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COMMUNICATIONS

STUDIES ON DEVELOPING RETINAL VESSELS

VIII. EFFECT OF OXYGEN ON THE RETINAL VESSELS OF THE RATLING*

BY

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IN 1953 it was found that exposure of kittens to high ambient concentrations of oxygen resulted in obliteration of their retinal vessels, and that subsequent return to air resulted in vasoproliferation (Ashton, Ward, and Serpell, 1953). It was later briefly reported that the retina of the ratling was considerably less susceptible to hyperoxia than that of the kitten, since only the posterior vessels were obliterated, but no opinion was given on vasoproliferation at that time as the ratlings had not survived on transfer to air (Ashton, Ward, and Serpell, 1954).

Other workers, however, have reported that the retina of the ratling behaves in an exactly similar way to that of the kitten (Patz, 1954a,b; Brands, Hofmann, and Klees, 1958); indeed ratlings were some of the chief experimental animals used by Patz, Eastham, Higginbotham, and Kleh (1953) and by Patz (1954a,b) in demonstrating retinal vasoproliferation due to oxygen exposure in their studies on retrolental fibroplasia. Patz (1954a) also reported complete obliteration of the retinal vessels of the rat after 3 days' exposure to hyperoxia and he obtained vasoproliferation in oxygen —a finding which was not confirmed by Brands and others (1958).

In view of these conflicting results and because the ratling would be a more convenient animal than the kitten for this type of work, we have carried out a further series of experiments to determine the effect of hyperoxia on the retinal vessels of the ratling.

ANATOMICAL CONSIDERATIONS

The anatomy and development of the retinal vasculature of the rat have been described in detail by Hesse (1880), Bruns (1882), Michaelson (1954), Janes and Bounds (1955), and more recently by Cairns (1959); apart from some minor differences their findings have been confirmed in the present study, which involved the examination of 53 normal animals of the following ages in days—4, 6, 8, 12, 21, 26, 31, 33, 36, 41, and adult. All specimens were examined by injection and 26 by histology (Table I, overleaf). In our experience the pattern and rate of growth of the vessels is much more variable in comparable animals than has been described by the above authors.

* Received for publication November 8, 1960.

TABLE I

NORMAL CONTROLS

Experiment No.	Number of Ratlings	Age at Death (days)	Posterior Deep Plexus	Vessels in Vitreous
3	10	4	0	—
11A	1	6	0	—
12A	1	6	0	—
21A	3	8	Developing (from venous side)	—
*5	10	12	+	Present
*19A	2	21	+	Present
20A	4	21	+	Present
*21B	2	26	+	Present
*19B	3	31	+	Present
20B	4	31	+	—
*21C	2	33	+	Present
*21D	1	36	+	Absent
*19C	4	41	+	Absent
20C	4	41	+	—
*22A	2	Adult	+	Absent

This Table shows the number and ages of normal animals examined, and indicates the groups in which the deep plexus was seen posteriorly in injected retinae, and those in which vitreal vessels could still be seen in sections.
* Experiments in which histological examination was carried out in addition to injection.

The retinal vessels arise at birth or shortly before from central vessels at the optic disc, and usually form six arteries and six veins,* which radiate symmetrically from the disc, alternating with each other, and initially forming a primitive capillary net in the nerve fibre layer. A peri-arterial capillary-free zone is evident throughout and persists in the adult pattern. The superficial vascular layer is complete by the 11th day, the peripheral vessels being the last to mature. The deep capillary net begins to form at the 8th to 9th day and is complete by the 15th day, when the final adult vascular arrangement is reached. The superficial capillary net, having lost its foetal pattern, is now seen to be almost entirely arterial in character, with no intercommunications or connexions with the veins in the superficial layer. The original venous connexions atrophy with the development of the deep net, although remnants are represented by occasional capillary communications between artery and vein. Branches from the superficial capillary net dip

* The number is variable: note there are ten vessels in Fig. 12b, thirteen in Fig. 7, and sixteen in Fig. 6.

down sharply into the retina to join the deep capillary nets on either side of the inner nuclear layer; the deeper of these nets, in contrast to the most superficial plexus, is entirely venous, and drains into tributaries, the largest being peripheral, which travel steeply towards the surface of the retina to the main veins (Figs 1, 2 and 3).

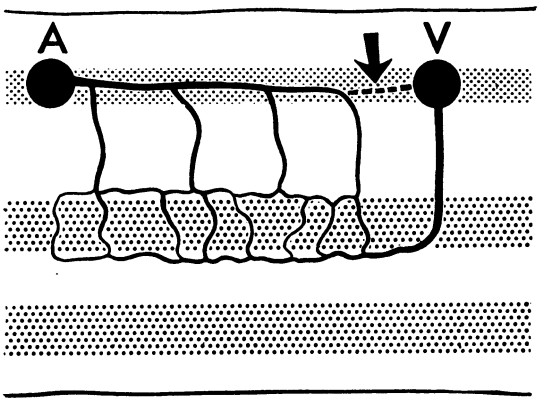


FIG. 1.—Diagram illustrating development of vessels of the rat retina. The deep capillary plexus, on either side of the inner nuclear layer, develops by downgrowth from the primitive superficial net, and the original venous connexions atrophy (arrow). The adult superficial net thus becomes entirely arterial, while the deeper vessels are predominantly venous.

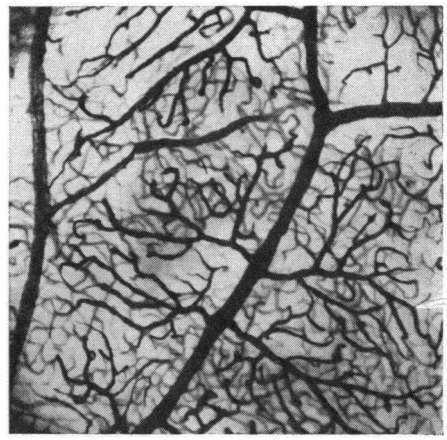


FIG. 2.—Flat preparation of injected adult rat retina, showing an artery on the right and a vein on the left. Note that the superficial plexus is almost entirely arterial and that the twig-like branches show no intercommunications or connexions with the veins at this level. The deep plexus can be seen faintly in the background. ×50.

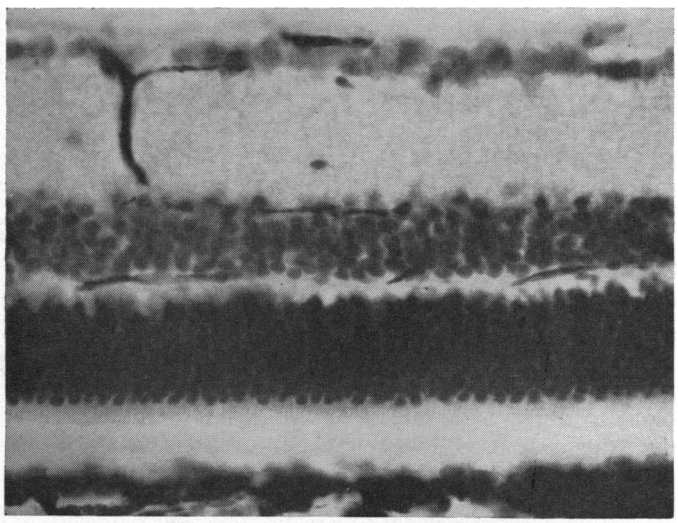


FIG. 3.—Section of injected rat retina, showing superficial plexus in stratum opticum communicating with the deep plexus on either side of the inner nuclear layer. ×280.

As will be emphasized again later, the developing eye of the ratling shows a dense meshwork of hyaloid vessels, well described and illustrated by Cairns (1959); their outer component lies close to the retinal surface, and these vessels may persist up to three and occasionally over four weeks—a point of some importance in the interpretation of vasoformation in sections (Table I).

Material and Methods

Albino rats of the Wistar strain were used in all experiments. The mother rats and weaned ratlings were given food compound 41B and water *ad lib.* The experimental ratlings were exposed to oxygen concentrations ranging from 60 to 80 per cent. in gas chambers at normal atmospheric pressure, as described by Ashton and others (1954), and at other times were kept in cages open to the normal atmosphere.

Controls.—These were obtained by transferring a proportion of each litter to a foster mother in air, but this was not done in all experiments since the normal development had been fully investigated as a preliminary study.

Injection Techniques and Method of Examination.—At the end of every experiment the ratlings were injected intraperitoneally with a fatal dose of Nembutal, and were also injected *via* the left ventricle either by Indian ink, as described by Ashton and others (1954), or by a silver solution as described by Hausler and Sibay (1959). In the latter method the vascular system was first washed free of blood with normal saline, followed by 10 per cent. formol saline, and then by a solution consisting of 1 per cent. silver dinapthylmethane disulphonate ("Viacutan")* two parts, and 1 per cent. silver nitrate one part. The vessels were finally irrigated with distilled water and the eyes enucleated.

The anterior segment of the eye was removed and the retina exposed to ultraviolet light for 3 to 5 minutes until the vessels turned brown. The retina was then dissected out and mounted flat in glycerine jelly. The silver injection, when successful, gave a clearer definition of the vessels than Indian ink, but was too unreliable for general use. Histological studies were usually made on the fellow eye—injected or uninjected.

Experiments.—A total of 174 (51 controls) Wistar albino ratlings were used. The experimental animals were exposed to oxygen at ages varying from the newborn to 11 days, and they remained in oxygen for periods varying from three to 14 days. Some were then killed and examined immediately, while others were returned to air for 10 to 20 days before examination. An additional experiment was carried out to observe the effects of hyperoxia on the retinal vessels of the adult rat: eight rats were used, two being kept as controls and six being placed in 70–80 per cent. oxygen for 4 days. Three were killed on coming out of oxygen, and three were killed after 10 days in air. These animals were injected with Indian ink and the retinal vessels were studied as described above.

* Ward Blenkinsop & Co. Ltd., London.

EXPERIMENTAL FINDINGS

It is convenient to consider the experimental results under two main headings: those obtained in hyperoxia and those obtained in air after exposure to hyperoxia. These are analysed in Table II and in Table III (p. 332).

(1) EFFECT OF HYPEROXIA

Sixteen experiments involving 67 animals were carried out to study the effect of hyperoxia alone. As may be seen in Table II, which shows the effect of hyperoxia for various periods of exposure (from 3 to 14 days) with increasing age (birth to 11 days and adult), obliteration of the retinal vessels occurred as a regular phenomenon but was confined to the capillaries at the posterior pole, the main vessels and peripheral capillaries being entirely unaffected. In the *adult* animal no vascular changes were observed.

TABLE II
EFFECT OF HYPEROXIA

Experiment No.	Number of Ratlings	Age (days)	Oxygen Exposure (days)	Age at Death (days)	Posterior	
					Vaso-obliteration	Deep Plexus
1	2	0	4	4	±	Absent
2	12	0	4	4	±	Absent
7A	3	0	7	7	+	Absent
*4	3	1	12	13	+	Absent
18A	3	4	4	8	+	Absent
21E	3	5	3	8	+	Absent
*11B	4	6	4	10	+	Absent
*12B	3	6	4	10	+	Absent
17A	5	6	4	10	+	Absent
*13A	3	7	3	10	+	Absent
*19D	2	7	14	21	+	Absent
20D	3	7	14	21	+	Absent
16A	3	8	4	12	+	Absent
15A	3	11	4	15	+	Absent
14	12	11	4	15	+	Absent
22B	3	Adult	4	Adult	0	Present

The Table shows the number and ages of ratlings subjected to hyperoxia for varying periods, and indicates the vaso-obliteration obtained posteriorly. In all ratlings the deep plexus was absent posteriorly.
* Experiments in which histological examination was carried out in addition to injection.

The diagram in Fig. 4 shows the periarterial capillary-free zones in the normal retina of the ratling extending from the disc to the periphery in sharply defined clear margins, well seen in specimens injected with Indian ink. In comparison with the retina from an animal treated in oxygen, it will be seen that these periarterial capillary-free zones have widened considerably, mainly posteriorly and least anteriorly. As the vessels converge upon the disc, so these capillary-free areas gradually merge together, producing total capillary obliteration at the posterior pole and resulting in a star-shaped pattern of avascular tissue (Fig. 5).

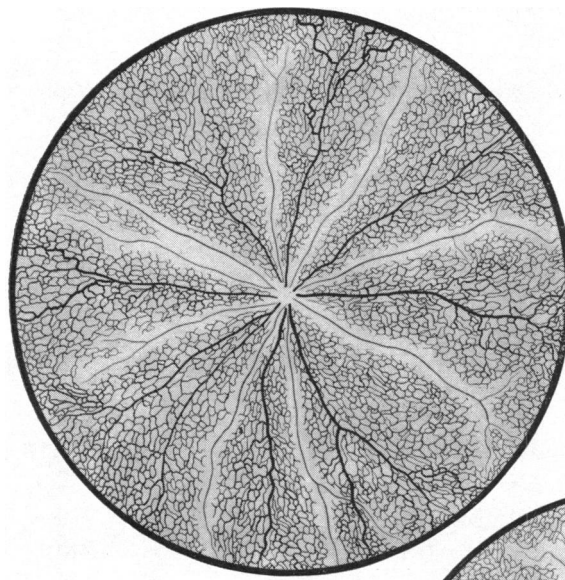


FIG. 4.—Diagram of normal ratling retina injected with Indian ink, showing the vascular pattern in an 8-day-old control animal. Note the peri-arterial capillary-free zones.

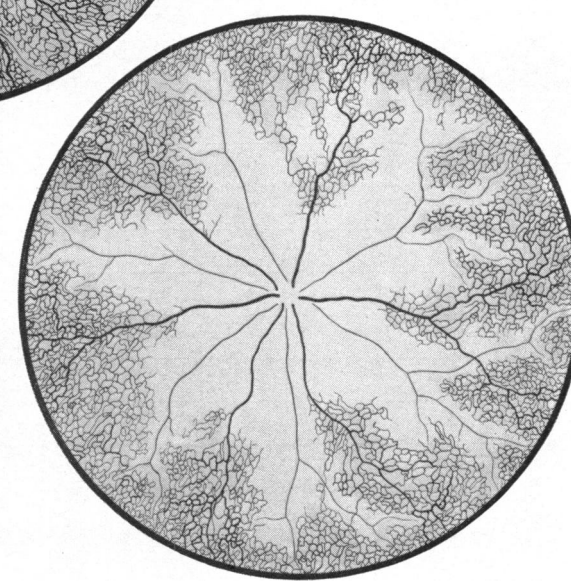


FIG. 5.—Diagram of retina of an 8-day-old ratling exposed to continuous hyperoxia for 3 days. Note that the peri-arterial capillary-free zones have widened considerably, mainly posteriorly and least anteriorly. As the vessels converge upon the disc, so these capillary-free areas gradually merge together, producing total capillary obliteration at the posterior pole, and resulting in a star-shaped pattern of avascular tissue.

These findings were much the same whether the rat had been exposed to

hyperoxia for 3 or 14 days (Figs 6, 7, 8), varying only with the extent of vascular development at differing ages.

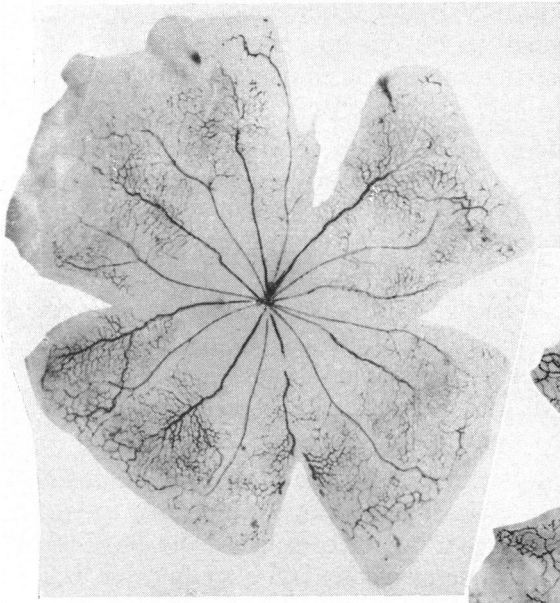
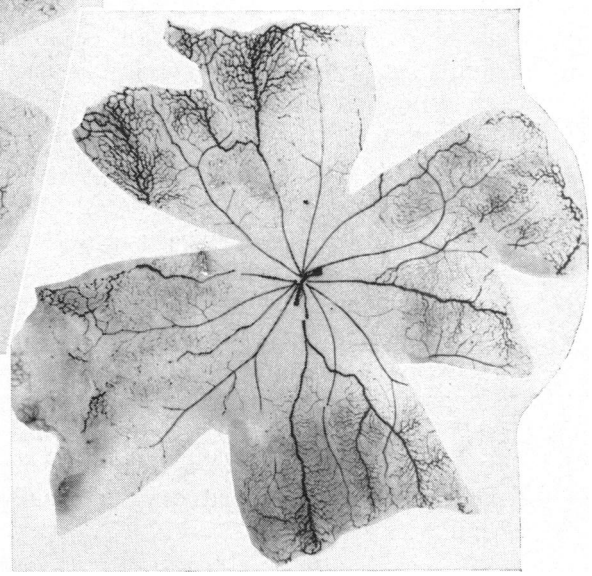


FIG. 6.—Photograph of injected retina from a 5-day-old ratling exposed to continuous hyperoxia for 3 days. Note typical star-shaped pattern of capillary obliteration. ×13·5.



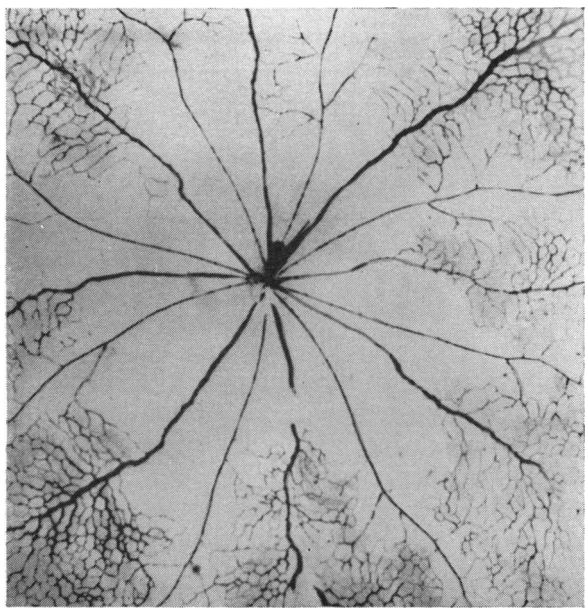


FIG. 7.—Photograph of injected retina from a 1-day-old ratling exposed to continuous hyperoxia for 12 days. The star-shaped pattern of capillary obliteration is very similar to that seen in Fig. 6. ×9·75.

FIG. 8.—High power of Fig. 6, showing total capillary obliteration around the disc. ×22·5.

The only exception was in Experiments 1 and 2, in which the injection results showed attenuated developing vessels but no complete obliteration. No abnormal proliferations were seen in these injected preparations in either test or control animals.

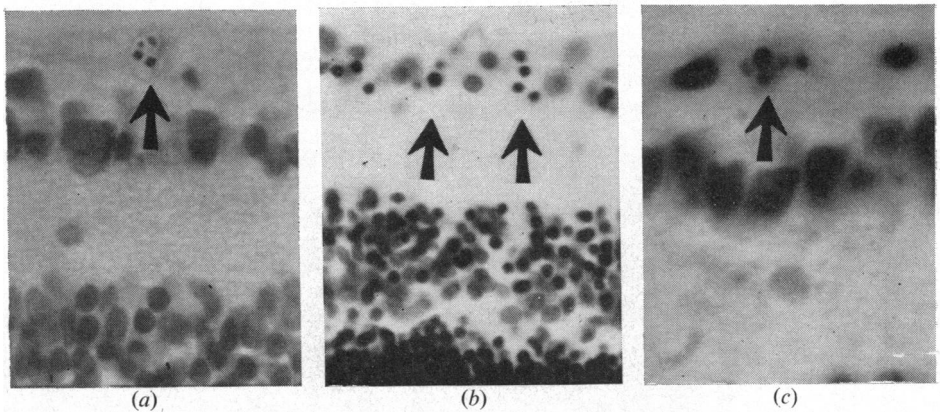
A similar pattern and extent of vaso-obliteration occurs also in the deep capillary net if this is present, as was shown, for instance, in Experiments 14 and 15A in which the deep net would have been well-formed at the age the animals were subjected to hyperoxia (11 days). In the injected specimens a star-shaped area of obliteration could be seen, not only in the superficial capillaries of the posterior pole but also in the deep net in that region. If the animal is subjected to oxygen before the deep net has developed, *i.e.* before 8 or 9 days of age, the net will not develop until the animal returns to air. In other words, hyperoxia will not only obliterate the posterior deep plexus but will also prevent its development.

On the other hand, hyperoxia does not prevent the growth of the main vessels or of the peripheral vessels—at least not to any appreciable extent. In Experiment 7A, for instance, a ratling exposed to oxygen from birth for 7 days showed, apart from the obliterative oxygen effects already described, a retinal vasculature as advanced anteriorly as that of a normal animal. Similarly the deep net will also develop in oxygen in areas where the superficial net, from which it arises, is not obliterated. This was shown in Experiment 20D in which a 7-day-old animal was kept in oxygen for 14 days: the deep net developed quite normally anteriorly where the superficial capillaries were patent.

Histology.—In five (15 animals) of the sixteen experiments shown in Table II, histological studies were made in addition to injections. In three of these experiments (Exps. 11B, 12B, and 13A) the animals were exposed to oxygen for short periods (3 or 4 days) and in two (Exps. 4 and 19D) for longer periods (12 and 14 days respectively).

The vaso-obliterative effects of oxygen already described in the injected specimens could be confirmed in that no patent capillaries were seen in the posterior pole of the retina. In some sections the endothelial cells of collapsed capillaries could be discerned and showed degeneration as evidenced by karyorrhexis and pyknosis (Fig. 9*a*, *b*, *c*). PAS-staining revealed no further differences between test and control animals.

No abnormal retinal vasoproliferation was found in any of the 75 sections examined, apart from a solitary doubtful nodule in one section (Exp. 19D, Fig. 10) which can be discounted in the presence of so many negative findings. Nor was a single retinal or vitreal haemorrhage seen. Hyaloid vessels were still evident in the vitreous in the oldest animals of the series (Exp. 19D; 21 days), but this is not abnormal. There was no abnormal proliferation of these vessels and no evidence of disorganization of the vitreous.



(*a*) (*b*) (*c*)

FIG. 9 (*a, b, and c*).—Sections of retinae of ratlings exposed to continuous hyperoxia for 4 to 7 days. Note karyorrhexis and pyknosis in degenerating endothelium of obliterated capillaries (arrows). ×700 ×480 ×1140.

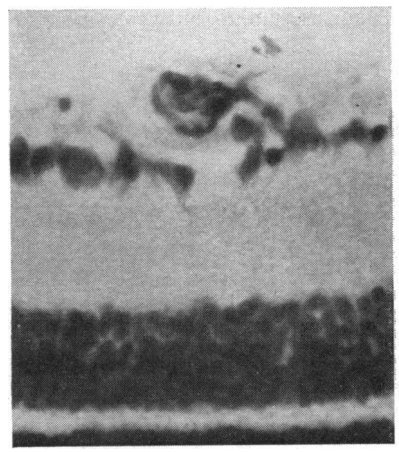


FIG. 10.—Section of retina of a ratling exposed to continuous hyperoxia for 14 days, showing a solitary nodule of endothelial cells in the stratum opticum. This was the only lesion found which could be interpreted as abnormal vasoproliferation. ×440.

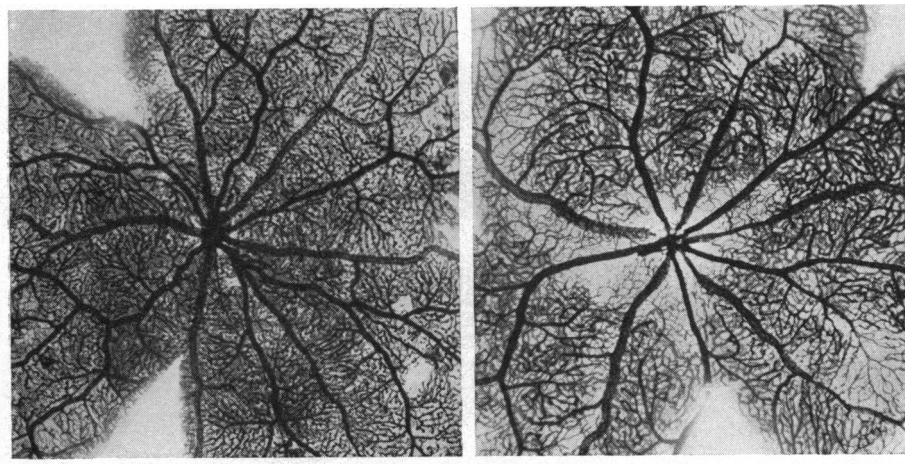
No degenerative cytological changes were found in the visual cells, the ganglion cells, or the cells of the inner and outer nuclear layers.

(2) EFFECT OF AIR SURVIVAL AFTER HYPEROXIA

23 experiments involving 62 animals were carried out to study the effect of varying periods of air survival (10 to 25 days) after varying periods of hyperoxia (3 to 14 days) with increasing age (birth to 11 days and adult). Table III (overleaf) shows that no abnormal vasoproliferations were found. Anteriorly, the periarterial capillary-free zone returned to its normal width, but the superficial capillary net, and deep capillary net if present at the time of exposure, remained permanently obliterated.

Since the deep capillaries normally develop from downgrowths from the superficial net, obliteration of these parent vessels at the posterior pole in

an animal of under 9 days, prevents the formation of the deep plexus in the immediately subjacent area. Eventually, however, vessels at the periphery of this avascular zone grow centripetally to re-form the deep net, and after 18 to 20 days in air this is virtually fully developed (Fig. 11*a*, *b*; Fig. 12*a*, *b*, opposite).



(*a*) (*b*)

FIG. 11 (*a*).—Control 31-day-old ratling retina injected by the silver method, showing normal vascular pattern. ×33.

FIG. 11 (*b*).—31-day-old ratling retina injected by the silver method (Exp. 19E). At 7 days old, the animal was exposed to continuous hyperoxia for 14 days and allowed to survive in air for 10 days. The retina shows permanent obliteration of the superficial capillary net at the posterior pole, and re-formation of the deep capillary net. ×33.

In older animals (over 9 days), in which the deep capillaries had been obliterated by oxygen, a similar process occurred, *i.e.* the deep plexus re-formed, although we cannot here exclude the possibility that some of the pre-existing vessels might have re-opened. No vessels, however, proliferated into the superficial peripapillary region which remained avascular for at least 25 days' air survival (Exp. 21G)—and would probably have done so permanently.

The final vascular pattern, therefore, was the same whether the animal was placed in oxygen at birth or up to 11 days of age, and whether it was kept in oxygen for 3 or up to 14 days.

Histology.—Of the 23 experiments shown in Table III (overleaf), histological studies were made, in addition to injection, in eleven experiments (41 animals; Exps. 6, 8, 9, 10, 11C, 12C, 13B, 19E, 19F, 21F, and 21G). The sections confirmed the findings already described in that no superficial net could be demonstrated in the posterior polar region of any of the ratling eyes. The deep net could be seen posteriorly in sections after 18 days' survival in air.

No abnormal vasoproliferations were seen in any of the 226 sections examined and only one solitary retinal haemorrhage was found (Exp. 13B).

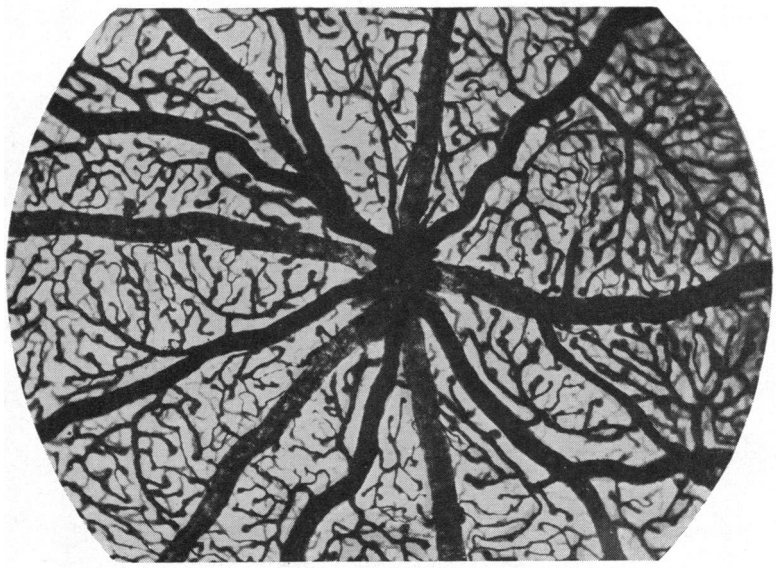


FIG. 12 (*a*).—High-power view of Fig. 11 (*a*). ×54.

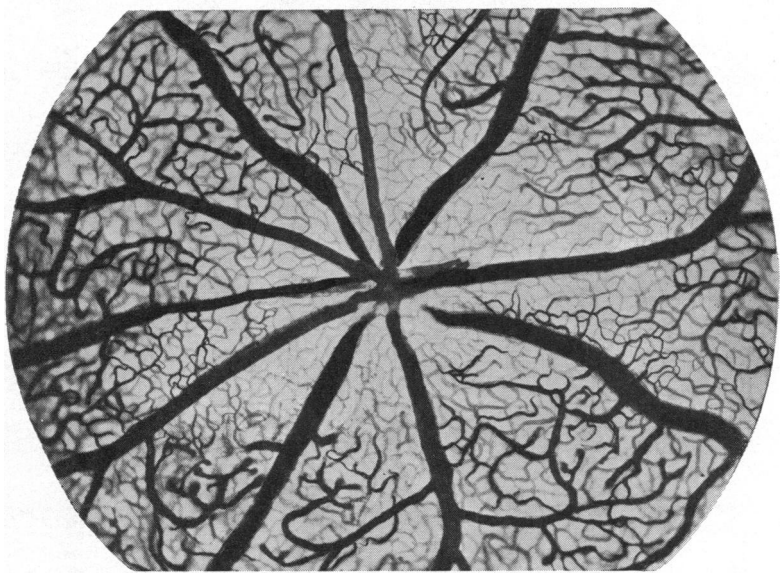


FIG. 12 (*b*).—High-power view of Fig. 11 (*b*). ×54.
Note zone of capillary obliteration in superficial retina around disc. The deep capillary plexus (seen faintly in the background) has re-formed by centripetal growth from the patent vessels at the margin of the area of obliteration.

Remnants of the hyaloid vessels still persisted in the vitreous in 33-day-old ratlings (Exp. 21G), but this is to be compared with the controls in which they were also seen at 33 days (Exp. 21C). We found no evidence that oxygen exposure resulted in any abnormal persistence of the hyaloid system.

No vitreous haemorrhages, no proliferation of vessels into the vitreous, and no disorganization of the vitreous were found.

TABLE III

HYPEROXIA WITH AIR SURVIVAL

Experiment No.	Number of Ratlings	Age (days)	Oxygen Exposure (days)	Air Survival (days)	Age at Death (days)	Posterior		Vasoproliferation
						Vasoobliteration	Deep Plexus	
18B	1	4	4	10	18	+	Absent	0
17B	2	6	4	10	20	+	Absent	0
20E	3	7	14	10	31	+	Absent	0
*19E	3	7	14	10	31	+	Absent	0
16B	1	8	4	10	22	+	?	0
15B	1	11	4	10	25	+	Absent	0
22C	3	Adult	4	10	Adult	0	Present	0
*6	4	1	7	11	19	Histology only		0
18C	1	4	4	16	24	+	?	0
17C	1	6	4	16	26	+	Present	0
16C	1	8	4	16	28	+	Present	0
15C	1	11	4	16	31	+	?	0
*10	6	0	5	18	23	?	Present	0
7B	3	0	7	18	25	+	Present	0
*8	6	2	5	18	25	+	Present	0
*9	6	2	5	18	25	+	Present	0
*21F	1	5	3	18	26	+	Present	0
*11C	4	6	4	18	28	+	?	0
*12C	2	6	4	18	28	+	Present	0
*13B	4	7	3	18	28	+	Present	0
20F	3	7	14	20	41	+	Present	0
*19F	4	7	14	20	41	+	Present	0
*21G	1	5	3	25	33	+	?	0

The Table shows the number and ages of ratlings subjected to varying periods of hyperoxia with increasing periods of air survival, and indicates the persistence of vaso-obliteration in the posterior fundus, the reappearance of the posterior deep plexus, and the absence of abnormal vasoproliferation.

* Experiments in which histological examination was carried out in addition to injection.

SUMMARY OF FINDINGS *

(*1*) *Hyperoxia without Air Survival*

(*a*) Widening of the peri-arterial capillary-free zone developed with obliteration of the superficial capillary net at the posterior pole; this resulted in a star-shaped pattern of avascular tissue.

(*b*) In younger ratlings the deep capillary net failed to develop in the same region; *i.e.* growth was arrested.

(*c*) In older ratlings, in which the deep capillary net was already present at the time of oxygenation, it closed together with, and to the same degree as the superficial net; *i.e.* these capillaries were also obliterated.

(*d*) The growth of the main retinal vessels and of the peripheral capillaries was not significantly affected.

(*2*) *Hyperoxia with Air Survival*

(*a*) Anteriorly the peri-arterial capillary-free zone returned to its normal width.

(*b*) The closed superficial capillaries at the posterior pole neither re-opened nor re-formed on return to air, irrespective of the age of the ratling.

(*c*) In contrast, the deep capillary net at the posterior pole re-developed (whether it had been arrested or obliterated), the vessels growing *centripetally* towards the optic disc in an orderly manner from the patent vessels at the margin of the affected area.

(*3*) *Hyperoxia, with or without Air Survival*

(*a*) No abnormal vasoproliferations were obtained into either retina or vitreous—even posteriorly where the superficial capillaries remained obliterated.

(*b*) No vitreal haemorrhages or disorganization occurred, and no abnormal persistence or proliferation of the hyaloid system was seen.

(*c*) The retinal vessels of the adult rat were unaffected.

DISCUSSION

Our findings differ considerably from those reported in the literature by other workers. It is possible, of course, that the experiments of each group are not in fact truly comparable, for there may have been variations of unrecognized significance in the experiments or in the strains of animals used, but the conflicting evidence appears to be largely attributable to differences in the interpretation of histological appearances.

In one of the earliest experiments carried out by Patz and others (1953), new-born ratlings were exposed to 80 per cent. oxygen for 21 days and then killed. The only retinal lesion found in the 27 oxygen-treated ratlings surviving at 21 days was prominent oedema of the inner layers of the retina in

* It should be emphasized that these are the results as assessed by injections and sections. No direct observations on the living animal were made as the eye proved too small for this type of study.

six eyes. Persistence and proliferation of the tunica vasculosa lentis and disorganization of the vitreous were found in seventeen eyes; 29 control animals were normal.

In a subsequent paper, however, Patz (1954a) described further changes. When " rats " (the age of these animals was not given) were exposed to 40–50 per cent. oxygen, a marked vasoconstriction of the retinal vessels was noted, but when the oxygen was maintained at 70–80 per cent. complete obliteration of the vessels resulted after 3 to 4 days' exposure. After subjection to hyperoxia for 12 to 15 days, capillary tufts resembling glomeruli were found on the surface of the retina and in sections these were found to be typical capillary proliferations. Abnormal neovascularization thus occurred in his animals while actually under high oxygen concentration.

There are therefore three main findings reported by Patz that we are unable to confirm: (1) *total* vaso-obliteration of the retinal vessels, (2) abnormal vasoproliferation in the retina, and (3) persistence and proliferation of the hyaloid vessels with disorganisation of the vitreous.

In the experiments carried out by Brands and others (1958), 4-day-old ratlings were exposed to 70 per cent. oxygen for 6 days continuously, or with 30 minutes in air each day, or with 1 to 5 hours in air each day. One group of ratlings was killed immediately after exposure to high oxygen concentrations, a second group was examined after 7 days' survival in air, and a third group after 17 days' survival in air; the eyes were examined in cross-section. In the first group no histological changes were found, but in the second and third groups the following abnormalities were noted: a blurred internal limiting membrane, vasoproliferation as evidenced by endothelial growth in the nerve fibre layer, capillary budding into the vitreous, retinal haemorrhages, and retinal oedema. The ratlings exposed to continuous oxygen and those allowed 30 minutes in air each day showed severe retinal changes, but those in air for 1 to 5 hours each day showed a much milder retinopathy.

There are, therefore, two main findings of Brands and others (1958) in rats returned to air after hyperoxia that we are unable to confirm: (1) retinal haemorrhages and oedema, and (2) abnormal vasoproliferation in the retina. We are in agreement with their finding that vasoproliferation does not occur in hyperoxia. All these results will now be considered below.

Differences with regard to Vaso-obliteration

As already stated, we have found that vaso-obliteration in oxygen is not complete but only *partial*, and is confined to the posterior polar region, while the main vessels and the peripheral vessels are apparently unaffected. Table II shows that we have demonstrated this at least fifty times. Patz (1954a) did not provide his full experimental data or report the number of

times he found total vaso-obliteration, so that we are at a loss to explain our divergent findings. Assuming that he used new-born ratlings, there are three comparable experiments in our series:

In Experiment 7A the new-born ratlings were kept in hyperoxia for 7 days; these showed the typical partial posterior closure.

In Experiments 1 and 2, which are exactly comparable to those of Patz in that the new-born animals were kept in hyperoxia for 4 days, the vessels were less advanced than normal but it was doubtful whether any obliteration had occurred.

It is in fact very difficult to assess vaso-obliteration in new-born ratlings kept in oxygen for only a few days, for it will be remembered that during this time the retinal vessels are growing from the disc to the equator, so that their absence after oxygen exposure could be attributed as well to arrest of growth as to obliteration. Indeed, in passing, we would stress that the exposure of new-born ratlings to oxygen is an entirely different experiment from exposing older animals with the retinal vasculature already established. It is only the exposure of older animals that is comparable to the situation in which premature infants are exposed to oxygen.

It is interesting that oxygen-induced vaso-obliteration should be only partial in the ratling, when at a comparable period of development it is complete in the kitten. It would perhaps be surprising if the ratling reacted to oxygen in exactly the same way as the kitten, as there are distinct differences in the development and anatomy of their retinal vessels. In the *rat* these vessels appear at about the time of birth and are nearly complete in 2 weeks, whereas in the *cat* they appear 3 weeks before birth and are not complete until 3 weeks after birth. The *rat* has about six pairs of vessels in communication with central vessels of the optic disc, whereas the *cat* has only three pairs of vessels, most of which are connected with the choroid. The arterial nature of the superficial capillary layer and the venous nature of the deep capillary layer of the *rat* is an arrangement not duplicated in the *cat* (in this opinion we differ from Michaelson, 1954). The developing retinal vessels of the kitten correspond much more closely to those of man.

The explanation of the differing reactions of the ratling and kitten is probably to be sought in the distribution of the retinal vessels themselves; certainly the comparative immunity of the anterior vessels of the ratling and their contrasting vulnerability in the kitten are not due to the density of the choroidal vessels, which in both animals is uniform throughout (although it may be related to differences in the distribution of choroidal arteries and veins). But, as we have already pointed out, the selective obliteration of capillaries in the posterior polar region is more probably due to the proximity of the large number of retinal arteries at the disc, which allows a high concentration of oxygen to be developed in the retinal tissue of this region, whereas in the anterior retina the venous system is predominant.

Differences with regard to Vasoproliferation

With regard to the second point of disagreement, namely, abnormal retinal vasoproliferation on transfer to air, we would first draw attention to the ways in which vasoproliferation may be simulated by artefact. For example, the relatively acellular stratum opticum can appear to contain buds of proliferating endothelial cells in the occasional section passing through the wall of a main artery (Fig. 13), or when the retina is cut obliquely (Fig 14), and capillary proliferation can be very closely mimicked by the proximity of

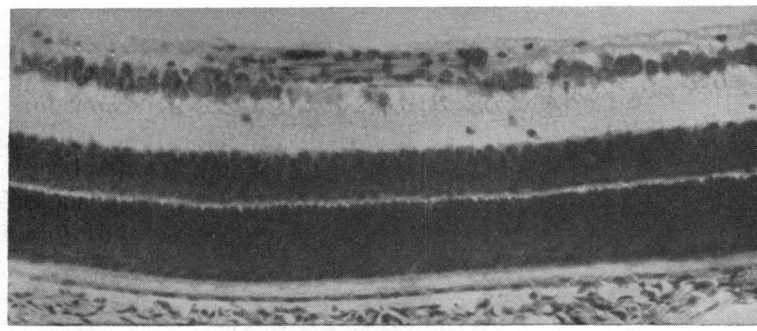


Fig. 13.—Section of normal 10-day-old ratling retina, showing a large vessel cut longitudinally in the nerve fibre layer. The appearances could be misinterpreted as abnormal vasoproliferation. Haematoxylin and eosin. ×280.

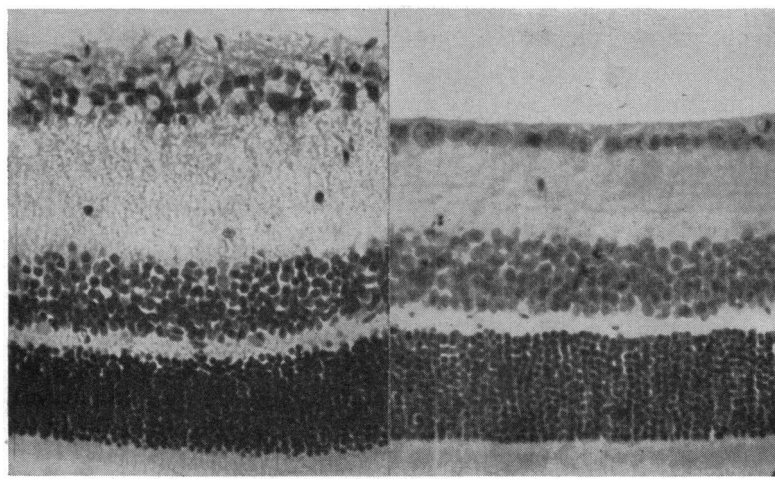


Fig. 14.—Two areas from the same section of the retina of a normal 26-day-old ratling. On the right the cross-section is truly vertical, whereas on the left it has passed obliquely through the tissue, producing a blurred internal membrane and the appearance of abnormal cellularity in the nerve fibre layer. Haematoxylin and eosin. ×280.

hyaloid vessels (Fig. 15, opposite). Furthermore, since the retinal vessels are developing in the ratling, it is not uncommon to encounter occasional endothelial buds in the normal animal. It is therefore essential that all these

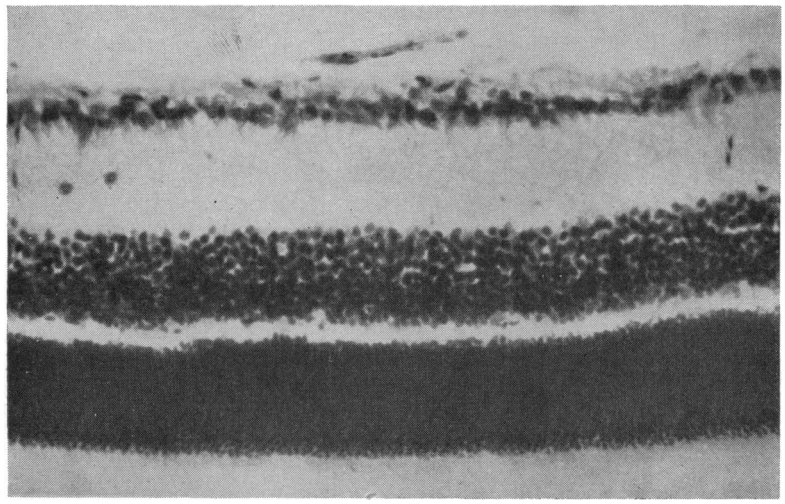


FIG. 15.—Section of normal 26-day-old ratling retina, showing a vestigial hyaloid vessel adjacent to the retinal surface which could be misinterpreted as abnormal vasoproliferation. Haematoxylin and eosin. ×280.

possibilities should be excluded before isolated proliferations are regarded as abnormal; in fact, one can be certain of this only when the vessels or vasoformative tissue are extremely well marked or are extending out of the retina into the vitreous. Not having seen the sections reported upon by Patz, we are in no position to comment upon his interpretations, but in his papers we can find only one illustration of such a lesion in the rat retina. This is comparable to the solitary lesion we found (Fig. 10), but no statistical data are provided to support his conclusion that these lesions are in fact attributable to oxygen, or could be regarded as a regular—or even usual—sequel to oxygen exposure. On the other hand, Fig. 12 in his Holmes Memorial Lecture (Patz, 1954b) clearly shows abnormal capillary tufts in an injected retina from a 16-day-old rat maintained continuously in 70 per cent. oxygen from the fourth day of life. This we have never found. Moreover, it is noteworthy that only one other group of workers (Gyllensten and Hellström, 1956) has produced vasoproliferation in oxygen alone, but this was after reducing the oxygen from 100 to 40 per cent.; moreover, the animals were mice and not rats. We conclude that abnormal vasoproliferation in rats kept in hyperoxia alone at *normal atmospheric pressure* must be a most exceptional development.

The abnormal vasoproliferations obtained by Brands and others (1958) with air survival after hyperoxia may be questioned by reference to their illustrations. In none of these examples is there certain evidence of abnormal vasoproliferation, for the appearance can all be duplicated in normal sections. In our view, the apparent abnormalities are due to obliquity of the sections

(which is supported by their finding of a " blurred internal limiting membrane "), or to the presence of hyaloid vessels near the surface of the retina. The obliquity of the sections is quite evident in their Figures 3, 5, 8, and 9, as judged by the width or irregularity of the internal nuclear layers alone. While not presuming to deny their claims, we nevertheless regard them as inadequately substantiated on the evidence provided.

In this connexion reference should be made to the careful and detailed reports of Gyllensten and Hellström, who in a large experimental series (1954; 1955; 1956; and Hellström, 1956) have presented more convincing evidence of abnormal vasoproliferations in the retinae of mice allowed to survive in air after exposure to oxygen. Since one might expect the rat and mouse to react in a similar way, our negative findings in the ratlings are somewhat discordant, but the explanation for this must await further investigation.

In a previous paper (Ashton, 1957) it has been shown that the vasoproliferative reaction results purely from obliteration of the vessels and is not itself directly concerned with oxygen exposure, so that one would at least have expected abnormal vasoproliferation posteriorly in the ratling's retina as a sequel to the capillary obliteration occurring in oxygen. It can be argued, however, that any demands of a vasoformative factor developing in this posterior avascular area when the animal is returned to air are met by the formation or re-formation of the deep capillary net. It would seem that this plexus is then adequate to supply the overlying or inner layers of the posterior retina, so that re-formation of the superficial net is not here required. Moreover, it may be of importance that in the ratling the main retinal vessels and the peripheral vessels continue to grow normally when the animal is subjected to hyperoxia, so that the normal vascular pattern is never destroyed. Any new vessels can grow within this framework and abnormal vasoproliferation is less likely to occur.

Differences with regard to other Intra-ocular Structures

With regard to the third group of findings (retinal oedema, retinal haemorrhages, disorganization of the vitreous, and persistence and proliferation of the hyaloid vessels), we can merely record that we failed to demonstrate them in our animals. No explanation can be offered for these differences until a more careful statistical study can be made, bearing in mind that retinal oedema is extremely difficult to assess in paraffin sections, that haemorrhages might be produced in removing eyes, and that the age at which the hyaloid vessels of the ratling disappear is not constant. We have, for instance, found vestigial hyaloid vessels in the vitreous as late as 30 days after birth, whereas Patz and others (1953) had regarded such vessels as abnormally persistent after 21 days.

In concluding this discussion of the peculiar reaction of the ratling to hyperoxia, with and without air survival, we express the view that only a

limited analogy can be made between the reactions of this animal and those of the kitten, while the reactions of the human retina as seen in retrolental fibroplasia are even more dissimilar. The reactions of the ratling, however, do show once again that vaso-obliteration is dependent upon highly critical levels of oxygen tension within the tissues and occurs only in the immature retina, the adult animal being completely immune.

SUMMARY

(1) Reports in the literature dealing with the reaction of the developing retinal vessels of the ratling to hyperoxia are conflicting. The problem has therefore been re-examined and the results of experiments subjecting ratlings of different ages to various degrees and periods of hyperoxia, with and without air survival, are reported.

(2) The findings may be summarized as follows:

(A) HYPEROXIA WITHOUT AIR SURVIVAL

(*i*) Widening of the peri-arterial capillary-free zone developed with obliteration of the superficial capillary net at the posterior pole; this resulted in a star-shaped pattern of avascular tissue.

(*ii*) In younger ratlings the deep capillary net failed to develop in the same region; *i.e.* growth was arrested.

(*iii*) In older ratlings, where the deep capillary net was already present at the time of oxygenation, it closed together with, and to the same degree as, the superficial net; *i.e.* these capillaries were also obliterated.

(*iv*) growth of the main retinal vessels and of the peripheral capillaries was not significantly affected.

(B) HYPEROXIA WITH AIR SURVIVAL

(*i*) Anteriorly the peri-arterial capillary-free zone returned to its normal width.

(*ii*) The closed superficial capillaries at the posterior pole neither re-opened nor re-formed on return to air, irrespective of the age of the ratling.

(*iii*) In contrast, the deep capillary net at the posterior pole re-developed (whether it had been arrested or obliterated), the vessels growing *centripetally* towards the optic disc in an orderly manner from the patent vessels at the margin of the affected area.

(C) HYPEROXIA WITH OR WITHOUT AIR SURVIVAL

(*i*) No abnormal vasoproliferations were obtained into either the retina or the vitreous—even posteriorly where the superficial capillaries remained obliterated.

(*ii*) No vitreal haemorrhages or disorganization occurred, and no abnormal persistence or proliferation of the hyaloid system was seen.

(*iii*) The retinal vessels of the adult rat were unaffected.

(3) These findings are considered in relation to other reports in the literature and some explanations are advanced for the peculiar reaction of the retinal vessels of the ratling. Finally it is pointed out that, as these reactions differ so much from those of the kitten and even more from those of man, the ratling is not a suitable animal for the study of retrolental fibroplasia. However, our findings do not contradict the theories previously advanced to explain vaso-obliteration and vasoproliferation and they further illustrate the specific sensitivity of growing retinal vessels to oxygen.

We are greatly indebted to Dr. J. Cairns for access to the specimens which he prepared in this Department while studying the rat retina. It is a pleasure to acknowledge the technical assistance of Messrs. G. Knight and A. McNeil, and Miss E. Robins, and the secretarial help of Miss E. FitzGerald and Mrs. M. Long.

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