decreases the absorbance of the effluent to less than 0.2 after a large peak with no polyphenol oxidase activity is eluted with the first 50 ml. Most of the activity (up to 50 %) is eluted with 0.080 *M* phosphate buffer, pH 8, as a colorless peak which trails off after the first 50 ml. A small additional elution is obtained with 0.10 *M* phosphate buffer, pH 8.

Step 7. The fractions containing a specific activity greater than 2000 units/ml/absorbance at 280 mμ are pooled and treated with $(NH_4)_2SO_4$, discarding the fraction insoluble at 35 % saturation and saving that insoluble at 50 % saturation. The solid is taken up in about 1.5 ml water and dialysed free of $(NH_4)_2SO_4$ against 0.005 *M* Na_2HPO_4. This solution may be used directly for studies in the ultracentrifuge.

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It has been subsequently observed that extremely slow elution can result in polyphenol oxidase preparations of even higher specific activity than reported here. A further complication arises from the finding of an inhibitor of this enzyme which accompanies it and appears in the 0.04 *M* phosphate eluate on chromatography (E. FRIEDEN AND Y. KARKHANIS, unpublished data).

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A method of desalting certain polypeptides

Corticotropin is an example of a polypeptide that has given difficulty in desalting because it can pass through cellophan membranes on dialysis. Extraction into organic solvents, precipitation, dialysis under special conditions and adsorption from solution have all been used to recover it in a salt-free state from buffer solutions, but each procedure has disadvantages and most are laborious. DIXON AND STACK-DUNNE[1] used the adsorption of corticotropin onto a carboxylic resin, but they displaced the corticotropin with alkali (ammonia) which converted some of the corticotropin A_1

to A_2. OTSUKA AND KIMURA[2] found, however, that acetic acid solutions eluted ox corticotropin from a carboxylic resin. The present method is based on this fact.

The corticotropin solution to be desalted is acidified with acetic acid and an excess added to give a concentration of about 5 % acetic acid. The solution is then allowed to run through a column 3 cm high, 2 cm diameter of a ground form of the carboxylic resin Amberlite IRC-50 (XE-64) as the free acid. A flow rate of 180 ml/h and a volume of 180 ml may be allowed on a column of this size. The column is then washed with dilute acetic acid until the effluent is salt-free. If Na^+ is the only cation present, 0.1 *N* acetic acid may be used, but if, for example, divalent cations of ethylenediamine are present, 5 % acetic acid should be used to elute them. When the effluent is salt-free, the corticotropin may be displaced by a wash of 35 ml 50 % (v/v) acetic acid. All acetic acid solutions to be added to the column should be shaken for a few seconds under reduced pressure beforehand, so that dissolved air will not come out of solution during passage through the column and block the flow. The solution obtained in acetic acid may be diluted and dried while frozen, or if the amount of corticotropin present is small, the solution may first be concentrated in a rotatory evaporator under reduced pressure. This avoids the risk that some of the polypeptide will be lost by being carried away in the stream of vapour, which may occur when very dilute solutions are dried while frozen. The liquid should not be allowed to rise above 40° during rotatory evaporation.

Rechromatography of material isolated in this way (Fig. 1) showed that most of it was chromatographically unchanged and the yield was almost quantitative. The absorption at 280 mμ showed 102 % recovery on the desalting step, and 83 % recovery as the main peak on rechromatography. Of the absorption at 280 mμ in the effluent of the rechromatogram, 95 % was in the unchanged peak.

This method of desalting is also suitable for both pig melanocyte-stimulating-hormones (α and β). It has also been applied to egg-white lysozyme, which also presents some difficulty in desalting[3]. A sample (Armour & Co., batch no. 20793) was chromatographed under conditions similar to those of TALLAN AND STEIN[3] (Fig. 2a) and the peak material collected. It was desalted by the above method and rechromatographed (Figs. 2b and 2c). The yield was at least 80 % and of the absorption at 280 mμ recovered from the rechromatograms, at least 87 % was chromatographically unchanged from the original lysozyme peak.

The capacity of the resin has not been fully investigated. In one experiment a column of the size described held 190 mg corticotropin A_1 without any loss. But on another occasion when a crude preparation of β melanocyte-stimulating hormone was put through a column of 2 cm × 2 cm, the column became saturated so that hormone activity was present in the effluent even though the column held only 100 mg material. The material in the effluent was adsorbed by a further column; the melanocyte-stimulating hormone had therefore not passed through the first column by forming a loose compound with unadsorbable material. It is probable that the capacity of the resin differs for different polypeptides.

The method may be applicable to other polypeptides. They do not need to have a basic isoelectric point, since β melanocyte-stimulating hormone has one of under 6, and they do not need to have many positively charged groups, since α melanocyte-stimulating hormone has only three. It may, however, be expected that less basic peptides will not be so strongly adsorbed by the resin. It seems, however, that poly-

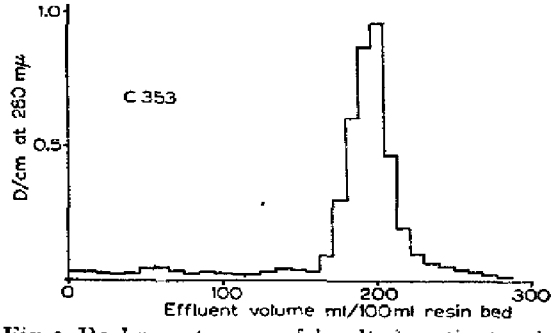

Fig. 1. Rechromatogram of desalted corticotropin A_1. Column of 25.6 cm × 2.15 cm Amberlite IRC-50 (XE-64), equilibrated with and developed with a buffer solution of 0.1 *M* Na_2HPO_4, 0.1 *M* NaH_2PO_4 and 0.25% chlorbutol. This system is similar to that of DIXON AND STACK-DUNNE[1]. The sample was the product of desalting a corticotropin A_1 peak whose absorption at 280 mμ was 34 units (D/cm × vol. in ml) (approx. 21 mg), dissolved in 2.6 ml 0.4 *M* H_3PO_4. To this there were added 2.0 ml 0.6 *N* NaOH and 1.1 ml of the buffer and of this 4.96 ml were transferred to the column.

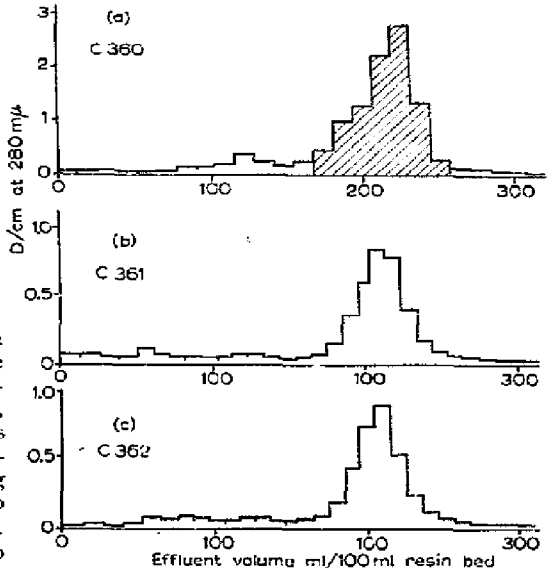

Fig. 2.

Fig. 2. Chromatogram of lysozyme and rechromatograms after desalting. The column used for each chromatogram was 28.7 cm × 1.1 cm of Amberlite IRC-50 (XE-64) in equilibrium with and developed with a buffer solution of 0.15 *M* Na_2HPO_4, 0.05 *M* NaH_2PO_4 and 0.025% toluene. This system is similar to that used by TALLAN AND STEIN[3]. (a) Sample of 18.7 mg egg-white lysozyme (Armour & Co) applied to the column in 1.04 ml buffer. (b) Rechromatogram of $^2/_5$ of the marked region of chromatogram (a) after desalting. The yield on desalting was 92% (by absorption at 280 mμ) and of the material applied 80% was recovered of which 95% was in the main peak. (c) Rechromatogram of $^1/_2$ the marked region of chromatogram (a) after separate desalting. The yield on desalting was 81% (by absorption at 280 mμ) and of the material applied 83% was recovered of which 87% was in the main peak.

peptides of greatly different properties may be desalted by adsorbing them from solution onto a carboxylic resin, eluting the salts with dilute acetic acid and finally eluting the polypeptides with a strong solution of acetic acid.

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