nitrogen for 10 min, the coverslip was flipped off quickly with a sharp scalpel and the slide was fixed in absolute methanol for 10 min at room temperature and dried in air. The slides were covered with diluted (1:1) Sakura NR-M_2 emulsion. After 2–4 weeks exposure, the slides were developed for 7 min at 17°C in Konidol-X developer and stained with hematoxyline eosin or May-Grünwald gimsa. For electronmicroscopical studies, the blastocysts containing ^{3}H-uridine were prefixed in 2.5% glutaraldehyde in 0.1 *M* PO_4 buffer (pH 7.3), postfixed in 1% osium tetraoxide, dehydrated, and embedded in Epon 812. Thin sections of the labelled blastocysts were cut on a Porter-Blum MT-II ultramicrotome. The sections were coated with Sakura NR-H2 nuclear track emulsion and the coated grids were stored for 4 weeks. The autoradiographs were then developed with Konidol-X, acid fixed and rinsed with distilled water. The sections were stained with lead citrate.

All the blastocysts collected on day 5 showed a heavy incorporation of ^{3}H-uridine. During delayed implantation, the incorporation of the precursor was seen in all parts of the blastocysts. 18 h after estrogen treatment, all the blastocysts showed marked increase in the incorporation of the precursor. It was particularly heavy in the parts of inner cell mass. Electron-microscopically silver grains were seen over the nucleolus, but the cytoplasm was unlabelled. The present data in the rat showed that the pre-implantation blastocyst synthesized RNA utilizing exogenous ^{3}H-urisine. Synthetic activity in the blastocysts was evident when the zona pellucida was still intact. In the delayed blastocyst, moderate RNA and protein synthesis with minimal DNA synthesis were observed. Jacobson et al.[4] showed that moderate RNA synthesis occured in the delayed blastocysts in vitro and estrogen administrated 30 h before autopsy markedly increased nuclear and nucleolar RNA synthesis. The mechanism by which estrogen initiates implantation of blastocyst has not been fully investigated. However, it has been suggested that RNA synthesized under the influence of estrogen may mediate the necessary requirement for further cell differentiation of the blastocyst and invasion of the endometrium by the trophoblast cells. Further electronmicroscopical autoradiographic studies on the pre-implantation embryos are going to investigate in our laboratory[5].

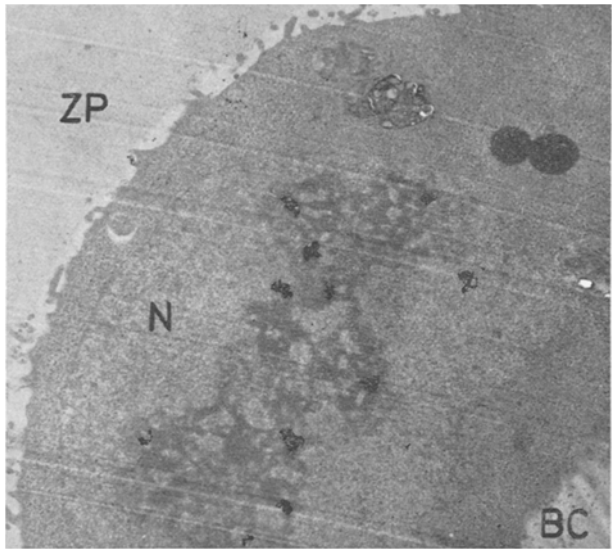

Fig. 3. Electronmicroscopical radioautography of blastocyst of rat on day 5 showing the incorporation of ^{3}H-uridine mostly in nucleus. ZP: zona pellucida; N: nucleus; BC: blastocoelic cavity. × 20,000.

Zusammenfassung. Die Rattenblastocyste synthetisiert, kurz bevor sich die Membrana pellucida auflöst, Nukleinsäuren, wie aus der Aufnahme von Uridin durch die Zellkerne elektronenmikroskopisch und autoradiographisch gezeigt wurde. Bei verzögerter Implantation wird Nukleinsäure in geringerem Ausmass aufgebaut.

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Synaptic Junctions in the Developing Chick Optic Tectum

The distribution of retinal fibres to the developing chick optic tectum[1–6] and the cytodifferentiation of the tectal neurons during embryogenesis (review of the literature by LaVail and Cowan[7]) have been extensively investigated. No data, however, are sofar available on the tectal synaptogenesis. In the present paper we report on the time of appearance and the distribution of the synaptic junctions in the optic tectum of the chick embryo.

Material. Electronmicroscopic observations were carried out on glutaraldehyde-osmium fixed samples taken from the anteroinferior, dorsomedial and posterodorsal quadrants of the optic tectum of chick embryos aged from the 3rd to the 20th incubation day. The specimens were prepared for examination in a Siemens Elmiskop IA electron microscope according to the technique described in a previous paper[8].

Results and discussion. Asymmetrical junctions between nerve endings and different dendritic segments were occasionally observed in a specimen from an 11-days embryo; these were constant findings, however, from the 12th incubation day (stage 38 according to

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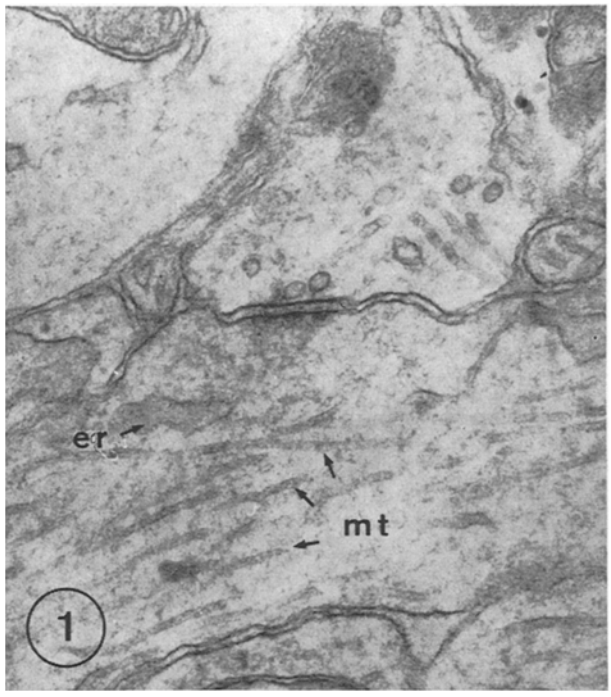

Fig. 1. A small bouton synapsing on a horizontal dendrite in the layer III. A few synaptic vesicles are present in the pre-synaptic terminal at the 12th incubation day. er, endoplasmic reticulum; mt, microtubules. ×60,000.

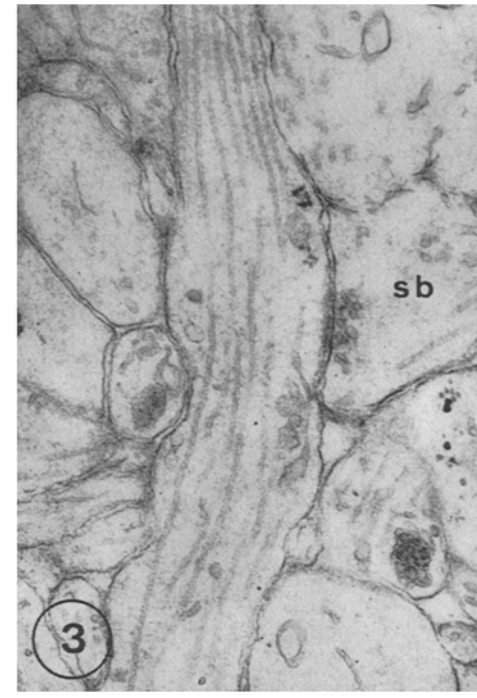

Fig. 3. Axodendritic junction in the layer X. A small bouton synapses on a dilatal portion of a radially oriented dendrite. ×40,000.

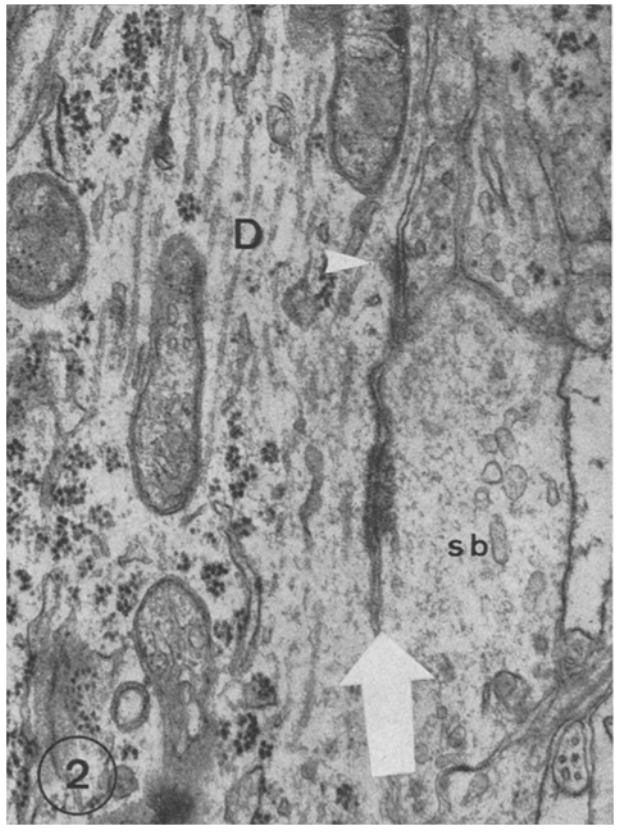

Fig. 2. A synaptic junction between a medium-sized bouton (sb) and the initial segment of a radial dendrite (D) in the layer VI at the 12th incubation day. White arrows point to 2 active zones. ×40,000.

HAMBURGER and HAMILTON[9]) onwards; they could be recognized in the following layers (in accordance to the terminology employed by LAVAIL and COWAN[10]): a) layers III and IV: junctions between small boutons and horizontally oriented dendrites (Figure 1): b) layers VI and VII: junctions between medium-sized boutons and the initial segment of radially oriented dendrites of piriform cells (Figure 2); c) layers X and XI: junctions between small boutons and peripheral segments of radially oriented dendrites (Figure 3).

The component morphology of these junctions was virtually uniform, irrespective of their location. The pre-synaptic terminals were in form of pearshaped boutons with a few vesicles of 40–60 nm, lying against a thickened part of the pre-synaptic membrane. The synaptic cleft was 15–30 nm in width and in extensive communication with the extracellular space. The post-synaptic membrane was parallel to the pre-synaptic one, both running straight and was thickened by a lining filamentous material. The peripheral part of pre-synaptic terminals often contained large empty vesicles as commonly present in the growing tips of axons in the same layers. Although only a few dendrites of group a) could be followed to their emergence from large horizontal neurons of the future stratum griseum centrale, their close morphological uniformity points to this major origin. The junctions detected in these layers were more numerous than those in other layers at the very first moment of appearance. The dendrites in group b) junctions continued the outer pole of middle-sized piriform cells in layer VI and of small piriform cells of layer VII. The dendrites of group c) junctions were thinner and synaptic terminals mostly contacted

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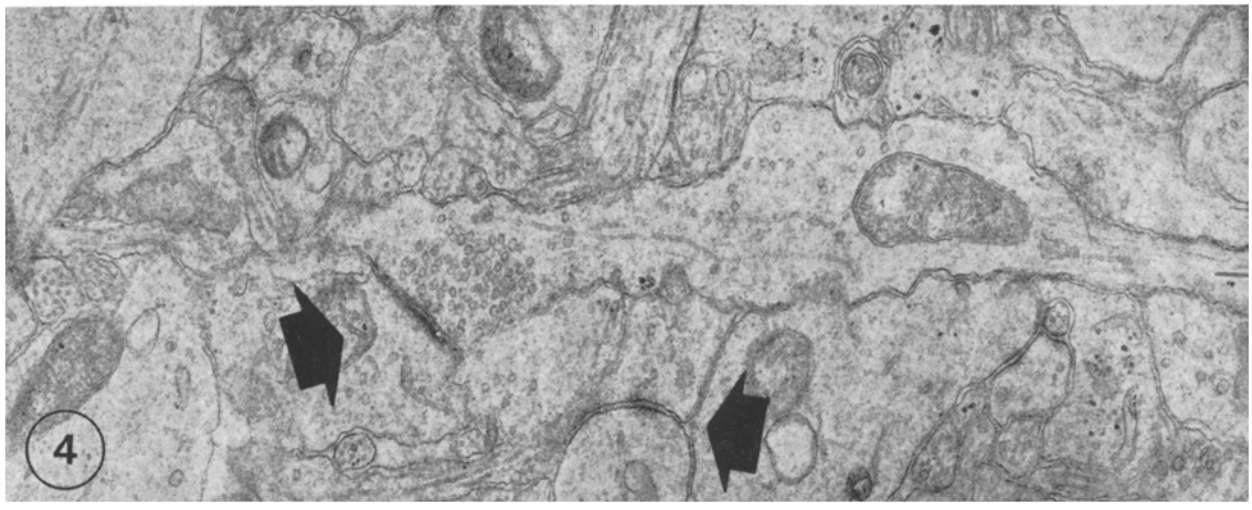

Fig. 4. Two synaptic junctions (arrows) in the layer XI of the optic tectum from an 18th incubation day chick embryo. ×31,000.

typical dilated portions. A small number of terminals in this group could be recognized as segments of fibres running in the stratum opticum and may be considered as retino-tectal synapses: the presence of synaptic terminals from other sources, however, cannot be excluded. The origin of the pre-synaptic terminals in group b) and c) junctions cannot yet be identidied. Whereas fibres of retinal origin are certainly not involved in the synapses of group a)[1, 10], they could represent at least part of those of group b). A quantitative evaluation of changes in synaptic density has not yet been done: however, synaptic junctions were few until the 14th incubation day; during the 14th–18th day period similar axodendritic junctions were observed throughout the other layers. A critical increase in number was then observed between the day 18 and the first hours after hatching (Figure 4).

Our observations may be summarized thus: the synaptic junctions in the developing chick optic tectum displayed the essential features of developing synapses previously observed in other parts of the CNS[11–13]. They appear at only a relatively late stage of tectal neuron maturation, particularly in the case of the large multipolar cells of the stratum griseum centrale. They could be recognized when cell proliferation has ceased in the neural epithelium[7] and the essential steps in the distribution of the migrating neurons into the 6 major strata of the tectum[14] have been accomplished. In addition, a marked delay between the time of arrival of retinal fibres and the appearance of retino-tectal synapses was observed.

Although the identification of most pre- and post-synaptic components is still difficult in many junctions, one may safely claim that fibres of very dissimilar origin (such as those of groups a) and c)) form synaptic connections in the same period of time.

Lastly, it should be noted that the first junctions between retinal fibres and the tectal neurons appear at an earlier stage than that in which the formation of intra-retinal synapses was demonstrated[15].

Riassunto. Giunzioni sinaptiche compaiono in differenti strati plessiformi del tetto ottico dell'embrione di pollo, a partire dal 12° giorno di incubazione: aumentano di numero fra il 12° ed il 18° giorno e, in maniera critica, fra questo e le prime ore dopo la schiusa.

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Human Spleen Inhibitor of Leukaemic Cell Growth

In recent years much information has been obtained to indicate that certain tissues contain substances termed chalones, which depress or inhibit cell division acting in a negative feedback mechanism[1–4].

The possibility that the spleen may produce a diffusiable substance with inhibitory or cytotoxic effects on haematopoietic cells has been suggested on the basis of clinical observations. We have been investigating whether or not such a factor was present in the spleens of 2 patients suffering from chronic myelocytic leukaemia (CML), 2 with primary hypersplenism and 4 normal persons undergoing surgery as a result of trauma. For comparison, 2 bovine spleens were also studied. Another important objective of our project was to develop long-term cultures of human CML cells and multiple myeloma cells to test the effect of the splenic inhibitor (SI). Each of the spleens were surgically removed, processed and tested separately. The spleens were homogenized in distilled