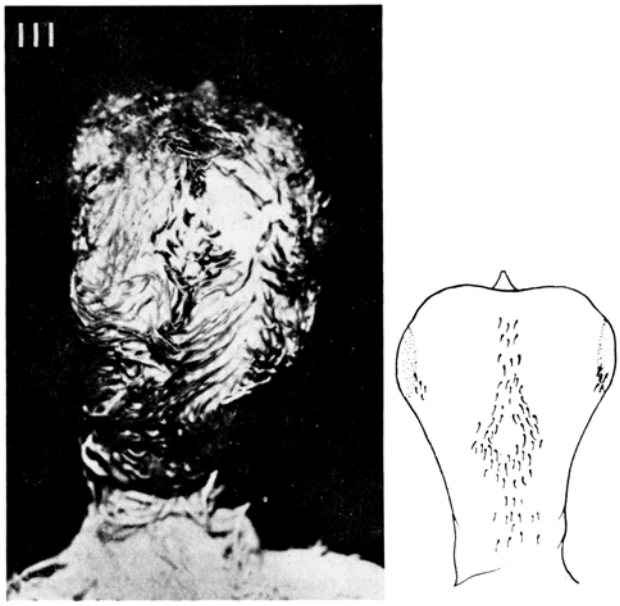

Fig. 3. No. 9067. 14-day-old female embryo, implanted with 2 pituitaries of 2-day-old ♂ chicks. Feathers brown; on occiput a diamond of black-brown feathers; area of black feathers extending from lateral angle of the eyes; blackening Degree 3.

coelomic cavity, exclusively stimulates the proliferation of black melanocytes in the occipital area. Further investigations are required to clarify this predilection.

As to the causative factor, weak positive results were obtained only by the administration of ACTH and α-MSH. CHEN et al.[6] demonstrated the melanophore-expanding hormone in embryonic chick pituitaries at first at 5 days of incubation. Therefore, if MSH is the causative factor, it is not surprising that 16-day-old pituitaries gave a positive result. Further support for this concept arises from the observation that the proliferation of black melanocytes is lacking in the area overlying the pineal body, which in the chick embryo is situated close under the skin. The melatonin, released by the pineal body, reverses the action of MSH (NOVALES[7,8]). Since both hormones act directly on the melanocytes (NOVALES[8]), it seems justified to ascribe the proliferation of black melanocytes to a production of MSH by the implanted pituitary and subsequent transportation to the occipital area. Further experiments are in progress to substantiate this concept[9].

Zusammenfassung. Hypophysen von embryonalen oder älteren Hühnern wurden in die Bauchhöhle von 5 Bruttage alten Hybriden der Hühnerkreuzung New Hampshire ♂ × Light Sussex ♀ implantiert. Vom 12. Bruttage an zeigen die Embryonen beider Geschlechter ein Gebiet mit schwarzer Daunenpigmentierung am Hinterkopf. Implantationen mit anderen Geweben und Injektionen mit synthetischen Hormonen blieben ohne Erfolg, ausgenommen ACTH und MSH, die schwach positiv waren. Die Ansicht, dass das Melanozyten-stimulierende Hormon (MSH) für die verfrühte Entwicklung des schwarzen Nackenkragens der Light-Sussex-Rasse verantwortlich ist, wird durch die Beobachtung erhärtet, dass das Gebiet über der Epiphysis cerebri von schwarzem Gefieder entblösst bleibt.

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Effect of Interrupting Retino-Hypothalamic Connections on the Melanophore Response in *Xenopus laevis*

The response of the melanophores in *Xenopus* to the colour of the animal's background is believed to be mediated by a reflex arc from the eyes[1] to hypothalamic centres innervating the pars intermedia of the hypophysis[2-5] and regulating the secretion of the melanocyte stimulating hormone (MSH). The nervous pathways from the retina to the hypothalamus are not known, but the possible involvement of direct retino-hypothalamic fibres must be considered, since such connections have been described in some[6-8], but not all[9] amphibian species. The present study was undertaken to determine whether the cutting of a retino-hypothalamic tract, if one exists in *Xenopus*, modifies the response of the melanophores to different backgrounds.

Operations were performed on 22 adult female toads under percutaneous urethane anaesthesia. In 12 animals, the optic chiasma was approached through a trans-buccal craniotomy and separated from the hypothalamus in such a way as to sever any direct optico-hypothalamic fibres without damaging the main visual pathways (Figure). Ten toads served as controls, in which the skull and dura were opened without any deliberate injury to the brain.

During the post-operative period, the experimental and mock-operated animals, along with a number of normal,

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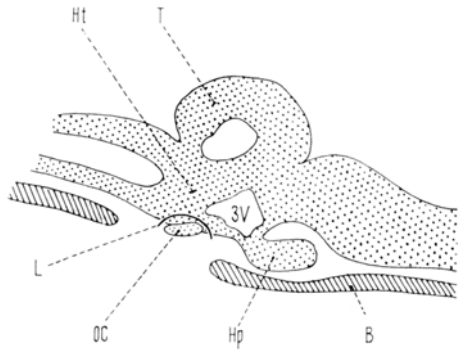
unoperated ones, were placed for periods of 3–4 days at a time on 3 different backgrounds: (1) white tank, (2) black tank, (3) in complete darkness. The white and black tanks received continuous illumination from above. At each changes of environment, the melanophore indices were measured[10]. The toads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