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COMMUNICATIONS

EFFECT OF INCREASED OXYGEN ON THE DEVELOPMENT OF THE RETINAL VESSELS*

AN EXPERIMENTAL STUDY

BY

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THERE is convincing clinical evidence that retrolental fibroplasia is the result of a disturbance of the oxygen conditions to which an infant is normally exposed. Direct experimental evidence of this was supplied by Ashton and others (1953), the anatomical studies of Michaelson (1948) and the physiological studies of Campbell (1951) having previously suggested the significance of the role of oxygen in the development of the retinal vessels. Ashton and his co-workers found occlusion of the retinal blood vessels in new-born kittens who were exposed to hyperoxic conditions. When the animals were returned to normal atmospheric or hypo-oxic conditions there was a subsequent disturbance of vessel growth and also new vessel ingrowth into the vitreous body. The following work had been planned in order to test the effect of hyperoxia on the developing vessels of the mouse retina. This animal was chosen because the development of the retinal vessels is confined almost completely to the period between the first and eleventh day of life, and because the mode of retinal vessel development and the definitive vessel pattern are very similar to those occurring in the human retina. A description of the normal development of the retinal vessels in the mouse followed by a description of their development after the animal had been for some time in an incubator at high oxygen concentrations.

Material and Methods

Development under Normal Conditions.—In order to investigate the normal development of the retinal vessels five litters of new-born mice were taken. Beginning on the first day of life and thereafter at intervals until about the 14th day, an animal from each litter was killed. In each case an injected preparation of the retinal vessel system was obtained by injecting the left ventricle with about 0.5 ml. Indian ink and thereafter carefully removing the retina from each enucleated eye. The retina was then mounted on a microscopic slide and the vascularization examined and measured by aid of a microscope. During the preparation care was taken to brush away—although not always with success—as much as possible of the hyaloid

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system of vessels which otherwise would tend to obscure the view of the underlying retinal vessels. In instances where the retinal vessel development was slight, it was useful, however, to be able to take the degree of injection of the hyaloid system as a criterion of a satisfactory injection.

Development under Experimental Conditions.—For this purpose several litters of mice were placed in a bacteriological incubator sealed hermetically and connected to a similarly sealed drum of 200 l. capacity. The air in both containers was replaced by an air-oyxgen mixture of the desired oxygen content. A circulation pump continually transferred air from the drum to the incubator, while a hose connection allowed the surplus gas in the incubator to flow back into the drum. The volume of air put at the disposal of the mice in the incubator was thus enough to obviate fluctuations in O_2 concentration caused by the consumption of oxygen between the adjustments made by adding pure oxygen twice a day. The fluctuations encountered in O_2 concentration were never higher than 3 vol. per cent. Oxygen concentrations were determined with the van Slyke manometric apparatus, using sodium anthraquinone betasulphonate and hydrosulphite. Moisture was reduced and CO_2 absorbed by $CaCl_2$ and soda-lime placed in the incubator. During hot days the incubator was cooled by ice-water in order to keep the animals comfortable.

Experiment 1.—Four mother mice with their litters were placed in the incubator for 7 days. For Litter A the oxygen concentration was 40 per cent., for Litters B and C 50 per cent., and for Litter D 50–70 per cent. Thereafter the animals were killed and the nature and extent of the retinal vessel system examined in the manner described above.

Experiment 2.—Litters F, G, and H were kept also in the incubator for 7 days and thereafter the retinal vessel system was examined and measured. This is described as a separate experiment from Experiment 1 because all the members of these litters were found to be much below weight at the end of the experiment.

Experiment 3.—Litter E was placed in the incubator for 2 days to a 50 per cent. concentration of oxygen, and the retinal vessel system was thereafter examined in the manner described above.

Experiment 4.—Litters I and J were kept in the incubator under hyperoxic conditions for 7 days and thereafter the animals were kept alive for periods varying between 7 and 28 days in ordinary atmospheric conditions. After each animal was killed the retinal vessels were injected and examined as above.

Results

Development under Normal Conditions.—The development begins on the first day of life when small capillaries can be seen passing over the edge of the disc (Fig. 1). The capillary net continues to grow peripherally in all directions and on about the 11th or 12th day the vessels have reached their definitive position close to the ora serrata and about 2 mm. from the disc (Figs 1–6). The capillary net around the disc remains more sparse than in the periphery until the completion of the development (Fig. 3). This is in keeping with the mode of development in the retina of the cat, Fig. 7 (Michaelson, 1953). On the 5th day it is possible to identify arteries from veins, the former having

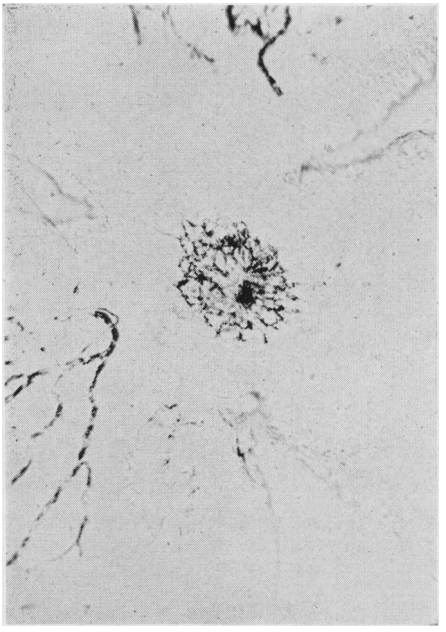

FIG. 1.—Injected retina of 1-day-old mouse. The retinal vessels can be seen passing over the edge of the disc. The hyaloid system of vessels, dissected from the region of the disc, can be seen at some distance from it. (×57).

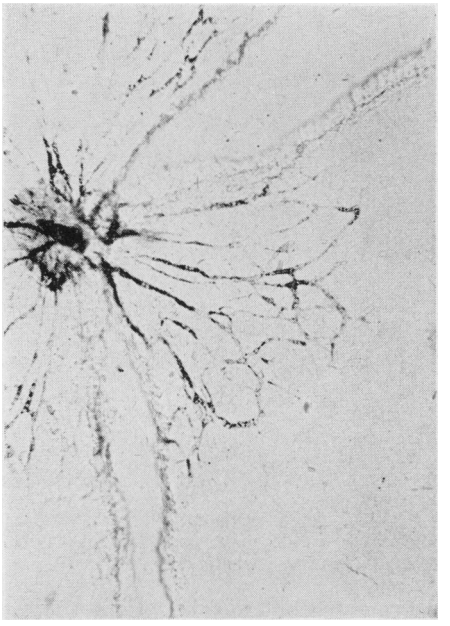

FIG. 2.—Injected retina of 2-day-old mouse. The retinal vessels have progressed 0·6 mm. beyond the disc margin. The retina has been cut in places to permit a flat preparation. (×57).

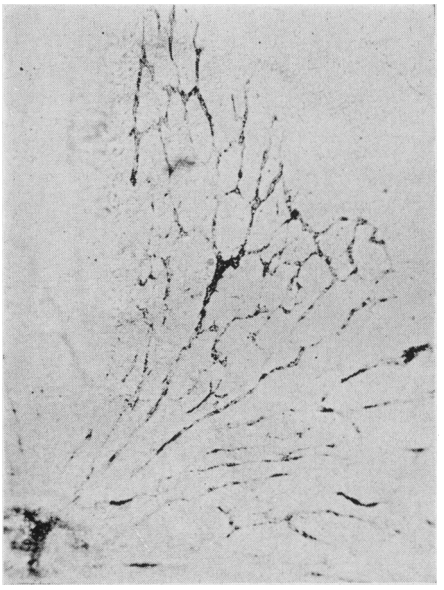

FIG. 3.—Injected retina of 4-day-old mouse. The retinal vessels have progressed 1·1 mm. beyond the disc margin. (×57).

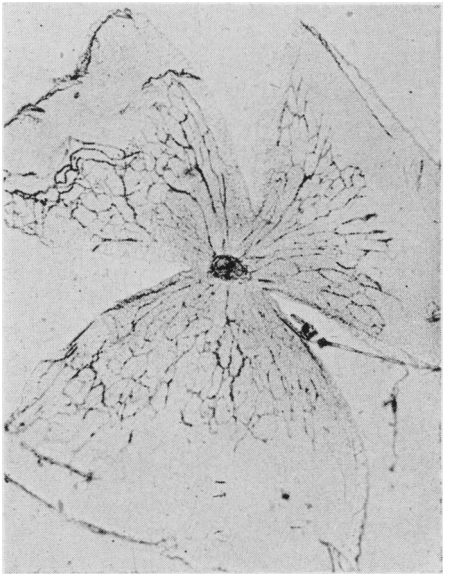

FIG. 4.—Injected retina of 4-day-old mouse. The preparation is that from which Fig. 3 was taken; the lower magnification gives a general view of the retina. (×25).

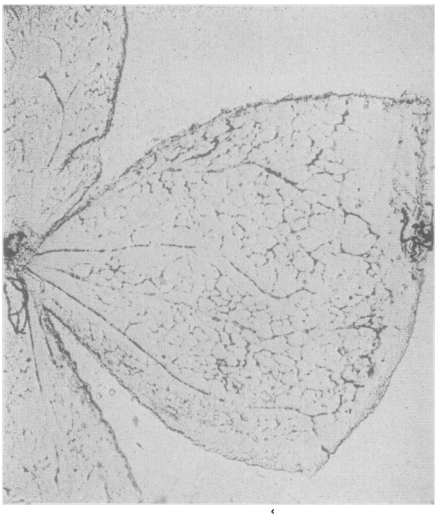

FIG. 5.—Injected retina of 6-day-old mouse. The retinal vessels have progressed 1·9 mm. beyond the disc margin. (×25).

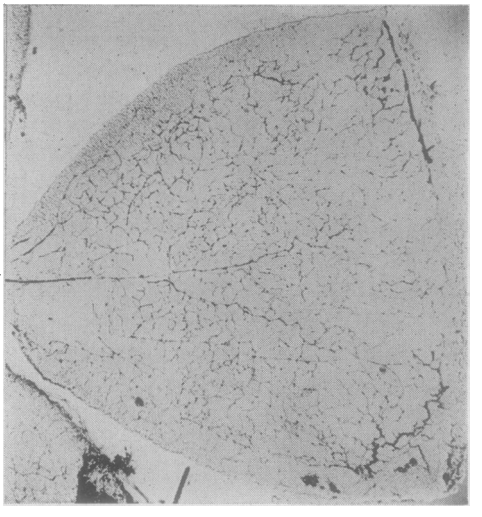

FIG. 6.—Injected retina of 11-day-old mouse. The retinal vessels have progressed 2·5 mm. beyond the disc margin. (×25).

a zone free of capillaries in their immediate neighbourhood. This is well seen in Fig. 8 taken on the 6th day. On that day the commencement of the deep capillary net can be seen growing downwards into the retina from the superficial net. The margin of the vascular area is frequently well-defined

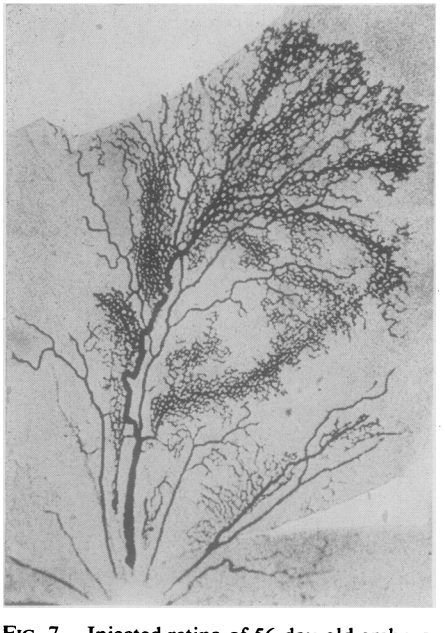

FIG. 7.—Injected retina of 56-day-old embryo of cat, showing lower temporal vessel complex. Note sparsity of capillaries in circumpapillary area.

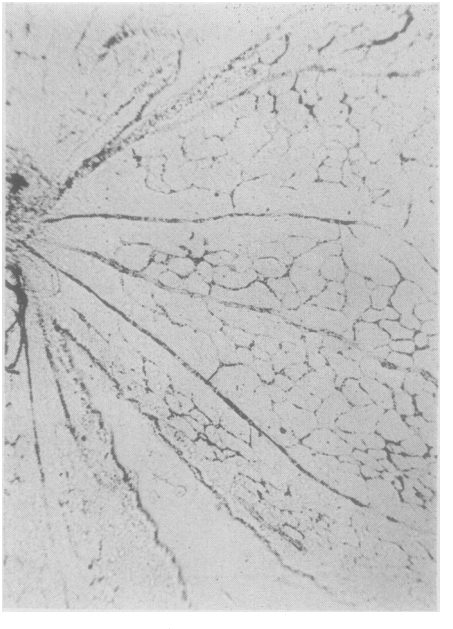

FIG. 8.—Injected retina of 6-day-old mouse. Portion of Fig. 5 showing capillary-free zone round arteries. (×50).

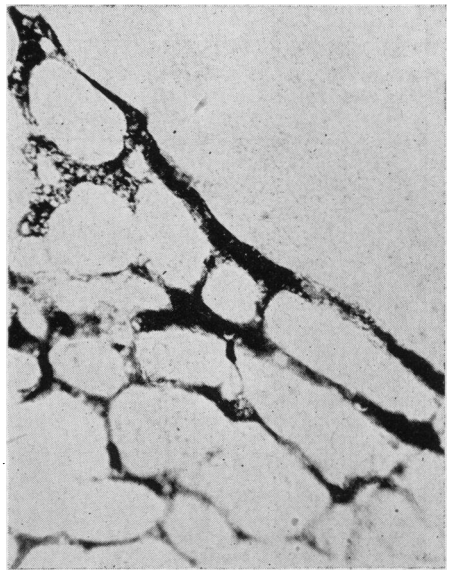

FIG. 9.—Injected retina of mouse showing peripheral vein limiting extent of vascularization. (×327).

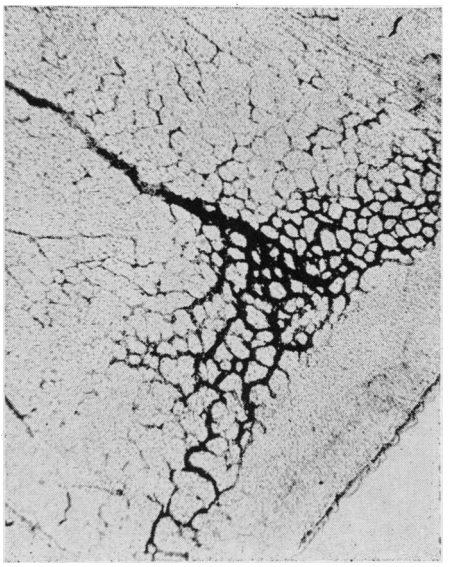

FIG. 10.—Injected retina of 8-day-old mouse, showing primitive type of retinal capillaries. (×65).

by a limiting vein (Fig. 9). During the process of development the peripheral capillaries tend to be of the primitive type; that is the mesh is smaller and the capillary broader than in the definitive type (Fig. 10). The hyaloid system of vessels is well marked at birth; it progressively atrophies during the first days of life, but is often still present in parts of the retina on the 11th day. A striking feature is the consistency in type and extent of the normal capillary growth in all animals having normal weight. This was so striking that in the tests illustrating development under experimental conditions it was not considered necessary to control the results by placing parallel members from each litter in ordinary air under the care of a foster mother.

The rate of growth of the extent of the capillary bed is represented by a graph (Fig. 11, overleaf). Five litters were used, varying in size from four to seven members.

Development under Experimental Conditions.

Experiment 1 (Animals maintained in incubator from first day of life till injection on 8th day).—The findings are shown in Table I (overleaf). Details of fifteen eyes from nine animals were obtainable. The litters were larger than suggested by this latter figure, but only those animals are included in which a completely satisfactory injection was obtained. Figs 12–14 (overleaf) illustrate the appearance of these retinae. The following conclusions can be drawn:

(*a*) The extent of the vessel outgrowth from the disc is not grossly affected by the change in oxygen concentration.

TABLE I

No. of animal	Litter	O_2 Content in Incubator (per cent.)	Condition and Weight before Injection (g.)	Extent of Vessel Outgrowth*	Extent of Circum-papillary Area Free of Capillaries*
1	A	40	well, 3·5	1·7	0·6
				1·7	0·5
2	B	50	well, 4·0	1·5	0·6
				1·5	0·6
3	B	50	well, 4·0	1·5	0·9
4	C	50	well, 3·5	1·5	0·8
				1·5	0·8
5	C	50	well, 3·5	1·1	0·5
6	C	50	well, 3·5	1·2	0·6
7	D	50–70	well, 3·5	1·8	0·9
				1·5	0·9
8	D	50–70	underd., 2·5	no vessels seen	
9	D	50–70	underd., 2·5	no vessels seen	

*In millimetres from disc margin.

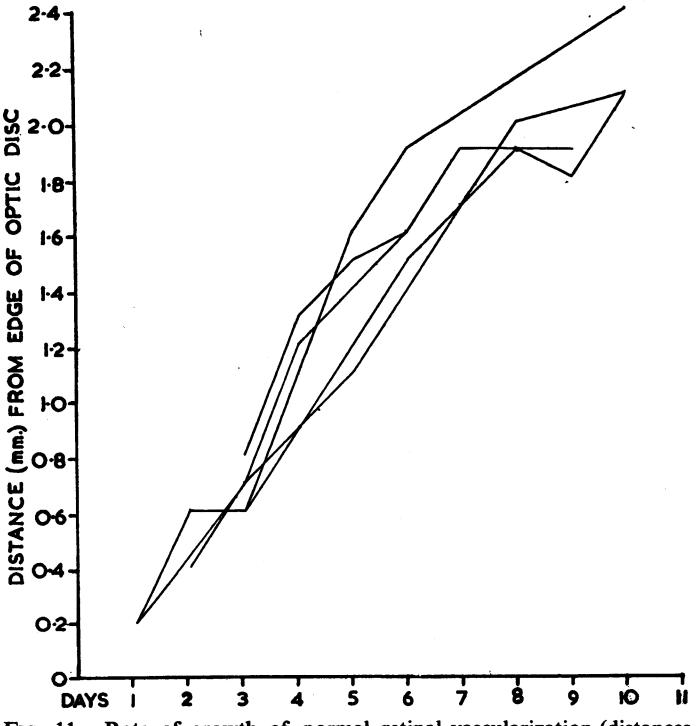

FIG. 11.—Rate of growth of normal retinal vascularization (distances measured from edge of optic disc).

(*b*) The most prominent change appears to be a delay in the formation of the capillary bed situated round the disc.

(*c*) In underdeveloped animals there is a complete absence of capillaries in the retina.

(*d*) Concentrations of oxygen of between 40 and 70 per cent. appear to give fairly comparable results.

Experiment 2 (Animals maintained in incubator from first day of life till injection between 6th and 8th day).—Table II (opposite) shows that this experiment confirms the fact that in underdeveloped animals there is a complete absence of capillaries in the retina (Fig. 15, overleaf).

TABLE II

No. of Animal	Litter	O_2 Content in Incubator (per cent.)	Condition and Weight (g.) before Injection	Extent of Vessel Outgrowth
10	F	50–70	underd., not weighed	no vessels seen
11	G	50–70	underd., not weighed	no vessels seen
12	H	40	underd., 1·6	no vessels seen
13	H	40	underd., 2·1	no vessels seen
14	H	40	underd., 2·6	no vessels seen
15	H	40	underd., not weighed	no vessels seen

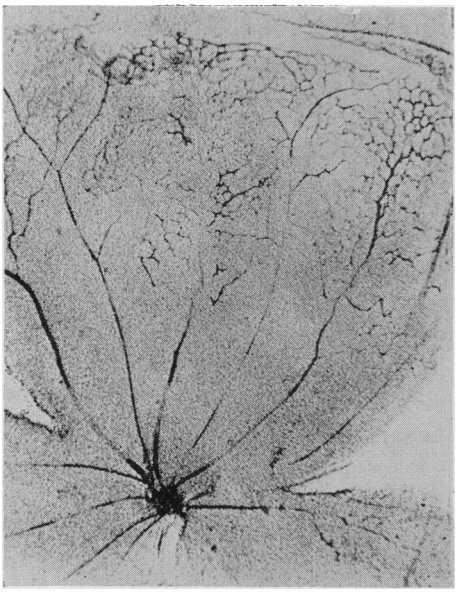

FIG. 12.—Injected retina of 8-day-old mouse, maintained in incubator from the first day of life exposed to an oxygen concentration of 50 per cent. Extent of vessel outgrowth from disc is 1·9 mm.; extent of circumpapillary area free from capillaries 0·8 mm. (×33).

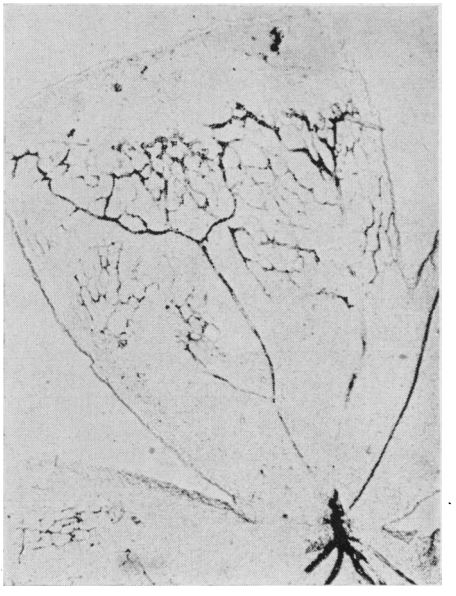

FIG. 13.—Injected retina of 8-day-old mouse, maintained in incubator from the first day of life exposed to an oxygen concentration of 50 per cent. Extent of vessel outgrowth from disc 1·8 mm.; extent of circumpapillary area free from capillaries 0·9 mm. (×33).

Experiment 3 (Animals maintained in 50 per cent. oxygen until injection on 4th day of life).—Only four eyes from two animals gave satisfactory injections. In each eye the extent of the vessel outgrowth from disc to periphery was 0.9 mm. and the extent of the circumpapillary area free from capillaries measured from the disc margin was 0.4 mm. This experiment shows that the defect in the capillary net in the neighbourhood of the disc can be observed earlier than on the 7th day. It would appear to be an exaggeration of the normal delay in capillary development in this region rather than an occlusion of capillaries after their formation (Fig. 16, overleaf).

Experiment 4 (New-born animals kept in hyperoxia for 7 days, and then in normal air until injected with Indian ink).—The normal capillary pattern

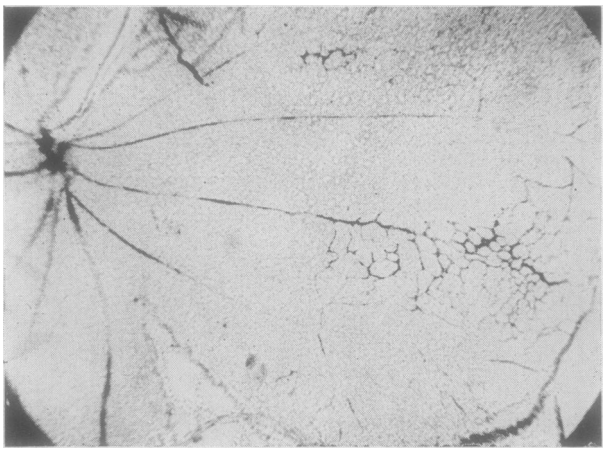

FIG. 14.—Injected retina of 8-day-old mouse, maintained in incubator from the first day of life exposed to an oxygen concentration of 50 per cent. Extent of vessel outgrowth from disc 1·8 mm.; extent of circumpapillary area free from capillaries 0·9 mm. (×40).

is established within a few days of the return of the animal to normal atmospheric conditions (Table III). There is no disturbance of this pattern during at least the subsequent few weeks. Some capillary buds were found in the vitreous at one or two places but appeared to us to be remnants of the hyaloid system.

TABLE III

No. of Animal	Litter	Days in Normal Air	O_2 Content in Incubator (per cent.)	Condition before Injection	Extent of Vessel Outgrowth*	Extent of Circumpapillary Area Free of Capillaries*
18	I	7	50	well	2·1	—
19	I	10	50	well	2·3	—
					2·8	—
20	J	13	40	well	2·0	—
					2·0	—
21	J	28	40	well	2·1	—
					2·0	—
22	J	28	40	well	2·1	—
					2·2	—
23	K	21	50–70	well	2·0	—
					2·1	—
24	K	21	50–70	well	2·0	—
					2·0	—

*In millimetres from disc margin.

Discussion

The description of the development of the retinal vessels is similar to that already reported (Michaelson, 1953). In the present report, however, attention has been drawn to the delay in the growth of the capillaries in the circumpapillary area.

The oxygen experiments described suggest that an increase of oxygen concentration to as low as 40 per cent. inhibits to some extent the growth of retinal capillaries during the first few days of life. If the animal is healthy it

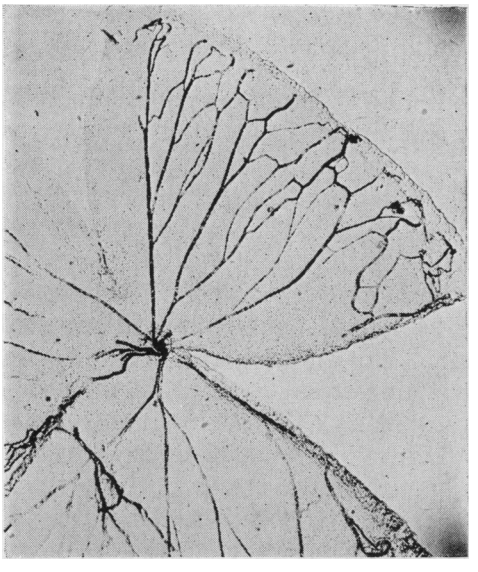

FIG. 15.—Injected retina of 5-day-old mouse, maintained in incubator from the first day of life exposed to an oxygen concentration of 50–70 per cent. The animal was underdeveloped. No retinal vessels present, injected vessels seen are those of the hyaloid system. (×33).

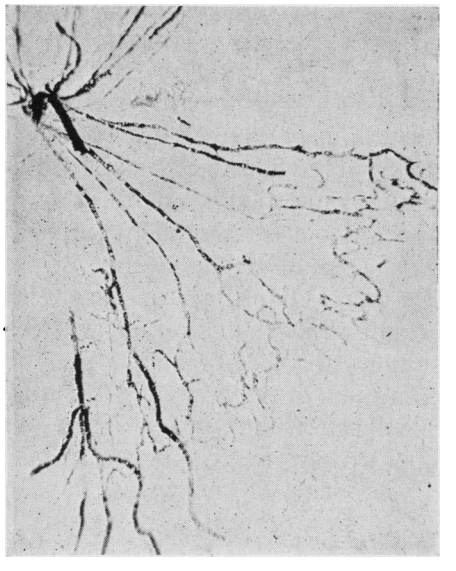

FIG. 16.—Injected retina of 4-day-old mouse, maintained in incubator from the first day of life exposed to an oxygen concentration of 50 per cent. Extent of vessel outgrowth from disc 0·2 mm., extent of circumpapillary area free from capillaries 0.5 mm. (×57).

does not greatly diminish the maximum outgrowth from the optic disc, but rather exaggerates in a gross manner the normal delay in the formation of capillaries in the retina around the disc. The process would appear to be one of inhibition rather than of occlusion, although probably this process may take place as Ashton (1953) observed in the retina of the cat. The subsequent completion of the capillary net on returning the animal to air was not accompanied by abnormality of the capillary net pattern or by capillary growth from the retina into the vitreous.

The hyperoxic condition leads in the first place to gross diminution of the number of retinal capillaries, particularly in the posterior part of the retina. This may be the first stage of the pathogenesis of the condition known as retrolental fibroplasia. It may serve as an explanation for these cases of retrolental fibroplasia in which clinically the fundus appearances consist chiefly of a milky opacity of the posterior portion of the fundus with subsequent pallor of the disc. The pallor is of the type seen in vascular occlusion. These cases frequently show no gross clinical evidence of neo-vascularization.

Among animals of equal age, the degree of diminution of capillary growth in the retina was related to the degree of development of the animal, in that absence of retinal capillaries was found only in underdeveloped animals. This might mean that the retinae of underdeveloped animals are particularly sensitive to hyperoxia; or else that the retina and the rest of the body were comparably affected by hyperoxia.

Among the animals preserved for several weeks after incubation in ordinary air, success was not obtained in producing the pathological neo-vascularization which is a notable feature of retrolental fibroplasia.

The capillary-free area in the neighbourhood of the disc in normal development and the exaggeration of it which is characteristic of animals placed in an increased concentration of oxygen are so striking as to arouse speculation. In this connection it is pertinent to recall that the need for retinal vessel growth appears to arise when the choroid becomes incapable of supplying the nutritional need of all the retina. In practically all the vertebrates, therefore, we find a period during development when the choroid can supply all the nutritive requirements of the retina, whereas in the later definitive stage the choroid supplies only a certain portion of the retina. For example, in man, the choroid continues throughout life to supply nutrition to all portions of the retina which are within 140 μ of the chorio-capillaris (Michaelson, 1953). It is thus perhaps permissible to speculate that the delay in the circumpapillary development of the retinal capillaries noted above in the normal mouse retina may be due to the greater capacity of the circumpapillary choroid to nourish the retina as compared with that of the more outlying portions. The exaggeration of this delay seen in the hyperoxic mice may be due to the capacity of the choroid in the circumpapillary region to transmit the additional oxygen to its adjacent retina.

Summary

(1) A description is given of the normal development of the retinal blood vessels in the mouse.

(2) A description is given of the development of these vessels after the animals had been for some time in an incubator in high concentrations of oxygen.

(3) The following findings were noted in the retinae of animals exposed to high oxygen concentrations.

(*a*) The extent of the vessel outgrowth from the disc is not grossly affected by the change in oxygen concentration.

(*b*) The most prominent change appears to be a delay in the formation of the capillary bed situated round the disc.

(*c*) In underdeveloped animals there is a complete absence of capillaries in the retina.

(*d*) Concentrations of oxygen between 40 and 70 per cent. appear to give fairly comparable results.

(*e*) The normal capillary pattern was established within a few days of the return of the animal to normal atmospheric conditions.

(4) It is suggested that the gross diminution in the number of retinal capillaries resulting from hyperoxic conditions may be the early stage of the pathology of retrolental fibroplasia, and may be an explanation of these

cases in which milky pallor of the fundus and optic atrophy are signs more outstanding than neovascularization.

(5) Mention is made of the association of underdevelopment with the retinal vessel changes found in hyperoxia.

(6) Speculation is made regarding the role of the choroid in the production of retinal vessel abnormalities due to hyperoxia.

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