THE PREPARATION AND COMPARATIVE PHYSIOLOGICAL ACTIVITIES OF BEEF, HOG, AND SHEEP ADRENAL CORTEX EXTRACTS

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Our knowledge of the chemical and physiological properties of compounds occurring in the adrenal cortex is based on investigations conducted almost exclusively with extracts and isolation products of beef adrenals. More than twenty-five pure steroids have been isolated but only a relatively few have been found to be biologically active. The amounts and proportions of active steroids present in the adrenals of different species have not been thoroughly determined. We are at present engaged in the fractionation of adrenal cortex extracts prepared from beef, hog, and sheep glands. This report is concerned with the preparation of extracts from each of these species and with a comparative study of their biological activities.

The preparations were assayed in adrenalectomized rats by the following two methods: (a) The survival-growth test, as previously reported from this laboratory (1), is a useful method for determining the complete cortical hormone activity of the whole extracts. All of the known steroids which have the property of replacing, at least in part, the functional activity of the adrenal cortex have been found active by this test. (b) The work performance test in adrenalectomized rats is believed to be specific for the 11-oxygenated sterols which affect carbohydrate metabolism. The previously described method (2, 3) has been modified for the purpose of quantitative assay. A detailed description of the apparatus and procedures will be published in a separate paper. 1 unit of activity is defined as the work equivalent of 0.2 mg. of 17-hydroxy-11-dehydrocorticosterone.

EXPERIMENTAL

The hog, beef, and sheep glands were collected and frozen at the packing house and shipped to the laboratory packed in dry ice. In each case the glands were finely ground while frozen and transferred immediately to acetone and processed as previously described for beef adrenal glands (4). For the beef and sheep glands, this procedure, which consists of acetone extraction for 5 days, filtration from gland residue, evaporation of acetone, extraction of the aqueous solution with petroleum ether of boiling point 60–70°C, followed by extraction with ethylene dichloride, yields an extract.
containing between 35 and 45 gm. of solids per 1000 pounds of gland processed.

In the attempt to work up hog adrenals by this procedure, considerable difficulty was encountered because of the exceedingly high content of fat. Before concentration of the acetone extract, it was necessary to dilute with sufficient water to make a 50 per cent acetone solution. A liquid fat fraction separated from this aqueous acetone which was separated by decantation. This fat was reextracted with 40 per cent acetone and the combined aqueous acetone solutions were concentrated in vacuo to remove acetone. The aqueous solution was then extracted with petroleum ether, followed by extraction with ethylene dichloride to obtain the active fraction. The ethylene dichloride-soluble material was transferred to 95 per cent alcohol and more fat and cholesterol were removed by extraction of aqueous alcohol solutions with petroleum ether, which was done first from 70 per cent ethyl alcohol, then from 50 per cent methyl alcohol, and finally from 30 per cent methyl alcohol. In the case of the beef extract the distribution between 30 per cent methanol and petroleum ether resulted in all the material remaining in the 30 per cent methanol layer. The hog and sheep extracts, however, were not completely free of fat until distribution was carried out between 30 per cent methanol and petroleum ether. The dilute methanol solutions free of petroleum ether-soluble material were concentrated to remove alcohol and the cloudy aqueous solutions were extracted with ethyl acetate. Acidic and basic substances were removed by washing the ethyl acetate solution with sodium carbonate followed by 0.5 N HCl and distilled water as earlier described (5).

Results

The entire fat fraction from hog gland amounted to about 175 pounds per 1000 pounds of gland, whereas the beef and sheep fat fractions weighed only 20 pounds per 1000 pounds of gland processed. The ethylene dichloride extract of hog gland also contained 10 times as much gland extractives as either the sheep or beef extract.

The results of the fractionation with particular reference to the amount of solids at the various steps, beginning with the ethylene dichloride solution, are summarized in Table I.

In Table II the comparative biological activities of the different fractions by the two assay methods are recorded. In order to present data on the recovery of activity after removal of acidic and basic material, assays of the 30 per cent methanol solutions are also given. The biological activity is given in units by the muscle contraction test of Ingle, in which 1 unit is equivalent to 0.2 mg. of 17-hydroxy-11 dehydrocorticosterone, and in units by the survival test, in which the unit, as previously defined
(1), is the minimum amount of hormone necessary to maintain 80 per cent of adrenalectomized 4 week-old rats for an injection period of 20 days and produce an average growth per rat of at least 20 gm. over this period. All assay samples were made up in sesame oil and the volume of oil injected was between 0.1 and 0.2 cc. per day in the survival test and 1.0 cc. in the muscle contraction test.

As can be seen from Table II, the biological activity of the hog extract was considerably higher than that of the beef or sheep extracts. By the survival test in adrenalectomized rats the hog extract was 50 per cent more active than the beef extract and 100 per cent more active than the sheep extract. By the muscle contraction test it was found 64 per cent more potent than the beef and 89 per cent more potent than the sheep extracts. Vars, Taylor, and Pfiffner (6), comparing the yields of "cortical hormone" from 5 pound quantities of gland by the dog assay method (7), reported that both hog and sheep extracts were about 40 per cent more active than beef extracts. Our results by the two different assay methods substantially confirm the findings of Vars et al. with respect to the hog extract, but not with respect to the sheep extract; the latter was found by us to be somewhat less active than the beef extract.

Complete recovery of activity, after removal of acidic and basic material, was found by both assay methods. There was indeed a slight increase by the muscle contraction test, which was greater for the sheep extract than for the hog. We attributed this increase, if significant at all, to the removal of toxic constituents.

The results by the muscle contraction test indicate that the high activity of the hog extract is due to its high content of those adrenal steroids which bear an oxygen atom at carbon atom 11, and which are active in carbohydrate metabolism. The increased activity of the hog extracts by the growth-survival test may therefore also be due to the higher concentration of these compounds.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Beef</th>
<th>Hog</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene dichloride</td>
<td>70</td>
<td>900</td>
<td>75</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>34</td>
<td>212</td>
<td>50</td>
</tr>
<tr>
<td>50% methanol</td>
<td>25</td>
<td>49</td>
<td>41</td>
</tr>
<tr>
<td>30% &quot;</td>
<td>39</td>
<td>39</td>
<td>31</td>
</tr>
<tr>
<td>Neutral ethyl acetate</td>
<td>18.73</td>
<td>25.88</td>
<td>14.82</td>
</tr>
</tbody>
</table>
TABLE II
Comparison of Biological Activity from Beef, Hog, and Sheep Adrenal Extracts

900 kilos of glands were extracted in each case.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight gm.</th>
<th>Biological activity</th>
<th></th>
<th>Weight gm.</th>
<th>Biological activity</th>
<th></th>
<th>Weight gm.</th>
<th>Biological activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Muscle test units per mg.</td>
<td>Total units mg.</td>
<td></td>
<td>Muscle test units per mg.</td>
<td>Total units mg.</td>
<td></td>
<td>Muscle test units per mg.</td>
<td>Total units mg.</td>
</tr>
<tr>
<td>30% methyl alcohol</td>
<td>25.96</td>
<td>0.94</td>
<td>24,400</td>
<td>39.0</td>
<td>1.03</td>
<td>40,000</td>
<td>31.0</td>
<td>0.55</td>
<td>17,050</td>
</tr>
<tr>
<td>Neutral ethyl acetate</td>
<td>18.73</td>
<td>1.37</td>
<td>25,700</td>
<td>25.88</td>
<td>1.64</td>
<td>42,443</td>
<td>14.82</td>
<td>1.51</td>
<td>22,378</td>
</tr>
</tbody>
</table>

* The 30 per cent methyl alcohol fraction and the neutral ethyl acetate fraction were assayed at levels of 12, 8, 6, 4, 3, and 2 gm. equivalents of gland per rat per day. In both fractions there was found to be 1 unit per 4 gm. of gland, since all rats receiving more than this were well maintained and all receiving less were not maintained.

† The sheep extracts were assayed at levels of 12, 8, 6, and 4 gm. equivalents of gland per rat per day. The rats failed at levels of 6 and 4 but were maintained at 12 and 8 gm. equivalents; so that there is 1 unit per 8 gm. of gland.
SUMMARY

Beef and sheep adrenal cortex extracts were prepared by the standard procedure previously reported (4). Because of the high content of fat in hog adrenal glands, this procedure was modified slightly for processing these glands. A comparison of the biological activities of these extracts was made by both the rat survival test and by the Ingle muscle contraction test. It was found that the hog adrenal extract was considerably more active by both tests than either beef or sheep adrenal extracts, and it was concluded that this higher activity may be due to increased amounts of 11-oxygenated sterols in the hog extract.

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