osmotic GABA[3], it seemed pertinent to compare the effects ot these two substances on the water content of the brain using a sensitive radio-isotopic method.

Tritiated water (^{3}HOH, specific radioactivity, 25 mC/g, New England Nuclear Corp.) was injected i.p. (in 0.9% NaCl) in a dose of 0.1 μC/g of fasted mouse. Mice were sacrificed at various times after administration of the ^{3}HOH, and homogenates of brain (above the level of the colliculi, and not including the cerebellum) were prepared in 80% ethanol solution (v/v) and centrifuged; radioactivity was determined in aliquots of the resulting supernatants. Results shown in the Table indicate that ^{3}HOH exchanges very rapidly with brain water and that a large amount of the radioactivity remains in the brain 5 h after injection. In the Table it is shown also that i.p. injections of hyper-osmotic GABA and DL-α-alanine (1M solutions in 0.9% NaCl; 25 mmoles/kg), given 30 min before sacrificing the animals, produce a significant dehydration of the brain.

Entry of i.p. injected ^{3}HOH into the brains of mice; effects of hyper-osmotic GABA and DL-α-alanine on the entry of ^{3}HOH

Time of injection of ^{3}HOH (0.1 μC/g)	Hyper-osmotic treatment	dpm/g brain ($\times 10^{-3}$)	
1 min	none	297.7 ± 13.44	(8)
5 min	none	541.9 ± 9.44	(8)
5 h	none	482.4 ± 3.16	(8)
5 h	GABA[a]	433.8 ± 10.31	(8)[b]
5 h	DL-α-ALA[a]	461.7 ± 3.63	(8)[b]
24 h	none	287.8 ± 6.87	(16)

[a] Hyper-osmotic treatments (25 mmoles/kg) were given i.p. 30 min before sacrificing animals. These values are to be compared with the 5 h untreated control value. Means ± standard errors; numbers of mice in parentheses; [b] indicates a p-value <0.001 with respect to controls (Student's t-test, one-tailed).

These results support further the finding[3] that the mechanism of the anti-convulsant effect of hyper-osmotic treatments is related to the brain dehydration produced, and not to a specific 'inhibitory' action of GABA. Although it seems likely that the hyper-osmotic fluid injected causes a loss of water from other tissues as well as from brain, due to the increased osmolarity of the serum which occurs[3], it is thought that the anti-convulsant effect is related mainly to the loss of brain water. It is suggested that DL-α-alanine, a substance which does not exert potent inhibitory actions on CNS neurones, acts by a mechanism similar to that of GABA in protecting against convulsions when administered as an hypertonic solution[5].

Résumé. Utilisant une technique radioactive avec le ^{3}HOH, nous avons montré que les injections i.p. des solutions hyperosmotiques de l'acide γ-aminobutyrique et de DL-α-alanine, deshydratent de manière significative les cerveaux des souris.

F. V. DEFEUDIS[6]

Department of Psychology Purdue University, Lafayette (Indiana, USA) 22 March 1971.

[1] J. D. WOOD, W. J. WATSON and F. M. CLYDESDALE, J. Neurochem. *10*, 625 (1963).
[2] J. D. WOOD and W. J. WATSON, Can. J. Physiol. Pharmac. *42*, 641 (1964).
[3] F. V. DEFEUDIS and K. A. C. ELLIOTT, Can. J. Physiol. Pharmac. 45, 857 (1967).
[4] F. V. DEFEUDIS and K. A. C. ELLIOTT, Can. J. Physiol. Pharmacol. *46*, 803 (1968).
[5] This study was supported by the U.S.P.H.S.
[6] Present address: Institute of Psychiatric Research, Indiana University Medical Center, 1100 West Michigan Street, Indianapolis (Indiana 46202, USA).

Apparent Activation Energy of the Nerve Impulse Conduction[1]

In this communication we calculate the apparent activation energy of the nerve impulse propagation, as expressed by the temperature dependence of the conduction velocity in the nerve fibres. We call this activation energy 'apparent', for we do not refer, as is usually done, to a chemical reaction, but to a complex physico-chemical process, the nerve impulse propagation, considered as a whole.

The spread of the excitation along the nerve fibre obviously means the transition of certain physical structures from a 'resting' to an 'active' state; it is likely that these structures are located in the axon membrane. From this point of view, the propagation of the action potential appears to be associated, at a molecular level, with transitions of certain macromolecular components of the nerve fibre. There is some evidence that, during the action potential, some vibrorotational transitions of the protein macromolecules take place in the axon membrane[2]. Even neglecting the concrete nature of the transformation and the macromolecules which undergo it, from a physico-chemical point of view, the nerve impulse propagation implies the 'transformation' of some components from a certain state 1 (resting state) to another state 2 (active state). It thus appears that the conduction velocity of the nerve impulse represents, or at least is proportional with, the transformation rate of the components from state 1 into state 2.

The activation energy (E) of this 'reaction' is given by the wellknown Arrhenius' formula[3]: $E = -R\,(\delta(ln\,k)/\delta(1/T))$, where k is the rate constant and R, T have their usual meanings.

The conduction velocity, that is the rate of the reaction $1 \rightarrow 2$, is: $v = k \cdot C_1^{\nu_1} \cdot C_2^{\nu_2} \cdots$, where c_1, $c_2 \cdots$ are the 'concentrations' of the components undergoing the transition $1 \rightarrow 2$, and ν_1, ν_2 are the stoichiometric coefficients. As: $(\delta v/\delta T) = (\delta k/\delta T)$, we can write: $E = -R\,(\delta(ln\,v)/\delta(1/T))$. Using finite differences instead of differentials, after a few simple operations we obtain: $E \approx R\,(T_1\,T_2/(T_2 - T_1))\,ln\,(v_2/v_1)$. Here v_1 and v_2 are the conduction velocities for the absolute temperatures T_1 and T_2. The Celsius temperatures are noted with t_1, t_2.

[1] This paper is based on an investigation in progress which will be submitted by D.-G. MĂRGINEANU in partial fulfilment of the requirements for a Doctoral Degree in Biophysics under the leadership of Prof. Dr. V. VASILESCU.
[2] D. MOISESCU and D. MĂRGINEANU, Biophys. J. *10*, 482 (1970).
[3] For all the problems of chemical kinetics reference is made to H. EYRING and E. M. EYRING, *Modern Chemical Kinetics* (Reinhold, New York 1963).

Table I.

t_1	t_2	Myelinated fibre				Unmyelinated fibre			
		E_{dt}	$\Delta H^{\neq}_{dt}$	E_{it}	$\Delta H^{\neq}_{it}$	E_{dt}	$\Delta H^{\neq}_{dt}$	E_{it}	$\Delta H^{\neq}_{it}$
15	20	9.33	8.75	25.72	25.14	11.06	10.48	11.83	11.25
20	25	7.54	6.95	14.20	13.61	12.19	11.60	11.47	10.88
25	30	4.65	4.05	12.05	11.45	8.56	7.96	12.46	11.86
30	35	4.30	3.69	10.22	9.61	3.48	2.87	16.32	15.71

Table II.

t_1	t_2	E_{I}	E_{II}	$\Delta H^{\neq}_{\mathrm{I}}$	$\Delta H^{\neq}_{\mathrm{II}}$
12	16	20.84	21.00	20.27	20.42
16	20	17.72	17.15	17.14	16.57
20	24	14.31	20.42	13.72	19.83
24	28	12.03	18.10	11.43	17.50
28	32	9.50	16.85	8.90	16.14
32	36	6.95	17.23	6.33	16.62

With the above formula we calculated the activation energy E, using the experimental data[4,5] which give the temperature dependence of the conduction velocity. The activation energy represents, generally speaking, the necessary amount of energy for a couple of molecules to react; in the case considered here, E represents the necessary energy for the transition $1 \rightarrow 2$ of a single membrane component. At the same time it is useful to calculate the thermodynamical parameters: $\Delta G^{\neq}$, $\Delta H^{\neq}$, $\Delta S^{\neq}$. These are respectively the activation free energy, enthalpy and entropy of the process, as defined in the absolute rate theory[3].

The activation enthalpy is: $\Delta H^{\neq} = E - RT$ and we shall use: $\Delta H^{\neq} \approx E - R\,((T_1 + T_2)/2)$.

In order to calculate $\Delta S^{\neq}$ and $\Delta G^{\neq}$ it is necessary to know the values of the rate constant k, with a view to obtain by extrapolation the intercept on the ordinate of the curve: $k = f(T)$. But, without elaborating a certain hypothesis on the concrete nature of the components which undergo the transition $1 \rightarrow 2$, one cannot define the rate constant of the nerve impulse propagation. As this is beyond the scope of this note, we shall restrict ourselves to calculate only E and $\Delta H^{\neq}$.

Results. Table I contains the E and $\Delta H^{\neq}$ values, calculated with the data of FRANZ and IGGO[4], figures 11B and 11C, for myelinated and unmyelinated fibres, isolated from cat saphenous nerve. The index *dt* marks the values corresponding to decreasing temperatures and *it* those for increasing temperatures. In both the Tables I and II, E and $\Delta H^{\neq}$ are expressed in kcal/mole.

Table II contains the E and $\Delta H^{\neq}$ values calculated with the data of PAINTAL[5], Figure 4, concerning the conduction velocity in myelinated fibres isolated from cat vagus nerve. The indices I and II reveal that the values correspond respectively to an impulse and to a second impulse applied immediately after the absolute refractory period of the first.

The possible sources of errors which alter the values calculated by us are: a) the inherent errors of the experimental measurements, b) errors introduced by the use of the data from the graphs and c) utilisation of approximations admitted. All these possible errors are, however, much under the differences between the calculated values, so that they do not affect the following conclusions.

Conclusions and discussion. 1. In all the cases, the apparent activation energy of the nerve impulse propagation is not independent of temperature, as considered in chemical kinetics, but shows generally an approximately linear decrease as the temperature increases. This fact suggests a certain labilization in the structure of the membrane components which undergo the transition $1 \rightarrow 2$, produced by the temperature. This possible labilization seems to be rather important, because in a range of only 20°C, the activation energy and enthalpy decrease sometimes as much as 50% of the initial values.

2. With respect to the apparent activation energy of the nerve impulse propagation, the differences between the myelinated and unmyelinated fibres of the same nerve are smaller than those between the myelinated fibres of 2 different nerves from the same animal species. This fact agrees well with the view that the molecular mechanism of the impulse propagation is essentially the same in the two kinds of fibres.

3. When a second impulse is applied immediately after the absolute refractory period of a first one, the activation energy of the second is less dependent on temperature than that of the first impulse. A possible explanation would be that the propagation of the second impulse takes place in a structure which is already labilized to some extent.

There are some very promising theoretical attempts to approach the excitablility of the biological membranes, considering that they consist of oligomeric functional subunits which undergo conformational transitions[6,7]. In this paper we did not consider the concrete nature of the subunits and their conformational transitions. It is, however, noteworthy that the calculated values for E and $\Delta H^{\neq}$ correspond to 1 mole of such unidentified functional subunits. As the energy of the hydrogen bond is about 4.5 kcal/mole, the data calculated by us suggest that during the nerve impulse propagation, the structural modifications which take place are equivalent, from an energetical point of view, with the break-down of 1 to 4 hydrogen bonds per functional subunit.

Résumé. On définit la notion d'énergie apparente d'activation de la transmission nerveuse et on calcule les valeurs de l'enthalpie d'activation de ce processus. Les résultats sont discutés par rapport au mécanisme moléculaire de la conduction.

D.-G. MĂRGINEANU and ILCA MĂRGINEANU

Department of Biophysics, Faculty Medicine, Bd. P. Groza 8, Bucuresti, and 'Fundeni' Clinical Hospital, Bucuresti (Romania), 13 April 1971.

[4] D. N. FRANZ and A. IGGO, J. Physiol., Lond. *199*, 319 (1968).
[5] A. S. PAINTAL, J. Physiol., Lond. *180*, 20 (1965).
[6] J. P. CHANGEUX and J. THIERY, in *Regulatory Functions of Biological Membranes* (Ed. J. JÄRNEFELT; Elsevier Publ. Comp., Amsterdam 1968).
[7] R. KILKSON, in *Symmetry and Function of Biological Systems at the Macromolecular Level* (Eds. A. ENGSTRÖM and B. STRANDBERG, Almqvist and Wiksell, Stockholm 1969).