not coagulated by thrombin, but which are precipitated by TCA. The enzyme is much less active for fibrin and casein. The former is lysed to give a product only about 20% of which is soluble in TCA. The product from casein, which is not coagulated by rennin, is also largely precipitated by TCA, although soluble to the extent of about 20%.

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The Depolarization of Nerve by Organic Compounds.* (20812)

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One of the perplexing aspects of the action of narcotic agents on nerve has been noted by Laget *et al.*(1), by Bishop(2), and by Brink(3). This is the fact that while the lower alcohols of the paraffin series depolarize nerve before a conduction block ensues, the higher alcohols and many other narcotic agents block conduction before there is any detectable depolarization. Indeed, there seems to be a slight increase in the membrane polarization associated with the action of the higher alcohols and with cocaine. In order to investigate this problem in more detail, we have measured the depolarization produced by a variety of organic reagents, and we have attempted to explain the results obtained in terms of a model.

Methods. In most experiments, bullfrog (*R. catesbiana*) sciatic nerves were used, although a few measurements were made on *R. pipiens* nerves. The general arrangement of the chamber for mounting nerves was similar to that used by Lorente(4). In most cases, the depolarization produced by 0.11 M KCl at one region along the nerve was compared with the depolarization produced by an experimental solution at another region along the nerve. Such measurements permitted the calculation of a depolarization rate relative to the rate of KCl depolarization as a standard. Measurements were made with a recording potentiometer so arranged as to record the potential between the KCl-Ringer segment and between the narcotic agent-Ringer segment at 16 sec. intervals. The sensitivity of this instrument with the nerve in the external circuit was about 0.1 mV, and the current drawn at balance, 10^{-9} amperes. The usual technic for measurement was to mount a nerve, previously dissected and kept in Ringer solution for 2 hours, in the chamber with Ringer in all electrodes. This was a check for any initial depolarization. Next, KCl, 0.11 M was added to electrode 1. Ringer to electrode 2, and test agent dissolved in Ringer to electrode 3. Recording was initiated, and continued usually for several hours after which all electrodes were changed to Ringer, and recording was continued to follow the course of repolarization. Throughout the course of the experiments, 95% O_2-

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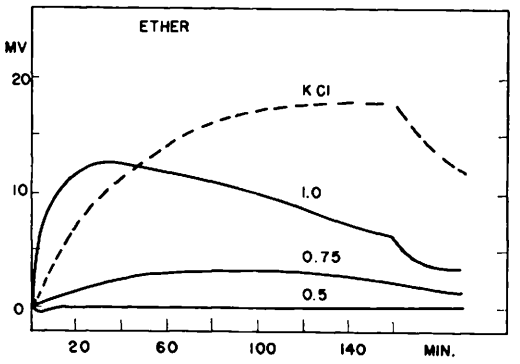

FIG. 1. Depolarization produced by ethyl ether at various thermodynamic activities plotted against time. Dotted line gives time course of KCl depolarization, measured on another segment of same nerve. At 160 min. solutions were changed to Ringer, and repolarization commenced.

5% CO_2 was allowed to flow slowly through the chamber. The ambient temperature was 20 ± 1°C, and the composition of the Ringer solution: NaCl 116 mM, KCl 2 mM, $CaCl_2$ 1.8 mM.

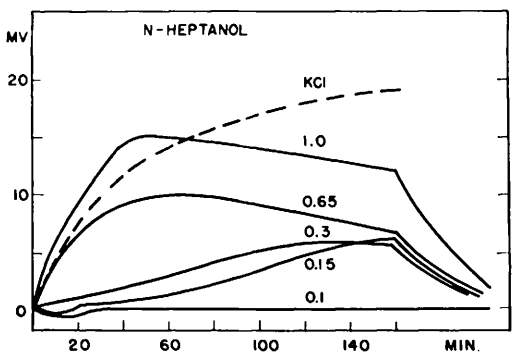

FIG. 2. Depolarization produced by n-heptanol at various thermodynamic activities plotted against time. Dotted line gives time course of KCl depolarization measured on another segment of same nerve. At 160 min. solutions were changed to Ringer, and repolarization commenced.

Results. A series of experiments was undertaken to determine the time course of depolarization produced by various organic substances, *viz.* diethyl ether, dichloromethane, chloroform, acetone, dioxane, n-pentane, and n-heptanol, as compared with KCl. Fig. 1 and 2 show some records for the depolarization produced by ether and by n-heptanol respectively at various concentrations. Table I gives a summary of the values obtained for other substances. The thermo-dynamic activity coefficients (γ_{H_2O}), for sparingly water soluble substances were computed by assuming a linear relationship between activity and saturation of an aqueous solution. Because the activities employed were generally high (greater than 0.5), this approximation is felt to involve no great error. For acetone, the activity at the various concentrations used was computed from vapor pressure-composition data as given in International Critical Tables. For dioxane, activity figures were approximated from freezing point depression data (Mellan(5)).

It appears clear that with the exception of n-pentane, which does not depolarize nerve, of chloroform which only rarely can produce a slight depolarization and n-heptanol which depolarizes at rather low values for the thermodynamic activity, all other substances tested depolarize nerve appreciably only at concentrations representing a thermodynamic activity greater than 0.5. This figure is to be compared with the thermodynamic activity for nerve block, $A_{nar} = 0.05$. Several features of the time course of depolarization are worth comment. The rate of depolarization by a saturated aqueous solution of ether is about 5 times faster than that produced by isosmotic KCl. Furthermore, the depolarization produced by ether is not maintained, but declines gradually to about half its maximum

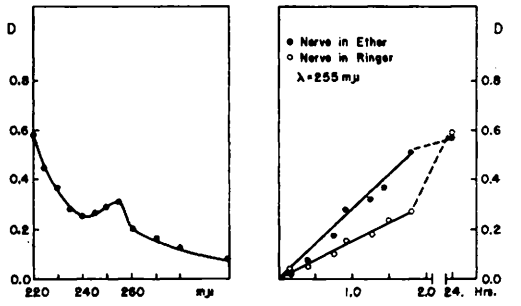

FIG. 3. On left is plotted optical density vs. wavelength for difference between a sol. containing 1.0 M ether in Ringer, plus a frog sciatic nerve, as compared with a Ringer sol. containing a frog nerve. Curves were obtained after one hour's immersion of nerve. Volume in both cases was 5 ml, and ether added to control Ringer sol. before measurement. On right, optical density plotted at 255 mμ vs. time a 1 M ether sol. was in contact with nerve; curve is relative to a control nerve in Ringer.

TABLE I. Depolarization of Frog Nerve by Various Substances.

Substance	Concentration M/L	Concentration A	Initial rate of depolarization, mV/min. Narcotic	Initial rate of depolarization, mV/min. KCl	Ratio: Narcotic/KCl
Ethyl ether	1.0	1.0	2.5	.5	5
	1.0	1.0	2.0	.4	5
	1.0	1.0	2.0	.4	5
	1.0	1.0	2.3	.5	5
	.8	.8	.7	.4	2
	.75	.75	.0	.4	—
	.75	.75	.02	.4	—
	.75	.75	.07	.4	—
Dichloromethane	.24	1.0	.8	.4	2
	.24	1.0	.8	.4	2
	.24	1.0	.7	.4	2
	.19	.8	.0	.5	—
1,4 Dioxane	2.5	.3	.02	.6	—
	2.5	.3	.05	.5	.1
	3.0	.4	.13	.4	.3
	1.3	.2	.0	.5	—
Acetone	5.2	.4	.9	.4	2
	3.0	.3	.5	.4	1
	1.6	.18	.0	.5	—
n-Pentane	.005	1.0	.0	.4	—
	.005	1.0	.0	.4	—
Chloroform	.084	1.0	.0	.5	—
n-Heptanol	.0078	1.0	1.0	.4	2.5
		1.0	1.0	.7	1.4
		1.0	1.0	.4	2.5
		1.0	1.1	.4	2.6
		1.0	.9	.7	1.3
		.65	.8	.5	1.6
		.3	.6	.4	1.5
		.15	.6	.4	1.5
		.10	.0	.5	—
		.05	.0	.4	—

value. Recovery from such depolarization is seldom complete, and after 2 hours there is a permanent depolarization about 5 mV below the initial level of the membrane potential. There is a rough equivalence between the initial rate of depolarization and the maximum value of the demarcation potential. The former measurement has been taken by us as being a more reproducible index of the action of substances in producing depolarization.

We have made some measurements in which a nerve was immersed in ether-saturated Ringer solution for some time, and a control paired nerve was kept in Ringer alone for the same period. The solutions were then placed in quartz cuvettes, and the ultraviolet absorption curve of each determined, using ether-saturated Ringer as a blank in one case, and Ringer in the other. The results of such experiments are shown in Fig. 3 and in Table II. In all cases where there was depolarization produced by an organic substance, there was in increased release of substances absorbing light at 255 mμ. An observation such as this can be related to the report that frog muscle loses a great deal of nitrogenous material when it is first immersed in Ringer solution(6), and that this loss is decreased by raising the external K^+ concentration and increased by anoxia. Since nerves depolarized in KCl do not show this increased release of material absorbing at 255 mμ, one may conclude that there is more than just a quantitative difference between the depolarization produced by narcotic agents and by KCl.

Discussion. The interpretation of the experimental results obtained presents two different problems. In the first, some explana-

TABLE II. Effect of Organic Substances on Loss of Material from Nerve.

Substance	Time of treatment of nerve, hr	Conc., M/L	% transmission at 255 mμ. Narcotic minus Ringer nerve sol.
KCl	18.5	.11	.5
Pentane	18.0	.005	1.3
Dioxane	18.0	3.0	28.2
Ether	1.0	1.0	50.0
Acetone	17.0	5.2	35.8
Acetone	17.0	3.0	32.5

tion must be found for the fact that ether at 0.75 M gives either a slight depolarization or none at all while at 1.0 M, it gives a depolarization many times faster than that of KCl. Such an observation is in marked contrast with studies on the kinetics of chemical reactions where inhibition or acceleration of the rate by various reagents takes place over a range of concentration of 2 log units. The second problem is to explain the markedly different concentrations (or activities) necessary for depolarization by the alcohols, as contrasted with less polar organic molecules. An explanation of the first point, namely the narrow range of concentration of aqueous solutions of ether that produce depolarization must lie in some physical change in such a solution as the concentration of ether is changed from 0.8 to 1.0 M. Such a change, taking place mainly in this concentration range, is the variation of the surface tension of the solution. Ether is not a particularly surface active substance, and low concentrations of ether in water have practically no effect on the surface tension of the solution. Dichloromethane has less, and chloroform even less surface activity; indeed a saturated aqueous solution of chloroform has a surface tension only 13 dynes/cm less than that of pure water. Pentane is completely without surface activity, while both dioxane and acetone are appreciably surface active only at very high aqueous concentrations. Fig. 4 summarizes our measurements and correlates them with the surface tension depression produced by the various organic reagents. If the depolarization produced by these substances were somehow related to the amount of each substance partitioned to the cell membrane, we should expect(3) that they would all act at some constant value of thermodynamic activity. Actually, at an activity of 1.0, ether depolarizes rapidly and chloroform not at all. The alcohols, by contrast with these other agents, are surface active even at low concentrations and the interpretation of the depolarization produced by the alcohols will be more complex.

The application of the notion of thermodynamic activities (A_{nar}) in narcosis(7,8) has been very helpful in explaining the aqueous concentrations required for nerve block(3); the determination of the activities for the alcohols in aqueous solutions is based on the data of Butler(9,10) who, however, has shown that the first 3 n-alcohols exhibit greater deviations from ideality when dissolved in benzene, than when dissolved in water. This is also true in heptane(11). Since a constant thermodynamic activity for nerve block implies an ideality of behavior of the narcotic agent in the non-aqueous system to which it is supposed to be partitioned, it is apparent that one can expect anomalous behavior from the alcohols, in that higher aqueous activities will be required to produce a given mole fraction in the non-aqueous phase. Heptanol, which we have studied, behaves ideally enough

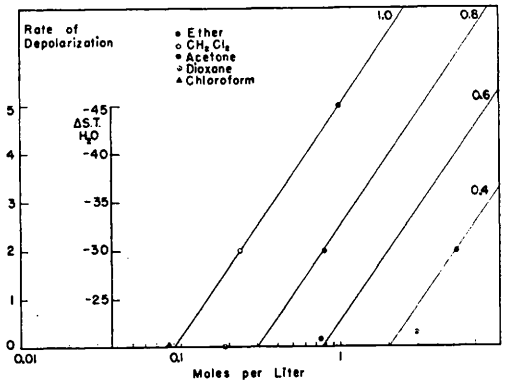

FIG. 4. Rate of depolarization, relative to KCl, plotted against aqueous concentration in moles/liter of various organic substances. Lines represent various thermodynamic activities for a particular solute, and are intended to show that a certain concentration is required to produce depolarization at any given activity. Superimposed ordinate $\Delta S.T._{H_2O}$ is the depression of surface tension of water in dynes/cm that a solution of given substance causes, at concentration shown on abscissa.

so that conduction is blocked at $A_{nar} = 0.07$ while depolarization starts at $A_{dep} = 0.2$-.3. The lower alcohols behave less ideally in benzene, and more ideally in water. For n-propanol, the activity coefficients in both benzene and water are the same, 15, while at the interface benzene:water it is probable that the C_3H_7-part of propanol has unit activity coefficient, as does the –OH part of the molecule in water. Such a notion finds confirmation in a study(12) showing that alcohols from C_6-C_{12} at equal concentrations show equal interfacial tension reductions at the benzene:water interface. The Traube rule does not hold for this interface, suggesting, indeed, that there is a constancy of activity coefficient from one alcohol to another. We might, therefore, expect that the alcohols will show equal degrees of depolarization at equal aqueous concentrations, while narcosis will demand equal thermodynamic activity of the narcotizing molecules (as a first approximation). Just how these various factors are related, is illustrated by Fig. 5, where we have combined the data of Laget(1) on depolarization and narcosis of frog sciatic nerve, with our own for the depolarization produced by heptanol. The line X_{dep} represents the constant aqueous mole fraction necessary for a depolarization of about 2 mV, the line $X^{H_2O}_{nar}$ represents the aqueous mole fraction of the alcohols necessary for narcosis (conduction block). The curve $X^{C_6H_6}_{nar}$ is the computed mole fraction of alcohol in benzene for each aqueous mole fraction. The line X_{nar} represents the assumed threshold mole fraction required for narcosis and is drawn constant to butanol, and then rises. Methanol and ethanol have non-aqueous mole fractions below those necessary for narcosis but both alcohols have aqueous mole fractions well above the non-aqueous.

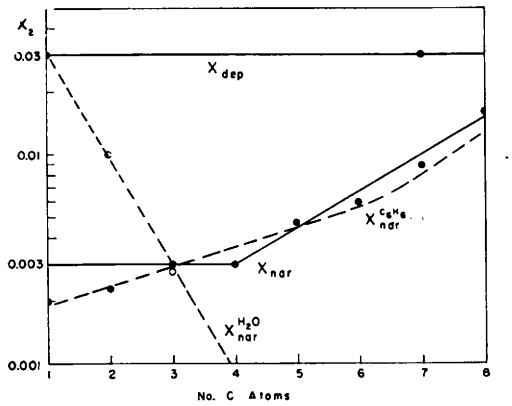

FIG. 5. Dotted line $X^{H_2O}_{nar}$ represents aqueous concentrations (as mole fraction) necessary for narcosis of frog sciatic nerve with various n-alcohols shown as abscissae. Dotted line $X^{C_6H_6}_{nar}$ represents computed mole fractions in benzene that give narcosis. Data are from Laget *et al.*(loc. cit.). Solid line X_{nar} represents assumed mole fraction required for narcosis. Initial value is 0.003 and is constant through n-butanol. This places the points for methanol and ethanol below the necessary mole fraction for narcosis. Line X_{dep} represents mole fraction necessary for depolarization of a certain degree. The point for methanol (open circles indicate aqueous mole fractions, solid circles benzene mole fractions) and the point for heptanol are experimentally determined. We are unable to compare the methanol with heptanol depolarizations directly, but curve is drawn parallel to abscissa on the assumption that tendency to accumulate at aqueous:non-aqueous interface is a constant for all alcohols. Other X_{dep} curves could be drawn, parallel and below the 0.03 mole fraction curve, these would represent lesser degrees of depolarization.

The data of Wilbrandt(14) on the effects of the dialkylamines on nerve depolarization suggest that surface activity of a compound is a factor of importance since dimethyl amine is without surface activity and does not depolarize while diamyl amine is both surface active and is equivalent to K^+ in depolarizing power. Agents which are highly surface active such as the salts of fatty acids also depolarize (13) and unmyelinated fibers appear to be more easily affected than do myelinated fibers. The data in the literature, and our present observations are not inconsistent with the view that the depolarization of nerve fibers can be produced in three, experimentally distinct, ways: (a) by anoxia, or metabolic poisons which, by interfering with sodium pumping, produce a slow depolarization; (b) KCl, by abolishing the K^+ gradient across the membrane; (c) by surface active agents which act, presumably, by disorganizing the membrane.

Summary. Depolarization produced by a variety of organic reagents acting on frog sciatic nerve has been measured. With the alcohols, the fact that the lower alcohols depolarize before blocking nerve, whereas higher

alcohols block conduction before depolarizing, is referable to the abnormally high activity coefficients of methanol and ethanol in non-aqueous solvents, and hence to the high aqueous mole fractions necessary for narcosis. While the alcohols depolarize at aqueous activities of about 0.15-0.30, other organic substances, of a relatively non-polar nature, depolarize only at activities of about 0.8. Such substances show a surface tension depression similar to that of the alcohols at these elevated thermodynamic activities. The distinction is made between narcosis, which requires a given mole fraction of a substance in the non-aqueous phase of the fiber, and depolarization which requires a given depression of the interfacial tension.

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Influence of Protein Intake on Magnesium Requirement during Protein Synthesis.* (20813)

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The deleterious effect of magnesium deficiency on protein synthesis was reported by Menaker and Kleiner(1). At a 9% level of protein intake (150 to 160 mg N daily), their rats gained about 40% more in 10 days on a complete ration than on a magnesium-deficient one (30.5 vs 21 g) with virtually isocaloric intake. They concluded that magnesium deficiency is approximately as deleterious to protein synthesis as is potassium deficiency, since Cannon *et al.*(2) had found that rats, on complete rations of 216 mg N daily, gain about 35% more in 10 days (about 46 g vs 34 g) than on an essentially isocaloric potassium-deficient ration. Frost and Sandy (3) recently reported that on 120 mg N daily, rats gain virtually as much on a Mg-deficient as on a complete ration the first few weeks, but in 11 weeks rats on a complete ration gain 25 to 30% more weight (about 90 g vs 70 g) than those on a Mg-deficient one. At this low level of N-intake, their rats on a complete ration gain less in 10 days than Menaker's and Kleiner's rats on a Mg-deficient ration of 150-160 mg N daily. An analysis of these data led the author to test whether the Mg requirement is influenced by the level of protein intake.

Method. 24 female Wistar albino rats, depleted 20-25% below initial 250 g (approx.), were divided into 4 groups. Group 1 received 7 g casein per 100 g basal ration plus complete mineral supplement. Group 2 received same basal ration (7% protein) but Mg-deficient supplement. Group 3 received 14 g casein per 100 g basal ration (14% protein) plus complete mineral supplement. Group 4 received this basal ration (14% protein) but

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