

Chromatography of Secretin on Carboxymethyl Cellulose

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In 1957 Ward and Guillemin¹ described a method for the purification of vasopressin in which the hormone is adsorbed on carboxymethyl cellulose from a solution of the crude material in 0.02 M ammonium acetate and selectively eluted by developing the column with a gradient to an ammonium acetate solution of higher concentration and pH. We decided to try a similar technique for the purification of secretin. Carboxymethyl cellulose has been used for the purification of secretin by Newton, Love, Heatley and Abraham² who adsorbed the activity from crude preparations on columns of carboxymethyl cellulose buffered with barium acetate at pH 4, eluted impurities with 0.02 N acetic acid and then a highly purified secretin with 0.1 N acetic acid.

We found, working with highly purified preparations, that secretin may be adsorbed on carboxymethyl cellulose* from solution in 0.02 M ammonium bicarbonate and eluted by increasing the concentration of the latter to 0.04 M. Further work showed,

however, that it was unnecessary to change the concentration of the eluting solution. If elution was continued for a sufficiently long time with the 0.02 M bicarbonate the secretin emerged from the column in good yield and in a high state of purity. Large quantities of impurities were both eluted from the column before and left on it after the secretin. The impurities left on the column could be eluted by increasing the concentration of the ammonium bicarbonate to 0.2 M.

On lyophilizing the most active fractions of the eluted material with 150 000 Hammarsten cat units** per mg has been obtained. The purity of the preparations is thus at least as good as that of preparations recently obtained on electrophoretic fractionation of highly purified material³.

Experimental. Starting material. Crude secretin was prepared as described by Jorpes and Mutt⁵ in 1956. For preliminary purification of this the technique outlined by Mutt³ was followed until the extraction of the activity into methanol. The methanolic solution

* Prepared according to Peterson and Sober⁴, obtained from the Brown company, New Hampshire, USA.

** One Hammarsten cat unit (HCU) is that amount of secretin activity which on intravenous injection stimulates the pancreas of the cat to the secretion of 0.1 ml 0.1 N alkali.

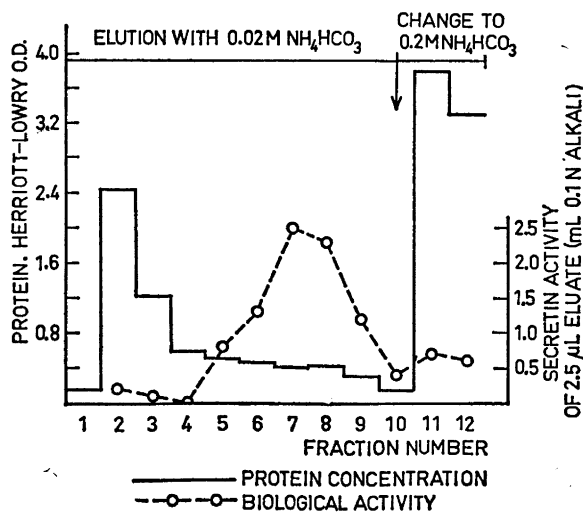


Fig. 1.

was brought to pH 7.5 with NaOH (glass electrode). Precipitated impurities were filtered off and discarded. The clear filtrate was acidified to pH 5.5 with HCl and the secretin precipitated with ether. After reprecipitation from water with sodium chloride at saturation it was collected on a suction filter and sucked as dry as possible. In repeated preparations the moist material had an activity of about 7 000 HCU per mg. This material was dissolved in water and the pH of the solution brought to 7.2. Three volumes of acetone were then added to the solution. The precipitate that formed was collected by centrifugation and discarded. The secretin was thrown out of solution by adding nine more volumes of acetone to the clear supernatant. The precipitate was collected by centrifugation and dissolved in water. The pH of the solution was adjusted to 5.5 with acetic acid and the secretin lyophilized from it. The lyophilized material had an activity varying in different preparations from 10 000 to 15 000 HCU per mg. The yield was 0.05–0.1 mg per hog.

Chromatography. 45 mg of a preparation with 12 000 HCU per mg was dissolved in 9 ml of 0.02 M ammonium bicarbonate and the solution allowed to sink into a column (7.5 × 1.4 cm) of 3 g of carboxymethyl cellulose which had been equilibrated with 0.02 M ammonium bicarbonate. Elution was carried out with 0.02 M ammonium bicarbonate. Fractions of 10 ml were collected. The flow rate was 40 ml/h. Collection of the fractions was started from the time when the secretin solution was applied to the column. Between the tenth and the eleventh fraction the eluting solution was changed to 0.2 M ammonium bicarbonate.

The approximate relative concentration of proteinaceous material in the fractions was determined by the Herriott reaction⁶ as adapted by Lowry *et al.*⁷, except that the copper salt containing stock solution of the latter authors was made up in 0.1 N NaOH instead of in water. The secretin activity was determined by injecting appropriate aliquots of the fractions intravenously into cats and titrating the amount of alkali secreted through a cannula in the main pancreatic duct in response to the injection.

The results are shown in Fig. 1.

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Polymorphism in Calcium Tantalum(V) Oxide CaTa_2O_6

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It was recently reported by Gasperin¹ that an equimolecular mixture of tantalum(V)oxide and calcium carbonate upon heating at about 1 700°C gives a product possessing the composition CaTaO_3 with a multiple-cell, deformed perovskite structure, *a* of the pseudocubic subcell unit being 3.88 Å. The formation of the new compound should evidently be accompanied by a reduction of tantalum to the tetravalent state. Such a process however, seems rather unlikely in view of the high reluctance to reduction shown by tantalum(V)oxide and of the white colour of the specimen which rather suggests the presence of the state of maximum valency. The density reported for the product (7 g.cm⁻³) likewise is not in good agreement with that calculated for the formula CaTaO_3 (7.6 g.cm⁻³) but is in fair accordance with that of a compound such as $\text{Ca}_{0.5}\text{TaVO}_3$ (7.1 g.cm⁻³). The validity of the latter formula was actually demonstrated by Gasperin in a subsequent analytical investigation of the