80 mm Hg ändert sich das Bild (Figur c): es gelingt nun, Sauerstoff sowohl von aussen durch Anblasen mit reinem Sauerstoff als auch von innen durch pO_2-Erhöhung im Blut zuzuführen. Dieses unterschiedliche Verhalten können wir noch nicht deuten. Vielleicht kommt es durch den erhöhten Druck zu einem zusätzlichen Flüssigkeitstransport in der Arterienwand. Es könnten auch die vasa vasorum eine Rolle spielen, wenn diese bei niedrigem Blutdruck nicht mehr perfundiert werden. Hier sollen weitere Versuche Klärung schaffen.

Summary. By means of local pO_2 measurements with Pt-needle electrodes an oxygen impermeable zone in the arteria carotis of the cat has been found.

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The Mode of Action of Insulin upon the Electrical Activity of Mammalian Retinas in vitro

Insulin has been known to affect blood glucose concentration in a decreasing manner when administered i.v. But recent electrophysiological studies on this polypeptide have proved that insulin affects resting membrane potential of other kinds of cells rather than retinal[1–7]. This study involved the direct action of insulin upon the retina employing the recently developed perfusion methods of keeping up the high activity of mammalian retinas in vitro, not the insulin effect upon the retina through its inducing hypoglycemia. In this study the *a*-, *b*-waves and oscillatory potential of electroretinogram (ERG) were used as indicators of retinal activity.

Materials and methods. The retinas of white rabbits weighing about 2 kg each were used as experimental materials. The excision techniques and perfusion methods of the rabbits' retinas have already been presented in detail in our previous reports[8–10].

As an incubating medium, the Ames solution[11,12] was used.

As an insulin source insulin regular, Iszilin (Takeda Pharmaceutical Co. Ltd., Japan), was used. Besides purified insulin extracted from cow pancreas and crystallized, this reagent contains 0.2 w/v/100 ml of tricresol (*orth*-: 33.3%, *meta*-: 33.3%, *para*-: 33.3%) for keeping it from bacterial contamination and 1.6 w/v/100 ml of glycerin as a stabilizing solvent. The insulin crystal, whose biological activity was proved to be 40 Unit/mg, was dissolved into this reagent at the ratio of 1 mg of crystal to 1 ml of solvent.

The concentration of insulin in the incubating medium was regulated by mixing this reagent with the pure Ames solution in various volume ratios. For example, in the case of Figure 1, the ratio of the insulin solution to the Ames solution was 1:20, and in the case of Figure 3, it was 1:200. Accordingly, the mixed incubating medium contained a very low concentration of tricresol and glycerin carried from the insulin solution.

Results and discussion. The traces in Figure 1 represent typical changes which the administration of 2.0 U/ml of insulin induced. The *b*-wave and the oscillatory potential in this figure show a tendency to increase in amplitude with the lapse of time. After administration an oscillatory potential of more than 10 peaks appeared (Figure 2) while the retinal preparation incubated in the pure Ames solution usually reveals the oscillatory potential of 5 to

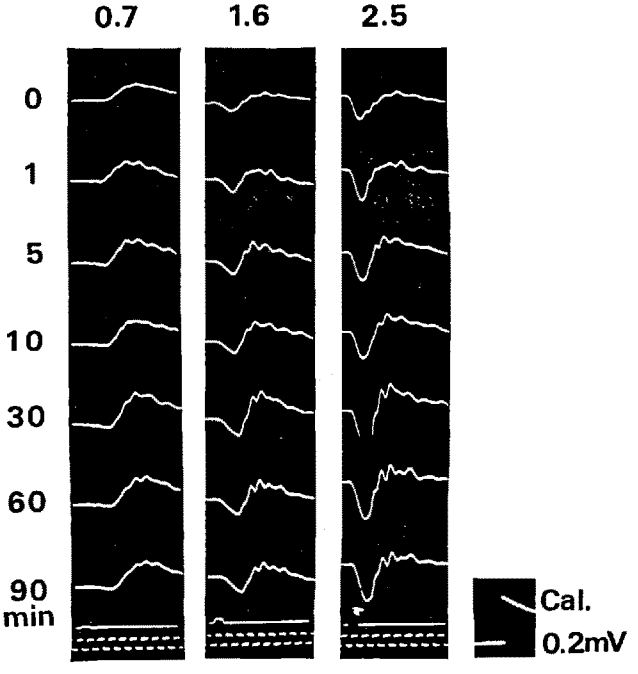

Fig. 1. The effects of 2.0 U/ml of insulin upon the action potential of a rabbit retina in vitro are illustrated with a series of photographs obtained from a typical case. The numbers on the left represent the time in minutes which elapsed after the start of incubation with the Ames solution containing insulin. Accordingly, the traces at zero time were photographed immediately before the pure Ames solution was substituted for the mixed solution. The numbers at the top of each column represent relative numbers of stimulus intensities in the logarithmic scale. See our previous reports[8,9] about their absolute numbers. The sweep speed of each tracing is identical, and the time marks on the bottom are 100 c/sec. The time constant of the amplifier was 0.03 sec. The positivity of the active electrode which was placed on the vitreous side of the retina was recorded as an upward deflection. The upward deflection on the second lower trace represents duration of photo-stimulus, 10 msec.

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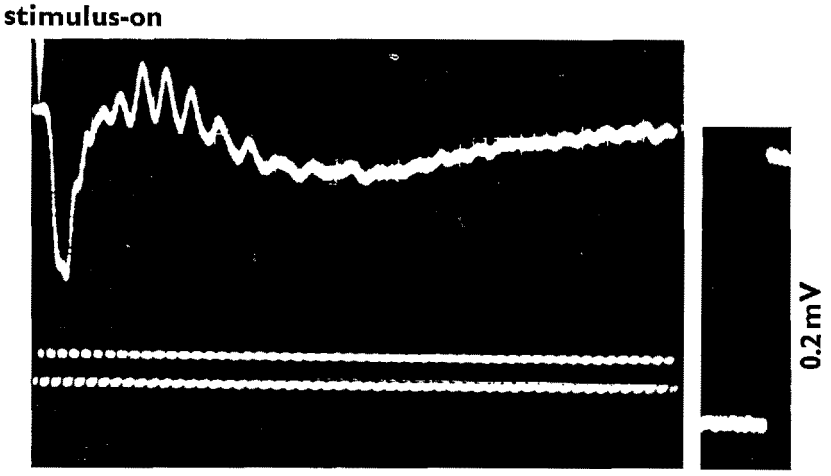

Fig. 2. The accelerating effect of insulin upon the appearance of the oscillatory potential is emphatically illustrated. More than 10 peaks can be observed in this case. The time constant of the amplifier was 0.03 sec. This photograph was obtained 30 min after the incubation with the Ames solution containing 1.0 U/ml of Insulin. The photo-stimulus intensity, log I, was 2.5, and the photo-stimulus duration was 10 msec.

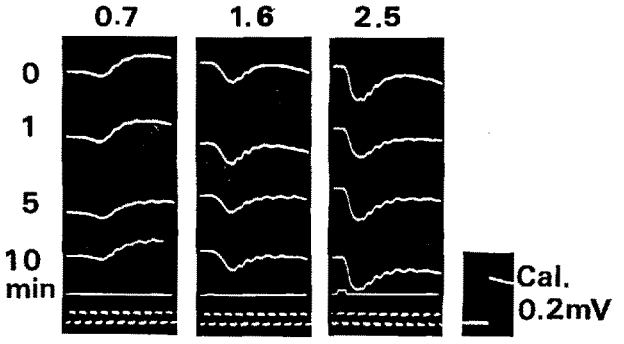

Fig. 3. The effects of 0.2 U/ml of Insulin upon the action potential of a rabbit retina in vitro are illustrated. The accelerating effects of insulin upon the appearance of the *b*-wave and the oscillatory potential were inconsiderable at this concentration and were not traceable at lower ones. The time constant of the amplifier was 0.3 sec.

7 peaks. The *a*-wave remained unaffected, even after the administration. A sporadic effect of insulin was shown when this polypeptide was applied in a concentration of 0.2 U/ml (Figure 3). Smaller concentrations of insulin than this resulted in no recognizable increase of the *b*-wave and the oscillatory potential.

The minimal concentration of insulin which revealed the accelerating effect upon the appearance of both waves, approximated the concentration which was proved effectively to increase the resting membrane potential of some kinds of cells other than retinal[1-3]. However, the minimal concentrations of effective doses shown here are higher than that proved with skeletal muscle from hypophysectomized rats (0.01 U/ml)[4], and than that demonstrated with rat epididymal adipose tissue (1×10^{-6} U/ml)[5,6]. In contrast, dealing with carp retinas in vitro, MITARAI et al.[13,14] have reported that the minimal dose of insulin affecting the S-potential of cold-blooded retinas was 2.0 U/ml. This value is higher than our minimal concentration.

As the glucose concentration of Ames solution (10 m*M*/l) was considered to be strong enough to support the retinal metabolism of mammals[11,12], and was not altered before and after the administration of insulin with very limited diluting effects by insulin reagent, this effect upon the in vitro preparation of mammalian retinas might result from the direct action of insulin upon some kinds of retinal cells.

The accelerating effect of insulin presented here is very interesting when considered in comparison with the fact that the oscillatory potential has been proved to decrease in amplitude and number in diabetic retinopathy[15-17]. It is conceivable that the decreasing tendency of the oscillatory potential in diabetic retinopathy may result from the lowered concentration of insulin within the retina.

This conclusion agrees with the results of recent clinical studies on diabetic retinopathy where locally administered insulin was observed to contribute towards ceasing the progress of diabetic retinopathy[18,19].

The glucose permeability and ion transport, especially potassium ion transport, through cell membranes have already been considered to be affected by insulin with other kinds of cells rather than retinal[7,20]. It can be proposed that the electrical activity of any kind of retinal cells is also affected by the local application of insulin[21].

Zusammenfassung. Die Wirkung des Insulins auf das Elektroretinogramm der in vitro überlebenden Kaninchennetzhaut ergibt eine Vermehrung der Zahl und Amplitude der *b*-Wellen, ein Effekt, welcher von der direkten Wirkung des Insulins auf die Netzhautzellen abhängig ist.

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