nephrine. This metabolite occurred in very much smaller concentration than 3-methoxy-4-hydroxymandelic acid and has the following R_f values: isopropyl alcohol ammonia, 0.22; benzene propionic acid, 0.12. Authentic dihydroxymandelic acid (*3*) has R_f values of 0.25 and 0.19 in the afore-mentioned solvent systems. This radioactive metabolite, occurring in trace quantities, is tentatively considered to be 3,4-dihydroxymandelic acid.

The results of these experiments (*7*) clearly indicate that iproniazid treatment in man inhibits the action of monamine oxidase, but does not influence those enzymes which are responsible for the O-methylation of epinephrine.

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Electrical Activity of Isolated Single Electroplax of Electric Eel as Affected by Temperature

In the last decade it has been shown that the permeability characteristics of the nerve membrane change during activity; the resistance decreases, and the electric currents propagating nerve impulses are carried by movements of Na^+ and K^+. Whereas there is little disagreement about this aspect, there are strongly opposing views about the mechanism by which these ion movements are controlled. Nachmansohn has persistently maintained the view that chemical processes must control this permeability change, and he and his associates have accumulated evidence that the acetylcholine system is inseparably associated with the elementary processes of nerve function—that is, the generation of bioelectric potentials (*1*). Support in favor of his views is the recent demonstration that lipid-soluble analogs of acetylcholine produce a depolarization of the active membrane (*2*).

On the other hand, purely physical processes are assumed by many leading physiologists to be responsible for the action potential; chemical reactions are considered to provide only the energy for restoring the ionic concentration gradients in the recovery period (*3*). The small initial heat production has been attributed to the mixing of Na^+ and K^+. Only a few measurements of temperature coefficients have been reported in the world literature (for reference, see *4*).

In view of the general interest in the problem whether or not the generation of bioelectric potentials requires chemical processes, and in view of the scarcity of data on temperature coefficients of conduction, we have evaluated the Q_{10} and the energy of activation over a wide range of temperatures on a recently developed preparation, the isolated single electroplax of the electric organ of *Electrophorus electricus* (*5, 6*). These organs are the most powerful electric generators created by nature, and they are highly specialized in their function; moreover, the preparation offers a favorable material for these studies. The duration of (i) the action potential, (ii) the latency period, and (iii) the postsynaptic potential has been studied as a function of temperature.

The duration of all three phenomena decreases with rise of temperature, whereas the amplitude of the spike and the postsynaptic potential remain unchanged (Figs. 1 and 2). Since there is a marked transitory change of permeability (*7*) during the action potential, the duration of the spike is a good measure of this change and pertinent for the question whether or not chemical reactions are involved in the process. If the logarithm of the reciprocal of the half-width of the spike is plotted against the reciprocal of the temperature according to Arrhenius, a straight line is obtained. This enables us to assign the energy of activation to the rate-controlling step in these processes.

The action potential elicited with direct stimulation has been studied at temperatures between 9° and 39°C. The Q_{10} has been found to be around 3.6, the energy of activation to be 21.000 cal/mole. The Q_{10}'s of the latency period and of the postsynaptic potential are very close to 2.6, and the energy of activation is around 16.000 cal/mole. An interesting observation in these experiments is the fact that it is impossible to elicit a postsynaptic potential and an indirect spike at temperatures above 32°C. This may indicate that the nerve action potential must have a certain duration above a critical level in order to be able to transmit the message across the synapse. The data support the conclusion that the three phenomena are dependent on chemical reactions. This conclusion is consistent with A. V. Hill's recent observation on the initial heat in nerve fibers (*8*). The latency period is frequently considered to be the result, partly at least, of the diffusion of a chemical transmitter from the tip of the axon to the postsynaptic membrane. Diffusion cannot have a Q_{10} of much greater than 1. Therefore, the high Q_{10} indicates that, if a diffusion process occurs, it is not the rate-limiting factor, but that chemical processes are responsible for the synaptic delay.

The Q_{10} found in the electroplax for the action potential is very close to that found in other conducting tissues. From the results published by Nastuk and Hodgkin (*9*), it is possible to calculate the Q_{10} for the duration of the action potential in the frog sartorius; its value is about 3.

The generation of bioelectric currents, the primary event in nerve conduction, is the only manifestation of living cells for which at present a purely physical

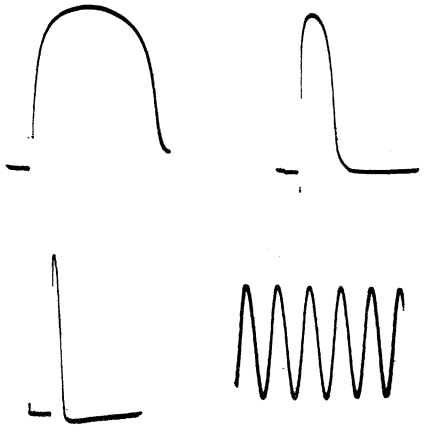

Fig. 1. Spike elicited by direct stimulation recorded with extracellular electrodes from a single isolated electroplax (*Electrophorus electricus*) at various temperatures. From upper left to lower right: 12°; 24°; 39°C; calibration, 50 mv, 2 msec.

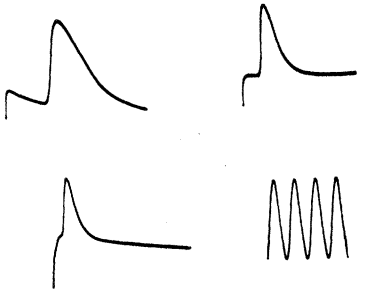

Fig. 2. Postsynaptic potential recorded with extracellular electrodes from a single isolated electroplax (*Electrophorus electricus*) at various temperatures. From upper left to lower right: 15°; 25°; 32°C; calibration, 5 mv, 1 msec.

process is offered as explanation and strongly supported by leading biologists. I consider the high values of the energy of activation reported here as incompatible with this view and as a support for those theories which postulate chemical processes as being responsible for the specific changes in permeability of conducting membranes during activity.

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Circulating Antibody Directed against Penicillin

Detection, by in vitro serological techniques, of circulating antibodies directed against penicillin has not been reported. In the past few months, however, sera from certain individuals have been encountered which appear to react specifically against penicillin. It is the purpose of this report to describe the system in which this reaction is demonstrable and to report studies on the characteristics and specificity of the antibody.

Addition of penicillin to erythrocyte suspensions is frequently a routine procedure in the preparation and preservation of red cells used in specificity panels in blood-bank laboratories. In August 1957, during routine testing, the serum of a prospective transfusion recipient was found to agglutinate all of such a panel of erythrocytes prepared with penicillin; if the same erythrocytes were not exposed to penicillin, this serum caused no agglutination. Furthermore, the exposure of the patient's own erythrocytes to penicillin made them agglutinable by his own serum.

On further study it was found that human erythrocytes of all blood groups, by exposure to appropriate concentrations of penicillin G, O, or K, could be sensitized to react with this serum, and with sera of similar characteristics later discovered in other individuals. Once the erythrocytes were sensitized, they remained sensitized as long as they remained useful for testing—that is, until they began to show marked hemolysis—usually after at least 2 or 3 weeks. The sensitization was not affected by additional exposure of the sensitized cells to penicillinase for periods up to 4 hours, nor was it altered by exposure of the cells to 0.5-percent papain or 0.1-percent ficin.

Thus far, no human red cells have been shown to resist this "penicillinization."

Substitution of penicillinase, papain, or ficin for penicillin in the sensitization procedure gave negative results. Furthermore, in an attempt to see whether other antibiotics would sensitize erythrocytes for this reaction, approximately equal weights (about 10 mg) of the following antibiotics (*1*) were each dissolved in 1 ml of phosphate buffer (final *p*H 7.2 to 7.4) and then incubated wtih erythrocyte suspensions: streptomycin, dihydrostreptomycin, polymyxin B, bacitracin, neomycin B, ristocetin, viomycin, oleandomycin, synnematin B, and the penicillins G, O, and K. The only preparations which sensitized the red cells to react with the particular sera were the penicillins G, O, and K and synnematin B, which is another penicillin derivative.

Penicillin which had been inactivated by the addition of penicillinase (*2*) was no longer able to produce sensitization of erythrocytes.

Studies on the effects of varying the time of the exposure of the erythrocytes to varying concentrations of penicillin G were performed. It was found that the degree of sensitization of the red cells, as measured by their agglutinability by weakly reacting sera, varied directly with the time of exposure of the cells to penicillin and with the concentration of penicillin in the incubation mixture. For example, a 25-percent suspension of erythrocytes could be sensitized to approximately the same degree either by incubation for 24 hours with a concentration of penicillin of 3000 units/ml or by incubation for 10 minutes with a concentration of penicillin of 50,000 units/ml. Concentrations of penicillin of less than 3000 units/ml produced weak and irregular sensitization. Incubation times of more than 24 hours enhanced the sensitization to a slight or negligible degree. As a matter of convenience, therefore, the usual method of preparing "penicillinized" cells for the study of reactive sera has been to add about 8 ml of an equal-part mixture of whole blood and Alsever's solution directly to a vial containing 200,000 units of powdered penicillin G. After incubation at 37°C for 1 hour, an aliquot is withdrawn from the vial, the erythrocytes are thrice washed with isotonic saline and made up to a 4- to 10-percent suspension in saline. Since the sensitization proceeds at all temperatures from 6° to 37°C, the temperature of exposure does not seem to be critical.

Table 1. Inhibition of hemagglutination by prior addition of penicillin to reactive serum. (i) Penicillin + reactive serum = mixture; (ii) mixture + sensitized red blood cells → agglutination.

Concn. of penicillin added to reactive serum (units/ml)	Agglutination
0	2+
100	2+
370	2+
750	2+
1,500	+
3,000	±
6,000	±
12,000	0
25,000	0
50,000	0
100,000	0
200,000	0

Certain sera can be shown to react with erythrocytes prepared in such a fashion. With some sera the reaction can be demonstrated only by the antiglobulin technique. More strongly reacting sera, however, may agglutinate the sensitized erythrocytes directly from a saline suspension in a test tube, or even on a slide.

Sensitized cells exposed to these sera have been heated for 15 minutes at 54°C in saline to elute the antibody. The consequent eluate was demonstrated to react with other penicillinized erythrocytes.

This antibody is stable for at least several weeks at ordinary refrigeration temperatures and resists degradation by a temperature of 56°C for 2 hours.

Additional demonstration of the specificity of these sera was obtained by inhibition tests. In these, an attempt was made to see whether prior incubation of a reactive serum with penicillin would so bind the presumed antibody that the serum would no longer react with sensitized erythrocytes.

Solutions of penicillin G were made up in AB serum in concentrations from 100 to 200,000 units/ml. Equal volumes of each penicillin solution were added to