STUDIES ON THE ADRENAL CORTEX

I. THE EFFECT OF A LIPID FRACTION UPON THE LIFE-SPAN OF ADRENALECTOMIZED CATS

W. W. SWINGLE and J. J. PFIFFNER

From the Biological Laboratory, Princeton University, and Biological Laboratory
Cold Spring Harbor, New York

Received for publication September 30, 1930

It is a well established fact that in cats and dogs, bilateral adrenal extirpation results in death within a few days. The animals exhibit a train of symptoms which has come to be recognized as typical of adrenal insufficiency. Experiments have shown, moreover, that death is due to removal of the cortical part of the suprarenal complex, and not to interference with, or loss of the medulla or adrenalin containing portion. (Biedl, 1913; Wheeler and Vincent, 1917; Houssay and Lewis, 1923; Wislocki and Crowe, 1924; Zwemer, 1927; Swingle, 1927.)

Administration of adrenalin to bilaterally operated animals has been repeatedly tried but without success, since animals so treated derive no benefit from the injections and die with characteristic symptoms of adrenal insufficiency. We have attempted numerous times to prolong the life-span of adrenalectomized cats by injections of adrenalin, employing various dosage but in no case have we noted any improvement in the condition of the animals or been able to prevent the onset of symptoms. In fact, it is our impression, although serious efforts to test the point were not made, that cats lacking adrenals are somewhat more sensitive to adrenalin than are normal animals. However this may be, it is certainly a conservative statement to say that in so far as the maintenance of life is concerned, it is the cortical and not the medullary portion of the suprarenal of mammals which is important.

Efforts to keep double operated animals alive by substitution therapy with suprarenal cortical tissue or extracts have not yielded decisive results. Very few investigators have reported success by transplantation methods (Jaffe, 1927), and but two reports have been made claiming success with extracts of fresh glands when tested upon bilaterally adrenalectomized animals.

Hartman, Brownell, Hartman, Dean and MacArthur (1928) extracted beef cortex by shaking the ground tissue with water for 15 to 20 minutes. Following isoelectric precipitation the proteins remaining in the filtrate
were salted out with sodium chloride. The salted out proteins, dissolved in water represented the extract. One cubic centimeter of the most concentrated extract was equivalent to 5 grams of fresh beef cortex. By subcutaneous semi-daily injections of the extract Hartman and co-workers stated that they were able to keep adrenalectomized cats alive for varying periods over and beyond the normal life-span of untreated adrenalectomized control animals.

Stewart and Rogoff (1929) using extract prepared according to the method of Hartman and collaborators, were unable to confirm Hartman's results. They were not able to obtain prolongation of the life-span of bilaterally adrenalectomized cats or dogs. Their data indicated that the extract-treated cats did not survive any longer than control untreated animals. On the basis of their negative findings Stewart and Rogoff concluded that Hartman's extract contained little, if any, cortical hormone.

These same investigators reported on an extract of the suprarenal glands of beef and sheep which they prepared. No data are given regarding the method of extraction, or whether or not the preparation does or does not contain adrenalin. They state that the extract when given intravenously to adrenalectomized dogs prolongs the life-span, but is ineffective when given subcutaneously or by mouth. However, examination of the data presented in their paper (1929) leads one to doubt whether their extract contained any more of the cortical hormone than that of Hartman. The average survival period of 32 adrenalectomized dogs treated daily with intravenous injections of sheep and beef adrenal cortex was 13 days (table 4, page 259). The average survival period for a series of 36 adrenalectomized control dogs not treated with extract was 9.6 days (table 2, Stewart and Rogoff, 1928).

It is our opinion that the difference between the survival periods of control and extract-treated dogs in these experiments is entirely too small to be of any significance. The quantity of active cortical hormone present in the extract must have been negligible.

So far as the writers are aware, Hartman and co-workers, and Stewart and Rogoff are the only investigators who have made any claim to have obtained an extract of the suprarenal cortex which has any effect upon the life-span of adrenalectomized animals. Critical examination of their data, however, leaves one with the conviction that the extracts contained little or no activity.

In the present confused state of our knowledge of the functional significance of the suprarenal cortex, the only reliable criterion for testing the activity of an extract is its effect upon the life-span of adrenalectomized animals. This criterion is severe and requires much time and effort, but at any rate is an excellent index of potency. If an extract fails to keep a series of double operated animals alive and in normal condition at least for
a considerable period of time over and beyond the maximum limit of the life-span of control non-treated adrenalectomized animals, we regard it as inactive. The maximum limit of the life-span of control operated cats in our colony is fifteen days, the average life-span being 7.7 days. Very rarely have we encountered accessory glands in the several hundred cats we have used in our various experiments. In our experience about 3 per cent of the animals have accessories. (Swingle, 1927.) All bilaterally adrenalectomized animals are autopsied and careful search made for accessory cortical glands.

During the past two years the writers have prepared and tested a large number of different types of cortical extracts. Varying degrees of activity were obtained from several of the extracts and one, a lipid fraction of the cortex, revealed a considerable degree of potency.

In the earlier work we employed acid agents such as acetic, hydrochloric, and sulphuric in different strengths, with and without heat; alkaline agents such as sodium hydroxide, sodium carbonate, sodium bicarbonate and ammonium hydroxide, with and without heat; acid and alkaline alcoholic extraction; desiccated cortical tissue extracted with various solvents, etc., etc. None of these various methods proved successful since but little of the active hormone was obtained. It is not improbable, however, that certain of these methods might yield potent extracts providing extensive experimentation were carried out. In our experience the extracts obtained by the methods enumerated were very weak in potency compared to the lipid fraction described in this paper. This fraction is the basis of all of our further fractionation procedures and the starting point of our most active preparations. A brief note regarding the active lipid fraction has been published. (Pfiffner and Swingle, 1929; Swingle and Pfiffner, 1929.)

Each extract was tested on at least three animals simultaneously and for some of our relatively inactive extracts fifteen or twenty animals were used before the method was definitely discarded. For obvious reasons data collected in testing the many methods of extraction which were later abandoned are not included in this paper.

The method used in preparing the active lipid fraction is as follows:

The beef adrenal glands packed in ice are received in the laboratory within twenty-four hours after collection at the abattoir. After removing extraneous fat and connective tissue the glands are cut lengthwise and dissected as free as possible from medullary tissue.

The ground cortical tissue is extracted from 24 to 72 hours at room temperature with 2.5 volumes of 95 per cent ethyl alcohol. During the course of extraction the material is occasionally stirred. The extraction alcohol is removed by straining through muslin and filtering. The gland residue is pressed as dry as possible in a tincture press, ground and extracted at room temperature for 24 to 72 hours with 2 volumes (calculated from the
weight of fresh cortex) of 80 per cent ethyl alcohol. The extraction alcohol is removed in the same manner as in the first extraction. The alcoholic filtrates are concentrated individually in partial vacuo at an external temperature of 50–60°C. to approximately one-fifteenth of their original volumes. Each concentrate is then mixed with an equal volume of benzene and set aside in the refrigerator. The benzene solution of lipids is removed and the aqueous residues re-extracted two or three times with similar quantities of benzene. The last benzene washing is colorless or only faintly yellow. The benzene washings are combined and the benzene is removed in partial vacuo at an external temperature of 45–50°C. The last traces of benzene are removed by the addition of 50 to 100 cc. portions of absolute alcohol and continuing the distillation to dryness.

The lipid residue is taken up in corn oil or olive oil for injection with the aid of absolute alcohol. The alcohol is removed by distillation in partial vacuo at 45–50°C. Sufficient oil is incorporated so that 1 cc. represents 30 grams of fresh cortical tissue. The extract was stored in the refrigerator at 6°C. and was made up fresh each week unless otherwise indicated. It was warmed to body temperature for injection.

The lipid fraction which is being used in further purification studies is obtained by the described technique with the exception that the cortical tissue is extracted four to six days.

The cats, both control and extract-treated, were kept under the same conditions. They were housed in thermostatically heat-regulated rooms at about 78–80°F., the temperature not varying more than three degrees during the twenty-four hours. The matter of temperature control is important in any experiments involving adrenalectomized cats since such animals are very susceptible to colds and respiratory infections. The diet consisted of canned salmon fed five days a week, raw liver or kidney once each week, and milk twice a week, the latter being given on the days when salmon was fed. A few drops of cod liver oil were administered about once each week. The animals were fed but once each day and given as much as they could eat.

It has been our experience that cats kept for considerable periods in the laboratory should be treated with a vermifuge. Many of our animals were found to be infested with hook-worm. It is part of our routine procedure to give all animals coming into the laboratory 0.5 cc. of tetrachlorethylene and to repeat the dose after five days. All of our animals were operated by the same individual.

The establishment of the normal life-span of adrenalectomized control animals is the first requisite to work on cortical extracts involving length of life. It has been repeatedly pointed out by various workers that double operated animals (cats and dogs) generally remain normal for the first few days following gland extirpation. The survival period differs in different
individuals and may vary anywhere from five to fifteen days, although the majority of our animals die between the 5th and 8th day after operation.

The survival period of thirty-eight untreated control adrenalectomized cats of our series ranged from 4 to 15 days with an average of 7.7 days.
These figures compare favorably with those of the best survivals reported in the literature. Table 1 shows the survival periods of our control animals. The time is given in days and is figured from the hour of operation. Fractions of days have not been recorded in the table. For instance if an animal lives five days (figured from hour of operation) and several hours beyond this time it is set down as having survived five days. If it lives five days and an additional twelve hours it is still recorded as living five days. If the animal is last seen alive at 9 p.m. and is found dead at 8 a.m. the following morning it is recorded as having died at 9 p.m. the hour when last seen alive. The exact number of hours an animal survives double adrenalectomy is of little importance—any survival period under four days is a poor case and any period over five days is a good case in so far as cats are concerned, providing the animal recovers quickly from the operation and eats and behaves normally for at least forty-eight hours before showing any symptoms.

There are two points brought out by examination of table 1, which are not without interest. The sex of the animal has no influence on the survival period, nor does the interval between first and second adrenal extirpation exert an appreciable influence on the life-span. It has been our custom to allow at least a seven day interval to elapse between removal of right and left glands.

In table 2 are shown the results of treating twenty-three double operated cats with a lipid fraction of the cortex. The survival periods vary greatly ranging as they do from 16 to 50 days. If table 1 is compared with table 2 the effect of the lipid extract upon the life-span is clearly brought out. None of our control cats survived over fifteen days, and none of the extract-treated animals survived less than sixteen days. The average life-span for controls was 7.7 days (38 animals) the average for the extract treated was 27.8 days (23 animals).

The extract-treated animals were normal, they ate, played about and remained active up until a few days before death (protocol 1). The onset of adrenal insufficiency symptoms was in general quite abrupt and when symptoms appeared we were unable by increasing the dosage to bring the animals back to normal. Death invariably occurred within two or three days from the date of first refusal of food. All extract-treated cats received two injections daily, one-half the daily kilogram dose being administered in the morning, the other half in the evening, except G 31, G 32, G 33 and G 34 of table 2. These animals received the entire daily dose in one injection.

The injections were begun twenty-four hours after the second operation in all except two cases (G 31 and G 32, table 2), when the first injection was given at the end of the fourth day following removal of the second gland. All of the animals later died of adrenal insufficiency aggravated by abscess formation at the site of the injections.
It is interesting to note that after the potent fraction is prepared and placed in oil it retains its potency for at least three weeks if kept at 6°C. Several cats were used to test the keeping quality of the extract in oil.

**TABLE 2**

Survival period of adrenalectomized cats treated with subcutaneous injections of a lipid extract

<table>
<thead>
<tr>
<th>SERIES</th>
<th>SEX</th>
<th>INTERVAL BETWEEN OPERATIONS</th>
<th>SURVIVED AFTER SECOND OPERATION</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>days</td>
<td>days</td>
<td></td>
</tr>
<tr>
<td>G. 11</td>
<td>Male</td>
<td>8</td>
<td>24</td>
<td>1 cc. extract per kilo daily. Abscess formed</td>
</tr>
<tr>
<td>G. 12</td>
<td>Male</td>
<td>20</td>
<td>29</td>
<td>1 cc. extract per kilo daily. Abscess formed</td>
</tr>
<tr>
<td>G. 13</td>
<td>Male</td>
<td>8</td>
<td>20</td>
<td>1 cc. per kilo daily. Abscess formed</td>
</tr>
<tr>
<td>G. 14</td>
<td>Male</td>
<td>8</td>
<td>17</td>
<td>1 cc. per kilo daily. Abscess formed</td>
</tr>
<tr>
<td>G. 15</td>
<td>Male</td>
<td>8</td>
<td>20</td>
<td>1 cc. per kilo daily. Abscess formed</td>
</tr>
<tr>
<td>G. 16</td>
<td>Female</td>
<td>8</td>
<td>16</td>
<td>1 cc. per kilo daily. Abscess formed</td>
</tr>
<tr>
<td>G. 17</td>
<td>Female</td>
<td>8</td>
<td>26</td>
<td>1 cc. per kilo daily. Abscess formed</td>
</tr>
<tr>
<td>G. 18</td>
<td>Female</td>
<td>8</td>
<td>28</td>
<td>1 cc. per kilo daily. Abscess formed</td>
</tr>
<tr>
<td>G. 19</td>
<td>Male</td>
<td>8</td>
<td>32</td>
<td>1 cc. per kilo daily. Abscess formed</td>
</tr>
<tr>
<td>G. 20</td>
<td>Female</td>
<td>8</td>
<td>39</td>
<td>1 cc. per kilo daily</td>
</tr>
<tr>
<td>G. 21</td>
<td>Female</td>
<td>7</td>
<td>44</td>
<td>1 cc. per kilo daily. Extract 3 weeks old at end of 44th day</td>
</tr>
<tr>
<td>G. 22</td>
<td>Male</td>
<td>7</td>
<td>50</td>
<td>1 cc. per kilo daily. Extract 3 weeks old at death of animal. Abscess formed</td>
</tr>
<tr>
<td>G. 23</td>
<td>Male</td>
<td>7</td>
<td>18</td>
<td>1 cc. per kilo by mouth daily</td>
</tr>
<tr>
<td>G. 24</td>
<td>Male</td>
<td>19</td>
<td>18</td>
<td>1 cc. per kilo daily</td>
</tr>
<tr>
<td>G. 25</td>
<td>Female</td>
<td>15</td>
<td>42</td>
<td>0.5 cc. per kilo daily. Died pneumonia</td>
</tr>
<tr>
<td>G. 26</td>
<td>Male</td>
<td>7</td>
<td>44</td>
<td>0.5 cc. per kilo daily</td>
</tr>
<tr>
<td>G. 27</td>
<td>Female</td>
<td>12</td>
<td>21</td>
<td>0.5 cc. per kilo daily</td>
</tr>
<tr>
<td>G. 28</td>
<td>Female</td>
<td>10</td>
<td>20</td>
<td>1 cc. per kilo. Cat sacrificed. Excellent condition to death. See protocol in text</td>
</tr>
<tr>
<td>G. 29</td>
<td>Male</td>
<td>10</td>
<td>28</td>
<td>1 cc. per kilo. Cat killed by adrenalin injections</td>
</tr>
<tr>
<td>G. 30</td>
<td>Male</td>
<td>26</td>
<td>19</td>
<td>0.5 cc. per cat daily. 1st injection 4 days after 2nd operation. Pneumonia</td>
</tr>
<tr>
<td>G. 31</td>
<td>Male</td>
<td>26</td>
<td>20</td>
<td>0.5 cc. per cat daily. 1st injection 4 days after 2nd operation. Pneumonia</td>
</tr>
<tr>
<td>G. 32</td>
<td>Female</td>
<td>28</td>
<td>23</td>
<td>1 cc. per cat daily.</td>
</tr>
<tr>
<td>G. 33</td>
<td>Female</td>
<td>12</td>
<td>33</td>
<td>0.5 cc. per kilo daily</td>
</tr>
</tbody>
</table>

The usual extract was prepared and injected daily, the same sample of extract being used until the animals died. One animal survived 19 days (G 31, table 2) and received but 0.5 cc. of extract per day. The extract was not injected until the fourth day following the second operation when the cat first refused food. Another animal operated the same day survived...
20 days and received the same dose. It also first received the extract on 
the fourth day after operation (G 32, table 2). A third animal operated the 
same day as the previous two cats, survived 23 days on the same batch of 
extract but received 1 cc. daily (G 33, table 2) and received the first 
injection twenty-four hours after operation.

The keeping quality of the hormone in ethyl alcohol was tested on two 
animals. The cats were started on a sample of extract. After some days' 
treatment the extract was used up and another lot made up from cortex 
tissue which had been standing in alcohol at room temperature for two 
weeks. This lot of extract kept the animals in excellent condition for a 
little over three weeks. The animals died within two days of one another. 
The survival period of the two animals was 42 days (G 26) and 44 days 
(G 27). These cases offer strong evidence that the active principle of the 
cortex remains stable in alcohol for at least two weeks at room temperature 
and when prepared in extract form in oil retains its potency for approxi-

mately three weeks.

In all of our cases complications arose following repeated injections of the 
extract, due to formation of abscesses at the site of injection. Animals 
which survived twenty-five to thirty days generally had several such 
abscesses, some open sores, some large lumpy masses. When large areas 
of the subcutaneous tissues became involved the cats developed symptoms 
of adrenal insufficiency and died. Autopsy revealed that little if any of the 
later injected extract had been absorbed. Most of it could be recovered 
at the site of injection. Consequently we have found it extremely difficult 
to keep animals alive for periods longer than 45 days with this crude extract.

Adrenalectomized animals are sensitive to any type of infection such as 
colds, or abscess formation such as just described. In later work we used 
olive oil instead of corn oil as a medium for injecting the extract. As a 
result of changing oils, abscess formation was materially reduced and the 
cats remained in better condition.

The presence of appreciable quantities of adrenalin in cortical tissue has 
proven to be a source of serious difficulty, especially so since adrenalecto-
mized animals appear to be somewhat hypersensitive to adrenalin. It was 
thought that this difficulty might be surmounted by dissection of the glands 
at the slaughterhouse immediately after removal from the animals. The 
data presented in table 3 show quite clearly that no advantage would be 
gained by such a procedure, since cortical tissue dissected at the slaughter-
house contains almost as much adrenalin as cortical tissue obtained from 
glands which were dissected 20 hours after removal from the animal. It 
should be noted that the glands used in the above analyses were handled 
very carefully so as to keep diffusion processes at a minimum. In routine 
work in the laboratory it is impractical to dissect very carefully large 
numbers of adrenal glands. Consequently the tissue used in the prepara-
tion of our extracts contains more adrenalin than the data in table 3 would indicate. The ground cortical tissue in routine practice assays about 2 to 2.5 mgm. of adrenalin per gram of tissue using the colorimetric method of Folin, Cannon, and Denis (1913). Approximately 30 to 40 per cent of the adrenalin present is extracted by the alcoholic treatment. As a result the aqueous concentrates after the removal of alcohol are highly toxic. Because of its toxic properties we were unable to demonstrate the presence of the cortical hormone in this material.

About 3 to 5 per cent of the adrenalin present in the cortical tissue is found in the benzene soluble fraction. Adrenalin is insoluble in benzene. However, it is soluble to a certain extent in a solution of lipid materials in that solvent. The chief disturbing factor in this connection appears to be phospholipid. Neutral fat, free fatty acids and cholesterol have a slight influence. This change in solvent properties probably accounts for the presence of adrenalin in the benzene soluble fraction. On the other hand, it is possible that a fraction of the adrenalin may exist in the tissue in a form which is soluble in benzene. Similar solubility behaviour of adrenalin was encountered with the many other solvents examined including ether, petroleum ether, chloroform, ethyl acetate, acetone, hexane, butyl alcohol, carbon disulfide, and carbon tetrachloride.

One cubic centimeter of the crude extract used in this study contained on average 2 mgm. of adrenalin. This quantity of extract is equivalent to 30 grams of cortical tissue which contained on average 60 mgm. of adrenalin. In the procedure used therefore from 96 to 97 per cent of the adrenalin is removed.

The quantity of adrenalin present in the crude extract is capable of inducing physiological changes in the organism such as elevation of blood pressure, rise in blood sugar, etc. However, the extract is given subcutaneously in oil and is absorbed rather slowly over a considerable period. We never give more than 1 cc. per kilo per day per animal and have not noted any changes in the behaviour of the animals following

| TABLE 3 |
| Adrenalin content of adrenal glands (beef) |
| Cortex dissected from medulla while glands still warm (about 20 minutes after death of animal) | Cortex | 0.7 |
| Medulla | 10.0 |
| Whole glands stored in refrigerator (8°C.) for 20 hours before dissection | Cortex | 1.0 |
| Medulla | 12.0 |
injection. However, if large doses are given, doses of 5 to 6 cc., the animals display typical symptoms of adrenalin overdosage. They become sluggish and lose their appetites.

It should be emphasized that our primary object in this study was the conclusive demonstration of the presence of the cortical hormone in the crude lipid fraction. No attempt was made to determine the minimum dosage or the minimum frequency of injection necessary to maintain life.

**SUMMARY AND CONCLUSIONS**

By use of an alcoholic-benzene extraction process a crude lipid fraction of the suprarenal cortex of beef has been obtained, which when injected subcutaneously will prolong considerably the survival period of bilaterally adrenalectomized cats.

The cortical hormone is stable in ethyl alcohol for a period of at least two weeks, and the crude lipid fraction when prepared in oil retains its potency for about three weeks when kept at 6°C.

The active lipid fraction is not adrenalin-free.

In so far as the writers are aware this is the first conclusive demonstration that the hormone of the suprarenal cortex which is essential for the maintenance of life can be extracted with lipid solvents.

*Protocol G #9, female.* 2-1-'29, r. adrenal removed. 2-10-'29, l. adrenal removed. Quick recovery from ether. Given 2 cc. daily subcutaneously of lipid extract suprarenal cortex in two equal doses. 2-10 to 2-12, cat perfectly normal, eats heartily, plays about, and is very active. 2-18 to 3-3, animal in such excellent condition that presence of accessory gland suspected. 3-3, extract discontinued, 3-6, animal first refused food, and showed first symptoms of adrenal insufficiency. 3-10, animal prostrate and verging on coma at 9 a.m. Died 4 p.m. Autopsy revealed no traces of accessory adrenal tissue. Total survival period 29 days.

*Protocol.* Cat G #8, male. 7-8-'29. Weight 2540 grams. R. adrenal removed. 7-15-'29. Weight 2535 grams. L. adrenal removed. 7-16-'29. Ate well. One and one-fourth cubic centimeter extract given subcutaneously twice daily unless otherwise noted. 7-27-'29 refused food, cat "droopy." 7-28-'29. Appetite returned, ate heartily, appears normal, plays about in lively fashion. 8-12-'29 extract discontinued for three days. Cat normal. 8-15-'29 injections resumed since cat refused food. Abscesses formed at site of injection. 8-25-'29 injections discontinued for two days. Cat normal. Injections resumed on 27th. 8-27-'29, cat shows typical adrenal symptoms—weakness, loss of appetite, lethargy. Several large abscesses at site of previous injections present. 8-28-'29 animal presented marked symptoms adrenal insufficiency. Bled (cardiac puncture) for urea and sugar, 8 a.m.; cat died 12 m., weight 2055 grams. Autopsy revealed no accessory tissue. Blood sugar four hours previous to death, 45 mgm. per cent; urea 24 mgm. per cent. No sugar in urine at time of death. Lived 44 days after second adrenal removed. Diagnosis—adrenal insufficiency aggravated by abscess formation.

This work was begun at the Zoological Laboratory of the State University of Iowa, continued on through the summer of 1929 at the Biological
Laboratory at Cold Spring Harbor, Long Island, and is now in progress at the Biological Laboratory of Princeton University. We take pleasure in acknowledging our indebtedness to Dr. Oliver Kamm, of Parke, Davis & Company, for his very generous cooperation.

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