slow decrease was again noticed. This could be due to some distortion of the normal metabolic pathway of the catecholamines produced by prolonged exposure to light[9].

Resumen. Una depleción inmediata de la adrenalina retiniana ocurre cuando sapos adaptados a la oscuridad son expuestos a una luz relativamente fuerte, alcanzándose un máximo en aproximadamente 5 min. La repleción de la adrenalina es más rápida cuando los animales, después de ser expuestos por 5 min a la luz, son devueltos a la oscuridad. En un período de 24 h la adrenalina alcanzó aproximadamente 85% de su valor original. Por otra parte, cuando los animales se mantienen expuestos a la luz, la repleción es más lenta y la adrenalina retiniana alcanza un máximo de sólamente 65% de su valor original en adaptación a la oscuridad.

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Protein Synthesis in the Brain of Rats Thyroidectomized at Birth

The important role that the thyroid plays in the development of the central nervous system is readily recognized by the severe mental retardation which ensues from early thyroid deficiency. The disorders which occur during the maturation of the thyroid hormone-deficient brain involve the growth of the nerve cell perikarya and their processes[1], the configuration of the capillary bed[2] and the deposition of myelin[3]. The metabolic changes underlying the severe clinico-pathological findings of the thyroid deficiency are, however, still unresolved. Biochemical studies have indicated that increased de novo synthesis of enzymes is detectable in the liver after thyroid hormone treatment[4] while in the hypothyroid brain the activities of succinate dehydrogenase and acetylcholinesterase are reduced[5]. Quantitative histochemical studies of enzymes concerned in the major metabolic pathways of oxidation-reduction, energy metabolism, nerve transmission, phospholytic and hydrolytic reactions have, however, failed to localize changes to any one system[6].

This report describes 2 experiments. First, since microsome preparations are known to contain the bound and free polysomes thought to be involved in the synthesis of protein essential for the development maintenance and renewal of cell processes[7], the ability of these structures to synthesize protein in the developing brain of thyroidectomized rats has been investigated. Second, the sedimentation profile of these aggregates on the sucrose density gradient has been examined.

Sprague Dawley albino rats were thyroidectomized by a single i.p. injection of 150–200 μC of ^{131}I on the day of birth. Animals were decapitated at 10 days and at 30 days of age and the whole brains or cortex homogenized in a medium of 0.15 *M* sucrose, 0.025 *M* potassium chloride, 0.01 *M* magnesium chloride and 0.035 *M* *Tris* buffer pH 7.8. Heavy and light microsomes were prepared by centrifuging the 15,000 *g* postmitochondrial supernatant at 25,000 *g* and 105,000 *g* for 1 h respectively. The polysomal pellets were incubated for $2^1/_2$–15 min under conditions favourable for the synthesis of protein[8] using the 'pH 5.0 enzyme' fraction obtained from the control animals and ^{14}C-phenylalanine as precursor. The reaction was arrested with 10% TCA and the protein purified by extraction of the acid soluble, lipid and nucleic acid fractions. The dry protein precipitates were dissolved in hyamine, transferred to vials, mixed with dioxane-containing scintillator and counted in a Nuclear Chicago liquid scintillation spectrometer using the channel ratio method for quench correction.

The 'pH 5.0 enzymes', used with the microsomal fractions from both the control and thyroidectomized animals, do not contain RNA fractions defined as cytoplasmic messenger when the extracted macromolecular RNAs are pulse labelled and sedimented on the sucrose density gradient (unpublished observation). The in vitro protein synthesis, therefore, reflects the function of the protein synthesizing machinery of the brain membrane preparations. The similarity between the protein-synthesizing ability of preparations made from the brains of both normal and thyroidectomized rats, whether at 10 or 30 days old (Table), suggests that early deprivation of

L-3-Phenylalanine-C^{14} incorporation into protein of heavy and light microsomal fractions of 10- and 30-day-old thyroidectomized and normal rat brain cell-free homogenates

Fraction	Mean ratio: thyroidectomized/control of (cpm/mgRNA)/15 min incubation	
	10-day-old rats	30-day-old rats
Heavy microsomes	0.960	0.97
Heavy microsomes + poly U[a]	1.06	0.96
Light microsomes	0.90	0.94
Light microsomes + poly U	0.83	0.92

[a] Polyuridylic acid.

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thyroid hormone does not give rise to changes in the amount of endogenous messenger in central nervous tissues. Neither did any significant change emerge when these preparations were incubated with an artificial coding agent (polyuridylic acid). The single ribosomes from the bound and free polysomes of the young brain easily combine with polyuridylic acid and stimulated the incorporation of phenylalanine. The rate of polyphenylalanine formation, however, was exactly the same as that of the control.

In the second group of experiments using ribonucleoprotein particles the brains were homogenized in a medium of 0.25 *M* sucrose, 50 m*M* KCl, 5 m*M* $MgCl_2$ and 25 m*M* *Tris* buffer (pH 7.8) at 0 °C and centrifuged at 15,000 *g* for 15 min. The post-mitochondrial supernatant was then treated with 1.3% sodium deoxycholate for 5 min at 0 °C and fractionated in a 10–30% linear sucrose density gradient buffered as above and centrifuged at 63,000 *g* for 2 h at 2 °C. Ribosomal fractions were collected from the bottom of the tubes and the optical density read at 260/280 nm.

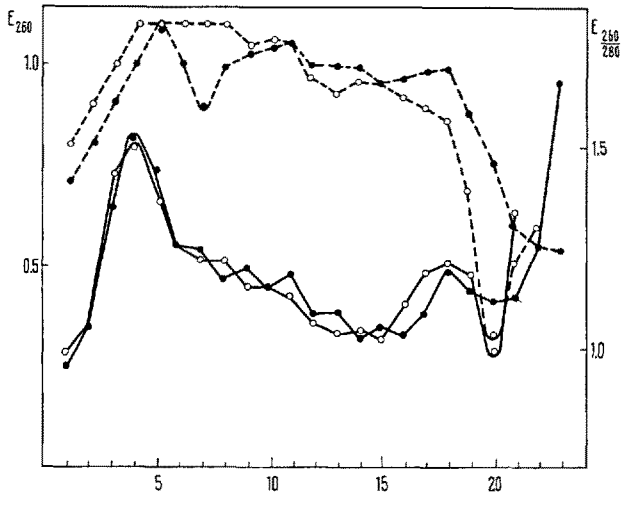
Results similar to those for protein synthesis were obtained whether the ribonucleoprotein aggregates were prepared from the brain of control or of thyroidectomized rats for the identical sedimentation profile on the sucrose density gradient showed no differences in the size distribution of the ribonucleoprotein aggregates isolated from either tissue (Figure).

Since the ribosomes are held together by messenger strands the sedimentation profile may reflect an amount and distribution of messenger in the brains of hypothyroid rats which is similar to the condition characteristic of those in the control animals. It is unlikely that nascent peptide contributed to the heavy aggregates since the UV optical density ratio of 260/280 nm throughout the gradient was at an acceptable level, demonstrating the presence of highly purified RNP aggregates free of contamination by membrane protein. Furthermore, these aggregates completely broke down to single ribosomes as a result of treatment with a trace amount of ribonuclease. A similar observation was made when pulse labelled messenger-RNA fractions associated with the ribosomal-RNAs were sedimented on the sucrose density gradient. It was also demonstrated that the heavy heterogeneous RNA fractions (heterogeneous in respect of their sedimentation rate and labelling time) characteristic of the messenger-RNA in the brain of normal young rats were also detected in the thymroidectoized littermates. Control processes involving the other components of the microsomal preparations of the ribosomes have recently been investigated in the liver of thyroidectomized rats.[9].

The severe histo-pathological and behavioural changes arising as a result of thyroid deprivation contrast strongly with the undamaged function of the microsomes. In view of the current concept of protein synthesis the function of the isolated microsomes reflects the whole molecular process of the renewal of the cell components. A single dose of thyroid hormone given to hypothyroid rats increases the rate of messenger RNA, ribosomal, and mitochondrial protein synthesis in the liver[10,11]. The absence of an effect on isolated nuclei[10,12] indicates that this organelle may not be involved in the earliest stages.

It is difficult to compare the effect of giving an acute dose of thyroid hormone with that of the severe degeneration during prolonged thyroid deficiency of the brain. Recently, however, the primary effect of a single injection of thyroxin on mitochondrial phosphorylation has been detected[13] and changes in the mitochondrial protein synthesis have also been demonstrated[12].

Our in vitro results do not exclude the possibility that in vivo ribosomal protein synthesis or the mitochondria might have been affected. They show, however, that severe hypothyroidism during the critical stages of cerebral development[14] does not appear to damage the polysomes of the brain cells which function at optimal conditions when the necessary components of the cytoplasm, i.e. the ATP generating system, GTP, amino acids and enzymes are provided[15].



Distribution of ribonucleoprotein (RNP) particles in 10-day-old normal and thyroidectomized rat brain. The arrow represents the direction of centrifugation and the abscissa the number of fractions. Optical density of RNP particles of control brain: ●——●; optical density of RNP particles of hypothyroid brain: o——o; optical density ratio $E_{260\,nm}/E_{280\,nm}$ of control brain: ●---●; optical density ration $E_{260\,nm}/E_{280\,nm}$ of hypothyroid brain: o---o.

Zusammenfassung. Im Rattenversuch wurde die Möglichkeit, dass eine Störung in der Proteinsynthese für die geistige Retardierung beim Kretinismus verantwortlich sei, untersucht. Ein Beweis dafür, dass die Funktion neuronaler Polysome durch Thyroidektomie bei der Geburt geschädigt wird, wurde nicht erbracht.

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