tion, node 13 for the second, node 20 for the third, and node 27 for the fourth. Intervening nodes bore pistillate flowers and all treated plants reverted abruptly to gynoecious habit. The 118 plants grown in this isolation cage produced 4.2 lbs of seed, demonstrating that ample pollen was provided by two, three, or four weekly foliar spray applications of gibberellin at 1500 parts per million.

In a genetic analysis of sex expression there are obvious advantages in being able to self gynoecious plants in segregating generations and to establish homozygosity for gynoecious habit. In established gynoecious inbreds there is evidence of genetic variability, in that some plants have remained gynoecious even after two applications of 2000 parts per million gibberellin (Table 1). Other gynoecious plants have produced staminate flowers following two applications of 250 parts per million. It is possible to use gibberellin as a selection device to classify plants for ease of staminate flower induction, and subsequently determine how such plants differ genetically.

Sex expression in *Cucumis sativus* can be explained on the basis of response to specific chemical substances synthesized by the plant. These syntheses are controlled by genes. Some genotypes depend upon environment for their expression while others are not sensitive to environment. It is possible that staminate flower production depends upon synthesis in the plant of a gibberellin-like growth substance, and that this synthesis does not occur in gynoecious plants. The establishment of a wide range of sex types under close genetic control should provide material for precise studies of biochemical and physiological mechanisms involved in sex determination (*10*).

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In vitro Studies of Single Isolated Beating Heart Cells

Abstract. Rat heart cells, separated by trypsin treatment and grown attached to glass in a liquid medium, exhibit periodic contractions similar to a whole beating heart. The rate of beating, which is up to 150 beats per minute, is affected by cardiac drugs and by metabolic substrates and inhibitors.

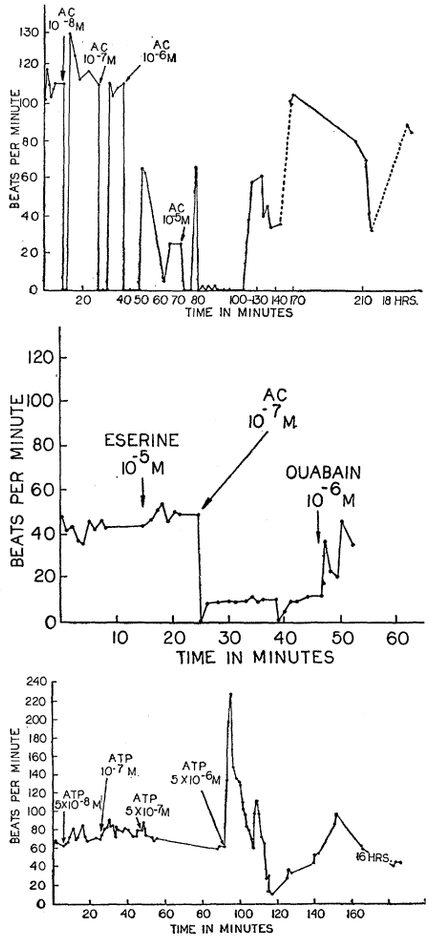
As part of a continuing study of the process of so called "dedifferentiation" in mammalian cell cultures, the cells of the rat heart were separated and grown in vitro. Upon examination of the cultures, it was seen that several of the separated cells exhibited a distinct and rhythmic contraction similar to a heart beat. Some of these cells were observed to beat for as long as 40 days.

The cultures were prepared by separating the cells of coarsely minced young rat heart by incubation with 0.10 percent trypsin for 30 to 60 minutes at 33° to 36°C (*1*). The resultant cell suspensions were centrifuged and suspended in a modified Puck's medium (*2*). The suspensions were made dilute enough so that contact between cells in the cultures was minimized. At first the cells were spherical, but within a few hours many of the cells settled to the bottom of the petri dish and became attached to the glass.

After 3 days at 37°C in an atmosphere of 5 percent CO_2 and 95 percent air, the cells appear to be either typical fibroblasts or myofibrils. Both of these types have been observed to beat. The cells are attached at first by a simple flattening at the bottom cell surface. Eventually the cells may flatten entirely to form either a symmetrical or an irregular shape. Long ameboid or thin filamentous processes increase the irregularity in the shape of the cell. The upper surfaces of the beating cells bulge upwards spherically or are stretched flat between what appear to be the strongly attached processes. It should be emphasized that the morphology of these cells varies and that no correlation can be made between their ability to contract and their anatomy. The lack of a definite histological identity, however, does not alter the fact that they are clearly functional heart cells. Most of the cells contain only one nucleus, although some multinuclei have been observed. In most cases only a small proportion of the cells have been observed to beat and the beating ranges all the way from intermittent, irregular twitches to steady, deep, rhythmic contractions at rates up to 150 per minute. Most of the cells, however, are within the range of 30 to 80 beats per minute. When two or more single beating cells have been observed in the same microscopic field they appear to beat independently.

During the first few days the increase in area covered on the glass seems to be due to the spreading out of the cells. Within a week an increase in the number of cells occurs. After three transfers, in which dilute trypsin was used to detach the cells from the glass, the suspensions were counted in a hemocytometer. An over-all fourfold increase in the number of cells has been observed. After transfer of these cells to another dish with enough dilution so that separate cells were again seen, no beating cells were found, and at no time in any of the transferred cultures were beating cells found.

The effect of certain drugs was tested on these cells to determine how the behavior of the cells compares with that of the intact heart. Figure 1 shows the



Figs. 1–3. Effect of cardiac drugs and metabolic substrates and inhibitors on rate of beating of rat heart cells. The substances were added at times shown by arrows, with final concentrations indicated. Fig. 1 (top), effect of acetylcholine (AC). Fig. 2 (middle), effect of eserine and AC. Fig. 3 (bottom), effect of adenosine triphosphate (ATP).

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.

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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