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THE REVERSIBLE ALTERATIONS OF THE ELECTRO-RETINOGRAM OF THE RABBIT AFTER OCCLUSION OF THE RETINAL CIRCULATION

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The blood flow in the retinal vessels is *partly* determined by changes in the intraocular pressure. By artificially changing the latter the blood supply to the retina and choroid can be controlled; thus, raising the intraocular pressure reduces the blood inflow and vice versa. Not until the pressure inside the eyes equals that of the blood in the main arteries entering the eye does the retina become ischaemic.

This paper describes the changes in the electroretinogram (e.r.g.) during ischaemia and after restoration of the retinal circulation. In several preliminary experiments the e.r.g. was recorded under conditions of reduced blood flow, but the main results are concerned with complete ischaemia and subsequent recovery.

METHODS

The experiments were performed on rabbits under light urethane anaesthesia (1·75 g/kg body weight in 25% solution). The eyes had been atropinized for 24 hr beforehand in order to eliminate changes in the pupillary aperture. Contraction of the orbicularis muscle was prevented by infiltration of the muscle and its nerve supply in an area lateral to the outer angle of the orbit using 1 ml. 2% lignocaine.

The femoral blood pressure and intraocular pressure were continuously recorded as previously described by Greaves & Perkins (1952), amethocaine drops being instilled into the eye 1 min before insertion of the needle for the latter purpose.

The needle inserted into the anterior chamber and used for recording the intraocular pressure also served as the active electrode for recording the e.r.g. It was insulated with polythene except for the part inside the eye. A brass plate, screwed to the skull to allow firm fixation of the head, also formed the indifferent electrode. The two leads were fed into a commercial electroretinograph of the type employed by Karpe (1945).

The retina was stimulated with light from a compact electric source (6 V 48 W lamp), the filament being imaged in the plane of the iris by means of a condensing lens which was thus 'seen' by the animal in Maxwellian view, the lens subtending 30° at the eye. A compur shutter was interposed and adjusted to give a stimulus duration of $\frac{1}{10}$ sec.

Calibration of the light source. The brightness of the light source was measured by placing an observer's eye in the position of the rabbit's. It was found that the brightness of the lens was matched by that of a magnesium oxide screen illuminated by $1{\cdot}892 \times 10^9$ lm. Measurements were made of the magnification of the images formed on the sclera of albino rabbits and the posterior nodal distance calculated. In four animals this varied from 11·2 to 9·75 mm with a mean of 10·2 mm. The diameter of the homatropinized pupil showed similar variation and averaged 10·55 mm. The retinal light flux was then calculated and found to be $1{\cdot}573 \times 10^6$ lux, neglecting optical imperfections and light loss in the optic media. All experiments were carried out in a blacked-out laboratory, after 20 min preliminary dark adaptation of the animal.

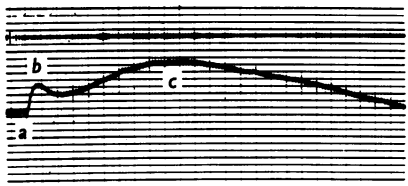

Fig. 1

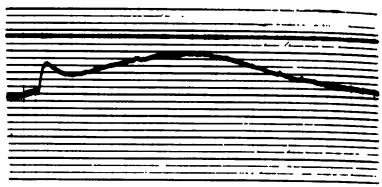

Fig. 2

Fig. 1. Normal rabbit e.r.g. (a-, b- and c-waves).

Fig. 2. e.r.g. taken 30 min after the intraocular pressure (I.O.P.) had been raised to 80 cm saline. Note essentially identical with Fig. 1.

RESULTS

The normal electroretinogram. Under the conditions of the experiment, the measured e.r.g. had a well-marked a-wave, a rounded b-wave and a fairly large c-wave (Fig. 1). There was no off-effect. In agreement with other authors (see, for example, Noell, 1954) we have found that the height of the c-wave varies, ranging in our experiments from about 50 to 200% of the height of the b-wave in different animals. In any one animal, however, the relative heights of a-, b- and c-waves remained constant.

Effect of raising the intraocular pressure. In a preliminary series of experiments the intraocular pressure was raised to and kept at 80, 100 and 120 cm saline in three animals for periods ranging from 20 to 40 min; in each animal the blood pressure was 110–120 mm Hg. In spite of the embarrassment to the retinal circulation under these conditions, the e.r.g. was unaffected (Fig. 2).

Sudden occlusion of the retinal circulation. By raising the level of intraocular pressure to equal that of the general blood pressure, all blood flow into the eye is prevented. Under these conditions the e.r.g. is rapidly extinguished, the component waves disappearing in the following order. First the c-wave, which is most susceptible, is greatly reduced within 1 min (Fig. 3*b*), and after it has gone the e.r.g. shows a slower negative potential after the b-wave. The a-wave also disappears rapidly, within $1\frac{1}{2}$ min of occlusion of the blood supply (Fig. 3*d*). The b-wave is more resistant, declining gradually over 3–4 min, the late negativity declining with it.

Restoration of blood flow. Although easily extinguished by retinal ischaemia, the e.r.g. returns rapidly when the blood flow is restored, and even after a 10 min occlusion a normal e.r.g. can reappear. In our first experiment where e.r.g.'s were taken throughout, the a- and b-waves returned to normal, the latter in about 20 min. However, the c-wave was much increased (see Fig. 4 *a*, *b*).

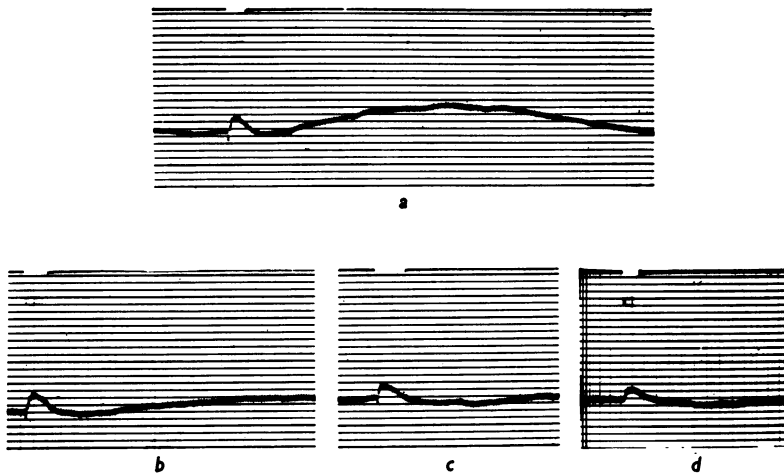

Fig. 3. The e.r.g. in ischaemia: I.O.P. 150 cm saline. *a*: before I.O.P. raised (normal); *b*: 30 sec after I.O.P. raised (c-wave reduced); *c*: 60 sec after I.O.P. raised (note late negativity); *d*: 2 min after I.O.P. raised (a-wave absent, b-wave reduced).

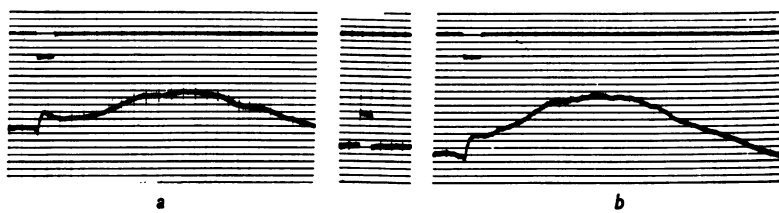

Fig. 4. Recovery from ischaemia. *a*: before I.O.P. raised (normal e.r.g.); *b*: 17 min after end of 10 min period of ischaemia (note increased c-wave). Calibration 500 μV (centre). Top tracing: stimulus marker, $\frac{1}{10}$ sec.

In the next experiment, no e.r.g.'s were taken during the period of ischaemia, and though the a- and b-waves returned to normal, the c-wave was not bigger than before. It therefore seemed possible that the size of the c-wave during the recovery period might depend upon the amount of light falling on the retina while it was ischaemic. This hypothesis was tested in the following way. An animal was dark adapted for 20 min and a series of e.r.g.'s taken to establish the normal. The stimulating light was then shone upon the eye for

10 sec before, during or after a 10 min period of ischaemia. No further e.r.g.'s were taken until the blood supply had been restored, when the recovery of the retina was followed for the next 20 min.

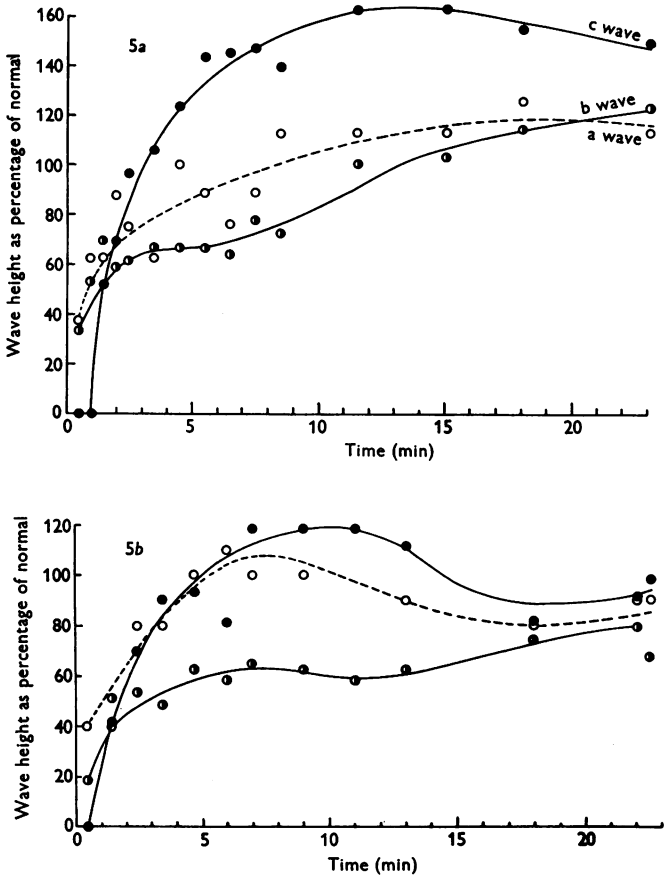

Fig. 5. Recovery of the e.r.g. after ischaemia. Ordinate: magnitude of components as percentage of normal. Abcissa: time in min after end of ischaemia. ○, a-wave; ◑, b-wave; ●, c-wave. *a*: test light shone on retina for 10 sec, 1 min *before* I.O.P. raised; *b*: test light shone on retina for 10 sec, 5 min *after* I.O.P. raised (note increase in c-wave in *a* compared with *b*).

The recovery process has been represented in Fig. 5*a*, *b*; the ordinate indicating the magnitude of the e.r.g. components in percentages of their original (normal) values, and the abscissa the time in minutes after the return of the intraocular pressure to its initial value (restoration of blood flow). E.r.g.'s were taken, and the eye was then exposed to the test light for 10 sec. One minute later the intraocular pressure was increased to 150 cm saline. After 10 min it was reduced to 20 cm saline, and e.r.g.'s were taken at 30 sec

intervals for the next 20 min. Immediately after the restoration of the circulation the e.r.g. was absent. Within 1 min the a- and b-waves returned and were soon followed by the c-wave. Over the succeeding minutes the b-wave gradually returned to normal, while the other components, increasing at a much greater rate, soon attained supernormal height. This was not, however, maintained and the height of the c-wave began to fall after about 15 min, when the b-wave had almost fully recovered.

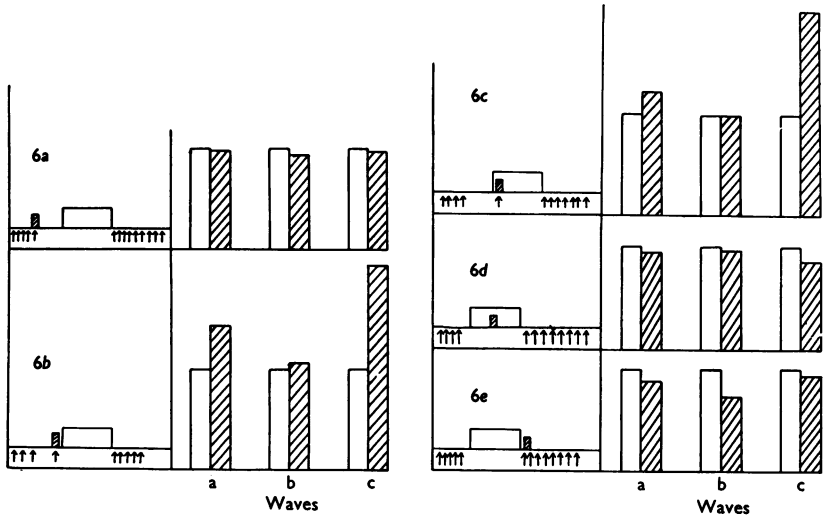

Fig. 6. Comparison of the e.r.g. before and after ischaemia. Left side of each diagram indicates plan of experiment: ↑ = e.r.g. taken. Shaded rectangle shows the 10 sec period of exposure of the eye to test light. Unshaded rectangle represents period of ischaemia. Right side shows heights of a-, b- and c-waves, the shaded portion representing the post-ischaemic height as a percentage of the pre-ischaemic. *a*: test light on 5 min before I.O.P. raised (period of ischaemia 10 min); *b*: test light on 1 min before I.O.P. raised (note a- and c-waves become supernormal); *c*: test light on 30 sec after I.O.P. raised when e.r.g. still present (note a- and c-waves again supernormal); *d*: test light on 5 min after I.O.P. raised when e.r.g. absent; *e*: test light on 30 sec after I.O.P. lowered when e.r.g. absent.

In the experiment represented in Fig. 5*b* the eye was exposed to the test light for 10 sec in the middle of a 10 min period of ischaemia. In the period immediately following on ischaemia the e.r.g. was absent. The separate component waves then appeared as before, the b-wave slowly, the a- and c-waves rapidly, but this time the c-wave did not become so markedly supernormal.

The collected results are expressed as histograms in Fig. 6, and the heights of the waves are the means of the e.r.g.'s taken at 14, 15 and 16 min after the end of ischaemia. Each result is the mean of three experiments except Fig. 6*d* in which four animals were used. In the column on the left the plan of the experiment is summarized. In the first group (Fig. 6*a*) of experiments the test light was shone on the eye 5 min before the onset of ischaemia. During

recovery the a-, b- and c-waves returned almost to the base-line level. Below this are the results of experiments where the eye was exposed to the test light 1 min before the onset of ischaemia (Fig. 6*b*). In this group only the b-wave has returned to normal in the post-ischaemic phase, the a- and c-waves having both increased in size.

The same is true in the next group where the eye was exposed to the test light 30 sec after the commencement of ischaemia and *whilst the full e.r.g. was present* (Fig. 6*c*). However, if the eye was exposed to the test light 5 min after the onset of ischaemia and *after the e.r.g. had been extinguished*, when the intraocular pressure was again restored to normal all the component waves of the e.r.g. returned almost to their pre-ischaemic levels without becoming supernormal (Fig. 6*d*). This is also true of the experiment where the eye was exposed to the test light 30 sec after the end of the ischaemic period (Fig. 6*e*).

It can be seen that if light is shone on the eye 1 min before, or shortly after, the occlusion of the circulation, the c-wave in the recovery phase is supernormal. Further, it is to be noted that the c-wave is increased only when exposure of the eye to the test light results in an e.r.g.

DISCUSSION

Experiments upon the visual system of the rabbit frequently yield unrepeatable results. For example, Noell (1954) found that the relative heights of the b- and c-waves varied enormously. Tansley (personal communication) has suggested that this may be correlated with the frequency with which abnormal retinal histology is found in normal rabbits; a partial atrophy of the inner nuclear layer is a common finding. Although in our experiments the relative heights of the components of the e.r.g. were more or less constant from rabbit to rabbit, it is just possible that the results might be vitiated by abnormal retinae, although unlikely, as Bornschein & Zwiauer (1952) have also noticed this phenomenon. They evoked e.r.g.'s in rabbits under conditions where no c-wave normally appeared. During the recovery phase after retinal ischaemia, however, a c-wave was evoked; it disappeared when recovery was complete. We have also performed a few similar experiments with cats and monkeys with results essentially similar to those described above on the rabbit.

Components of the electroretinogram

The best known and most generally accepted analysis of the electroretinogram is due to Granit (1947). In this he divided vertebrate e.r.g.'s into two classes, 'E' and 'I'. The 'E' or rod-dominated e.r.g. is found in its purest form in the rabbit. Here there is little off-effect, a small a-wave, large c-wave and, even at high intensities of stimulation, a rounded b-wave. However, the present experiments do not, in some respects, support Granit's analysis. Granit attempted to asphyxiate the retina of cats (also an 'E' retina) by

compressing the carotid arteries. He found that the order of disappearance of the components of the e.r.g. was b, then c and last of all, a. We find that in the rabbit the a- and c-waves disappear rapidly, the b-wave being more resistant; similarly, the different waves reappear in the reverse order. This may be a species difference but probably the discrepancy lies in the intensity of the light used to stimulate the eye. Granit found that after the b-wave had disappeared a strong stimulus could still evoke it. His asphyxia was not complete, blood still reaching the eye from the vertebral arteries. We find much the same picture as he did during the recovery phase. It is therefore probable that the b-wave (Granit's process P II), is less sensitive to asphyxia than the other components and not more sensitive as Granit thought. This makes it unlikely to be a manifestation of late retinal activity.

Relation of a- and b-waves. In the experiments described above it was shown that the b-wave could be produced under conditions where the a-wave was extinguished. Noell (1952) has found the same phenomenon to occur in iodate poisoned eyes. It therefore seems unlikely that the a- and b-waves are in any way causally related.

Relation of a- and c-waves. On the other hand, the a- and c-waves are extinguished and recover with much the same time-course. In addition, the a-wave is increased in size in those recovery experiments (Fig. 6) where the c-wave is supernormal, and in no others. Therman (1938) found that this correspondence occurred in frogs' eyes which had been treated with adrenaline, both a- and c-waves being increased in size. Noell seems to have encountered a similar phenomenon when, in azide poisoned retinae, the a- and c-waves increased in size, independently of the b-waves.

The c-wave in ischaemia and recovery

The experiments described above show that if the eye is exposed to light early in the ischaemic phase, the c-wave during the subsequent recovery period becomes supernormal. This, however, is not the case if light is shone on the eye some time after the ischaemia has become established; that is, when the e.r.g. has been extinguished. The decomposition of visual purple when exposed to light continues independently of the presence of circulating blood; the c-wave therefore cannot be concerned directly with the breakdown of visual purple. It must be related to the subsequent processes which give rise to the other components of the e.r.g. Further, if the eye is exposed to light immediately before the occlusion of the circulation, the c-wave is again increased in the recovery period; in fact, much the same happens as when the eye is exposed to light during ischaemia.

It seems therefore that when light falls on the retina it does not return to the resting state until more than one minute has elapsed—long after the end of the e.r.g. This alteration in the retina, though not connected with any electrical

activity which we could record, can be indirectly demonstrated by the experiments reported above.

We do not know the nature of this recovery, but (1) it occupies more than one minute; (2) it is interfered with by retinal ischaemia and is probably therefore a metabolic recovery in that part of the retina associated with the e.r.g.

The c-wave, of all the components of the e.r.g., seems to be most closely connected with this recovery. Whether this restorative process is directly related to an oxidative system is not clear but the early disappearance of the c-wave in ischaemia supports this idea.

Noell showed that the intravenous injection of azide into rabbits resulted in an increased c-wave. Azide raises the d.c. potential of the eye even when the receptors have been destroyed by iodo-acetic acid, but not when the pigment epithelium has been destroyed by iodate. Noell's inference was that the c-wave 'originates across' the pigment epithelium. However, since the iodate used in these experiments destroyed both receptors and pigment epithelium, Noell could not put this inference to experimental test (as none of the poisons damaged only the pigment epithelium). Our experiments support the idea that the c-wave is a metabolic potential which accompanies activity of the retina. It may be that the restoration *in statum quo* depends in part on the integrity of the pigment epithelium.

SUMMARY

1. The normal rabbit's e.r.g. is described. It is unaffected when the intraocular pressure is raised to 120 cm saline. When the intraocular pressure is raised above the arterial pressure, the e.r.g. is extinguished within 4 min. The c-wave is most sensitive, the b-wave least, to this occlusion of the retinal circulation.

2. After 10 min occlusion a normal e.r.g. can be evoked when the circulation is restored.

3. If light is shone on the eye immediately before, or within 4 min of the beginning of the period of retinal ischaemia, the a- and c-waves become supernormal when the circulation is restored.

4. This does not happen if light is shone on the eye more than 4 min after the beginning of ischaemia, i.e. when there is no electrical evidence of retinal activity.

5. These phenomena are discussed in relation to the origin of the e.r.g.

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