EXTRACTION STUDIES ON THE ADRENAL CORTICAL HORMONE

I. METHODS OF PREPARATION*

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(Received for publication, June 13, 1934)

A method of extraction for the adrenal cortical hormone was described some time ago (1, 2). In this early work the length of the survival period of the adrenalectomized cat served as the biological criterion of physiological potency. Some further observations on extraction technique and chemical properties of the hormone were made, the relief of the symptoms of adrenal insufficiency in the same species being used as a guide (3). Neither of these biological criteria was satisfactory from a chemical standpoint, since the results obtained had only a qualitative significance. The development of a method of assay based upon the minimum maintenance requirement of the adrenalectomized dog (4) made it possible to obtain quantitative information concerning the preparation of the hormone. These quantitative data are summarized in this report.

Method of Assay—The dog method of assay was used (4). Details of the technique as employed in this laboratory have been reported (5). The animals were standardized against a single

* This investigation was supported by a grant from the Josiah Macy, Jr., Foundation.

We wish to express our appreciation to the management and research staff of Parke, Davis and Company for their generous cooperation.

An abstract of a portion of these data was read before the American Society of Biological Chemists at Philadelphia, April 28-30, 1932 (Pfiffner, J. J., Vars, H. M., Bott, P. A., and Swingle, W. W., J. Biol. Chem., 100, lxxviii (1933)).
TABLE I

Yield of Cortical Hormone Obtained from Beef Adrenal Glands

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Starting material and method of extraction*</th>
<th>Yield per kilo tissue</th>
<th>Assay results</th>
<th>Average Fraction weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>dog units</td>
<td>mg.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Dissected cortex</td>
<td></td>
<td>&gt;300 &lt;400</td>
<td>350 80</td>
</tr>
<tr>
<td>2</td>
<td>“ “</td>
<td></td>
<td>&gt;2640 &lt;3300</td>
<td>2870 73</td>
</tr>
<tr>
<td>3-a</td>
<td>“ “</td>
<td></td>
<td>&gt;3330 &lt;5500</td>
<td>4400 83</td>
</tr>
<tr>
<td>3-b</td>
<td>Medulla</td>
<td></td>
<td>&lt;500</td>
<td>&lt;500 80</td>
</tr>
<tr>
<td>3-c</td>
<td>Whole gland</td>
<td></td>
<td>3330</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Chilled and packed in ice 24 hrs.</td>
<td></td>
<td>&gt;2000 &lt;2900</td>
<td>2500 59$</td>
</tr>
<tr>
<td>5</td>
<td>Frozen 30 days at -5°</td>
<td></td>
<td>&gt;1500 &lt;3000</td>
<td>2250 60</td>
</tr>
<tr>
<td>6</td>
<td>“ 6 mos. “ -5°</td>
<td></td>
<td>&gt;1000 &lt;2000</td>
<td>1500 63</td>
</tr>
<tr>
<td>7</td>
<td>“ and packed in dry ice 3 days</td>
<td></td>
<td>&gt;2000 &lt;3000</td>
<td>2500 55</td>
</tr>
<tr>
<td>8</td>
<td>Autolyzed 48 hrs. at room temperature (23°)</td>
<td></td>
<td>&gt;1000 &lt;2000</td>
<td>1500 42</td>
</tr>
<tr>
<td>9</td>
<td>Ethyl alcohol</td>
<td></td>
<td>&gt;1700 &lt;2700</td>
<td>2200 59</td>
</tr>
<tr>
<td>10</td>
<td>Ethyl alcohol + 0.5% acetic acid</td>
<td></td>
<td>&gt;1000 &lt;2000</td>
<td>1500 80</td>
</tr>
<tr>
<td>11</td>
<td>Ethyl alcohol + 0.5% acetic acid</td>
<td>&lt;1000</td>
<td>&lt;1000 133</td>
<td>&lt;8</td>
</tr>
<tr>
<td>12</td>
<td>Ethyl alcohol made 0.08 N with H₂SO₄</td>
<td>&gt;380 &lt;750</td>
<td>615 242</td>
<td>&lt;3</td>
</tr>
<tr>
<td>13</td>
<td>Ethyl alcohol made 0.08 N with H₂SO₄ (neutralized before distillation)</td>
<td>&gt;750 &lt;1500</td>
<td>1125 38</td>
<td>30</td>
</tr>
<tr>
<td>14</td>
<td>Kutz (6)</td>
<td>&gt;125 &lt;250</td>
<td>187 200§</td>
<td>&lt;1</td>
</tr>
<tr>
<td>15</td>
<td>Kendall et al. (7, 8)</td>
<td>&gt;750 &lt;1500</td>
<td>1125 395§</td>
<td>&lt;3</td>
</tr>
<tr>
<td>16</td>
<td>Gland residue from standard method extracted by Kendall’s method</td>
<td>&lt;100</td>
<td>&lt;100 88</td>
<td>&lt;2</td>
</tr>
<tr>
<td>17</td>
<td>Aqueous residue from standard method extracted by Kendall’s method</td>
<td>&gt;50 &lt;100</td>
<td>75 106</td>
<td>&lt;1</td>
</tr>
<tr>
<td>18</td>
<td>Neutral acetone ¶</td>
<td>&gt;1000 &lt;2000</td>
<td>1500 100</td>
<td>15</td>
</tr>
<tr>
<td>19</td>
<td>Grollman and Firor (9)</td>
<td>&gt;500 &lt;1000</td>
<td>750 160§</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

* 4 kilos of glands were used as the starting material in each experiment. Our standard method of extraction referred to in the text was used with the modifications or exceptions noted.
TABLE I—Concluded

† Calculated from the yields and relative weights of cortex (73 per cent) and medulla (27 per cent).
‡ The results recorded are the averages obtained from three experiments in which glands collected at various times during a 6 month interval were used. The respective average yields were 2250, 2400, and 2750 dog units. The fraction weight ranged from 50 to 67 mg.
§ The active fractions obtained in Experiments 4, 15, 16, and 20 from 1 kilo of whole beef adrenals contained 0.01, 0.10, 0.02, and 0.10 mg. of adrenalin, respectively. The adrenalin was determined by bioassay (blood pressure reaction in anesthetized dog).
¶ The yields of adrenalin obtained in Experiments 10, 11, 12, and 13 were as follows: 0.14, 0.18, 0.19, and 0.22 per cent.
¶‡ Tissue extracted for 24 hours with 2.5 volumes of acetone; extractives fractionated by standard method.

preparation of hormone. The error of determination under the conditions employed is approximately ±25 per cent.

Source of Glands—Beef adrenals were used. In the early work the glands were received in the laboratory packed in ice on the day following collection at the abattoir. Later the glands were collected at the abattoir for a period of several days, frozen, and shipped to the laboratory packed in dry ice.

Methods of Extraction—4 kilos of glands served as the starting material in each experiment with the exception of those in which dissected cortex or medulla was employed. 3 kilos of dissected cortex and 1 of dissected medulla were used. The method of extraction employed unless otherwise specified was that described earlier (2, 3).

Relative Yield from Cortex, Medulla, and Whole Gland—The first assays of dissected cortex extract prepared in this laboratory were carried out at the Johns Hopkins Hospital by Drs. Harrop and Weinstein. Their assays showed a yield of 200 to 300 dog units per kilo of cortex (4). When assay work was started in this laboratory in the fall of 1931, their results were confirmed (Experiment 1, Table I). Further work showed that whole gland extracts had from 5 to 10 times this activity, which seemed to indicate the possible presence of a high concentration of hormone in the medulla. In Experiment 3, 4 kilos of whole gland were dissected and the medulla and cortex extracted separately and assayed. In this, as well as in all later experiments, dissected cortex yielded as
Preparation of Adrenal Hormone

much hormone as whole gland.¹ We are unable at the present time to offer an explanation for the differences in yield obtained from dissected cortex. Both the technique of extraction and assay were, so far as we are aware, held constant.

No significant variation could be detected in the yield of hormone obtained from glands collected during the various seasons of the year.

Effect of Previous Handling of Glands—The necessity of great precaution in handling the adrenal glands prior to extraction has been emphasized by various workers. Data are presented on the yield obtained from glands handled in a variety of ways. Glands can be frozen for prolonged periods without an appreciable effect on the yield. Tissue frozen for 6 months assayed 1500 dog units as compared to 2200 dog units for recently collected material. The good yield of hormone from autolyzed material is particularly interesting in view of the extensive precautions taken by Grollman and Firor (9) to avoid the destructive influence of enzymatic processes in the cell.

Desiccated whole adrenal gland has long been used orally in the treatment of various types of adrenal deficiency. The beneficial effect observed by many investigators must have been due to some substance other than the cortical hormone, since the yield of hormone from whole adrenal glands desiccated at a low temperature is less than 250 dog units per kilo of fresh whole gland.

Efficiency of Extraction Procedure—The magnitude of the losses encountered in the various steps is presented in the accompanying diagram. The major portion of the hormone is readily extracted

¹ A sample of the whole gland extract prepared in Experiment 4 (Table I) was submitted to Dr. G. A. Harrop of the Johns Hopkins Hospital for assay in March, 1932. He reported (private communication) a yield of approximately 2000 dog units per kilo of gland. A sample was also submitted to Drs. Oliver Kamm and N. North of the Research Laboratories of Parke, Davis and Company for assay on their colony of adrenalectomized dogs. They found the yield to be greater than 1000 dog units per kilo of gland but did not carry the assay further (private communication). Hence the high yield of hormone obtained by whole gland extraction was confirmed on three different groups of adrenalectomized dogs in as many laboratories. In every instance the yield was found to be many times (5 to 10) greater than that obtained previously from dissected cortex.
Flaw Sheet of Fractionation Procedure for Preparation of Adrenal Cortical Hormone with Fraction Weights and Losses Encountered at Each Step

Whole beef adrenal, 1 kilo
2.5 liters 5 per cent alcohol, 2-7 days

Alcoholic extract
Tissue residue
2 liters 80 per cent alcohol, 2-7 days

Alcoholic extract >200 <400 dog units
Tissue residue (discard) <125 dog units

Concentrate to 0.05 volume and extract 5 times with equal volume of benzene

Benzene-soluble fraction, 25 gm.
Remove benzene and extract with acetone twice

Acetone extractives, 6 gm.
Acetone removed and fraction partitioned twice between 70 per cent alcohol and petroleum ether

Phospholipid fraction

70 per cent alcohol-soluble fraction, 0.4 gm.
Filtered through 10 gm. permutit

Filtrate, 0.1 gm.
Permutit-adsorbed fraction (discard)

Water-soluble fraction, 0.04 gm., 2000-3000 dog units
Water-insoluble fraction (inactive, discard)

Filter and ppt. with NH₄OH

Ppt. of crude adrenalin, 1.4 gm.
Filtrate (discard)

Petroleum ether-soluble fraction (discard) <100 dog units

Concentrated, water added, and brought to desired volume
with a single alcohol treatment. An additional 300 dog units can be obtained with a second alcohol extraction. A negligible amount of hormone remains in the gland tissue after two such extractions. The alcohol extracts are concentrated about 20 times. The exact degree of concentration is of no particular consequence, since the hormone is readily extracted from the concentrate with benzene, even though as much as 20 per cent of alcohol is present in the concentrate. On extracting the concentrate with benzene, emulsions are formed which break slowly. This emulsion formation is an advantage, since the interface of the two phases is increased tremendously over periods of hours without repeated shaking. As the benzene extraction proceeds, the tendency to form emulsions becomes less. 8 to 12 hours are allowed for the separation of the two phases. After five benzene extractions very little if any emulsion remains. The aqueous residue, including the small amount of emulsion, retains only about 30 dog units of cortical hormone. All of the hormone is readily extracted from the benzene-soluble fraction with two acetone treatments; the first acetone extraction is carried on by rubbing and allowing the material to stand overnight in the refrigerator. The lipid mass becomes friable and can be transferred readily to a mortar for the second extraction. This step affects a 4-fold concentration of activity. In the early work the acetone-soluble fraction was distributed four times between 70 per cent alcohol and petroleum ether. Less than 100 dog units are lost in two distributions, with proper technique. The activity is concentrated about 15 times. A 4-fold concentration is effected by permuthit filtration with no loss of hormone. The final water-soluble fraction constitutes about 50 per cent of the fraction passing through the permuthit filter. It varies considerably in weight in different preparations, ranging from 30 to 60 mg. per kilo of gland. This depends upon the length of time taken for the original alcohol extraction.

The mechanical losses in the various fractionation steps total about 300 dog units, with a yield of 2000 to 3000 dog units per kilo. 75 per cent of hormone added to inactivated adrenal material can be recovered. Recovery experiments were carried out as follows: 4 kilos of glands were extracted twice with alcohol as usual. The alcohol extracts were fractionated as indicated in the flow sheet. All the discarded fractions were saved, combined, and
inactivated in a boiling water bath for 5 hours. This inactivated material was added to the thoroughly extracted tissue. To this gland mixture, which represented adrenal starting material minus the cortical hormone as closely as it could be reproduced, was added a definite number of dog units of cortical hormone and the mixture subjected to the complete fractionation procedure. In the first experiment 176 mg. of a fraction containing 6800 dog units were added and a fraction weighing 160 mg. and containing 5000 dog units recovered. In the second experiment 8000 dog units were added and 6000 dog units recovered.

*Stability of Hormone in Various Stages of Extraction*—Considerable emphasis has been laid by some workers on the necessity of very rapid extraction of the hormone from the gland. Ground glands can be stored in 2.5 volumes of 95 per cent alcohol for 3 weeks at room temperature with no appreciable loss in activity. However, the yield is less than 500 dog units after a 10 week period. The benzene-soluble fraction can be stored at room temperature for 11 weeks without loss of activity. The permutit-purified fractions preserved in 95 per cent ethyl alcohol at 6° for 2 ½ years still retain their initial activity. The finished aqueous extracts, if preserved with 0.1 per cent benzoic acid,² are equally stable. The pH of these aqueous solutions is usually between 4.0 and 5.5.³

In the several fractionation steps involving concentration of relatively large volumes such concentration has been effected by distillation under reduced pressure at an external bath temperature of 45–55°. The permutit-purified fraction can be refluxed in 95 per cent alcohol for 20 minutes without loss of activity, while no deterioration occurs in aqueous solutions of the permutit-purified fraction when held at 80° for the same length of time.

*Influence of Acid Extraction on Yield*—The effect of acid was studied in an effort to increase the yield of adrenalin reclaimable as a by-product. The results obtained with acetic and sulfuric acid were found to be unsatisfactory. The use of benzoic acid as a preservative for aqueous solutions of the hormone was suggested by Dr. Oliver Kamm.

² In preparing the permutit-purified fraction for injection it is preferable to add the sodium chloride and benzoic acid before subjecting the suspension to Seitz filtration. The reduction in pH in the presence of sodium chloride causes the suspended material to coalesce, thereby increasing the rate of filtration. Cleghorn (10) observed a similar effect.
acids are summarized in Experiments 10 to 14 (Table I). The addition of acetic acid affected the yield of adrenalin favorably but had the opposite effect on the yield of cortical hormone. 0.5 per cent acetic acid decreased the yield of cortical hormone by more than 50 per cent. The amount of impurities present in the final extract was increased markedly. After the completion of the foregoing experiments with acetic acid, Kendall et al. (7, 8) stated that sulfuric acid-acetone extraction increased the yield of cortical hormone. Experiments 13 and 14 show that the yield is decreased with sulfuric acid-alcohol extraction, either with or without neutralization before concentration (see p. 633).

Other Methods of Extraction—Several methods for the preparation of the cortical hormone have been described (6–9, 11–17). Some procedures were suggested on the grounds of increased yield and greater purity of product. Fractionation steps were employed which in our work are shown to be inefficient. Therefore, the yields obtained with several of the proposed methods were compared under standardized assay conditions.

Hartman and Brownell (11) extracted dissected cortex with ether. The ether-soluble fraction was purified by freezing inactive material from various concentrations of ethyl alcohol and water. No specific fractionation step was used for the separation of adrenalin from the cortical hormone. Their method is inadequate for the preparation of extract from whole beef adrenal gland because of the toxic substances present in the medulla (15, 16).

A simplified method of extraction was suggested by Kutz (6). Ground beef adrenal glands were extracted with acetone. The aqueous sludge obtained on concentration of the acetone extract was extracted with benzene. The concentrated benzene washes were freed of adrenalin by washing with 4 per cent NaHCO₃. The benzene-soluble fraction was suspended in water and the water-insoluble material removed by filtration. This method gave a yield of 200 dog units per kilo of gland (Experiment 15). The hormone is readily extracted from the gland with acetone, as demonstrated in Experiment 19. In this process the major share of the hormone is lost in the bicarbonate washes.

Grollman and Firor (9) have described a method which they recommend because of its simplicity and large yield of hormone obtained. It is essentially the same as that described by Kutz
Following the bicarbonate wash of the benzene-soluble fraction as in the Kutz procedure, Grollman and Firor wash with \( \text{N} \) HCl before suspending the benzene-soluble fraction in water. They emphasize the necessity of obtaining glands immediately after slaughter and of working at a very low temperature to prevent enzymatic destruction. We were able to obtain 750 dog units per kilo of gland by their method (Experiment 20). This is about 50 per cent of the yield which can be obtained with our method from autolyzed glands and less than 50 per cent of the yield which can be obtained from fresh tissue. The low yield obtained with the method of Grollman and Firor is due to the losses in the discarded fractions. We recovered 600 dog units per kilo with our method from the tissue residue and aqueous residue after extracting with acetone (including refluxing) and benzene respectively. This does not take into consideration the loss encountered in the bicarbonate washes.

Kendall and his coworkers (7, 8) extracted with acetone made 0.2 \( \text{N} \) with \( \text{H}_2\text{SO}_4 \). The object of the acid was to liberate the cortical hormone from an assumed complex, for they state (7): "The hormone exists only in small part in a freely extractable form; most of it is firmly bound to the protein fraction and is only liberated in the presence of free acid." The yield of hormone obtained with their technique was 1100 dog units per kilo. The yield was less than that which was obtained with neutral alcohol or acetone (compare Experiments 10, 16, and 19). Tissue which had been extracted twice with neutral alcohol failed to yield a significant amount of activity when further extracted either by the method of Kendall et al. (Experiment 17) or by our method (see diagram). These results demonstrate that all of the cortical hormone is freely extractable with neutral alcohol or acetone. Kendall et al. (7) state further that the benzene-soluble fraction contains only "insignificant traces of the hormone." Aqueous residues from our standard method (see diagram) were fractionated by Kendall's procedure. Only an additional 75 dog units were obtained (Experiment 18), showing clearly that all the activity was in the benzene-soluble fraction.

Separation of Adrenalin from Cortical Hormone—In most of the methods now in use for the preparation of the cortical hormone the bulk of the adrenalin is separated by selective distribution between
immiscible solvents. Kendall et al. (7, 8) remove most of the adrenalin by basic lead precipitation. The really significant fractionation step, however, is the separation of the cortical hormone from the last traces of adrenalin which carry through the fractionation procedures. We have employed permunit for this purpose. As has already been mentioned, Kutz (6) removed adrenalin by washing a benzene solution of the active fraction with sodium bicarbonate solution, while Grollman and Firor (9) wash with both sodium bicarbonate and hydrochloric acid. A serious objection to such a separation is the loss of cortical hormone in the washes as evidenced by the relatively low yield. In distribution studies we have found the coefficient between benzene and aqueous bicarbonate or acid to be 1:3 or 1:4. Kendall et al. (7, 8) removed the last traces of adrenalin by grinding an acetone solution of the active fraction with lead nitrate and potassium carbonate. The remaining fraction still contains impurities which are readily removed with permunit. Hartman and Brownell (15) stated that much of the physiological potency was lost when permunit was used to separate adrenalin and the cortical hormone. No data have as yet been presented to substantiate this claim. If permunit is used properly, the loss of cortical hormone cannot be detected by available methods of determination. Permumit enjoys the distinct advantage in that it readily removes many impurities other than adrenalin from the active fraction. The relative efficiency of the several methods discussed is brought out in the data recorded under Experiments 4, 15, 16, and 20. In earlier work (3) we suggested the separation of adrenalin from the cortical hormone by washing an ether solution of the active fraction with 0.1 N NaOH. This fractionation step was abandoned when results with the present method of assay showed that most of the hormone was destroyed by this concentration of alkali.

Recovery of Adrenalin—Adrenalin can be recovered from the aqueous residue following benzene extraction. It has been our practise to concentrate the first and second alcohol extracts separately, since only the first extract contains sufficient adrenalin to make the recovery worth while. The yield of adrenalin based on the weight of the fresh glands is 0.12 to 0.17 per cent.

On the Nature of Impurities Present in Adrenal Extract—In
Houssay and Marenzi (18) pointed out that choline was absent from adrenal extracts prepared by our method. We had never made direct tests for choline, since it is readily removed from alcoholic solution by permutit (19). Eagle (20), apparently unacquainted with the work of Houssay and Marenzi (18), concluded that this type of adrenal extract contained considerable quantities of choline. He based this conclusion upon results obtained with "2 microscopic tests, a precipitation test, and a color test" but neglected to specify the exact nature of the tests. It is a well recognized fact that choline has no specific chemical reaction by which it can be identified (21, 22). The two most characteristic reactions of choline are (1) splitting off of trimethylamine on alkaline hydrolysis, and (2) formation of the characteristic choline periodide crystals (23). Our extract gives a negative trimethylamine test. It gives several of the non-specific reactions which are also given by choline; i.e., precipitation with Staněk's reagent, a positive alloxan reaction, precipitation in alcohol with mercuric chloride or platinic chloride, and an atypical positive test with Kraut's reagent. The first two reactions can be obtained equally well with a solution containing the mild oxidation products of adrenalin (0.1 per cent). The precipitate obtained with mercuric chloride or platinic chloride does not give the Rosenheim periodide test, which can be obtained readily with the corresponding salts of choline. To prove the presence of choline in adrenal extract it is necessary to isolate it in the form of one of its salts and to obtain analytical data for its identification. This we have attempted to do.

25 cc. of adrenal extract representing 1 kilo of fresh beef adrenal glands were concentrated to semidryness on a water bath following the addition of 0.5 cc. of 0.1 N HCl. The residue was brought to constant weight (63.7 mg.) in a vacuum over sulfuric acid and extracted thoroughly with a total of 10 cc. of absolute ethyl alcohol. To 8.5 cc. of the filtered alcohol extract (representing 850 gm. of whole beef adrenal) 400 mg. of platinum chloride dissolved in 1 cc. of absolute ethyl alcohol were added. Precipitation was complete in 30 minutes. The light tan, amorphous

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4 The extract used in this experiment had no sodium chloride or preservative added. This sample was prepared from aliquots of five individual preparations of 4 kilos each.
precipitate was collected at the centrifuge, washed three times with 2 cc. portions of absolute ethyl alcohol, and dried in a vacuum over sulfuric acid. It weighed 11.6 mg. It gave a negative Rosenheim periodide reaction for choline. Analysis\(^6\) of the chloroplatinate gave the following results.

\[(\text{C}_3\text{H}_6\text{N}_2\text{OCl})_2\cdot\text{PtCl}_4\]  
Calculated. N 4.55, Pt 31.67  
Found. " 8.74, " 29.33

These analytical data show clearly that the chloroplatinate is not choline chloroplatinate. The negative Rosenheim periodide reaction shows that the precipitate does not consist of a mixture of choline chloroplatinate and other chloroplatinates which might conceivably account for the analytical figures. In control experiments, the contamination of the chloroplatinate with choline chloroplatinate could be detected by this reaction when as little as 0.002 per cent choline hydrochloride was added to the original adrenal extract. These data demonstrate that choline is not present in our extract in detectable amounts.\(^6\) Further work on the nature of this nitrogenous fraction is in progress. The quantities of chloroplatinate obtained from a series of extracts varied from 3.0 to 13.7 mg. per kilo of gland. Chloroplatinates were obtained in a similar manner from extracts prepared by the methods of Grollman and Firor (9) and Kendall et al. (7, 8). Both of these products, however, gave a positive Rosenheim periodide reaction for choline.

Cleghorn (10) described histamine-like reactions with extracts which he prepared. He observed the typical depressor effect of histamine after the extract had been exposed for 1 hour to 1 N NaOH. We obtained a negative Pauly reaction with 8.4 mg. of the water-soluble fraction equivalent to 90 gm. of cortex, and a negative Knoop-Hunter reaction with 14.0 mg., equivalent to 150 gm. of cortex. In numerous adrenalin bioassays (blood pressure reaction in the anesthetized dog and cat) on our extracts no evidence was obtained for the presence of histamine or a histamine-like

\(^6\) Microanalytical data were obtained through the courtesy of Dr. Oskar Wintersteiner.

\(^6\) Three samples of eschatin (Parke, Davis and Company, lot Nos. 004896-A, 854234, and 295) were examined in a similar manner. In no case were we able to establish the presence of choline.
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substance. The product from as much as 4 kilos of glands when administered in a single intravenous injection to a 10 kilo dog had no untoward effects.

**SUMMARY**

A quantitative study has been made of the extraction of the cortical hormone of the adrenal gland. Assays were conducted on a standardized series of adrenalectomized dogs. The maximum yield of cortical hormone which can be obtained from beef adrenal gland by any available method is about 2500 dog units per kilo. The method giving this yield is approximately 75 per cent efficient. All of the hormone can be extracted from the gland with neutral alcohol or acetone. Methods of separating the two hormones of the gland and the nature of the impurities present in the extract are discussed.

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