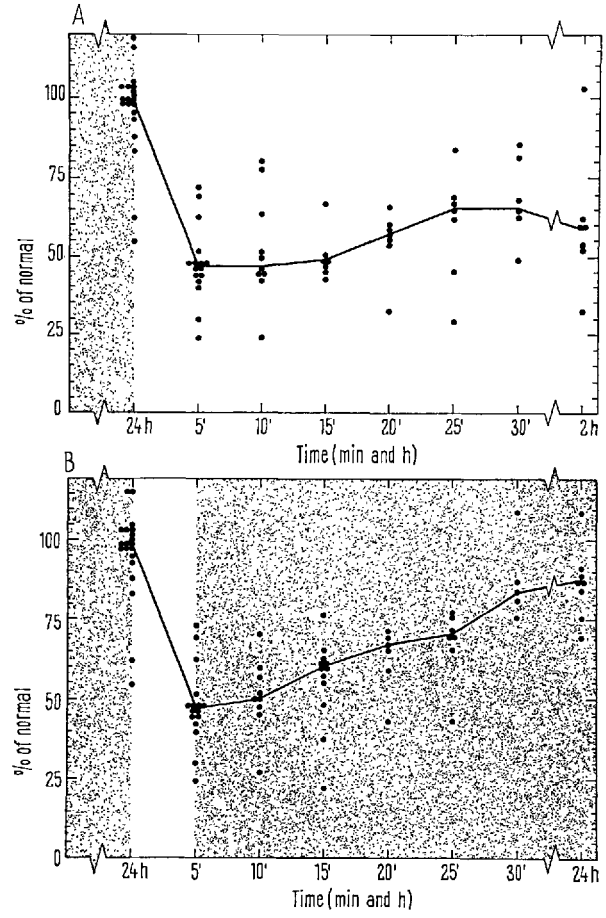
Adrenaline Depletion Induced by Light in the Dark-Adapted Retina

Compounds such as reserpine and many of its congeners, as well as SU 9064 (Ciba), α-methyl-dopa, R04-1284 (Hoffmann-La Roche), and Win 18501-2 (Winthrop), are able to lower the catecholamines content in nervous tissue[1–4]. Electro-shock and cold-shock also produce depletions of catecholamines in the brain[2,5]. Since it has been demonstrated that the levels of catecholamines in the retina depend upon the state of light adaptation[6,7], it was considered of interest to investigate whether a short duration of exposure of a dark-adapted animal to strong light would also modify the catecholamine levels in the retina.

Toads (*Bufo marinus*) weighing 100–150 g were used in the experiments. A relatively strong light was applied in the following way: dark-adapted toads were put into glass containers which were surrounded by 4 light sources, each consisting of a 150 W reflector flood bulb at a distance of 1 m and directed toward the animals. The decapitation of the animals and fast removal (this operation does not exceed 2 min) of the dark-adapted retinae were performed in a dim red light. The tissue was kept frozen prior to the adrenaline determination done by the fluorescence method of DRUJAN et al.[8]. The deproteinization is carried out with trichloracetic acid, the adrenaline was adsorbed on alumina columns, eluted with acetic acid and converted to the highly fluorescent trihydroxyindol derivatives with alkaline ascorbate. The fluorescence was measured in an Aminco Bowman spectrophotofluorometer, the excitation and fluorescence wave-lengths being 430 and 510 nm, respectively. A recovery curve with added adrenaline was made for each run. The adrenaline content in dark-adapted toad retinae, including the pigment epithelium, was found to be 0.60 ± S.D. 0.09 μg/g tissue wet weight. In the light-adapted retinae was 0.49 ± S.D. 0.11 μg/g tissue wet weight.

The experimental results showed that when dark-adapted animals were exposed to strong light the adrenaline content in the retina was rapidly reduced. Exposure to light of 5 min duration depleted over 50% of the total adrenaline content in the retina, including its pigment layer. The Figure shows that after 5 min the adrenaline content of the retina reaches a minimum and that after 5–10 min recovery begins. A study of the repletion process of adrenaline revealed that in the animals exposed to light for 5 min and then placed in darkness (Figure B) an immediate repletion of retinal adrenaline started and after 24 h a level corresponding to 85% of the original dark-adapted value was reached. However, when the animals were kept continuously in strong light (Figure A) the repletion was much slower, starting after 15 min of exposure and reaching a maximum in 25–30 min. At the end of this time a slow decrease was noticed and after a further 2 h the adrenaline content of the retina was about 60% of that found in the dark-adapted state.

When strong light was applied to dark-adapted toads, the depletion of adrenaline from the retina took place instantaneously. HESS et al.[1] showed that the catecholamine depletion produced by drugs depends on the dose used and that the depletion continues as long as the substance is administered. On the other hand, in the case of light-induced adrenaline depletion we have shown that light has an optimum in depletion ability. After 5 min the depletion ceased and recovery began. This suggests that in our experiments a strong light was only able to liberate adrenaline from one specific pool and that there exists an adrenaline fraction which cannot be depleted in this way. The adrenaline recovery in toads placed in darkness suggests that light has a short-acting effect which is not followed by any prolonged biochemical or physiological changes. However, in the case of a continuous exposure to strong light the repletion of adrenaline in the retina ceased after 30 min and the beginning of a



Depletion effect of light on the adrenaline content of the dark-adapted toad retina (including pigment epithelium) and its recovery in subsequent light (A) and darkness (B). Each point represents the value obtained from a pool of at least 6 retinae. Ordinates give the adrenaline value in percentage of the level found in dark-adaptation. Abscissae give time in min and h. Shadowed areas indicate dark conditions, while the other areas correspond to illumination. The adrenaline content in dark-adapted toad retinae with its pigment epithelium (controls) is 0.60 μg/g wet weight.

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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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