THE EVALUATION OF THYROID FUNCTION IN EXPERIMENTAL DEHYDRATION OF RATS

by

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INTRODUCTION

The effects of various types of stress stimuli upon the thyroid gland have been studied in recent years with little agreement concerning the reactions of the thyroid gland toward these stimuli. This study was undertaken to determine, using histological criteria, whether the thyroid gland responds to the stress of severe dehydration and the secondary stressor agent, starvation.

LITERATURE REVIEW

Selye (1936) has shown that different types of stress, both physical and emotional, produce the general adaptation syndrome. However, little work has been done concerning the effects of dehydration on the thyroid gland.

Depressed thyroid activity followed various types of stress according to Bogoroch and Timaris (1951) who used formalin injections, forced exercise and severence of the spinal cord to affect severe stress in rats. They found that the thyroid gland uptake of radioactive iodine was less in the experimental animals than it was in the thyroid gland of the control animals. Thus, it was concluded that the activity of the thyroid gland was depressed by these stimuli.

Brown-Grant, et. al. (1954) using such stressor agents as subcutaneous electrical shocks, tying the animals down for twenty-four to seventy-two hours and intraperitoneal injections of turpentine, also found a reduced level of radioactive iodine uptake by the thyroid glands of rabbits. Here too, the conclusion was that the activity of the thyroid was depressed by these stressor agents.

D'Angelo (1951) and Meites and Wolterink (1950) both used severe starvation as a stress stimulus in rats and mice. It was found that there was a lower level of thyrotropic hormone in the blood stream of the experimental animals as contrasted with the controls. Thus, it was concluded by these investigators that the activity of the thyroid gland was lowered by the lowered level of thyrotropic hormone.

Intramuscular injections of ten percent formalin were used by Paschkis, et. al. (1950) as a stress stimulus in rats. Thyroid activity was judged by the radioactive iodine uptake of the thyroid glands. The rate of uptake was decreased, with the conclusion that the thyroid gland activity was diminished.

Cortisone and A.C.T.H. were administered to rats by Bondy and Hagewood (1952) in order to simulate a stresslike condition. Using protein-bound iodine determinations as an indicator of thyroid function, it was found that these injections lowered the level of protein-bound iodine in the blood. It was concluded from this data, that since A.C.T.H. and cortisone had the effect of depressing the activity of the thyroid gland, stresses should also have this same effect.

Cohn (1958) found that hypothyroidism occurred as a result of forced feeding of normal rats. Hypothyroidism was diagnosed because of a lowered uptake of radioactive iodine by the thyroid glands of experimental animals.

A decrease of activity, as measured by thyroid gland uptake of radioactive iodine, occurred when rats were subjected to dehydration and starvation. From this data, Reichlin (1957) concluded that the activity of the thyroid glands was decreased.

Using some of the same stress stimuli as were used in the preceding experiments, the following investigators found that the thyroid gland was stimulated to produce more thyroxine under various conditions of stress.

Puntriano and Meites (1951) used the stimulus of continuous light or continuous darkness as a stressor agent on mice. It was concluded that an environment of continuous light caused a reduction of thyroid gland weight and uptake of radioactive iodine, whereas, an environment of continuous darkness caused an increase in these phenomena.

Cold was used as a stimulus by Starr and Roskelley (1940) and Peterson and Young (1955), on rats and guinea pigs. Hypertrophy of the gland and a heightened epithelium were the responses which were noted in these experiments. It was concluded that there was an increase in thyroid function in both cases.

Williams, et. al. (1949) used starvation, cold and adrenalin injections as stress agents in rats. Using both radioactive iodine and protein-bound iodine determinations, it was found that the activity of the thyroid glands was heightened by these stimuli.

Using prolonged sodium chloride ingestion as a stressor agent, Isler, et. al. (1958) observed that the level of circulating thyroid hormone was increased in the mouse, by means of the protein-bound iodine determination. An accelerated level of thyrotropic hormone release by the pituitary gland was felt to be the reason for an increased thyroxine production.

Tourniquet shock was used on rats as a stress stimulus by Hamolsky, et. al. (1951). As diagnosed by radioactive iodine uptake of the thyroid glands, it was found that in some cases an increase in uptake of radioactive iodine occurred, while in other cases a decrease was found in the uptake of radioactive iodine. Adrenalectomy of the experimental animals did not alter these results.

Ackerman and Arons (1958) administered epinepherine and norepinepherine to dogs as stress stimuli. It was found that the level of thyroid hormone in the blood stream was significantly increased by these stimuli.

The response of the human thyroid gland to stress appears to be the same as that of experimental animals'. Goldenberg et. al. (1954) found that patients had increased thyroid hormone in their blood stream immediately after an operation.

Hertzel et. al. (1952a; 1952b) used the protein-bound iodine determination as a diagnostic agent for the amount of thyroid hormone present in the blood streams of human patients who were undergoing or had undergone stressful life experiences. Thyroxine levels in the blood were raised during and after such experiences.

MATERIALS AND METHODS

The experimental animals were two-month old, female, albino rats of the Sprague-Dawley strain. After arriving from Madison, Wisconsin, they were kept for a week to recover from the trip. The rats were then divided into two groups and were fed Purina Laboratory Chow, ad libidum.

Thirty-two rats were used in the first experiment. Sixteen of these received food and water ad libidum. The other sixteen received food but were deprived of water. The rats were kept in an air conditioned room. Two rats from each group, control and experimental, were sacrificed each day for eight days, by means of an overdose of ether.

The rats were weighed, the body cavity opened and the thyroid gland with an attached segment of the trachea and the adrenal glands were removed. The thyroid and the right adrenal glands were placed in Bouin's fixative, while the left adrenal gland was fixed in ten percent neutral formalin.

The thyroid glands and the right adrenal glands were infiltrated by the dioxane method, embedded in paraffin, and sectioned at five to six micra. At least two slides from each thyroid gland were made. A modification of Mallorys' triple stain was used in the staining procedures. The embedded adrenal glands were not sectioned, but were preserved for study at a later date, and were not incorporated in this study.

The second experimental group consisted of forty-eight rats which were likewise divided into two equal groups. One group received food and water, while the other group was deprived of water. Three rats from each group were sacrificed each day for eight days. The thyroid and adrenal glands were removed and prepared as in the previous experiment.

The third experiment involved eighteen animals. Twelve rats were placed in the experimental group while the remainder served as controls. The experimental rats were dehydrated for seven days, at which time, six rats were sacrificed from this group, along with three control animals. On the eighth day, the remaining six experimental and the three control animals were sacrificed. The same procedures for removal and treatment of the thyroid and adrenal glands were followed in this experiment as in the preceding experiments.

In the fourth experiment, sixteen animals were used. Twelve rats were placed in the experimental, dehydrated group, and the other four were used as controls. On the fourth day of dehydration, four experimental animals were anaesthetized with ether and weighed. The body cavity was opened and the blood was removed by opening the bottom of the heart and allowing the blood to flow into a centrifuge tube. The blood was permitted to clot after which it was centrifuged. The serum was transferred to vials and frozen, and later used for protein-bound iodine determinations. The adrenal glands were quick-frozen in solid carbon dioxide, and were later used in ascorbic acid determinations. The thyroid glands were removed, fixed in Bouin's fluid, infiltrated, embedded and sectioned as outlined in the previous experiments.

On the sixth day of dehydration, four control animals and four experimental animals were sacrificed and on the eighth day of dehydration the remaining four experimental animals were sacrificed, following the procedures as before.

In addition to the general microscopic observations of the sectioned glands, measurements of specific cells were taken. All measurements were made using high power (538 X) magnification. An ocular micrometer was used as a measuring device. Each slide was first observed under low power, then the following measurements were taken using high power: the height of the alveolar epithelium, the height of the alveolar epithelium plus the cell extensions and the approximate colloidal area of the largest alveoli. Four random measurements of the alveolar epithelium were made on each slide, giving eight measurements for each thyroid gland.

A similar method was used in measuring the epithelial height plus the cell extensions, resulting in eight readings per gland.

In measuring the approximate colloidal area, the gland was first scanned with low power in order to determine the six largest alveoli. The microscope was then changed to high power and the length and width of the colloid filled area within these alveoli were measured. Thus, twelve measurements for each gland were recorded.

The four measurements of the epithelial height alone and with the cell extensions taken on each slide were added together and an average was taken. In the approximate colloid area measurements, the height was multiplied by the width to give an index to the area of the colloid.

A list of all of the seperate measurements and of the averaged measurements was made and sent to the Statistics Department for a statistical analysis.

The frozen serum samples and the frozen adrenal glands were taken to the Lattimore-Fink Laboratories in Topeka, Kansas, for the protein-bound iodine and the ascorbic acid determinations.

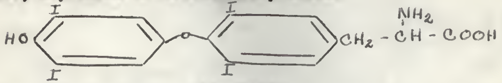
EXPERIMENTAL RESULTS

The thyroid glands of rats are small and so closely associated with the larynx and trachea that excision of the glands alone was difficult. In order to obtain thyroids for sectioning, a portion of the trachea was removed and fixed along with the thyroid tissue. The resulting sections were of the trachea with a lobe of thyroid tissue on each side.

Control Groups

The thyroid glands of the control animals were composed of numerous alveoli. In section, each alveolus was ovoid in shape and lined with a single layer of cuboidal epithelium. Each alveolus was filled with colloid, a substance composed largely of thyroglobulin, a protein complex of thyroid hormones. A basement membrane surrounds the epithelial cells to complete an alveolus, the functional unit of the thyroid gland. The periphery of the thyroid contains somewhat larger alveoli than are present in the center of the gland.

The epithelial cells are the secreting portion of the gland, with the colloid acting as a storage space for accumulated thyroid hormone, thyroxine or tetraiodothyronine.



Thyroxine

The epithelial cells are somewhat triangular in outline with the apex of the triangle toward the colloid and the base of the triangle surrounded by the basement membrane. The majority of these epithelial cells had cell extensions, or small globules of clear, nonstaining material projecting from the apex of the cell into the colloid. No correlation has thus far been discovered between these cell extensions and the secretory mechanism of the epithelium, or in any other function of the thyroid gland.

Experimental Groups

Casual observation under low or high magnification failed to show any striking differences in the morphology of the thyroid glands of the experimental and control groups. The alveoli were of the same shape and distribution within the gland and there seemed to be no difference in the amount of colloid within alveoli.

By the analysis of varience method, the experimentally dehydrated animals showed a small, but significant, difference in the height of the cuboidal epithelium. (P=>0.001). Thus, there was a direct correlation between the height of the cells and the length of the dehydration period. As the dehydration period increased, the height of the epithelial cells increased. This relationship is shown on Plate I.

No significant differences could be found in the measurements of the epithelial height plus the cell extensions or in the approximate colloidal area of the six largest alveoli per section studied.

Starvation, as a direct result of dehydration, must be taken into consideration as a secondary stressor agent. Feeding virtually stopped after the second day of dehydration, adding to the stress to which the animals were subjected.

Body weights of the experimental animals decreased progressively as the period of dehydration and subsequent starvation continued. The blood, became noticably more viscous by the fourth day, the middle of the dehydration period.

Body weights of the control animals showed a progressive increase of approximately five grams per day during the experimental period. This relationship may be seen in Plate II.

Protein-bound Iodine

Serum protein-bound iodine determinations are used as a measure of thyroid gland activity. McClendon and Foster (1941) and Chaikoff, et. al. (1947), both stated that this determination is one of the most accurate diagnostic methods of determining thyroid function known at the present time. The protein-bound iodine determination was done by the method outlined by Chaney (1940).

There are two types of iodine within the body, the inorganic iodine and the organically protein-bound iodine. The protein-bound iodine determination measures the amount of organically bound iodine found in the serum. Thyroxine and the triodothyronines the main thyroid hormones, are organically bound. Thus, there is a direct correlation between the activity of the thyroid gland and the amount of organically bound iodine to be found in the serum.

In this experiment the organically bound iodine increased steadily from the fourth to the sixth day of dehydration, after which time it began to decrease. By the eighth day of dehydration, the organically bound iodine had fallen to a level below that of the control animals. (Plate III).

From these results, it may be concluded that the thyroid gland continually increased its output of thyroxine until the sixth day of dehydration, at which time its activity decreased to a level below that of the normal thyroid gland.

Ascorbic Acid

The ascorbic acid determination has been used as a measurement of the activity of the adrenal glands. This determination was done by the method outlined by Roe and Kuether (1943).

Sayers and Sayers (1947) found that various types of stress including exposure to heat and cold, and the injection of epinephrine and histamine caused a reduction in the ascorbic acid content of the adrenal gland of the rat.

There are at least two theories concerning this lowered ascorbic acid content of the adrenal gland. This phenomenon has been attributed to a transport mechanism utilizing the ascorbic acid, or to the utilization of ascorbic acid in the actual formation of the adrenocortical steroids. There appears to be no agreement as to which of these theories is more nearly correct.

Kimura, (1954) using the deprivation of food and water as a stress stimulus in rats, found that the ascorbic acid level of the adrenal glands was higher than it was in those of the normal control animals.

Elton, et. al. (1959) found that several species of animals, when subjected to such stressor agents as cold exposure, showed an increase in ascorbic acid levels. Such animals as frogs, cats, mice and chickens showed a significant increase in their ascorbic acid levels.

Done, et. al. (1953) found that in spite of the virtual absence of ascorbic acid concentrations in guinea pigs, the adrenocorticosteroids were at a much higher level in the scorbutic guinea pigs than they were in the normal control animals.

Stewart, et. al. (1952) found that the function of the adrenal cortex in scorbutic monkeys was normal and often increased over those of the normal animals.

Results of this experiment show that the adrenal ascorbic acid level is increased from the second to the sixth day of dehydration, at which time, the levels fall to below those of the control animals' ascorbic acid. This relationship may be seen on Plate III.

DISCUSSION

The histological results of this experiment are in agreement with those of Starr and Roskelley (1940), in that the alveolar epithelium was increased in height, as the result of a stress situation. This data alone was felt to be sufficient to indicate that the activity of the thyroid gland was increased. As supporting evidence, however, protein-bound iodine determinations were used. Results from the protein-bound iodine determinations are in agreement with those made by Williams et. al. (1949), in their work with rats and the stress syndrome.

Ascorbic acid determinations were used as a means of determining the amount of stress to which experimental animals were subjected by Sayers and Sayers (1947). Studies of ascorbic acid levels in experimental animals in early stages of the stress syndrome, showed a depletion of adrenal ascorbic acid as one reaction to stress in general. In recent years, however, there have been various studies questioning the validity of this hypothesis.

The results of this experiment agree with those of Elton, et. al. (1959) and Kimura (1954). These investigators found that there was an increase in adrenal ascorbic acid as a result of stress stimuli on rats and various other experimental animals. No explanation has been proposed to explain the discrepancy in these results, and those of Sayers and Sayers (1947). The animals in this experiment were definitely thought to be in a stress condition as the result, primarily, of the dehydration and secondarily, of the resulting starvation.

SUMMARY

The effects of experimental dehydration of rats were studied and evaluated.

Thyroid glands from experimental and control animals were removed, embedded, sectioned, stained and studied under the microscope. Blood was removed from one group of rats, and was used for protein-bound iodine determinations. Adrenal glands were also removed and used for ascorbic acid determinations.

The following results were obtained:

1. A significant (P= 0.001) increase in the height of the alveolar epithelium of the dehydrated rats, as compared with the control animals, was noted.
2. No significant differences were found between the control and experimental groups in the measurements of the alveolar epithelium and the cell extensions. The approximate colloidal areas of the six largest alveoli were not found to show any significant differences between the experimental and control groups.
3. Protein-bound iodine levels of the dehydrated rats increased from the fourth to the sixth day of dehydration. Following the sixth day, and until the eighth day of dehydration, the iodine level dropped to below the level of that of the control animals. These results indicate that there was an increase in serum thyroxine until the sixth day of dehydration, then a decrease to below normal

levels by the eighth day of dehydration.

4. Ascorbic acid levels of the experimental animals increased from the fourth to the sixth day of dehydration. The levels decreased from the sixth to the eighth day of dehydration to below the level of that in the control animals.

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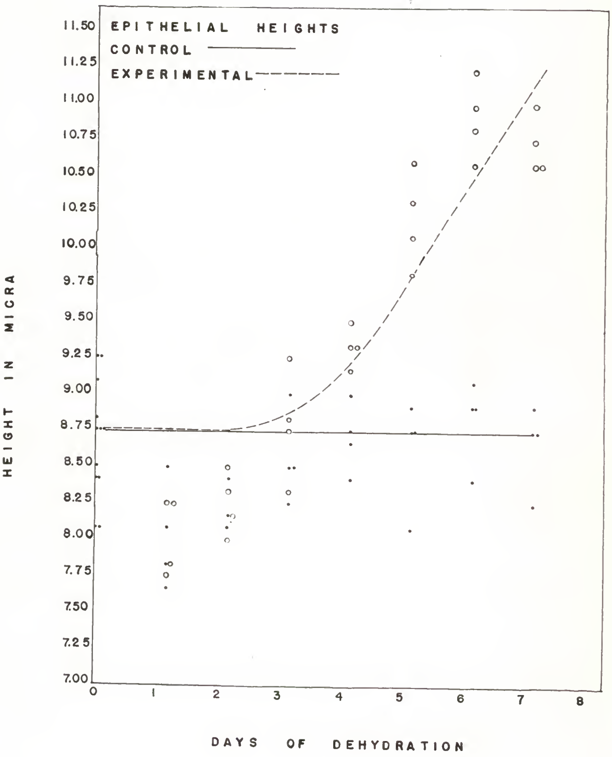
APPENDIX

EXPLANATION OF PLATE I

This graph represents the height of epithelial cells of both the control and the experimental animals from the first experimental group. The solid line shows the height of the alveolar epithelium of the control animals. The dotted line shows the increase in the height of the alveolar epithelium of experimental animals over the period of dehydration.

Plate I


EPITHELIAL HEIGHTS
CONTROL
EXPERIMENTAL
HEIGHT IN MICRA
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10.75
10.50
10.25
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9.75
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9.00
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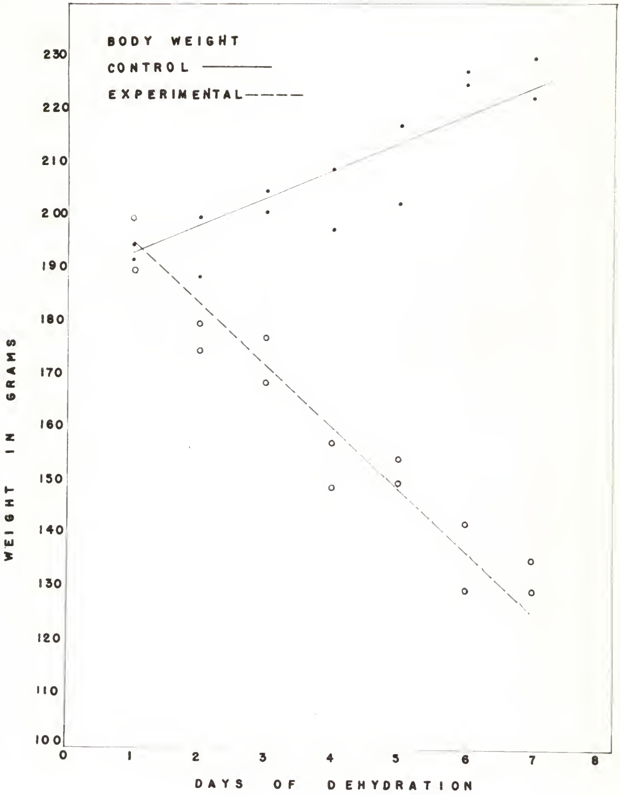
8.25
8.00
7.75
7.50
7.25
7.00
0
1
2
3
4
5
6
7
8
DAYS OF DEHYDRATION

EXPLANATION OF PLATE II

This graph shows the body weights of both the control and experimental animals over the dehydration period. The solid line represents the body weights of the control animals. The dotted line represents the body weights of the experimental animals during the dehydration period.

Plate II


BODY WEIGHT
CONTROL
EXPERIMENTAL
WEIGHT IN GRAMS
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220
210
200
190
180
170
160
150
140
130
120
110
100
0
1
2
3
4
5
6
7
8
DAYS OF DEHYDRATION

EXPLANATION OF PLATE III

Figure I is a graph showing the levels of ascorbic acid over the dehydration period. The solid line shows the ascorbic acid levels of the control animals. The dotted line represents the ascorbic acid levels of the experimental animals over the dehydration period.

Figure 2 is the graph of the protein-bound iodine levels over the dehydration period. The solid line shows the level of the protein-bound iodine in the control animals. The dotted line represents the protein-bound iodine levels of the experimental animals.

Plate III

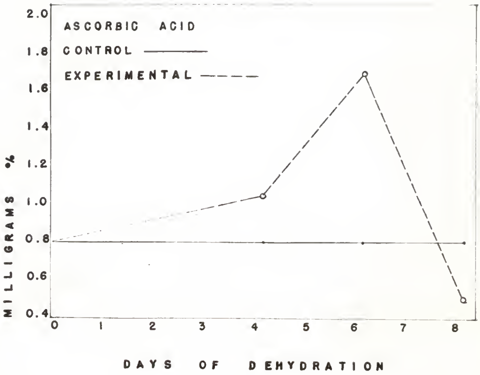

Fig. 1

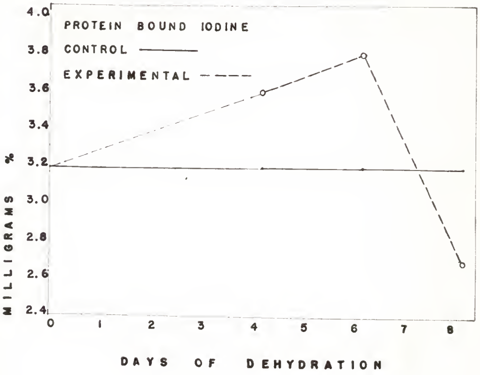

Fig. 2

EXPLANATION OF PLATE IV

Figure 1 is a photomicrograph of the rat thyroid gland as seen under low power. (X 125 approximately).

Figure 2 is a photomicrograph of the same thyroid gland as in Figure 1 but under high power. (X 540 approximately).

Plate IV

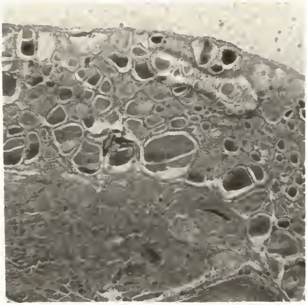

Fig. 1

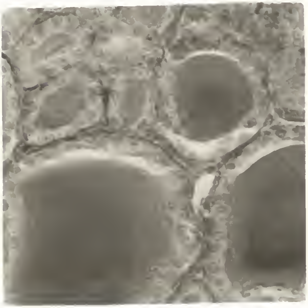

Fig. 2

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This study was undertaken to discover the histological changes taking place in the thyroid gland of rats subjected to severe stress, in the form of dehydration. Also an evaluation of the function of the thyroid glands during this stress period.

Four groups of experimental animals were randomly allotted. The first three groups were comprised of control and experimental animals from which the thyroid and adrenal glands were removed over a period of eight days. The fourth group consisted of experimental and control animals from which blood and the adrenal glands were removed. The blood serum and adrenal glands were frozen and later used for protein-bound iodine and ascorbic acid determinations respectively.

In addition to the general microscopic observations of the sectioned thyroid tissue, the measurements of specific tissues and cells were recorded. An ocular micrometer was employed as a measuring device.

The following results were observed:

1. The height of the alveolar epithelium increased as the period of dehydration contimued. This growth was believed to be correlated with an increased production of thyroxine.
2. Measurements of the alveolar epithelium plus cell extensions and of the approximate area of the colloid of the six largest alveoli were not found to be statistically significant.
3. Protein-bound iodine determinations showed an increased level of thyroxine in the blood stream of the dehydrated animals, until

the sixth day of dehydration, after which time, the levels fell to below those of the control animals by the eighth day of dehydration.

4. Ascorbic acid determinations showed an increased level of adrenal ascorbic acid until the sixth day of dehydration, after which time the levels fell until on the eighth day of the dehydration period, the level was below that of the normal control animals.