of oleic, linoleic, linolenic or arachidonic acid. No similar growth inhibitory effect by albumin has been observed in the case of tubercle bacilli.

These different phenomena are illustrated in Table I in which are compared the growths of a strain of avian tubercle bacilli and of micrococcus C in a casein hydrolysate medium to which oleic acid, stearic acid, glucose and serum albumin were added as indicated.

More extensive data concerning the effect of different fatty acids on bacteria—both in liquid and on agar media—will be presented in a forthcoming publication; it will be shown also that, at equal concentrations of long chain fatty acids, the water soluble esters are more efficient than the corresponding soaps in supporting bacterial growth.

It appears worth pointing out at this time that, under the proper cultural conditions, the amount of growth yielded by micrococcus C seems to be directly related to the amount of unsaturated fatty acids present in the medium (between 0.00001 and 0.0001%). This property suggests that the culture might lend itself to the development of a microbiological assay method for these lipids.

15492

Heart Rate of the Albino Rat.*

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Hoskins, Lee and Durrant[1] counted the heart rates of rats by stethoscope while the animals lay dozing in their cages, and they reported a basal rate of 281±18 beats per minute. Fishburne and Cunningham[2] wrapped their rats in a towel for auscultation of the heart sounds; the rates they obtained fell in the neighborhood of 300 beats per minute. Meyer and Yost[3] held their rats in the crook of the arm while palpating the apex beat, and they found the rates of adult rats to vary between 300 and 330 beats per minute.

Drury, Harris and Maudsley,[4] Robertson and Doyle,[5] and Leblond and Hoff[6] recorded electrocardiograms in rats and thus avoided subjective errors, but they did so while forcibly restraining their rats in the supine position to allow insertion of needle electrodes through the skin. Kniazuk[7] described an oscillographic method for obtaining electrocardiograms from rats placed in specially constructed cages. The method developed independently by the present author follows the same principle as that of Kniazuk but has the advantage of making inexpensive permanent records. It involves registration of the electrocardiogram from the surfaces of the rat's feet.

At first, the record was transcribed with the usual electrocardiograph, but, aside from the cost of bromide paper and the time lost in developing the film, that procedure was unsatisfactory in that it failed to tap the heart's potential through plantar callouses that the rats acquired in their mesh-floored cages.

The apparatus finally adopted was that developed for the recording of brain waves

* Publication No. 596, research series, the University of Arkansas.

1 Hoskins, R. G., Lee, M. O., and Durrant, E. P., *Am. J. Physiol.*, 1927, **82**, 621.

2 Fishburne, M., and Cunningham, B., *Endocrinology*, 1938, **22**, 122.

3 Meyer, A. E., and Yost, M., *Endocrinology*, 1939, **24**, 806.

4 Drury, A. N., Harris, L. J., and Maudsley, C., *Biochem. J.*, 1930, **24**, 1632.

5 Robertson, E. C., and Doyle, M. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 139.

6 Leblond, C. P., and Hoff, H. E., *Am. J. Physiol.*, 1944, **141**, 32.

7 Kniazuk, M., *J. Lab. Clin. Med.*, 1937, **22**, 868.

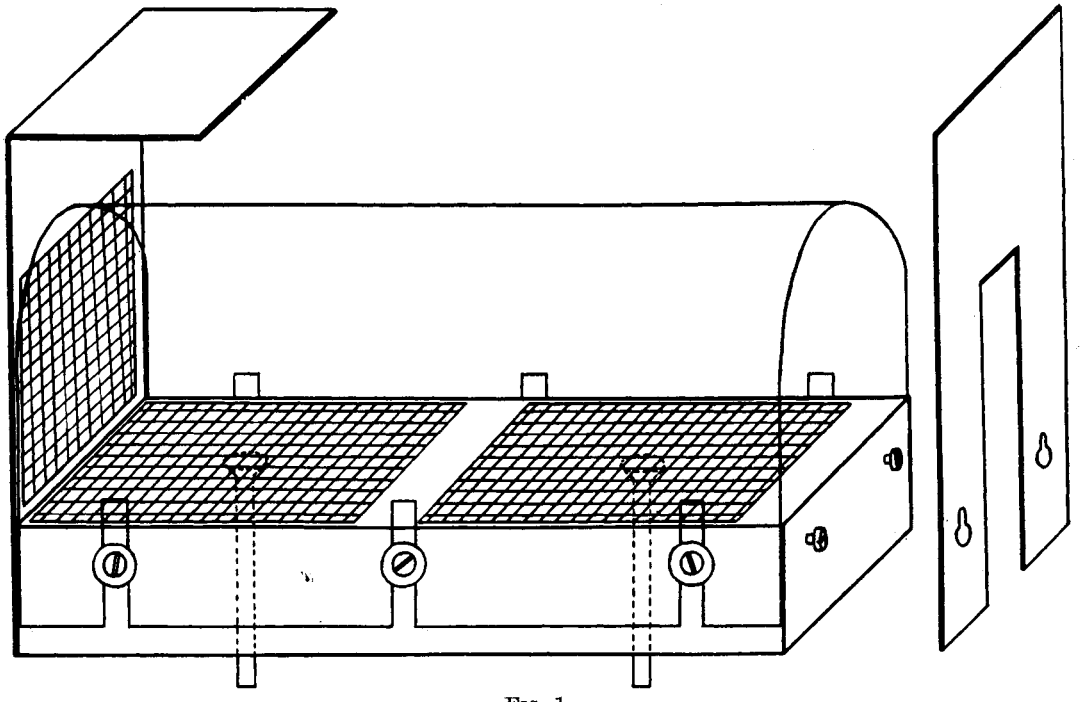

FIG. 1.
Rat holder for heart rate determination. Approximately one-fourth actual size. Description in text.

by Gerard and his associates. In this method, the impulse is picked up by contact electrodes in the form of copper floor plates, stepped up by Offner's[8] 3-stage push-pull amplifier, and inscribed on adding machine tape by the piezo-electric crystograph of Offner and Gerard.[9] Since the tape is moved by a constant speed motor, the heart rates can be measured accurately by the use of dividers.

Construction of the animal holder is shown in Fig. 1. The floor is a 1″ x 2½″ x 6″ hard rubber block into which copper plates are countersunk to act as the pick-up electrodes, one for both forefeet and the other for the hind feet. To simulate the rough floor to which rats are apparently partial, a square of copper screening is soldered to the surface of each of the copper plates. A long iron screw, soldered to each plate, passes through the hard rubber floor to dip into a mercury cup set into the shelf on which the cage rests. The potentials are led from these mercury cups into the amplifier and crystograph. Animal, cage, and wiring are shielded from 60-cycle hum by enclosure in a large copper screen chamber, appropriately grounded.

Neither electrode paste nor saline is required to facilitate transmission from the rat to the electrodes on which he is standing.

To make each cage adjustable for rats of all sizes, the sides and roof are formed by a single bent sheet of heavy celluloid, measuring 7″ x 6″. Slots cut into the celluloid sheet in place of screw holes thus make it possible to alter the volume of the cage at will.

The front gate is constructed of heavy sheet metal, 2¾″ x 6½″, with a large copper screen window for ventilation. The top of this front gate is bent to form a 2-inch projection to shade the animal's head. (This author has found no merit to the almost universal assumption that bright light induces rats to sleep; it probably irritates their eyes,

[8] Offner, F., *Rev. Sci. Instruments*, 1937, **8**, 20.
[9] Offner, F., and Gerard, R. W., *Science*, 1936, **84**, 209.

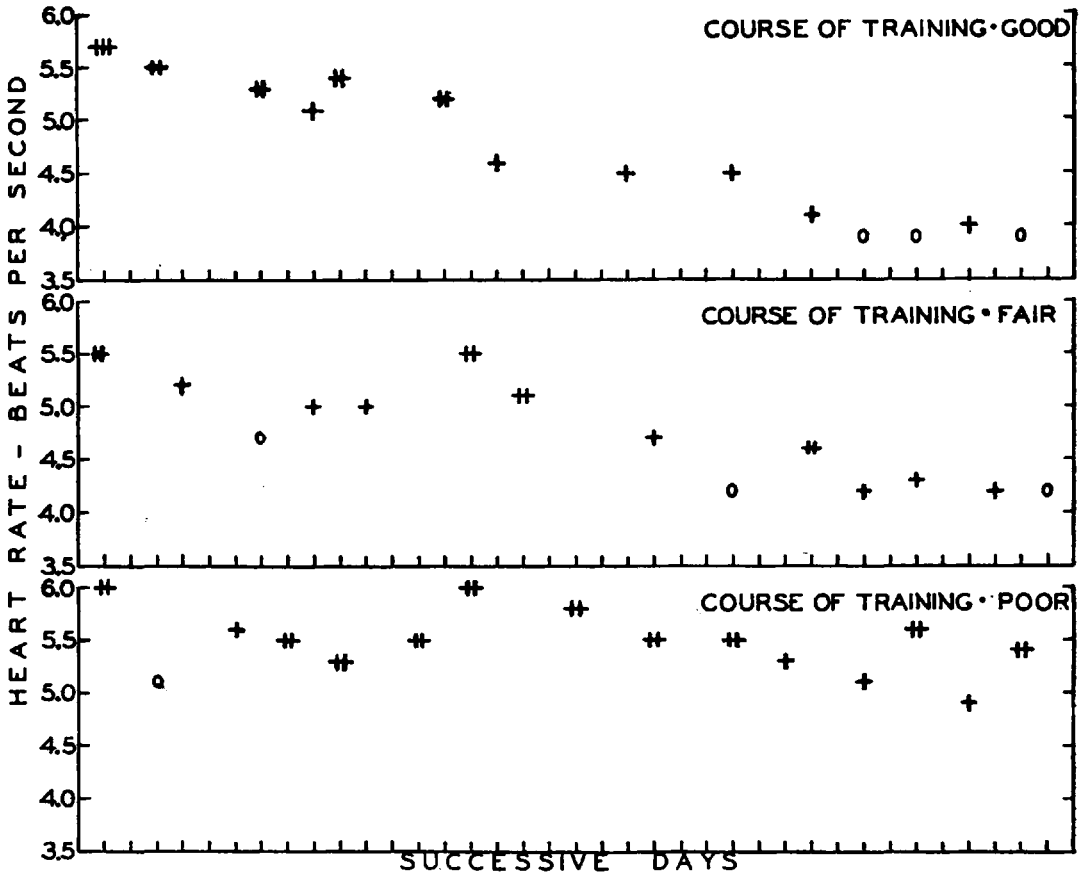

FIG. 2.

Decline in heart rate with progressive training. Evaluation of activity: ++++, extreme and prolonged; +++, intermittent but great; ++, moderate; +, occasional and mild; 0, insignificant; ∞, prolonged inactivity.

which are unprotected by pigment. Actually, it was observed that coating the shiny front screen with flat black paint aids significantly in producing relaxation).

The tail gate is also of heavy sheet metal, its dimensions 2¾″ x 4″. A large slot is cut into this gate, from below upward, to allow protrusion of the tail and testicles. A small plate is used to close this slot in those instances, early in the training period, when a rat succeeds in turning around in the cage; without such a device, he is likely to gnaw at the edge of the tail gate.

For the most part, adult male rats have been used in this study, and most of the observations regarding heart rate levels and training regimes were made upon them. The training was facilitated by use of a battery of heart rate cages, since a preliminary rest of 15-20 minutes was found necessary before the heart rate reached basal levels. Generally, the records taken for experimental purposes, after training, were made on each rat separately, involving a 10-minute run following a 10-minute rest. Later, it was found that a restless animal could be prevented from communicating his mood to a neighbor by interposing a partition between adjacent cages. With this modification, a number of rats can be rested simultaneously and then each cage switched in sequence into the circuit for recording.

The heart rate adopted from any given run is the lowest one found in that tracing. The trend of succeeding determinations is followed from the curve of daily findings; that value indicated by a leveling off of the curve is taken as the "predictable,"

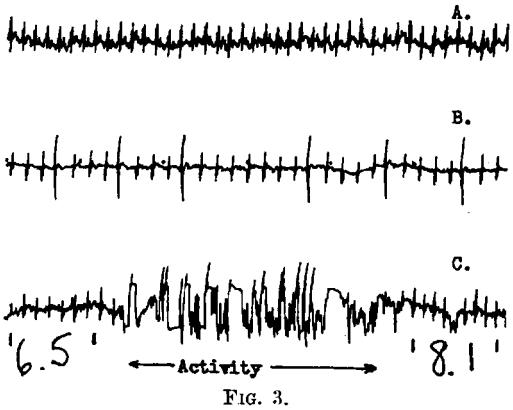

FIG. 3.
Illustrative tracings. Approximately one-half actual size.
a. Fairly complete ECG.
b. Ventricular extrasystoles (large, premature QRS complexes).
c. Effect of activity on heart rate (early phase of training).

if not always true basal, heart rate. The resultant values on 113 adult males, ranging from 200 to 370 g body weight, averaged 4.4±0.30 beats per second, or 264±18 per minute.

The attainment of a predictable heart rate level usually was found to require at least a month of training.

The course of training is fairly consistent in practically all instances, as indicated by 3 curves selected to bring out the average and the 2 extremes (Fig. 2). In general, the rats become progressively more and more accustomed to the regime, and, though only a rare individual goes to sleep in the cage, most come to accept the restriction with equanimity. The symbols used on the curves are a purely subjective evaluation of activity as determined by inspection of the tracings.

The tracings obtained do not usually constitute complete electrocardiograms. A fair proportion of them do show the customary 3 major deviations of the cardiac cycle (Fig. 3a), but most record only a triphasic QRS complex. No doubt, establishment of better contact between the feet and the electrodes would suffice in all instances to bring out the P and T waves, if desired.

Occasionally, an individual rat exhibits cardiac arrhythmia, varying from a slight irregularity to a series of extra-systoles of apparently ventricular origin (Fig. 3b). In none of these animals was there noted any other sign of impaired health.

Any bodily activity is marked by large, irregular oscillations of the crystograph pen and so is easily distinguished on the tracing. In general, the heart rate varies with the degree and violence of movement (Fig. 3c).

However, it soon became clear that physical activity is not the only factor causing variations in heart rate, nor is it the most important factor. In a large proportion of subjects, neither the "basal" rate of an individual tracing nor the rates obtained on different days jibe as completely as one might predict with the state of rest or activity (Fig. 4b). Since a spontaneous quickening of the pulse often precedes a movement and since, in other instances, activity is neither accompanied nor followed by cardio-acceleration (Fig. 4a), it seems that greater variance is induced by the state of the emotions than by that of the musculature. The relative importance of this factor is further stressed by the uniform finding that increases in heart rate with body movement are of great degree only during the early phases of the training period.

Temperature changes likewise exercise an important influence on the heart rate. Much of this study was carried out in a constant temperature room at the University of Chi-

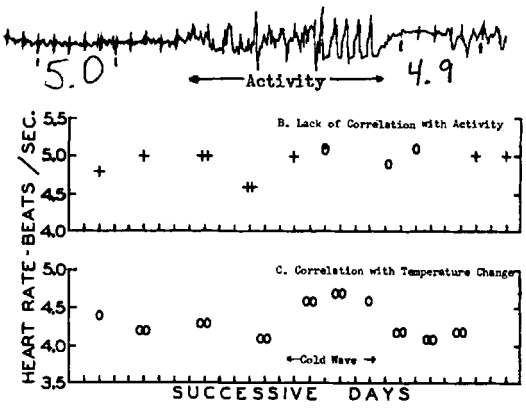

FIG. 4.
Factors altering heart rate.
a. Lack of effect of activity (late phase of training period).
b. Lack of day-to-day correlation with activity.
c. Effect of room temperature.

cago, and occasionally in wintertime, during a severe cold wave, the room temperature declined by several degrees. All heart rates taken during such periods were greatly elevated, even in rats which were resting quietly (Fig. 4c). In the investigation for which these heart rate studies were made,[10] one highly effective method used to accelerate the heart was chilling of the animal by depilation.

From these evidences it seems clear that dependable normal pulse rate determinations can be made in the unanesthetized rat, providing the extreme excitability of the animal and the great variability with temperature changes are recognized and steps taken accordingly. Early indications of studies in progress are that the training period may be cut to a week or two by the use of roomier cages than were formerly deemed necessary.

Summary. 1. A method is presented for accurate determination of the normal, conscious, resting heart rate in the albino rat. 2. The average value obtained on 113 adult males of 200-370 g body weight was 4.4 ± 0.30 beats per second, or 264 ± 18 per minute. 3. Emotional states were found to cause greater variation in heart rate than muscular activity. 4. Alterations in environmental temperature proved to exert a decided influence on the rat heart rate.

[10] Moses, L. E., *Am. J. Physiol.*, 1944, **142**, 686.

15493 P

Effect of Alloxan upon External Secretion of the Pancreas.

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The report of Goldner and Gomori[1] that in dogs made "diabetic" by alloxan injection the intralobular duct cells of the pancreas showed vacuolization suggested to us the possibility that this might serve as a means of investigating whether these duct cells perform a secretory function.

Methods and Results. A standard preparation of secretin concentrate designated S_1[2] was used in these experiments. It is known to contain both secretin and pancreozymin. The particular lot of material used for these experiments was tested on 2 normal dogs and the threshold dose[3] was found to be 0.3 and 0.5 mg respectively, indicating that the material had standard potency. In 4 dogs rendered "diabetic" for 18 to 30 days by the intravenous injection of 75 mg of alloxan per kg of body weight, the threshold dose of this same lot of secretin concentrate was found to be 2, 4, 5 and 10 mg. The "diabetic state" was attested by the strongly positive sugar reaction of the urine accompanied by moderate decline in body weight.

Two dogs which were similarly treated with alloxan but failed to become "diabetic" showed a threshold of 0.4 and 0.8 mg secretin concentrate, respectively. In the 2 normal dogs mentioned above, which were used to establish the potency of the secretin preparation, the injection of alloxan in a dose of 75 mg per kg of body weight after the threshold response had been determined did not significantly alter their response to a subsequently administered threshold dose of secretin concentrate within 3 hours.

Amylase determinations by the method of Schmidt[4] were performed on all samples of

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[1] Goldner, M. G., and Gomori, G., *Endocrin.*, 1943, **33**, 297.

[2] Greengard, H., and Ivy, A. C., *Am. J. Physiol.*, 1938, **124**, 427.

[3] Ivy, A. C., Kloster, G., Drewyer, G. E., and Lueth, H. C., *Am. J. Physiol.*, 1930, **95**, 35.

[4] Schmidt, C. R., Greengard, H., and Ivy, A. C., *Am. J. Digest. Dis.*, 1934, **1**, 618.