Die Nachweisgrenze für Phosphinoxyde liegt bei 1 γ. Da viele der untersuchten Substanzen[1] auch bei einer Auftragung von 500 γ nach der Entwicklung keine "Schwanzbildung" zeigen, kann man bei genügend unterschiedlichen R_F-Werten Verunreinigungen von 1 % noch gut erkennen. In Tabelle I werden einige R_F-Werte verschiedener Substanzgruppen angegeben (zur Anfärbung Permanganat–Schwefelsäure).

Da die Permanganat–Schwefelsäure sogar Vaseline oxydiert, kann die beschriebene Methode zur Untersuchung auch von Kohlenwasserstoffen aller Art Anwendung finden.

Eine weitere sehr empfindliche Reaktion auf bestimmte organische Verbindungen, die dreiwertiges Eisen zu zweiwertigem reduzieren (z.B. Phenole), gründet sich auf den Nachweis von Eisen(II) als Berliner Blau. Man besprüht die entwickelte Platte mit 5–10 % $FeCl_3$-Lösung, dann mit 5 % Kaliumcyanoferrat(III)-Lösung; die Substanzflecken färben sich tiefblau.

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The chromatography of barley ribonuclease

Ribonucleases with limited specificity may prove highly valuable in elucidating the structure of ribonucleic acids. Purity, however, is almost an absolute prerequisite for such a purpose.

Several proteins with a slightly basic character have successfully been chromatographed on an Amberlite XE-64 column using elution analysis, mostly in the presence of phosphate ions. We have been able to purify considerably a ribonuclease from barley (B.RNase) with a similar system, but avoiding the interaction of a polyvalent anion, which tends to increase the negative net charge of a protein.

The cation exchanger is purified according to HIRS[1]. 0.2 *M* cacodylate, adjusted to pH 6.00 with NaOH and to $\Gamma/2$ 0.2 with NaCl, is used for equilibrating the column and the enzyme solution, and for elution. The column is loaded with 1 ml of an enzyme solution partially purified by $(NH_4)_2SO_4$-fractionation[2] and containing approximately 8 mg protein.

It is seen that the RNase-activity is eluted in two peaks (Fig. 1), named succes-

sively B.RNase B and B.RNase A, in analogy with the pancreatic enzymes. B.RNase B moves only slightly behind the solvent front, and is not separated from the non-specific phosphatase, the phosphodiesterase, the 3'-nucleotidase and the bulk of the

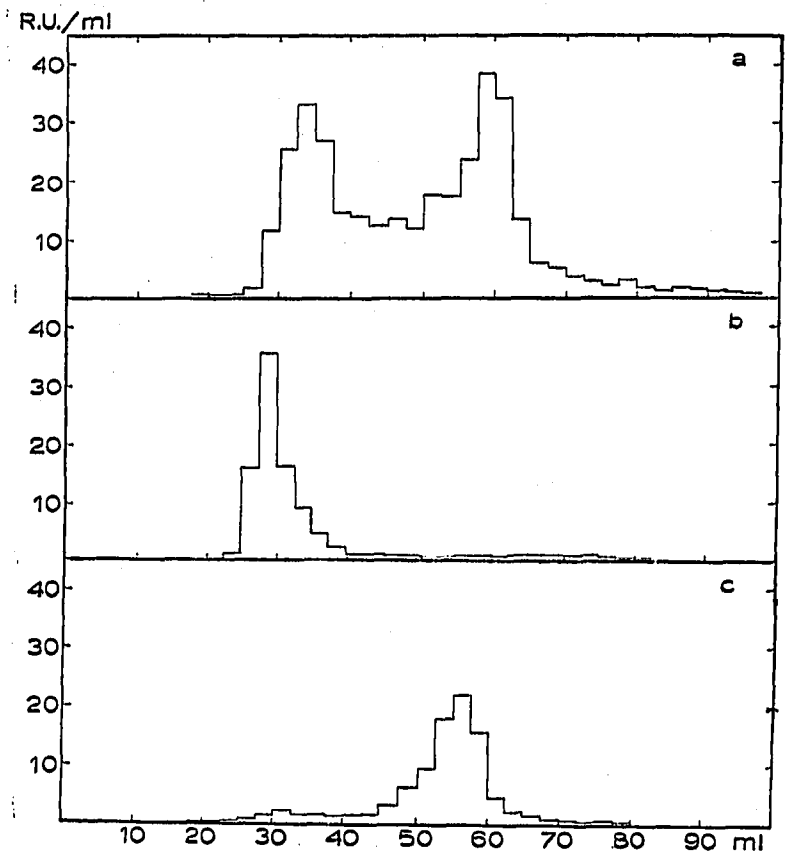

Fig. 1. Chromatography of B.RNase on a 1.07 × 50 cm Amberlite XE-64 column. Ordinate: RNase units/ml. Fraction volume 2.5 ml; flow rate 1 ml/h; temp. 3°. (a) Partially purified B.RNase; (b) rechromatography of B.NRase B; (c) rechromatography of B.RNase A.

proteins brought on to the column. B.RNase A on the other hand emerges after approximately three hold-up volumes, and contains usually more than 50% of the recovered enzymic activity. Considering the high selectivity of this chromatographic procedure, the latter enzyme preparation is probably nearly pure. The protein content of these fractions is below the detection level. The over-all purification obtained is at least 70 if based on the colorimetrically determined protein content, or 175 if based on the absorbancy at 280 mμ.

Increasing the pH to 6.5 or the $\Gamma/2$ to 0.3 results in loss of resolution. The recovery is very low if the $\Gamma/2$ is decreased to 0.082 (same buffer, but NaCl omitted). The sodium cacodylate buffer cannot be replaced by an ammonium acetate buffer of equal pH and $\Gamma/2$.

The total recovery of enzymic activity ranges from 55 to 77%, the remaining being irreversibly lost. The activity in the eluates slowly disappears, at least partly because of surface adsorption. Triton X-100 is added to the pooled eluate, corresponding to each RNase-fraction, to a final concentration of 0.005%. The solution is dia-

lyzed against dilute $(NH_4)HCO_3$-buffer pH 7.0 and lyophilized. The yield of several chromatographic separations is combined, taken up in cacodylate buffer pH 6.00 and rechromatographed. Both B.RNase B and B.RNase A move as a single component (Fig. 1), indicating that each enzyme really corresponds to a different protein entity. Both peaks, however, emerge slightly ahead of their respective positions in the original chromatography; this effect is probably due to the high viscosity of the initial enzyme solution.

The RNase-activity in the eluate between the two B.RNase-peaks does not decrease to zero, even when the column length is increased. Tailing of B.RNase B is unlikely to be the cause, as the other enzymes assayed as well as the RNases upon rechromatography emerge in nearly symmetrical peaks. As yet not enough material from this intermediate region could be collected to allow rechromatography.

The separation of two active B.RNases is similar to several reported heterogeneities of enzymic activity, mostly demonstrated by means of elution analysis. Chemical and enzymological studies indicate that B.RNase B and A are closely related. Moreover, the chromatographic behaviour is not changed upon addition of 0.01 *M* thioglycol to the eluent. The proteolytic activity in the extract is negligibly small. Hence the most likely explanation for the observed heterogeneity is that B.RNase A contains one or a few more amide functions than B.RNase B, although the formation of a strongly bound complex is not excluded.

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"Window" origin spots in paper electrophoresis and chromatography

In this communication a method is described for preparing nearly identical origin spots for use in paper electrophoresis or chromatography. The difficulty of obtaining nearly identical spots by the conventional method adversely affects separation and quantitative results, where the latter are required.

In the following method the origin positions are marked by a pencil dot, and a