Influence of Temperature on Myocardial Healing-Over

It is known that depolarization caused by injury of heart muscle is quickly reversed but it is re-established by another lesion applied near the previous one[1] (healing-over). Studies of membrane potential and resistance in Purkinje fibers[2] showed, indeed, no change near the cut end. Although the precise mechanism of sealing has not been established in heart muscle, as yet, evidence has been provided that the insulating barriers which hold up the spread of depolarizing current from the damaged area are not previously established but are created after lesion[2,3]. More recently, the possibility has been discussed that a decrease in conductance of the intercellular junctions located at the limit of the lesion could be the major explanation for the healing-over process in myocardrial fibers and that the morphological integrity of these junctions is essential for the sealing process[3].

Calcium is essential for the healing-over in Purkinje fibers[4] and myocardial cells[3] since in Ca-free solution no sealing has been found. Experiments with EDTA[3] also supported these observations. It is quite possible that the binding of Ca to injured areas and intercellular junctions nearby the lesion led to a process of coagulation of the cytoplasm or to a drastic change in junctional conductance[3]. In relation to this hypothesis, it is important to know how dependent is the healing-over process on temperature. To answer this question studies were made on muscle strips of toad ventricle (*Bufo marinus*) immersed in Ringer's solution at different temperatures. The rate of the sealing process was determined by cutting a small group of fibers and measuring the change in amplitude of injury potentials as a function of time, as described elsewhere[2]. The roving electrode technique[5] was used to measure the size of the injury potentials. All the experiments were performed on quiescent muscle strips immersed in Ringer's solution. The muscles were kept initially at room temperature (25°C) and then transferred to solutions at 15°C and 5°C. Figure 1, which represents the average result from 8 muscles, shows the influence of tempreature on the rate of sealing of myocardial cells. At 25°C complete reversion of the injury potentials was accomplished in about 12 min. The reduction of temperature of Ringer's solution to 15°C decreased significantly the rate of healing. 30 min after lesion about 3.5% of the initial potential difference was still present

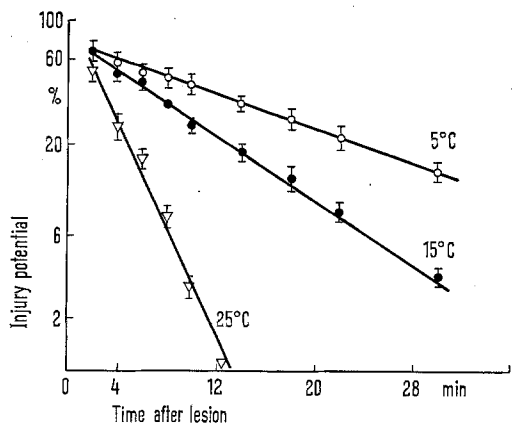

Fig. 1. Graph showing the influence of temperature on the rate of sealing of toad ventricular muscle. Ordinates: percent of maximal amplitude of injury potential plotted in semi-logarithmic scale. On these experiments 100% was the size of injury potentials recorded immediately after damage. Abscissae: time after lesion. Each line represents the average from 8 muscles (24 determinations). The lines drawn vertically through the points are equal to S.E. of the mean.

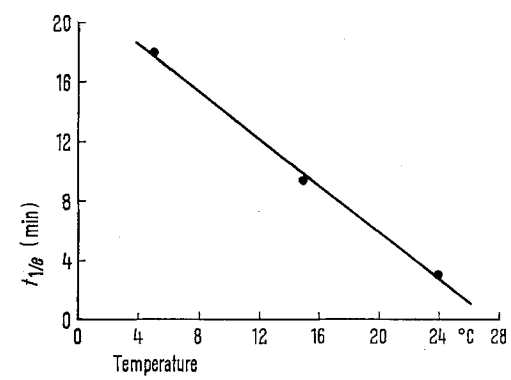

Fig. 2. Time constant (t^1/e) of the rate of healing calculated from data presented in Figure 1. Ordinates: time constant in min. Abscissae: temperature in degrees centrigrade.

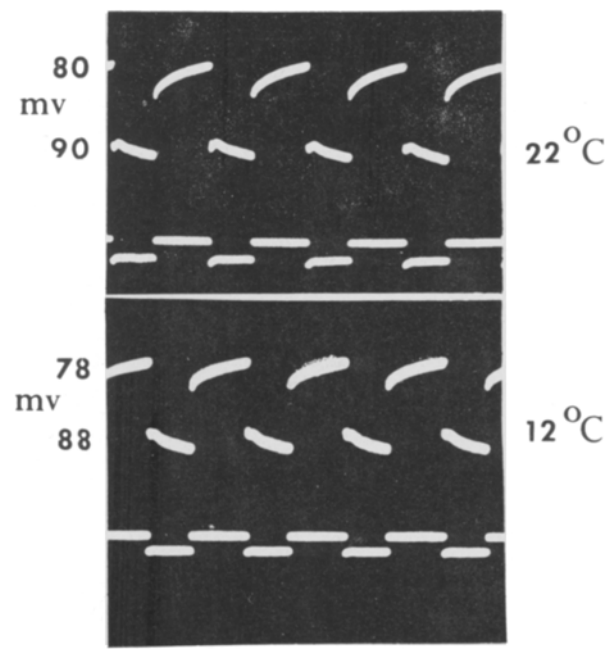

Fig. 3. Electrical coupling between adjacent heart cells of toad's ventricle at 22 °C and at 12 °C. Top traces show the electrotonic potentials recorded when an inward pulse of current (10^{-7}A) (200 msec duration)-lower traces, was injected into a neighbour cell through a microelectrode. Numbers at the left indicates voltage calibration. Top numbers show value of resting potential.

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(see Figure 1). Further reduction on temperature of saline solution to 5°C caused an even greater decrease on the rate of healing; about 15% of the initial injury potential remained for 30 min after lesion (see Figure 1). From the curves presented in Figure 1, the time constant of the rate of healing was determined for each group of experiments. As shown in Figure 2, such time constant proved to be a linear function of the inverse of temperatur. The temperature coefficient (Q_{10}) of the rate of healing, measured in this way, between 25° and 15°C is 4.7 indicating a high activation energy for this process.

Measurements of resting potential of non-damaged cells performed with intracellular microelectrodes also indicated that at low temperature the depolarization produced by lesion is not totally reversed in the non-injured cells located 500 μm or more from the lesion. On the other hand, it was found that the resting potential of toad's ventricular fibers at 25°C was 80 mV (S.E. ±1.5) at 15°C–78 mV (S.E. ±1.2) and at 5°C –76 mV (S.E. ±1.8). Similar results have previously been reported in frog ventricular muscle[6] in which changes on temperature from 0.3 to 24°C caused neglegible variation in resting potential. The fact that injury potentials can easily be elicited at low temperatures is another indication that membrane polarization is not drastically altered. In salivary glands the failure of the sealing process found at low temperature seems to be related to cell uncoupling[7]. The influence of temperature on the electrical coupling between heart cells was investigated by impaling 2 microelectrodes close together (50 to 70 μm); hyperpolarizing pulses of current were injected through 1 electrode and the voltage changes were recorded with the other electrode. The results obtained indicated that the electrical interaction between myocardial cells of toad is slightly altered by reducing the temperature (see Figure 3).

The present results indicate that the healing-over process in toad's myocardium is largely dependent upon temperature and that the depression on the rate of healing is not due to cell uncoupling as in epithelia[8,9].

Résumé. L'influence de la température dans le phénomène de suppression des potentiels de lésion «healing-over» du muscle cardiaque de *Bufo marinus* est édudiée avec des électrodes extracellulaires. A la température de 15°C ou 5°C, une réduction marquée de la vélocité de suppression est observée.

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Release of Fibrinolytic Activators from Human Tumours Cultured in vitro[1]

Tumours possess coagulative properties[2,3] and the ability to develop fibrinolytic activity[4–7]. Fibrin degradation procucts (FDP) have been demonstrated in the serum and in very high concentration in ascitic fluid from patients with malignant ovarian tumours[8]. These findings are presumably connected with the fibrinolytic activity of the neoplasms.

This paper reports the release of fibrinolytic activators from human oral tumours cultured in vitro (Table I). The tumours were excised under local anaesthesia (Lidocain® 2% with Exadrin® 12.5 μg/ml) and divided under sterile conditions. One part was set aside for histopathological examination, while the other was studied for its capacity to release fibrinolytic agents in organ culture with the method described previously[9]. Explants from the tumours were cultured as organ cultures on gelatine foam (Spongostan®) in Leighton tubes containing 1 ml Parker 199 (SLB, Stockholm) synthetic medium and a preformed clot obtained by adding 1 ml human fibrinogen (Kabi Stockholm 1% in distilled water) to 0.02 ml of thrombin (Topostasin® 75 NIH U/ml saline). Penicillin and streptomycin was added to the Parker solution in order to prevent infection.

Each tube contained 6 tumour explants distributed on 2 slices of gelatine foam. Every 24th h after the beginning of the culture, a small volume (0.06 ml) of medium was aspirated and assayed quantitatively for fibrin degradation products (FDP) according to an immunological method[10].

At the end of the culture period (3 days), the explants were examined histochemically for activation of fibrinolysis by a modified fibrin slide technique[11]. Some explants were fixed in Bouin's solution and examined by routine histology.

In all tumours examined, FDP appeared in progressively increasing amounts. A culture experiment is shown in Table II. The mean values of FDP found in the culture medium are given in Table I. There was a considerable difference in the fibrinolytic activity of different tumours. The highest activity was found for epulis angiomatosa. Addition of Tranexamic acid (AMCA) in the culture medium completely inhibited fibrinolysis suggesting that the agent liberated is an activator of plasminogen since AMCA mainly affects fibrinolysis by exerting a competi-

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