of X-ray irradiation, and does not constitute any specific alteration per se. This objection may be waved, however, on the basis of such alterations as the splitting and formation of blebs of the internal leaflet of the nuclear membrane. Moreover, the fixation in situ did not favour the mechanical damage of the cell due to the preparative procedure. The chromatin of irradiated KIRKMAN-ROBBINS tumour cells did not demonstrate any changes suggestive of the separation of histone from DNA contrary to findings on irradiated thymocytes[2,3]. It may be supposed that the irradiated chromatin of KIRKMAN-ROBBINS tumour cells remains intact because nuclear membrane of these cells lacks the respiratory enzymes, but we cannot furnish any experimental data for this assumption. It is noteworthy, however, that in tumour cells, as in other cellular systems[8], the primary target of the ionizing radiation is a nuclear membrane.

Riassunto. L'osservazione al microscopio elettronico del tumore di KIRKMAN-ROBBINS irradiato con raggi X dimostrava danni nucleolari, frammentazione, assottigliamento e scollamento dei foglietti della carioteca. Inoltre, nel citoplasma si osservava cromatina fuoriuscita dal nucleo.

W. DJACZENKO[9], H. STARZYK and Z. RZUCIDŁO

Department of Immunologiy of Tumours, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw (Poland), 29 May 1972.

[8] H. BRAUN, Strahlentherapie *122*, 248 (1963).

[9] Present address: Istituto di Microbiologica dell'Università, I-00100 Roma (Italy).

Autoradiographic Studies on the Rat Embryos in the Pre-implantation Stages

Although considerable attempts have been made to investigate the metabolic process of the early mammalian embryos in the preimplantation stage, our understanding of the events is rather fragmentary[1,2]. The present investigation was designed to explore autoradiographically the pattern of nucleic acid synthesis in the blastocyst during normal and delayed implantation stages in the rat. The rats used were adult virgin females of the Wister Imamichi strain ranging in weight from 180 to 240 g. Cyclic females in pro-estrus were caged with normal males and left overnight. Delayed implantation was induced following the method of COCHRANE and MEYER[3]. On the third day of pregnancy, mated female rats were bilaterally ovariectomized and each rat was treated with 4 mg of progesterone subcutaneously per day. The precursor was instilled directly into the uterine lumen 15 min before autopsy from the tubal end. The precursor used was ^{3}H-uridine (specific activity 5.0 c/mmole, Daiichi Kagaku Yakuhin K.K.; 5 μc/uterine horn in 0.05 ml sterile physiological saline). In the course of normal pregnancy, rats were autopsied 15 min after instillation of the precursor on the fifth or sixth day of pregnancy. In the delayed implantation the precursor was instilled into the uterine lumen of the ninth day of delayed implantation and in another group, 1 μg of estradiol-17β was injected s.c. on the eight day of delayed implantation and after 16 h the precursor was instilled into the uterine lumen. 2 or 3 blastocysts were together transferred to a slide glass and squashed gently with a cover slide. After the slides were rapidly frozen in liquid

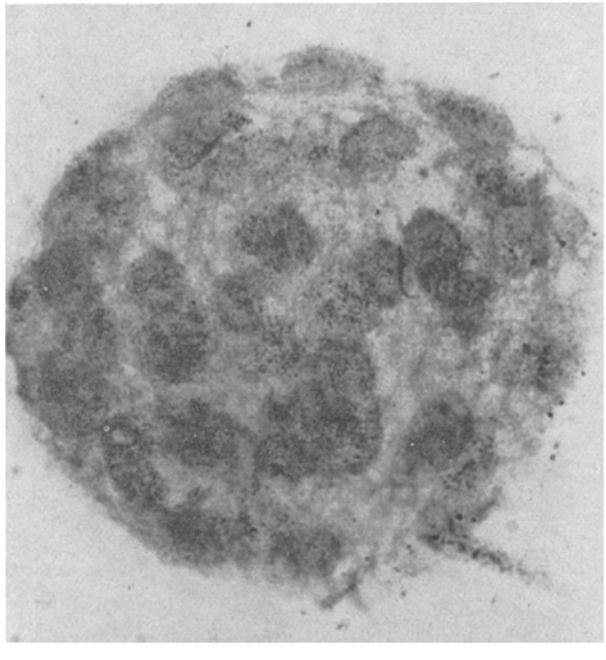

Fig. 1. Blastocyst of rat on day 5 showing the incorporation of ^{3}H-uridine. ×600.

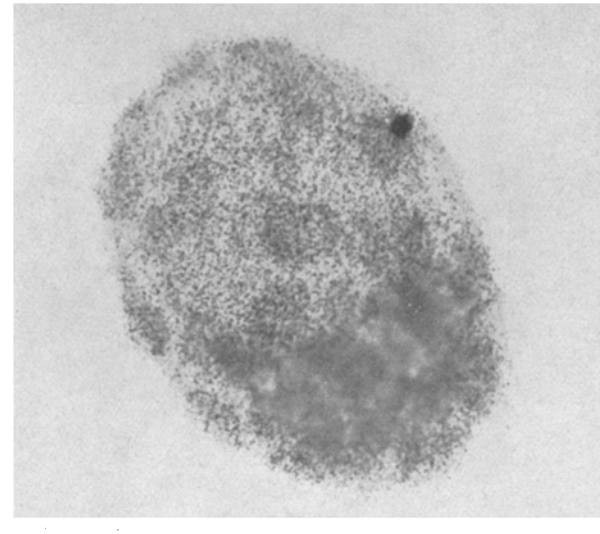

Fig. 2. Blastocyst of rat on day 5 showing the incorporation of ^{3}H-phenylalanine uniformly in all regions. ×600.

[1] S. SUZUKI, M. INOUE, Y. HAMADA and K. KAMI, 4th Annual Meeting of the Society for the Study of Reproduction (Abstr.), Boston 1971.

[2] M. INOUE, Acta obstet. gynec. Japonica *18*, 4 (1972).

[3] R. L. COCHRANE and R. K. MEYER, Proc. Soc. exp. Biol. Med. *96*, 155 (1957).

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].

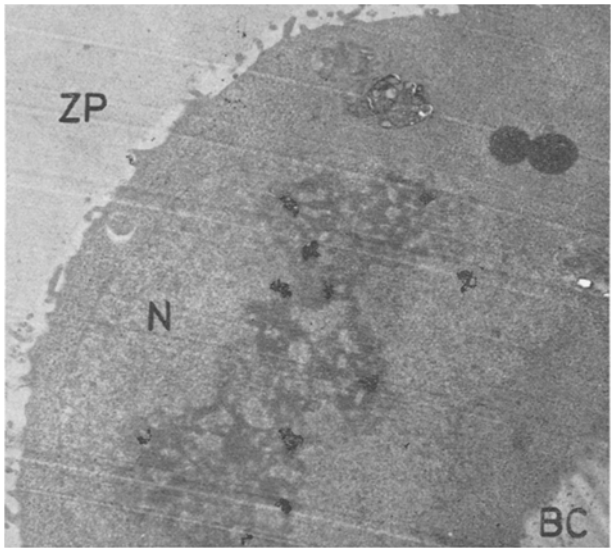

Fig. 3. Electronmicroscopical radioautography of blastocyst of rat on day 5 showing the incorporation of ^{3}H-uridine mostly in nucleus. ZP: zona pellucida; N: nucleus; BC: blastocoelic cavity. × 20,000.

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.

S. SUZUKI, M. INOUE, H. MIZUMOTO, T. FUJIWARA, K. ITAKURA, Y. HAMADA, H. MITANI, T. ITOH, K. SEKI, K. KAMI and M. HAMADA

Department of Obstetrics and Gynecology, School of Medicine, Keio University, Tokyo (Japan), 23 May 1972.

[4] M. A. JACOBSON, M. K. SANAYL and R. K. MEYER, Endocrinology *86*, 982 (1970).

[5] This work was supported by Grants Nos. M67-79, M69-144 and M72-80 from the Population Council, New York, USA.

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

[1] F. TELLO, Trab. Lab. Res. biol., *21*, 1 (1923).
[2] C. R. DELONG and A. J. COULOMBRE, Expl. Neurol. *13*, 351 (1965).
[3] S. LEGHISSA, Z. Anat. EntGesch. *120*, 247 (1958).
[4] F. A. HAMDI and D. WHITTERIDGE, Q. J. exp. Physiol. *39*, 111 (1954).
[5] J. I. MCGILL, T. P. S. POWELL and W. M. COWAN, J. Anat. *100*, 5 (1966).
[6] C. R. DELONG and A. J. COULOMBRE, Expl. Neurol. *16*, 513 (1967).
[7] J. H. LAVAIL and W. M. COWAN, Brain Res. *28*, 421 (1971).
[8] D. CANTINO and L. SISTO DANEO, Brain Res. *38*, 13 (1972).