PREPARATION OF HIGH POTENCY OXYTOCIC MATERIAL BY THE USE OF COUNTER-CURRENT DISTRIBUTION*

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The active principles of the posterior lobe of the pituitary gland have been separated and concentrated in several different laboratories by a variety of procedures. In a recent review (1) the details of many of these procedures and the potencies of the products obtained have been compared. Among the methods used for separating and concentrating the oxytocic and pressor factors, differential precipitation has been the most widely used. Electrophoresis of active material has been used effectively in concentrating the pressor factor (2), but this technique has not yet been applied to the purification of the oxytocic substance. The pressor and oxytocic factors have also been separated by chromatographic adsorption (3). Further purification (4) of the material eluted from the chromatographic column has yielded pressor and oxytocic material having a higher potency than that obtained by any of the other methods used up to that time. Although the various concentration procedures have yielded oxytocic and pressor material of quite high potency, there has been no evidence that any of the preparations have been pure.

In this Laboratory the counter-current distribution technique of Craig (5) has recently been applied to the purification of the oxytocic factor. By the use of this technique much inert material has been removed, and there is some evidence that the most potent preparations obtained by this method may be very nearly pure. Starting with material having a potency of 20 units per mg., a product having an activity of 850 units per mg. or higher has been obtained repeatedly. This activity is in the same range as that reported by Potts and Gallagher for the oxytocic material which they prepared (4), which had a potency of 700 units per mg.

Since the oxytocic hormone is soluble in 1-butanol and 2-butanol as well as in water, it is possible to distribute it between one of these alcohols and an aqueous solution. The partition coefficients of the oxytocic material between 2-butanol and various aqueous solutions were determined, and, from the solvent pairs tested, two were selected for use in the purification

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procedure described here. These solvent pairs were 0.05 per cent acetic acid-2-butanol, in which the partition coefficient of the active material was found to be 0.4, and 2-butanol-0.01 M ammonium hydroxide, in which the partition coefficient was found to be 1.8.

During the determination of the partition coefficients in various solvents, it was found that, when 2 M phosphate buffer at pH 6.8 was used as the aqueous phase, about 85 per cent of the oxytocic activity but only 50 per cent of the solids went into the 2-butanol layer. Use was made of this fact in effecting a preliminary purification of the starting material. In this way the potency was increased from 20 to around 200 oxytocic units per mg. This partially purified material was then put through several distributions in the Craig counter-current machine (5). The most potent material obtained in this manner had an activity of 865 oxytocic units per mg. when assayed by the chicken blood pressure method (6).

The close agreement of the counter-current distribution curves of weight and activity with the calculated theoretical curve for a single pure substance makes it appear either that the oxytocic material of this high potency is very nearly pure or that, if the material is contaminated, the impurity has a distribution coefficient identical with that of the oxytocic factor.

EXPERIMENTAL

Starting Material—The pitocin preparation which was used in this work possessed 20 units of oxytocic activity per mg. It was obtained in solution1 containing 5.6 mg. of dry solids per ml. The original activity of this solution in November, 1940, was 200 oxytocic2 and 4 pressor units per ml. By January, 1947, when the present research was begun, the activity had decreased to 100 units per ml.2 Assays on this material were made at intervals from January to June, 1948, with the chicken blood pressure method. The average activity was found by this method to be 120 oxytocic units per ml. or about 20 units per mg. of solids.

Partition Coefficients—The partition coefficients (K) of the oxytocic activity were determined with 2-butanol and a number of aqueous solutions. Weighed samples of pitocin (12 to 16 mg.) were equilibrated between the two solvents (3 ml. of each solvent), the layers were separated, and the activity and weight of material in each phase were determined. The results are shown in Table I.

Phosphate buffer (2 M)3 at pH 6.8 was also tried as the aqueous phase

1 We are indebted to Dr. Oliver Kamm of Parke, Davis and Company, who furnished us with this material.
2 This assay was carried out in the laboratory of Dr. Oliver Kamm of Parke, Davis and Company by the guinea pig uterine strip method.
3 Made up from 2 M NaH₂PO₄ (379 ml.) and 2 M K₂HPO₄ (621 ml.).
with both 1-butanol and 2-butanol as the organic phase. In both cases
the oxytocic activity was found to be largely insoluble in the buffer. In
the case of 1-butanol 100 per cent of the activity and 49 per cent of the
total solids were found in the alcohol layer. In the case of 2-butanol 85
per cent of the activity and 47 per cent of the solids were found in the
organic phase.

Counter-Current Distribution of Starting Material—212 mg. of dry pow-
dered material obtained by lyophilization of the Parke, Davis and Com-
pany “Pitocin” solution were distributed in a 54 tube Craig counter-current
machine between 2-butanol and 0.05 per cent acetic acid at 20° with 10
ml. of each solvent per tube. After twelve transfers had been applied,

<table>
<thead>
<tr>
<th>Aqueous phase</th>
<th>pH of aqueous phase</th>
<th>Partition coefficient (K)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% acetic acid</td>
<td>3.51</td>
<td>0.6</td>
</tr>
<tr>
<td>0.05% acetic acid</td>
<td>5.24</td>
<td>0.5</td>
</tr>
<tr>
<td>0.01 M pyridine</td>
<td>5.71</td>
<td>0.5</td>
</tr>
<tr>
<td>0.01 M ammonium hydroxide</td>
<td>7.31</td>
<td>0.5</td>
</tr>
<tr>
<td>0.05 M “ “</td>
<td>9.70</td>
<td>0.4</td>
</tr>
<tr>
<td>0.1 M “ “</td>
<td>10.01</td>
<td>0.4</td>
</tr>
<tr>
<td>0.1 M triethylamine</td>
<td>9.81</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* K, ratio amount in upper phase to amount in lower phase.
† Only the material in the butanol phase was assayed. K was calculated from
the difference between the total activity of the starting material and the activity
found in the butanol phase.

the solution in each tube was evaporated to dryness in a vacuum desiccator
at the water pump and was finally dried in vacuo over P₂O₅. The resultant
glassy product was then weighed, dissolved in 0.05 per cent acetic acid
(1 ml. per mg. of solids), and assayed. The results of this distribution are
shown in Fig. 1.

A large amount of inert material was found in Tubes 0, 1, and 2, while
the active material was found chiefly in Tubes 4 to 8. In this distribution
the activity was increased from 20 units per mg. to 188 units per mg. (in
Tube 6). The activity recovered from this distribution amounted to 82
per cent of the starting activity.

Preliminary Purification of Starting Material—A preliminary purification
procedure for obtaining material with an activity of 200 units per mg. was
devised on the basis of our early experience with the distribution of solids
and oxytocic activity between 2-butanol and 0.05 per cent acetic acid and between 2-butanol and 2 M phosphate buffer at pH 6.8.

100 ml of Parke, Davis and Company "Pitocin" solution were lyophilized to a gummy product. This material was dissolved immediately in 28 ml of 0.05 per cent acetic acid which had been previously saturated with 2-butanol. This solution was then extracted with six portions of 2-butanol (saturated with 0.05 per cent acetic acid) equal in volume to the acetic acid solution. The butanol extracts were combined and washed three times with equal volumes of 2 M phosphate buffer. The butanol solution was then dried over dry, powdered magnesium sulfate (1 gm. per 10 ml. of solution) at 5° for 3 to 18 hours. The dry butanol solution was then evaporated in vacuo in a water bath at a temperature of not over 25°, and the residue was taken up in 10 ml. of 0.05 per cent acetic acid and lyophilized.

In a typical experiment the lyophilized product was a fluffy white powder which weighed 49 mg. and had an activity of 220 oxytocic units per mg. This is a total of 10,800 units or 90 per cent of the activity present in the starting material. This procedure removed 91 per cent of the solids present in the starting material.
Preparation of Material of 600 Units per Mg. by Counter-Current Distribution of Material of 200 to 250 Units per Mg. (A) Between 2-Butanol and 0.05 Per Cent Acetic Acid—188 mg. of material were prepared from 22.4 gm. of material of 20 units per mg. by the procedure described above. This material had 250 units of oxytocic activity per mg. (total 47,000 units) and was distributed between 2-butanol and 0.05 per cent acetic acid in a 53 transfer distribution at 20°. The solutions from each tube were dried at room temperature in vacuo, weighed, and dissolved in 0.05 per cent acetic acid for assay.

The results of this distribution are plotted in Fig. 2. The peak of the activity corresponds with the peak of the major part of the solids. The material in Tube 15 was the most potent, having an activity of 575 units per mg. The total activity recovered from this distribution was 41,000 units or 87 per cent of the starting activity.

(B) Between 2-Butanol and 0.01 M Ammonium Hydroxide—234 mg. of oxytocic material having a potency of 205 units per mg. (total 48,000 units) were distributed between 2-butanol and 0.01 M ammonium hydroxide in a 53 transfer distribution at 20°. The solutions were dried and assayed in the usual way. The distribution of solids and activity are shown in Fig. 3. The peak of the activity was in Tube 32. This material had a
potency of 530 oxytocic units per mg. The total activity recovered from this distribution amounted to 40,600 units or 84 per cent of the starting activity.

Preparation of Material of 700 Units per Mg. by Counter-Current Distribution of Material of 400 Units per Mg.—The material from Tubes 9 through 20 of the 53 transfer distribution between 2-butanol and 0.05 per cent acetic acid was dried to a powder by lyophilization. The product weighed 80 mg. and contained a total of 38,600 units of oxytocic activity (483 units per mg.). This material was distributed between 2-butanol and 0.05 per cent acetic acid for 100 transfers. The solutions were dried, weighed, and assayed. The peaks of both the activity and solid curves were in Tube 30. The most active material had 700 units of oxytocic activity per mg.

A similar 100 transfer distribution was carried out on the material from Tubes 25 through 38 of the 53 transfer distribution between 2-butanol and 0.01 M ammonium hydroxide. The combined material from these tubes weighed 105 mg. and contained 39,300 units of oxytocic activity (375 units per mg.). When this material was distributed between 2-butanol and 0.05 per cent acetic acid for 100 transfers, the peak of the activity and of the solids was in Tube 26. The material in this tube had a potency of 660 units per mg.
Preparation of Material of 865 Units per Mg. by Counter-Current Distribution—157 mg. of material accumulated from several distributions, and having an oxytocic activity of 335 units per mg. (total 52,500 units), were put through a 53 transfer distribution between 2-butanol and 0.05 per cent acetic acid. Instead of being dried in the usual manner at room temperature, the butanol-acetic acid mixtures were frozen and lyophilized. The resultant white powders were stored in vacuo over P₂O₅ until they were dissolved in 0.05 per cent acetic acid and assayed. From this distribution 87 mg. of material having a potency of 550 units per mg. were obtained.

85 mg. of this 550 unit material were distributed again between 2-butanol and 0.05 per cent acetic acid in a 53 transfer distribution. The distribution of solids and activity are shown in Fig. 4. The material at the peak of the curve (Tube 16) had a potency of 865 units of oxytocic activity per mg.

Ultraviolet Absorption Spectrum—The ultraviolet absorption spectrum of a solution of purified oxytocic material in 0.05 per cent acetic acid is shown in Fig. 5. Strong end-absorption was found below 240 μ and there was a small peak at 275 μ. This peak coincides with the absorption peak of tyrosine and is probably due to tyrosine present in the material (4).

Assay Procedure—The oxytocic material was assayed by a modification of the method of Coon (6). Chickens weighing about 2 kilos were anesthetized with sodium phenobarbital intramuscularly. Injection of oxytocic material was made through a Kaliski needle fastened into one of the large
wing veins. The blood pressure recordings were made with a mercury manometer, the cannula being inserted into a wing artery and connected to the manometer with a rubber tube containing 0.9 per cent sodium chloride. Heparin was used in the saline solution to prevent clotting. A standard solution of oxytocic material was prepared from U. S. P. posterior pituitary reference standard and was made up according to U. S. P. XII directions (7). Injections of this standard solution, which contained 0.5 oxytocic unit per ml., were alternated with injections of unknown oxytocic solutions. The unknown solutions were diluted serially until they contained approximately 0.5 oxytocic unit per ml.

The authors are indebted to Miss Janet Gruschow for assistance with the assays.

SUMMARY

The counter-current distribution principle of Craig has been applied to the purification of the oxytocic fraction of the posterior pituitary gland.  

![Ultraviolet absorption spectrum of purified oxytocic material](http://www.jbc.org/)

**Fig. 5.** Ultraviolet absorption spectrum of purified oxytocic material
By the use of this procedure, material having a potency of 865 units of oxytocic activity per mg. has been obtained. The characteristics of the distribution curve of this high potency material suggest either that this material is very nearly pure or that, if any impurity is present, it has a distribution coefficient almost identical with that of the oxytocic factor.

BIBLIOGRAPHY

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