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# THE EFFECT OF OCCLUDING THE RETINAL AND CHOROIDAL CIRCULATIONS ON THE ELECTRO-RETINOGRAM OF MONKEYS

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Several methods have been tried to isolate the photoreceptors of the retina in normal physiological condition in order to learn more about their contribution to the e.r.g. One such approach is that of Brown & Watanabe (1962) who blocked the retinal circulation in cats and monkeys by inserting a stainless-steel rod through the vitreous humour and pressed it against the retinal vessels at the optic disk. Since the retinal vessels extend only to the outer plexiform layer, it is generally assumed that the inner layers of the retina are nourished by the retinal circulation while the photoreceptors are maintained by the choroidal circulation.

When the retinal circulation was occluded as described, Brown & Watanabe reported a selective loss of the b-wave, leaving a response which appeared identical to the PIII component of Granit (1933). The response had its maximum amplitude when the micro-electrode was at the distal tip of the receptors and in the fovea, and Brown & Watanabe have designated it as a receptor potential.

That asphyxia of the eye can abolish the b-wave and leave the PI and PIII components was shown by Granit (1933). Arden & Greaves (1956) produced retinal ischaemia by raising the intraocular pressure above systolic pressure and reported that the b-wave was the most resistant wave in the rabbit.

The effects of choroidal occlusion on the e.r.g. has not been reported. Because the b-wave is more sensitive than PIII to most agents, the retention of the PIII component and loss of the b-wave may indicate little about the site of origin of these two components.

Thus it was felt that a comparison of the changes produced in the e.r.g. by independent retinal and choroidal occlusion would provide information on the role played by them in maintaining normal electrical activity of the retina. Surgical methods were used to occlude each circulation independently and reversibly, and it will be shown that during the early stages

the b-wave was the first to be lost with either retinal or choroidal occlusion. The PI and PIII components which remained, however, could be maintained for at least 60 min only when the choroidal circulation was intact.

## METHODS

The experiments were carried out on 31 animals—26 *Saimiri sciurea*, two *Aotes trivirgatus*, two *Macaca irus* and one *Rhesus macaque*. All were performed under Nembutal (pentobarbitone sodium) anaesthesia with local infusion of Xylocaine (lidocaine hydrochloride) around the surgical wounds. The operative procedures used for isolating and occluding each circulation will be presented with the results.

E.r.g.s were recorded with a cotton-wick electrode placed on the anaesthetized cornea. The pupils were dilated by 1 % atropine sulphate and 10% neo-synephrine (phenylephrine hydrochloride). The over-all time constant of the recording system was 1 sec, and permanent records were made on a Grass Polygraph. Stimuli were provided by a car headlamp bulb focused in the plane of the pupil. The last lens was 115 cm from the pupil. Its diameter was 6·5 cm, and its luminance as seen in Maxwellian view was $7 \times 10^5$ ft.L. The duration of the stimulus was approximately 1 sec.

At the completion of each experiment, the integrity of the circulation was checked by the injection of Indian ink into the circulatory system. In the early experiments, the ink was injected into the carotid artery but the results were difficult to interpret because the exact pressure of the injection was not known. Thereafter, the ink was injected slowly into the left side of the heart and the ink was carried to the eye under the action of the heart alone. The use of Indian ink was reliable and proved essential for the interpretation of the results.

The eyes were placed in Kolmer's fixative and routine paraffin sections 7 $\mu$ thick were made. Sections from representative areas of the retina were examined for ink in the blood vessels.

## RESULTS

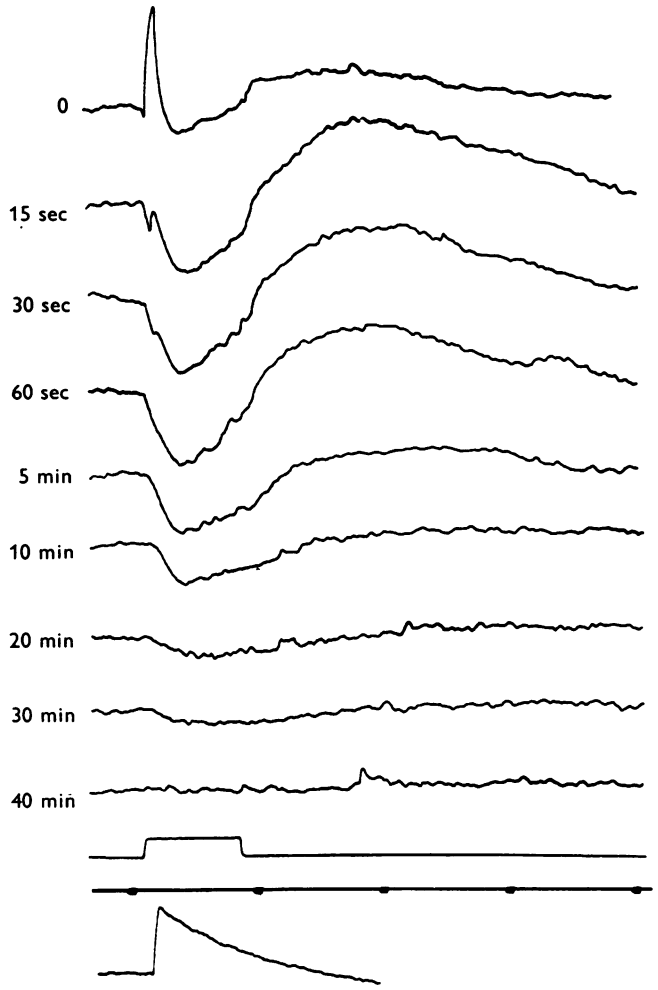
### *Effect of occluding both retinal and choroidal circulations*

The anaesthetized animal was placed in a head-holder and dark-adapted for 15–30 min. After the control e.r.g.s were recorded, the eye was enucleated under dim red illumination and placed back into the socket. The changes in the e.r.g. were then followed and the results of one experiment are shown in Text-fig. 1. The other eye served as the control and remained normal throughout the experiment.

Immediately after removing all blood flow to the eye, the b-wave rapidly decreased in amplitude and was completely abolished after 60 sec. The remaining PI and PIII components gradually decreased, and PI was not present in records after 5 min. The loss of PIII varied from 20 to 40 min after the occlusion of all blood flow.

Note the increase in PI after the enucleation. This enhancement was usually seen when there was an interference with the choroidal circulation.

It is interesting to note that the off-response disappeared significantly earlier than the on-response. This was not due to dark-adaptation for a stimulus of long duration, 20–30 sec, still did not evoke an off-response.



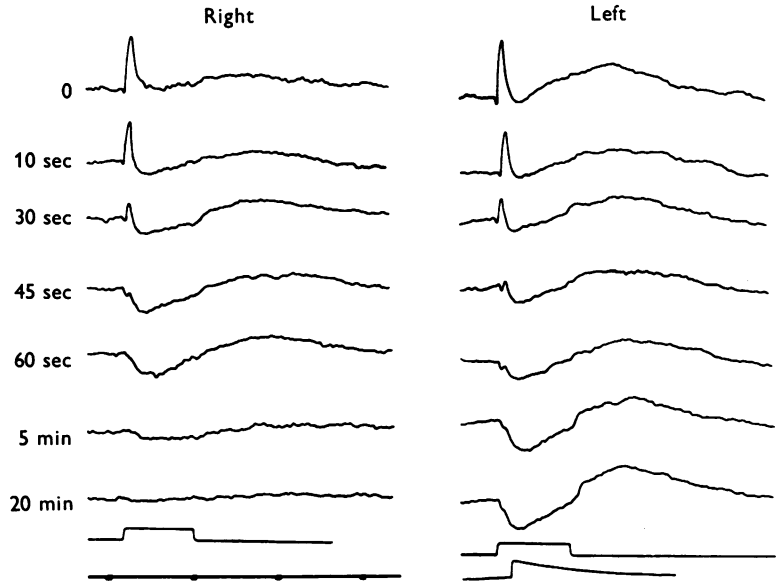

Text-fig. 1. Effect of occluding all blood flow to the eye (enucleation) on the e.r.g. of one *Saimiri* monkey. Numbers at left represent times after the enucleation. Calibration, 100 $\mu$V; time, 1 sec.

### *Effect of occluding the retinal circulation*

In order to isolate the retinal circulation, the intraorbital portion of the optic nerve was dissected free from the surrounding tissues and blood vessels. All the extraocular muscles were cut 2–3 mm from their insertions. With the aid of an operating microscope, a fine thread was passed around the optic nerve close to the globe, special care being taken not to injure or interfere with the ciliary vessels. The ends of the thread were passed through narrow polyethylene tubing which was then pushed up to the optic nerve.

The animal was placed in the head-holder and the control e.r.g.s obtained. To occlude the retinal circulation, the tubing was pushed gently against the optic nerve to compress the nerve and ophthalmic vessels. This resulted in an immediate reduction in the amplitude of the b-wave similar to that seen following enucleation. After 45–60 sec, the b-wave was completely abolished and only the PI and PIII components remained.

Releasing the pressure on the optic nerve allowed the blood to return to the retina and within 5–10 sec a small b-wave appeared. The e.r.g. returned to the control level after about 1 min.




Text-fig. 2. Comparison of the effect of occluding both the retinal and choroidal circulation (right eye) with that following occlusion of the retinal circulation (left eye) in a *Rhesus* monkey. Numbers at left represent times after the occlusion. Calibration, 100 $\mu$V; time, 1 sec.

To study the long-term effects of retinal circulation occlusion, stronger pressure was exerted on the tubing and the optic nerve and vessels cut through. In Text-fig. 2 the effects of occluding the retinal circulation (left eye) are compared with those following occlusion of both the retinal and choroidal circulation by the polyethylene tubing method (right eye). In this animal the anterior segment of the eye with the crystalline lens was removed to eliminate any possibility that the procedures were raising the intraocular pressures (see choroidal occlusion).

It can be seen that the b-wave was the first wave to be affected by both procedures. The b-wave was almost completely abolished after 60 sec

leaving small PI and large PIII components. With further time, PI and PIII were depressed in the eye without any circulation while the eye with only choroidal blood flow maintained PI and PIII responses for at least 20 min. Other experiments showed that the PI and PIII components could be maintained for at least 60 min by the choroidal circulation.

Histological examination of the left eye showed Indian ink in the choroidal blood vessels and complete absence from the retinal vessels. As expected, no ink was present in either the retinal or the choroidal circulations of the right eye.

From these two types of experiments it can be seen that while the b-wave requires the retinal circulation to be intact, the PI and PIII components can be maintained by the choroidal circulation alone.

*Occlusion of the choroidal circulation*

The surgical procedures used for isolating the ciliary vessels were similar to those for isolating the retinal circulation. A fine thread was passed around the optic nerve with the aid of the operating microscope, but this time the ends were pulled away from the optic nerve to surround the ciliary vessels and surrounding tissues. Again, all the extraocular muscles were cut close to their insertions.

In the early experiments when the polyethylene tubing method was used to compress the ciliary vessels, it was found that the Indian ink entered neither the choroidal nor the retinal vessels. Subsequently it was found that the tubing was either pressing against the globe to raise the intraocular pressure above systolic pressure or was pressing against the optic nerve to block retinal blood flow. In either case, the flow to the retina and choroid was blocked and the results were unsatisfactory.

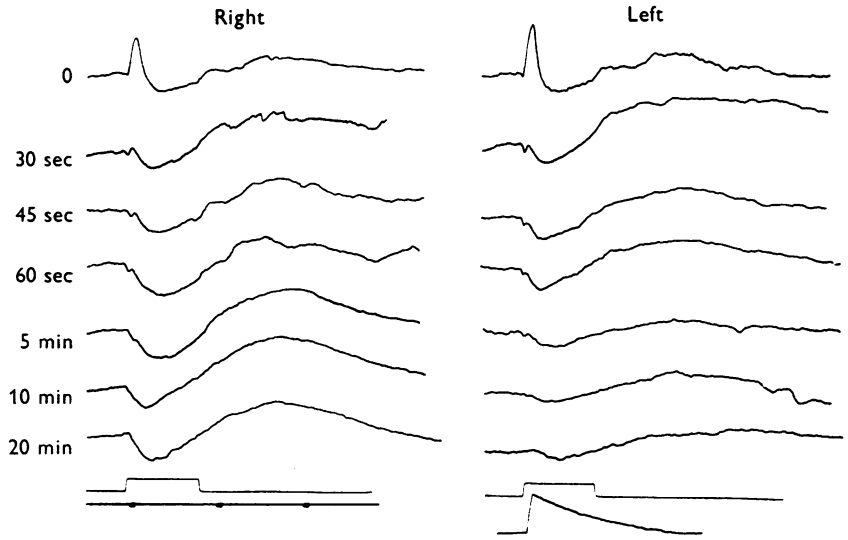
Another method attempted for choroidal occlusion should be mentioned here although the results were not satisfactory. The four vortex veins were dissected free and threads passed around them. When these vessels were tied off there was a marked reduction in the b-wave and an increase in the a- and c-waves. However, even after 45 min a small e.r.g. with all components depressed could be evoked from the eye. Histological examination of the retinas showed all the retinal vessels filled with Indian ink but a substantial amount also present in the choroid.

The method which was found to be most satisfactory for occluding the choroidal circulation without affecting the retinal blood supply was to cut the ciliary vessels. The thread was passed around the ciliary vessels as described and was used to pull all the ciliary vessels away from the optic nerve. All the vessels were cut with care so as not to injure the optic nerve.

Immediately after cutting the ciliary vessels of the left eye (Text-fig. 3),

there was a rapid loss of the b-wave which left the PI and PIII components. The retinal circulation was occluded in the right eye as described above, and it can be seen that as before the b-wave was the first to be lost.

With time, however, the PI and PIII components gradually decreased in the left eye until only a small response could be elicited at 20 min. The right eye at this time responded with large PI and PIII components.




Text-fig. 3. Comparison of the effect of occluding the retinal circulation (right eye) and choroidal circulation (left eye) in a *Saimiri* monkey. Numbers at left represent times after the occlusion. Calibration, 100 $\mu$V; time, 1 sec.

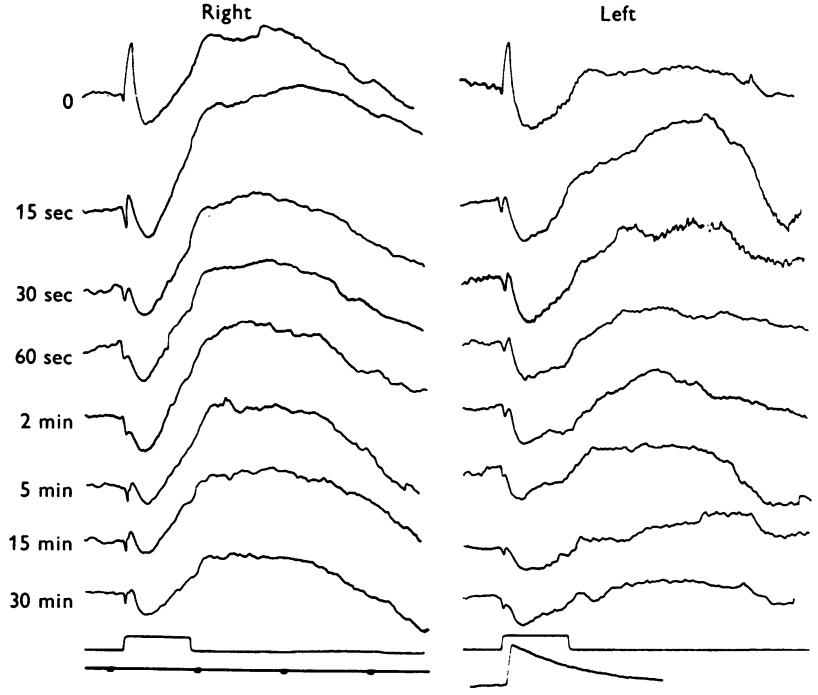
In both eyes, the off-response disappeared much sooner than the on-response. Photomicrographs from the eyes of this animal are shown in Pl. 1. In order to demonstrate the Indian ink better the melanin pigment of the choroid was bleached by 0·5 % potassium permanganate solution. Note the presence of Indian ink in the small capillaries of the retina of the left eye (Pl. 1*a*), and the large amounts of Indian ink in the choroidal vessels of the right eye (Pl. 1*b*). The corresponding circulations, the choroidal of the left eye and the retinal of the right eye, were empty.

Choroidal occlusion was attempted in 18 eyes of which only three showed complete occlusion with complete absence of Indian ink in the choroidal circulation. The more usual observations are shown in Text-fig. 4. The ciliary vessels were cut in both eyes and again the b-wave was the wave first affected. In the right eye the small b-wave which remained after 2 min increased with further time and at the termination of the experiment, at 30 min, all components of the e.r.g. were present although much

reduced. The same changes were seen in the left eye with the b-wave remaining very small throughout the experiment.

In both eyes, there was a great increase in the amplitude of the c-wave following the reduction of the choroidal blood flow.

Examination of the retinas showed Indian ink in the retinal circulation of both eyes. The choroidal circulation in both eyes showed small amounts of Indian ink in capillaries close to the head of the optic nerve with the left eye showing less Indian ink than the right.




Text-fig. 4. Changes in the e.r.g. following the cutting of all ciliary vessels in both the right and left eyes of a *Saimiri*. Note that all components of the e.r.g. were present at the termination of the experiment. Numbers at left represent times after the operative procedures. Calibration, 100 $\mu$V; time, 1 sec.

Careful histological study of the sections through the optic nerve showed a branch of the central retinal artery which passed to the choroid and ink could be followed in this collateral to the choroid. Examination of neoprene-injected eyes showed this retino-choroidal collateral very clearly.

## DISCUSSION

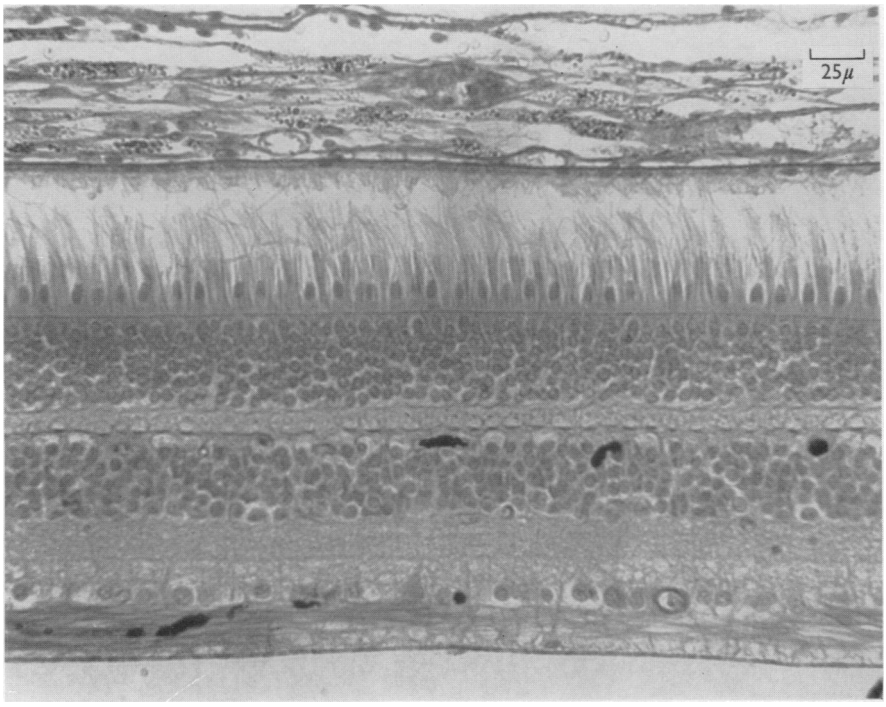
The results of this study demonstrate the role of the retinal and choroidal circulations in maintaining normal electrical activity of the retina. It was shown that while the b-wave required both the retinal and choroidal circulations to be intact, the PI and PIII components could be maintained adequately for at least 1 hr by the choroidal circulation.

The changes in the e.r.g. produced by the occlusions of all blood flow to the eye were identical to those reported by Granit (1933) in the cat. The enhancement of the c-wave following interference with the ocular circulation was also noted by Granit. The time of abolition of all components of the e.r.g. varied from 20 to 40 min, and probably depended on the condition of the eye and the animal at the time of occlusion of blood supply.

Assuming that the anatomical distribution of the blood vessels does separate physiologically the elements of the retina then it might be expected that occlusion of the retinal blood supply would abolish all electrical responses from elements in the inner layers of the retina. Thus if the b-wave arises from the bipolar cells as the evidence at the moment indicates (see Tomita, 1963), then occlusion of retinal blood flow would be expected to abolish the b-wave. This was indeed what was found here and previously by Brown & Watanabe (1962).

The remaining PI and PIII components should then arise from elements in the outer layers of the retina and the choroid. And it might be expected that the primary effect of occluding the choroidal blood supply would be on the PI and PIII components with the concurrent loss of the b-wave secondary to this. But the results showed that choroidal occlusion also affected the b-wave significantly earlier than the other two components. Hence, the early loss of the b-wave merely indicates that the elements which give rise to it are more sensitive to changes than those giving rise to the PI and PIII components.

The PIII component, on the other hand, reacted differently depending on the particular circulation occluded. The loss of PIII when the choroidal circulation was occluded demonstrated that it was sensitive to alterations in the blood-flow pattern, and thus its maintenance by the choroidal circulation alone indicated that it probably arises from the outer layers of the retina or the choroid. The choroid and pigment epithelium can be ruled out inasmuch as the PIII component can be obtained from the isolated retina without these structures. Therefore, the receptors with their nuclei appear to be the most likely elements involved, and the observations support Brown & Watanabe's conclusion that the PIII component arises from the receptors.

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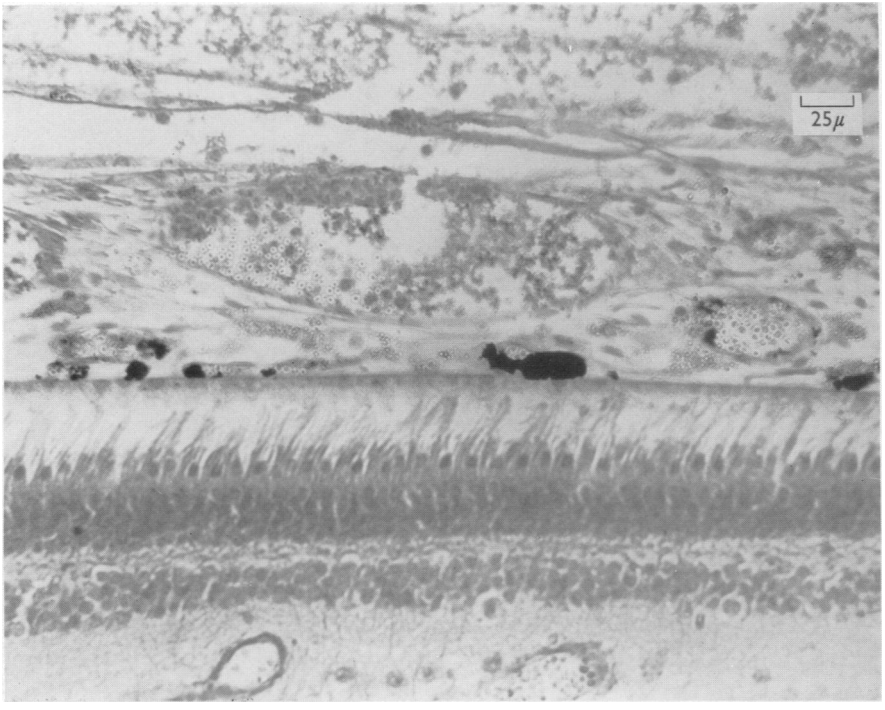


*b*

SUMMARY

1. When all blood flow to the eye was blocked, there was an immediate loss of the b-wave leaving the PI and PIII components. PI was lost after 5 min, and PIII 20–40 min after the occlusion.

2. Retinal circulation occlusion resulted in an immediate depression of the b-wave followed by its complete abolition in 60 sec. The remaining PI and PIII components could be maintained for at least 60 min by the choroidal circulation.

3. Choroidal occlusion also resulted in a loss of the b-wave at times comparable to that seen with retinal occlusion. The remaining PI and PIII could not be maintained by the retinal circulation and were lost after 20–40 min.

4. The observation that the b-wave was the first wave to be lost under all experimental conditions demonstrated the high sensitivity of the elements which give rise to it to alterations in blood-flow patterns. Little can be said about the identity of these structures.

5. The results support Brown & Watanabe's (1962) conclusion that PIII arises from the receptors.

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EXPLANATION OF PLATE

(*a*) Right retina and choroid of squirrel monkey. Note the presence of Indian ink in the choriocapillaries and the complete absence from the two retinal vessels shown. The choroidal melanin pigment was bleached with 0·5% potassium permanganate. E.r.g.s from this eye shown in Text-fig. 3.

(*b*) Left retina and choroid of squirrel monkey. Note the presence of Indian ink in the retinal vessels and the complete absence from the choroidal vessels. The choroidal melanin pigment was bleached with 0·5% potassium permanganate. E.r.g.s from this eye shown in Text-fig. 3.