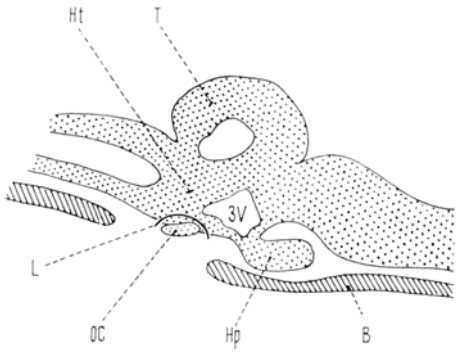
unoperated ones, were placed for periods of 3–4 days at a time on 3 different backgrounds: (1) white tank, (2) black tank, (3) in complete darkness. The white and black tanks received continuous illumination from above. At each changes of environment, the melanophore indices were measured[10]. The toads were killed 1–2 months after operation and the effects of the surgical procedures were histologically confirmed.

The melanophores in the unoperated animals behaved in the normal and expected manner throughout[1]. In 5 out of the 10 mock-operated toads and in 5 out of the 12 in which the chiasma had been separated from the hypothalamus, a state of permanent dispersion of the melanophores developed. The melanophores of the remaining operated and mock-operated toads responded to the different backgrounds in the same way as the unoperated controls.

If a retino-hypothalamic connection were responsible for controlling the secretion of MSH, those toads in which it had been severed should have assumed an intermediate colour with the melanophores in a state midway between full concentration and full dispersion, as occurs in blinded animals or in ones kept in total darkness[1]. The state of permanent full dispersion of the melanophores which developed in some of the experimental and control animals is evidently an inconstant effect of exposing the base of the brain.

It is suggested that a direct retino-hypothalamic tract, if indeed there is such a pathway, is not essential to the reflex secretion of MSH in *Xenopus* laevis.



Sagittal section through brain and base of scull of *Xenopus*, showing position of lesion separating optic chiasma from hypothalamus. T, optical tectum; OC, optic chiasma; Ht, hypothalamus; 3V, third ventricle; L, site of lesion; B, base of scull, showing extent of craniotomy; Hp, hypophysis.

Résumé. On a estimé possible que les axones rétino-hypothalamiques décrits chez certains amphibiens jouent un rôle dans le contrôle neuro-endocrinien de la pars intermedia de l'hypophyse. Mais si on sectionne chez des *Xenopus* cette voie supposée, ces animaux peuvent néanmoins modifier la couleur de leur peau lorsqu'on les place sur les terrains différents. Il n'est donc pas vraisemblable que ces connections fassent partie d'un arc réflexe contrôlant la sécrétion de l'hormone mélanotrophique.

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Department of Anatomy, Medical School, Birmingham, 15 (England), 11 December 1967.

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Drosophila Alcohol Dehydrogenase Isozymes: Identity of Molecular Size[1]

In *Drosophila*, the enzyme alcohol dehydrogenase (ADH) occurs in multiple electrophoretic forms[2–4]. In the course of an investigation of the causes for these isozymic forms, we were interested to know whether the electrophoretically different forms represented a series of polymers or aggregates differing in size or whether, alternatively, all isozymes were of the same size. A modification of RAYMOND's[5] two-dimensional acrylamide technique was used for answering this question.

ADH was purified from a mixture of *Drosophila* ADH-genotypes I, II and III[4] according to the method of SOFER and URSPRUNG[6]. These genotypes are all wild-type stocks, but differ from one another in the electrophoretic mobilities of ADH molecules; the mixture contains a total of 5 electrophoretically distinguishable varieties of the enzyme. The preparation used for the electrophoretic analysis had a specific activity of 22,000 units/mg protein. A total of 7,200 units ADH, contained in 0.2 ml, was applied to each starting well. Gels contained a 5% or 8% solution of Cyanogum – 41 made up in a 1/150 dilution of EBT buffer, pH 8.7 with 0.4 g ammonium persulfate and 0.4 ml N,N,N′,N′-tetramethylethylenediamine/300 ml as catalyst. A vertical slab electrophoresis apparatus (E-C Apparatus Corp., Philadelphia, Pa.) was used for electrophoresis. The buffer reservoirs contained EBT buffer, pH 8.7, diluted 1/150. The stock EBT buffer contained EDTA (0.02 *M*), boric acid (0.5 *M*), and *Tris* (0.9 *M*). No spacer or sample gels were used. To each gel, a voltage gradient of 16.7 V/cm was applied, with a current of about 65 mA. Electrophoresis was discontinued after 18 h (5% gels), or 24 h (8% gels), and the gels stained for ADH activity[4], or for protein using a 1% solution of Amido-schwarz in 7% acetic acid.

A comparison of the ADH patterns in the 5% and 8% gels shows readily that the 5 isozymes present in this preparation migrate as a cluster of more or less equally spaced bands (Figure 1). The rate of migration in the 8% gel is clearly reduced, indicating that the pore size of this gel has become limiting to molecules of the size of *Drosophila* ADH. The fact that the ADH bands still maintain the same relative spacing strongly suggests that they are of identical size. This is borne out by the behavior of the other proteins used as internal standards. The ratio of electrophoretic migration of egg albumin and pepsin and of egg albumin and lactate dehydrogenase is clearly different in the 2 gels (Figures 2 and 3). This shows that in

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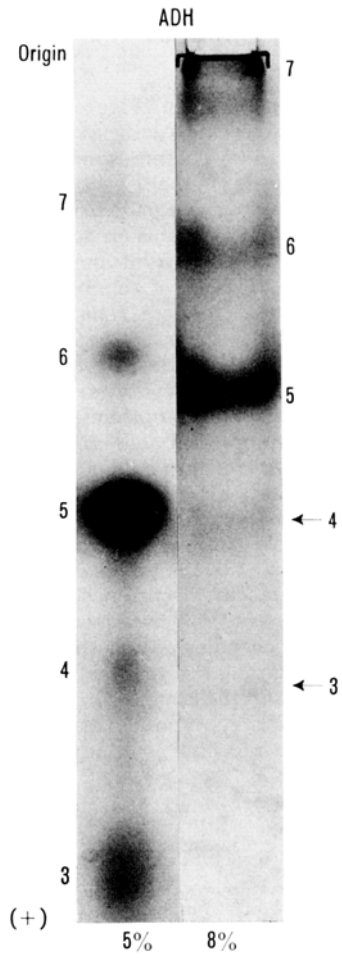

Fig. 1. Migration of *Drosophila* ADH in 5% and 8% acrylamide gels. The isozymes are numbered 3–7[4].

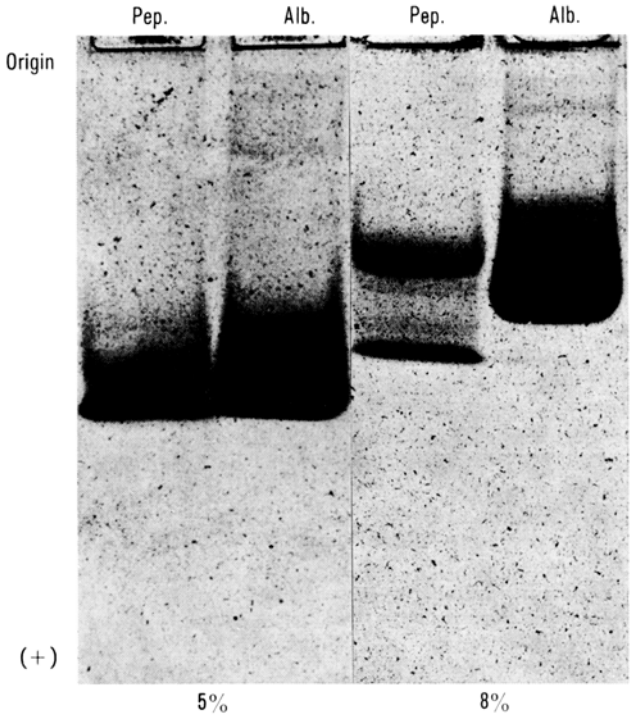

Fig. 2. Difference in migration of pepsin and egg albumin in 5% and 8% acrylamide gels, respectively.

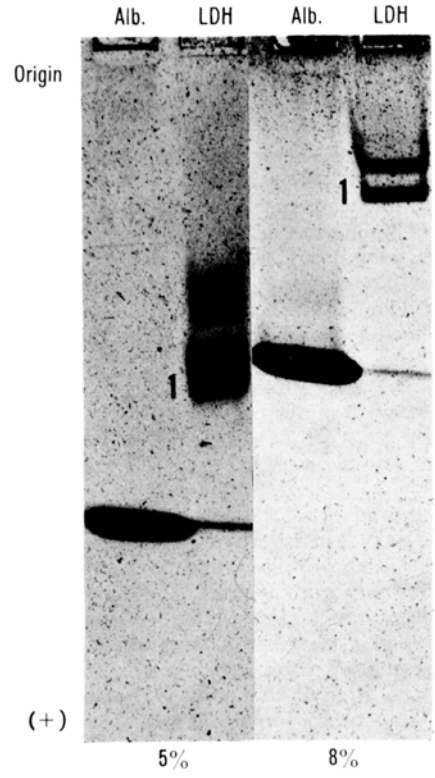

Fig. 3. Differences in migration of egg albumin and pig heart lactate dehydrogenase in 5% and 8% acrylamide gels, respectively. The ratio of migration of albumin and LDH-1 is 1.45 in the 5% gel and 2.2 in the 8% gel.

the 8% gel a molecule of molecular weight (MW) 140,000 (lactate dehydrogenase) is more retarded than one of MW 44,000 (egg albumin), which in turn is more retarded than one of MW 35,000 (pepsin). *Drosophila* ADH has a MW of 40,000–50,000 on the basis of Sephadex chromatography and sucrose density gradient centrifugation[6]. Polymers or aggregates of this molecule would be expected to be increasingly retarded in the 8% gel. Since this was not observed, the various ADH molecules must have identical size. This conclusion is supported furthermore by the observation that ADH migrates in a monodisperse fashion in the analytical ultracentrifuge[6]. The same conclusion was also reached by Dr. K. B. Jacobson of the Oak Ridge National Laboratory in a related investigation[7].

Zusammenfassung. Drosophila besitzt Alkoholdehydrogenase in mehreren elektrophoretisch trennbaren molekularen Formen. Durch Anwendung von Acrylamid-Elektrophorese bei 2 verschiedenen Gel-Konzentrationen haben wir nachgewiesen, dass diese verschiedenen Isozyme sich nicht in ihrer Molekulargrösse unterscheiden und deshalb nicht Aggregate darstellen.

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