URINARY GLUCOCORTICOID EXCRETION

BY

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In recent years two comparatively simple methods have been described for the assessment of urinary glucocorticoid excretion. Reddy, Jenkins, and Thorn in 1952 gave details of a method for estimating urinary "17-hydroxycorticoids." This involved extraction of the urinary glucocorticoids (cortisone and hydrocortisone) and their metabolites with n-butyl alcohol and the use of the Porter-Silber colour reaction. The second method is the measurement of urinary 17-ketogenic steroids (Norymberski, Stubbs, and West, 1953). This is done by oxidizing the glucocorticoids and their metabolites to 17-ketosteroids with sodium bismuthate and estimating the newly formed and pre-existing 17-ketosteroids by the Zimmermann reaction. If at the same time the 17-ketosteroid content of the untreated urine is measured, the increase following bismuthate oxidation is an index of the glucocorticoid content of the urine.

For the investigation of patients suspected of having adrenal dysfunction, it would be of considerable value if the urinary glucocorticoid content could be assessed by a method suitable for use in a hospital biochemical laboratory. From the original descriptions of these methods either appears suitable for this purpose. We have used them in the investigation of patients with endocrine disease, after adrenalectomy and in other connexions, and have encountered certain difficulties. This has led us to modify the original Norymberski method and to adopt some of the features of the urinary 17-hydroxycorticoid estimation described by Smith, Mellinger, and Patti (1954).

Using these modifications we have tried to ascertain whether these methods really are an index of urinary glucocorticoid excretion. The 17-hydroxycorticoid and 17-ketogenic steroid content of more than 400 urines have been measured and the results compared. Recoveries of cortisone and hydrocortisone added to urine have been studied and the glucocorticoid excretion of adrenalectomized patients receiving different amounts of hydrocortisone and of cortisone acetate have been measured.

URINARY 17-HYDROXYCORTICOIDS

The first difficulty encountered with this method was in the purification of n-butyl alcohol. The methods suggested by Reddy (1954) and Smith et al. (1954) proved disappointing. We are indebted to Mr. R. W. H. Edwards, B.Sc., of the Courtauld Institute of Biochemistry of the Middlesex Hospital, for suggesting the method described in the next section.

The other difficulty was with the Porter-Silber reaction. 17, 21-Dihydroxy-20-ketosteroids produce a yellow colour with phenylhydrazine and strong sulphuric acid, but when the reaction is applied to urine extracts allowance must be made for the colour produced by strong sulphuric acid without phenylhydrazine. Like Smith et al. (1954) we found that the 62% sulphuric acid used in the original method gave very high readings; their modification using 56% sulphuric acid and allowing longer time for the colour to develop has proved more satisfactory.

Method of Estimating Urinary 17-Hydroxycorticoids

Reagents.—The following are used:

n-Butyl Alcohol (A.R.).—This is purified by adding 2 g. phenylhydrazine hydrochloride to 2 l. butanol, acidifying with a few drops of glacial acetic acid and allowing to stand for seven days. It is then distilled twice, collecting the fraction boiling at 117° C. The purified butanol is kept in dark bottles. Satisfactory blank readings were of the following order (using a "unicam S.P. 500" spectrophotometer and a 1 cm. cuvette): butanol+phenylhydrazine-sulphuric acid reagent 0.038 and butanol+sulphuric acid reagent 0.033.

Sulphuric Acid Reagent.—For this 1.27 vol. concentrated sulphuric acid (A.R.) is added to 1 volume distilled water.

Phenylhydrazine-Sulphuric Acid Reagent.—Phenylhydrazine hydrochloride (A.R.), 65 mg., is dissolved in 100 ml. of the sulphuric acid reagent. This may be kept in a refrigerator for one week; if only infrequent estimations are made, it should be prepared freshly on each occasion.
Technique.—The urine is collected without preservative. If the 24-hour volume is less than 2 l., distilled water is added to bring it to 2 l. A 20 ml. sample is adjusted to pH 4.5 with glacial acetic acid, added to 0.5 g. acid-washed kieselguhr in a 40 ml. ground-glass stoppered test-tube and shaken for five minutes in a Kahn shaker. After centrifuging at 2,000 r.p.m., the urine is poured off and adjusted to pH 1–1.5 with 50% sulphuric acid.

The urine is then shaken with 10 ml. purified butanol for four minutes, using a fresh 40 ml. ground-glass stoppered test-tube and a Kahn shaker. The butanol is separated in a separating funnel and centrifuged for five minutes to remove the remaining water. The butanol extract is poured off or removed with a pipette and placed in a test-tube containing approximately 0.5 g. anhydrous sodium sulphate (A.R.). It is allowed to remain in contact with this for a few minutes and then filtered (Whatman No. 41) on to 0.5 g. anhydrous sodium carbonate (A.R.) in another stopped tube. This is shaken by hand for one minute, and immediately after 1 ml. of extract is added to each of two tubes, one containing 4 ml. of phenylhydrazine-sulphuric acid reagent and the other 4 ml. of sulphuric acid reagent. The contents of the tubes must be well mixed. At the same time duplicate blank and standard tubes are prepared, the former containing 1 ml. of butanol and the latter 1 ml. of a standard solution of cortisone alcohol in butanol (3–5 μg./ml.), with 4 ml. of the reagents. The tubes are placed in a water-bath at 60 to 1°C. for 42 minutes. At the end of this time they are removed and cooled in iced water for five minutes. They are read against butanol at 410 μm in a "unicam S.P. 500 spectrophotometer." 1

Calculation.—The output of 17-hydroxy-corticoid is calculated as follows: Corrected optical densities (C.O.D.) of the blank, standard, and extract are obtained by subtracting the optical density of the tube containing sulphuric acid reagent from the corresponding tube containing phenylhydrazine-sulphuric acid reagent.

Urinary 17-hydroxy-corticoid (μg./ml.) =

\[
\frac{C.O.D. \text{ Extract} - C.O.D. \text{ Blank}}{C.O.D. \text{ Standard} - C.O.D. \text{ Blank}} \times \text{Cortisone concentration of standard (μg. ml.)} \times 2
\]

Results

Recoveries of cortisone and hydrocortisone added to urine are shown in Table I. (The steroids were added dissolved in ethyl alcohol, the volume of which did not exceed 0.015 ml. per ml. of urine.) These are fairly satisfactory, but the glucocorticoids and their metabolites found in the urine are mostly in the form of conjugates, and it is more important to know about the recovery of conjugated steroids than the free substances. This has been studied by giving adrenalectomized subjects different doses of cortisone acetate and hydrocortisone alcohol by mouth and measuring the urinary excretion of 17-hydroxycorticoids. The results of one investigation of this type are shown in Fig. 1. The cortisone was given six-hourly and the dose was changed every third day; glucocorticoid excretions were measured on the second and third day at each level. When no cortisone was being given the patient received 0.5 mg. 9α-fluorohydrocortisone per day. The coefficient of correlation between the cortisone acetate dose and 17-hydroxycorticoid excretion is 0.98, and the relation-
ship between them is expressed by the following equation:

\[
\text{Cortisone acetate dose} = 3.52 \times 17\text{-hydroxycorticoid excretion} - 3.7
\]

or

\[
\text{Cortisone (alcohol) dose} = 3.14 \times 17\text{-hydroxycorticoid excretion} - 3.3
\]

In a group of 20 patients who had undergone total adrenalectomy (10), had severe Addison's disease (2), or who had adrenal suppression from prolonged cortisone therapy (8), the correlation coefficient between cortisone acetate dose and 17-hydroxycorticoid excretion was 0.89 and the regression equation

Dose of cortisone acetate = 3.48 \times 17\text{-hydroxycorticoid excretion} - 8.2

A study of 17-hydroxycorticoid excretion was made in one adrenalectomized patient while she was taking oral hydrocortisone (free alcohol) in amounts ranging from 0 to 120 mg./day. Here the correlation coefficient was 0.975 and the regression equation

Dose of hydrocortisone = 6.5 \times 17\text{-hydroxycorticoid excretion} - 15.5

Urinary 17-hydroxycorticoids were in all cases expressed as milligrams of cortisone. This is of some importance because the intensity of the colour developed by equimolar solutions of the glucocorticoids and their metabolites in the Porter-Silber reaction is not the same. Reddy (1954) gives the following figures for colour development: cortisone 1.0, tetrahydrocortisone 0.88, hydrocortisone 0.87, tetrahydrohydrocortisone 0.58. The low figure for the latter probably explains the smaller "17-hydroxycorticoid" excretion of the patient receiving hydrocortisone. We have not adopted the suggestion made by Di Raimondo, Orr, Island, Rinfret, and Forsham (1955) that hydrocortisone should be used as the standard on some occasions and cortisone on others in view of the known interconvertibility of these two steroids in the body (Cope and Hurlock, 1954).

Reddy (1954) has recently described a very simple extraction procedure for urinary 17-hydroxycorticoid measurements. We have performed a number of parallel extractions with the method described above and the simplified method; in all cases the technique for the Porter-Silber reaction suggested by Smith et al. (1954) has been used. The simplified method of preparing the butanol extract is as follows:

A 10 ml. sample of urine is adjusted to pH 1 by the addition of 50% sulphuric acid. It is placed in a 40 ml. ground-glass stoppered test-tube with 2.5 to 3 g. anhydrous sodium sulphate (A.R.) and 10 ml. of purified butanol. The tube is shaken for five minutes in a Kahn shaker and then centrifuged for five minutes at 2,500 r.p.m. Then 5 ml. of butanol is pipetted off into a fresh stoppered test-tube and 0.5 g. anhydrous sodium carbonate added. The tube is shaken thoroughly, allowed to stand for five minutes, and two 1 ml. portions taken for the colour reaction. The Porter-Silber reaction and calculation are carried out as in the previous method except that the 17-hydroxycorticoid output in \(\mu g./mL\) of urine is

\[
\text{C.O.D. Extract} - \text{C.O.D. Blank}
\]

or

\[
\text{C.O.D. Standard} - \text{C.O.D. Blank}
\]

Recoveries of cortisone and hydrocortisone added to normal urine using the simplified procedure are shown in Table II.

<table>
<thead>
<tr>
<th>Steroid Added</th>
<th>Amount (mg./ml.)</th>
<th>Cortisone* Equivalent (mg./ml.)</th>
<th>Number of Experiments</th>
<th>Recovery Range (mg./ml.)</th>
<th>Mean Recovery (mg./ml.)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisone</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>9.0-10.1</td>
<td>9.5</td>
<td>90-101</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15</td>
<td>5</td>
<td>13.7-15.4</td>
<td>14.4</td>
<td>92-103</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>10</td>
<td>8.7</td>
<td>5</td>
<td>6.5-7.4</td>
<td>5.9</td>
<td>75-85</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>17.4</td>
<td>5</td>
<td>12.6-13.8</td>
<td>13.2</td>
<td>72-79</td>
</tr>
</tbody>
</table>

* See Reddy (1954).

The 17-hydroxycorticoid content of 152 different specimens of urine have been estimated by both methods; the correlation between the two was 0.906 and the relationship given by the equation

Simplified extraction = 1.46 \times \text{original extraction} - 1.48

**URINARY 17-KETOGENIC STEROID EXCRETION**

Norymberski et al. (1953) used 8 ml. samples of urine, and, after treatment with sodium bismuthate and hydrolysis, extracted with 20 ml. ethylene dichloride. After washing and filtering two 5 ml. samples were evaporated and the residue redissolved in 0.2 ml. alcohol for the Zimmermann reaction. We have found that values for urinary 17-ketosteroids determined by the method described by Norymberski et al. (1953) were considerably lower than those given by the Medical Research Council method (1951). Recoveries of added dehydroepiandrosterone in the 17-keto-steroid procedure were inconsistent and often of the order of 50%; with the 17-ketogenic steroid procedure they were even less. Using carbon tetrachloride instead of ethylene dichloride failed to improve the recovery figures (see Norymberski and Stubbs, 1954), and ether proved unsatisfactory as a solvent on account of evaporation.
It seemed that the original technique recommended by Norymberski et al. (1953), involving extraction of very small amounts of steroid and in which the residue was taken up in 0.2 ml. alcohol, was too delicate for use as a routine method. We have therefore adopted a larger scale extraction based partly on the M.R.C. method (1951) and partly on that in use in the routine biochemical laboratory of the Courtauld Institute of Biochemistry of the Middlesex Hospital.

Method of Estimating 17-Ketogenic Steroids

The urine is collected without preservative, and if the 24-hour volume is less than 2 l. it is made up to that figure with distilled water.

Estimation of 17-Ketosteroids.—Urine, 30 ml., 10 ml. glacial acetic acid (A.R.), 7.5 ml. concentrated hydrochloric acid (A.R.), and 25 ml. benzene (A.R.) are placed in a 250 ml. round-bottomed flask and boiled under reflux for 30 minutes. The A.R. benzene has not required any purification, and blanks run with 30 ml. of distilled water instead of the urine have consistently given zero readings. After boiling the flask is cooled and the aqueous layer removed in a 100 ml. separating funnel with a ground-glass stopper. The aqueous layer is shaken in a second separating funnel with two further 25 ml. portions of benzene. The extracts are combined and washed twice with 15 ml. N sodium hydroxide, once with 15 ml. N/2 hydrochloric acid and three times with 15 ml. distilled water. Washing is done in a 100 ml. stoppered separating funnel, shaken by hand. The washed benzene extract is transferred to a 100 ml. round-bottomed flask and evaporated to dryness under reduced pressure in a boiling water-bath. The benzene distillate is collected and may be used again. The flask is placed in a desiccator overnight.

The contents of the flask are redissolved in 1 ml. ethyl alcohol (2 ml. may be used if a very high reading is expected). We have used "R.R. quality" alcohol, without further purification, and agree with Munson and Kenny (1954) that none of the usually recommended methods for alcohol purification is really effective. We have usually had to be satisfied with an alcohol which will give a Zimmermann reading of 0.060 at 520 mμ in a 1 cm. cuvette in a "unicam S.P. 500" spectrophotometer. In performing the Zimmermann reaction, the M.R.C. method (1951) has been followed using a standard containing 0.05 mg. dehydroepiandrosterone in 0.2 ml. and applying the colour correction recommended.

Estimate of 17-Ketosteroids + 17-Ketogenic Steroids.—A 20 ml. sample of urine, 20 ml. glacial acetic acid (A.R.), and 4.5 g. sodium bismuthate (A.R.) are placed in a 50 ml. ground-glass stoppered test tube and shaken for 30 minutes in the dark in a Kahn shaker. The stoppers are removed and the tubes centrifuged for five minutes at 2,000 r.p.m.; the supernatant is poured into another tube and again centrifuged; then 25 ml. of the supernatant is placed in a 250 ml. round-bottomed flask. Fifteen drops freshly prepared 5% solution of sodium metabisulphite is added and the flask shaken, then 25 ml. distilled water, 7.5 ml. concentrated hydrochloric acid, and 25 ml. benzene are also added. The contents of the flask are boiled for 30 minutes and treated as in the ketosteroid procedure.

In calculating the urinary steroid output, if 1 ml. alcohol is used for dissolving the residue, the 0.2 ml. taken for colour development represents 6 ml. of urine in the ketosteroid procedure and 2.5 ml. in that for estimation of 17-ketosteroids + 17-ketogenic steroids.

Results

Recoveries of dehydroepiandrosterone, cortisone, and hydrocortisone added to normal urine are shown in Table III. The figures for the glucocorticoids are not so good as those reported by Norymberski et al. (1953), but are satisfactory for clinical purposes. Recovery of metabolites and conjugates was studied by estimating the output of 17-ketosteroids in adrenalectomized patients given cortisone and hydrocortisone. In one patient (Fig. 2) the correlation coefficient between dose of cortisone acetate and 17-ketogenic steroid excretion was 0.99, and the relationship between them given by the equation

Cortisone acetate dose = 2.54 x 17-ketogenic steroid excretion - 1.0

or

Cortisone dose = 2.26 x 17-ketogenic steroid excretion - 0.9

In the group of 20 patients given cortisone acetate by mouth after adrenalectomy or in the presence of Addison's disease or adrenal suppression the

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\(^{1}\) Distillers Co. Ltd.
correlation coefficient between dose and output was 0.97 (106 observations); the regression equation was:

\[
\text{Dose of cortisone acetate} = 2.74 \times 17\text{-ketogenic steroid output} - 4.5
\]

or

\[
\text{Dose of cortisone} = 2.44 \times 17\text{-ketogenic steroid output} - 4.0
\]

The adrenalectomized patient given oral hydrocortisone gave the following relationship (correlation coefficient 0.98):

\[
\text{Hydrocortisone dose} = 2.91 \times 17\text{-ketogenic steroid output} - 2.5
\]

The 17-ketogenic steroid excretion, like that of the 17-hydroxycorticoids, is a little lower with hydrocortisone than with a similar dose of cortisone. The oxidation products of the glucocorticoids and their metabolites vary in the amount of colour produced in the Zimmermann reaction (Brooks and Norymberski, 1953). Dehydroepiandrosterone is used as the standard, and the oxidation products of 10 µg. cortisone correspond to 7.8 µg. of dehydroepiandrosterone, of 10 µg. hydrocortisone to 5.5 µg. and the oxidation products of 10 µg. tetrahydrocortisone to 10.2 µg. of standard (from figures given by Norymberski et al., 1953).

Appleby, Gibson, Norymberski, and Stubbs (1955) have recently described a method of estimating 17-ketogenic steroids directly instead of by difference between 17-ketosteroids + 17-ketogenic steroids and 17-ketosteroids. Sodium borohydride is used to destroy the pre-existing 17-ketosteroids and then the urine is treated by bismuthate oxidation and the 17-ketogenic steroids alone estimated. The preliminary treatment with sodium borohydride renders an additional group of steroids 17-ketogenic (21-deoxy-17:20 ketols), but except in the adreno-genital syndrome these are only present in small amount. There are other ways in which the steroids estimated with this technique differ from the original 17-ketogenic steroids, and Appleby et al. (1955) have suggested referring to the new method as determination of the "total urinary 17-hydroxycorticosteroids." Although this new procedure may provide a more direct and accurate means of assessing glucocorticoid excretion, in cases of adrenal hyperfunction it will still be necessary to estimate the 17-ketosteroids for the diagnostic purposes.

**CORRELATION BETWEEN URINARY 17-KETOCORTICOSTERONE AND 17-HYDROXICORTICOSTERONE**

The excretions of 17-hydroxycorticoids and 17-ketogenic steroids have been determined on more than 400 urine collections. In general the correlation between the two indices of glucocorticoid output is good (coefficient 0.89) and the relationship is given by the equation:

\[
\text{Urinary 17-hydroxycorticoids} = 0.664 \times \text{urinary 17-ketogenic steroids} - 0.66
\]
With some urines there was quite a big difference, and the range of urinary 17-hydroxycorticoids, corresponding to different levels of 17-ketogenic steroid excretion, are shown in Fig. 3.

RESULTS IN NORMAL SUBJECTS AND PATIENTS WITH ENDOCRINE DISEASE

The results of estimations of urinary 17-hydroxycorticoids and 17-ketogenic steroids in normal adults and patients without any evidence of adrenal dysfunction are shown in Table IV.

<table>
<thead>
<tr>
<th>TABLE IV</th>
<th>URINARY 17-HYDR OCYTICOID AND 17-KETOCORTICOID EXCRETION IN NORMAL ADULTS AND PATIENTS WITHOUT EVIDENCE OF ADRENAL DYSFUNCTION (MG./24 HOURS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Range</td>
<td>No. Range</td>
</tr>
<tr>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>17-Hydroxycorticoids</td>
<td>30</td>
</tr>
<tr>
<td>17-Hydroxycorticoids (simplified extraction method)</td>
<td>19</td>
</tr>
<tr>
<td>17-Ketogenic steroids</td>
<td>30</td>
</tr>
</tbody>
</table>

In cases of Cushing's syndrome the excretion of 17-ketogenic steroids varied from 16 to 60 mg./day and that of 17-hydroxycorticoids from 12 to 42 mg./day.

Few patients with untreated Addison's disease have been available for study; in one case the 17-ketogenic steroids were from 0 to 2.1 mg./day and 17-hydroxycorticoids 0.4 to 2.9 mg./day. A patient with Addison's disease on 9α-fluorohydrocortisone 0.5 mg./day excreted between 1.1 and 1.5 mg./day 17-ketogenic steroid and from 0 to 2.7 mg./day 17-hydroxycorticoid. The outputs in 15 urine collections from three adrenalectomized patients on 0.5 or 1.0 mg. 9α-fluorohydrocortisone per day were 17-ketogenic steroids 0-2.0 mg./day and 17-hydroxycorticoids 0-2.8 mg./day.

In four untreated cases of panhypopituitarism the urinary 17-hydroxycorticoids were 1.9, 2.5, 0.5, and 1.8 mg./day; corresponding figures for 17-ketogenic steroids were 1.5, 2.0, 1.8, and 4.0 mg./day.

DISCUSSION

Laboratory investigations are essential for the diagnosis of adrenal dysfunction and are desirable for the control of corticotrophin therapy. In many hospitals the only direct measurement that can be undertaken is the urinary excretion of 17-ketosteroids. These are derived principally from adrenal androgens; in males testicular androgen makes an important contribution, and a small proportion is derived from glucocorticoid. The urinary 17-ketosteroids are not entirely satisfactory as an index of adrenal cortical activity. Glucocorticoid production can usually only be assessed by indirect evidence such as the level of circulating eosinophils, insulin resistance, and in some instances by changes in the urinary sodium/potassium ratio (Nabarro, 1954).

Results of the investigations in adrenalectomized patients receiving varying amounts of cortisone and hydrocortisone show that the urinary 17-ketosteroids and 17-hydroxycorticoids are closely related to glucocorticoid excretion. In most cases figures obtained by the two modified methods described in this report are in good agreement. Choice of method depends on certain practical considerations. The 17-hydroxycorticoid determinations can only be made if a spectrophotometer giving reliable readings at 410 mμ is available. On the other hand it is quicker, and if Reddy's simplified technique (1954) is used it is easily possible to perform eight duplicate estimations a day. The limit with the 17-ketogenic steroid method described above is three duplicate estimations and these have to be read the next day. On the other hand, in many cases of adrenal dysfunction, urinary 17-ketosteroids must be determined as well as glucocorticoids, and this is included in the 17-ketogenic steroid estimations.

An additional consideration in individual cases is that certain urinary constituents interfere with the measurements. For example 17-ketogenic steroids cannot be estimated on a urine containing more than 0.5% of glucose; iodides, chloral hydrate, paraformaldehyde (Marks and Leftin, 1954), and ketone bodies (Di Raimondo et al., 1955) interfere with 17-hydroxycorticoid estimations.

SUMMARY

(1) Urinary glucocorticoid estimations are often required in the investigation of patients with adrenal dysfunction and in the control of corticotrophin therapy.
(2) The urinary excretions of 17-ketogenic steroids (Norymberski) and 17-hydrocorticooids (Reddy-Jenkins-Thorn) have been studied.
(3) Modifications of the methods are suggested, which will, it is believed, prove suitable for routine use.
(4) Recoveries of added steroid by these modified methods are satisfactory.
(5) Estimation of urinary 17-ketogenic steroids and 17-hydrocorticooids in adrenalectomized patients receiving cortisone and hydrocortisone shows that they do indicate glucocorticoid excretion.
ADDENDUM

Since this paper was submitted for publication we have obtained further experience with the total 17-hydroxycorticosteroid method of Appleby et al. (1955). In this method the pre-existing ketosteroids are destroyed with sodium borohydride, and the glucocorticoids are then oxidized with sodium bis-muthate. The urinary glucocorticoids are therefore determined directly and not by difference as in the case of ketogenic steroids. In our hands the original method has proved inconsistent and we have modified it as with our ketogenic steroid procedure, the first step being to add 100 mg. sodium borohydride to the 20 ml. of urine and allow the urine to stand at room temperature overnight. The next day we proceed exactly as indicated on page 354. There is considerable frothing when the borohydride is added, but this can be stopped by the addition of a few drops of ether. If the urine contains more than 0.5% of sugar we add 0.75 g. of baker’s yeast to 25 ml. of urine and keep in an incubator (37° C.) from six to 24 hours until the sugar concentration is 0.5% or less; the urine is then centrifuged and a 20 ml. sample taken for treatment with borohydride.

Using this method the normal ranges are for males (16) 8.0–21.8 mg./24 hr. and for females (21) 4.6–17.0 mg./24 hr. Sixty-seven determinations have been made on adrenalectomized subjects taking between 0 and 150 mg. cortisone acetate per day by mouth. The correlation coefficient between dose of steroid and urinary total 17-hydroxycorticosteroids was 0.95 with a regression equation:

\[
\text{Cortisone acetate dose} = 2.62 \times \text{steroid excretion} + 0.7
\]

We now use this modification of the Appleby et al. (1955) method for all urinary glucocorticoid estimations and are convinced that at this time it is the most satisfactory method available and that it is suitable for use in hospital biochemistry laboratories.

We wish to acknowledge the help we have received from Dr. A. E. Kellie, Ph.D., and his associates in the Courtauld Institute of Biochemistry. We are extremely grateful to the Clinical Research Committee of the Middlesex Hospital for the provision of laboratory facilities and for a personal grant to J. D. N. N. The steroids used in the recovery studies and 9α-fluorohydrocortisone were provided by the Medical Research Council, the hydrocortisone alcohol by Roussel Laboratories. The figures were prepared by Mr. V. K. Asta.

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