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.

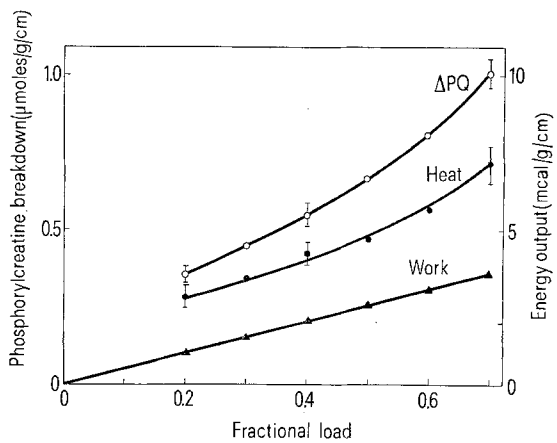

Fig. 1. Relation between total energy output and phosphorylcreatine breakdown in isotonic tetani. The heat recordings have been corrected for contributions from the stimulus heat and the thermoelastic heat transients upon quickly reducing the muscle tension to the value corresponding to the chosen external 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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[5] C. GILBERT, K. M. KRETZSCHMAR, D. R. WILKIE and R. C. WOLEDGE, J. Physiol., Lond. *218*, 163 (1971).

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

[1] The first communication in this series is: D.-G. MĂRGINEANU and I. MĂRGINEANU, Experientia *27*, 1285 (1971).
[2] K. S. COLE and H. J. CURTIS, J. Gen. Physiol. *22*, 649 (1939).
[3] L. B. COHEN, R. D. KEYNES and B. HILLE, Nature *218*, 438 (1968).
[4] I. TASAKI, A. WATANABE, R. SANDLIN and L. CARNAY, Proc. natn. Acad. Sci., USA *61*, 883 (1968).
[5] G. N. BERESTOVSKY, E. A. LIBERMAN, V. Z. LUNEVSKY and G. M. FRANK, Biofizika *15*, 62 (1970).
[6] R. W. TSIEN and D. NOBLE, J. Membrane Biol. *1*, 248 (1969).
[7] J. W. WOODBURY, Adv. chem. Physics *21*, 601 (1971).
[8] G. ROY, J. Membrane Biol. *6*, 329 (1971).

surface on which excitation spreads in a time interval dt. For a nerve fibre with a diameter D, it is obvious that: $ds = \pi \cdot D \cdot dl$, where dl is the linear distance covered by excitation in the time interval dt. As: $dl/dt = \theta$ is the conduction velocity of the nerve impulse, we have: $V = n \cdot \pi \cdot D \cdot \theta$.

As the subunits are spatially located, since they are part of the membrane structure, it follows that the transition R $\rightarrow$ A is a monomolecular like reaction[9], so that: $V = K \cdot n$, K being the rate constant. From the last two relations we have:

$$K = \pi \cdot D \cdot \theta \tag{1}$$

Using the wellknown Arrhenius' formula[10]:

$$K = A \cdot \exp(-E/RT) \tag{2}$$

one can calculate the activation energy: $E = -R\,\delta(\ln K)/\delta(1/T)$. Here R and T have their usual meanings. From (1) it is clear that: $\delta K/\delta T = \delta\theta/\delta T$, so that:

$$E = -R\,\partial(\ln\theta)/\partial(1/T) \tag{3}$$

The activation enthalpy of nerve impulse conduction is:

$$\Delta H^{\neq} = E - RT = -R\,[T + \partial(\ln\theta)/\partial(1/T)] \tag{4}$$

In the activated complex theory[10] one demonstrates that the term A in the Arrhenius' equation is given by:

$$A = (kT/h)\exp(\Delta S^{\neq}/R + 1) \tag{5}$$

where k and h are the Boltzmann and Planck constants and $\Delta S^{\neq}$ is the activation entropy. After several simple calculations, from (1), (2) and (5) one obtains the activation entropy of nerve impulse propagation:

$$\Delta S^{\neq} = R\,[\ln(\pi \cdot D) + \ln(\theta) - \ln(kT/h) + E/RT - 1] \tag{6}$$

Now, one can calculate the Gibbs free energy of activation for impulse propagation:

$$\Delta G^{\neq} = \Delta H^{\neq} - T \cdot \Delta S^{\neq} \tag{7}$$

The relations (3), (4), (6) and (7) show clearly that all the activation parameters of the nerve impulse propagation can be computed knowing solely the diameter of the nerve fibre and the temperature dependence of the conduction velocity.

Results. The Table summarises the values of the activation parameters of impulse conduction in various types of nerve fibres. In the last column of the Table, the reference sources of the conduction velocities at 20 °C and 22 °C are listed. The diameters of nerve fibres have been obtained both from the indicated sources and from HURSH data[11]. In order to facilitate the comparison with chemical reactions, the activation parameters are expressed in cal and kcal per mole, in this case '1 mole of reactant' meaning a membrane area bearing a number of subunits equal with Avogadro's number.

Conclusions and discussion. The large positive values of $\Delta G^{\neq}$ indicate that the resting state of the subunits, and consequently of the membrane, is stable. A stimulus, no matter its nature, will produce the excitation of the nerve fibre if, and only if, it is able to transfer to the membrane an energy greater than $\Delta G^{\neq}$. This observation could be the basis for a unitary approach of the various kind of stimulations (electrical, chemical, thermal, etc.) offering a physical meaning at molecular level for the threshold condition, quite artificially imposed by the current theories of nerve excitation. One can see that $\Delta G^{\neq}$ displays only small variations from one type of nerve fibre to another, these variations being 100 times smaller than those between the conduction velocities. As no correlation can be established between $\Delta G^{\neq}$ and the fibre diameter and no marked difference exists between the myelinated and the unmyelinated fibres, it is allowed to conclude that, at the molecular level, there is a unique mechanism of the functioning of all axonal membranes. A value of $\Delta G^{\neq} \approx 20$ kcal/mol could thus be considered as a general defining parameter of the nervous conduction.

If the membrane subunits absorb an extrenal energy during the excitation, it is to be expected that, when returning to the resting state, they will release energy, a part of this serving to induce the transition in the neighbouring subunits and another part being released as electromagnetic emission. A very good experimental support of this view arises from the fact that an energy as

[9] A first order kinetics could be considered as only one of several possible working hypotheses, but it seems to be the most reasonable.

[10] For all the problems of chemical kinetics reference is made to: H. EYRING and E. M. EYRING, *Modern Chemical Kinetics* (Reynhold, New York 1963).

[11] J. B. HURSH, Am. J. Physiol. *127*, 131 (1939).

[12] N. A. HUTCHINSON, Z. J. KOLES and R. S. SMITH, J. Physiol., Lond. *208*, 279 (1970).

[13] B. FRANKENHAEUSER and B. WALTMAN, J. Physiol., Lond. *148*, 677 (1959).

[14] D.-G. MĂRGINEANU, unpublished results.

[15] A. S. PAINTAL, J. Physiol., Lond. *180*, 20 (1965).

[16] D. N. FRANZ and A. IGGO, J. Physiol., Lond. *199*, 319 (1968).

[17] J. V. HOWARTH, R. D. KEYNES and J. M. RITCHIE, J. Physiol., Lond. *194*, 745 (1968).

[18] A. L. HODGKIN and B. KATZ, J. Physiol., Lond. *109*, 240 (1949).

Activation parameters of nervous conduction calculated on the basis of conduction velocities at 20 and 22 °C

Type of fibre	Nerve	Animal species	D (μm)	θ20 °C (m/s)	E	$\Delta H^{\neq}$ (kcal/mol)	$\Delta G^{\neq}$	$\Delta S^{\neq}$ (cal/mol·°K)	Reference source of θ 20 °C values
Myelinated	sciatic	*X. laevis*	3	5.40	5.57	4.99	23.04	−61.62	12
Myelinated	sciatic	*X. laevis*	12	21.60	5.53	4.94	23.91	−58.24	12
Myelinated	sciatic	*X. laevis*	22	39.60	5.43	4.85	20.71	−54.14	12
Myelinated	sciatic	*X. laevis*	30	67.70	2.59	2.01	20.21	−62.14	13
Myelinated	sciatic	*R. temporaria*	20	33.20	6.83	6.25	20.83	−49.60	14
Myelinated	vagus	cat	6	12.90	11.19	10.60	22.20	−39.46	15
Myelinated	saphenous	cat	7	22.60	7.94	7.36	21.68	−48.73	16
Unmyelinated	saphenous	cat	0.50	0.53	9.14	8.55	25.43	−57.72	16
Unmyelinated	vagus	rabbit	1.20	0.51	12.45	11.86	25.05	−44.86	17
Giant axon		squid	500	[a]	6.82	6.24	19.08	−44.27	18

[a] In this case, the calculations are based on the conduction velocities at 13.3 and 20.2 °C.

$\Delta G^{\neq}$ and $\Delta H^{\neq}$ corresponds to quanta with wavelength of ≈ 2 μm. This value lies just in the domain in which the electromagnetic emission of the active nerves was detected[19].

The fact that $\Delta S^{\neq}$ always has large negative values shows that the active state of the membrane is a more ordered one. It appears that, during the excitation, the entropy of the membrane firstly decreases, after which it increases, while the entropy of the axoplasma and the interstitial fluid only increases due to ionic fluxes[20]. These opposite entropy changes offer a basis for the understanding of the biphasic form of the initial heat production associated with a nerve impulse[17,21]. All the attempts to explain this fact solely on the basis of ionic fluxes failed. The optical changes associated with nerve impulse propagation[3-5] indicate the occurence of structural changes in the membrane and even seem to indicate a decrease in entropy. The calculations presented here clearly show such a decrease, and we can get an idea of the magnitude of structural changes, remembering that the formation of a hydrogen bond is associated with $\Delta H = 4.5$ kcal/mol and $\Delta S = -12$ cal/mol °K. Accordingly, the structural changes occuring in each membrane subunit are energetically equivalent with the formation of up to 5 hydrogen bonds.

Résumé. La théorie du complexe d'activé est appliquée à la description de la propagation de l'influx nerveux. On déduit les formules donnant tous les paramètres d'activation de ce processus et on les calcule pour différent types de fibres nerveuses. L'entropie d'activation est grande et négative, ce qui prouve que la membrane excitable est dans un état plus ordonné pendant l'excitation. L'énergie libre d'activation est presque la mème pour toutes les fibres nerveuses. Elle peut donc être considérée comme un paramètre fondamental de la conduction nerveuse.

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[19] A. FRASER and A. H. FREY, Biophys. J. *8*, 731 (1968).
[20] D. MĂRGINEANU, Biophysik *6*, 327 (1970).
[21] B. C. ABBOTT, A. V. HILL and J. V. HOWARTH, Proc. R. Soc. *148 B*, 149 (1958).
[22] Permanent address (for inquiries): Department of Biophysics, Faculty of Medicine, Bd. P. Groza No. 8, București (Romania).

Embryonic Megaloblastosis

A consistent feature of embryonic development is the change from a megaloblastic haemopoietic cell line to one which is normoblastic. This has been well documented in the chick embryo[1], but few studies – other than morphological ones – have been undertaken to determine the cause. The question was asked whether megaloblastosis in the embryo could be related to the metabolic defect occurring in orotic aciduria, where there is very little activity of the enzymes, orotidylate pyrophosphorylase and decarboxylase, and resultant megaloblastosis[2]. The developing chick embryo was used as a model.

Fertilized fowl eggs were incubated at 37 °C for time intervals up to 4 days. Blastoderms dissected from the eggs were classified according to the stages defined by HAMBURGER and HAMILTON[3]. Embryonic or blood island tissue was homogenized for estimation of combined orotidylate pyrophosphorylase and decarboxylase levels by the release of radioactive CO_2 from orotic acid, labelled in the carboxyl group[4].

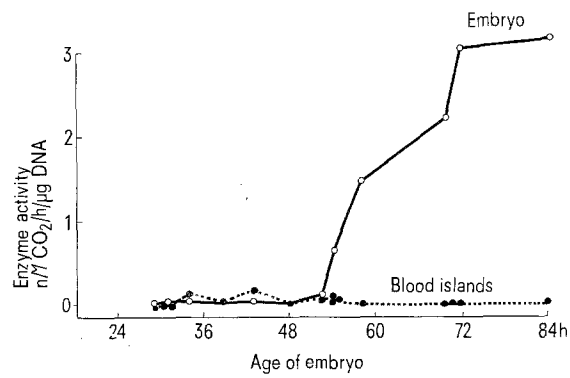

Fig. 1. Combined orotidylate pyrophosphorylase and decarboxylase activity in chick embryonic (open circles) and blood island (closed circles) tissues, at different stages of development.

The results of estimation of enzymatic activity in embryonic or blood island tissue at different stages of development from 24 to 84 h are shown in Figure 1. In the embryo, enzymatic activity was barely detectable by this method until 55 h, when there was a striking increase. In contrast, in the blood islands, activity remained at a low level until 55 h, but the subsequent rise in activity was masked by a parallel rise in DNA content – that is, relative activity in the blood islands did not alter with age. Similar trends were obtained when enzymatic activity was compared with protein.

The relative lack of this essential step in de novo pyrimidine biosynthesis during the early stages of chick embryogenesis is interesting in view of the active DNA synthesis occurring at this time. DNA synthesis may depend on other sources, such as the salvage pathway (uridine kinase). In view of these findings, orotidylate pyrophosphorylase and decarboxylase activity was compared with that of a control enzyme in the purine pathway, hypoxanthine-guanine phosphoribosyl transferase (HGPRTase), which converts hypoxanthine to inosine monophosphate. HGPRTase is also a salvage enzyme. Figure 2 shows that this enzyme was present from the beginning, and its activity was gradually reduced in both embryonic and blood island tissue at all stages of development.

[1] A. B. DAWSON, Z. Zellforsch. *24*, 256 (1936).
[2] L. H. SMITH, C. M. HUGULEY and J. A. BAIN, in *The Metabolic Basis of Inherited Disease* (Eds. J. B. STANBURY, J. B. WYNGAARDEN and D. S. FREDRICKSON; McGraw-Hill, New York 1966), p. 739.
[3] V. HAMBURGER and H. L. HAMILTON, J. Morph. *88*, 49 (1951).
[4] L. H. SMITH, M. SULLIVAN and C. M. HUGULEY, J. clin. Invest. *40*, 656 (1960).