have the picture of normal haem—that is, iron protoporphyrin—together with masses of uroporphyrin I and coproporphyrin I, the end stages of the abnormal synthesis proceeding in some erythrocytes.

To understand this situation in more detail it is necessary to recall what is now known about the biochemistry of porphyrin synthesis, and this I have summarized in Fig. 2. Glycine and succinic acid are the two simple materials from which the complex ring structure of porphyrin is built up. An initial condensation, for which pyridoxine and coenzyme A appear to be essential, results in the formation of δ -aminolaevulinic acid. Two molecules of this substance are then combined in such a way that a pyrrolic substance is formed. This is porphobilinogen, the urinary excretion of large quantities of which, it is interesting to note, is the biochemical abnormality characterizing acute intermittent porphyria, a disease involving the liver rather than the haemopoietic system. Porphobilinogen can be transformed either chemically or enzymatically into uroporphyrin, and in each case it is the unsymmetrical series III isomer which is the principal product.

Many schemes have been put forward to explain the way in which combination of four identical porphobilinogen units could result in a porphyrin of this configuration, and I shall not discuss the merits and demerits of each hypothesis here. It is sufficient to note that this is the normal course of events. We (Booiij and Rimington, 1957) have found, however, that if our enzyme system, extracted from red blood cells, is heated for some time at about 60° its character is altered so that when incubated with porphobilinogen it now forms exclusively uroporphyrin I, the symmetrical uroporphyrin isomer, which, as I have already said, is excreted in large quantities by congenital porphyria. Not only this, but if we prepare an enzyme system from the erythrocytes of animals or human patients with congenital porphyria and incubate it with porphobilinogen *in vitro*, we find that both uroporphyrins I and III are formed and the series I isomer may even predominate (Rimington and Booiij, 1957).

It is thus clear that by mild heat treatment of the enzyme system from normal cells we have made it resemble that of the congenitally abnormal cells of this rare familial disease. Just what happens as a result of heating is under investigation at the present moment in my department. When we know, we shall, I feel convinced, understand also the inborn error responsible for congenital porphyria.

Conclusion

In this survey I have tried to view haemoglobin biosynthesis as a whole and then to indicate how experimental findings over a wide field are slowly but steadily being related to various steps in this vital process. Nearly 100 years have elapsed since Körber (1866) first noticed the difference between foetal and adult haemoglobins, and we cannot flatter ourselves on the speed of our progress; nevertheless the record of the past few years holds out some promise that before another century dawns we may have achieved understanding and control of some, at least, of the medical problems associated with haemoglobin biosynthesis.

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THE PRODUCTION RATE OF CORTISOL IN MAN

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Hitherto the great majority of proposed measures of adrenal cortical activity have involved the estimation of a particular chemical group of metabolites in the urine. These have all suffered from the disadvantage that it has not been possible to check their validity by comparison with any more direct measure of adrenal activity, and the relation between the amount of excreted compounds and the actual production rate by the adrenal cortex has been a matter of considerable conjecture. Indeed, some tests have relied for their justification on little more than the observation that they give low readings in clinical hypoadrenalism and high readings in Cushing's syndrome.

One of the major products of the adrenal cortex is now proved to be cortisol (hydrocortisone), and measurement of the production rate of this hormone must therefore be regarded as one of the major indices of adrenal activity.

The recent introduction of radioactive cortisol, labelled with ^{14}C in ring A, has made possible a much more direct estimation of the rate of production of endogenous cortisol than could be achieved before. Peterson and Wyngaarden (1956) have pioneered its use for this purpose. By injecting trace amounts of ^{14}C -labelled cortisol into human subjects and by making serial estimations of the changing ^{14}C content (specific activity—S.A.) of the circulating cortisol they were able to calculate turnover rates by methods conventional in such isotope studies. In nine normal subjects they reported a mean daily cortisol production of 21 mg.

The estimates of these authors were based on events occurring during an observation period of two to four hours, and daily output was calculated on the assumption that the observed production rate persisted throughout the 24 hours, an assumption which is probably not justified. While such an estimate of production over a short period is valuable for many purposes, a simpler method capable of indicating the daily output without recourse to difficult serial analyses would also be of very great clinical value.

We suggest here an alternative method of using ^{14}C -labelled cortisol which is believed to give a useful estimate of the daily production of cortisol by the subject, which is relatively easy to apply clinically even to ill patients, and which does not make excessive demands on the time and skill of laboratory staff. Results in a variety of clinical conditions using this method are presented.

Principle

The principle of the measurement is the well-established one of isotope dilution. If a small amount of ^{14}C -labelled cortisol is introduced into the body it may be expected to be metabolized in precisely the same manner as the endogenous cortisol. It will thus enter equally into all the metabolites, known and unknown, of cortisol and will appear in these in the urine.

The radioactive cortisol will be diluted by the endogenous non-radioactive cortisol, and likewise all the metabolites will be diluted by the corresponding non-radioactive products of endogenous cortisol breakdown. The extent of this dilution can be found by determining the ^{14}C content (S.A.) of a representative excreted metabolite and comparing this with the ^{14}C content (S.A.) of the radioactive steroid administered. The ratio between the two specific activities indicates the degree of dilution, and if the mass of the radioactive cortisol dose be known the weight of endogenous cortisol and its metabolites necessary to effect this dilution can be simply calculated, being the dose times the dilution.

The method thus involves the separation of a suitable cortisol metabolite from the urine. Cortisol itself is present in urine but is not well suited for such an analysis because it is present only in low concentration averaging less than 50 μg . a day in normal urines (Cope and Hurlock, 1953).

Two major breakdown products of cortisol metabolism, tetrahydrocortisol and tetrahydrocortisone, are, however, present in urine in relatively large amounts, averaging together more than 2.5 mg. in normal urines. These tetrahydro compounds are excreted mainly in the conjugated form with glucuronic acid, and can be extracted from the urine by chloroform only after they have been hydrolysed with the enzyme β -glucuronidase. They can be separated from the crude chloroform extract by paper chromatography, and provide very suitable representative metabolites of cortisol, the specific activity or ^{14}C content of which can readily be determined, as they are relatively easily estimated by sensitive colour reactions.

Method

The radioactive cortisol was used as received from the National Institute of Health, Bethesda, U.S.A., without added carrier. Its stated specific activity was 1.47 mC per m.mol or 4.06 μC per mg. A small quantity of this was dissolved in pure ethanol and its ^{14}C content determined by direct plating of a small aliquot. The test dose was measured out by pipette and was diluted with 40 to 50 ml. of water. About 40 μg . was used in each test, equivalent to about 20,000 counts per minute (c.p.m.) in our counting system.

For the test the patient first empties the bladder and then drinks the whole test dose, taking care to avoid any loss. Urine is then collected for the next 24 hours. Two measurements are required from this sample: (1) a direct measurement of the total ^{14}C content of the 24-hour urine sample, and (2) determination of the specific activity of the tetrahydrocortisone.

Total ^{14}C content is determined by direct plating of 1- or 2-ml. samples of the urine on planchettes, counting for one to two hours and correcting for the weight of urine residue against a calibration curve specially constructed with varying quantities of urine. With the doses of radioactivity used counts are sometimes low, with consequent impairment of counting accuracy. In such cases accuracy can be improved by making a butanol extract of 5 to 10 ml. of urine and plating this on planchettes for direct counting. About 90% of the total ^{14}C is usually extracted in this way, and the contamination with inert material is much reduced. With these butanol extracts, however, greater care is needed to prevent creeping during plating.

^{14}C Content of the Tetrahydrocortisone

Liberation of the tetrahydrocortisone and tetrahydrocortisol from their glucuronide conjugates is achieved by incubating one-twelfth of the 24-hour sample—that is, two hours—with 500 units per ml. of a suitable β -glucuronidase

preparation. Our own has been made from the livers of young rats by the method of Talalay, Fishman, and Huggins (1946). Incubation at pH 4.8 with acetate buffer for 48 hours at 37° C. liberates the tetrahydrocortisone. After incubation the steroids are extracted with chloroform by manual shaking. The chloroform extract is washed with a small volume of N/10 soda followed by water, and is then evaporated to dryness. The residue is transferred with a small volume of fresh chloroform to the starting line of a paper chromatogram and is developed for four days or more with the toluene/propylene glycol system of Burton, Zaffaroni, and Keutmann (1951) with a 20- μg . cortisol standard running in a separate lane. After drying the chromatogram thoroughly it is scanned for zones of radioactivity. Two large peaks are regularly found in the regions corresponding to tetrahydrocortisol and tetrahydrocortisone, which lie about 23 and 37% respectively of the distance from the starting line to the cortisol zone.

One or both of these zones are cut out, cut into small pieces with scissors, and eluted by standing the pieces overnight in a test-tube with 4 ml. of pure redistilled ethanol. Then 2 ml. of this eluate is plated on a planchette for ^{14}C counting, and 0.2 to 2 ml. of the remainder is assayed for reducing steroid by the method of Chen, Wheeler, and Tewell (1953), using tetramethylammonium hydroxide as the alkali. For the chemical analysis a paper blank of the same area from a part of the paper free of reducing steroid is also eluted and treated similarly.

From the results the specific activity of the tetrahydrocortisone is calculated and expressed as counts per minute per microgram of reducing steroid.

Calculation of the Cortisol Production Rate

If $S.A._U$ be the observed specific activity or ^{14}C content of the representative metabolite tetrahydrocortisone, and if C_U be the total ^{14}C content of the 24-hour urine sample, both expressed as counts per minute by the same counter, then the total mass of cortisol metabolites of all forms, known and unknown, in this urine will be $C_U/S.A._U$, provided only that the ingested radioactive steroid is handled in the body in the same manner as the endogenous cortisol.

If all the breakdown products of cortisol production were excreted ultimately in the urine, then under steady conditions of adrenal activity the mass of metabolites in the 24-hour urine would equal the daily production of cortisol. Such complete excretion of metabolites in the urine cannot, however, be assumed in the individual case. The efficiency with which the cortisol is actually excreted is, however, indicated in each case by the behaviour of the administered radioactive cortisol, for the ratio $C_U/\text{Dose} \times 100$ indicates the percentage excretion of cortisol metabolites during the 24 hours under observation.

Many factors are operative in determining the apparent excretion of orally ingested ^{14}C cortisol. Among these, loss of part of the dose or part of the urine sample, and impaired alimentary absorption, are factors which can give an apparent excretion efficiency lower than the true excretion of the endogenous cortisol metabolites. Since the magnitude of these factors will often be unknown, the true excretion efficiency must be presumed to lie between the observed figure C_U/Dose and the theoretical maximum of 100%. The estimate of the daily cortisol production will therefore lie between the two figures $\text{Dose}/S.A._U$ and $C_U/S.A._U$ dependent on the two extreme possibilities: (a) that the whole of the dose given was absorbed but that only a part was excreted in the urine, and (b) that only part of the dose was absorbed but that this was completely excreted in the urine. The mean of these two results will usually give a closer estimate than either extreme.

When it can be shown in the individual case that at least 80% of the dose administered has appeared in the 24-hour urine, then this provides satisfactory evidence that losses in absorption or of part of the urine sample have been of minor degree. It indicates, moreover, that the excretion

of metabolites is at least 80% complete, and thus should lead to an estimate of daily cortisol production which will lie between 80 and 100% of the true figure. The higher the observed recovery in a given case, the greater the accuracy of the estimate of cortisol production is likely to be.

Results

In a representative series of 12 normal subjects convalescent from diseases not likely to cause any adrenal stimulation, the mean recovery in the 24-hour urine sample was 87% of the ^{14}C of an administered dose of ^{14}C cortisol, the range being from 68 to 95 (Table I). In a larger series of 44

TABLE I.—Resting Normal Subjects

Case No.	Dose (c.p.m.)	% Excretion 24 Hours	S.A. _U	Estimated Cortisol Production between $C_U/S.A._U$ and Dose/S.A. _U (mg. day)	
1	19,500	95	1.3	14.2	15.0
2	19,500	92	2.4	7.5	8.1
3	17,200	78	1.8	7.5	9.5
4	18,100	86	0.65	24.0	27.9
5	27,500	95	1.2	21.8	23.0
6	18,000	68	2.5	4.9	7.2
7	16,700	84	1.1	12.7	15.2
8	18,000	82	1.6	9.2	11.2
9	18,000	95	1.17	14.6	15.4
10	16,400	87	1.2	11.9	13.6
11	19,370	88	2.0	8.5	9.7
12	18,300	92	1.03	16.4	17.8
Mean ..		87	1.50	12.8	14.5

miscellaneous hospital patients, including some with endocrine disorders but excluding all those with gross renal functional damage, the mean excretion rate was 79% in 24 hours and 89% in 48 hours (see Chart). In 33 of these

Total urinary excretion in 48 hours of ^{14}C after an oral dose of ^{14}C cortisol. Each square represents the excretion in one of the 44 cases.

cases (72%) the excretion was in excess of 80% in 48 hours. The observed specific activity of the tetrahydrocortisone isolated from the 12 normal subjects ranged from 0.6 to 2.5 counts per minute per microgram in the normal group, with a mean of 1.5. These specific activities corresponded to estimated daily cortisol outputs which ranged between 4.9

and 25.3 mg., with mean of 12.8 mg. when based on a ratio $C_U/S.A._U$ and varied between 7.2 and 27.9 mg. per day when based on the ratio Dose/S.A._U, the mean then being 14.5 mg. (Table I). Though some of these outputs were much lower than had been expected the mean estimate of the group is in close correspondence with the clinical estimate based on replacement therapy in Addison's disease and after adrenalectomy.

In each of five subjects comparison was made of cortisol output estimates obtained on one occasion by oral and on another by intravenous administration of the ^{14}C cortisol. The mean of the estimates by the oral route was 8.1 mg. and the mean of those obtained by the intravenous injection 9.8 mg. This comparison thus suggests that no significant error is introduced by giving the ^{14}C cortisol into the portal blood stream.

(Table II). In this series no attempt was made to achieve maximum stimulation, and higher outputs could no doubt have been achieved with larger doses of corticotrophin.

TABLE II.—Corticotrophin Stimulation

Case No.	Dose (c.p.m.)	% Excretion 24 Hours	S.A. _U	Estimated Cortisol Production between $C_U/S.A._U$ and Dose/S.A. _U (mg. day)	
25	19,100	84	0.2	82	97.5
26	17,600	87	0.08	192	225
27	18,100	70	0.32	39.6	56.6
28	18,000	72	0.23	59.5	83
29	18,000	77	0.26	53	69
30	18,000	77	0.07	207	257
31	18,000	75	0.62	21.8	29
32	18,000	66	0.1	119.5	180
33	16,400	74	0.09	135	182
34	18,400	89.5	0.11	150	167
35	18,400	58	0.21	49	86
36	18,400	88	0.21	77	88
Mean ..		76.4	—	99	127

In contrast, a series of four cases of gross hypoadrenalism due to hypopituitarism or Addison's disease gave a mean 24-hour recovery of ^{14}C of 81% of the dose and indicated a mean daily cortisol production of between 0.8 and 1 mg. A similar low output of only 1 mg. daily was recorded in an adult subject with pseudohermaphroditism due to adrenal hyperplasia whose treatment with cortisone had been temporarily suspended (Table III).

TABLE III.—Hypoadrenalism

Case No.	Disease	Dose (c.p.m.)	% Excretion 24 Hours	S.A. _U	Estimated Cortisol Production between $C_U/S.A._U$ and Dose/S.A. _U (mg. day)	
37	Addison's	16,280	87.5	20	0.7	0.8
38	Hypopit.	27,500	84	24	1.0	1.1
39	"	16,800	72	20.7	0.6	0.8
40	"	18,400	81	15.0	1.0	1.2
41	Pseudohermaphroditism	18,400	84	17.2	0.9	1.07
Mean ..			81.7	—	0.8	1.0
Inhibition with						
43	9- α -Fluoro-cortisol	17,200	82	7	2.3	2.8
44	Prednisone	27,500	92	10.6	2.4	2.6
45	"	18,400	72	8.5	1.56	2.16
Mean ..			82	—	2.09	2.52

With the method proposed here it is sometimes possible to estimate cortisol output in persons whose adrenal activity is inhibited by prednisone or other similar steroid. When prednisone is given, a large part of it is converted into prednisolone and appears as such in the urine. Most of this can be removed by an initial chloroform extraction before treatment with glucuronidase, but small amounts may persist in the glucuronidase extract and will run on the chromatogram at a rate similar to that of tetrahydrocortisone, so that a proper separation of the two is not readily achieved. But no such contamination occurs with the tetrahydrocortisol, which can therefore sometimes be used to determine the specific activity of the metabolites. In three subjects on prednisone in whom cortisol production rate was measured in this way outputs of 2 to 2.8 mg. were recorded (Table III). However, Caspi and Pechet (1957) have identified tetrahydrocortisol among the metabolites of prednisone, so that estimates based on the specific activity of tetrahydrocortisol in persons on prednisone may be too high. The actual outputs in the three cases studied will probably therefore be below the recorded figures.

It is now well recognized that in many active disease states adrenal stimulation often occurs. In a series of six such cases estimates of cortisol production varying from 10 to 31.9 mg. daily were recorded, the mean production rate being between 18.1 and 22.2 mg. daily. In three further subjects with well-marked hyperthyroidism the mean estimated cortisol production lay between 21.9 and 25.3 mg.

daily. In three subjects with advanced hepatic cirrhosis but without ascites the mean estimates were between 6.7 and 9.5 mg. daily (Table IV).

TABLE IV.—*Disease States*

Case No.	Disease	Dose (c.p.m.)	% Excretion 24 Hours	S.A.v	Estimated Cortisol Production C.v/S.A.v and Dose/S.A.v (mg./day)
<i>Active Diseases</i>					
13	Lymphosarcoma	18,500	86	0.7	22.7
14	Carc. breast	19,200	84	1.3	12.4
15	Rheum. fever	17,200	90	1.0	15.5
16	Advanced tuberculosis	19,100	81	0.6	25.8
17	Advanced Hodgkin's	18,000	58	1.04	10.0
18	Rheum. fever	18,000	86	0.69	22.4
Mean					18.1
<i>Hyperthyroidism</i>					
19		17,600	99	1.02	17.1
20		18,100	94	0.65	26.1
21		18,100	73	0.59	22.4
Mean					21.9
<i>Hepatic Cirrhosis (with Endocrine Change)</i>					
22		16,800	84	2.3	6.1
23		16,800	54	1.5	6.1
24		18,000	79	1.8	7.9
Mean					6.7

Discussion

The calculation of the cortisol production rate proposed here involves two stages. First is the estimation of the total mass of adrenal cortisol metabolites in the urine. The reliability of this depends solely on the entry of the ^{14}C of the ^{14}C cortisol equally into all the metabolites. Although this has not been strictly shown, except in a few instances (Cope and Black, 1958), it is difficult to see why it should not be so, since ^{14}C cortisol must be treated in the body precisely as endogenous cortisol. The dose administered will not appreciably alter the prevailing concentrations in the blood, since it represents a quantity normally to be found in less than 500 ml. of venous blood.

The second stage is the determination of the relation between the total mass of excreted metabolites in the urine and the actual cortisol production. For this also the behaviour of the ^{14}C provides the necessary information of the proportion excreted in the urine. The excretion observed in the 24-hour urine sample is less than the total excretion, since further small amounts are excreted during the second 24 hours. With the small dose of ^{14}C cortisol used here it is difficult to measure reliably the ^{14}C content of the second 24-hour urine sample, but it usually contains about 5% of the dose.

Administration of the ^{14}C cortisol by the oral route introduces certain ambiguities about the completeness of absorption, and the absorbed cortisol enters the portal blood stream. Intravenous administration of the dose would avoid the possible objection that steroid entering the portal blood is not necessarily treated in the same manner as cortisol in the systemic circulation, and would, furthermore, eliminate uncertainty over the completeness of absorption. We have preferred the oral route, however, because it is simpler, because in most cases absorption is rapid and complete (Peterson and Wyngaarden, 1956), because it is likely to give a slow and steady introduction of the dose into the blood stream, and because it avoids psychological trauma which may readily cause an adrenal stimulation. We have not found it easy to be sure of the correct introduction of the whole dose intravenously without using a prolonged saline drip which may upset the patient and his adrenal activity. The relatively good correspondence we have obtained between estimates of cortisol production based on oral and intravenous ^{14}C cortisol

administration justifies the belief that the oral route, with its greater simplicity, introduced no systematic errors.

Because of the uncertainty which will frequently exist over the completeness of ingestion or of absorption of the dose, or over the completeness of the 24-hour urine specimen, the estimate of output must be a range of values based on the two extreme possibilities mentioned earlier. But in the majority of cases studied, in which an excretion of over 80% of the dose can be demonstrated, the two estimates will not be widely separated, and a mean of the two should reduce the maximum error due to this ambiguity to little more than 10%.

Because the specific activity of the cortisol metabolite and not its total amount is the measurement required, only total urine ^{14}C and the final analysis of the eluate from the chromatogram need to be accurate. Losses in extraction of the urine, inadequate hydrolysis of the glucuronide, losses during transfer of the steroid extract to the paper chromatogram, and incomplete elution from this chromatogram will not at all impair the accuracy of the test.

The demands made by the method on the technical skill of laboratory staff are therefore relatively small.

The method measures the actual output during the 24 hours and not the production rate for a short period. For its principle to be valid a reasonably steady state must prevail, and it is not suited to the measurement of rapid changes in output either during the day or from day to day.

The validity of the method is dependent on the ability to obtain the tetrahydrocortisone sufficiently free from interfering impurities. Although no attempt is made to isolate this metabolite in a state of chemical purity, evidence that the relatively simple technique employed here yields a product pure enough for clinical purposes has been presented elsewhere (Cope and Black, 1958).

Previous attempts to estimate the daily cortisol production rate are summarized in Table V.

TABLE V

Authors	Method	Circumstances	Output Daily (mg.)
Knowlton (1952) ..	Replacement therapy	Normal	12.5-25
Dorfman (1954) ..	? Excretion of metabolites	"	Men 21. Women 18
Silber (1955) ..	17-OHCS excretion	"	30-60
Bondy and Altrock (1953)	Adrenal vein F	Stress	21
Moxham and Nabarro (1956)	{ 17-KGS excretion 17-OHCS excretion	Normal	Men, 8.5 to 49 Women, 5.5 to 40
Samuels <i>et al.</i> (1957)	Rate fall blood F	{ Normals Corticotrophin	Men, 14 to 45 Women, 9.5 to 36
Peterson and Wyngaarden (1956)	Fall of S.A. blood F	{ Normals Evening Corticotrophin	36 250
Hardy and Turner (1957)	Adrenal vein F	Stress	17 to 29 12 160
Cope and Black (1958)	S.A. of tetrahydrocortisone	{ Normals Corticotrophin	34
			5 to 25 Up to 257

The needs of the Addisonian or adrenalectomized subject, which vary from 12.5 to 25 mg. daily, suggest an output from the normal adrenal of this order. Several attempts have been made to correlate the excretion of certain groups of cortisol metabolites with the actual production rate, by observing the proportion of an ingested dose which is subsequently excreted in the form of such groups of compounds. Dorfman (1954) and Silber (1955) have made estimates in this way, that of the former being 18 to 21 mg. and of the latter the much higher figure of 30 to 60 mg. daily. Moxham and Nabarro (1956) have correlated oral cortisol dosage with both ketogenic steroid and 17-hydroxycorticosteroid excretion, and have thereby provided data which suggest an excretion between 5.5 and 49 mg. judged by ketogenic steroids and from 9.5 to 45 mg. judged by the 17-hydroxycorticosteroids. The disadvantage of such estimates is that one must assume that the observed average

ratio between cortisol dose and the metabolite will apply to each individual case, an assumption which is scarcely justified in disease states.

Both Bondy and Altmann (1953) and Hardy and Turner (1957) have obtained much more direct estimates in selected individuals by actual collection and analysis of the adrenal vein blood. This is clearly a procedure of very limited application and will inevitably introduce a condition of stress which is likely to raise the adrenal activity.

The rate of disappearance of injected cortisol has been employed by Samuels *et al.* (1957) to estimate cortisol destruction rate, but when cortisol levels in the blood are greatly raised there is considerable likelihood that the turnover rate or destruction rate will be increased. It is for this reason especially that the radioactive steroid is preferable, for it permits the use of quantities so small that the blood levels do not exceed the normal physiological range.

The method of Peterson and Wyngaarden (1956) is especially suited to estimates of cortisol turnover during relatively short periods, but is not well suited to observational periods greater than a few hours. For general clinical purposes, however, an average excretion rate over at least a day is likely to be of much more diagnostic value, and that is more conveniently obtained by the method used in the present paper.

The estimates of cortisol production obtained by our method, with a mean of 13 to 14 mg. daily, are appreciably lower than the estimates obtained by Peterson and Wyngaarden (1956), which are the only comparable estimates of which we are aware. Their results, with a mean output of 21 mg. daily, are, however, based on data obtained in a three-hour period during the morning hours when there is a strong probability that adrenal cortical activity is at the higher levels of the diurnal rhythm, and are based on the assumption that the observed adrenal activity continues throughout the 24 hours, which is improbable. In contrast the estimates obtained here are the result of observation of the whole 24-hour period, and include the period of sleep when probably adrenal activity is at its lowest. It is of considerable interest in this connexion that in the one case where Peterson and Wyngaarden made their test at 9 p.m. instead of in the morning they obtained their lowest estimate of cortisol production rate, an output of 12 mg. daily, and this figure is in good agreement with our own average.

The wide variation in outputs of cortisol in patients on corticotrophin is of considerable interest, for all were subjects on corticotrophin therapy of normal dosage for the treatment of disease. In one case no appreciable stimulation had been achieved; in other cases a fifteenfold increase in output had occurred. The higher outputs are comparable in magnitude to estimates made in single cases by Samuels *et al.* (1957) and by Peterson and Wyngaarden (1956). They do not represent the highest attainable outputs.

Unlike most estimates and analyses of endocrine function, the accuracy of this test is not appreciably reduced at the lower levels of adrenal activity. The amount of ^{14}C to be found in the final eluate is dependent on the dose of ^{14}C cortisol administered, and is not at all affected by the degree of adrenal activity. In persons with very low adrenal activity the amount of tetrahydrocortisone in the urine is greatly reduced, but sufficient for analysis can usually be obtained by increasing the volume of urine extracted after glucuronidase hydrolysis.

Because of this undiminished sensitivity at the lower levels of activity, the method is likely to be of special value in determining the severity of cases of hypopituitarism. Thus outputs of less than 1 mg. daily in cases of complete hypopituitarism can be distinguished from outputs between 2 and 5 mg. which have been found in less complete hypopituitarism. This ability to measure low cortisol outputs has already proved of some clinical value in assessing the degree of pituitary damage produced by irradiation of the gland with yttrium implants in the treatment of metastatic carcinoma.

The outputs in hyperthyroidism are all at the upper limit of the quiescent normal range. They are not surprising in view of the increased metabolism which occurs in such subjects.

The figures recorded in cirrhosis, however, suggest that output in severe liver damage is reduced to 50 to 60% of the normal. Samuels *et al.* (1957) have also obtained evidence suggesting that turnover in hepatic cirrhosis is reduced. This may be due to slower destruction of the hormone by the liver, so that less fresh cortisol is needed to maintain normal plasma concentrations.

Summary

A method has been developed, using ^{14}C -labelled cortisol, for measuring the daily production rate of cortisol by the adrenal cortex in man.

In 12 quiescent and convalescent subjects, free of endocrine or other active disease, the range of estimated production rates was from 4.9 to 27.9 mg. daily, the mean figure being 12.8 or 14.5 mg. daily according to the method of calculation.

In six subjects with severe active but non-endocrine diseases values of 10 to 31.9 mg. were estimated, with a mean of 20.1 mg. daily.

The response to routine corticotrophin therapy was very variable, the highest estimated output being about 257 mg. daily and the lowest 21.8 mg., a figure within the normal unstimulated range.

In three subjects with advanced hepatic cirrhosis production rates were low, averaging 8.1 mg. daily; and in three patients with hyperthyroidism the cortisol production rates were high, averaging 23.6 mg. daily.

In hypoadrenalism due to Addison's disease or to hypopituitarism estimated cortisol production rates were between 0.6 and 1.2 mg. daily, with a mean of 0.9 mg., and in three subjects whose adrenals were inhibited by prednisone or 9- α -fluorocortisol therapy estimated production rates averaged 2.3 mg. daily. A cortisol production of only 1 mg. daily was found in a pseudo-hermaphrodite, confirming the low output believed to occur in these patients.

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The 37th annual meeting of the British Cardiac Society was held at Leeds University on April 17 and 18, under the chairmanship of Dr. JOHN TOWERS. On the first day there were short papers followed by demonstrations in the afternoon, and a symposium on extracorporeal circulation, in which Professor A. C. BROM, of Leyden, and Dr. L. R. RADIGAN, of Indianapolis, took part as guest speakers. On the second day there was a symposium on diuretics followed by more short papers. The annual dinner was held in the university refectory, with Dr. Towers in the chair.