STUDIES ON DEVELOPING RETINAL VESSELS*

IV. EFFECT OF IONIZING RADIATION

BY

CHRISTOPHER PEDLER

Department of Pathology, Institute of Ophthalmology, University of London

THE EFFECT of hyperoxia on the vasculature of the immature retina is now well established, and it has been repeatedly demonstrated that when a newly-born kitten is subjected to an ambient oxygen concentration exceeding approximately 35 per cent. a total and permanent vaso-obliteration will develop within a period of 8 to 36 hours (Ashton, Ward, and Serpell, 1954). This observation has provided the basis for much of the significant work carried out on the aetiology of retrolental fibroplasia, and may in fact provide useful information about other diseases of the retina. In view of this, it is clearly essential to assemble more information about the mechanism by which oxygen is able to produce vaso-obliteration in the immature retina.

In recent years a considerable amount of work has been published suggesting that the toxic effects of both oxygen and ionizing irradiation may be brought about by the inhibition or destruction of common enzymatic systems. For example, reduction of metabolism in the experimental animal by cooling, provides some protection against oxygen poisoning, whereas raising the body temperature enhances oxygen susceptibility (Grossman and Penrod, 1949; Stadie, Riggs, and Havgaard, 1944). Similarly it appears that a lowered metabolism decreases the amount of damage incurred by a given dose of radiation (Ord and Stocken, 1953). Furthermore, both agents affect some of the enzymes of intermediary metabolism in a comparable fashion; for instance, the sulphhydryl enzymes, pyruvic oxidase and succinate dehydrogenase, are both inactivated by high oxygen concentration (Dickens, 1946), and the activity of diphosphoglyceric-aldehyde dehydrogenase is almost completely destroyed *in vitro* by a radiation exposure of 500 r. (Barron, Dickman, Muntz, and Singer, 1949). These reactions are very probably produced by the interaction of various oxidizing free-radicals transiently elaborated by ionising radiation from the breakdown of water, for there is considerable evidence to show that irradiation acts indirectly by forming toxic peroxide and hydroperoxyl ions (Rajewsky, 1952; Dainton, 1951).

In fact the yield of these substances is very considerably enhanced by increasing the quantities of molecular oxygen present; indeed, the incidence of chromosomal aberrations in inflorescences of algae can be made to rise as the oxygen concentration increases (Giles and Riley, 1950), and certain animal tumours can be made as much as three times more radiosensitive if they are irradiated in atmospheres of high oxygen concentration (Gray,

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Conger, Ebert, Hornsey, and Scott, 1953). Conversely, it has been shown that an anoxia produced by breathing 5 per cent. oxygen for 8 minutes protected rats from otherwise lethal doses of whole body radiation (Dowdy, Bennet, and Chastain, 1950). These facts have been applied to the treatment of human malignant disease with some success (Churchill-Davidson, Sanger, and Thomlinson, 1955; Hultborn and Forssberg, 1954).

Thus ionizing radiation and oxygen are apparently synergistic in their toxicity and both agents almost certainly act through similar enzyme systems. These arguments form the basis of the following experiments which were carried out to determine whether irradiation of the immature retina might parallel the vaso-obliterative effects of oxygen.

Technique

The irradiation source in these experiments was a cylinder of Cobalt 60, 4 mm. in diameter and 4 mm. in length. Its gamma equivalent was 1.4 milli-electron volts and its half life 5.3 years. The cylinder which was screened to emit gamma rays only, was enclosed in a lead bomb in such a way as to produce a surface contact dose rate of 240r/hr and a 5-mm. rate of 100r/hr.

In the first group (Table, opposite), each of the five animals was exposed to a different dose of irradiation, killed at once, and the retinal blood vessels injected with Indian ink. In the second group, four animals were exposed to the same dose of irradiation but kept for much longer periods before being killed and injected. In the third group, an attempt was made to watch the vessels whilst irradiation was in progress. For this it was necessary to modify the apparatus we had constructed for the direct observation of retinal vessels. The cobalt container was mounted on a swinging arm above a perspex box containing the kitten, so that it could be moved away and lowered into a protective lead surround in a very few seconds. The Zeiss stereoscopic microscope used for these observations was also mounted on a rotating arm, so that, when the cobalt was swung away into its safe position, the microscope could be moved very rapidly into place over the animal to observe the retina. In this way it was possible to see the retinal vessels at regular intervals throughout the irradiation. Before the commencement of the experiment, the kitten was anaesthetized with Nembutal and a limbal window inserted into the front of one eye using the same technique as that described by Ashton and Cook (1954). The head of the animal was then fixed in a rigid clamp, so that the radiation beam passed through one eye only, the other eye acting as a control and receiving approximately 10 per cent. of the total dose administered.

Results

In Group I, only one animal (k1) showed any abnormal vessel pattern after injection. In this case there was no injection of the vessels on the temporal half of the retina; however, it was impossible to decide whether this disappearance was due to the irradiation or to a thrombus in one of the main arteries.

In Group II, the long-term survival experiments, the vessels of both control and irradiated eyes appeared very similar in all animals.

In Group III, the size and general appearance of the vessels were recorded during the actual irradiation. In one animal (k10) the retinal arteries and veins were seen to become grossly dilated, but this was thought to be a result of its terminal condition. In the other two animals, no change at all was observed in the retinal vasculature during the course of the irradiation.

TABLE

Animal No.	Age (days)	Dosage Rate (r/hr)	Total Dose (r)	Estimated Control Eye Dose (r)	Survival Time (hrs)	Group	Experiment
k1	6	100	50	5	$\frac{1}{2}$	I	<i>Short Survival</i> Killed and injected with Indian ink
k2	8	100	100	10	1		
k3	8	240	480	48	24		
k4	8	240	960	96	$8\frac{1}{2}$		
k5	9	240	960	96	$4\frac{1}{2}$		
k6	13	240	960	96	7 days	II	<i>Long Survival</i> Killed and injected with Indian ink
k7	12	240	960	96	10 days		
k8	7	240	960	96	21 days		
k9	10	240	180	18	$6\frac{1}{2}$	III	<i>Direct Observation</i>
k10	13	100	500	50	$7\frac{1}{2}$		
k11	13	240	1200	120	$2\frac{1}{2}$		

The results of these experiments were completely negative. That is to say, we found no consistent change in the appearance or calibre of the vessels as a result of the irradiation doses employed. Any change that was observed was fairly easily explicable on the grounds of a variation in one or more of the experimental conditions.

Thus, these experiments provide no evidence to suggest that the biochemical systems known to be affected by both oxygen and irradiation are essentially involved in the vaso-obliterative effects of oxygen.

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