SOME STUDIES OF THE CHEMISTRY AND PHARMACOLOGY OF ADRENOCORTICOTROPHIC HORMONE

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The final establishment of certain biochemical actions of the adrenocorticotrophic hormone (ACTH) has aroused the interest of clinicians, physiologists, pharmacologists and chemists alike, and has made it desirable that larger quantities of the hormone should be obtainable. It is unfortunate, however, that various reports have been apt to mislead in the matter of potential supplies of this hormone. We believe that the opinion that only minute amounts of the hormone can be produced at high cost needs rectifying in the light of our own experience. Sufficient hormone for therapeutic use should be available if the maximum yield attainable is achieved and the hormone is used in the optimal way from the clinical and pharmacological point of view.

Yields

It has been known for many years that the adrenocorticotrophic hormone concentration of pig pituitaries is far higher than that of cattle or sheep pituitaries. Although 1 kg. of frozen pig pituitaries contains only 20 times as much adrenocorticotrophic hormone as 1 kg. of cattle pituitaries, in practice one can prepare at least 100 times more, adequately purified for clinical use, from the pig pituitaries, than is obtained from the cattle pituitaries. This is due to the fact that the same extraction methods which are used to prepare the crude preparation yield, from pig pituitaries, a preparation which is 20 to 100 times purer than the preparation from the cattle pituitaries. In order to get from cattle pituitaries adrenocorticotrophic hormone of purity similar to that of the preparation from pig pituitaries, additional purification steps have to be introduced which are sometimes connected with very considerable losses.

The only yield calculation we can find published so far comes from Fishman[1], who has worked in connection with the White-Sayers group. He prepares from 1 kg. of pig pituitary 6·5 g. of crude extract, from which about 1 g. of purified preparation can be obtained. According to this conception only forty 25 mg. ampoules for clinical use could be prepared from 1 kg. of pig pituitary. Most of the purification methods described so far are only of academic interest. Their adoption as routine methods for the production of the pharmaco-therapeutic preparation would make the clinical use of adrenocorticotrophic hormone prohibitive.

The procedures to be described below give the following calculated yield. From 1 kg. of frozen pig pituitary 35 to 40 g. of a crude preparation which is already free from gonadotrophic, thyrotrophic or growth hormones is obtained. Its potency is at least one-third as high as that of

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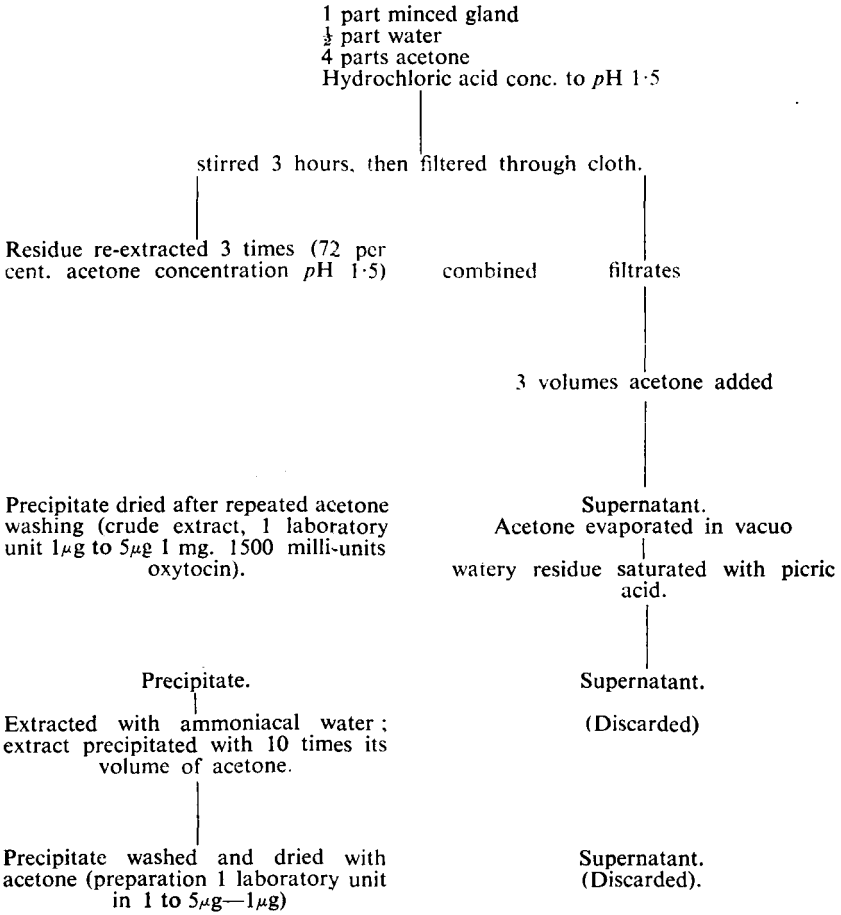
some of the pure preparations described, such as the Armour standard preparation La-I-A. Only the very low (nearly negligible) content of lactogenetic hormone and the posterior lobe hormone present in the preparation must be removed before it can be used therapeutically. These procedures are connected with the loss of about 25 per cent. of the active material. It should be possible to prepare 600 to 800 ampoules of an activity equivalent to that of 25 mg. of standard hormone from 1 kg. of pig pituitaries.

Chemical Preparation

The crude hormone preparation has been made by a process similar to that of Lyons[2]. The glands were extracted with strong acid acetone for 3 hours. This was carried out in accordance with the process shown

TABLE I



in Table I. The high acetone concentration used does not extract the gonadotrophic and thyrotrophic hormones. The extraction is repeated

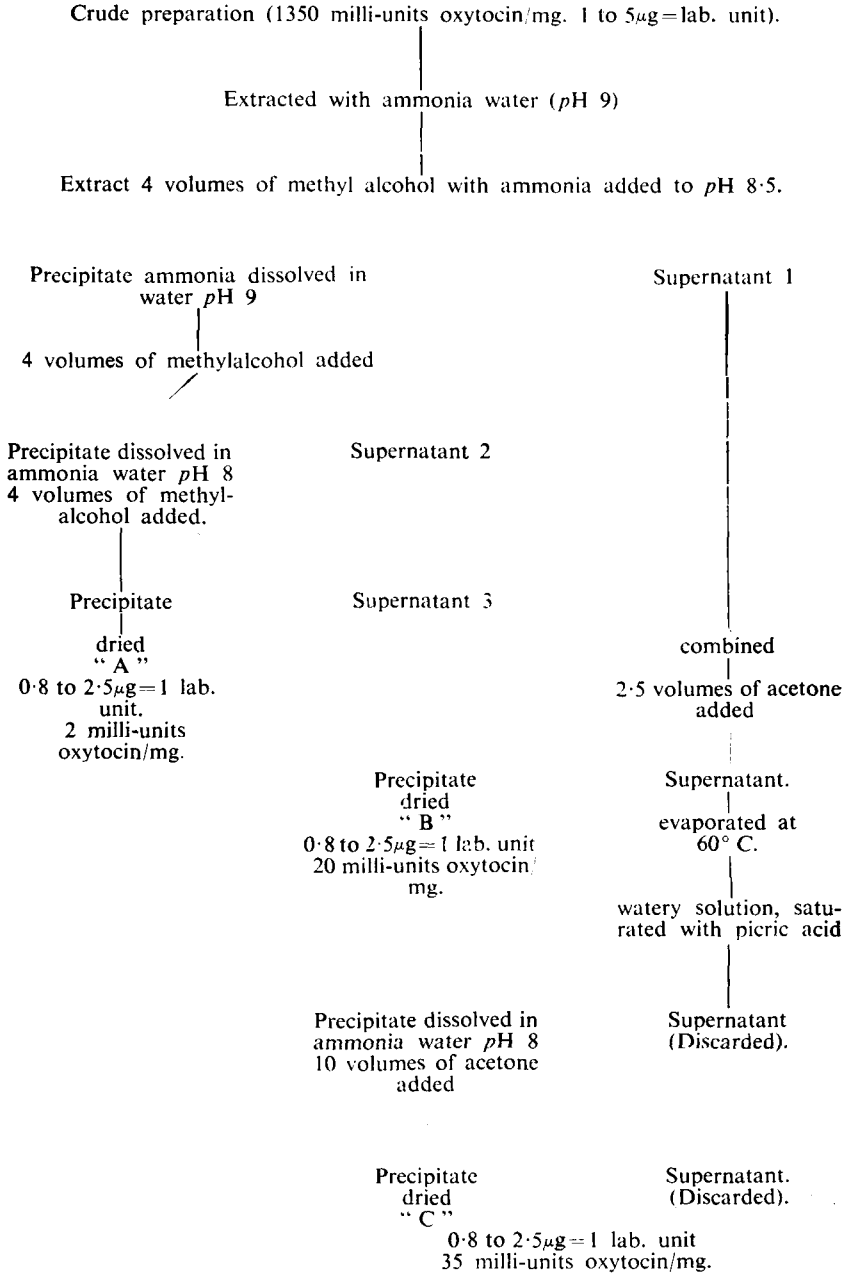
3 or 4 times. After precipitation of the bulk of the adrenocorticotrophic hormone with 95 per cent. acetone some activity still remains in solution and the hormone can be precipitated by picric acid, after evaporation of the acetone. The biological activity of this picrate is similar to that of the bulk of the preparation and constitutes about 5 per cent. of the total yield. It consists apparently of an adrenocorticotrophic hormone of smaller molecular size than is present in the 95 per cent. acetone precipitate.

Removal of the lactogenic hormone from the crude extract is best carried out in accordance with the various American methods by precipitation with sodium chloride at *p*H 3·0 (Li, Simpson and Evans[3]).

The main difficulty of the whole purification procedure, however, is the removal of the posterior lobe hormone contamination. This is done in all published methods by destruction of the posterior lobe hormone by adding large amounts of concentrated ammonia, but it is difficult to emphasise sufficiently how wasteful this method is, as not only posterior lobe hormones, but also very considerable amounts of adrenocorticotrophic hormone are destroyed by this procedure. A purification method (shown in Table II), based on the fact, found in our investigations, that the posterior lobe hormones are more soluble in alkaline methyl alcoholic solution than the adrenocorticotrophic hormone, allows not only the removal of the posterior lobe hormones, but also the separation of the total adrenocorticotrophic activity into three different hormone fractions. The crude powder is extracted with ammoniacal water and by adding methylalcohol to 80 per cent. concentration a precipitate deposits which is twice more re-precipitated and finally dried with acetone. This preparation (" A") is highly active in the ascorbic acid test and nearly completely free from posterior lobe hormones. It comprises about 25 per cent. of the total yield of the purified preparation. The combined supernatants are mixed with two and a half times their volume of acetone, whereupon the main fraction precipitates. This preparation (" B ") too exhibits only very little oxytocic activity (about 20 milli-units per mg.) and is equal in potency to the preparation " A " as shown by the adrenal ascorbic acid test. The supernatant liquid is evaporated until nearly all the acetone and methyl alcohol are removed. The watery solution is saturated with picric acid, the resulting picrate dissolved in ammoniacal water at *p*H 8 and precipitated with 10 volumes of acetone. The precipitate is then washed and dried with acetone. It has the same activity as shown by the ascorbic acid test as the " A " and " B " fractions and only contains small amounts of oxytocic activity. This last fraction constitutes at least 3 per cent. of the total yield.

It is not intended to deal here with further chemical characteristics of these apparently distinct adrenocorticotrophic hormone fractions, nor has the investigation as to the possibility of different biological actions of these fractions been completed. It is only conceivable that different molecular sizes or different sizes of protein carriers of the same active prosthetic hormone group may have been separated in the three different fractions.

TABLE II



Certain parallelisms with the relations between, for instance, thyroglobulin and thyroxine or dialysable and undialysable adrenocorticotrophic hormone are obvious. We have reported several years ago (Reiss

and Golla[4]) on the high alcohol solubility of an adrenocorticotrophic hormone fraction prepared from cattle pituitaries.

The preparation "B" has been used on our patients clinically for adrenal response tests and for treatment. Concentrated solutions can be Seitz-filtered or irradiated by 30 watt ultra-violet germicidal lamp λ 2537, without any loss of activity. The resulting sterile* solution is filled into ampoules and freeze-dried.

One of the shortcomings of clinical treatment with adrenocorticotrophic hormone is its speedy disappearance from the circulation. We have found that the heating of the powder to 180° to 200°C. does not reduce its biological activity if it is kept under a high vacuum during this procedure, but its solubility is considerably diminished, and it remains to be seen whether such powders used as implants will not make the clinical use of adrenocorticotrophic hormone more economical.

Biological Assay

The biological assay of adrenocorticotrophic hormone has been greatly simplified since the adrenal ascorbic acid depletion method of Sayers and Sayers[5] was introduced.

For the purpose of comparison in our purification attempts the dose that reduced the ascorbic acid content of the adrenal of hypophysectomised rats by 20 per cent. was taken as the laboratory unit. This laboratory unit is approximately equivalent to a 1 μg. dose of the Armour standard La-I-A and is also equivalent to about 1/30th to 1/75th of the sudanophobic repair unit (Reiss, Balint, Oestreicher and Aronson[6]: Simpson, Evans and Li[7]). The animal unit is only used for following the course of the preparation of the hormone. A final estimation of the potency of a preparation is always made by a simultaneous test of the preparation against the one which has been adopted as a temporary standard of reference (La-I-A Armour standard).

Sayers, Sayers and Woodbury's[8] arrangement of the ascorbic acid depletion method appeared to us to be the most satisfactory. We quote, however, in Table III the results of an assay which show that one is not likely to achieve the degree of precision required using the small group of animals recommended by the authors.

TABLE III

La-I-A				Unknown Preparation B			
No. of rats	Dose	Mean Resp.	S.D.	No. of rats	Dose	Mean Resp.	S.D.
14	1·0	106	27·4	14	1·0	75	22·0
11	0·25	67	20·2	11	0·25	48	24·2

* The sterility test was carried out in our Pathological Laboratory by Mr. G. Pope. The contents of ampoules chosen from each batch at random were dropped into suitable media such as peptone water or broth and examined after 24 to 72 hours.

	La-I-A	Preparation B	Combined
b	64·78	44·85	54·81
s	24·5	23·0	23·8
$\lambda = \frac{s}{b}$	0·3782	0·5128	0·4342

M = 1·53075; ratio of potency Prep. B/La-I-A = 0·3447 with Standard (La-I-A) at 100 per cent. Preparation B = 34·5 per cent. Sm = ± 0·1559; limits of error at p = 0·95 are 49·5 to 202·8 per cent. Range of potency Pre. B/La-I-A in 95 cases out of 100 is 16·78 to 68·75 per cent.

In Table III are given: the number of rats used for each dose, the dose expressed in μg./100 g. of bodyweight, the mean response to each dose (i.e., the average of the differences between right and left adrenal ascorbic acid in mg/100 g. of fresh tissue) and the standard deviation of a single observation of the response to each dose; b is the slope of the log dose response curve for each preparation and for the two combined, S is the standard deviation for each preparation (derived from both doses) and the standard deviation for both preparations. λ = an index of precision, M = the log. of the ratio of the potency of the preparation B to La-I-A, Sm = the standard error of M. The statistical analysis of the results was carried out according to the method of Irwin as given by Pugsley[9].

The slope of the log dose-response curve obtained in the standardisation is less steep than that mentioned in the example published by Sayers, Sayers and Woodbury[8] although twice the number of animals were used in the experiment described in the table. The slope described here is typical of a series of our standardisations and it remains to be seen whether an improvement of the experimental arrangement of the method or (what appears to us at the moment more probable) a considerable increase in the number of experimental animals used will improve the exactitude of the method.

Clinical Diagnostic and Therapeutic Technique

The introduction of the clinical adrenal cortex response test, as worked out by Thorn[10], Pincus[11] and others, is of great importance. Clinical treatment with adrenocorticotrophic hormone will only prove successful if it is applied in suitable selected cases. Treatment with this hormone can only be considered in patients with an adrenal cortex which responds to it. There again, it will be necessary to distinguish between patients who show a positive glucose tolerance or cold test and those who do not. Or in other words, between patients in which the pituitary is able to produce endogenous hormone and patients in whom it cannot do so. Only in those cases where the adrenal cortex can react to this hormone; but who are unable to produce enough of it themselves, is adrenocorticotrophic hormone therapy indicated.

Doses and duration of treatment must also be considered very carefully by the clinician in co-operation with the pharmacologist. Prolonged treatment with high doses of adrenocorticotrophic hormone might not only be wasteful but contra-indicated. The hormone given over a long

period may produce considerable enlargement of the suprarenal cortex, and finally an exhaustion of the hormone-producing capability of the gland. Unpleasant side effects too, have been reported which were due to the increased mobilisation of some adreno-cortical compounds, the physiological excretory balance of which was disturbed by the dose of adrenocorticotrophic hormone administered.

It still remains to be seen whether there do not exist different fractions which are responsible for the mobilisation of the different adrenocortical compounds.

In our experience the 17-ketosteroid excretion of patients can sometimes, after a few days' treatment with the high 25 mg. doses, rise to figures which have been seen by us so far only as a sequel to severe adrenal cortex hyperfunction and hyperplasia, or even adenoma. We have further seen a mental patient who improved considerably during the first seven days of treatment with the high dose, and deteriorated again when the injections were continued for a further 10 days. On the other hand, one injection of the high dose as given sometimes for the purpose of the response test can initiate an improvement which occasionally lasts for many weeks.

Future experience might still show whether prolonged treatment with small 1 or 2 mg. doses, as we carried out some years ago (Hemphill and Reiss[12]) will not prove the method of choice.

Considering in how many physiological processes the hormones of the adrenal cortex participate, it can scarcely be foreseen how manifold the indications for the use of adrenocorticotrophic hormone may be, and it is therefore imperative to start to define its pharmaco-dynamic qualities.

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