(Figure 3), as already noted by several investigators[7–9], and of nearly the same order of magnitude as those of the electrode filled by the glass fibre method (Figure 1). The effect of acid treatment of glass tubing on the TP values is also clearly seen in Figure 3.

Since many laboratories use capillaries and glass fibres straight form a shelf, some observations were made on the microelectrodes prepared by the glass fibre method without any acid treatment. The TP values of such electrodes observed in PBS showed considerable variations from −3.4 to 18.4 (means, around −9) mV. Such a high TP was markedly reduced by the use of acid-treated glass tubing and glass fibre or of acidified 3 *M* KCl. The TP values in the latter were around −2 mV (−0.3 ∼ −3.7 mV) and of nearly the same order of magnitude as those in the former (Figure 1).

All these results suggest that something happened to the tips while the electrodes were immersed in the neutral 3 *M* KCl solution, and that this could be prevented to a considerable extent by the acid treatment of glass, and/or the acidification of filling solution. It has been suggested that the tip potential is the result of contamination[5,10]. In our experiments, the filling solution was freshly filtered, so that gross contamination can be ruled out. If the accumulation of miro-dirt causes the TP formation, the fluctuation of the TP values observed with different electrodes would be expected to be much greater than that seen in Figures 1 and 3, and the effect of acid treatment shown above would be difficult to explain. Our results are in general consistent with the view that fixed negative charges on the glass wall will bring about tip potential, as already suggested[11,12], for the acid treatment of glass as well as the acidification of the filling solution could to a certain extent counteract the formation of fixed negative charges.

Whatever the mechanism of generating the potential at the tip, the smaller the tip potential of an electrode, the more accurate is the membrane potential obtained. In view of the result shown in Figure 2, storing in 3 *M* KCl of the microelectrodes over 3 days should be avoided. Acidification of the filling solution up to pH 2 provides a simple means of preparing microelectrodes of low tip potential[7–9], if the high concentration of H^+ in the capillaries does not affect the membrane potential measurements. The glass fibre method is far superior to the alcohol method in order to obtain low tip potential. The direct filling method may be also applicable for microelectrodes of a little larger tip diameter, having the resistance of around 10 *MΩ*.

Zusammenfassung. Glasmikroelektroden, mit 3 *M* KCl gefüllt, wurden verschiedenartig präpariert und ihr Spitzenpotential mit 0.01 ∼ 3.0 *M* KCl gemessen. Die Resultate sprechen dafür, dass die negativen elektrischen Ladungen, an der Glaswand fixiert, eine entscheidende Rolle bei der Entstehung des Spitzenpotentials spielen.

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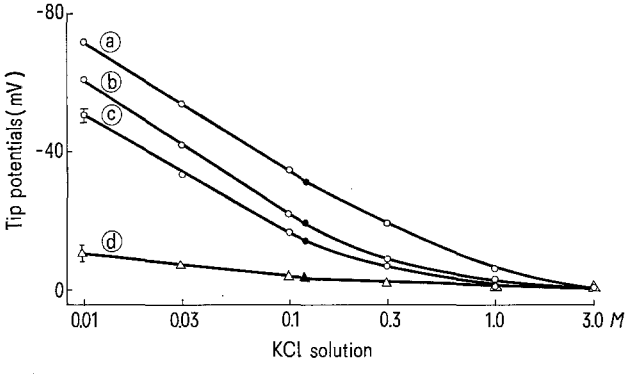

Fig. 3. Effect on the tip potential of acid treatment of glass tubing and acidification of filling solution. All electrodes tested were prepared under the same pulling condition (heater current, 16.0 A) and filled with the alcohol method. a) without any acid pre-treatment (electrode resistance was 7.2 ± 0.11 *MΩ*); b) pre-treated with 1% HCl for 32 h (7.2 ± 0.34 *MΩ*); c) pre-treated with H_2SO_4-dichromate mixture for 72 h (7.5 ± 0.34 *MΩ*); d) pre-treated with H_2SO_4-dichromate mixture for 72 h and filled with 3 *M* KCl adjusted to pH 2 with HCl (14.6 ± 0.83 *MΩ*). The vertical bars represent standard errors on either side of average of 5 electrodes tested.

[7] J. A. WILLIAMS, C. D. WITHROW and D. W. WOODBURY, J. Physiol., Lond. *212*, 101 (1971).
[8] K. T. WANN and M. W. GOLDSMITH, Nature, Phys. Sci. *238*, 44 (1972).
[9] J. RIEMER, C. MAYER and G. ULBRECHT, Pflügers Arch. ges. Physiol. *349*, 267 (1974).
[10] F. FRANK and M. C. BECKER, in *Physical Techniques in Biological Research V* (Ed. W. L. NASTUK; Academic Press, New York and London 1964).
[11] M. LAVALLÉE, Circulation Res. *15*, 183 (1964).
[12] G. KAWABATA and Y. NAKAMURA, Bull. Yamaguchi med. School *12*, 1 (1965).
[13] Acknowledgments. The authors wish to thank Miss MARIKO OHARA for assistance with the manuscript.

Influence of Temperature on the Electric Activity of the Isolated Dog Retina

In the last few years, the influence of temperature on the function of isolated organs has been investigated to an increasing degree in order to get some information about kinetic and thermodynamic quantities of biological reactions. The temperature as a parameter of the retinal cell system has been examined many times, the measurements being carried out also on the isolated retina[1–5]. As corresponding experiments with the isolated dog retina have not yet been reported, the influence of temperature on the dog's retinal activity after light stimulation (ERG) was investigated. The dependence on temperature of the latency of restoration after a light stimulus was measured by means of double-stimuli.

[1] P. DETTMAR and R. HANITZSCH, Acta biol. med. german. *14*, 720 (1965).
[2] A. VON LÜTZOW and H. BORNSCHEIN, V. Graefes Arch. Ophthal. *170*, 332 (1966).
[3] R. HANITZSCH and A. VON LÜTZOW, V. Graefes Arch. Ophthal. *173*, 217 (1967).
[4] Y. HONDA, Acta Soc. opthal. jap. *73*, 1865 (1969).
[5] U. BORCHARD, Experientia *29*, 1349 (1973).

Methods. The measurements were carried out using the method of the isolated retina similar to that described by AMES and GURIAN[6]. Under pentobarbital narcosis (25 mg/kg), the central retinal artery of the dog was ligated, the eyeball enucleated and transferred to a cooled modified Tyrode solution. Under dim red light little cylindrical pieces of the retina, 8 mm in diameter, were prepared and transferred to modified Tyrode solution at room temperature. The solution was made up as follows (m*M*/l): NaCl, 120; KCl, 3; $MgCl_2$, 0.3; $CaCl_2$, 0.15; NaH_2PO_4, 1.5; Na_2HPO_4, 15 and glucose 5. The pH was 7.7. The time of preparation was 5–8 min. The retina was fixed by a ring to a Monodur-net of a carrier and inserted into the chamber of a double-wall apparatus made of glass which was filled with 10 ml Tyrode solution. The space between the walls was connected to a thermostat so that the solution of the chamber could be adjusted to a fixed temperature within a few seconds. Convection of the nutritive medium was provided by purified air bubbling through the solution. The intensity of the light stimulus could be varied by means of neutral filters from 40 to 1.3×10^{-6} lux, corresponding to extinctions from 0 to 7.5. The intensity of illumination was calibrated by means of a lux-meter (Bruno Lange). The duration of the light stimulus was 1 sec for all experiments. The measurements were carried out in dark adaptation. The ERG was led off by Ag/AgCl-electrodes and recorded on an oscilloscope and a penwriter. A schematic drawing of the polyphasic potential with a denomination of its waves and components is illustrated in Figure 1.

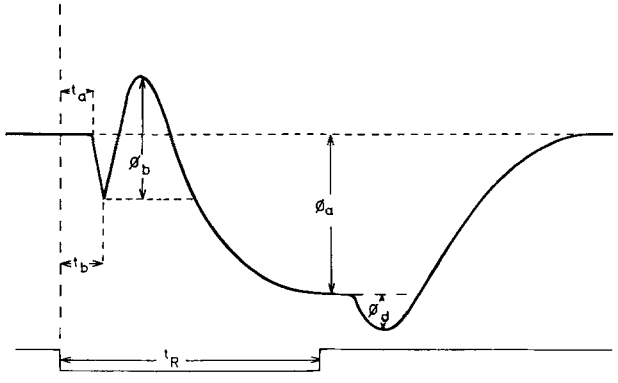

Fig. 1. Schematic illustration of the electroretinogram (ERG) of the isolated dog retina. t_R, duration of the light stimulus; t_a, t_b, latencies of a- and b-wave. The polyphasic potential is characterized by the amplitudes of the late RP (ϕ_a), b- (ϕ_b) and d-wave (ϕ_d).

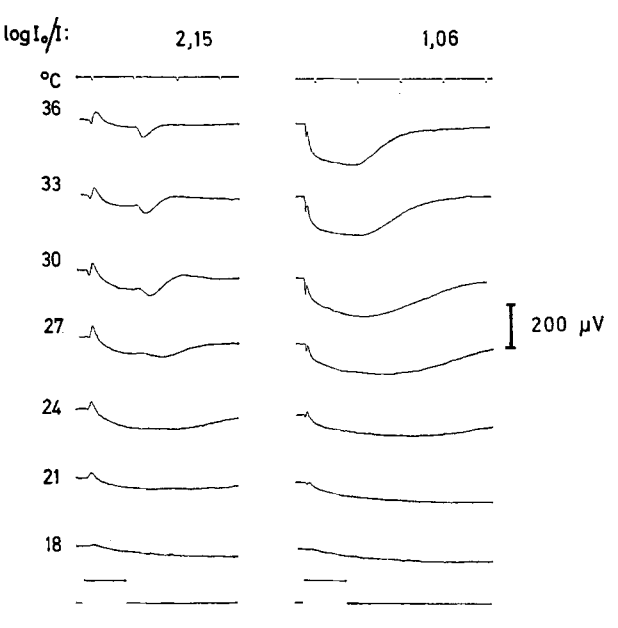

Fig. 2. Original recordings of the dog ERG at varying temperatures. The maximum light intensity was 40 lux. First line: time intervals 1 sec. Last line: light stimulus 1 sec.

Results. The influence of temperature on the retinal activity after light stimulation is represented in Figure 2 which shows original recordings. The temperature was changed in steps from 18 to 36 °C at intervals of 15 min. At low temperatures there is a prolongation of the latencies and a decrease of all amplitudes so that the result is a long flat response. With increase in temperature and at high intensities of illumination, the course of the ERG is mainly marked by the late receptor potential[7] (late RP). Below 12 °C the ERG disappeared. The loss of potential by cooling of the preparation turned out to be fully reversible.

In Figure 3 the influence of temperature on the function $\phi_a = f(I)$ is plotted on a semilogarithmic scale. While the threshold intensity of the late RP lies at extinctions from 3.5 to 3, the maximum increase in potential can be observed between 2.5 and 1. At a fixed temperature the linear part of the log-curve illustrating the light intensity-effect-characteristic may be described by the following relation[5] $d\,\phi_a/d\,\log I = k_a$ or by the integral $\phi_a = k_a \cdot \log I + K$. K is the integral constant. The value k_a may be interpreted as reaction constant for the process of building up the late RP. As can be seen from Figure 3, k_a changes with temperature in a characteristic way. According to ARRHENIUS, the connection between the activation energy A, the absolute temperature and the reaction constant k is characterized by[8] $\log k = \log k_o - A/2.303\,RT$. Log k_a was plotted against 1/T and from the linear slope of the curves in a temperature range from 21 to 36 °C the gross activation energy was calculated. The mean value based on 30 curves was 5.38 ± 0.32 (S.D.) Kcal corresponding to a Q_{10}-value of 1.34 at a mean temperature of 28.5 °C.

Furthermore, the results show that the latencies of the a- and b-wave, t_a and t_b (cf. Figure 1), increase if the temperature is lowered. Figure 4 illustrates the influence of temperature on the latency of restoration of the late RP and b-wave measured by means of double stimuli. The interval until a second potential reaches the size of the first one is increased with decreasing temperature. The restoration of ϕ_b is much slowed compared to that of ϕ_a.

Discussion. A comparison of the results for the isolated dog retina with the shape of the dog ERG in vivo reveals many areas of agreement[9,10]. Parts of the retina behave like the whole retina as regards their electric activity. The extensive increase of the late RP with rising light intensity and temperature is characteristic of the dog's ERG. Because of the slow decrease of the late RP after the cessation of illumination, and because of the polarity of the off-effect (ϕ_d), the polyphasic potential corresponds to a predominantly rod ERG[7]. PARRY and TANSLEY[10] already concluded from their experiments in vivo that

[6] A. AMES III and B. S. GURIAN, J. Neurophysiol. *23*, 676 (1960).
[7] K. T. BROWN, Vision Res. *8*, 633 (1967).
[8] H. NETTER, *Biologische Physikochemie* (Akad. Verlagsges. Athenaion, Potsdam 1951).
[9] H. B. PARRY, K. TANSLEY and L. C. THOMSON, J. Physiol., Lond. *115*, 48P (1951).
[10] H. B. PARRY, K. TANSLEY and L. C. THOMSON, J. Physiol., Lond. *120*, 28 (1953).

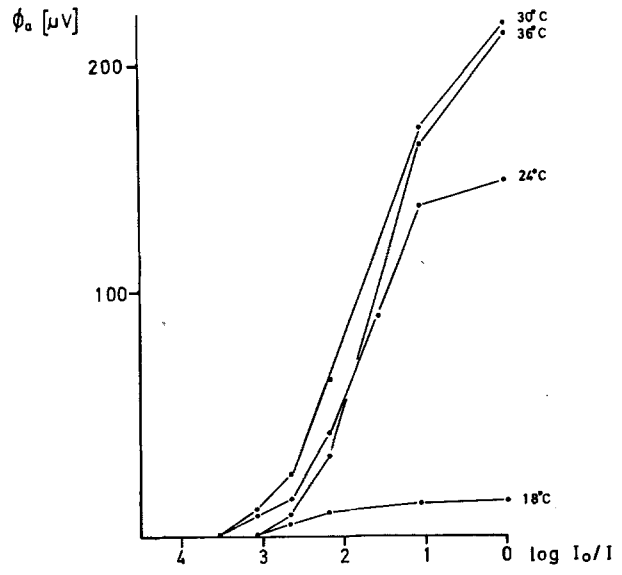

Fig. 3. Dependence on temperature of the function $\phi_a = f(I)$. The maximum light intensity was $I_0 = 40$ lux.

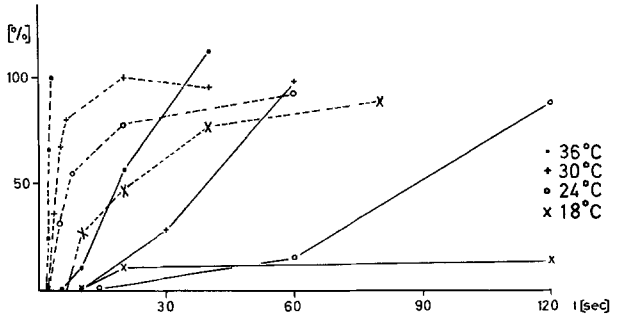

Fig. 4. Influence of temperature on the latency of restoration, measured by means of double stimuli at different intervals. Ordinate: Second potential as percentage of the first; dashed line: late RP (ϕ_a); continuous line: b-wave (ϕ_b). Abscissa: Interval between first and second light stimulus. Intensity of illumination 40 lux, duration 1 sec.

the dog ERG might be attributed to the activity of rods. The influence of temperature on the off-effect is different from that on the b-wave (cf. Figure 2). Below 24 °C, ϕ_a is extinguished while ϕ_b still exists. Therefore the 2 waves seem to originate in different retinal elements. The investigation of early positive components of the rabbit ERG by LÜTZOW[11] showed that a b-wave could only be registered above 28 °C. Our experiments were carried out without adding plasma to the perfusion medium, which seems to play an important role for the isolated rabbit retina[2,3].

The isolated retina of the poikilothermic frog shows maximum potential values at temperatures from 17 to 20 °C, while below 6 °C a b-wave can no longer be registered[1,5]. CORNU and CLOTTES[12] calculated Q_{10}-values between 1.6 and 1.7 for the a-wave of the frog ERG at temperatures from 10 to 30 °C. If one compares the gross activation energies for the b-wave of the frog ERG[5] and the late RP of the dog ERG, their equal size demonstrates that the processes which lead to the formation of both potentials may be ascribed to analogous physicochemical mechanisms. The size of the corresponding Q_{10}-values agrees with the observation that the formation of the potentials is mainly triggered by diffusion processes[13]. The difference between the retina of the homoiothermal dog and the poikilothermal frog is that the first appearance of a potential at low temperatures as well as the range of temperature for the validity of the linear ϕ — log I-relation for the dog retina are shifted by 9 °C to higher temperatures. Comparing the morphology of frog and dog retina, it is striking that the frog retina has a broad inner and small outer nuclear layer, whereas the situation for the dog retina is the reverse. Comparing the corresponding potentials, the frog ERG is characterized mainly by the b-wave and the dog ERG by the late RP.

Zusammenfassung. Grösse und Form des Belichtungspotentials der isolierten Hunderetina stimmen mit den Ergebnissen in vivo überein und lassen sich vorwiegend der Stäbchenaktivität zuschreiben. Ein Vergleich zwischen dem späten Rezeptorpotential des homoiothermen Hundes und der b-Wellenamplitude des poikilothermen Frosches ergibt, dass die Bildung beider Potentiale analogen thermodynamischen Gesetzmässigkeiten folgt, wobei der Temperaturbereich bei der Hunderetina um 9 °C zu höheren Temperaturen verschoben ist.

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[11] A. VON LÜTZOW, Vision Res. *10*, 1035 (1970).
[12] L. CORNU and A. CLOTTES, C. r. Soc. Biol., Paris *146*, 463 (1952).
[13] A. CAVAGGIONI, R. T. SORBI and S. TURINI, J. Physiol., Lond. *222*, 427 (1972).

The Use of Crocetin in Experimental Atherosclerosis

Several past studies[1–3] have indicated that atherosclerosis may be initiated by hypoxia at the vascular wall. However, atherosclerosis is a very common disease[4], and how can hypoxia by so universal? Some investigators have suggested that it may be induced by increased levels of carbon monoxide in the blood[5]. We have previously suggested that such hypoxia may instead be due to a decreased rate of diffusion of oxygen from the red blood cells to the vascular wall[6].

A decreased rate of oxygen diffusion could be due to several factors. We have found, in vitro, that increases in plasma protein levels[6] or of serum glucose levels[7] cause large decreases in the diffusion rate of oxygen through

[1] K. KJELDSEN, J. WANSTRUP and P. ASTRUP, J. Atheroscler. Res. *8*, 835 (1968).
[2] C. GARBARSCH, C. M. MATHIESSEN, P. HELIN and I. LORENZEN, J. Atheroscler. Res. *9*, 283 (1969).
[3] A. L. ROBERTSON, JR., Progr. Biochem. Pharmac. *4*, 305 (1968).
[4] *Arteriosclerosis*, A Report by the National Heart and Lung Institute Task Force on Arteriosclerosis (National Institutes of Health, Bethesda, Maryland, USA, June 1971), vol. 2.
[5] K. KJELDSEN, Thesis, University of Copenhagen (Munksgaard, Copenhagen 1969).
[6] G. M. CHISOLM, J. L. GAINER, G. E. STONER and J. V. GAINER, JR., Atherosclerosis *14*, 327 (1972).
[7] G. M. CHISOLM and J. L. GAINER, in *Oxygen Transport to Tissue. Pharmacology, Mathematical Studies and Neonatology* (Eds. D. F. BRULEY and H. I. BICHER; Plenum Press, New York 1973), p. 729.