EXTRACTION STUDIES ON THE ADRENAL CORTICAL HORMONE

II. YIELD FROM GLANDS OF VARIOUS SPECIES*

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In earlier work (1, 2) the maximum yield of hormone obtained from fresh whole beef adrenal glands was shown to be about 2500 dog units per kilo. In recovery experiments it was demonstrated that the extraction method was 75 per cent efficient; i.e., 75 per cent of previously assayed hormone added to adrenal tissue devoid of hormone activity could be recovered in the form of an extract suitable for assay purposes. It seemed desirable to determine the yield of hormone from the adrenal glands of other species. The present report is a summary of the results obtained with the glands of man, horse, ox, sheep, hog, dog, and shark.

Material

The adrenals from man were supplied to us by Dr. R. S. Ferguson of the Memorial Hospital, New York. The adrenal tissue was collected at 107 necropsies. Death was due to a variety of causes. No glands from cases of cancer or pregnancy were included. The glands were frozen after dissecting off the excess fat. The collection extended over a period of 80 days. The glands were received January 23, 1933.

The horse glands were collected at the abattoir of Chappel Brothers, Inc., Rockford, Illinois, through the courtesy of Dr. A. E. Meyer and were supplied to us April 29, 1933, by Dr. S. J.

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Cortical Hormone from Various Species

Martin of the Medical School, University of Wisconsin. After trimming off the excess fat the glands were chilled thoroughly before shipping.

The ox, sheep, and hog adrenals were collected from animals at time of slaughtering. These glands were frozen after trimming off the excess fat and shipped to the laboratory packed in dry ice. The ox glands were received on March 16, 1932, and the sheep and hog glands on March 2, 1933.

The dog glands were collected from the carcasses of animals sacrificed at the Trenton dog pound. The dogs were killed by the municipal authorities with carbon monoxide. The glands were removed within 1 hour after death. After chilling and removing extraneous tissue, the glands were frozen at \(-10^\circ\). The collection extended over a period of 66 days, the period ending July 17, 1933.

The interrenal tissue of the shark (Galeus vulgaris) was collected at the Staatliche Biologische Anstalt auf Helgoland under the direction of Dr. A. Hinrichs during the late summer of 1933. The animals (75) were brought to the laboratory alive and the interrenal tissue removed at the time of killing. The tissue was finely chopped and shipped in 2.5 volumes of ethyl alcohol.

**Table I**

Yield of Cortical Hormone from Adrenal Glands of Various Animals

<table>
<thead>
<tr>
<th>Animal</th>
<th>Estimated average body weight</th>
<th>Average weight of adrenal tissue</th>
<th>Weight of glands extracted</th>
<th>Average yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg.</td>
<td>gm.</td>
<td>gm.</td>
<td>dog units</td>
</tr>
<tr>
<td>Man</td>
<td>70</td>
<td>14.0</td>
<td>0.20</td>
<td>1500</td>
</tr>
<tr>
<td>Horse</td>
<td>500</td>
<td>42.2</td>
<td>0.08</td>
<td>2500</td>
</tr>
<tr>
<td>Ox</td>
<td>420</td>
<td>20.0</td>
<td>0.05</td>
<td>4000</td>
</tr>
<tr>
<td>Sheep</td>
<td>60</td>
<td>2.7</td>
<td>0.05</td>
<td>2345</td>
</tr>
<tr>
<td>Hog</td>
<td>110</td>
<td>5.4</td>
<td>0.05</td>
<td>2250</td>
</tr>
<tr>
<td>Dog</td>
<td>9</td>
<td>1.2</td>
<td>0.13</td>
<td>395.7</td>
</tr>
<tr>
<td>Shark</td>
<td>13.5</td>
<td>2.2*</td>
<td>0.16</td>
<td>163.6</td>
</tr>
</tbody>
</table>

* Interrenal body.
Methods of Extraction and Assay

The tissues were extracted by the usual method (3) with certain minor modifications discussed more recently (2). Since the quantities of available tissue varied appreciably, 163.6 to 4000 gm., the yield of hormone obtained from varying quantities of ox adrenal glands was determined as a check on extraction technique. It was found that the yield of hormone from 500 gm. of beef adrenal glands with the technique employed was essentially the same as that obtained from a 4000 gm. lot. The dog method of assay was employed (4). Each assay was checked on at least two animals. All test animals were standardized against a single preparation of hormone, that of the ox, recorded in Table I.

DISCUSSION

The figures recorded in Table I under the heading “Average yield” represent the average of the “holding” and “failing” dosage levels employed in the assay; i.e., if in the course of the assay the test animals were maintained adequately on a dosage level corresponding to 3000 dog units per kilo of tissue but lapsed into insufficiency on reduction of the dosage level to that corresponding to 4000 dog units, the “average yield” was accepted as 3500 dog units per kilo. The yield of hormone obtained from the glands of horse, ox, sheep, hog, and dog are of the same order of magnitude (1500 to 3500 dog units per kilo of gland). The tissues of man and the shark contained negligible amounts in comparison with the glands of other species, <7 and <20 per cent that of the ox. With the available material only a maximum limit could be established for the hormone content of the glands in these two species. The amount of hormone present may have been zero. In the case of man there are several possibilities to explain the low yield. We are inclined to feel that the glands were depleted of their hormone content at the time of death. One could explain the low yield by assuming destruction of the hormone by autolytic processes if it were not for the fact that a good yield of hormone can be obtained from ox glands after 48 hours autolysis at room temperature (about 23°) (1). The yield from a clear cell hypernephroma (weight 537 gm.), supplied to us by Dr. R. S. Ferguson, was no greater than that obtained from other human autopsy.
material. Studies on the hormone content of adrenal tumors should be made with tissues removed at operation rather than at necropsy.

The only obvious time for the destruction of the hormone in the interrenal tissue was while en route (about 2 weeks). Control experiments demonstrate that beef adrenal glands can be ground and stored in 2.5 volumes of ethyl alcohol for 2 weeks at room temperature without a detectable loss in potency. Cleghorn (5) was unable to demonstrate the presence of the hormone in extracts which he prepared from the interrenal body of skate (Raja clavata). He used the survival of the adrenalectomized cat as the physiological criterion of potency and demonstrated the adequacy of his extraction technique by the preparation of physiologically potent extracts from ox adrenals. Grollman, Firor, and Grollman (6) criticized Cleghorn's work on the grounds (1) that the hormone was probably destroyed before the tissue was dissected from the fish and (2) that the extraction technique was inadequate. These workers prepared extracts from the interrenal tissue of the skate (Raja stabuliforis, Raja diaphanes, and Raja erinacea), using a method of their own which is a modification of one described by Kutz (7). They presented data on six adrenalectomized rats (three injected and three control). The three experimental rats injected daily for a period of 7 days with relatively large doses gained an average of about 7 gm. and died 10, 11, and 12 days following operation. The three control rats survived 5, 6, and 8 days and showed no gain in weight. These data were interpreted as conclusive proof of the presence of the cortical hormone in interrenal tissue. From the extirpation experiments of Biedl (8) and Kisch (9) it is extremely probable that the cortical hormone is present in the interrenal body. We do not feel that its presence has been established adequately by any extraction and substitution data presented to date.

Most workers consider the interrenal body to be adrenalin-free. The material with which we worked gave several of the usual colorimetric tests for adrenalin.

The estimated average body weights accepted in Table I for the ox, sheep, and hog are those recorded for animals at slaughtering (10); that for the horse was made at the abattoir (11); the average body weight of the shark was determined at killing; that
of the dog is the average of body weight recorded by Rogoff and Stewart ((12) Tables I and II). The average weight of adrenal tissue found by these authors in a series of 67 dogs was 1.21 gm. In the present work the average weight of the adrenal tissue was 1.23 gm. in a series of 322 dogs. It seems justifiable therefore to accept the average body weight of 9 kilos for purposes of calculation, although it is realized that even in the same species there is no strict correlation between body and adrenal weight.

In comparing the species on the basis of the amount of adrenal tissue per unit of body weight it will be seen that the horse, ox, sheep, and hog fall into one group while man, dog, and shark fall into another. The relatively smaller amount of adrenal tissue per unit of body weight in the first group may be due to the fact that the animals in the group, with the probable exception of the horse, are for the most part fattened for slaughter.

Another interesting comparison of the various species can be made on the basis of the yield of hormone per kilo of body weight. The values for the horse, ox, sheep, and hog are reasonably constant, 0.12 to 0.17 dog unit per kilo of body weight. The values for man and the shark are very low, <0.03 to <0.07 dog unit, whereas the value for the dog is 0.33.

The data on the dog are of particular interest, for they give an indication of the rate of synthesis of the hormone in the adrenal cortex. The dog unit is the minimum daily kilo dose required for maintenance under standard conditions. Since the dog gland at any one time contains only one-third of this amount, the period of depletion in the absence of continued synthesis would be about 8 hours. If we assume the hormone requirement of the other species to be comparable to that of the dog, then the depletion periods calculated with the data available would be as follows: man <43 minutes, horse 3.6 hours, ox 2.9 hours, sheep 3.8 hours, hog 4.1 hours, dog 8 hours, and shark <1.7 hours.

SUMMARY

Extracts were prepared from the adrenal glands of man, horse, ox, sheep, hog, and dog, and from the interrenal body of the shark. The extracts were assayed for their cortical hormone content on a standardized series of adrenalectomized dogs. The rate of synthesis of the cortical hormone in the adrenal gland is discussed.
BIBLIOGRAPHY

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