stained using the periodic acid fuchsin technique and counterstained with haematoxylin. 6 animals were used of the hybrid type and 8 of the inbred; 500 tubule cross-sections were scored per animal. The observer did not know the identity of the animals.

Table III. Duration of each stage of the seminiferous epithelium based on the assumption of a cycle length of 207 h

Stage	Duration of stage (h)			
	C3H inbred mice	F_1 C3H ♂ C57 Bl ♀	F_1 ARR ♂ C3H ♀	F_1 C3H ♂ 101 ♀ (OAKBERG)
I	12.4	13.6	15.6	22.2
II	10.4	7.3	16.7	18.1
III	26.7	25.9	30.0	8.7
IV	17.8	13.3	19.9	18.6
V	12.4	10.7	15.9	11.3
VI	9.7	10.4	9.7	18.1
VII	14.9	13.3	10.7	20.6
VIII	27.5	31.2	15.9	20.8
IX	26.5	29.2	27.4	15.2
X	14.1	15.8	12.3	11.3
XI	13.7	15.3	12.9	21.4
XII	21.1	21.1	20.0	20.4

Results. The results obtained are reported for the C3H inbred mice in Table I. Because of an unexpected, very high frequency of stage III, we decided to repeat the scoring of the animals at various times. The results obtained are reported in the same table.

The first scoring was done by determining the frequency of the various stages in 500 randomly selected cross-sections from each of the 8 mice used for this experiment. An additional scoring of 1000 tubule cross-sections from each of 2 mice used in a previous experiment was made. Since no difference in the tubule frequency was observed, the results were pooled together. From the results shown in Table I, there is a very good reproducibility in the tubule frequency determination.

In Table II are reported the results obtained using the F_1 hybrid mice. Also for these animals a higher frequency of tubules in stage III was found. Some significant variations in frequency for some stages such as stage II were also observed. Assuming that the duration of 1 cycle is 207 h, as determined by OAKBERG, we have evaluated the duration of each stage and the values obtained are reported in Table III. Differences in the duration of the various stages have been found for the 3 strains of mice used and also between our values and the ones reported by OAKBERG for mice F_1 hybrid between C3H ♂ and 101 ♀[1] (see Table III).

Consequently, it seems necessary to evaluate the frequency of the tubules at the different stages before any study of the radiation sensitivity of selected spermatogonial cells can be carried out in an accurate way.

Further Studies on the Effect of 6-Hydroxydopamine in Retinal Development

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Summary. Neonatal albino rats treated with 6-hydroxydopamine revealed depletion of rhodopsin and monoamine oxidase in their retinas.

6-Hydroxydopamine, a drug which causes prolonged depletion of the catecholaminergic terminals[2–4], has been receiving increased attention. In 1974, YEW et al.[5] reported malformations in the developing chick retinas after treatment with this agent. This effect may be attributed to the depletion of the catecholaminergic neurotransmitters. This is a study made to evaluate what effect this agent might have on the developing mammalian retina, since catecholamines were also reported in this system[6–8]. The model of the retinas of albino neonatal rats was chosen, because the young rats do not begin to develope inner segments in the retinas until around the 8th or 9th postnatal day, and outer segments until around the 14–15th postnatal day[9].

Materials and methods. Simonsen albino neonatal rats were injected s.c. with 0.2 mg of 6-hydroxydopamine (Sigma, USA) in 0.1 ml ascorbic acid vehicle solution (0.1% ascorbic acid in chilled saline) in the neck region on the 9th postnatal day and injected with a booster dose of 0.1 mg 6-hydroxydopamine in 0.1 ml ascorbic acid vehicle solution in the same region on the 14th postnatal day. Control animals were injected with only 0.1 ml ascorbic acid solution twice following the above schedule. Both the experimental animals and the control

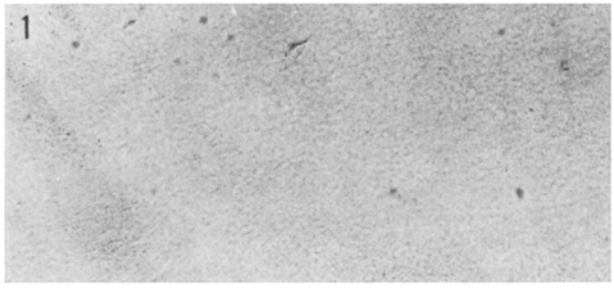

Fig. 1. Monoamine oxidase (MAO) histochemical reaction on the 6-hydroxydopamine-treated retina (wet mount). Note very few granular deposits (positive MAO sites). × 250.

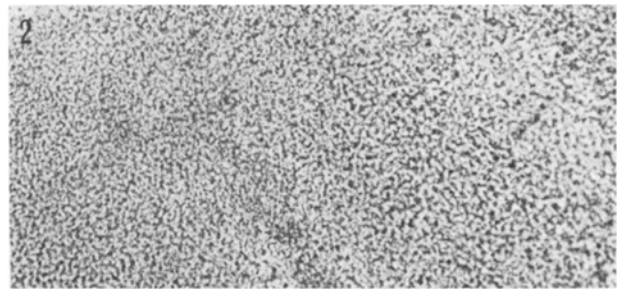

Fig. 2. Monoamine oxidase (MAO) histochemical reaction on the control retina (wet mount). Note a lot of granular deposits (positive MAO sites). × 250.

animals were kept under the same lighting conditions. At the end of the first postnatal month, both treated and untreated rats were decapitated, the retinas of each group were dissected out and divided into 3 subgroups. The 1st subgroup retinas were processed for light microscopy (Hematoxylin and Eosin staining). The 2nd subgroup retinas were used for monoamine oxidase histochemistry[10] and the retinas after the reaction were mounted (wet mount) on the slides and observed under the light microscope to determine the intensity of the reaction. The 3rd subgroup retinas were cut into equal size pieces for biochemical rhodopsin determination[11].

Results. Histologically, no conspicuous difference was observed between the treated and untreated retinas. All the retinal layers were observed in both the control and experimental retinas. Focal areas of pyknosis, karyorrhexis, karyolysis and necrosis were absent in the nuclear layers of the treated and untreated retinas. However, the nuclear layers (expecially the outer nuclear layer) in the control retinal sections were more basophilic than the corresponding layers of the treated retinas under the routine Hematoxylin and Eosin staining. Histochemical demonstrations of monoamine oxidase (MAO) showed considerable deviation in amount between the treated and untreated retinas. There was a considerable depletion of this enzyme in the 6-hydroxydopamine-treated retinas as compared with the control retinas (Figures 1 and 2).

Biochemical determination of rhodopsin revealed a concomitant depletion of the visual pigment in the 6-hydroxydopamine-treated retinas (69.7% transmission as measured by the spectrophotometer at 505 nm) as compared with that of the control retinas (45.2% transmission as measured by the spectrophotometer).

Discussion. Our results indicated that 6-hydroxydopamine caused similar effects on the development of the visual system of the neonatal rats as that in the embryonic chick retinas[5], although the effect is less drastic in the mammalian model. The depletion of MAO in the treated retinas supports the idea there has been a decline in amount in catecholamines in those specimens. The concomitant depletion of rhodopsin in the treated retinas, on the other hand, appears to strengthen the former hypothesis that outer segment formation (i.e. rhodopsin formation at the same time) may be depended on the amount of catecholaminergic neurotransmitters.

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Intercellular Bridges of Chick Blastoderm Studied by Scanning and Transmission Electron Microscopy[1]

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Summary. Chick blastoderms were studied by scanning and transmission electron microscopy to identify by both methods a type of thread-like structure lying on the epiblast. The structure was identified by transmission microscopy as a long telophase bridge containing mid-body and spindle remnant. It apperas to provide cytoplasmic continuity between only 2 cells.

In the study of the chick blastoderm by scanning electron microscopy (SEM) a number of investigators have noted the appearance of long, thread-like cords stretching along the surface of the epiblast, and connecting together 2 cells frequently located some distance apart[2-6]. Jacob et al.[6] observed such 'connecting cords' bridging up to 5 cells and regarded them as a special form of intercellular connection. They thought it likely that the connecting cords resulted from the separation of previously contiguous cells. Bancroft and Bellairs[3] thought it possible that the threads may function to provide communication between cells that are not immediate neighbours.

Unfortunately the true identity of these structures has not been established previously because TEM micrographs were not usually prepared and those published by Bancroft and Bellairs[3] were not of adequate quality to provide clues to the identity of the structures. In light of the uncertainty about the nature of hese structures, we undertook to examine them by TEM as well as by SEM.

Methods. Chick blastoderms of Hamburger-Hamilton stages 4 to 9 were removed by a filter paper ring, fixed in 2% cacodylate buffered glutaraldehyde for 20 min, osmicated, dehydrated with ethanol, and dried by the critical point method with CO_2. After coating with silver, the specimens were examined at 20 KV in an Hitachi HHS 2R scanning microscope. Other specimens were

[1] Supported by a grant from The Medical Research Council of Canada.

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