and to slow the rate by dilution of the enzymes. The results are presented in Fig. 3 and show that inclusion of pancreatic inhibitor decreased the rate of insulin destruction.

The hypothesis that trypsin inhibitor is of physiological significance in facilitating the intestinal absorption of proteins (insulin) has been confirmed by a direct experiment (*13*).

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11 December 1957

Iproniazid Treatment and Metabolism of Labeled Epinephrine in Schizophrenics

Previous work in this laboratory (*1, 2*) showed that when epinephrine labeled with carbon-14 in the beta position was infused into schizophrenic patients and normal subjects, essentially all of the radioactivity was recovered in the urine. When epinephrine labeled with carbon-14 in the methyl group of the side chain was infused, approximately one-third of the radioactivity was recovered in the urine. In both cases, the excretion of biologically active material represented only 1 to 5 percent of the infused epinephrine. A total of 14 infusions were performed. The urine of patients infused with beta-labeled epinephrine was selectively extracted and subjected to paper chromatographic analysis. A major radioactive metabolite was obtained, which possessed the solubility properties of a phenolic acid and had the same R_f values as authentic 3-methoxy-4-hydroxymandelic acid (*3–5*). This radioactive metabolite could not be demonstrated in the urine of patients infused with epinephrine labeled with carbon-14 in the methyl group of the side chain (*2*).

These data suggest the following hypotheses concerning the metabolic transformations of epinephrine: (i) The beta carbon atom remains attached to the benzene ring, and (ii) approximately two-thirds of the molecules of epinephrine lose the methyl group of the side chain. If one assumes that the methyl group of the side chain is lost, together with the amino group, under the influence of amine oxidase, then iproniazid treatment should result in more molecules of epinephrine retaining their methyl groups in the side chain. If this is the case, then more radioactivity should be recovered in the urine of patients receiving iproniazid and infused with methyl-labeled epinephrine.

Three female, chronic schizophrenic patients were placed on iproniazid, 100 mg/day, on 20 June 1957. The dosage was increased to 150 mg/day on 12 August. The first patient was infused with 0.5 mg of methyl-labeled *dl*-epinephrine on 3 September, the second on 18 September, and the third on 9 October. Fifty-nine, 74, and 63 percent of the infused radioactivity was recovered in the urine of these three patients, respectively. This is in contrast to 34 ± 3 percent recovered in the urine of four non-iproniazid-treated schizophrenic patients infused with the same amount of methyl-labeled *dl*-epinephrine. Both types of patients demonstrated typical cardiovascular responses to the infused epinephrine.

Two to 3 weeks after the cessation of iproniazid treatment, the first and second patients were again infused with methyl-labeled epinephrine. Fifty and 43 percent of the infused radioactivity was recovered in the urine of these two patients, respectively. This indicates that approximately half of the effect of iproniazid on monamine oxidase activity, as reflected by the metabolism of exogenously administered epinephrine, was still evident 2 to 3 weeks after the cessation of iproniazid therapy. Thus, approximately twice as many molecules of infused epinephrine retain the methyl group of the side chain when the patient is under iproniazid treatment in the dosages mentioned above as when he is not. These three patients varied in their psychiatric responses to iproniazid therapy. Nevertheless, all three patients showed a remarkably similar alteration in the metabolism of exogenously administered epinephrine.

The question then arose whether the increase in number of molecules retaining the methyl group following iproniazid treatment represents nondegraded, biologically active epinephrine or a stage in metabolism prior to amine oxidase action. Recently, Axelrod (*6*) reported the presence of methoxyepinephrine in the urine of rats, which was found in a greater amount following the intraperitoneal administration of iproniazid and epinephrine.

The following experiments were performed in our laboratory. The urine from patients was collected following the infusion of either beta-labeled or methyl-labeled epinephrine. The urine samples were lyophilized and stored at 0 to 5°C. The lyophilized urine was reconstituted with water and extracted for phenolic acids, according to the procedure of Armstrong *et al.* (*4*). The extracts were concentrated down to a small volume, *in vacuo*, at 45°C. An aliquot of the concentrated extract was chromatographed in the butanol : acetic acid : water system (4:1:5). Another aliquot was chromatographed in the two-phase solvent systems of Armstrong *et al.* (*4*). The phenolic acids were visualized by spraying with diazotized *p*-nitroaniline reagent. Autoradiograms were made from the chromatograms, in order to visualize these metabolites, which were derived from the infused labeled epinephrine. The urine which had been extracted for phenolic acids was hydrolyzed and selectively extracted for methoxyepinephrine in accordance with the procedures outlined by Axelrod (*6*). The extracts were concentrated down to a small volume, *in vacuo*, at 45°C and subjected to paper chromatographic analysis, as outlined above.

The following results were obtained. The urine of non-iproniazid-treated patients infused with beta-labeled epinephrine consistently showed a major radioactive metabolite, which was a phenolic acid having the same R_f value as authentic 3-methoxy-4-hydroxymandelic acid. Very little methoxyepinephrine could be extracted from the urine of these patients. The urine of iproniazid-treated patients infused with methyl-labeled epinephrine consistently showed a major radioactive metabolite, which was a phenolic amine having the same R_f value as authentic methoxyepinephrine (*6*). The increase in excretion of radioactivity by the ipronized-treated patients infused with methyl-labeled epinephrine could be accounted for by the accumulation of methoxyepinephrine with a decrease in formation of 3-methoxy-4-hydroxymandelic acid.

The autoradiograms of urine obtained from patients infused with beta-labeled epinephrine showed the presence of another phenolic acid metabolite of epi-

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

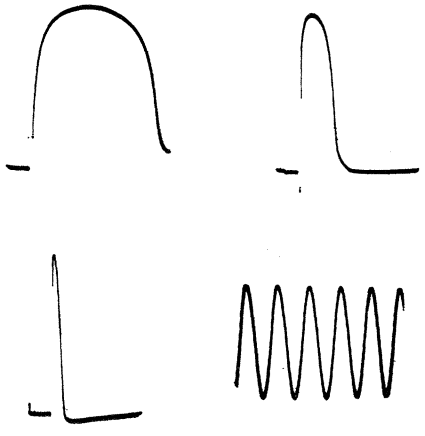

Fig. 1. Spike elicited by direct stimulation recorded with extracellular electrodes from a single isolated electroplax (*Electrophorus electricus*) at various temperatures. From upper left to lower right: 12°; 24°; 39°C; calibration, 50 mv, 2 msec.

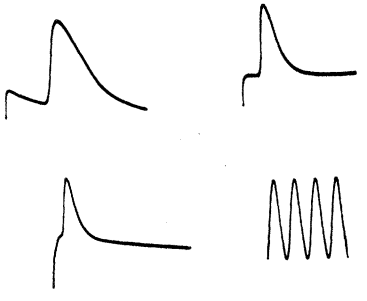

Fig. 2. Postsynaptic potential recorded with extracellular electrodes from a single isolated electroplax (*Electrophorus electricus*) at various temperatures. From upper left to lower right: 15°; 25°; 32°C; calibration, 5 mv, 1 msec.