ings (which were swollen and contained vacuoles) were observed. Both elements, however, retained their synaptic contacts (Figure 1).

The organization of the IPL relative to receptors suggested a different reaction to prolonged illumination for the various types of synapses. Therefore the synaptic density for each type of contact was determined (Table). Since after 8 h daily illumination for 2 months (group IA) the visual receptors were well preserved, the synaptic densities in the IPL of this group were taken as the reference value and data of all other groups were expressed as a percentage of this value (Figure 2). The density of the amacrine/amacrine contacts was only slightly affected by the length of light exposure, and a mild decrease in the density of dyads occurred. However, the density of amacrine/bipolar synapses was considerably decreased after prolonged illumination, and that of amacrine/dendritic contacts was significantly increased (Figure 2).

Discussion. Since bipolar elements are directly connected with visual receptors they can be expected to reflect the degree of damage to the receptors. This transsynaptic effect manifests itself by a loss of contacts in which the bipolar endings participate either pre- or postsynaptically (amacrine/bipolars and dyads). Similarly, severing the optic nerve induced transsynaptically a decrease in the synaptic density of the visual cortex[12].

Amacrine cells are not directly connected with the receptors, so that the loss of the latter does not affect contacts involving exclusively amacrine endings (amacrine/amacrine synapses). However, the amacrine endings become, by the loss of bipolar endings, deprived of one of the main sites of termination. They seem to make new contacts instead on dendrites and the formation of amacrine/dendritic synapses is inversely related to the density of bipolar synapses. Such a translocation would require formation of new dendritic surface to accomodate the amacrine endings. Since bipolar terminals contact, besides the amacrine endings also the ganglion cells and their dendrites, both amacrine and ganglion cells become partly denervated after the degeneration of the bipolar terminals. Denervated tissue has been found to be a strong stimulus for growth and can cause either axonal sprouting or a change in dendritic morphology[13].

Two systems in the rat brain can serve as examples of the first mechanism, axonal sprouting. The septal nuclei receive 2 main inputs: from the hippocampus and from the hypothalamus. A long-term lesion in one system induces the spread of terminals of the other system to sites originally occupied by the first, and vice versa[14]. Similar synaptic rearrangement occurs in the superior colliculus after long-term unilateral enucleation[15]. Altered dendrites of anterior horn cells observed proximal to the site of hemitranssection of the spinal cord could serve as an example of the second mechanism[16]. Varicosities developing on the dendritic tree increase the potential synaptic sites. Since the amacrine and ganglion cells become partially denervated structures, either of the above mechanisms could then trigger a process resulting in an increased number of amacrine/dendritic contacts.

Zusammenfassung. Nachweis, dass die Netzhautrezeptoren der weissen Ratte durch Lichtexposition beschädigt werden. 16 h Beleuchtung täglich verursacht nach 6 Monaten eine drastische Rezeptorenabnahme, wodurch die synaptische Organisation der inneren plexiformen Schicht geändert und die Dichte der Synapsen vermindert wird.

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On the Presence of Glycogen in the Rhombencephalon of Chick Embryos During Development

Although the distribution of glycogen in the nervous system of adult and embryonic animals has been investigated in many vertebrate species, few studies on its distribution in birds are available. GAGE[1], using the iodine reaction and salivary digestion, found that glycogen appears late in the development of the nervous system in chick embryos with respect to Petromyzon and Amblystoma.

Glycogen is abundant in the medulla oblongata and the lumbosacral region of the spinal cord from the 6th to the 10th day of development, although its precise location has not been reported. GAGE states that the production and utilization of glycogen is a property that the protoplasm of nervous cells shares with the protoplasm of any other cell. Glycogen is essential to nervous tissue, as it is to all other tissues during histogenesis, especially in the transition to their definitive and functional state. Glycogen is an energy reserve, and its appearance in all vertebrate tissues depends, at least in part, on the time at which the tissue becomes functional. It therefore appears early in the development of Amblystoma and later in the chick embryo.

In the nervous system of higher vertebrates, Gage did not find demonstrable quantities of glycogen after the embryonic period, since after this stage liver and muscle assume the principal glycogenetic functions.

The accumulation of glycogen in nervous tissue during the proliferative process, and its disappearance during histological differentiation as reported by GAGE[1] in the chick embryo and by JANOSKY and WENGER[2] in Amblystoma, has not been confirmed by SACCANI and MARINI[3] in urodele amphibians.

MASAI[4] found seasonal variations of glycogen content in the nervous system in amphibians, fish and reptiles. In

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birds (Columba, Passer, Uralancha) seasonal variations were not found, but glycogen was found just under the ventral surfaces of the mesencephalon and rhombencephalon. The intercellular substance (neuropile) of the brain in birds gives a weaker glycogen reaction than that of poikilothermic animals. This is interpreted as due to the more intense aerobic respiration in the bird brain than in mammalian brain. Birds give a weak reaction for glycogen in large areas of the central nervous system when compared to amphibians, fish and reptiles. However, some nervous cells in birds contain glycogen and also a PAS-positive substance resistent to digestion by salivary amylase.

The data regarding glycogen in the central nervous system of birds are scanty, with the exception of those of GAGE[1] on embryos and of MASAI[4] on adult animals.

Since we have observed a characteristic localization of PAS-positive material in the rhombencephalon during our studies on the development of the otic bud in chick embryos, we have carried out a systematic histochemical study on glycogen localization during the development of the rhombencephalon.

Materials and methods. The investigation was carried out on chick embryos after 3, 4, 5, 6, 7, 9, 11, 13, 15 and 18 days of incubation; 6 embryos were fixed in each developmental stage, 3 in Zenker's fixative and 3 in Rossman's fixative. In the early stages of development, whole embryos or the whole cephalic region were fixed. In more advanced stages (from 13 to 18 days) the brain and part of the spinal cord were dissected prior to fixation.

After inclusion in paraffin, 7 micron sections were cut and alternate sections were mounted on slides.

Some of the slides were stained directly with PAS, others were first digested in 1% α-amylase in phosphate buffer pH 6 for 1 h at 37 °C (LISON[5]), after which the PAS reaction was carried out.

In both cases sections were counterstained with 1% aqueous Toluidine blue for contrast.

Results. We observed an accumulation of glycogen in the bud of the medulla oblongata and pons, located on the median and paramedian line, extending from the dorsal face of the pons and medulla oblongata to the anterior ventral fissure.

Glycogen, which appears distributed in droplets, is particularly abundant from the 7th to the 13th day of development; however, a small quantity is observed in 4th and 15th day embryos. No PAS-positive material was observed in 3rd day embryos, and in only a few 18th day embryos were small quantities of glycogen observed. Zenker's is the fixative of choice since more glycogen was seen.

After predigestion with α-amylase, the embryos of all stages resulted completely negative, indicating that the material stained by PAS was glycogen. It is localized in the ependymal cells of the median and paramedian line of the bud of the medulla oblongata and pons, in their prolongation and in the surrounding neuropile.

In many cases drops of PAS-positive material are also present in the endings of the glial processes which make up the external limiting membrane.

What is the significance of the accumulation of glycogen in the rhombencephalon in these developmental stages? An accumulation of glycogen in the ependymal cells of the ventral sulcus of the rhombencephalon[3] and in the ependymal cells of the spinal cord[4,6,7] has been shown in Triturus.

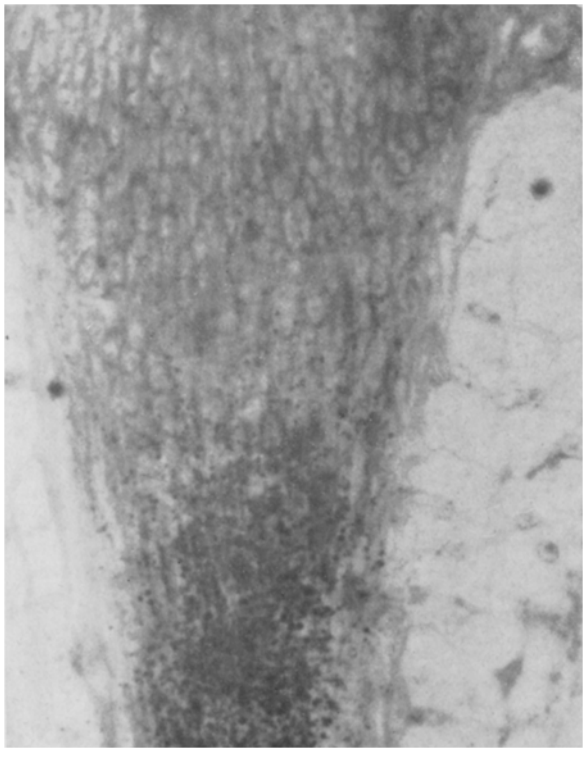

Fig. 1. Section of medulla oblongata of 7 days chick embryo. PAS reaction. Note the presence of PAS-positive granules in the ependymal cells, in their prolongations and in the surrounding neuropile. × 560.

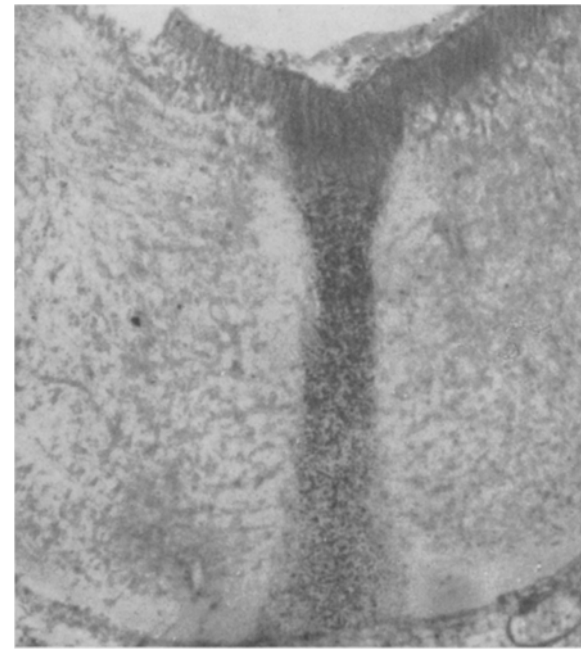

Fig. 2. 9 days chick embryo. Note the accumulation of glycogen in the median and paramedian line of the bud of the medulla oblongata. PAS reaction ×140.

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SHIMIZU[8] has reported the occurrence of variable quantities of glycogen in the area postrema of the mammalian brain located in the intercellular fibrillar structures around small vessels.

KAPPERS[9] describes in Amblystoma mexicanum the presence of glycogen in the cytoplasm and in the long thin processes of the ependymal gliocytes of the ventricular wall, especially in the lateral ventricle. The endings of these processes, which make up the external limiting membrane, also contain glycogen. He thinks that the paraphysis cerebri secretes glycogen in the ventricular cavity in Amblystoma. Such glycogen would then be adsorbed by the ependymal gliocytes and carried along their processes into the nervous parenchyma.

The transporting capacity of ependymal gliocytes of Amblystoma[9] and of guinea-pigs[10,11] has been demonstrated by inoculating India ink into the ventricles.

The glycogen deposited in the nervous parenchyma would have both a metabolic (as an energy source) and a plastic function.

The evidence reported for amphibians and mammals suggests that the accumulation of glycogen in the ependymal cells and in the surrounding neuropile of the bud, of the medulla oblongata and pons in birds can be correlated with the transport of glycogen, formed either by direct production by these cells or by adsorption.

This transport can be considered as a trophic function with regard to the nervous parenchyma. There is expression of a prevalence of anaerobic metabolism[12] in this area at the time of neuron differentiation, when a large energy supply is needed. Since anaerobic glycolysis contributes a low energy yield, a large glycogen reserve is required to satisfy the energetic needs.

Riassunto. Viene condotto uno studio sulla distribuzione del glicogeno nel romboencefalo dell'embrione di pollo durante lo sviluppo. I risultati delle indagini condotte hanno messo in evidenza una caratteristica localizzazione del glicogeno nell'abbozzo del ponte e del bulbo più evidente in embrioni dal 7° al 13° giorno di incubazione. Tale reperto viene discusso nel suo significato.

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Intracellular Localization of Glutamine-Aminohydrolase in Normal and Malignant Tissues

Glutamine aminohydrolase has been reported to be a mitochondrial enzyme in normal tissues[1-4] and is present in various organs of the animal body[5]. Glutaminase in the tumour tissue has been found to be a dialysable enzyme[6,7] and a possible leakage of this enzyme from the transplanted tumour into the blood stream of the animal has also been postulated[8]. This lead us to investigate the intracellular localization of this enzyme in tumour and the host tissues of the tumour transplanted animals.

Materials and methods. A mouse fibro-sarcoma (MFS)[9], Ehrlich's ascites tumour and Sarcoma 180 strains used, were maintained in Swiss mice in our laboratories. A 10% (w/v) tissue homogenate was prepared in 0.25 *M* sucrose in cold and centrifuged at 3000 *g* for 10 min using Sorvall RC-2B centrifuge. The supernatant thus obtained was recentrifuged at 12,000 *g* for 15 min to obtain mitochondrial and supernatant fractions. The supernatant was collected and mitochondrial pellet was resuspended in 0.25 *M* sucrose and centrifuged at the same speed for 10 min to get a washed mitochondrial preparation. Glutaminase assays were done, as described by BRAGANCA et al.[10]. Incubation mixtures used were as described by HOROWITZ and KNOX[11] for the liver and kidney type glutaminases. The incubation mixture used for tumour enzyme assay was as used for the kidney[11]. Protein estimations were carried out according to the method of LOWRY et al.[12]. Succinic dehydrogenase was assayed at 400 nm by the method of SLATER and BONNER[13], using a Zeiss spectrophotometer. S.c. injection of Actinomycin-D (100 γ/kg body wt.) was started 30 min after the transplantation of tumour and was continued on every alternate day until the tumour attained maximum size. The animals were sacrificed 24 h after every injection of Actinomycin-D.

Results. It can be observed from Table I that the glutaminase activity was mainly found to be present in the supernatant fraction in both types of tumours studied. There was, however, some difference in the 2 tumours, in that the enzyme activity was completely

Table I. Distribution of glutaminase activity in tumour cell-fractions

Tumour type	Homogenate	Mitochondria	Supernatant
MFS	3.0	0.0	3.3
Ehrlich's solid tumour	4.5	1.0	3.8

All values expressed as μmoles ammonia produced/mg protein/min and are an average of 4 experiments.

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