THE CORTICAL HORMONE REQUIREMENT OF THE
ADRENALECTOMIZED DOG, WITH SPECIAL REF-
ERENCE TO A METHOD OF ASSAY*

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(Received for publication, October 24, 1933)

A method of assay for the adrenal cortical hormone utilizing the
blood urea level of the adrenalectomized dog was suggested by
Harrop, Pfiffner, Weinstein, and Swingle (1, 2) as a result of earlier
cooperative work on the effect of the hormone in the normal and
adrenalectomized animal. In those studies it was found that the
cortical hormone had no demonstrable effect in the normal dog.
A rise in blood urea was observed to be one of the first manifesta-
tions of insufficiency in the adrenalectomized dog on the gradual
withdrawal of extract. A dog unit was defined as "the minimum
daily kilogram dose of cortical hormone necessary to maintain
normal physiological conditions in the adrenalectomized dog for a
period of 7 to 10 days; the two criteria of normal physiological
condition being maintenance of body weight and blood level of
non-protein nitrogen (or urea)." Rogoff and Stewart (3) had
found in their studies on the effect of adrenalectomy in the dog
that a significant rise in the blood urea usually terminated the
"period of good health" and occurred some time (usually at least
several days) before death.1 All other methods of assay so far

* In the collaborative studies on the adrenal cortex carried on in this
laboratory the writers have specialized in certain phases of the experiments.
The physiological aspect of the problem is the responsibility of the senior
author (W. W. S.); the biochemical work of extraction and purification of
the hormone, that of one of us (J. J. P.). An abstract of this paper was read
before the American Society of Biological Chemists at Cincinnati, April
10-12, 1933.

These investigations have been aided by a grant from the Josiah Macy,
Jr. Foundation.

1 Marshall and Davis (4) were the first to observe an increase in the blood
urea following adrenalectomy. These observations were made on the cat.
suggested have employed either the rat or cat. A discussion of these techniques and the factors affecting the results are contained in the literature cited (5-15).

Knowledge of the influence of various factors, both physiological and environmental, on the hormone requirement of the individual animal, is necessary for extraction and fractionation studies. This paper is concerned with the quantitative effect of these factors.

Adrenal Extracts Employed—The experimental work (with a few exceptions noted in the text) was carried out with a single lot of extract (Extract 31632) prepared by methods previously described (16) from 40 kilos of whole beef adrenals which were received in the laboratory between February 17 and March 16, 1932. The adrenalin concentration of this particular extract was 1:1,000,000. In those instances in which an extract other than this lot was employed the number of the extract is specified. Unassayed stock extract was used to maintain the animals from operation to experimentation, in realimentation, and maintenance between experimental runs. These extracts were prepared so that 1 cc. represented 40 gm. of whole gland. All dosages of extract are expressed in terms of gm. of whole adrenal gland per kilo of body weight per day unless otherwise stated.

Care and Maintenance of Adrenalectomized Dog—The present study was conducted on a series of ten adrenalectomized adult mongrel dogs, nine males and one female. The adrenal glands

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2 We wish to thank Mr. L. W. Rowe of the Research Laboratories of Parke, Davis and Company for the adrenalin bioassays conducted on the extracts employed in this study.

3 Unassayed stock extract was prepared by the usual methods (16). The yield of cortical hormone as indicated by bioassay of representative batches was 2000 dog units or more per kilo of whole beef adrenal. Since September, 1932, stock extract was prepared by the so called "half method" (17). Assays conducted on samples drawn from various lots prepared by this method during a period of 12 months production showed the yield to run consistently between 1000 and 2000 dog units per kilo of gland. Methods of extraction will be discussed more fully elsewhere.

4 At the present writing (October 23, 1933) six of the animals (Dogs 2, 7, 12, 23, 42, and 92) are alive and in good condition, 853, 785, 755, 652, 524, and 272 days, respectively, since adrenalectomy. The other four dogs died 119 to 329 days following the removal of the second adrenal. Autopsy in these four cases failed to reveal any accessory tissue. These data demonstrate
were removed in two stages, first the right and then the left gland. For several days following the removal of the left gland the animals received a dose of 20 to 40 gm. of extract, gradually dropping to a level of 4 to 8 gm. This was the usual dose for maintaining animals between assays. In bringing animals out of an insufficiency period a dose of 10 to 40 gm. was employed, depending upon the severity of the condition. Extract was administered subcutaneously in divided doses unless otherwise stated. Animals in very severe insufficiency were treated intravenously. In the present study a 7 day period was employed for each dosage level.

The animals were housed in individual cages in heated quarters. They received no exercise other than that which they could obtain in their cages. They were fed once a day usually between 10 and 12 a.m. The maintenance diet consisted of prepared dog foods, Diet B, and Diet K, or a mixture of both to which the following supplements were added three times weekly, 10 gm. of yeast, 5 cc. of cod liver oil, 5 gm. of Cowgill's salt mixture (18), and 0.4 gm. of iron and ammonium citrate. The animals were allowed approximately 80 calories of food per kilo of body weight per day.

that adrenalectomized dogs can be maintained for periods of 2 years or longer in apparently normal physiological condition even though they have been allowed to pass repeatedly into insufficiency by withdrawal of extract. It perhaps should be pointed out, however, that two abnormalities have been recognized in this colony of adrenalectomized dogs. In all cases an anemia of varying degree of severity has been observed to develop within the first few months following adrenalectomy. Unoperated control dogs have maintained a normal hemoglobin level upon the same dietary regimen. The quantity of blood taken from these assay animals is negligible when compared to the amount found necessary by Whipple and others to produce hemorrhagic anemia. Parasitic infection has been ruled out as a possible source of hemoglobin loss. Thus far various forms of therapy have failed to correct the condition.

The other abnormality observed in adrenalectomized dogs maintained in good physical condition with ample doses of cortical hormone is an abnormal glucose tolerance curve. The foregoing points will be discussed in detail elsewhere.

6 Bal Ra, Valentine Meat Juice Company, Richmond, Virginia.
6 Ken-L-Ration, Chappel Brothers, Rockford, Illinois.
7 Northwestern Yeast Company, Chicago.
8 In connection with the high concentration of vitamin C in the adrenal cortex as demonstrated by Szent-Györgyi and others, it is of interest to note here that the diet employed in these experiments contains very little
A daily record of food consumption was kept for each animal. Those which lost weight were allowed to recover their weight losses between assays. Blood samples were obtained at least 15 to 18 hours after all food had been withdrawn.

The method of Folin and Svedberg (19) was used in determining the blood urea nitrogen with the exception of some of the more recent figures which were obtained with the gasometric hypobromite method of Van Slyke (20). Hemoglobin was estimated by Newcomer’s method. Blood sugar was determined on a tungstic acid filtrate with the Somogyi (21) modification of the Shaffer-Hartmann reagent.

Hormone Requirement As Influenced by Physiological Factors—An assay on a series of nine adrenalectomized dogs, eight males and one female in anestrus, shows what variation may be expected in the hormone requirement of individual animals. A summary of the data appears in Table I, Series A. Occasionally an animal brought into insufficiency by gradual reduction of dosage level will show a marked fluctuation in the level of blood urea while exhibiting no clinical symptoms of insufficiency other than a somewhat variable appetite. A comparison was made, therefore, of assay results based upon a 100 per cent urea rise and upon clinical failure. The minimum maintenance dose9 determined by the urea rise in the eight dogs in which this was determined ranged from 0.2 to 0.5 gm., with an average of 0.38 gm. On the basis of clinical failure which includes anorexia, asthenia, and usually vomiting, the minimum maintenance dose for 9 days ranged from 0.2 to 0.4 gm., average 0.29 gm. The same dog showed a variation in minimum maintenance dose by these two criteria of 0 to 108 per cent. There is no correlation between minimum maintenance dose and

if any vitamin C as demonstrated in feeding experiments with guinea pigs. Several assay animals have been maintained on this dietary régime for periods of 2 years and longer without their adrenal glands but with adrenal extracts containing no demonstrable quantity of vitamin C. Large quantities of vitamin C (decitrated and concentrated lemon juice) orally administered had no effect on the cortical hormone requirement of adrenalectomized dogs. Experimental details will be published elsewhere.

9 The minimum maintenance dose is the dose the animal was receiving during the period prior to the 100 per cent rise in urea nitrogen or prior to clinical failure as the case may be.
the time interval elapsing between operation and assay. The results are also independent of the number of previous assays. The average weight loss during an assay was 10 per cent. However, dogs can exhibit marked insufficiency and die at a peak weight.

**Table I**

*Cortical Hormone Requirement of a Series of Nine Adrenalectomized Dogs*

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Series</th>
<th>1st day of assay</th>
<th>Date from second operation to assay</th>
<th>Previous insufficiencies</th>
<th>Maximum and final body weight during assay</th>
<th>Minimum maintenance dose</th>
<th>Failing dose</th>
<th>Diet plus supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>92</td>
<td>A</td>
<td>Feb. 21, 1933</td>
<td>27</td>
<td>0</td>
<td>10.9 - 9.5</td>
<td>10.2 - 11.0</td>
<td>0.34</td>
<td>K</td>
</tr>
<tr>
<td>42</td>
<td>&quot;</td>
<td>July 20, 1932</td>
<td>62</td>
<td>1</td>
<td>8.1 - 7.3</td>
<td>8.0 - 11.0</td>
<td>0.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>25</td>
<td>&quot;</td>
<td>May 13, 1932</td>
<td>98</td>
<td>3</td>
<td>14.0 - 11.6</td>
<td>14.0 - 11.0</td>
<td>0.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>23</td>
<td>A</td>
<td>May 3, 1932</td>
<td>113</td>
<td>4</td>
<td>9.5 - 8.8</td>
<td>9.0 - 11.0</td>
<td>0.5</td>
<td>&quot;</td>
</tr>
<tr>
<td>20</td>
<td>&quot;</td>
<td>Apr. 20, 1932</td>
<td>118</td>
<td>3</td>
<td>9.5 - 9.1</td>
<td>9.1 - 11.0</td>
<td>0.5</td>
<td>&quot;</td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>July 22, 1932</td>
<td>204</td>
<td>5</td>
<td>10.2 - 9.6</td>
<td>10.0 - 11.0</td>
<td>0.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>10</td>
<td>A</td>
<td>May 3, 1932</td>
<td>224</td>
<td>5</td>
<td>15.0 - 11.0</td>
<td>15.0 - 11.0</td>
<td>0.2</td>
<td>BH</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
<td>3, 1932</td>
<td>6</td>
<td>10.2 - 9.0</td>
<td>10.0 - 11.0</td>
<td>0.2</td>
<td>K</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>May 2, 1932</td>
<td>314</td>
<td>6</td>
<td>13.2 - 11.2</td>
<td>13.0 - 11.0</td>
<td>0.28</td>
<td>&quot;</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>July 21, 1932</td>
<td>6</td>
<td>12.7 - 11.0</td>
<td>12.0 - 11.0</td>
<td>12.0 - 11.0</td>
<td>0.28</td>
<td>B</td>
</tr>
</tbody>
</table>

* The following dosage levels were used: in Series A, 4.0, 2.0, 1.0, 0.5, 0.4, 0.34, 0.28, 0.24, 0.22, 0.2 gm. of gland per kilo per day; in Series B, 4.0, 0.4, 0.34, 0.28, 0.24, 0.22, 0.2 gm.

† The maximum weight is recorded rather than the initial weight since several of the animals gained weight during the early part of the assay.

‡ Diet K, Ken-L-Ration; Diet B, Bal Ra; Diet BH, beef heart.

§ Not determined.

‖ A 10 to 29 day period elapsed between check assays.

¶ Female in anestrus; all other dogs males.

The duration of the assay periods, in Table I, ranged from 20 to 70 days. This variation in time period had no striking influence upon the results.
The sex of the animal is of no significance provided the female is in anestrus. Rogoff and Stewart (22) were the first to demonstrate that the bitch, if adrenalectomized while in heat, could survive as long as 65 days. This observation has been confirmed in this laboratory by withdrawing extract from adrenalectomized bitches when in heat.

On completing the above assay five of the dogs were used to recheck the results. The check assays, Table I, Series B, show the results when one or the other stock diet was fed exclusively. These animals would eat either ration with equal readiness when in good physical condition. Dogs coming into insufficiency and subsisting on Diet B alone usually show an aversion to food earlier than when receiving Diet K. In Series A the animals were started upon the former and gradually worked over to the latter. (This is the feeding schedule in routine assay work.) Inspection of the data, Series B, shows that there was no striking difference in results obtained on the same dog when using the different diets. With Dog 12, who was fed a ration of beef heart, the failing dose was 0.4 gm. in contrast to 0.2 gm. on Diet K. The animal did not relish the ration, and refused the food for the last 5 days of the assay. At the time of realimentation it exhibited no asthenia (spasticity), but did show a continued refusal to eat this diet, somewhat lessened activity, and a markedly elevated blood urea and pulse. The experiments on fasting, Table III, indicate that this early failure of Dog 12 can be accounted for by the voluntary fast, and not the beef heart diet, per se.

The check assays on Dogs 23 and 25 indicate that a loss of weight is not necessarily an integral part of the syndrome of cortical insufficiency in the dog. Dog 25 died at a peak weight. Autopsy findings were typical of death from insufficiency. This dog as in some others when subsisting on a dose approximating the minimum maintenance dose always exhibited a fluctuating blood urea, occurring without concomitant changes in clinical condition. Similar fluctuations in blood urea occurred in Dog 20, when held for a period of 33 days upon a minimum maintenance dose of 0.4 gm. It should be emphasized that all blood samples were obtained at least 15 hours after all food had been withdrawn. The possibility remains that these variations of blood urea in animals on the verge of insufficiency may be due in part to a delayed absorption. Food records
for these periods show some correlation between food ingested and blood urea. Whenever an animal took very little food for a day or two, a marked rise in blood urea would occur—being followed by a drop when the ration was again ingested. The great variability in the appetite of these dogs chronically at a level of insufficiency remains unexplained.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Date of operation</th>
<th>Dosage prior to assay*</th>
<th>1st day of assay</th>
<th>Extract No.</th>
<th>Minimum maintenance dose</th>
<th>Insufficiencies between check assays</th>
<th>Time interval†</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Aug. 31, 1931</td>
<td>15.0†</td>
<td>Sept. 23, 1931</td>
<td>813-A</td>
<td>3.0</td>
<td>&lt;2.3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.0†</td>
<td>Apr. 6, 1932</td>
<td>31632</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8</td>
<td>May 3, 1932</td>
<td></td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.0</td>
<td>Nov. 21, 1932</td>
<td></td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>Sept. 2, 1933</td>
<td></td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Sept. 30, 1931</td>
<td>10.0</td>
<td>Jan. 26, 1932</td>
<td>1532-B</td>
<td>0.5</td>
<td></td>
<td>7</td>
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<tr>
<td></td>
<td></td>
<td>0.4§</td>
<td>Mar. 7, 1933</td>
<td></td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Jan. 11, 1932</td>
<td>8.0</td>
<td>May 3, 1932</td>
<td>31632</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.2</td>
<td>Apr. 14, 1933</td>
<td></td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>May 19, 1932</td>
<td>4.0</td>
<td>June 11, 1932</td>
<td>D-218</td>
<td>2.0</td>
<td></td>
<td>5</td>
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<tr>
<td></td>
<td></td>
<td>0.75§</td>
<td>Dec. 12, 1932</td>
<td></td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Unassayed stock extract. Estimated that 0.5 gm. represents 1 dog unit.
† Number of days which elapsed between the termination of an assay and the beginning of the check assay.
‡ Dissected cortex. Estimated that 5.0 gm. represent 1 dog unit.
§ Previously assayed. Amount indicated known to represent 1 dog unit.

In Table II are tabulated data on several check assays conducted on the same dog at intervals of 164 to 359 days, the same sample of extract being used in each instance. During this interval the dogs experienced from three to seven periods of insufficiency. Comparable findings on the minimum maintenance dose were obtained

10 Preserved with benzoic acid (0.1 per cent) and stored in the refrigerator at 6°.
in all cases with the exception of the first check assay on Dog 7. The sample of extract was exhausted before the check assay could be completed. One point of particular interest brought out in the assays on Dogs 12 and 42, Table II, is that the minimum maintenance dose is quite independent of the dosage level on which the dog was maintained prior to assay. Dog 12 was receiving an estimated daily dose of 8 dog units per kilo prior to the first and 1 dog unit per kilo for a period of 7 days prior to the check assay. It has been found that the dosage level prior to complete withdrawal of extract has no striking influence on the length of the survival period. Since apparently the hormone cannot be stored and since an overdosage effect of the hormone seemingly cannot be demonstrated, it would appear that all excess of injected hormone over and above the animal’s immediate requirement is either excreted or further metabolized.

Experiment shows that cortical hormone added to normal dog urine can be recovered quantitatively. When a massive dose (2340 dog units) of cortical hormone was injected subcutaneously into a normal male dog, less than 200 dog units could be detected in the urine collected for the following 53 hours. The possibility remains that a certain amount of hormone may be excreted by the kidney if large quantities are administered intravenously. The return from insufficiency to normal condition, based on blood urea figures, was about equally rapid by either subcutaneous or intravenous therapy, usually requiring 24 to 72 hours with the doses employed.

Britton, Flippin, and Silvette (23) from their work on adrenalectomized cats expressed the opinion that the oral administration of the cortical hormone held out promising clinical possibilities. Comparative assay findings on the dog demonstrate that in this species the subcutaneous administration of the hormone is more than 12.5 times as efficient as the oral route. The minimum maintenance dose (subcutaneous) was determined on Dog 42 as 0.4 gm. This dog came promptly (7 days) into the state of insufficiency when treated orally with the same extract at a dosage level of 5 gm. (extract administered in gelatin capsules).

It would seem that the therapeutic method of choice would consist in small frequent subcutaneous injections except in emergency when the intravenous route would be indicated.
Hormone Requirement As Influenced by Environmental Factors—
The early failure of Dog 12 (Table I) on a beef heart diet, a ration which the animal did not relish, suggested that the nature of the diet as reflected in the voluntary fasting of the test animal may influence assay results. The data recorded in Table III demonstrate the effect of fasting on the hormone requirement. It is of particular interest to note (Period 2) that the dog while on one minimum maintenance dose was thrown into mild insufficiency by withdrawal of food but that the symptoms of insufficiency were readily alleviated by allowing the animal to eat. During 5 days fasting the blood urea became elevated as did also the pulse rate and hemoglobin. The blood sugar dropped to a fasting level. During the period of fasting the water intake was only about 10 per cent of the intake during the control period. The urea, glucose, and hemoglobin returned to the original levels during the post-fasting period. During the period of fasting, on excess dosage of hormone (Period 1), the urea nitrogen level remained in the normal range, the glucose dropped to a fasting level, the pulse rate did not change significantly, while the hemoglobin level rose 29 per cent. In Period 3 the animal was given water by stomach sound in an amount comparable to the total daily water intake during the control period. The hemoglobin and pulse rate became elevated but the urea level remained in the normal range. After a week of fasting with the administration of water by sound the animal failed to recover promptly on refeeding. It seemed to be on the verge of insufficiency judging from the slightly elevated urea and hemoglobin levels even though it was eating well and gaining in weight. On the morning of January 30 the dog was found in a state of collapse. The blood sugar was 45 mg. per cent. It recovered promptly with the treatment indicated. In Period 4 it is seen that orally administered sodium chloride with the accompanying voluntary water ingestion maintained the blood urea and hemoglobin at the control levels during the 7 day period of fasting. During this interval the animal was receiving one minimum maintenance dose of hormone. Two other adrenalectomized dogs, Dogs 7 and 23, were used in these studies on fasting. They reacted in essentially the same manner as Dog 12, except as regards the results obtained on forcing water during fasting (the amount of water used being comparable to the quantity normally
TABLE III

**Effect of Fasting and Ingestion of Water or Salt during Fast on Adrenalectomized Dog Subsisting on Minimum Maintenance Dose of Cortical Hormone**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Date</th>
<th>Body weight</th>
<th>Food offered</th>
<th>Total water intake</th>
<th>Urea N (mg.)</th>
<th>Glucose (mg.)</th>
<th>Hb (gm.)</th>
<th>Pulse</th>
<th>Dosage per kilo per day</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>10-11-32</td>
<td>11.2</td>
<td>0</td>
<td>615(4)</td>
<td>19</td>
<td>84</td>
<td>13.5</td>
<td>72.8</td>
<td>Clinically normal</td>
<td></td>
</tr>
<tr>
<td>10-18-32</td>
<td>9.6</td>
<td>+</td>
<td>95(7)</td>
<td>23</td>
<td>60</td>
<td>17.4</td>
<td>52</td>
<td></td>
<td></td>
<td>throughout period</td>
</tr>
<tr>
<td>10-25-32</td>
<td>11.0</td>
<td>+</td>
<td>660(7)</td>
<td>25</td>
<td>84</td>
<td>13.5</td>
<td>68</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Period 2. Effect of fasting and refeeding while receiving 1 minimum maintenance dose**

|          |          |             |              |                   |              |               |           |       |                        |                          |
|          |          |             |              |                   |              |               |           |       |                        |                          |
| 11-21-32 | 11.0     | +           |              | 28                | 93           | 11.0          | 80        | 0.4   | Clinically normal       | throughout period         |
| 11-25-32 | 11.0     | 0           | 690(20)      | 29                | 11.3         | 16.5          | 80        |       |                        |                          |
| 11-30-32 | 9.9      | +           | 70(5)        | 50                | 78           | 16.8          | 52        |       |                        |                          |
| 12-1-32  | 10.2     | +           |              | 36                | 80           | 15.8          | 72        |       |                        |                          |
| 12-10-32 | 11.0     | +           | 800(10)      | 34                | 84           | 14.2          | 76        | 0.5   | Dosage level raised     | 25 per cent               |
| 12-20-32 | 11.6     | +           | 795(10)      | 31                | 87           | 11.3          | 70        |       |                        |                          |

**Period 3. Effect of administration of water by sound during fasting while receiving 1 minimum maintenance dose**

|          |          |             |              |                   |              |               |           |       |                        |                          |
|          |          |             |              |                   |              |               |           |       |                        |                          |
| 12-29-32 | 11.6     | +           | 550(9)       | 18                | 81           | 11.7          | 72        | 0.4   | Clinically normal until noted below |                            |
| 1-2-33   | 11.7     | 0           | 670(3)       | 21                | 91           | 12.0          | 88        |       | Distilled water by sound                            |
| 1-9-33   | 10.4     | +           | 960(7)       | 21                | 78           | 16.5          | 132       |       |                            |
| 1-19-33  | 11.0     | +           | 1015(10)     | 33                | 14.5         | 96            | 0.5       |       | Dosage level raised 25 per cent                           |
| 1-30-33  | 11.3     | +           |              | 30                | 48           | 16.2          | 172       |       | Found 9 a.m. in collapsed state                          |
| 2-14-33  | 11.1     | +           |              | 14                | 83           | 10.0          | 72        |       |                            |

**Period 4. Effect of salt ingestion during fasting while receiving 1 minimum maintenance dose**

|          |          |             |              |                   |              |               |           |       |                        |                          |
|          |          |             |              |                   |              |               |           |       |                        |                          |
| 2-1-33   | 11.1     | +           |              | 14                | 83           | 10.0          | 72        | 0.4   | Clinically normal throughout period                       |
| 2-7-33   | 11.0     | 0           | 505(2)       | 18                | 84           | 10.4          | 76        |       | NaCl administered**                                    |
| 2-24-33  | 10.2     | +           | 382(7)**     | 17                | 51           | 10.4          | 48        |       |                            |
| 2-25-33  | 10.4     | +           |              | 24                | 87           | 10.2          | 56        |       |                            |
| 2-27-33  | 10.4     | +           | 655(3)       | 27                | 82           | 10.1          | 80        |       |                            |
TABLE III—Continued

Effect of administration of water by sound during fasting while receiving 1 minimum maintenance dose

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Date</th>
<th>Body weight</th>
<th>Food offered*</th>
<th>Total water intake</th>
<th>Urea</th>
<th>Glucose</th>
<th>Hb</th>
<th>Pulse</th>
<th>Dosage per day</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>12-29-32</td>
<td>10.8 +</td>
<td>17</td>
<td>85 11.2</td>
<td>116</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td>Clinically normal</td>
</tr>
<tr>
<td>1-2</td>
<td>2-33</td>
<td>10.5 0</td>
<td>24</td>
<td>100 14.3</td>
<td>144</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Water (900 cc.)†† daily until Jan. 7, 1933</td>
</tr>
<tr>
<td>1-6</td>
<td>3-33</td>
<td>10.2 0</td>
<td>19</td>
<td>93 16.2</td>
<td>112</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Convulsions‡‡</td>
</tr>
<tr>
<td>1-7</td>
<td>7-33</td>
<td>10.1 0</td>
<td>22</td>
<td>99 17.3</td>
<td>204</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anemia§§</td>
</tr>
<tr>
<td>1-23</td>
<td>23-33</td>
<td>10.9 +</td>
<td>18</td>
<td>82 7.9</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clinically normal</td>
</tr>
<tr>
<td>23</td>
<td>2-20-33</td>
<td>11.3 +</td>
<td>16</td>
<td>93 7.9</td>
<td>88.4</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>2-23</td>
<td>2-33</td>
<td>11.1 0</td>
<td>23</td>
<td>101 8.9</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Water (900 cc.)†† daily until Feb. 20, 1933</td>
</tr>
<tr>
<td>2-28</td>
<td>3-33</td>
<td>10.7 0</td>
<td>21</td>
<td>92 9.8</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Convulsions</td>
</tr>
<tr>
<td>3-1</td>
<td>1-33</td>
<td>+</td>
<td>20</td>
<td>84 7.2</td>
<td>108</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Asthenic</td>
</tr>
<tr>
<td>3-23</td>
<td>2-33</td>
<td>11.2 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clinically normal</td>
</tr>
</tbody>
</table>

* 0 in the food column indicates the complete withdrawal of the food on that date. The fasting period continued until the following entry and was terminated with one-half ration of food. On Jan. 7, 1933 food was offered Dog 7 but none was taken.
† The average total daily intake (water ingested plus water in food consumed) for the number of days (in parentheses) prior to the entry date.
‡ Dosage levels stated were continued through the experimental period indicated or until a different dosage level was adopted in the same experimental period with exceptions noted.
§ Extract 1532-N previously assayed on this dog. 8 gm. = 10 dog units.
|| During the 7 days indicated 900 cc. of water were administered by sound in three equal portions daily at 10 a.m., 2 p.m., and 5 p.m.
¶ Unable to walk; intravenous injection of 40 gm.; copious bloody and watery stools; 100 cc. of 50 per cent glucose were administered by mouth in a.m. and p.m. Much improved on the following day. Normal blood and clinical findings 3 days later on dosage level of 20 gm.; then dropped to 10 gm. for remainder of period.
** During the 7 days indicated 6 gm. of sodium chloride were administered orally (in gelatin capsules) in three equal portions daily at 10 a.m., 2 p.m., and 5 p.m.
†† By sound in three equal portions at 10 a.m., 2 p.m., and 5 p.m.
TABLE III—Concluded

†† This blood sample obtained at 10.30 p.m. when the animal was found in convulsions. Complete prostration. Intravenous injection of 40 gm.
§§ Intravenous injection of 40 gm. at 9 a.m. Started to take food on Jan. 8, 1933. 40 gm. subcutaneously until Jan. 14, 1933. Recovery gradual. Appeared clinically normal on Jan. 14, 1933. Reduced dosage to 20 gm. on this date for remainder of period.
¶¶ This blood sample obtained at 3.50 p.m. when animal appeared quiet and weak. Found in convulsions at 5 p.m. Hemoglobin 11.9 gm. per cent. Intravenous injection of 40 gm. Dosage of 10 gm. for remainder of period. Clinically normal on Feb. 29, 1933 and for remainder of period.

ingested during control periods). The pertinent data are recorded in Table III. Dog 7 was found in convulsions on the 4th day and Dog 23 on the 5th day of this regimen. The convulsions came on very abruptly. The clinical picture was quite different from that seen in adrenal insufficiency and resembled those described by Rowntree (24) and others as occurring in water intoxication. The amounts of water which brought on these symptoms were very small compared to the huge quantities required to produce water intoxication in the normal dog. At the time the convulsions occurred both dogs exhibited normal urea and glucose levels, whereas the hemoglobin was elevated in both instances. Both animals recovered with large doses of cortical hormone. These results indicate that the increased hormone requirement is apparently more directly concerned with the failure of the animal to ingest the salts contained in the usual ration. The observations of Loeb, Atchley, Benedict, and Leland (25) and Harrop, Soffer, Ellsworth, and Trescher (26) on the base balance of the adrenalectomized dog seem to offer the explanation for these findings. These studies on fasting emphasize the importance of a diet in assay work which is relished by the test animals in order to avoid the introduction of errors due to voluntary fasting.

It is a common observation that adrenalectomized animals are very sensitive to infection. Assay data are of little or no significance unless the animal is free of all infective processes. This is more important than having the animal in optimal nutritive condition. For example, a dog suffering from a mild respiratory infection had a minimum maintenance dose of at least 4 gm. After complete recovery repetition of the assay showed the minimum maintenance dose to be 0.5 gm., a difference of 800 per cent. In
cases of more severe respiratory infection relatively huge doses (10 to 40 dog units per kilo) are necessary to keep the blood urea at a normal level. Animals can die of these infections with a normal blood urea.

Dogs used for assay work were kept free of intestinal parasites. They were treated with a vermifuge before the second operation. Completely adrenalectomized dogs when treated with a vermifuge require larger amounts of hormone. For example, Dog 11, receiving ten or more minimum maintenance doses daily was clinically normal with a blood urea of 18 mg. per cent. The day following treatment the urea was 50 mg. per cent and marked asthenia developed. On the next day the urea was 22 and there was definite clinical improvement. Similar effects can be demonstrated after ½ hour of morphine-ether anesthesia. The validity of assay data collected on animals which are being subjected to any abnormal strain would be obviously open to question.

In using the elevation of blood urea alone as a guide in assay work sufficient control data should be available to demonstrate the absence of nitrogen retention due to causes other than adrenal insufficiency. In a normal dog that developed spontaneous nephritis (following a severe distemper) prior to adrenalectomy the urea rose from a previous normal of 25 to a value averaging 47 mg. per cent. In assays conducted with this animal (and controlled by simultaneous assays on two other dogs) the results checked very well when compared on the basis of clinical failure. No comparison could be made on the basis of blood urea alone. The clinical picture of insufficiency was not noticeably influenced by the previous kidney damage.

Remarks on Method of Assay—In early assay work (2) the end-point of the assay consisted in a rise in blood urea nitrogen or non-protein nitrogen of 15 mg. or more, accompanied by a loss in body weight, and clinical failure as evidenced by asthenia, vomiting, etc. As a result of the comparative studies reported in this communication the end-point of the assay as now being utilized in this laboratory consists of a rise in blood urea nitrogen of 100 per cent. There may or may not be a weight loss. When it occurs it seldom exceeds 10 per cent of the body weight. Definite

11 Tetrachloroethylene (Nema worm capsules, Parke, Davis and Company) followed with 10 gm. of magnesium sulfate.
asthenia is encountered only rarely. A somewhat lessened activity and a failing appetite of varying degree are usually the only clinical manifestations of abnormality. The former is usually apparent only to those familiar with the behavior of the test animal. In a series of test animals the percentage variation in the minimum maintenance dose based on clinical failure was less than the variation encountered in the minimum maintenance dose based on a 100 per cent blood urea rise. The somewhat greater degree of accuracy, however, that may be attained by the former method is more than outweighed by the decrease in the risk of losing the test animal in the latter procedure as well as by the saving in time in reconditioning the animals for further assay work. The accuracy of the method is limited both by the differences encountered in the hormone requirement of individual animals and by the magnitude of the differences between successive dosage levels employed in the assay. The error due to these variables has been kept at a minimum by employing the same dogs and a uniform gradation of dose in assaying a series of fractions. Experience in conducting assays during the past few years under the above conditions indicates the error of determination to be approximately ±25 per cent. In extraction and fractionation studies two animals are usually employed in ascertaining the potency of any given sample.

**SUMMARY**

The cortical requirement of a series of nine adult adrenalectomized dogs was determined. The individual requirement was found to vary by approximately 100 per cent. In check assays conducted under presumably optimal conditions the requirement of the same animal was found to vary about 25 per cent. The hormone requirement was found to be independent of the time period which elapsed between adrenalectomy and assay. In the adult dog the hormone requirement remains relatively constant over as long a time period as studied (16 months). It is not influenced by sex provided the female is in anestrus; nor is it influenced by the level of hormone dosage prior to the assay. The number of insufficiency periods which the adrenalectomized animal has experienced does not affect the assay results. By adjusting the level of hormone therapy adrenalectomized dogs were maintained in chronic insufficiency for periods of 30 to 40 days.
The cortical hormone requirement of the dog is increased in fasting. The increased requirement is apparently concerned with the failure of the animal to ingest the salts contained in the ration. The diet used in assay work should be relished by the test animals in order to avoid errors due to voluntary fasting.

During fasting the adrenalectomized dog subsisting on a minimum maintenance dose of hormone is sensitive to relatively small amounts of water administered by sound. A clinical condition resembling water intoxication was produced in two of three animals studied.

Orally administered hormone is less than 8 per cent as efficient as when subcutaneously administered.

Cortical hormone injected subcutaneously is not excreted in the urine by the normal dog. Since there is no demonstrable storage in the organism of the dog the hormone is either metabolized or excreted by some other route.

Three adrenalectomized dogs have been maintained on cortical extracts for a period of more than 2 years.

Acknowledgment is due to Mr. A. R. Taylor for his assistance in the preparation of the adrenal extracts used in this study.

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