Isolation of a melanophore-stimulating peptide from pig pituitary gland

The melanophore expanding activity present in the pituitary of vertebrates has received increasing interest during recent years, which in part has been stimulated by a presumed relationship between this principle and the adrenocorticotrophic hormone. Investigations so far reported have been carried out with relatively impure preparations. Recently, however, LERNER AND LEE[1] claim to have obtained an electrophoretically homogeneous substance possessing a very high potency. By a milder procedure than that used by these authors, we have isolated a peptide which differs substantially from theirs[3] and which has a lower molecular weight.

Acetone-dried pig posterior lobe powder, kindly supplied by Dr. J. LENS, Organon, Holland, was purified according to the method of LANDGREBE AND MITCHELL[2]. The initial material was of relatively low potency, containing about 1.5 "International" units/mg when tested in *Xenopus laevis* and the method yielded a preparation containing 300–350 "I.U."/mg. This preparation was fractionated by zone-electrophoresis in a vertical column using the apparatus described by one of us[4]. The tube (45 × 3 cm) was packed with cellulose powder according to the directions given by FLODIN AND KUPKE[5]. Pyridinium acetate solution was chosen as the conducting medium in order to easily recover the fractionated material in a salt-free condition. Very satisfactory fractionation of 57 mg crude material was obtained by using 0.1 molar pyridinium acetate (pH 4.8). A current of 30 mA was passed through the column for 60 hours with 175 V applied across the electrodes.

No loss of activity was observed but a considerable amount of inactive material left the column in both directions. The active substance together with some inert material was removed from the column and collected in 4 ml portions. The distribution of solid matter was estimated using the ninhydrin reaction and the portions were pooled into larger fractions as indicated in Fig. 1. In Fraction II, 17 mg was recovered containing 80% of the total amount of activity applied to the column (approximately 900 I.U./mg). This fraction was subjected to a new run under the same conditions as before except for longer duration (100 hours). A sharp peak containing all the activity but only 65% of the solid matter was obtained. The distribution of material within the peak was perfectly symmetrical according to ninhydrin, ultraviolet absorption (of pyridin-free fractions) at 240 mμ and 280 mμ as well as amino acid distribution. This product contained 1500 "I.U."/mg and is about 1000 times as active as the original pig posterior lobe powder. By proper packing of the column, a sharper separation was achieved with this type of long duration zone-electrophoresis than with electrophoresis on paper. In fact, substances which cannot be distinguished by their electrophoretic behaviour on paper can often be fractionated on satisfactorily prepared columns.

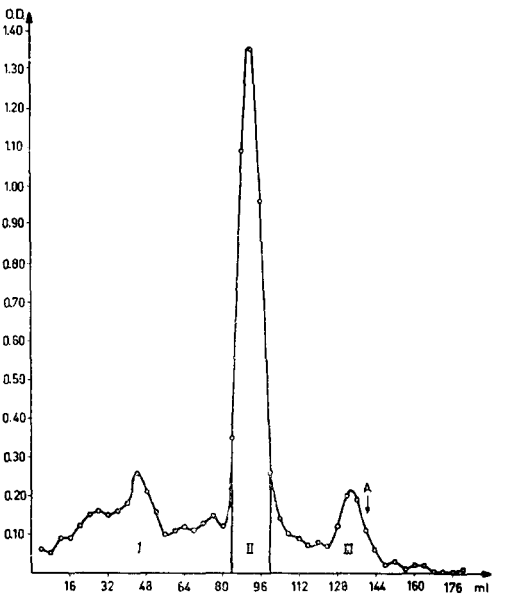

Fig. 1. Distribution of ninhydrin positive material obtained in an electrophoretic run on column as described in the text. Ordinate: Optical density at 570 mμ. Abscissa: Volume of displaced solution from the column. The anode-direction corresponds to increasing displacement volumes. The arrow at A indicates the volume of fluid between the filter plate at the bottom and the starting position of the original zone.

The most active preparation which apparently is a peptide was shown to be electrophoretically homogeneous at pH 3.5 (pyridinium formate 0.1 molar with respect to formic acid) and at 7.0 (sodium phosphate u 0.2). Due to the instability of the activity in alkaline solution, no run was performed at a higher pH than 7.0. The isoelectric point of the peptide in 0.05 molar phosphate[6] solution was found to be 5.2 as determined by a procedure similar to that by KUNKEL AND TISELIUS but using glucose and proline as reference substances to determine the electroosmotic flow.

An approximate molecular weight of 3000 was found by equilibrium centrifugation, which also yielded additional evidence of the homogeneity of the preparation. Among the products of hydrolysis the following amino acids have been identified: aspartic and glutamic acids, serine, glycine, alanine, valine, proline, phenylalanine, tyrosine, histidine, lysine and arginine. Certain peptide linkages appear to be particularly resistant to acid hydrolysis. The peptide reacts with Ehrlich's reagent and ultra-

violet absorption in alkaline solution reveals the presence of one mole of both tryptophane and tyrosine. No sulphur-containing amino acid residues have been found to be present.

When comparing the data of LERNER AND LEE with ours, the following striking differences are encountered: The isoelectric point of our peptide is about pH 5 as compared with pH 10.5–11 and is devoid of cystine, leucine and threonine. It is difficult to compare the potencies of the two preparations since they were assayed differently. We assayed our material according to the method of LANDGREBE AND WARING[3] to an accuracy of 10% against a standard material. LERNER AND LEE, on the other hand, used a much less accurate method of assay based on the response of isolated frog skin. Methods of this kind have been adequately criticised elsewhere[7,8].

A possible explanation of the divergence is, in our opinion, that LERNER AND LEE have isolated a basic aggregate of the active peptide and inert material. We have observed a basic component in the material eluted from oxycellulose, the isoelectric point of which appears to agree with that reported by LERNER AND LEE. As complete recovery was found when assaying material not including this component its activity was not determined.

A more detailed communication concerning the isolation and physical, chemical and biological properties of the peptide will be published later.

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Influence de la ribonucléase sur la teneur en adénosinetriphosphate (ATP) et la consommation d'oxygène des amibes vivantes

Un travail récent de BRACHET[2] a montré qu'un traitement approprié à la ribonucléase (RNase) permet de faire varier, presque à volonté et dans le même sens, la teneur en acide ribonucléique (ARN) et l'incorporation des acides aminés dans les protéines, chez les amibes vivantes (*Amoeba proteus*). Nous avons cherché à voir si la RNase modifie la teneur en ATP et la consommation d'oxygène des amibes ainsi traitées, afin de préciser si l'enzyme n'altère pas l'anabolisme protéique en inhibant la production d'énergie.

L'ATP a été dosé suivant la technique de STREHLER[7], dans les conditions décrites récemment par BRACHET[3]*.

Nous avons fait agir la RNase cristallisée (G.B.I.) pendant des temps variables, en vérifiant chaque fois l'activité enzymatique des solutions par la méthode de KUNITZ[5]; la concentration était de l'ordre de 0.1 mg/ml. Les variations observées au cours de 8 séries d'expériences, pendant des temps allant de 15 à 18 h, ne sont pas très importantes: la différence entre les moyennes est de l'ordre de 25% (Tableau I). Cependant, au bout d'une demi-heure à une heure, on peut noter une légère augmentation de la teneur en ATP, bientôt suivie d'une chute. Si on les remet dans leur milieu habituel (liquide de Chalkley) ou dans de l'ARN (Schwarz 1.4 mg/ml), les amibes retrouvent leur taux d'ATP normal.

TABLEAU I

TENEUR MOYENNE EN ATP (μg/100 amibes) DES AMIBES NORMALES ET TRAITÉES À LA RNASE

Amibes normales	*Amibes traitées par l'RNase*								
	15′	*½ h*	*¾ h*	*1 h*	*1 h ½*	*2 h*	*18 h*	*+Chalkley*	*+ ARN*
0.175	0.186	0.196	0.220	0.205	0.172	0.170	0.165	0.183	0.177

* Toutes ces mesures ont été faites avec le concours du Dr. M. ERRERA auquel vont mes plus vifs remerciements.