THE EFFECTS OF SODIUM DEPRIVATION ON
THE ANIMAL ORGANISM

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Numerous investigations on the mineral requirements of the animal organism have emphasized the necessity of an adequate supply of salts in the diet. Thus far only certain effects of specific mineral deficiencies have been investigated. On the basis of their classic work in 1918, Osborne and Mendel (1) concluded that the "law of the minimum" holds for all essential salts in the diet, failure of growth in the whole body resulting where the limiting factors are deficiencies in the salts of chlorine, sodium, magnesium, potassium, calcium and phosphorus. These investigators found that rats grew normally on a ration containing 0.035 per cent sodium. Mitchell and Carmean (2), Miller (3), St. John (4), Schoorl (5) and Sjollema (6) reported a decreased rate of growth when the sodium content in the food is low. In addition, Miller (3) observed sterility of the female rat on this type of diet. St. John (4) demonstrated further effects of sodium deficiency. On such diets reproduction was abnormal and the eyes of many of the rats were seriously affected, blindness resulting in many cases.

Although the above-mentioned experiments have elucidated to some extent the importance of sodium, the diets contained considerable amounts of sodium and, almost without exception, they were obviously deficient in some other dietary essentials. In this report it will be shown that these difficulties have been largely overcome. The sodium content of the experimental diet has been reduced to 0.002 per cent and other nutritive essentials have been provided in adequate amounts as demonstrated by the growth rate of rats restricted to this diet supplemented with sodium bicarbonate. This marked reduction in dietary sodium has been effected by the use of a new method (7) of extracting the vitamin B complex from yeast.

EXPERIMENTAL. A total of ninety experimental and fifty control rats were used in this investigation. Weaned rats weighing 35 to 40 grams were placed on the diets, the composition of which appears on the following page.
Sodium-low Diet

Acid washed, alcohol extracted casein .................................. 18.0
Methyl alcohol-gaseous HCl yeast extract = to dry yeast. 5.0
Salt mixture no. 18a ........................................... 5.3
Commercial sucrose ........................................... 66.7
Sweet butter fat .............................................. 5.0
Viosterol, 15 drops per kilo...

Salt Mixture no. 18a

CaCO₃ .................................................. 2.0
KCl .................................................. 1.0
MgO .................................................. 0.2
KH₂PO₄ ........................................... 1.5
Ferric citrate .......................................... 0.5
CuSO₄·5 H₂O ......................................... 0.1

5.3

Control Diet

Acid washed, alcohol-extracted casein .................................. 18.0
Salt mixture no. 19 .......................................... 0.7
Methyl alcohol-gaseous HCl yeast extract = to dry yeast. 5.0
Commercial sucrose ........................................... 65.3
Sweet butter fat ........................................... 5.0
Viosterol, 15 drops per kilo...

Salt Mixture no. 19

NaHCO₃ ........................................... 1.4
CaCO₃ .................................................. 2.0
KCl .................................................. 1.0
MgO .................................................. 0.2
KH₂PO₄ ........................................... 1.5
Ferric citrate .......................................... 0.5
CuSO₄·5 H₂O ......................................... 0.1

6.7

In preparing the methyl alcohol-gaseous HCl extract, in order to be sure of obtaining an extract of the activity described in the original paper (7) it is of utmost importance to have the proper hydrogen-ion concentration and, furthermore, after completion of the methyl alcohol-gaseous HCl treatment, the acidified mixture should be heated to boiling and filtered. The residue is washed with absolute methyl alcohol. The filtrate and washings are then allowed to stand in the cold overnight. If these precautions are not observed the extraction of the vitamin is incomplete.

At about this time crystalline B₁ and lactoflavin had become available. It seemed worthwhile, therefore, to investigate the effect of the same sodium-low diet substituting as the source of the vitamin B complex, Merck's crystalline vitamin B₁ (20 y per rat per day), lactoflavin (40 y per rat twice weekly), and vitamin B₆ in the form of Peter's eluate (1 cc. per rat per day) (8). Twelve rats on the experimental and six on the control diet were used for this purpose. The symptomatology produced was similar to that when the methyl alcohol-gaseous-HCl extract was used as the source of the vitamin B complex in the ration. The methods for preparing the casein and the diet, and the care of the animals are the same as those described in a previous publication (9).

The animals on the sodium-low diet ate well and their food intake was normal as compared with the control rats. The experimental animals grew fairly rapidly for a few weeks and then began to lose weight (chart I). No noticeable symptoms were observed until about the sixth to eighth week, when the difference in appearance of the experimental and control animals was more striking than the difference in growth. At this time the experimental animals began to show eye changes. Although some of the
Rats had normal corneae at this time, others began to exhibit a bluish-grey cornea; some had perforated ulcers and all showed a thin sanguinolent secretion covering both eyes (Table I).

During the next two weeks, practically all experimental animals developed the following progressive eye changes: thickening and bluish-grey appearance of the corneae, sanguinolent secretion, corneal ulceration, perforation of the ulcers, hypopyon, marked bulbar and ciliary injection, edema of the lids, loss of hair of lids, loss of lashes and abscess of the anterior segment of the eyes.

At this point, the eighth week of the experiment, nineteen rats were selected for histological studies of the eye. They were divided into two groups, A and B. The animals of group A were killed, their eyes were removed and prepared for histological examination by fixing in Bouin solution, imbedding in paraffin, sectioning and staining in hematoxylin and eosin. Those of group B were continued on the experimental diet. These animals died in the twentieth week of the experiment, their eyes showing perforated ulcers and abscess of the anterior segment of the eye. Microscopic studies of the eyes of this group were also made.

The histological changes of the eyes of the rats eight weeks on the sodium-deficient diet consisted in some cases of corneal lesions with extensive anterior synechiae, also posterior synechiae and beginning capsular cataract. Wandering cells were present in the posterior chamber. In others, the corneal epithelium was thickened in places and showed areas of ulceration. The whole thickness of the cornea was infiltrated with wan-
dering cells of all types and there was apparently perforation, for the iris was adherent to the center of the cornea in this region. There were some round cells on the surface of the iris in the posterior chamber. The lacrimal gland appeared to be normal.

The sections of the eyes of the rats twenty weeks on the experimental diet showed the following changes: in a number, there was intense infiltration throughout the cornea, which had evidently been associated with perforation of an ulcer, inasmuch as the iris was adherent to the back of the cornea over its whole extent. The corneal epithelium is composed of thin, basal cells which are flattened. The superficial two-fifths are keratinized; in certain animals there was intense purulent keratitis with perforated ulcer and hemorrhage into the anterior chamber. In places, the corneal epithelium was greatly thickened. Basal cells were irregular in pattern, the superficial layer was keratinized. There was a thick plaque of hyaline material over the ulcer which contained colonies of bacteria.

<table>
<thead>
<tr>
<th>RAT NUMBER</th>
<th>LUNGS</th>
<th>HEMORRHAGIC SECRE-</th>
<th>LACRIMATION</th>
<th>CORNEA</th>
<th>CILIARY AND CONJUNCTIVAL APPEARANCE</th>
<th>ULCE-</th>
<th>HYPOPTON</th>
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<tbody>
<tr>
<td>6th week</td>
<td></td>
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<tr>
<td>1 Normal</td>
<td>Present</td>
<td>Slight increase</td>
<td>Clear</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2 Normal</td>
<td>Present</td>
<td>Slight increase</td>
<td>Clear</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3 Edema</td>
<td>Present</td>
<td>Normal</td>
<td>Bluish-white opaque</td>
<td>Marked congestion</td>
<td>Present and keratocon-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Marked edema</td>
<td>Present</td>
<td>Normal</td>
<td>Grey, opaque</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5 Marked edema</td>
<td>Present</td>
<td>Normal</td>
<td>Grey, opaque</td>
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<td>7th week</td>
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<tr>
<td>1 Edema</td>
<td>Present</td>
<td>Normal</td>
<td>Bluish-grey</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2 Slight edema</td>
<td>Present</td>
<td>Normal</td>
<td>Left-clear right-opaque</td>
<td></td>
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<tr>
<td>3 Edema</td>
<td>Present</td>
<td>Normal</td>
<td>Left-grey and thickened</td>
<td></td>
<td></td>
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<tr>
<td>4 Marked edema</td>
<td>Present</td>
<td>Normal</td>
<td>Grey</td>
<td></td>
<td></td>
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<tr>
<td>5 Marked edema</td>
<td>Present</td>
<td>Normal</td>
<td>Grey</td>
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<tr>
<td>8th week</td>
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<tr>
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<td>Normal</td>
<td>Grey</td>
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<tr>
<td>2 Marked edema</td>
<td>Present</td>
<td>Normal</td>
<td>Grey, opaque</td>
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<tr>
<td>3 Marked edema</td>
<td>Present</td>
<td>Normal</td>
<td>Opaque</td>
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<tr>
<td>4 Marked edema</td>
<td>Present</td>
<td>Normal</td>
<td>Grey</td>
<td></td>
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<tr>
<td>5 Marked edema</td>
<td>Present</td>
<td>Normal</td>
<td>Grey</td>
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The sections of the eyes of the control animals were all normal. Since keratinization of the corneal epithelium was found in the sodium-depleted rats and since keratinization of epithelial structures is characteristic of vitamin A deficiency, the histopathology of the eyes of the sodium-deficient animals was studied and compared with that of the vitamin A-deficient animal. As is noted elsewhere in this paper, the diet contained sufficient amounts of vitamin A. Various investigators agree that in vitamin A deficiency there is diminished lacrimation and dryness of the eyes. In this experiment, however, the eyes in every case appeared to be bathed in a normal amount of tears (table 1), and in some instances lacrimation increased. Furthermore, Wolbach and Howe (10) state that in vitamin A deficiency they "have not a single example of ulceration of the cornea or of
hypopyon" which is in marked contrast to the findings in this study. It is obvious that the eye disturbances of the rat occurring as a result of sodium deprivation are markedly different from the changes in the eye produced by vitamin A deficiency.

As the eye condition progressed, the rats rapidly declined in weight, many of them dying. There was 100 per cent mortality after 18 to 21 weeks on the experimental diet. Postmortem examinations were made of all the animals. The body was lean; the adipose tissue when present was very sparse. There was marked atrophy of muscle tissue. The lungs were badly infected. The liver was a darker reddish brown than normal and was also mottled. The spleen was very dark and greatly reduced in size. The kidneys were darker and occasionally showed mottling. The suprarenals were orange in color as contrasted with the pale pink of the controls. Frequently the bladder was distended, and the stomach and intestine were filled with gas. Abscesses or cysts were observed on the small lymph glands situated in front of the submaxillary glands in a large number of the rats.

The bones were fragile, softer and cut more easily than the controls. When stained with silver nitrate, the femurs and tibiae appeared to be slightly rarified and osteoporotic, which phenomena were not exhibited by the control animals. Microscopic examination of the tibiae of the rats showed very little cartilage and little osteoid tissue. The cartilage band was narrow and flat. The picture of these bones is very similar to that seen in hypothyroid animals.

The testes of rats dying between the 18th and 20th week on the diet were smaller than those of the controls. The epididymides of these animals appeared to be atrophied.

Reproduction. A series of experiments to investigate the effect of sodium deprivation on reproduction was set up. First, the oestrous behavior of rats on the experimental diet was studied. Maturity was somewhat delayed, occurring on the average about the 66th day of life in contrast to the 58th day of life of the control rats. Vaginal smears were made following the technic of Long and Evans (11). A group of twelve rats on the experimental diet, and another group of eight on the control one, were used for this purpose. After establishment of the vaginal canal, smears were taken daily for a period of 56 days. The control animals showed no abnormality in the vaginal smears. These rats exhibited an oestrous cycle of approximately 4½ days, which is within the normal range. The ovulation rhythm of the sodium-depleted rats was gravely affected. The experimental rats behaved as follows during this period: for an average of 14 days the ovulatory performance of the sodium-low rats was approximately normal. Thereafter a characteristic disturbance was observed in
the oestrus of the depleted animals resembling that of vitamin A deficiency. It consisted in prolongation of the oestrous desquamation stage in the vaginal epithelium, the smears showing almost exclusively large, flaky, cornified cells which normally characterize the actual period of oestrus and ovulation, but which in this case occurred throughout the entire deple

tion period. In addition to the cornified cells, there were present small amounts of mucus; in some cases, excessive numbers of leucocytes; and in others, a few cornified, nucleated epithelial cells were seen. This abnormality in the vaginal smears of the depleted rats appeared about the same time as the initial changes in the eyes but preceded the growth decline.

In spite of the fact that the diet contained butter fat in amount known to be sufficient, due to the similarity in the vaginal picture to that of the smear in vitamin-A depleted rats, it seemed advisable to rule out the possibility of a borderline vitamin A deficiency resulting from impaired utilization of this vitamin. Therefore, both butter fat and carotene respectively were administered orally. However, repair of the vaginal epithelium was not demonstrated following vitamin A therapy although these substances were administered in large doses.

In order to further determine the effect of sodium depletion on the reproductive systems of both males and females, mating experiments were carried on with rats maintained on the sodium-low diet from the time of weaning and with rats from the stock colony whose reproductive histories were known. During a period of about ten weeks following the attainment of sexual maturity, sodium-depleted females were mated with males from the stock breeding colony. The rats were housed in individual cages. Each female was introduced into the cage of the stock male for a period of 10 to 12 hours out of each 24 hours. During this time the rats had no access to food. During the remainder of the 24 hours both the stock males and the sodium-depleted females were returned to their individual cages where they were fed their respective diets.

Although these females were under observation during the day throughout the experimental period, copulation was not observed. However, from about the 13th day of the experiment there was a slight increase in weight which was suggestive of pregnancy and which was maintained throughout the remainder of the survival period. Postmortem examination at the end of the ten week period revealed no signs of pregnancy in ten of the sodium-depleted animals. The remaining two females, nos. 73 and 81, behaved somewhat differently. They gained weight slowly but to a greater extent than the other sodium-low rats. During a period of 43 days, rat 73 gained 46 grams and rat 81, 49 grams. At that time they were sacrificed. In rat 73 but one fetus was found. Rat 81 had five fetuses which were all located in one uterine horn. These fetuses appeared
to be normal except in size. The one in rat 73 weighed 3.6 grams. Of those in rat 81, one weighed 4.5 grams, another 2.2 grams, and the total weight of the remaining three was 1.7 grams. If these findings are the result of sodium depletion, it may indicate that sodium deficiency causes a prolongation of the gestation period.

In order to study the effect of sodium depletion on fertility in the male rat, twelve males were selected which had been restricted to the experimental diet for a period of 6 weeks after weaning. The sperm motility test, gross examination of the testes at autopsy and mating tests with female rats from the stock breeding colony whose fertility records were known, were the tests used for this purpose. Testicular smears were made at 2 week intervals starting with the 40th day on the experimental diet and were continued to the 110th day. The motility continued to be normal till about the 96th day on the diet, when in five of the animals studied there was a decrease in motility. Simultaneously the epididymides appeared somewhat atrophied and there seemed to be a decrease in their fluid content. Although the testes were smaller than those of the controls, they were normal in proportion to the body weight. No abnormality was noted in their appearance.

Six other males were used for the mating experiments. The procedure for mating, housing, feeding, and general care was similar to that followed in mating the sodium-depleted females with stock males. Vigorous females which had been reared on the stock diet and were known to be fertile were introduced into the individual males' cages. Vaginal smears were run on these females in order to observe the mating behavior. Frequent matings were made for a period of about 8 weeks. In this case the females became impregnated. Two females delivered first litters 22 days, another 27 days, after mating. The litters were normal in size, varying from 9 to 11 young per litter. The young were normal in weight and appearance. These experiments were discontinued after each female delivered two litters, which was before the time when the testicular smears of males on the diet began to show signs of degeneration. In addition to motility, these observations prove that the sodium-depleted male remains fertile for at least 75 to 80 days after restriction to the sodium-deficient diet.

Effect of diets deficient in chlorine and sodium chloride. It seemed to be of importance in connection with this investigation to observe the effect of the deprivation of chlorine alone, and of both sodium and chlorine. The symptomatology produced by these rations was compared with that caused by the sodium-low diet. The composition of these diets was the same as the one deficient in sodium only with the exception that the formulae for the salt mixtures were as follows:
Eight rats were placed on each of these diets. In both cases growth was retarded (chart II). The animals on the chlorine-low diet exhibited no other symptoms although the experiments were carried on for 90 days. The rats on the sodium and chlorine-deficient diet grew more slowly than the chlorine-depleted animals. However, they were not as retarded in growth as the sodium-depleted rats. After the third week on the diet there were pronounced denuded areas about the shoulders and back. At this time the animals were very apprehensive. At the end of the first month, the mouth, ears, nose and forepaws were bloody; the urine was very dark brown in color. In the fifth week the loss of hair became more marked and extended over larger areas. This increased in intensity through the experimental period. However, it seemed to be restricted to the anterio-dorsal parts of the body only. In relation to the effects of sodium deprivation it is of special interest that during the entire experimental period of 90 days, the eyes of these rats were in normal condition.

On postmortem examination the thymus and liver indicated possible internal hemorrhage. The testes appeared too small for the size of the animal, but otherwise seemed to be in good condition as far as could be judged by gross examination. The adrenals were noticeably different from the controls; they were smaller in every case. The bone cut hard as in the controls, whereas the bone of the sodium-depleted rats cut very easily.

Discussion. The results of the effect of sodium deprivation on the animal organism present several points of interest. The symptomatology as demonstrated by the staphyloma of the eyes, lymph gland abscesses and lung infection indicates that the sodium-depleted animals have a decreased resistance to infection, which condition may not be due directly to sodium depletion, but may be rather an indirect effect of the deficiency. Apparently the experimental animals die of an upper respiratory infection.

The retardation in growth cannot be attributed to a decrease in the food intake since the food consumption of the experimental and control animals was practically the same. This fact suggests that the decrease in growth may be the result of a disturbance in the metabolism of the animal.
Perhaps the outstanding symptoms were the effect of sodium depletion on the adrenals and eyes of rats. It is well known that the aqueous humor maintains the nutritive state of the lens. The composition of the inorganic constituents of the aqueous humor is essentially the same as that of the blood, the Na-Ca-K balance being the same in the lens as in the blood. In the diseased lens this salt balance probably is disturbed. Since the lens is extremely sensitive to salt balance, and to changes of osmotic pressure, it is possible that the abnormal condition of the eyes may be attributed to a disturbance of the sodium-calcium-potassium balance. A second possibility is that resistance to infection of these animals is decreased and may be the cause of the disturbance observed.

The preliminary microscopic findings of the bones of sodium-depleted animals are similar to those of bones of other animals which are retarded in growth. As microscopic examination showed, there was little cartilage in the tibiae of the depleted rats. Since normally cartilage is especially rich in sodium, the withdrawal of it from the organism apparently produces the marked effect observed. The tentative assumption is made that the effect on the bone is not a specific one of sodium deficiency but is due to a general slowing down of growth.

In evaluating the results of the experiments on reproduction the fact is emphasized that the mineral content of the diet is of great importance in reproduction as well as growth. The studies presented in this paper demonstrated that sodium depletion gives rise to serious disturbances of reproductive function. The sodium-depleted male rat is fertile for at least 75 to 96 days after restriction to the experimental diet as is proven by sperm motility and mating tests. In the case of the female, however, the changes produced by the deprivation of sodium appear to be entirely different in etiology and nature from the much simpler disturbance characteristic of vitamin A or vitamin E deficiency.

It is evident that sodium depletion may postpone the attainment of sexual maturity of the rat and that this deficiency is inimical not only to growth but to the normal rhythm of ovulation. Since the continuance of oestrus rhythm in the ovary and vaginal epithelium represents cell activity and growth, it may be assumed that sodium deprivation provokes an abnormal physiology of the reproductive system. The mating experiments apparently indicate that reproduction on the sodium-deficient diet is impossible after a certain time due to sterility of the female. These findings support Miller's and St. John's observations on the effect of sodium-low diets on reproduction. The results obtained in the two instances where impregnation did occur are difficult to interpret. However, since ovulation appears to be abnormal it may be deduced that the reproductive processes of the female rat are affected by sodium depletion.
Summary

Restriction of rats to a diet containing only 0.002 per cent sodium but adequate amounts of other nutritive essentials produces a series of symptoms which is terminated by death. The symptomatology comprises retarded growth, disturbances of the eyes and reproductive function.

Extensive corneal lesions, ulceration, hypopyon, hemorrhage and bulbar and ciliary injection are observed in the sodium-depleted rats. Keratinization is seen in the cornea but the lacrimal gland is normal.

The sodium-depleted male remains fertile for at least 75 to 96 days after restriction to the sodium-deficient diet.

In the female on this diet sexual maturity is delayed, the oestrous rhythm and reproductive processes are affected.

In mating the sodium-depleted females with stock males, copulation is not observed. In most cases no signs of pregnancy are exhibited.

It is shown that the disturbances in the eyes and oestrus occurring in the sodium-depleted rats are different from similar disturbances produced by vitamin A deficiency.

Postmortem examination shows that practically all the tissues are affected. The change in the color of the adrenals is especially noteworthy. The bones of these rats are soft and easy to cut.

A chlorine-low diet and a sodium and chlorine-low diet respectively lead to a symptomatology considerably different from that exhibited by the sodium-low diet. Growth is retarded in both cases, but not to the extent of that in sodium deprivation alone. On the sodium and chlorine-low diet alopecia is observed, which is restricted to the anterior-dorsal part of the body. The eyes and bones of the rats on these diets are normal.

References