of ICSH potency, as compared with the HCG preparation[8]. It remains to explain why, after a post-hypophysectomy regression period, the testicular events are FSH plus testosterone dependent, whereas testosterone or HCG, if given immediately after the extirpation of the pituitary, maintains a rather normal spermatogenesis[1,2,7]. Long term experiments are in progress in order to find out if this androgen replacement therapy can go on indefinitely with full success, or if spermatogenesis is restricted by a time factor when it is generated by androgen therapies only. In other words, it must be studied if testosterone becomes ineffective without the FSH synergism after about 48 days. This period of time is supposed to cover four 12-day cycles of the seminiferous epithelium which is believed to be necessary to complete once all events of spermatogenesis in the rat[9].

*Zusammenfassung.* Nach einer einjährigen Involutionsperiode im Anschluss an die Hypophysektomie gelang es mit Hilfe eines PMS-Präparates bereits nach 24tägiger Applikationsdauer, die völlig atrophierten Testes zu annähernd normalen Gewichten mit vollständiger Spermatogenese zu restaurieren. Im Gegensatz hierzu war ein HCG-Präparat, das keine messbaren FSH-Potenzen aufwies, praktisch nur in der Lage, die Leydigschen Zwischenzellen selektiv zu stimulieren. Diese Ergebnisse deuten an, dass bei Ratten die Hodenfunktion trotz maximaler Atrophie wieder völlig hergestellt werden kann, sofern ein Präparat eingesetzt wird, das sowohl ausreichende ICSH- als auch FSH-Potenzen enthält.

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[8] Measurements of relative potency (with juvenile hypophysectomized rats, ventral prostate test[6], 3 dose factorial assay, including the covariance procedure) revealed that the ICSH potencies of PMS and HCG were identical when I.U. were compared.

[9] C. P. LEBLOND, E. STEINBERGER and E. C. ROOSEN-RUNGE, in *Mechanisms Concerned with Conception* (Ed. C. G. HARTMAN; Pergamon Press, New York – London 1963), p. 1.

## Development of Black Down Feathering in Hybrid Chick Embryos (Cross New Hampshire ♂ × Light Sussex ♀) after Pituitary Implantation

The male embryos and newly hatched chicks of the cross New Hampshire ♂ × Light Sussex ♀ are white, i.e. have no down pigmentation; the females develop a reddish-brown down pigmentation from the eleventh day of incubation onwards.

In previous communications (GROENENDIJK-HUIJBERS[1,2]) evidence was presented in favour of the concept that the formation of the red pigment in the female embryos is bound to an adequate level of gonadal hormones, either ovarian or testicular. While studying the influence of endocrine glands on the formation of the red pigment, an unexpected phenomenon was observed after pituitary implantation. The preliminary results of this study are reported here.

*Material and methods.* The hybrid eggs of the above cross were 'windowed' on the fourth day of incubation according to a method already described (GROENENDIJK-HUIJBERS[3]). On the fifth day, 1–3 pituitaries from chick donors of various ages were implanted into the coelomic cavity of the host embryos according to DOSSEL's method[4]. The age of the donors varied from 16 days of incubation to 6 weeks after hatching. As sham implants, fragments of embryonic heart, liver, thyroid and adrenal gland were used. Moreover, as a sham treatment, some embryos received on the fifth day of incubation a single dose of a synthetic hormone, such as testosterone-propionate (Orchisterone, Frosst; or Neohombreol, Organon), oestradiol-benzoate (Dimenformon, Organon), thyrotrophic hormone (Ambinon, Organon; or TSH, Sigma) or ACTH (Cortrophine and Cortrophine-Z, Organon). Finally, synthetic α-MSH (melanocyte-stimulating hormone) was administered either in 3 doses on the fifth, eighth and tenth day, or in 5 doses on the seventh to eleventh day of incubation. The drugs were administered by instilling the chorio-allantois through the shell window.

Autopsy was done at 12–15 days of incubation. The down on head, back, tail and wings was carefully examined with the aid of a dissecting microscope and photographed. The host's gonads and thyroids as well as the implants were removed and prepared for histological examination.

*Results.* The results are summarized in the Table and demonstrated in the Figures. When a sufficient amount of pituitary tissue has developed in the implant, a peculiar black-feathered area is found on the occiput of the host embryos of either sex. When only a few black feathers are present (which is arbitrarily designated as Degree 1: the number of black feathers ranging from 3–20), they are found along the median line (Figures 1 and 1a) just cranially and caudally to the pineal body, that in the 14-day-old chick embryo lies in direct contact with the scalp (Figures 2 and 2a).

When a larger amount of pituitary tissue has developed in the implant, some 20–40 black feathers embrace the pineal body in the form of a diamond and extend along the median line proximally and distally (Degree 2, Figures 2 and 2a). When the blackening is still more advanced, tiny black feathers are found in line with the lateral angle of the eyes (Degree 3, Figures 3 and 3a). Proximally the feathers extend as far as the vertex, close to the root of the comb.

As a rule the 'black feathers' are only partially black: the black pigment is arranged in longitudinal stripes along one side of the feather. In the females the fine-dotted brown pigment can be clearly distinguished lying side by side with the coarse black pigment (magnification × 40).

The degree of blackening is related to the amount of tissue developed in the implant rather than to the age of

[1] M. M. GROENENDIJK-HUIJBERS, Experientia *22*, 302 (1966).

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[4] W. E. DOSSEL, Science *120*, 262 (1954).

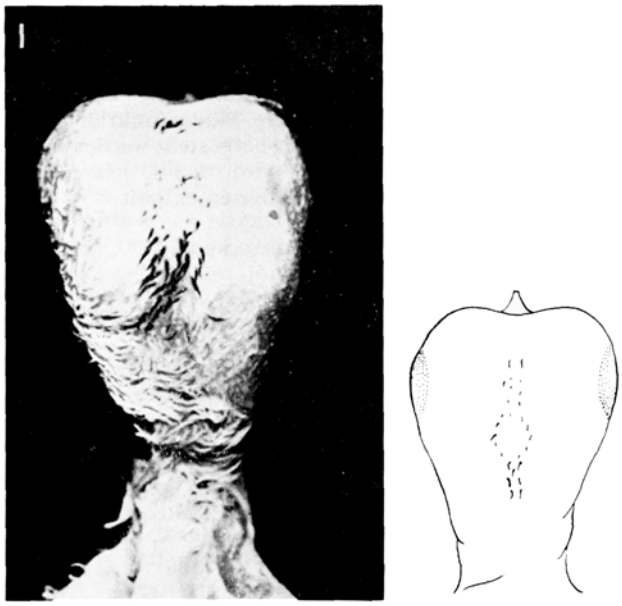

Fig. 1. No. 9058. 14-day-old male embryo, implanted with three 16-day-old embryonic chick pituitaries. Feathers white except on occiput, where a number of black feathers have developed; blackening Degree 2.

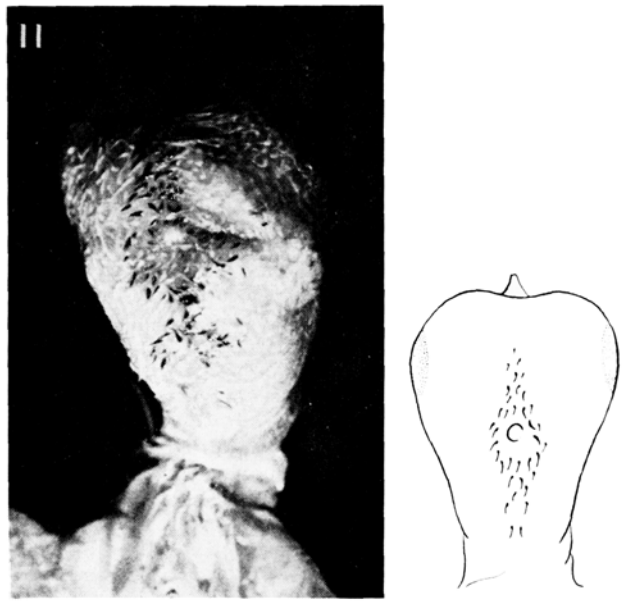

Fig. 2. No. 9050. 14-day-old female embryo, implanted with 1 pituitary of a 3-week-old ♀ chick. Feathers pale brown; on occiput black feathers surrounding the pineal body; blackening Degree 2.

Black down feathering after pituitary implantation in $F_1$-hybrid chick embryos (New Hampshire ♂ × Light Sussex ♀)

| Implanted pituitary | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Embryonic | | | | | | New-born | | | | | 3–6 weeks of age | | | | |
| Sex of host | Case No. | Implants No. | age | sex | Degree of blackening[b] | Case No. | Implants No. | age | sex | Degree of blackening[b] | Case No. | Implants No. | age | sex | Degree of blackening[b] |
| ♂ | 9058 | 3 | 16 di[a] | ♂ | 2 | 9061 | 1 | 2 days | ♂ | 1 | 9055 | 1 | 3 weeks | ♂ | 2 |
| ♂ | 9198 | 1 | 21 di[a] | ♀ | 1 | 9064 | 1 | 2 days | ♀ | 1 | 9056 | 1 | 3 weeks | ♂ | 2 |
| ♂ | | | | | | 9065 | 1 | 2 days | ♀ | 1 | | | | | |
| ♀ | 9196 | 2 | 20 di[a] | ♂ | 3 | 9067 | 2 | 2 days | ♂ | 3 | 9049 | 1 | 3 weeks | ♀ | 1 |
| ♀ | | | | | | | | | | | 9050 | 1 | 3 weeks | ♀ | 2 |
| ♀ | | | | | | | | | | | 9057 | 1 | 3 weeks | ♂ | 2 |
| ♀ | | | | | | | | | | | 9054 | 1 | 6 weeks | ♀ | 3 |
| ♀ | | | | | | | | | | | 9070 | 1 | 6 weeks | ♂ | 3 |

[a] di, days of incubation. [b] Degree of blackening: see text.

the implanted pituitary. This appears from the data presented in the Table: if, with the implantation of embryonic pituitaries, a similar degree of blackening is to be effected as with a 6-week pituitary, the number has to be raised from 1 to 3.

No black feathers developed after implantation of pieces of heart, liver, thyroid or adrenal, nor after administration of synthetic hormones except the following ones. After administration of ACTH (0.6–1.5 I.U. in a single dose on the fifth day of incubation), in 2 out of 26 cases, a few black feathers were found on the occiput only. After administration of synthetic α-MSH in 2 cases out of 14, 2 black feathers and in one other case 20 black feathers were found on the occiput only. A sham treatment with Hanks BSS or NaCl failed to bring about the development of black feathers.

*Discussion and conclusions.* The observation described raises 2 questions. Firstly, why do the black feathers develop in this particular area? Secondly, which is the responsible factor?

The Light Sussex breed is characterized by the possession of a crown of black feathers on the dorsal side of the neck. A close inspection of the adult bird reveals that the upper border of the crown reaches as far as the vertex and laterally approaches the lateral angle of the eyes. It therefore seems indisputable that the black-feathered area constitutes the prospective crown of black feathers characteristic of the Sussex breed. It has to be emphasized, however, that in the Light Sussex chick black pigmentation of the neck primaries does not appear until the sixth week of life, whereas black pigmentation at the base of wing and tail primaries appears during the first week after hatching (COHEN[5]). The pituitary implant, grown in the

[5] J. COHEN, J. Embryol. exp. Morph. 7, 361 (1959).

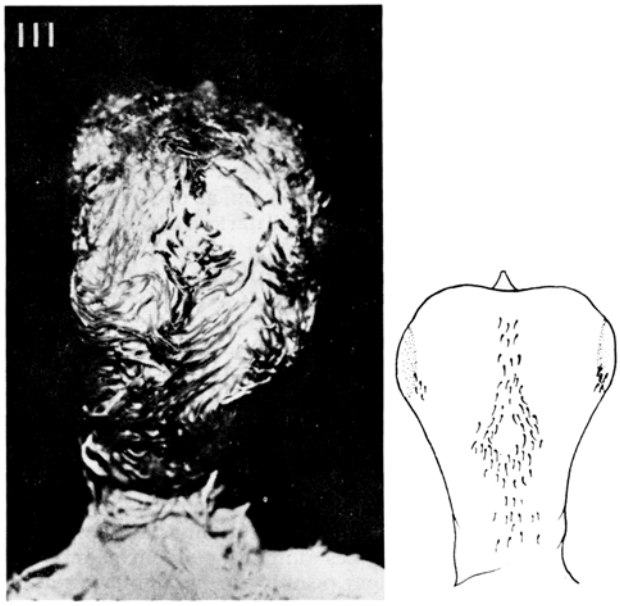

Fig. 3. No. 9067. 14-day-old female embryo, implanted with 2 pituitaries of 2-day-old ♂ chicks. Feathers brown; on occiput a diamond of black-brown feathers; area of black feathers extending from lateral angle of the eyes; blackening Degree 3.

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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[8] R. R. NOVALES, Ann. N.Y. Acad. Sci. *100*, 1035 (1963).
[9] I express my thanks to Dr. J. A. VLIEGENTHART and Dr. P. G. SMELIK for their generous gift of synthetic α-MSH, and to Frosst Company, Montreal and N.V. Organon, Oss, for their gifts of synthetic hormones.

## 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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