results of treatment with acetylcholine. A concentration of $10^{-6}M$ stopped the beating completely for 4 minutes. Upon recovery the beating increased about 30 percent over the original rate, then dropped to the original rate. Further addition of acetylcholine eventually led to a marked decrease in the rate, followed by recovery.

In Fig. 2 it is seen that eserine had no effect on a cell which beat at a rate of 50 times per minute. Addition of acetylcholine lowered the beat to a rate of 8 per minute. This rate was maintained until ouabain was added, at which time the rate was raised to about 30 per minute.

The recovery of the noneserinized cells after inhibition with low levels of acetylcholine may be a result of the release of inhibition by the action of cholinesterase. Thus the inhibition of the recovery by eserine could be due to the inhibition of cholinesterase which might not now release the inhibition by acetylcholine.

Several attempts are being made to determine the energy source in the heart cell, necessary for beating. Dinitrophenol (DNP), known to uncouple oxidative phosphorylation (*3*), was added to the medium at various concentrations. At a level of $5 \times 10^{-5}M$, after a short transient stimulation, the rate fell from 90 to 10 beats per minute. Since DNP is thought to act specifically by uncoupling phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP), the latter compound was added to the inhibited system. ATP at a concentration as low as $5 \times 10^{-8}M$ restores the rate to 30 to 40 beats per minute. At $5 \times 10^{-6}M$, ATP shows the greatest effect, increasing the beats to 100 to 150 per minute.

The effect of increasing concentrations of ATP is shown in Fig. 3. Not till $5 \times 10^{-6}M$ was reached was any effect noticed. The rate jumped from 60 up to 240 beats per minute, and this was followed by an inhibitory phase and then recovery to the initial rate. Similar experiments with ADP and adenosine monophosphate (AMP) at the same concentrations showed no effect. However, when the concentration reached $10^{-4}M$, ATP, ADP, and AMP all inhibited completely.

The effect of other metabolic inhibitors was studied. Monofluoracetate at $10^{-6}M$ inhibited completely, which indicated the importance of the tricarboxylic acid cycle (*4*) for the periodic contractions. Iodoacetamide at $10^{-3}M$ also inhibited the beating. This high concentration may affect the cell by binding sulfhydryl enzymes other than triosephosphate dehydrogenase. 2-Desoxy-D-glucose at $10^{-3}M$, which has been reported to inhibit glycolysis (*5*), had no effect. However, in the presence of the large amount of glucose present in the media, this concentration may not have been high enough to compete successfully.

These single beating cells isolated from rat heart may provide a unique system for the study of the requirements of the periodic contractility typical of mammalian hearts. Particularly, they may provide a means of determining the contribution of various metabolic pathways for the process, and for determining its nutritional requirements.

ISAAC HARARY
BARBARA FARLEY
Department of Nuclear Medicine and Radiation Biology and *Department of Physiological Chemistry, University of California, Los Angeles*

References and Notes

1. T. T. Puck, P. I. Marcus, S. J. Cieciura, *J. Exptl. Med.* **103**, 273 (1956).
2. P. I. Marcus, S. J. Cieciura, T. T. Puck, *ibid.* **104**, 615 (1956).
3. W. F. Loomis and F. Lipmann, *J. Biol. Chem.* **173**, 807 (1948).
4. R. A. Peters, *Biochem. J.* **50**, xiii (1952).
5. A. N. Wick, D. R. Drury, H. I. Nakada, J. B. Wolfe, *J. Biol. Chem.* **224**, 963 (1957).
6. This work was supported in part by research grant A-2135 from the National Institutes of Health, U. S. Public Health Service, and under contract No. AT-04-1-GEN-12 between the U. S. Atomic Energy Commission and the University of California at Los Angeles.

25 January 1960.

A Study of Thermoregulatory and Emotional Sweating in Man by Skin Ion Transfer

Abstract. Local introduction of atropine and dibenzyline into human skin was carried out by iontophoresis. Both thermoregulatory and emotional sweating were blocked by atropine but were not blocked by dibenzyline. It would seem that emotional sweating produced as a result of a physical stress situation is partly or predominately under cholinergic control.

It has been established that thermoregulatory sweating is mediated through cholinergic response of the sweat glands (*1*). Sweating can be produced by the systemic administration of cholinergic drugs and can be inhibited by the systemic administration of the belladonna alkaloids, with a significant rise in body temperature in man. Little is known, however, of the sweat glandular response to emotional stress. It has been a clinical observation, since the introduction of the adrenergic blocking agents, that the systemic administration of these agents will suppress the hyperhydrosis of the hands and feet of emotionally labile people.

The term "adrenergic blocking agents" designates those compounds which selectively inhibit the responses of effector cells to adrenergic sympathetic nerve impulses, and to epinephrine and related amines. The locus of action of blocking agents of this type is on effector cells, and is selectively distinguished from that of substances which prevent sympathoadrenal discharge by blocking nerve impulse transmission in autonomic ganglia, along peripheral nerves, or within the cerebrospinal axis.

Whether it is the adrenergic rather than the cholinergic response of the sweat glands which produces emotional sweating has not yet been established.

Introduction of atropine and dibenzyline into human skin was carried out by iontophoresis (*2*) in order to affect the sweat glands situated in the skin over a localized area. An aqueous solution of 0.25-percent atropine sulfate and 0.1-percent dibenzyline hydrochloride in 20-percent propylene glycol was freshly prepared each test day. Water was added to the solvent to facilitate ionization of the material. The positive electrode was used in each case, 10 ma for 20 minutes for atropine and 10 ma for 40 minutes for dibenzyline, over an area of 30 to 40 cm^2 of body surface. The much longer time used for the introduction of dibenzyline was found to be necessary in order to assure proper introduction. Testing was done 1 hour after introduction of atropine and 3 hours after introduction of dibenzyline.

Proof of introduction into the skin of dibenzyline was established by the intradermal injection of 0.1 ml of 1:1,000,000 epinephrine hydrochloride into the areas of iontophoresis and demonstration that the local skin blanching (*3*) at the site of the injection was not present over the dibenzyline treated areas, as opposed to the nontreated opposite part serving as a control. It was not deemed necessary to do similar testing with atropine, since a systemic reaction to atropine was observed in two patients, which in itself served as proof of introduction. No such systemic reaction was observed with dibenzyline. Electrophoresis of propylene glycol without the addition of dibenzyline was also carried out to be sure that the solvent had no blocking effects. None was observed. Sweat patterns were identified by the application of the established iodine-starch method. Five patients were tested, two males and three females.

In the thermoregulatory sweating trials both atropine and dibenzyline were alternately introduced into the volar surfaces of the forearms. The iodine-starch technique was applied to the areas treated. The patient was then covered with blankets and allowed to remain in an extremely warm room for

30 minutes until sweating was pronounced.

In the emotional sweating trials both atropine and dibenzyline were alternately introduced into the palms. The iodine-starch technique was applied to the areas treated. The patient was put in an air-conditioned, cool room for 30 minutes as a control period to be sure that no thermoregulatory sweating took place. A painful stimulus was then applied, either by performing a clumsy venipuncture or manipulating a body part until obvious pain and sweating was produced.

The following observations resulted from the above trials: Well delineated sweat patterns were observed on the areas tested after overheating and after stress. In all instances, both thermoregulatory and emotional sweating were blocked by the local introduction of atropine into the skin. In no instance was either thermoregulatory or emotional sweating inhibited by the local introduction of dibenzyline into the skin.

The sweat glands are anatomically under control of the sympathetic nervous system, but their function is modified by drugs which act on parasympathetically innervated effector cells. This was clarified by Dale and Feldberg (*4*) in 1934 when it was discovered that nerve impulses which cause sweating release acetylcholine at the neuroglandular junction. Although the nerve fibers involved traverse sympathetic pathways, they are functionally analagous to parasympathetic nerves. In man, most sweat glands are probably innervated by fibers which function via acetylcholine as the mediator, but there is indirect evidence that the sweat glands in certain areas may be supplied by fibers which release the sympathetic mediator at the periphery (*5*). Haimovici (*6*), in 1950, pointed out that sweating in man can be elicited by adrenergic agents and can be inhibited by an adrenergic blocking agent. He concluded that in addition to the known cholinergic fiber supplying the sweat glands, there is also an adrenergic component in the nervous mechanism of sweating in man.

Accordingly, there can be little doubt that there is both cholinergic and adrenergic response of the sweat glands under certain circumstances, but under the conditions existing during my experiments it seems that both thermoregulatory and emotional sweating remains under cholinergic control. There may be a quantitative difference in the type and amount of sweating, depending on the emotional state of the individual. Perhaps during a prolonged emotional stress period the adrenergic mechanism becomes more prominent, and this may account for the clinical observation that adrenergic blocking agents will control sweating to some degree (*6*).

Dibenzyline is thought to act as a blockade to one of the steps in the process of excitation by adrenergic agents. This blockade is interposed between the penetration of the cells of the effector organ by norepinephrine and secretion of the effector organ. If adrenergic sweating does in fact exist, perhaps the site of action or the mechanism of excitation at the effector organ is not as previously thought. Perhaps the site of action of systemic dibenzyline is different than that produced by local iontophoresis. More definitive work is necessary to further clarify this mechanism.

VICTOR CUMMINGS
Montefiore Hospital, New York, New York

References

1. L. S. Goodman and A. Gilman, *Pharmacological Basis of Therapeutics* (Macmillan, New York, ed. 2, 1955).
2. K. Harpuder, *Occupational Therapy and Rehabil.* **27**, 449 (1948).
3. A. C. Yard, H. F. Hardman, M. Nickerson, *J. Pharmacol. Exptl. Therap.* **106**, 427 (1952).
4. H. H. Dale and W. Feldberg, *J. Physiol. London* **82**, 121 (1934).
5. R. R. Sonnenschein, *Proc. Soc. Exptl. Biol. Med.* **71**, 654 (1949).
6. H. Haimovici, *J. Appl. Physiol.* **2**, 7 (1950).

4 February 1960

Movement of Radiosodium in a Chemically Stratified Lake

Abstract. The rapid horizontal dispersal of sodium-24 at an average rate of about 18 m/day has been observed near the bottom of a small thermally and chemically stratified lake. However, no appreciable vertical movement of the radioactivity was observed during a period of 6 days.

The discovery of a meromictic lake (Stewart's Dark Lake) in northwestern Wisconsin (T.33N., R.9W.) has provided us with the opportunity to study the limnological characteristics of meromixis. Moreover, we envisaged the use of this lake for pilot studies in aquatic science pertaining especially to problems of radioactive waste disposal.

Meromixis occurs in a lake in which the dissolved substances create a gradient of density differences, in depth, preventing complete mixing or circulation.

This type of lake is normally stratified in three arbitrary zones. The upper, freely circulating layer of water is termed the mixolimnion (*1*). The bottom, relatively very dense, noncirculating stratum is the monimolimnion (*2*) and the transition zone between, the chemocline.

Most temperate-zone lakes are stratified throughout the summer and winter because of density differences owing to a temperature gradient. However, during the spring and autumn when temperatures become homoiothermal from top to bottom, relatively low wind velocities can cause these lakes to "turn over" or circulate completely. Meromictic lakes may have a thermal stratification superimposed upon the chemical stratification. Nevertheless, it is the solute concentration that maintains the stability which persists from year to year, thereby inhibiting the intermingling of the monimolimnetic waters with the above water.

Stewart's Dark Lake is a bog lake with an area of approximately 2 acres. The maximum depth is 8.8 m, the average depth 4.3 m. The lake is maintained by seepage, for there is no inlet or outlet. Colloidal (humic) materials arise from decaying vegetation and "stain" the water a dark brown. The lake represents a highly restrictive habitat for organisms because the monimolimnion never contains measurable amounts of dissolved oxygen. This condition becomes extreme during the winter when the entire lake is characterized by the absence of dissolved oxygen for 2 months or more. In addition, the lower strata contain relatively high concentrations of sulfides.

From the limnological data obtained on the meromictic nature of this lake during the past 2 years, it was observed that under optimum conditions for "overturn," complete circulation extended only to the 6-m level. Thus the monimolimnion, by definition, exists continuously in at least the bottom 2.5 m of the lake. This represents 12.7 percent of the total volume of the lake. Carbonate levels as high as 96 mg/liter persist in the deep water as compared with a concentration of 5 mg/liter at the surface.

In order to obtain information concerning the extent to which this supposedly "stagnant" zone is isolated from the remainder of the lake, we used a radioactive tracer. On 1 July 1959 approximately 47 mc of sodium-24 in the form of NaCl in HCl solution were released within the lake at the 8-m level by smashing an 800-ml museum jar containing the radioactive solution.

Sampling was done along six transect lines radiating from above the release point. These were lines of polyethylene "floating" rope which spanned the lake.

A 2-inch sodium iodide crystal scintillation detector, enclosed in a watertight