Ocular Defects in Newborn Rats Treated with 5-Iododeoxyuridine (IUDR) (37222)

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(Introduced by A. S. Rabson)

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Recently there has been considerable interest shown in the use of 5-iododeoxyuridine (IUDR) as a treatment for viral infections. IUDR therapy has been utilized in human medicine for a variety of conditions including generalized herpetic infections (1, 2), encephalitis (3), and herpetic keratitis (4). However, developmental defects have been produced experimentally in animals treated with other halogenated uridines. Lesions have been described in the offspring of pregnant mice treated with 5-bromodeoxyuridine (BU DR) (5) and 5-fluorodeoxyuridine (FUDR) (6). In addition, developmental defects have been observed in mice given FUDR postnatally (7). The studies herein described were therefore initiated to determine the effects of IUDR when given to newborn rats.

Materials and Methods. IUDR (A Grade, Calbiochem, Los Angeles, CA) was prepared as a 0.5% solution in 5% dextrose as previously described (8). Two newborn Sprague-Dawley and two newborn Wistar rats were injected sc with IUDR at the rate of 400 mg per kg per day for 5 days beginning at 1 day of age. In addition, two newborn Sprague-Dawley rats were treated at the rate of 200 mg per kg per day for 5 days. Control rats were treated sc with identical volumes of 5% dextrose, and nontreated control animals were also included in the studies. All animals were killed by chloroform inhalation at 20 days of age. Rats were necropsied, and tissues for light microscopy were immersed in Carnoy's, Bouin's, or Zenker's fixatives. Specimens were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin or PAS stains. Organs examined histologically from each animal included central nervous system (CNS), eye, and kidney. Eyes for electron microscopy examination were fixed by immersion in 3% glutaraldehyde, washed in several changes of phosphate buffer, stained with osmium tetroxide, embedded in epon, sectioned with an LKB microtome, stained in uranyl acetate followed by lead citrate, and examined with a Siemens I electron microscope using an 80-kV accelerating voltage and a 50-μm objective aperture. Ocular tissue from one treated Wistar rat (397) and one treated Sprague-Dawley rat (443) together with tissues from one control animal from each strain were processed for electron microscopy.

Results. Growth rates were reduced in animals treated with IUDR compared to control littermates. In addition, the growth of the body hair was markedly retarded in treated rats killed at 20 days of age.

Histopathology. Eye. The eyes of newborn rats treated with IUDR at 200 mg/kg and 400 mg/kg were generally smaller than those of control littermates. The retina was the ocular tissue most commonly and severely affected, although vitreous hemorrhage, hypoplasia, and disorganization of the iris and ciliary body and proliferation of the lens epithelium were also observed. Retinal changes varied from slight disruption in the architecture of the outer and inner nuclear and ganglion cell layers but with individual cells appearing normal, to dysplastic rosette formation of the outer and inner nuclear layers and disorganization of the ganglion cell layer with severe pyknosis throughout the layers of the retina (Figs. 1 and 2). In one animal (395), a configuration resembling persistent primary vitreous was seen. The appearance of the retinal pigment epithelium was variable, ranging from absence to proliferation beneath both

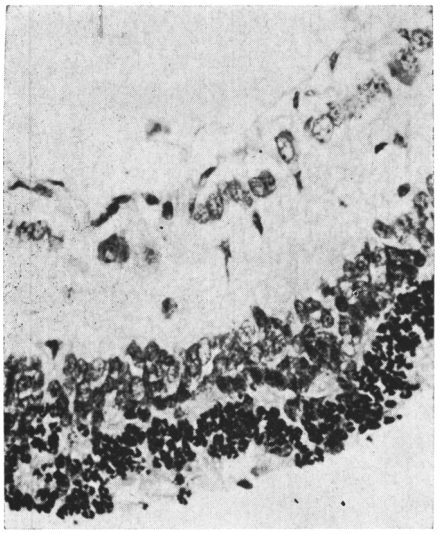

FIG. 1. Retina of rat No. 324 treated with IUDR at 400 mg/kg/day. The inner and outer nuclear layers and rods and cones (lower portion of photo) are poorly delineated and reduced in thickness with numerous undulations present in the outer nuclear layer. Note the rosette formation in this region (lower left). (H & E, ×250).

areas of normal retina and areas of retinal dysplasia. The dysplastic retina was in some areas pathologically detached, while in other regions the retina was attached to the underlying retinal pigment epithelium or choroid.

Electron microscopy of the dysplastic retina demonstrated photoreceptor cells, bipolar cells, and Müller cell processes within the rosettes seen on light microscopy (Fig. 3). The appearance of retinal pigment epithelium by EM was generally unremarkable. Lesions were not observed in the retinas from control rats processed for electron microscopy.

CNS. In newborn rats treated at 200 mg/kg and 400 mg/kg, the outer germinal layer of the cerebellum was prominent in some areas and sometimes up to 5–6 cells in thickness in the sulci of the lateral hemispheres. In control rats, the width of the outer germinal layer was usually 1–2 cells in thickness. There was frequently a minimal to moderate reduction in the number of cell nuclei in the inner granular layer compared to control rats at this age (Table I).

Kidney. In animals treated with IUDR, numerous foci were present in the subcapsular region of the renal cortex which consisted of poorly differentiated glomeruli and tubules. In addition, nests of mononuclear cells were present in the interstitial regions which were interpreted to be nondifferentiated primordial cells. Similar structures were not observed in control rats examined at this age (Table I).

Discussion. Retinal dysplasia has been described in animals infected perinatally with certain viruses (9–12). Although retinal lesions may be due in part to specific tissue tropisms of certain viruses, more significant may be the apparent affinity for many viruses for dividing cells. This has been demonstrated in newborn animals infected experimentally with feline panleukopenia virus (13) and Kilham's rat virus (14) which produced cerebellar dysplasia in susceptible species. Such viruses have been termed "mitolytic" agents since they replicate more readily in rapidly dividing cells with resultant damage to these tissues (14).

Similarly, substances such as IUDR are most damaging to rapidly dividing cells. IU-

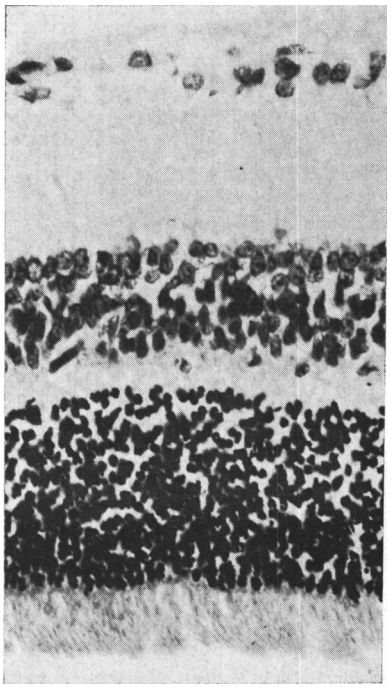

FIG. 2. Retina of control rat also at 20 days of age from approximately the same area as depicted in Fig. 1. Note the thickness of the retina compared to the treated animal. The inner and outer nuclear layers and the rods and cones (lower portion of the photo) are clearly delineated. (H & E, ×250).

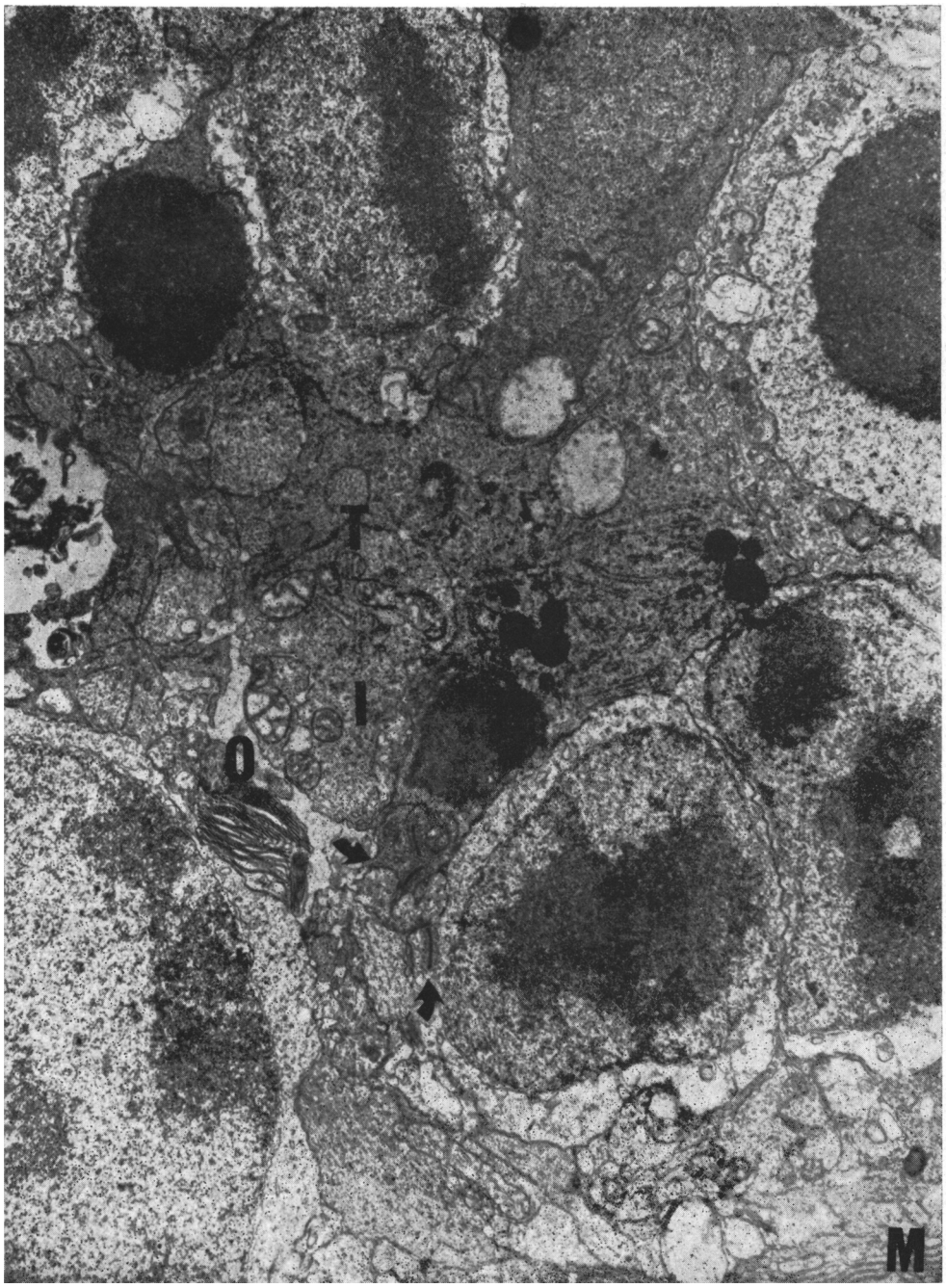

FIG. 3. Explanation of electron micrograph: electron micrograph of dysplastic rosette (Rat No. 397). Structure is composed of photoreceptor cells, Müller cell processes, bipolar cells, and structures interpreted to be processes of the outer plexiform layer. Within rosettes are (O) outer and (arrows) synapses, (I) inner segments, (T) terminal bar, and Müller cell processes; (M) microvilli of the retinal pigment epithelium are seen at the lower right corner of the picture (×7500).

TABLE I. Distribution of Developmental Defects in Newborn Rats Treated with IUDR.[a]

No.	Strain	Age (days)	Treatment[b]	Retina	Cerebellum	Kidney
395	Wistar	20	IUDR (400 mg/kg)	++	+	++
397	Wistar	20	IUDR (400 mg/kg)	++	+	++
394	Wistar	20	Control	—	—	—
396	Wistar	20	Control	—	—	—
324	S-D	20	IUDR (400 mg/kg)	++	±	++
443	S-D	20	IUDR (400 mg/kg)	++	±	+
344	S-D	20	IUDR (200 mg/kg)	+	±	±
388	S-D	20	IUDR (200 mg/kg)	++	±	+
479	S-D	20	5% Dextrose (0.08 ml/g)	—	—	—
478	S-D	20	5% Dextrose (0.04 ml/g)	—	—	—
442	S-D	20	Control	—	—	—
444	S-D	20	Control	—	—	—

[a] Legend: S-D, Sprague-Dawley; —, histologically normal; ±, questionable lesions; +, moderate lesions; ++, extensive lesions.

[b] Dosages of IUDR and dextrose were for 5 days beginning at 1 day of age.

DR replaces thymidine in the synthesis of DNA with the resultant formation of "fraudulent" DNA (8). Adverse side effects are therefore related to the capacity of IUDR to become incorporated into metabolically active cells with resultant impairment of maturation and/or cellular division. Because of this property, IUDR has been utilized not only in the treatment of virus infections (1–4) but also in cancer chemotherapy (8). The adverse side effects encountered with IUDR administration include leukopenia, thrombocytopenia, and alopecia. These effects are all related to the capacity of IUDR to inhibit rapidly proliferating tissues (8). Although such manifestations are generally reversible, there are still more critical cites of cellular division in the newborn animal. The cerebellar cortex is one region where considerable cellular division and differentiation occurs postnatally (13–15). The perinatal development of the retina has been described in human subjects (16), but to date the postnatal evolvement of the retina in other mammals has not received wide attention. Based on histopathological evaluation, it is apparent that considerable cellular division and differentiation occurs in the retina and renal cortex of the rat during the first few days of life. Treatment with substances such as IU DR during this critical period of organogenesis may then result in irreparable damage to these areas as demonstrated in the present studies. A variety of causes have been cited in naturally-occurring and experimentally-induced retinal dysplasia (9, 10, 17–19). It is now apparent that IUDR should be included as a substance capable of inducing retinal dysplasia when given parenterally to the newborn rat.

Summary. Newborn Wistar and Sprague-Dawley rats were treated parenterally with IUDR at the rate of 400 mg/kg or 200 mg/kg per day for 5 days beginning at 1 day of

age. Treated and control animals were killed at 20 days of age and tissues processed for light and electron microscopy. In treated rats, developmental defects were observed in the eye, cerebellum, and kidney. Ocular lesions were most extensive in the retina and were characterized by disruption of the architecture and dysplastic rosette formation in the outer and inner nuclear cell layers. Although used as an antiviral substance, the ability of IUDR to induce developmental defects in the newborn rat emphasizes the need for further investigation.

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