small part in the death of animals. Additional evidence in respect to the toxicity of the Na-ion in NaCl was later given by Joseph and Meltzer (1910). They showed that the convulsive twitching of the limbs arising from large doses of sodium chloride is not owing to "salt action," but can be attributed to the toxic action of the Na-ion on the nervous system.

METHODS

The comparison of the toxicity of magnesium, calcium, potassium and sodium chlorate and chloride was made by administering to animals solutions of these salts per os or injecting them intraperitoneally. Such methods of administering the salts obviated the necessity of giving an anesthetic which might obscure the action of the cation constituent of these salts, particularly the anesthetic action of the Mg-ion. In a few instances intravenous injections were used in order to observe any difference in the systemic effects produced by this method and the others that were employed. As experimental animals, large healthy albino rats, guinea pigs, rabbits, and cats were used. The solutions of salts were given per os to guinea pigs and albino rats by means of a flexible silk catheter and a hypodermic syringe. The salts were given in solutions containing one or two mols or a fraction of one mol. With the exception of the sodium salt, a M/2 solution of the salts was used for injection and a M/1 was given them per os. In order to avoid the necessity of giving the sodium salt in too great an amount of fluid, owing to its lesser toxicity, a M/1 solution was injected and a 2 M solution given per os. All these solutions of salts are hypertonic. For intravenous injection, a M/7 solution was used which is nearly isotonic with the blood.

A comparison of the toxicity of the chlorates and the chlorides necessitates the finding of an average lethal dose for each salt which would produce acute poisoning and death in one hour. Such a dose was believed to have been determined when from 90 to 95 per cent of deaths within this time exceeded survivals. An effort was made to see if average lethal doses of chlorates approximated in toxicity that of equimolecular amounts of the chlorides.

As far as it was conceivably possible, there was determined the effect of these salts on the heart and respiration.

RESULTS

Invariably convulsions ensue before death from hypertonic solutions of the chlorates and chlorides, and it is evident that they arise from the effect of such solutions on the nervous system, or a part of it, particularly on the respiratory centers. These centers are stimulated to greater activity or they are noticeably depressed. Under certain conditions, the heart is first affected before respiration. This is generally true when hypertonic solutions of salts are intravenously injected.

Potassium chlorate and chloride

The action of the constituent K-ion in KCl and in other potassium salts has often been studied, particularly on the circulation. Mathison (1911) showed that an intravenous injection of KCl and other potassium salts of varying strengths produces in all cases a fall in aortic pressure. Slow injections and weak solutions of these salts produce a fall in pressure often followed by a temporary rise. The fall in pressure results from a depression of the heart, the rise from a stimulating action of potassium on the spinal, on the vaso-motor centers, and on the peripheral vessel walls. Rapid injections as well as large doses of these salts produce a permanent fall in aortic pressure with a stoppage of the heart in diastole. Similar results were not obtained from sodium salts.

Little or no interest on the other hand has been shown in regard to the action of the K-ion in $KClO_3$ on the circulation. Podcopaew (1865) reported that death from an intravenous injection of $KClO_3$ in dogs was owing to the effect of potassium on the heart. Meltzer (1900) injected the salt intracranially in rabbits and concluded that death was due to the action of potassium on the nervous system: it paralyzes the inspiratory centers. He does not consider the action of this salt on the heart.

These statements lead one to believe that the K-ion in $KClO_3$ and in KCl exert a similar influence on the circulation in animals.

FIG. I. CAT IX. ♀. WEIGHT 2320 GRAMS. M/7 KCl

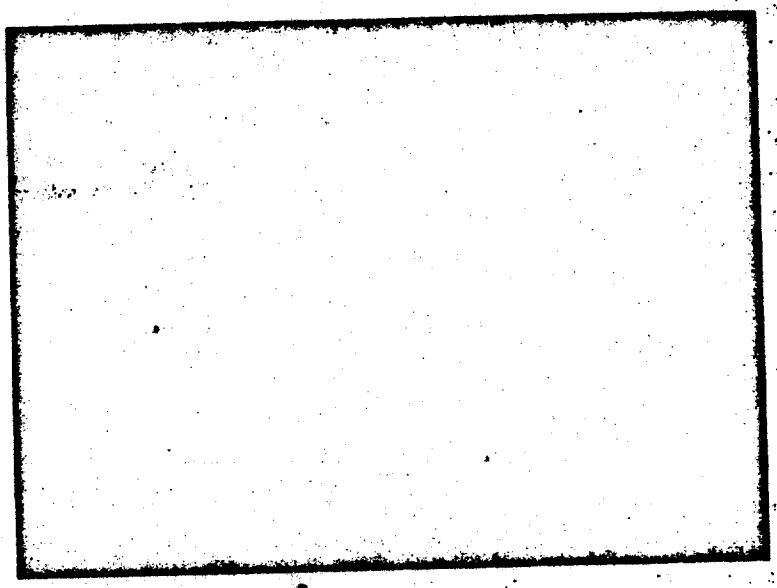

FIG. II. CAT VIII, ♂. WEIGHT 2350 GRAMS. M/7 $KClO_3$

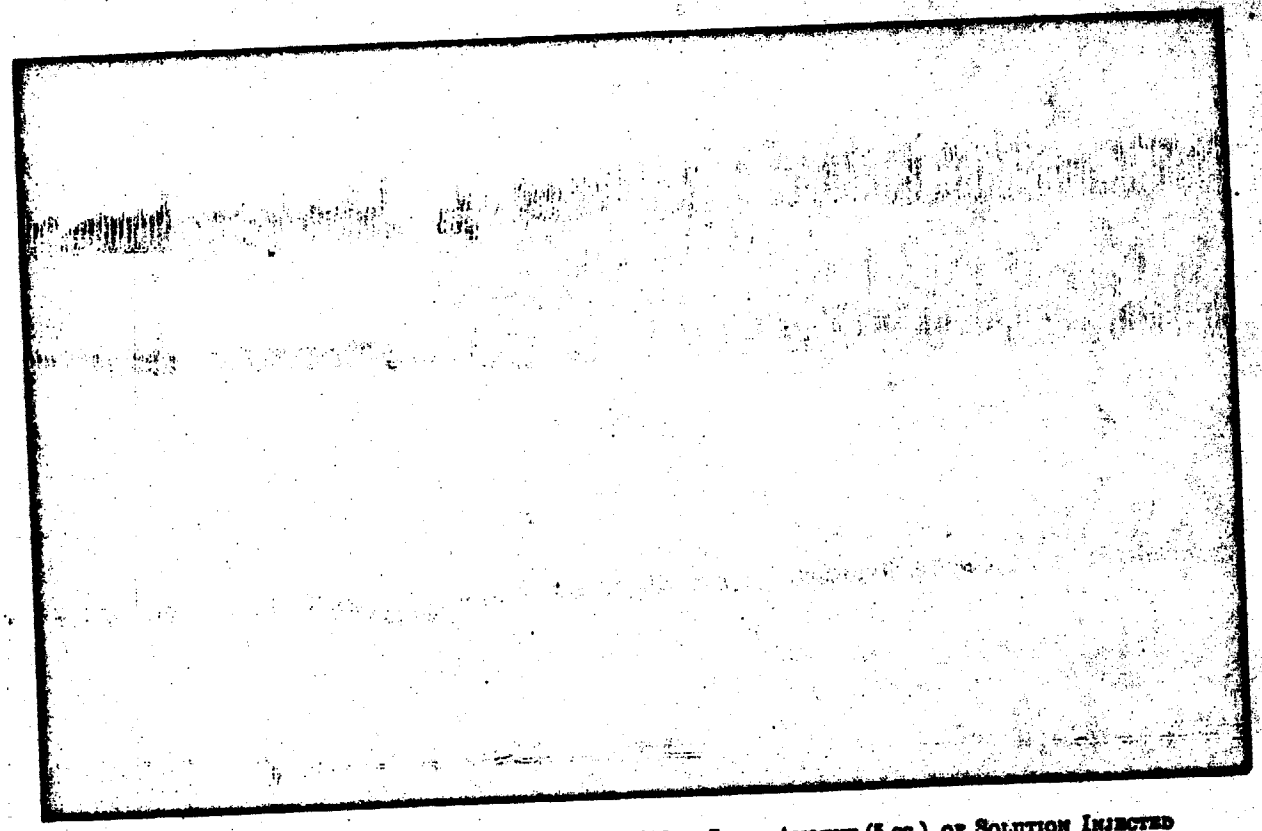

FIG. III. RABBIT III, WEIGHT 2350 GRAMS. M/7 $KClO_3$. SMALL AMOUNT (5 CC.) OF SOLUTION INJECTED
Upper tracing, thoracic respiration; lower, abdominal

An injection of 10 cc. of a M/7 solution of $KClO_3$ into the femoral vein of rabbits and cats within approximately 40 seconds produces the same effect as a M/7 solution of KCl, namely, a permanent fall in aortic pressure, and an almost immediate stoppage of the heart much before the injection is complete. Usually respiration stops a short interval later. A slower injection or a considerably lesser amount of these solutions of $KClO_3$ and KCl results in a temporary fall in pressure. In some instances the effect they produce upon respiration may appear greater than that upon the heart. A similar strength of $NaClO_3$ in a M/1 solution produces no change in circulation or in respiration.

Similar evidence of the action of the K-ion in $KClO_3$ and KCl is also to be had after administering these salts in hypertonic solutions per os or intraperitoneally. A noticeable difference, however, exists in the action of this ion when it is administered by these methods and when it is intravenously injected. The former methods result in completely depressing respiration before the heart stops beating. This difference arises from the fact that these salts when they are intravenously injected reach the circulation directly and affect the heart almost immediately, whereas when they are administered per os or intraperitoneally they reach the circulation after absorption from the intestine, and, before they affect one specific part of the body, the respiratory centers produce general systemic effects such as convulsions. The time before death supervenes is also different. Death is immediate after giving large doses of these salts intravenously, whereas it occurs much later when they are administered per os or injected intraperitoneally. In the last instances, if fatalities ensue within fifteen to forty-five minutes, acute poisoning is manifest and, if they occur somewhat later, in several hours or days, sub-acute poisoning occurs. At times animals show wide variation in the effects produced by $KClO_3$ and KCl. Survival from an average lethal dose may take place. The average lethal doses administered to different animals are given in tables 1 and 2.

The symptoms produced in acute poisoning by $KClO_3$ and KCl are in every respect the same. Soon after administering

TABLE 1

Average lethal doses

Salts	Number of animals used	Average weights	Injection				Feeding			
			Solution used	Anhydrous salt in grams per kilogram animal	In these amounts: Cations are represented by:	In these amounts: Anions by:	Solution used	Anhydrous salt in grams per kilogram animal	In these amounts: Cations are represented by:	In these amounts: Anions by:
Rats										
$Ca(ClO_3)_2$	25	223	M/2	0.625	0.121	0.504	M/1	4.500	0.871	3.629
$CaCl_2$	14	195	M/2	0.500	0.181	0.319	M/1	4.000	1.445	2.555
$Mg(ClO_3)_2$	25	222	M/2	1.100	0.140	0.960	M/1	5.250	0.668	4.582
$MgCl_2$	21	214	M/2	0.225	0.057	0.168	M/1	2.300	0.715	2.085
$KClO_3$	29	218	M/2	1.500	0.479	1.021	M/1	7.000	2.233	4.767
KCl	12	184	M/2	0.825	0.433	0.392	M/1	2.430	1.275	1.155
$NaClO_3$	28	206	M/1	6.000	1.296	4.704	2 M	12.000	2.593	9.407
NaCl	11	220	M/1	5.000	1.967	3.033	2 M	8.000	3.147	4.853
Guinea pigs										
$KClO_3$	32	689	M/2	1.800	0.578	1.223	M/1	7.500	2.393	5.107
KCl	14	649	M/2	0.900	0.472	0.428	M/1	2.500	1.311	1.189

TABLE 2

Rats

Chlorates	Solutions used	Average lethal doses of chlorates	Weight of equimolecular doses of the corresponding chlorides	Chlorides
Injection				
$Ca(ClO_3)_2$	M/2	0.625	0.335	$CaCl_2$
$Mg(ClO_3)_2$	M/2	1.100	0.543	$MgCl_2$
$KClO_3$	M/2	1.500	0.913	KCl
$NaClO_3$	M/1	6.000	3.296	NaCl
Feeding				
$Ca(ClO_3)_2$	M/1	4.500	2.413	$CaCl_2$
$Mg(ClO_3)_2$	M/1	5.250	2.615	$MgCl_2$
$KClO_3$	M/1	7.000	4.259	KCl
$NaClO_3$	2 M	12.000	6.592	NaCl

these salts, respiration and heart action are increased. The first indication of toxic symptoms is difficult breathing, evidenced in an opening of the mouth. These movements pass into a raising of the head and occasionally into asphyxial convulsions with forced movements and opisthotonus. These manifestations give way to a short comatose state and death. In the comatose state inspiratory movements are intermittent. Heart action, though feeble, continues often after respiration has practically stopped. Intracranial injections of these salts produce the same effect on respiration. Electrical stimulation of animals at any time before death reveals that the reflexes are not depressed. Large doses of $NaClO_3$ and NaCl do not produce asphyxial convulsions.

In sub-acute poisoning, the symptoms produced by potassium salts are different. After being given these salts, animals remain inactive and generally refuse food and water. Gradually a long comatose state ensues, sometimes interrupted by convulsions, but these are not of the asphyxial type. Electrical stimulation of the skin covering the limbs shows that the reflexes are depressed. In some instances an hour or so before death the nervous system is so depressed that an animal may be operated upon without evoking reflex disturbances.

A comparison of the toxicity of $KClO_3$ and KCl reveals that the chloride is more toxic. Average lethal doses of these salts that produce acute poisoning in albino rats are 1.5 grams per kilogram animal for $KClO_3$ and 0.82 gram for KCl, both injected in a M/2 solution. This dose of 0.82 gram of KCl is smaller than the one corresponding to the equimolecular quantity of $KClO_3$, which is 0.91 gram of KCl. When these salts are administered per os, KCl is also more toxic than $KClO_3$. An intraperitoneal injection of 1.8 grams of $KClO_3$ and one of 0.9 of KCl into guinea pigs produced approximately the same number of fatalities. Administration of these salts per os to the last animals showed again that KCl is more toxic.

Sodium chlorate and chloride

The common neutral sodium salts are regarded as almost nontoxic. Large doses of them are necessary to produce toxic effects.

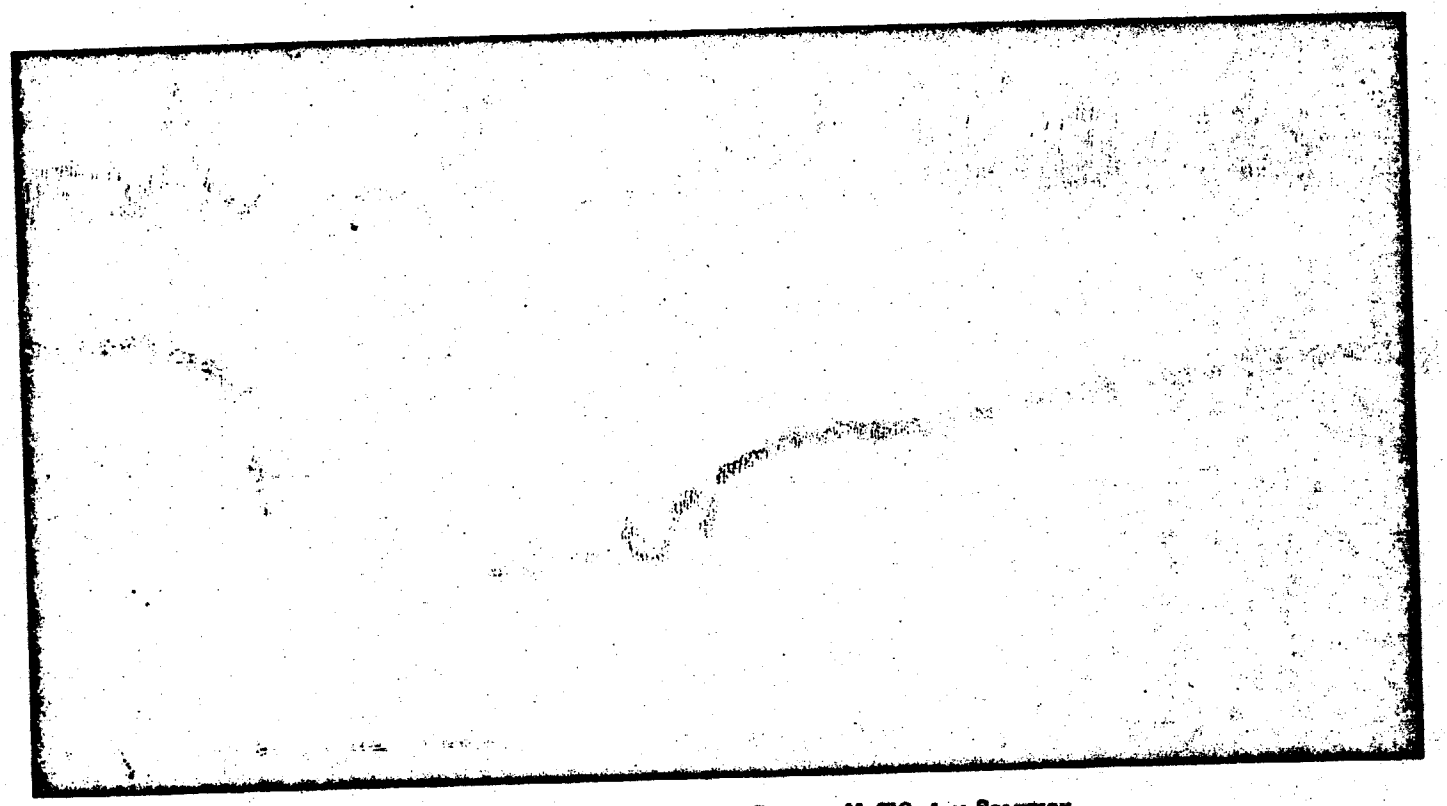

FIG. IV. CAT XII, 9, 1685 GRAMS. $NaClO_3$ 1 M SOLUTION

The reason sometimes given for their low toxicity, particularly NaCl, is that the Na-ion is abundant in the fluids of the body. Sollmann (1927) states that large amounts of NaCl first stimulate and then paralyze the central nervous system. The toxicity of $NaClO_3$ has been investigated only in so far as it forms methemoglobin, and accordingly, its ClO_3-ion is believed to be toxic and not the Na-ion.

In this investigation it was found that intravenous injections of either $NaClO_3$ or NaCl produce similar results. A M/7 solution of either of these salts injected within fifteen seconds in the femoral vein of cats and rabbits produces a permanent fall in aortic pressure with a stopping of the heart. Respiration stops somewhat later. Slower injection within thirty seconds results in a slight rise in aortic pressure or a temporary fall. Respiration may be slightly affected. As was the case with potassium salts, intravenous injections of sodium salts can have a pronounced effect upon the heart.

When $NaClO_3$ and NaCl are administered per os or injected intraperitoneally in large doses, respiration, as death nears, is permanently depressed before the heart. Before this depression occurs marked convulsions of a non-asphyxial type are common and they appear rather suddenly. As the convulsions gradually diminish, twitching of the limbs and body musculature, observed also by Joseph and Meltzer (1910) and attributed by them to the toxic action of the Na-ion is invariably manifest. Methemoglobin is unfailingly formed in the blood by $NaClO_3$.

Comparing the average lethal doses of NaCl and $NaClO_3$ one finds that NaCl is comparatively more toxic than $NaClO_3$. Thus a dose of 6.0 grams of $NaClO_3$ per kilogram animal in a M/1 solution administered intraperitoneally and one of 5.0 grams of NaCl in the same concentration are of the same comparative toxicity. Similar results are observed upon administration per os. Survival from the average lethal dose of NaCl may occasionally occur, but not from one of $NaClO_3$, when methemoglobin is formed in abundance by this last salt. When a dose of 5.0 grams of $NaClO_3$, equal to that given for NaCl, is injected, many rats survive and in the case of those that survive, methemoglobin is

For the determination of the comparative toxicity of magnesium salts, rats were used. It was found that a dose of 1.1 grams of $Mg(ClO_3)_2$ and one of 0.22 grams of $MgCl_2$ in a M/2 solution given intraperitoneally are average lethal doses. The chloride salt is again more toxic than the chlorate. The same result was obtained when these salts were introduced per os in a M/1 solution. Also in whatever way these salts are administered, it was found that in equimolecular doses, $MgCl_2$ is more toxic than $Mg(ClO_3)_2$ (see below, under "Discussion".)

Calcium chlorate and chloride

The intravenous injection of $CaCl_2$ and $Ca(ClO_3)_2$ was not attempted, but Joseph and Meltzer (1909) (1910) report that when $CaCl_2$ is intravenously injected slowly into dogs, this salt first accelerates and then completely inhibits the heart. Respiration increases then decreases. In many cases the heart stops before respiration. Apparently, no investigation of the action of $Ca(ClO_3)$ in animals has been undertaken.

The symptoms observed by Joseph and Meltzer when $CaCl_2$ was intravenously injected were not found to be in every particular the same as when this salt was given per os or intraperitoneally. Invariably respiration is completely depressed before the heart. In other respects the symptoms they noted can be confirmed. Soon after administering $CaCl_2$ the heart is accelerated. Its rate is diminished as convulsions disappear. The convulsions are of the non-asphyxial type. Death occurs in six to forty-five minutes. Some animals show only sub-acute poisoning, live through a period of convulsions and die several hours later or survive after an attack of convulsions.

An investigation of the comparative toxicity of $CaCl_2$ and $Ca(ClO_3)_2$ shows that an average lethal dose of the last salt is smaller, and accordingly, it is more toxic. A dose of 0.62 gram of calcium chlorate in a M/2 solution given intraperitoneally kills rats in less than thirty minutes and calcium chloride produces in a 0.5 gram dose the same effect. Also when these salts are given per os, the last salt is more toxic. On the other hand $CaCl_2$ given in an equimolecular amount with the average lethal dose of $Ca(ClO_3)_2$ is less toxic.

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not formed in the blood; it may be found in the blood of those that die. When these salts are administered to rabbits per os, it was also found that NaCl is more toxic. Methemoglobin is as readily formed by $NaClO_3$ in the blood of rabbits as it is in that of rats. When an equimolecular amount of NaCl corresponding to an average lethal dose of $NaClO_3$ is administered to rats, the first salt is less toxic (see below, under "Discussion").

Magnesium chlorate and chloride

When magnesium salts are given per os in not too large doses, they act as osmotic cathartics and produce slight diuresis. Meltzer and Auer (1905) (1906) report that when these salts are injected hypodermically or intravenously in large doses close to the lethal one, they produce anesthesia without previous excitation. In fatal doses they depress the heart and nervous system, and death results from paralysis of the respiratory centers while the circulation shows little disturbance. Evidently, in large doses magnesium salts are readily absorbed into the blood stream. The action of $Mg(ClO_3)_2$ has apparently not been studied.

An intravenous injection of $Mg(ClO_3)_2$ and $MgCl_2$ was not attempted, for undoubtedly, if large doses of these salts are rapidly injected as was done with $NaClO_3$, the heart would be overcome. Systemic effects produced by large doses of these salts when injected intraperitoneally or given per os are the same. Purgation and slight diuresis occur when they are administered per os and at times when they are given intraperitoneally. Often marked purgation and diuresis indicated that animals would survive. If the heart and respiration are depressed, the latter stops invariably before the heart. In such acute poisoning animals die in convulsions and no evidence of anesthesia is present. But in sub-acute poisoning, when death usually supervenes in several hours, excitation, that is, mild convulsions, precedes what is apparently anesthesia before death. The period of anesthesia might be mistaken for coma, but a stimulation of animals evokes no reflex responses. Moreover, survival instead of death may occur after a long period of anesthesia. Toxic symptoms may be neutralized if $CaCl_2$ is injected.

In this study of the comparative toxicity of the chlorates and chlorides of the alkali and the alkaline earth metals, other facts than the one that the chlorides are more toxic may be noted. The degree of the toxicity of the chlorates either when administered per os or when injected intraperitoneally can be represented in a series in which $Ca(ClO_3)_2 > Mg(ClO_3)_2 > KClO_3 > NaClO_3$. The chlorides when injected intraperitoneally show a different scale of toxicity which runs $MgCl_2 > CaCl_2 > KCl > NaCl$. There is also a considerable difference in the scale of the chlorides when they are administered per os and intraperitoneally; their degree of toxicity when given per os runs $KCl > MgCl_2 > CaCl_2 > NaCl$.

DISCUSSION

When considering the toxicity of a substance, a dose in absolute weight is usually taken. In this paper when comparing the toxicity of two salts, toxic doses are always stated in terms of actual weight. It is evident, however, that an exact comparison of toxicities and doses demands a comparison of doses on the basis of number of molecules. In actual practice, this would be expressed as a fraction of a molecular weight of a salt, or number of cubic centimeters of a solution of standard molarity. To illustrate, it has been shown above that the lethal doses of NaCl and $NaClO_3$ are 5.0 and 6.0 grams respectively—thus demonstrating NaCl to be more toxic. However, the volume of M/1 solutions of these substances which were used were 86 cc. of NaCl (or 0.086 molecular weight) and 57 cc. of $NaClO_3$ (or 0.057 molecular weight) respectively. On this basis, it appears that a greater number of NaCl molecules are required, and therefore this substance is less toxic than $NaClO_3$. That a greater number of molecules of the chloride is required is indicated above when a comparative toxicity of equi-molecular amounts of chlorate and chloride is stated. In the same way, a comparison of KCl and $KClO_3$ shows that a greater volume of the last salt than of the first one, in the same concentration, is required. This would imply that KCl is more toxic than $KClO_3$.

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From this investigation of the comparative toxicity of magnesium, calcium, potassium and sodium chlorates and chlorides, it is apparent that only in large doses are these salts toxic and their toxicity is predominantly owing; not to the common anions Cl or ClO_3, but to their different cations. If osmotic "salt action" plays any part in acute poisoning by these salts in large doses, it is only a small part. This belief is supported by a distinct difference in the systemic effect produced by each salt and by a different toxic dose for each salt.

The most marked outward symptoms in acute poisoning produced by large doses of these salts are the convulsions appearing before death. They indicate that generally the central nervous system is powerfully stimulated which eventually leads to a depression of this system, for respiration is permanently depressed before the heart. In case of $KClO_3$ a depression or a paralysis of the inspiratory centers occurs at the same time as a stimulation of other nerve centers. In no other way does it seem that the asphyxial type of convulsions produced by $KClO_3$ and KCl can be explained. This statement is true only when these salts are given per os or intraperitoneally and not when injected intravenously; for this last method of administering the potassium salts leads to a depression, a stopping of the heart, before respiration is affected. A depression of the heart also ensues after intravenous injection of sodium salts. Probably if these salts are injected very slowly, resulting in turn in a slow absorption, a depression of respiration would ensue before any marked change is evidenced in the heart. Undoubtedly it is a slow absorption and a retention of chlorides and chlorates that produce, after a period of convulsions, a noticeable depression of the heart and the respiration, particularly the latter. A general depression of the nervous system is also apparent in subacute poisoning. Respiration invariably stops before the heart.

CONCLUSION

1. Definite results are presented which show that calcium, magnesium, potassium and sodium chlorate are less toxic than the chlorides of these metals.

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2. Only in large doses in hypertonic solutions are these chlorates and chlorides toxic.

3. The toxicity of these salts is owing evidently to the depression they eventually produce, particularly on the nervous system.

REFERENCES

(1) Mathison, G. C.: Jour. Physiol., xlii, 471 (1911).
(2) Joseph, D., and Meltzer, S. J.: Proc. Soc. Exper. Biol. Med., v, 85 (1907); Jour. Pharmacol., i, 26 (1909–1910).
(3) Joseph, D., and Meltzer, S. J.: Proc. Soc. Exper. Biol. Med., viii (1910–1911).
(4) Meltzer, S. J.: Proc. Physiol. Soc. Sec., 1899, Amer. Jour. Physiol., ix, 3 (1900).
(5) Meltzer, S. J., and Auer, J. J.: Amer. J. Physiol., xiv, 366 (1905), xvi, 233 (1906), xxxiii, 141 (1908).
(6) Podcopaew: Virchow's Arch., xxxiii, (1865).
(7) Sollmann, T.: Manual of Pharmacology, 3rd ed., 849 (1927).