Effects of Triiodothyronine on the Cerebellar Cortex of the New-Born Rat in Tissue Culture

It is well known that the thyroid hormones are necessary for the normal functional and morphological development of the nervous system[1], and it is likely that the hormones act by stimulating the synthesis of proteins in the cells of immature nervous tissue[2–5]. In adult rats, regeneration of severed axons in the spinal cord has been shown to occur following treatment of the experimental animals with thyroxine[6] and it is possible that this effect may also be the result of an increased rate of synthesis of axoplasmic protein. This communication reports some morphological observations of the effects of various ambient levels of triiodothyronine upon the cultured cerebellar cortex of the neonatal rat, with special reference to the axons.

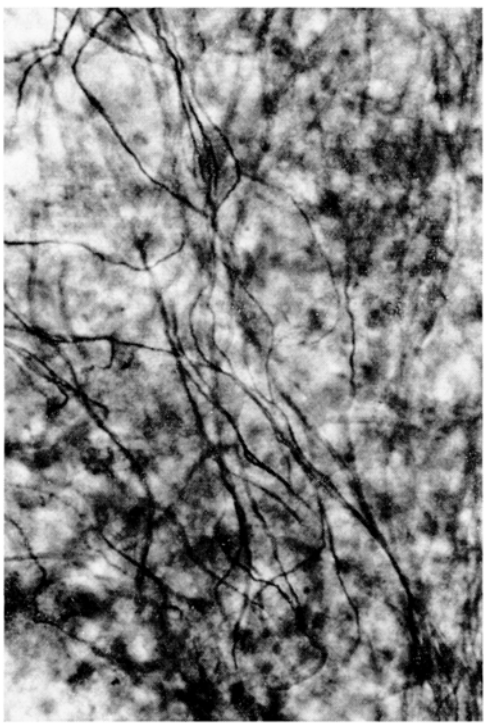

Fig. 1. Control culture, showing numerous axons of normal appearance. Urea-silver nitrate method. ×800.

Materials and methods. 40 fragments, each of approximately 1.0 mm³, were taken from the cerebellar cortices of new-born Wistar rats of both sexes. The fragments were explanted individually onto strips of cellulose acetate which were placed in roller tubes containing 1.0 ml of culture medium. The medium used consisted of Medium 199, buffered with 0.01 *M* HEPES[7], and containing 20% calf serum and added glucose to bring the final concentration to 600 mg per 100 ml[8]. 3, 5, 3′-triiodo-L-thyronine sodium (T3), in concentrations of 1.0, 10, 100 and 1000 ng per ml was incorporated into the media of 4 respective groups of 8 cultures. The remaining eight cultures served as controls. All media were changed on alternate days.

After culturing for 6 days the explants were washed briefly with balanced salt solution and fixed for 3 h in formol-acetic-alcohol[9]. After fixation the cultures, still on their cellulose acetate strips, were stained by a urea-silver nitrate method[10], dehydrated, cleared in terpineol and xylene and mounted onto slides. Some explants were stained with cresyl violet and mounted in the same way.

Observations. In the control cultures, argyrophilic axons, of completely normal appearance, were present in abundance (Figure 1). In the control preparations stained with cresyl violet, Purkinje cells, granule cells and fibroblast-like cells were easily recognized. A few pyknotic nuclei were also seen in the control cultures.

The explants cultured in 1.0 ng per ml of T3 also contained numerous normal axons, but pyknotic nuclei occurred more frequently than in the controls.

With 10 ng per ml of T3, some of the axons were beaded and fragmented (Figures 2 and 3). Pyknotic nuclei were present in numbers comparable with those in cultures grown in 1.0 ng per ml of T3.

At levels of 100 and 1000 ng per ml, T3 was clearly toxic to the cultures. Fragmented axons and argyrophilic debris occurred abundantly (Figure 4), most nuclei were pyknotic or karyorrhectic, and in some cultures treated with 1000 ng per ml of T3 the explants had disintegrated.

Discussion. While the responses to the 2 higher doses of T3 can only be considered as non-specific toxic effects, it is likely that the damage to axons and cells brought about by the 2 lower dosages (which are not greatly in excess of the normal circulating levels of T3[11]) parallels the similar regressive changes induced by thyroxine in

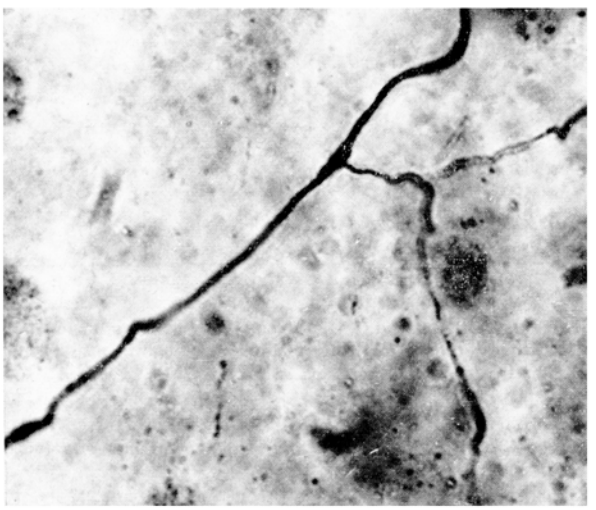

Fig. 2. Culture with T3, 10 ng per ml, showing slight beading of a branched axon. Urea-silver nitrate method. ×1625.

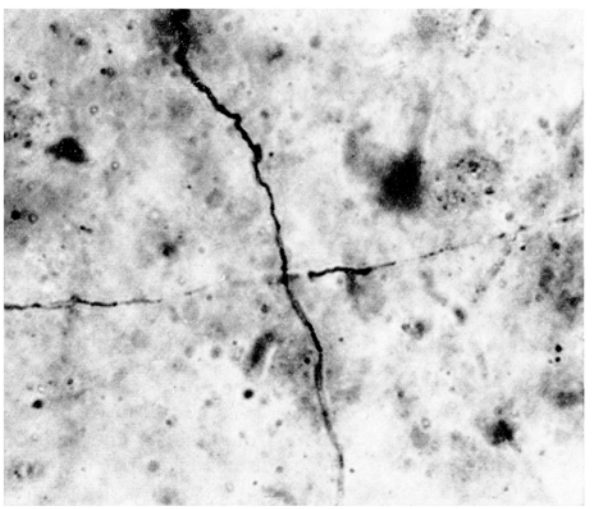

Fig. 3. Culture with T3, 10 ng per ml, showing beaded and fragmented axons. Urea-silver nitrate method. ×1625.

tissue cultures of the developing mammalian cerebellum[12-14]. However, treatment of young animals with thyroid hormones increases the sizes of the neurons and the density of axons in the central nervous system[15-17].

This difference between the effects of thyroid hormones in vivo and in vitro may be due to the fact that many of the afferent and efferent connections of the cultured neurons are necessarily severed by the process of explantation. In this connection it is of interest to note that thyroxine accelerates the degeneration of motor neurons in the spinal cord of the hypophysectomized tadpole, following amputation of the limbs[18]. It is possible that in normal ontogeny the thyroid hormones accelerate the regression of those neurons which differentiate but do not form functional connections.

While triiodothyronine causes degenerative changes in the axons of the cultured cerebellum, thyroxine stimulates the regeneration of axons in the crushed spinal cord of the rat[6]. It is unlikely that this difference represents a differential mode of action of the 2 hormones since these are in all other respects similar, except that T3 is about 10 times as potent as thyroxine[19]. The conflicting observations may, however, be reconciled by the hypothesis that the thyroid hormones accelerate the degeneration of cells whose peripheral connections have been removed. In the cultured fragments many of the afferent and efferent fibres to and from the neurons are severed, while in the crushed spinal cord only the axons are divided, the perikarya, dendrites and afferent synapses of the injured neurons being for the most part remote from the site of axonotmesis. According to the theory, therefore, one would expect treatment with thyroid hormones to produce predominantly regressive changes in culture and predominantly stimulatory effects in the animal with a single localized central nervous lesion.

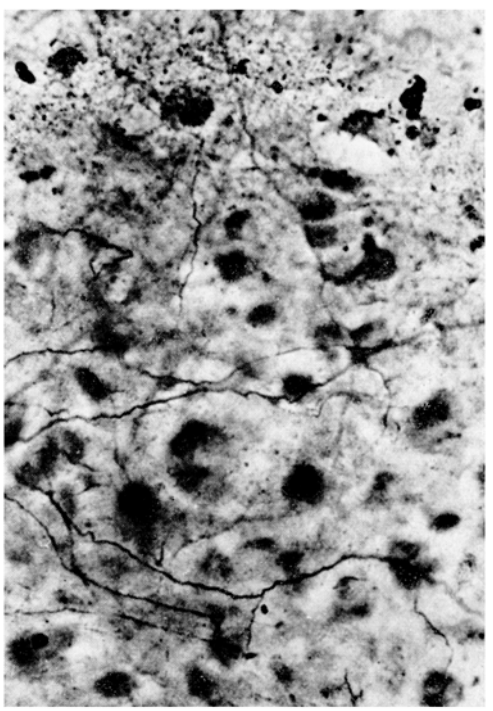

Fig. 4. Culture with T3, 100 ng per ml, showing axons, some of them degenerate, and much argyrophilic debris. Urea-silver nitrate method. × 730.

Résumé. La triiodothyronine produit in vitro des changements régressifs dans les cellules et dans les axones du cervelet du rat nouveau-né. Il est suggéré que les hormones thyroïdes provoquent une dégénérescence accélérée des neurones dont les connexions afférentes et efférentes ont été coupées, tandis qu'elles stimulent la croissance et la différenciation des neurones aux connexions périphériques intactes[20].

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Fibrinolytic Activity in Wall of Human Ductus Arteriosus

It is generally accepted that closure of the ductus arteriosus after birth is brought about by active contraction of the vessel wall followed by intimal proliferation[1-4]. This large shunt, which normally allows $^2/_3$ of the blood ejected from the right heart ventricle to by-pass the high-resistance pulmonary vascular bed[5], is essential for the large foetal cardiac output[6]. Physiological mechanisms for maintaining patency of the ductus in utero have received little attention.

An adequate flow of blood through the ductus is necessary not only for the foetal systemic circulation, but also for nutrition of the ductal wall. The adventitia

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