A progressive decrease in alkaline phosphatase thermostability with increasing pH values of the homogenizing media was shown. It was evident in all the tissues studied (Figure 1). As seen from the figure, bone and liver alkaline phosphatase resisted better the delirious effects of increased alkalinity. After only 3 min at 56 °C at pH 9.0 the intestinal alkaline phosphatase showed the lowest activity, as opposed to the enzyme in other organs. This difference was highly significant ($n = 10$, p 0.001). 15 min at 56 °C at pH 10.3 resulted in complete inactivation of alkaline phosphatases in all tissues. Thus, it appears that the enzyme thermostability depends upon the conditions of measurement. A comparison of tissue homogenates is complicated by the fact that the degree of enzyme purity affects its thermostability. As shown in Figure 2, the intestinal alkaline phosphatase is more thermostable in more concentrated homogenates. This finding, which requires further study, again shows the interrelationship between molecular conformation and pH, ionic strength[11] and the structure of membranes of cells[12].

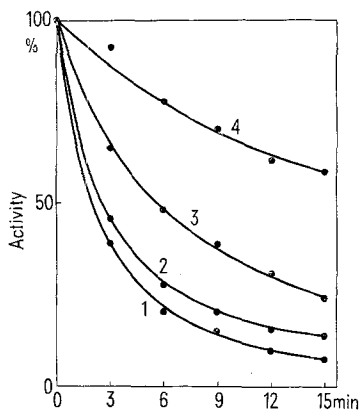

Fig. 2. Effect of dilution of tissue homogenates on susceptibility of alkaline phosphatases to inactivation by heat at 56 °C. 20% homogenate in isotonic NaCl was diluted by buffer at pH 7.4. All the activities were calculated for the initial (20%) homogenate. The resp. dilutions 1. 1:20; 2. 1:9; 3. 1:5; 4. 1:2.

The decreased thermostability of tissue alkaline phosphatases in alkaline buffers is considerable for selection of homogenizing media. Also the usual conditions for estimating alkaline phosphatase activity (dilution of an enzyme, high pH values and temperature of 37 °C) may well have an inactivating influence on the enzyme. The practical importance is demonstrated by the fact that only 80% of activity of intestinal alkaline phosphatase is preserved after 60 min of incubation at conditions used routinely for the activity estimation, i.e. 37 °C and carbonate-bicarbonate buffer at pH 9.8, even when the homogenate was diluted 1:2 (Figure 2). The factors mentioned should be considered for the interpretation of the thermostability of alkaline phosphatase, particularly under pathological conditions.

Zusammenfassung. Die Hitzeinaktivierung der alkalischen Phosphatase in Darm-, Leber-, Knochen- und Nieren-Homogenaten der Ratte erhöht sich mit steigenden pH-Werten, was für die übliche Bestimmung der Gesamtaktivität des Enzyms bedeutend ist.

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The Energetics of Frog Rectus Abdominis Muscle Shortening under Isotonic Load

There is considerable disagreement in the literature as to whether or not the large fraction of heat liberation during muscle shortening is accompanied by a hydrolysis of ATP or phosphorylcreatine. Particularly the data of Davies et al.[1,2], who analyzed the chemical changes in frog rectus abdominis for constant amounts of work and shortening, respectively, have been quoted as evidence for a mere correlation between mechanical work and chemical energy expenditure. In view of the importance of this kind of conclusions, both heat and chemical changes have been measured under steady state mechanical conditions, where the results are not affected by changes in the level of activation.

After dissection paired rectus abdominis muscles of *Rana esculenta* were kept in Ringer's solution containing 1 m*M* iodoacetate for 25 min at 20°C. This was followed by flushing with iodoacetate-Ringer gassed with 5% CO_2 and 95% N_2 at 1°C for another 20 min. The fresh muscle weight was obtained by hooking the tendon onto a spring balance. The individual muscle weight (M) varied between 165–180 mg. The muscles were fixed with Duco cement onto 2 anodized aluminium disks, being connected to a length and force transducer[3]. The initial free muscle length at very slight extension (l_0) varied between 4.5–4.7 cm. Isotonic experiments were carried out by reducing the maximal isometric tension at l_0+4 mm (which was initially developed by each muscle) through quick release to a level equal to any chosen external load, which in this way could be set at a well-defined fraction of the isometric tension. This fractional load then constitutes the new reference value for the servo-control mechanism of the apparatus[3].

For the heat measurements a Wilkie-type integrating thermopile[4] was used, the sensivity of which was increased by incorporating 10 chromel-constantan junctions. The gas was 99% O_2 and 1% CO_2. The temperature was maintained at 0°C in a Dewar cessel. The two muscles of the pair were stimulated simultaneously with supramaximal condenser discharges at a frequency of 10 Hz.

Chemical analyzes have been carried out in parallel experiments, so as to allow a rapid freezing in isopentane

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at −150°C. The changes in phosphorylcreatine (PC) and creatine (Cr) content were determined as described by GILBERT et al.[5]. To provide statistically secured average values 10 muscle pairs have been analyzed both for thermal and chemical changes under any fractional load. For sampling the population, only muscles have been included which could maintain an isometric tension (P_0) close to a mean value of $P_0 l_0/M = 1050$ g-cm/g.

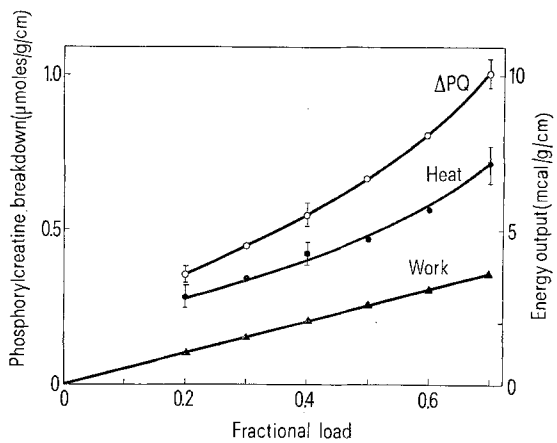

Fig. 1. Relation between total energy output and phosphorylcreatine breakdown in isotonic tetani. The heat recordings have been corrected for contributions from the stimulus heat and the thermoelastic heat transients upon quickly reducing the muscle tension to the value corresponding to the chosen external load. Each of the points is the mean value of 10 experiments. As the relative standard errors of the mean are rather similar, they have been indicated by the cross bars only for characteristic points. The great accuracy of the mechanical apparatus allows one to set any fractional load to within 0.1% of the isometric tetanic tension (P_0). To compare the large number of experiments all data have been normalized against the characteristic value of P_0 obtained in any one experiment.

To limit the considerations of the energy balance to the steady state, both muscles of the pair were initially stimulated tetanically for 4 sec under isometric conditions. At this time 1 of the muscles was frozen (not necessary in the thermal experiments) while the other was allowed to shorten from $l_0 + 4$ mm to $l_0 - 8$ mm under constant load. The PC and Cr content of the muscle shortening additionally by 12 mm was compared to the isometric twin frozen at $t = 4$ sec and the difference related to the amount of work and extra-heat produced by other muscle pairs shortening whilst stimulation continued for another 0.6–3.5 sec. The 4 sec isometric muscle represents an internal control as in this way the results are corrected for initial transient changes[5]. The results obtained with steady state isotonic shortenings at 0°C (Figure) indicate clearly that heat liberation plus work are almost exactly balanced by a breakdown of phosphorylcreatine. The mean quotient of 10.85 mcal energy liberated as work plus heat per μmole of PC split agrees closely with the in vivo free enthalpy change of 11.0 kcal/mole for PC hydrolyses[4].

Zusammenfassung. In parallelen Experimenten wurden die Wärmeentwicklung und Kreatinphoshatspaltung des M. rectus abdominis des Frosches bei verschiedenen isotechnischen Lasten untersucht. Die Resultate zeigen eine gute Übereinstimmung zwischen Gesamtenergieausgang (Wärme und Arbeit) und chemischem Energieverbrauch.

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Activation Parameters of the Nerve Impulse Conduction (II)[1]

The changes of membrane impedance[2] and the optical changes[3–5] associated with nerve impulse propagation clearly indicate that, during the spread of excitation in the nerve fibres, some important structural changes in the axon membrane take place. On the other hand, an increasing amount of data show that on the cell membrane surface there are proteic or lipo-proteic functional subunits.

These two observations suggest that the spread of excitation along a nerve fibre is associated with the transition of some (still) unknown kinds of membrane constituents from a 'resting' (R) to an 'active' (A) conformation. The aim of this paper is to introduce and to calculate all the activation parameters of the transition R → A, that is, the activation parameters of nerve impulse propagation. The procedure used in the previous communication[1] allowed the computation of only the activation energy and enthalpy.

In connection with the problem analyzed here, we have to mention some very promising attempts to describe the conductance changes in excitable membranes in terms of Eyring rate theory[6–8]. But, while these studies refer to ionic fluxes which cross the membrane during the action potential, we calculate here the activation parameters of the entire assembly of physico-chemical processes in which the nerve impulse consists, by referring to the rate limiting step of the spread of excitation. It is noteworthy that, even if the starting point in the definition of the activation parameters of nervous conduction is a purely phenomenological one, their knowledge gives useful information about the molecular mechanisms involved.

Theory. Let us consider that the membrane components, the hypothetical subunits, undergoing the transition R → A, are uniformly distributed on the membrane with a surface density n. The rate of the 'reaction' represented by this transition is: $V = n \cdot (ds/dt)$, ds being the membrane

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