Retinal Atrophy and Cataract in Rats Following Administration of *N*-Methyl-*N*-nitrosourea[1] (36083)

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(Introduced by Saul Malkiel)

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N-Methyl-*N*-nitrosourea (NMU) (NSC-23909), a nitroso compound, is a potent carcinogen, teratogen, and mutagen. It is not retained in the body for more than a few hours (1). A single dose induces neoplasms in many organs (2, 3). Alkylation of nucleic acids and proteins in animals treated with nitrosamines and nitrosoureas is well documented (4). However, the various biologic manifestations of these compounds do not appear to be dependent on alkylation (4–8).

There is a paucity of information on ocular changes occurring in animals treated with chemical carcinogens except for a pigmentary degeneration of the retina in Syrian hamsters treated with NMU (9). Myleran, a drug used in the chemotherapy of leukemia, and X-rays induce cataracts in rats (10, 11). An unexpected observation on the morphology and mineral composition of eyes of rats treated with NMU is reported here.

Materials and Methods. *N*-Methyl-*N*-nitrosourea[2] in saline was injected at a dose of 70 mg/kg body weight into the tail vein of a group of 30 day old Wistar rats (36 males, 36 females); untreated animals (10 males, 10 females) of the same age group served as controls. Apart from a mild chronic proteinuria and deaths from infection or leukemia, these animals maintained good general health until sacrifice despite development of a variety of tumors. Opacity, predominantly of the lens was apparent in eyes of most animals of both sexes, 4 months following injection of NMU, and increased in severity with time. Eyes from all animals were fixed in Tellyesniczsky's fluid (70% alcohol, acetic acid, and formaldehyde in the ratio 100:5.5:5), and processed for histologic examination. Mineral analysis was done by atomic absorption spectrometry (12) on one eye of each of 12 treated and 6 control rats killed 7-8 months after the start of the experiment.

Results and Discussion. In the eyes of animals killed 6 months after injection of NMU, retinal atrophy appeared to be basically focal, later becoming confluent and spreading in all directions. The anterior portions of the retina were less severely damaged than that around the optic nerve. Retinal atrophy was slower to develop in females than in males. Cataracts were present in all rats at 6 months, but were less severe in females. The earliest massive lenticular degeneration was noted in males at 7 months. Changes in the subcapsular epithelium were distinctly focal, and posterior to posterolateral in early manifestations. Retinal and lenticular changes were not completely parallel in severity; occasionally, retinal changes were fairly advanced in comparison with cataract development. Apart from animals developing leukemia and 3 with restricted chronic encephalitis or healed necrosis of brain, no central nervous system abnormalities were recognized histologically.

The following changes were observed in

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[2] NMU was kindly made available by Dr. Harry B. Wood, Jr., of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health.

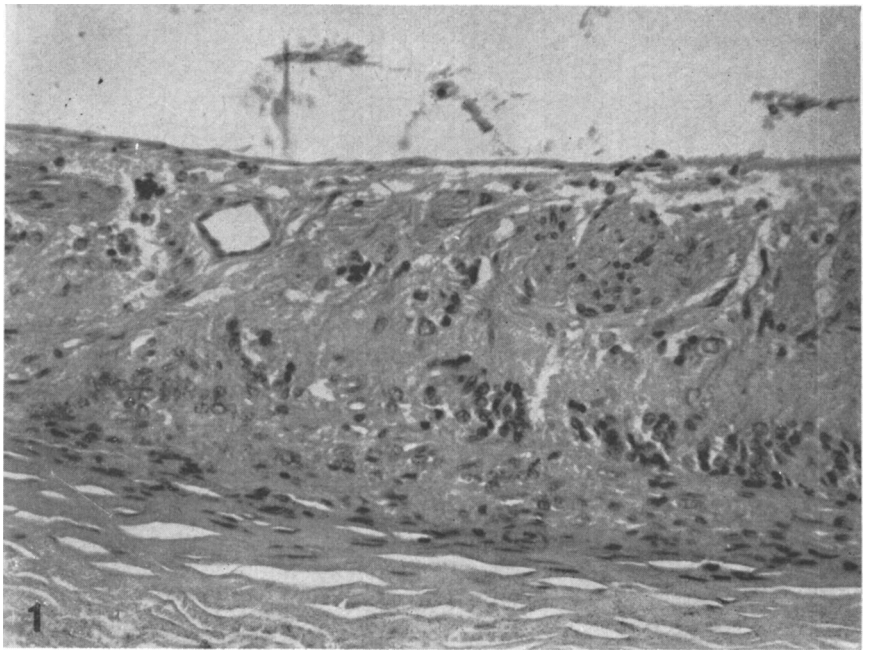

FIG. 1. Posterior retina of rat 7 months after NMU: A few remnants of internal nuclear layer persist. Fibers and nuclei are increased. Rods and cones not present; hematoxylin and eosin, ×145.

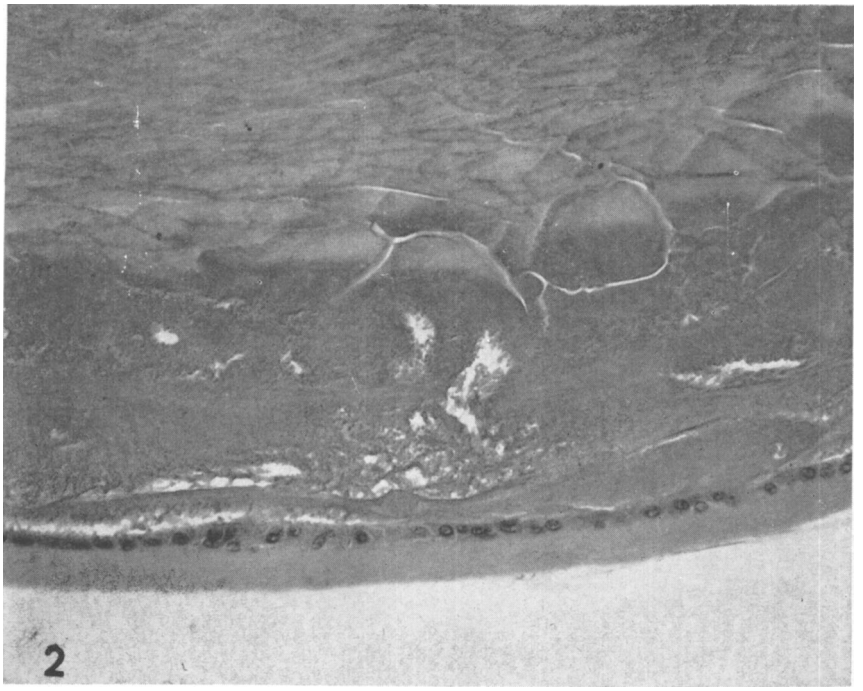

FIG. 2. Posterolateral lens of rat 7 months after NMU: Beneath hypertrophic and hyperplastic epithelium the lens fibers are swollen and vacuolated, with indistinct borders and contain scattered mineral deposits; hematoxylin and eosin ×145.

TABLE I. Weights and Mineral Composition of Eyes of Rats Treated with *N*-Methyl Nitrosourea.

Group	Animal no.	Wt (g)		Dry/wet wt	(μg/g of dry wt)					
		Wet	Dry	(%)	Sodium	Potassium	Calcium	Magnesium	Iron	Zinc
NMU treated	1	0.097	0.024	24.9	756	141	3146	217	41	48
	2	0.140	0.025	18.1	739	148	1970	230	35	45
	3	0.122	0.027	21.9	636	92	16269	462	41	56
	4	0.094	0.025	26.6	472	80	4020	151	35	37
	5	0.131	0.025	19.5	686	96	2979	147	60	43
	6	0.150	0.028	18.7	650	121	2225	208	33	34
	7	0.145	0.025	17.6	741	155	2744	269	25	33
	8	0.123	0.026	20.8	692	107	7605	254	47	37
	9	0.101	0.022	21.3	566	88	6584	169	439	52
	10	0.123	0.024	19.6	756	110	3726	217	18	35
	11	0.136	0.024	18.0	748	123	3280	215	8	39
	12	0.097	0.022	22.9	683	101	6048	246	18	38
	Median	0.123	0.025	20.2	689	108.5	3503	217	35	38.5
Controls	1	0.161	0.043	26.7	313	140	167	168	10	20
	2	0.185	0.045	24.1	364	168	188	176	20	37
	3	0.172	0.041	24.1	381	152	198	182	42	23
	4	0.171	0.042	24.8	348	143	222	150	6	47
	5	0.167	0.042	25.0	361	151	173	174	10	20
	6	0.161	0.044	27.4	346	158	163	170	16	16
	Median	0.169	0.042	24.9	354	151	180	172	13	21.5
	p			$<.02$	$<.002$	$<.02$	$<.002$	$<.1$	$<.1$	$<.05$

the eyes of treated animals used for mineral analysis: (i) Retina was atrophied. A few ganglion and inner nuclear cells were present and there were no rods and cones (Fig. 1). Choroidal epithelium could not be clearly distinguished. (ii) The anterior chamber contained a few mononuclear cells and a granular precipitate; similar changes were not present in the canal of Schlemm or in episcleral veins. (iii) Ciliary bodies were small and the epithelial cells were shrunken. (iv) The lens was the site of small anterior synychiae of equatorial subcapsular epithelial proliferation and liquefaction, which was most advanced posteriorly. There was a faint bluish granularity in the subjacent fibers and scattered mineral deposits (Fig. 2). At 8 months in some animals, the lens capsule was ruptured with round cell infiltrates in the atrophic lens and iris.

Table I summarizes the weights and mineral components in the eyes of the 2 groups of rats. [Statistical evaluations were done by Mann-Whitney *U* test (13)]. In the eyes of treated rats, weight, water content, and potassium content were decreased accompanied by a concomitant increase in sodium and calcium. These changes are similar to those reported in galactose-induced cataracts in rats (14) and in the sclerosed human lens (15). As in other cataracts, there appears to be a cation shift, which is ascribed to altered permeability. The significance of the increased zinc in the eyes of these animals is not clear. There was no alteration in the iron and magnesium content.

The mechanism of progressive degeneration of retina and lens, and early changes in chemical and mineral composition of eyes of rats treated with NMU is of interest, because nitroso compounds are potent carcinogens, can form in stomach and in intestine (16, 17), and are known to be present in the environment. Studies are in progress to define the early stages of the ocular abnormality.

Summary. Retinal atrophy and cataracts were present in eyes of rats 6 months after injection of *N*-methyl-*N*-nitrosourea at a dose of 70 mg/kg of body weight. Retinal and lenticular changes were less severe in females than in males. There was a decrease in weight, water, and potassium content and an increase in sodium and calcium in the eyes. Our findings are similar to those reported in galactose-induced cataracts in rats and in sclerosed human lens.

1. Swann, P. F., Biochem. J. **119**, 49 (1968).

2. Druckrey, H., Steinhof, D., Preussman, R., and Ivankovic, S., Z. Krebsforsch. **66**, 1 (1964).

3. Leaver, D. D., Swann, P. F., and Magee, P. N., Brit. J. Cancer. **23**, 177 (1969).

4. Magee, P. N., Ann. N.Y. Acad. Sci. **163**, 717 (1969).

5. Krüger, F. W., Ballweg, H., and Maier-Borst, W., Experientia **24**, 592 (1968).

6. Lijinsky, W., and Ross, A. E., J. Nat. Cancer Inst. **42**, 1095 (1969).

7. Krüger, F. W., Walker, G., and Wiessler, M., Experientia **26**, 520 (1970).

8. Magee, P. N., Cancer Res. **31**, 599 (1971).

9. Herrold, K. McD., Arch. Ophthalmol. **78**, 650 (1967).

10. Solomon, C., Light, A. E., and DeBeer, E. J., Arch. Ophthalmol. **54**, 850 (1955).

11. VanHeyningen, R., *in* "The Eye" (H. Davson, ed.), Vol. 1, p. 213. Academic Press, New York (1962).

12. Kopito, L., "Atomic Absorption Spectrometry in Clinical Chemistry," 100 pp. Fischer Scientific Co., Pittsburgh (1970).

13. Siegel, S., "Nonparametric Statistics for Behavioral Sciences," 312 pp. McGraw-Hill, New York (1956).

14. Kinoshita, J. H., and Merola, L. O., Invest. Ophthalmol. 3, 577 (1964).

15. Sailit, P. W., Arch. Ophthalmol. **30**, 255 (1943).

16. Klubes, P., and Jondorf, W. R., Res. Commun. Chem. Pathol. Pharmacol. 2, 24 (1971).

17. Montesano, R., and Magee, P. N., Int. J. Cancer. 7, 249 (1971).

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