

concerning the increasing aldehyde fixation on all the cell structures during the 1st h of incubation. The autoradiographic analysis, however, brought additional data: the preferential area of cellular fixation for acrolein was the nucleus; this fixation was stable for at least 2 days, which is not the case for cytoplasmic or chloroplastic fixation.

These results are in agreement with those obtained on regenerating rat liver *in vivo*<sup>11</sup>, where it was demonstrated that the acrolein was rapidly fixed on DNA and the fixation was stable for at least 24 h. *In vitro*, the acrolein had a great affinity for the DNA polymerase to which it could

be irreversibly attached<sup>12</sup>. This preferential and stable fixation of the acrolein to the nucleus may explain the high toxicity and the irreversibility of the cytotoxic effects of that molecule. These results raise the question of the reconsideration of ALARCON's<sup>13</sup> hypothesis which suggests that acrolein could be an element of an universal system of control for cellular growth.

<sup>12</sup> N. MUNSCH, A. M. DE RECONDO and C. FRAYSSINET, *Experientia* 30, 1234 (1974).  
<sup>13</sup> R. A. ALARCON, *J. theor. Biol.* 37, 159 (1972).

Frequency of the Various Stages of the Seminiferous Epithelium in Different Strains of Male Mice

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**Summary.** The duration of the various development stages of the seminiferous epithelium in different strains of male mice was determined by scoring the frequency distribution in randomly selected tubule cross-sections. The results obtained show a difference in the duration of the various stages between the different strains.

The duration of spermatogenesis in the mouse and of the stages in the cycle of the seminiferous epithelium has been determined by OAKBERG<sup>3</sup>.

Using the periodic acid Schiff technique, OAKBERG was able to distinguish 16 stages of spermatogenesis in the mouse, 12 of these corresponding to 1 cycle of the seminiferous epithelium. 4 cycles occur between the development of the first type A spermatogonia to the spermatozoa. According to OAKBERG, the duration of the spermatogenesis in the mouse is 34<sup>1</sup>/<sub>2</sub> days and the duration of each cycle is 207 ± 6.2 h.

The frequency distribution of tubules in randomly selected samples of non-irradiated animals was used to time each stage of the seminiferous epithelium development, and the results obtained have been of great help in the determination of the radiation sensitivity of the different

Table II. Frequency of the various stages of the seminiferous epithelium in F<sub>1</sub> hybrid mice

Stage	F <sub>1</sub> C3H ♂ C57 Bl ♀		F <sub>1</sub> ARR ♂ C3H ♀	
	No. of tubules per stage <sup>a</sup>	Relative frequency	No. of tubules per stage <sup>a</sup>	Relative frequency
I	197	0.066	227	0.075
II	106	0.035	244	0.081
III	377	0.125	439	0.145
IV	193	0.064	290	0.096
V	155	0.052	232	0.077
VI	151	0.050	141	0.047
VII	193	0.064	156	0.052
VIII	454	0.151	232	0.077
IX	425	0.141	400	0.133
X	229	0.076	179	0.059
XI	222	0.074	188	0.062
XII	306	0.102	291	0.096
Total	3008		3019	

Table I. Frequency of tubules by stages

Stage	C3H inbred mice			
	First scoring		Second scoring	
	No. of tubules per stage <sup>a</sup>	Relative frequency	No. of tubules per stage <sup>b</sup>	Relative frequency
I	358	0.060	264	0.067
II	298	0.050	205	0.052
III	775	0.129	476	0.120
IV	514	0.086	338	0.085
V	360	0.060	243	0.061
VI	285	0.047	195	0.049
VII	431	0.072	296	0.075
VIII	796	0.133	447	0.113
IX	769	0.128	560	0.141
X	407	0.068	268	0.068
XI	399	0.066	232	0.059
XII	613	0.102	433	0.109
Total	6005		3957	

<sup>a</sup> 500 randomly selected tubules scored in 8 mice + 1000 tubules scored in 2 mice. <sup>b</sup> About 500 randomly selected tubules scored in 8 mice.

<sup>a</sup> 500 randomly selected tubules scored for each of the 6 mice.

kinds of spermatogonial cells. No investigations have been reported, however, to determine the tubular frequency and to check whether variations exist in different strains of mice.

**Materials and methods.** Male mice C3H inbred, F<sub>1</sub> hybrid between inbred C3H ♂ and C57 Bl ♀, and F<sub>1</sub> hybrid between ARR ♂ and C3H ♀, 9 to 12 weeks old, were used. The animals were killed by cervical dislocation. The testes were fixed in Orth fluid, embedded in paraffin, and then cut in 5 µm sections. The sections were

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<sup>3</sup> E. F. OAKBERG, *Am. J. Anat.* 99, 507 (1956).

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 <sub>1</sub> C3H ♂ C57 Bl ♀	F <sub>1</sub> ARR ♂ C3H ♀	F <sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> hybrid between C3H ♂ and 101 ♀<sup>1</sup> (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<sup>2-4</sup>, has been receiving increased attention. In 1974, YEW et al.<sup>5</sup> 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<sup>6-8</sup>. The model of the retinas of albino neonatal rats was chosen, because the young rats do not begin to develop inner segments in the retinas until around the

8th or 9th postnatal day, and outer segments until around the 14-15th postnatal day<sup>9</sup>.

**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

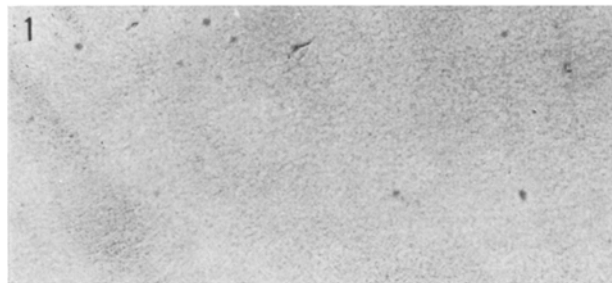


Fig. 1. Monoamine oxidase (MAO) histochemical reaction on the 6-hydroxydopamine-treated retina (wet mount). Note very few granular deposits (positive MAO sites).  $\times 250$ .

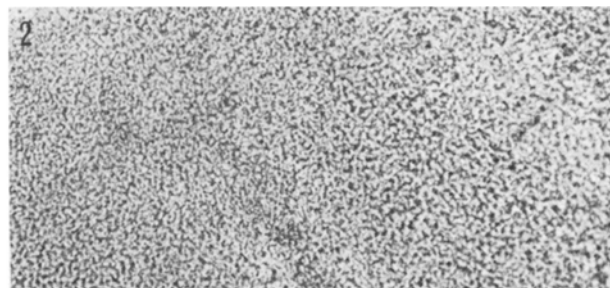


Fig. 2. Monoamine oxidase (MAO) histochemical reaction on the control retina (wet mount). Note a lot of granular deposits (positive MAO sites).  $\times 250$ .