SEPARATION OF THE HORMONES OF THE POSTERIOR PITUITARY FROM A CRUDE EXTRACT BY ELECTRO-CHEMICAL MEANS

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Received February 15, 1957

Pressor and oxytocic activities were separated from a crude extract of pig posterior pituitary tissue using a continuous electrophoretic technique. Both voltage and rate of application were kept constant. The material obtained during the first 24 hours was discarded; collections could then be made for up to 7 days. With a Whatman No. 2 filter paper, a rate of feed of 3·5 ml. of crude extract in 24 hours and 400 V., pooled activities had about 5 per cent of contamination.

IN 1928 Kamm, Aldrich, Grote, Rowe and Bugber[1] separated by means of a long and tedious chemical process two of the active substances of the posterior lobe of the pituitary. These active substances were isolated and a structure suggested for them by du Vigneaud and his colleagues[2,3] and by Fromageot, Acher, Clauser and Maier-Huser[4]. Taylor, du Vigneaud and Kunkel[5] found that a mixture of purified oxytocin and vasopressin could be separated electrophoretically using glass beads as supporting medium. Since it may well be many years before the pure polypeptides are commercially available some attempt was made to separate these hormones from a crude extract by an economical and reasonably quick method on filter paper.

MATERIALS AND METHOD

The apparatus used was one for "Continuous Electrophoresis on Paper" built upon the specifications published by Holdsworth[6], except that a pump was not necessary, the buffer being fed into the apparatus from a 20 litre aspirator at a rate of 250 ml./hour and the effluent allowed to flow down the sink. The aspirator was refilled daily. The crude extract is applied to the top corner of a sheet of filter paper supported vertically and the various components move in a direction which is the resultant of two forces. The two forces are: (1) a flow of material down the paper due to gravity, and (2) an electrical pull across the paper due to an applied voltage. The supported filter paper was serrated at its lower vertical end. Under each serration was positioned a collecting tube. The buffer solution plus separated activity having vertically traversed the paper would then drip from a serration into a collecting tube, of which there were 29 in number.

The effect of temperature on separation was not analysed and for the purpose of these experiments was kept approximately constant at 20°, no cooling device being necessary in the apparatus.

Pig posterior pituitary powder, kindly supplied by Dr. Tindall of Organon Limited, was the raw material. The potency of these powders

varied between—vasopressin, 1080 and 1500 I.U./g. and oxytocin 1680 and 2180 I.U./g. A crude extract was quickly made by boiling one g. of the initial powder with 10 ml. of 0·3 per cent acetic acid for 3 minutes, cooling rapidly and filtering. The material remaining on the filter paper was then washed with a further 5 ml. of 0·3 per cent acetic acid, and the two filtrates mixed. The buffer used in the apparatus was also 0·3 per cent acetic acid.

The assay for oxytocin was that specified by the B.P. 1953 for the "rat uterus method" with the following alterations. Magnesium chloride was omitted from the saline, which was aerated and kept at a temperature of 30°. The female rat taken was in early dioestrus.

The assay of vasopressin was as specified by the B.P. 1953 for the "pressor activity method", but an anaesthetised rat of minimum weight of 300 g. was used.

RESULTS

The crude extract was fed to the top corner of the filter paper on the anode side so that the polypeptides would be drawn across the paper towards the cathode, the hormones being positively charged. Alteration of the voltage applied alters the resultant paths of polypeptides. By increasing the voltage, whilst maintaining all other factors constant, the position of collection of both hormones was moved nearer to the cathode. An increase of about 10 volts was found to move the peak of any one activity one tube nearer the cathode. For this reason, it was found necessary to stabilise the voltage, the most suitable for separation being 400 volts.

Alteration of the rate of application of the crude extract also considerably altered the position of recovery of the hormones. By increasing the rate of application, the position of recovery of the activities was found to move towards the anode. Thus it was also necessary to stabilise the rate of application by a synchronously driven constant infusion syringe. Attached to the end of this was a capillary polythene tube which led to the top corner of the filter paper.

Differing kinds of filter paper also greatly affected the position of recovery of the activities. For example with Whatman No. 1 filter paper, and a voltage of 500, current 2·2 milliamps and a rate of application of 4 ml. crude extract in 24 hours, and numbering the collection tubes from 1 to 29 in ascending order from the anode, a peak value for oxytocin activity was found in tube No. 11. But with Whatman No. 2 filter paper under the same conditions, the peak value was found in tube No. 23. For the purposes of these experiments, the filter papers were termed "fast" or "slow", depending on the rate at which the materials seeped through them. For a given set of conditions, the "slower" the filter paper, the nearer to the cathode the activities are found.

All these variables affect the positions of recovery of the activities, but not the amount and concentrations recovered. There are two factors which affect the latter, namely, the length of time of running the apparatus and the quality of the initial powder.

The apparatus was normally run for 72 hours. For the first 24 hours of collection, the concentrations of activities collected continually varied. No significant difference was then found for collection made between 24–48 hours and 48–72 hours. It was found that the better the initial sample of pituitary powder the more the concentration of any one activity at its peak, and the less the spread of the activity around the peak.

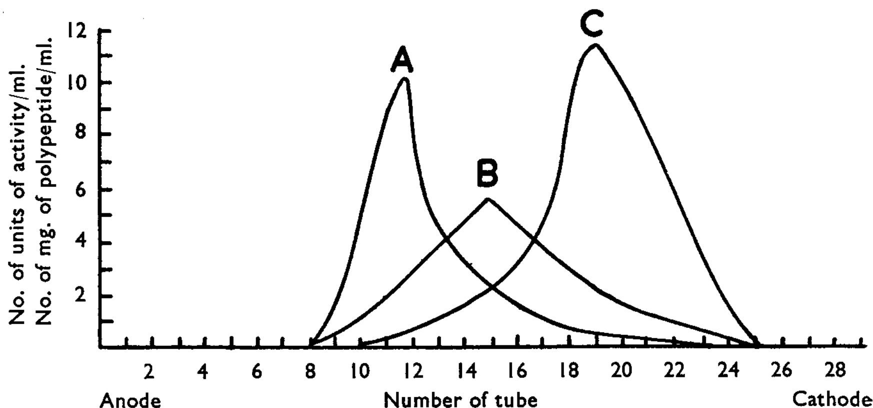

FIG. 1. Showing the distribution of activities of a typical run. A represents the distribution of oxytocic activity, C that for pressor activity and B shows the distribution of proteins and polypeptides.

Having found the most suitable conditions for separation (in this particular instance a Whatman No. 2 filter paper, 400 V., rate of application of 3·5 ml. crude extract/24 hours) the peaks of oxytocic activity and pressor activity are found. The activities in the neighbourhood of the peaks are then pooled so as to give a resulting solution of fairly concentrated activity, but lying within the B.P. limits of contamination, one with the other. For example, from the experiment recorded in Figure 1, tubes 9–12 would be taken in the collection of oxytocin. The two separate "pools" are then freeze dried. In the results obtained by us, from 2·5 ml. of such a "pool" of oxytocin, the activity recovered was about 25 I.U./mg. of freeze dried product.

DISCUSSION

At the "peak" tubes the concentration of either activity is about 10 I.U./ml.

To be economical the rate of application of extract, and hence the rate of collection of the individual activities, must be high. However, increasing the rate of application moves the separated activities towards the anode, whilst maintaining the same distance between the peaks. Thus there must be a compensatory increase of voltage to return the peaks to their original positions. But, now the two peaks move nearer to one another and the contamination of pooled activities increases as curves A and C of Figure 1 overlap. Thus there is an upper limit to the rate of application.

The overlapping of the activities can be limited in two ways. The first is by increasing the vertical length of the filter paper. Then the two directional forces mentioned above will act for a longer time on the polypeptides and hence their separation will be greater. The second is the "spread" of the activity along the tubes. This "spread" can be reduced by careful stabilisation of the voltage and rate of application.

From Figure 1 it can be seen that most of the protein and polypeptides were collected between the two activities. This suggests that the third hormone of the posterior pituitary, the melanophore-expanding hormone, might be found between the other two activities. This aspect of the work was not pursued.

It was found that after running the apparatus with the same paper for a long time, separation was adversely affected. This was probably due to the accumulation of inert material around the point of application of the crude extract.

References

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