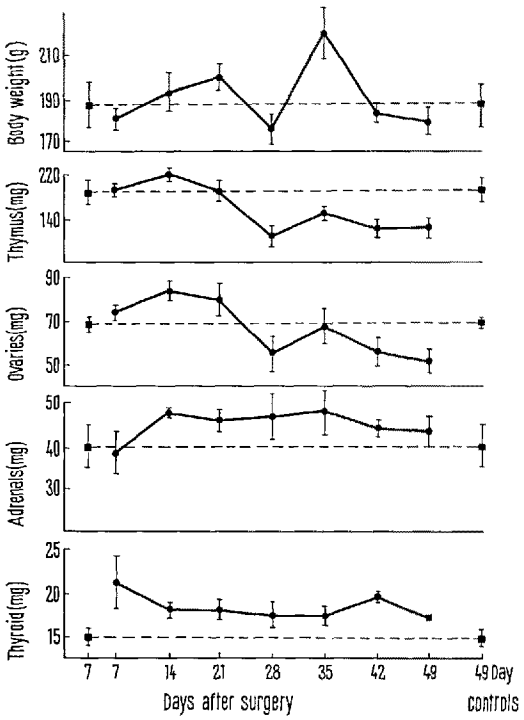
mized rats were less atrophic than those of hypophysectomized rats.

It is at present accepted that the pineal gland influences the function of gonads[17,18]. Ovarian and testicular weight increase after pinealectomy are not always found and are related to the time interval after the operation: it develops shortly after pinealectomy and has a tendency to disappear after several weeks[17]. Ovarian and testicular weight increase is found with few or no alterations in its histological structure in animals whose pineal is removed[18].

Similar features were found in the thyroid of our pinealectomized rats. The thyroid weight increase was found very soon after the operation and disappeared after several weeks. There were few histological modifications in the thyroids of which weight was increased.

The removal of the pituitary did not prevent the thyroid or ovarian weight increase after pinealectomy. This suggests a direct effect of the pineal gland upon the size of these organs[19].



Effects of pinealectomy on the weight of the endocrine glands in rats.

Zusammenfassung. Epiphysektomie bei verschiedenen Gruppen weiblicher Ratten ergab Zunahme des Schilddrüsengewichtes. Diese war postoperativ in der ersten Woche besonders stark und perennierte während 7 Wochen der Experimentdauer. Entsprechende Gewichtszunahme der Schilddrüse resultiert auch nach Epiphysektomie bei hypophysenlosen Tieren.

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Cholesterol Metabolism in the Myelin of Rat Brain

Studies of DAVISON et al.[1] and DAVISON and WAJDA[2] using labeled cholesterol in developing animals demonstrated the metabolic stability of cholesterol in brain white matter. Since the white matter is rich in myelin, the results suggested that once cholesterol is incorporated in the myelin membrane it is retained without apparent turnover. In a recent study on rat brain myelin, CUZNER et al.[3] suggested that the myelin lipids continue to turnover at the same rate throughout the life span of the animal. More recently SMITH[4], and SMITH and ENG[5], have demonstrated the incorporation of labeled acetate and glucose into myelin cholesterol of adult rat brain.

Myelin cholesterol accounts for about 70% of the total cholesterol in the adult rat brain[6], and is generally considered metabolically stable[1]; almost all of it is incorporated into the myelin during the early period of active myelination.

In the present investigation the uptake of glucose carbon into cholesterol and total lipids of brain myelin was studied beyond the period of active myelination. Since no blood brain barrier exists for glucose, it was hoped that injection of glucose through the i.p. route would serve as an efficient precursor if any cholesterogenesis continued in the CNS myelin beyond the period of active myelination.

Six-week- and 6-month-old female rats of the Sprague-Dawley strain (Charles River Laboratories, Wilmington, Mass.), weighing 135–145 and 335–365 g respectively, were used in these studies. The animals were given an i.p. injection of 4.8 μc U-^{14}C glucose/100 g body weight and killed after different intervals of time over a period of 200 days. The animals had free access to water and Purina Laboratory Chow prior to and after receiving the labeled substrate. The animals were anesthetized and decapitated. The brain was removed and washed in saline, blotted on filter paper and weighed. Myelin was isolated from the brain by a slight modification of the method of ADAMS et al.[7]. Only the crude mitochondrial fraction as isolated by KHAN and WILSON[8] was used for the separa-

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tion of myelin. The sediment from 11,000 *g* was suspended in 5 ml of 0.32 *M* sucrose solution containing 1.0 m*M* EDTA and 6.0 m*M* Na_2HPO_4 (pH 7.3) and layered over 0.80 *M* sucrose solution. It was centrifuged at 13,000 *g* for 45 min. The myelin was removed from the top of the tube. Total lipids were extracted from the myelin as described by EICHBERG et al.[9]. A small amount of washed total lipid extract was plated and radioactivity was determined. Cholesterol was separated and the radioactivity was determined as described by KHAN and FOLCH-PI[10].

The data on the incorporation and persistence of glucose carbon in the myelin cholesterol of young and mature rat brain is shown in Figure 1. In the young animals, after the initial incorporation, there was a slow decline in specific radioactivity of myelin cholesterol over a period of 200 days. In the mature animals, the initial incorporation was close to that in young animals and, subsequently, no significant decline in specific radioactivity was observed during the experimental period. In comparison, the data on incorporation and persistence of glucose carbon in the total lipids of myelin of the young and mature animals are shown in Figure 2.

In the young animals the wet weight of the brains increased by one-third (from 1.5–2.0 g) during the course of experiments. The faster decline in specific radioactivity in the total lipids of myelin of young animals, therefore, could be due to dilutions with unlabeled lipids as a result of increase in brain weight as well as to active turnover of lipids (Figure 2). Since in the mature animals there was no significant increase in brain weight during the experimental period, the slow decrease in specific radioactivity could be due principally to steady turnover of lipids. In the young animals, the extremely slow decline in specific radioactivity in the total lipids of myelin after 110 days (Figure 2) may be due principally to retention of label by stable components such as cholesterol and sulphatides.

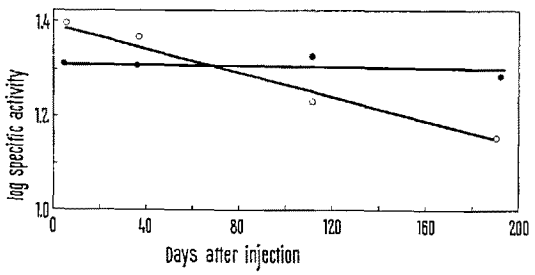

Fig. 1. Incorporation and persistence of glucose carbon in the cholesterol of rat brain myelin. Animals were given 4.8 μc U-^{14}C glucose/100 g body weight and killed over a period of 200 days. Young and mature animals were 6 weeks and 6 months old respectively. o——o, young animals; •——•, mature animals. Each point represents an average value of 2 animals.

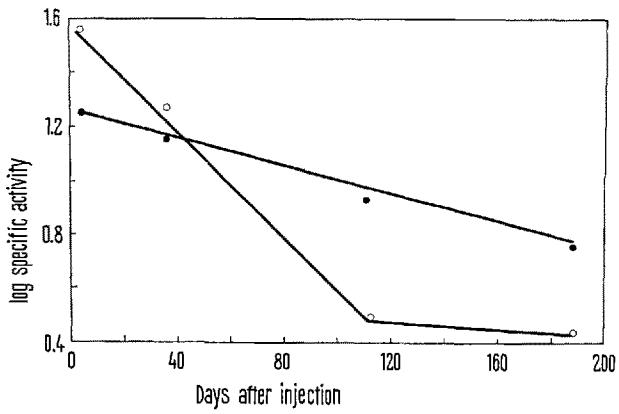

Fig. 2. Incorporation and persistence of glucose carbon in the total lipids of rat brain myelin. o——o, young animals; •——•, mature animals. Each point represents an average of 2 animals. Injections were the same as in Figure 1.

In rat brain the period of active myelination, during which most of the myelin is laid down, ends around 42 days after birth. The slow decline in specific radioactivity of myelin cholesterol in the 6-week-old rats probably reflects a dilution from accumulation of unlabeled cholesterol in myelin membranes formed at a slow rate after the period of active myelination, rather than active cholesterol catabolism. In the mature animals also, continued synthesis of small amounts of myelin membrane may largely explain the incorporation of label in myelin cholesterol, and may not actually be due to any cholesterol turnover. On the other hand, the lack of any significant decline subsequently in specific radioactivity indicates that the bulk of the cholesterol in mature rat brain myelin is metabolically stable. KISHIMOTO et al.[11] showed parallel accumulation of cholesterol and galactolipid in rat brain up to 275 days (beyond which they did not continue the study), which is suggestive evidence that the new myelin may continue to be formed at a slow rate in mature rat brain; my observation of incorporation of label in the total lipids and cholesterol of mature rat brain myelin is in accord with this. This new myelin may result from (1) lengthening of axons; (2) increase in thickness of myelin sheath of some axons; or possibly (3) some remodeling of parts of the sheath during the life span of the animal. Only detailed histological study would reveal such myelin accumulation in the mature animal[12,13].

Résumé. La persistance de la radioactivité incorporée dans le cholesterol et les lipides totaux de la myeline cérébrale a été mesurée pendant 200 jours après un injection i.p. de glucose U-^{14}C chez des rats de 6 semaines ayant donc précisément dépassé la période de myelinisation active et également chez des animaux de 6 mois. La radioactivité spécifique relativement faible incorporée dans le cholesterol de la myeline des animaux les plus jeunes a décru lentement tandis que chez les animaux adultes elle est restée pratiquement constante. L'incorporation de carbone de glucose dans le cholesterol de la myeline des animaux adultes ne reflète probablement pas le «turnover» du cholesterol mais pourrait indiquer plutôt que la myeline continue à être formée lentement tout au long de la vie de l'animal.

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[12] The author is grateful to Dr. HELEN H. HESS for her suggestions and criticism in preparing this manuscript.

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