THE 17-HYDROXYCORTICOSTERONE CONTENT OF HUMAN ASCITIC FLUID

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In spite of many investigations uncertainty still exists about the quantity of adrenal cortical hormone present in human blood and other human body fluids. Most work in this field has been done on animals, and particular attention has been paid to the blood leaving the adrenal gland by the adrenal vein, since the highest concentrations of adrenal hormones may be expected in such blood. Both chemical and biological methods of assay have been applied, but neither has been entirely satisfactory, since chemical methods have not in general been sufficiently specific, and the biological methods have not been sensitive enough to respond to the very small quantities of active hormone present in circulating blood.

The Literature

One of the earliest estimates was that of Hemphill and Reiss (1947), who used a chemical extraction of blood and subsequent estimation of the reducing power of the steroid fraction. They worked mainly on rats, but found 0.21 to 0.43 mg. per 100 ml. in human blood. This early estimate must now be regarded as much too high.

Paschkis, Cantarow, and Boyle (1949) reported the detection of small amounts of adrenal hormone in arterial plasma of the dog. They used the method of glycogen deposition in the liver of the adrenalectomized mouse which had been developed by Venning, Kazmin, and Bell (1946). In the following year Paschkis, Cantarow, Walkling, and Boyle (1950) reported finding values of 14 to 50 μ g. per ml. of dog adrenal-vein blood, the activity being expressed in terms of 11-hydrocorticosterone (compound A) acetate. They could also demonstrate biological activity in the peripheral blood of the dog.

In 1950 also, Nelson, Reich, and Samuels succeeded in isolating and identifying compound F of Kendall (17-hydroxycorticosterone; hydrocortisone) in the blood of the adrenal vein. Brownell, Hartman, and Liu (1951), who used the Venning glycogen deposition method, reported finding biological activity in dog adrenal-vein blood, but they were unable to detect any activity in the peripheral blood. Thus the earlier work of Paschkis and his colleagues on peripheral blood was not confirmed.

The relatively higher concentrations of hormone in adrenal-vein blood enabled more rapid progress to be made than was possible with peripheral blood, and Pincus, Hechter, and Zaffaroni (1951) were able to extract adrenal steroids from dog adrenal-vein blood in amount sufficient to allow of analysis by paper chromatography. In the dog the steroids identified were mainly 17-hydroxycorticosterone and corticosterone. The concentration of these after adrenal stimulation by A.C.T.H. rose as high as 55 μ g. of each per 100 ml. Cortisone was present in a concentration less than 10 μ g. per 100 ml., and was regarded as a metabolite, 17-hydroxycorticosterone and corticosterone being the main products.

Following up their identification of 17-hydroxycorticosterone in dog adrenal-vein blood, Nelson, Samuels, and Reich (1951) developed a chemical method based on the purification of the extracted adrenal steroids on a chromatographic column and subsequent measurement of the eluted adrenal steroids by means of a colour reaction with phenyl-

hydrazine and sulphuric acid. This colour reaction had been developed by Porter and Silber (1950) and had been shown by them to be specific for steroids having a 17-21-dihydroxy, 20-keto grouping. Both cortisone and 17-hydroxycorticosterone give the typical reaction, but it is given only weakly by corticosterone. Compounds giving this reaction are often called 17-hydroxycorticosteroids. The reaction cannot be regarded as entirely specific for active hormones, since some other products of adrenal steroid metabolism, notably tetrahydrocortisone, also give a strong reaction with the reagent but are biologically inert. Using the reaction on a micro-scale, Nelson and Samuels (1952), and Nelson, Willardson, Tyler, and Samuels (1951) found 4 to 6 μ g. of 17-hydroxycorticosteroid per 100 ml. of human venous blood and 9 to 11 μ g. in human arterial blood. Both figures rose after A.C.T.H. While some of this was probably 17-hydroxycorticosterone an unknown proportion may have been tetrahydrocortisone. This latter metabolite presumably occurs in blood, since it is present in large amount in urine, and has been identified in a peritoneal dialysate by Savard, Kolff, and Corcoran (1952).

Complication rather than simplification has ensued more recently. Thus Bush (1953) showed that the adrenal steroids differed in nature in different species of animal, some having much 17-hydroxycorticosterone and little corticosterone, and other species more of the latter than the former. Sweat and Farrell (1952), using a microchromatographic technique and subsequent detection of the steroids by fluorescence, developed a method sensitive to 0.25 μ g. They found 4 to 8 μ g. of adrenal steroid in the adrenal-vein plasma of stressed rats, but were unable to find any in peripheral blood of adrenalectomized rats. In the adrenal-vein plasma of dogs under stress they found 3 μ g. of a steroid resembling 17-hydroxycorticosterone and 1 μ g. of a corticosterone-like steroid.

At the same time Pincus, Romanoff, and Romanoff (1953) were producing data indicating the complexity of the problems involved in this and allied aspects of adrenal metabolism, for they were able to detect 35 different neutral lipoids in hydrolysed human urine, and similar compounds were found in human peripheral blood. Among the most important of these were corticosterone (compound B), 17-hydroxycorticosterone, cortisone, pregnane 3-11-17-21 tetrol 20-one, and pregnane 3-17-21 triol 11-20 dione (tetrahydrocortisone).

Zaffaroni and Burton (1953) have analysed a large volume of dog adrenal-vein blood from an animal stimulated by A.C.T.H. They found 130 μ g. of 17-dehydroxycorticosterone and 95 μ g. of corticosterone per 100 ml. of blood. Only traces of other compounds were found, but the method they used would not have detected some of the important inert metabolites such as tetrahydrocortisone.

The present position may therefore be summarized as follows.

Both corticosterone and 17-hydroxycorticosterone are present in appreciable quantity in adrenal-vein blood of experimental animals. Cortisone is not usually found. The most refined chemical methods indicate the normal presence of 4 to 6 μ g. of 17-hydroxycorticosteroid in 100 ml. of peripheral venous blood and 9 to 11 μ g. per 100 ml. of arterial blood. A considerable part of this seems to be 17-hydroxycorticosterone, but an uncertain fraction is metabolites which give the same colour reaction but are biologically inert—for example, tetrahydrocortisone. Biological assay indicates the presence of some active adrenal steroid in dog adrenal-vein blood, but no certain activity has been established in peripheral blood in either animals or man.

It seemed to us that useful light might be thrown on the problem by analysis of human ascitic fluid. Although ascitic fluid is not a normal body product it can be obtained in relatively large quantities, sufficient to make a biological assay feasible if the content in active hormones is 1.5 μ g. or more per 100 ml. We have found activity in excess of this figure in five out of ten ascitic fluid samples tested.

Methods

Samples of ascitic fluid have been obtained from suitable patients in volumes of 3 to 7 litres. These samples were extracted and the steroids concentrated into a volume small enough for injection into mice by a method employed by Bush (1952) for blood. The ascitic fluid was extracted by shaking four times with separate lots of chloroform, each representing 15% of the volume of the sample. The four extracts were combined and evaporated under reduced pressure at a temperature below 50° C. to small volume. To the residue of 5 to 10 ml. was added 200 to 400 ml. of acetone and a few drops of saturated magnesium chloride. The mixture was cooled overnight in the refrigerator. After filtration the acetone solution was distilled down to a small volume (3 to 5 ml.). This residue was taken up into 100 ml. of 70% aqueous ethanol and washed three times with 10-ml. portions of light petroleum to remove excess fats. The ethanol solution was evaporated under reduced pressure to dryness, dissolved in a small volume of chloroform, and transferred to a small tube in which it was evaporated to dryness by a stream of nitrogen to await injection.

For bio-assay the method described by Cope, Boysen, and McCrae (1951) has been used. This method makes use of the fact that cortisone-like hormones lower the number of eosinophil cells in the blood of adrenalectomized mice. An extract equivalent to 1,500 ml. of ascitic fluid was injected subcutaneously into each adrenalectomized mouse. For injection the extract was dissolved in propylene glycol, using 0.1 ml. for each 1,500 ml. of ascitic fluid represented. Four volumes of saline were then added, so that the final volume was such that each mouse received a 0.5-ml. injection of this 20% propylene glycol solution.

The eosinophil cell drop in the injected adrenalectomized mice was measured at four and six hours, the greater drop being taken as the significant one. The equivalent activity of cortisone was read off from a calibration curve prepared by injecting cortisone acetate in similar and constant volume of 20% propylene glycol, but in varying dosage into adrenalectomized mice.

Results

The results obtained with these extracts are summarized in the accompanying Table. Ten samples of human ascitic fluid have been separately extracted and tested, most of

Patient	Disease	Vol. per Mouse (ml.)	No. of Mice	Toxicity	Mean Drop Eosinophils	Equivalent Cortisone Content (µg. per 100 ml.)
1 Fr	Cirrhosis	1,500	3	++	No drop	Nil
2 Fr	"	1,500	3	+	"77%"	4.4
3 Re	"	1,700	2	0		
4 Pe	Carcinoma of peritoneum	1,600	3	++	62%	3.1
5 Pe	"	1,500	2	++	11%	<1.5
6 Du	Tricuspid incompetence	1,560	2	0	68%	3.8
7 Du	"	1,500	3	0	77%	5.0
8 Ha	Cirrhosis	1,560	3	0	74%	4.5
9 Du	Tricuspid incompetence	1,500	2	+	15%	<1.5
10 Du	"	1,500	3	+	20%	<1.5

+ toxicity = Well-marked sedation without loss of consciousness. ++ toxicity = A light coma from which the animals recovered in a few hours.

these being of sufficient volume to provide material for three mice. Five of these extracts showed unequivocal evidence of eosinopenic activity. Two extracts, both from the same patient, showed no trace of biological activity, and in the remaining three the presence of activity was equivocal.

Some of these extracts were rather toxic to the mice, producing lethargy and sometimes light narcosis. This effect was at first thought to be due to traces of the magnesium chloride used in the preparation of the extract being carried into the final residue, but efforts to remove this magnesium did not remove the toxicity. There was no relation between this toxicity and the magnitude of the eosinopenic response. Long-acting anaesthetics such as pentobarbitone sodium do not influence the eosinophil response to cortisone in the

adrenalectomized mouse. The degree of toxicity of the extracts is indicated in the Table.

It will be noted that although both ascitic fluid samples obtained from patient Fr were completely devoid of activity, yet in patients Pe and Du eosinopenic activity varied in samples of ascitic fluid removed from the same patient at different times. No explanation can be offered for this apparent variation, since the method of making the extracts was the same in all.

Discussion

The data obtained indicate that, although the amount of biologically active adrenal hormone extractable from human ascitic fluid appears to be variable, it does not usually exceed 5 µg. per 100 ml. In half the extracts examined it was much less than this, being below the level necessary for reliable detection by the methods used. Our previous experience with this bio-assay technique indicates that the variations in activity observed are much greater than could be due to errors in the method of assay.

Some information has been obtained on the nature of the hormone responsible for this biological activity. Extensive search by many investigators has failed to reveal any hormones other than cortisone and 17-hydroxycorticosterone, which lower the eosinophil count in the blood

Comparison of paper chromatograms of both urine and ascitic fluid extracts.

of adrenalectomized mice. Portions of four of the ascitic fluid extracts used in the present study have been submitted to analysis by paper chromatography, using the solvent systems developed by Burton, Zaffaroni, and Keutmann (1951). Whereas a substance behaving like 17-hydroxycorticosterone could be demonstrated in all of the extracts examined, cortisone was usually absent completely. It was indeed detected only in faint trace in a single extract. Urine, in contrast, usually contains both 17-hydroxycorticosterone and cortisone. Actual paper chromatograms of both urine and ascitic fluid extracts are compared in the accompanying illustration. The 17-hydroxycorticosterone found in these extracts was identified by the following points: (1) Its rate of flow in the chromatogram was identical with that of an authentic sample of free 17-hydroxycorticosterone. (2) It reduced the blue tetrazolium reagent like the authentic compound. (3) It absorbed ultra-violet light at 240 mµ. (4) The compound behaving identically with this which is present in urine has been injected into mice and shown to have strong eosinopenic activity.

Sufficient of one extract (No. 10) was available to permit a chemical estimate of the quantity of 17-hydroxycorticosterone present by measurement of the amount of tetrazolium reagent reduced at the appropriate point on the chromatogram. The estimate of 1.8 µg. per 100 ml. agrees well with the traces of activity found in the biological test, for larger quantities in the extract would have given an unequivocal biological response.

As corticosterone itself has been identified in the blood of many mammals, it is possible that this compound also is present in human ascitic fluid. No search for this substance was made in the present work, because no sample of

authentic corticosterone was available for comparison. Corticosterone, however, is reported to have only a slight eosinopenic effect, which would not contribute appreciably to the biological activity detected in these ascitic fluid extracts.

It may be concluded, therefore, that almost all of the biological activity observed is due to the content of 17-hydroxycorticosterone, and that this varies in different samples of ascitic fluid from zero to about 5 μ g. per 100 ml. Cortisone itself does not contribute to the biological activity in significant degree.

It is, of course, clear that one cannot assume that the hormone content of ascitic fluid is necessarily the same as that of circulating blood. Ascitic fluid may, however, be regarded as representing a form of extracellular fluid. Its hormone content may be indicative of the adrenal hormone content of extracellular fluid in general, a content which must be of more direct importance to living cells than is the hormone content of the blood. Our inability to demonstrate any hormonal activity in the two samples from patient Fr raises the possibility that conditions may sometimes exist in which adrenal cortical hormone diffuses with difficulty from the blood into the extracellular fluid. Such a state could account for the fact that rheumatoid arthritic tissues and joints respond promptly to cortisone or 17-hydroxycorticosterone therapy, although all the evidence suggests that adrenal cortical function is normal in this disease. While this suggestion is purely speculative at the present time, further investigation of the hormonal content of human ascitic fluid should prove of considerable interest, in so far as it is representative of an extracellular fluid.

Summary

The adrenal cortical hormone content of human ascitic fluid has been measured biologically, using the power of such hormones to lower the level of circulating eosinophil cells in adrenalectomized mice.

Of ten samples of ascitic fluid tested, five showed activity equivalent to between 3 and 5 μ g. of cortisone per 100 ml. In two samples from one patient no activity at all could be detected. In the remaining three activity was equivocal and less than 1.5 μ g. per 100 ml.

17-hydroxycorticosterone (compound F) was detected by chromatographic analysis in all four samples in which it was sought, but cortisone was found only as a faint trace in a single extract. The biological activity is apparently due almost entirely to the 17-hydroxycorticosterone, only a negligible part being due to cortisone.

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PIPERAZINE IN THE TREATMENT OF THREADWORMS IN CHILDREN

REPORT ON A CLINICAL TRIAL

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The successful use of piperazine in the treatment of threadworms was first recorded by Mouriquand, Roman, and Coisnard (1951) and later by Turpin, Cavier, and Savaton-Pillet (1952). Mouriquand *et al.* used piperazine hydrate in solution, while Turpin *et al.* chose piperazine diphenyl-acetate in the form of glutenized granules. In most instances oral dosage was supplemented by the use of piperazine suppositories. The clinical trials carried out by both groups of workers were preceded by successful preliminary experiments upon the mouse *Aspicularis* or *Syphacea*. In the clinical trials Mouriquand *et al.* reported a high proportion of cures in 18 patients, while Turpin *et al.* recorded one definite failure out of 41 patients.

We ourselves decided to carry out clinical trials following successful experiments with piperazine hydrate against *Aspicularis* in the mouse (Standen, 1953). As a comparative standard, gentian violet was also included, and, in addition, diphenan, which has achieved considerable popularity on account of its lack of toxic side-effects. Laboratory tests had failed to demonstrate oxyuricidal properties in diphenan. These results were in keeping with the clinical findings of Miller and Choquette (1950), Beaver and Young (1950), Dowsett and Brown (1953), Jung and Beaver (1953). In contrast, Killingsworth *et al.* (1952), using diphenan in conjunction with hexylresorcinol enemas, reported 77% cures, while Bumbalo *et al.* (1953) reported 30% cures with diphenan alone. Killingsworth *et al.* and Van Someren (1939) both quote Schulemann (1920) as stating that diphenan breaks down in the small intestine to form para-benzylphenol, and that this kills oxyurids in 5–10 minutes at 1:4,000 dilution. In fact, Schulemann was working with "Strudelwurmen," which are free-living turbellarians and not in any way related to oxyurids in particular nor to nematodes in general. It is considered that this mistranslation of Schulemann's work should be recorded, since it appears to be in danger of perpetuation in the literature.

Clinical Tests

The material used in the tests consisted chiefly of children admitted to hospital for medical or surgical reasons, and these were identified as cases of *Enterobius* infection by microscopical examination of adhesive "cellophane" swabs of the perianal area. Swabbing was carried out in the morning before the children were bathed or used the toilet, and was repeated for seven days unless a positive result was obtained earlier. In addition, previously established cases of oxyuriasis were included. For purposes of comparative drug effect the children were treated in four groups, each group receiving piperazine, gentian violet, diphenan, or a lactose placebo.