fibrin more susceptible to plasmin attack. These differences may be due to the removal of inhibitors to plasmin or plasminogen activators during the purification of the commercial fibrinogen. Alternatively, the bovine fibrinogen may have become slightly denatured during the purification process resulting in a fibrin more sensitive to plasmin digestion.

In addition to the estimation of bacterial activators and crude and purified urokinase preparations, the use of plasminogen-free fibrinogen in the quantitative fibrinolytic method now enables plasmin to be estimated, even though the plasmin preparation may be contaminated with plasminogen activators.

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[1] A. J. ANDERSON, *Biochim. Biophys. Acta*, 57 (1962) 154.
[2] F. M. DAVIDSON, *Nature*, 185 (1960) 626.
[3] A. J. ANDERSON, *Biochem. J.*, 83 (1962) 7P.
[4] A. J. ANDERSON AND N. F. MACLAGAN, *Biochem. J.*, 59 (1955) 638.
[5] B. KICKHÖFEN, F. E. STRUWE, B. BRAMESFELD AND O. WESTPHAL, *Biochem. Z.*, 330 (1958) 467.
[6] A. J. ANDERSON, unpublished results.
[7] M. W. MOSESSON, *Biochim. Biophys. Acta*, 57 (1962) 204.
[8] F. M. DAVIDSON, *Biochem. J.*, 76 (1960) 56.

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SC 2266

Chromatographic separation of a hind-brain inducing substance into mesodermal and neural-inducing subfractions

Starting with 9-day-old chick embryos we isolated protein and nucleoprotein fractions which cause the competent undetermined ectoderm of *Triturus alpestris* gastrulae to form regionally specific organ complexes. One of them induces chiefly spinocaudal structures (notochord, muscle, kidney and neural tube), while others induce preferentially deuterencephalic structures such as hind-brain and ear vesicles[1].

By further purification we obtained from the spinocaudal-inducing fraction a purely "mesodermal"-inducing protein. The inductions evoked by this protein consist of muscle, notochord and pronephros and not any neural tube nor any other neural tissue. It was concluded that spinocaudal inductions arise from cooperation of a mesodermal-inducing factor and a small amount of a neural-inducing factor[2].

The aim of the following investigations was to test whether the deuterencephalic-inducing fractions also consist of more than one factor. In the latter case it should be possible to separate the complex into at least two factors or groups of factors which induce different organs.

Whole 9-day-old chick embryos (the yolk sac was not included) were homogenized and extracted 3 times with 0.05 M pyrophosphate buffer (pH 9.5). Solid $(NH_4)_2SO_4$ was added to 0.4 saturation and the pH adjusted to 7.4 thereafter. The precipitate was again extracted with pyrophosphate buffer (pH 9.5). After dialysis against 0.066 M KCl plus 0.01 M Na_2HPO_4 (pH 8.6) 0.2 M NaH_2PO_4 was added to pH 6.8; then a large part of the ribonucleoproteins was precipitated with streptomycin sulphate (0.02 M final concentration). The precipitate was washed and then extracted with 0.05 M pyrophosphate (pH 8.4), and KCl (2 M final concentration) was added to the extract. The precipitate formed after 5 h contains the main part of the spinocaudal activity still present. The chiefly deuterencephalic-inducing supernatant is dialyzed against 0.01 M Na_2HPO_4 (pH 8.8) for 36 h, adsorbed on a DEAE-cellulose column and then eluted stepwise at increasing pH and increasing ionic strength. All procedures were performed at 0–2°. An example of such a chromatography is shown in Fig. 1 as well as the inducing capacity of the different fractions. As already mentioned the stock fraction before chromatography (E 310/6) has a high inductive ability and causes the formation of mainly deuterencephalic complexes (hind brain, ear vesicles, head muscle and small pieces of notochord), and, to a lesser extent, spinocaudal structures also. At 0.1 M NaCl plus 0.1 M phosphate a fraction is eluted which induces almost pure spinocaudal complexes (310/15, Fig. 1), and at 0.5 M NaCl plus 0.1 M phosphate a preponderantly archencephalic-inducing fraction with only a small deuterencephalic activity was obtained (310/17, Fig. 1). The latter fraction consists of a ribonucleoprotein and free RNA. In separate experiments, the

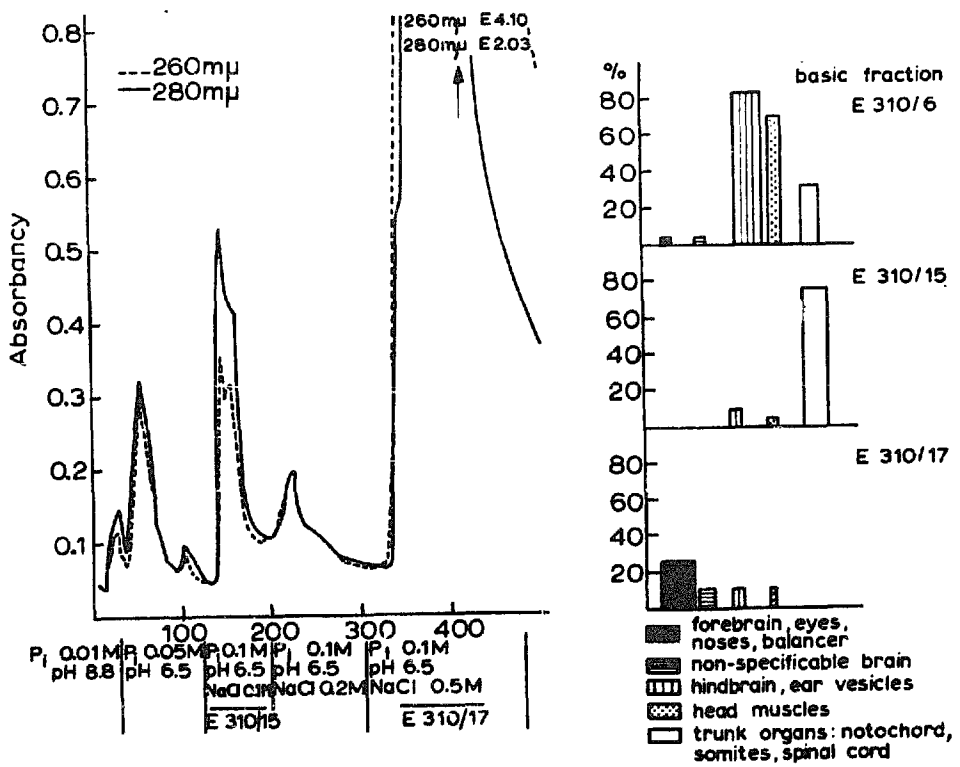

Fig. 1. Chromatography of fraction E 310/6 (65 mg protein) on DEAE-cellulose (3 g) at 0–2°. For testing, the fractions were pooled as indicated. After adding 3 mg γ-globulin, the solutions were dialyzed against distilled water, freeze-dried, and tested by the "Einsteckmethode"[5] in *Triturus alpestris*. The inducing capacity (percentage of positive cases) of the fractions is shown at the right side of the diagramm. The average size of the different regional inductions is indicated by the width of the columns.

ribonucleoprotein possessed the entire neural-inducing capacity, whereas RNA had only a very weak activity.

In a second series of experiments a deuterencephalic-inducing extract was prepared from ribosomes obtained from 9-day-old chick embryos[3]. The ribosomes* were prepared with deoxycholate in phosphate–$MgCl_2$ buffer, washed once, and then extracted with pyrophosphat–deoxycholate. The extract was dialyzed for 36 h against 0.01 M phosphate (pH 8.8), and adsorbed on DEAE-cellulose. The chromatogram and the inducing ability of the different fractions are shown in Fig. 2. The stock fraction (E 314/5) induces chiefly deuterencephalic, besides showing a small archencephalic, but no spinocaudal effect. At 0.05 M phosphate, a spinocaudal-deuterencephalic inducing fraction (E 314/8) and at 0.1 M phosphate plus 0.5 M NaCl a chiefly archencephalic-inducing fraction (E 314/11) is eluted. The samples eluted at 0.1 M phosphate and 0.1 M phosphate plus 0.2 M NaCl are not very active and have no particular regional specificity.

The chemical properties of the neural archencephalic-inducing ribonucleoproteid fractions have to be further investigated. Degradation of the RNA fraction to oligonucleotides with pancreas RNAase does not inactivate the fraction.

The experiments show that mainly deuterencephalic-inducing fractions can be partially separated into spinocaudal mesodermal-inducing ones containing the above-mentioned mesodermal-inducing factor, and into archencephalic neural-inducing fractions. The latter contain a neural-inducing factor.

There may be different reasons why the deuterencephalic-inducing fractions are

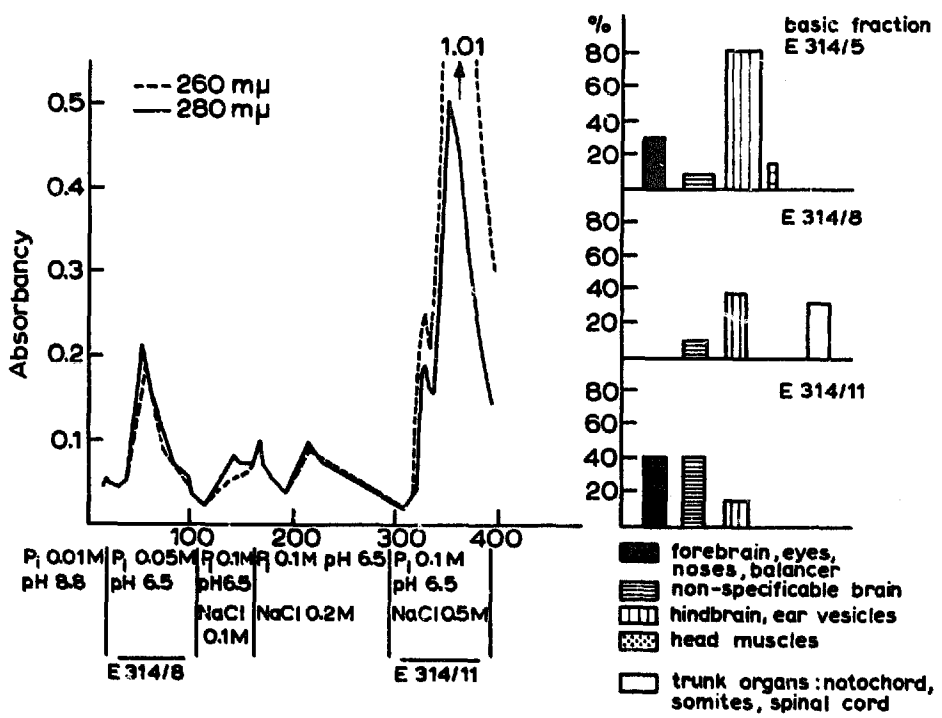

Fig. 2. Chromatography of fraction E 314/5 (35 mg protein) on DEAE-cellulose (3 g). For testing the fractions see Fig. 1.

* It depends on the method of preparation of the ribosomes (pH and ionic strength of buffer, deoxycholate concentration) whether they induce mainly deuterencephalic or rather archencephalic.

not completely separated. Firstly it may prove impossible to obtain a complete separation by a single chromatographic fractionation. Furthermore, the active principles in the different organs of 9-day-old chick embryos could behave somewhat differently in chromatography, and overlap partially.

The results suggest complex hind-head inductions to arise from the cooperation of the neural- and the mesodermal-inducing factors. SAXÉN AND TOIVONEN[4] came to a similar conclusion by testing heated (archencephalic-inducing) and non-heated (spinocaudal-inducing) HeLa cells together. Combination experiments with mesodermal and archencephalic neural-inducing fractions have furnished new arguments for this hypothesis*.

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[1] H. TIEDEMANN, K. KESSELRING, U. BECKER AND H. TIEDEMANN, *Biochim. Biophys. Acta*, 49 (1961) 603.
[2] H. TIEDEMANN, *Naturwissenschaften*, 46 (1959) 613.
[3] H. TIEDEMANN, K. KESSELRING, U. BECKER AND HILDEGARD TIEDEMANN, *Develop. Biol.*, 4 (1962) 214.
[4] L. SAXÈN AND S. TOIVONEN, *J. Embryol. Exptl. Morphol.*, 9 (1961) 514.
[5] O. MANGOLD, *Arch. mikroskop. Anat.*, 100 (1923) 198.

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Gas-chromatographic characterization of steroid ketones as *N,N*-dimethylhydrazones

Gas-chromatographic techniques have made it possible to separate microgram or submicrogram amounts of many steroids for purposes of identification and estimation. The identification or recognition of functional groups may be made by the determination of "steroid numbers"[1] (summations of values determined by the carbon content of the steroid nucleus and values characteristic of the functional groups of the steroid), by the determination of "*T* values"[2] (based on changes in retention-time behavior observed with changes in the liquid phase) and by the use of ΔR_{Mg} values[3] (by analogy with paper partition chromatography). It is equally possible to characterize or detect functional groups through the use of reagents which alter the gas-chromatographic properties of the compounds under study through derivative formation. This is not different in principle from the use of classical functional-group reagents to detect or identify specific functional groups, but the requirements for the reaction products are different. Classical techniques usually provide relatively insoluble crystalline

Abbreviations: SE-30, methyl silicone polymer; QF-1, fluorinated alkyl silicone polymer (10000 cs); CNSi, cyanoethylmethylsilicone polymer (65 mole % cyanoethyl).