

factor, since preliminary experiments replacing carotene by vitamin A in the diet reveal the same picture. The excessive vitamin A of the kidneys, never seen normally,⁹ excludes an absorption difficulty. Furthermore, the wealth of vitamin A in histologically normal as well as hemorrhagic kidneys would seem to exclude renal pathology as a fundamental cause. Whether the vitamin A deposits in the kidney are due to compensatory storage or urinary excretion cannot yet be answered.

Summary. Rats on a choline-poor diet containing liberal supplements of carotene develop fatty livers poor in vitamin A. The kidneys of these animals are extremely rich in vitamin A.

13514 P

Localized Maturation of Lid-closure Reflex Mechanism by Thyroid Implants into Tadpole Hindbrain.*

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The lid-closure (wink) reflex of amphibians—retraction of the eye bulb upon tactile stimulation of the cornea or surrounding skin—appears comparatively late in development. Urodeles display it first in advanced larval stages, anurans at or about the time of metamorphic climax. In the latter group its dependence upon the metamorphosing agents was demonstrated by experiments in which metamorphosis was either suppressed (hypophysectomy) or advanced (hyperthyroidism);¹ in the former case, the reflex failed to appear unless thyroid was administered, while in the latter case its onset was advanced almost, though not fully, as much as was the transformation of the body. Both the sensory (trigeminal) and motor (abducens) components of the reflex develop full functional capacity in very early larval stages, but their central linkage into a workable reflex mechanism is not effected until at the time of metamorphosis.

The close association with metamorphosis suggested that maturation of the central mechanism of the reflex might be under direct

⁹ Popper, H., and Greenberg, R., *Arch. Path.*, 1941, **32**, 11.

* This work was done under the direction of Dr. Paul Weiss. It has been supported by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

¹ Kollros, J. J., *J. Exp. Zool.*, 1942, **89**, 37.

control of the thyroid hormone. In order to test this possibility, an attempt was made to precipitate the development of the reflex relative to the metamorphic changes in the rest of the body by exposing the hind brain locally to supernormal concentrations of the hormone. As Hartwig² has shown, implantation of thyroxin-soaked agar under the fin skin of salamander larvae causes local metamorphosis of the surrounding tissues. A similar procedure was, therefore, adopted for the present experiments.

Agar pellets were soaked in thyroxin (1:1000) and then implanted into the fourth ventricle of tadpoles of *Rana pipiens*. In another series fresh rat thyroids were used. The grafts could be expected to exert both localized and diffuse effects, the latter to speed metamorphosis in general. Local effects resulting from higher concentration of the hormone at the site of the graft might register not merely in absolute precocity, but in definite advancement of the onset of the reflex relative to other accelerated body changes. Emergence of the fore limbs ("f.l.e.") was chosen as a criterion of body metamorphosis.

According to earlier observations,¹ the reflex in the normal tadpole antedates f.l.e. by about 4 days. In 29 control animals of the present series this period was found to be from 0 to 10 days (average 4.1 days). In animals precociously metamorphosed by diffuse thyroid treatment, the reflex usually lags behind f.l.e. by from 1 to 5 days. In contrast, 33 animals with thyroid implants in the fourth ventricle displayed the reflex first from 1 to 18 days (average 10.5 days) prior to f.l.e., indicating a definite local acceleration of functional brain differentiation in the vicinity of the graft. The treated animals fall into two classes according to age. The 12 youngest specimens (hind legs with beginning digitation), although initially stimulated, apparently received insufficient hormone from the implant to complete metamorphosis at an accelerated pace, and in these the reflex appeared at the usual time. If the computation is confined to the 21 cases treated at a later stage (all toes clearly outlined), the average advancement of the reflex relative to f.l.e. rises to 12.7 days (Fig. 1), with the reflex in all cases present at least 7 days prior to f.l.e. When the implants are made into animals with only an anticipated 10 or 11 days to go until f.l.e., the reflex appears over night, while the anticipated interval until f.l.e. is reduced by one-half.

Animals with thyroid grafts in the brain often exhibit striking asynchronism in the first appearance of the reflex in the 2 eyes. Whereas the 2 eyes of normal tadpoles are never more than one day

² Hartwig, H., *Biol. Zent.*, 1940, **60**, 473.

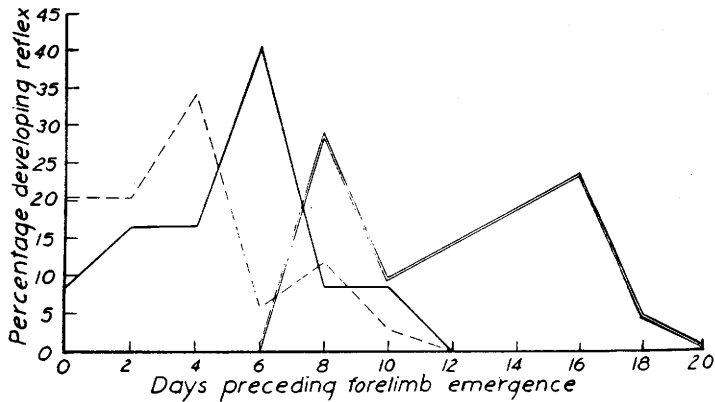


FIG. 1.

Frequency curves showing the percentage of animals developing the corneal reflex in successive 2-day intervals in the period preceding forelimb emergence; — — — normal controls, ——— animals having received thyroid implants at an earlier stage (hind limb undergoing digitation), ····· animals having received thyroid implants at a later stage (all digits distinguishable).

apart in showing the first signs of the reflex, some of the animals with implants showed a difference between the 2 sides of as much as 7 days. This difference is interpreted as resulting from an asymmetrical position of the graft in the ventricle, subjecting the 2 lateral halves of the brain wall to different hormone concentrations. This assumption is borne out by 2 cases studied microscopically in sections, in which the grafts actually were found to be situated nearer the side which had displayed the reflex first.

In conclusion, these experiments seem to prove a direct local effect of thyroxin on the setting into operation of the brain centers mediating the lid-closure reflex. The hormone exerts this effect only in older larvae. Its ineffectiveness during mid-larval stages (save for general metamorphic acceleration) indicates that the reflex mechanism itself differentiates independently of hormone action, obviously by self-differentiation within the brain centers.³ Only the final stages of this process and the release of the apparatus for actual operation seem to require the supplementary action of thyroid hormone.

³ Weiss, P., *Comp. Psychol. Monogr.*, 1941, 17, No. 4.