tion with acetone and alcohol. They found that acetone removed from the Norit more pressor substance than did alcohol extraction. The pressor substance apparently acted centrally because its action paralleled that of alpha lobelin, a central acting drug. Contrary to Bohn and Hahn[2] they found no relationship between the amount of pressor substance in the urine and the presence or absence of hypertension in the patient.

7655 C

Potentials in Embryo Rat Heart Muscle Cultures.

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As far as we have been able to discover, there have been no published investigations of action potentials in heart muscle cultures. This is a report on a method which it is hoped will furnish information on the mechanism of irritable tissues in general and particularly those which are excited spontaneously.

In general, the potential difference between the micro-electrodes making contact with a muscle culture is impressed on the grid of a vacuum tube and measured by the deflection of a string galvanometer in the plate circuit.

The cultures were prepared by the usual hanging drop technique. Small pieces of the ventricle of 16-day rat embryos were explanted in a medium of rat blood plasma and rat embryo extract. The ages of the cultures varied from 2 days to 2 months. The present records were taken from the older cultures containing outgrowths of muscle which had differentiated in the manner described by Goss[1] and were presumably without nervous tissue elements. Spontaneous contraction occurs in practically all cultures at one time or another and may be rhythmic or irregular. There is a great variation in the duration of the resting and acting periods and also in the frequency of rhythmic activity.

The electrode systems are micro-pipettes, 2-5 mu in diameter at the tip, with chloride coated silver wire coils placed in the large ends and the whole filled with mammalian Ringer solution. These pipette

[2] Bohn, H., and Hahn, F., *Z. f. klin. Med.*, 1933, **123**, 558.

[1] Goss, C. M., *Arch. f. Exp. Zellforsch.*, 1932, **12**, 233.

electrodes are mounted in micro-manipulators[2] and enter the moist chamber, on which the culture cover slip is placed, through a water trap as used by Chambers.[3] Such an electrode system has a high electrical resistance which precludes the direct use of a rapid recording instrument even if polarization difficulties could be overcome. The electrodes are connected to the grid circuit of a type 32 vacuum tube, operating at its free grid potential so as to draw a minimum of grid current. The Einthoven string galvanometer as used has a response time of about 0.01 sec. and is connected in the plate circuit of the tube. The steady plate current is by-passed through the usual Dowling shunt. A standard tuning fork driven time wheel giving time in 0.04 sec. is used and the camera is of the usual electrocardiograph type. To eliminate interference all electric power lines in the room are disconnected, the camera driven by an air motor, and a one microfarad condenser connected across the tuning fork contacts.

The ground electrode was usually placed in the culture medium at a short distance from the culture and the grid electrode was used for exploration.

As the exploring electrode approached the culture, a small but increasing negative potential relative to ground was found. Upon traversing the culture, one and sometimes 2 or 3, well demarcated areas about 10-20 mu in diameter were found where the negative potential was markedly higher, sometimes as much as 20-30 millivolts. These areas seemed sometimes to lie in the vicinity of elongated cells.

With the exploring electrode near one of these negative centers, contraction of the culture is coincident with a positive "action" potential, *i. e.*, a decrease of the negative resting potential. The record of Fig. 1a is from a particularly sensitive culture which was stimulated to activity by tapping the microscope with the electrode in position near an active center. In another culture, Fig. 1b, stimulation was effected by moving the electrode slowly toward an active center, showing an increase of the negative resting potential before the initiation of activity. After stimulation, the action potentials assume a characteristic form which is repeated at a constant or slightly decreasing frequency until quiescense. The form is that of a quick positive rise followed by a less rapid and rather uniform fall until near the resting potential which it reaches more slowly as

[2] Microdissection and microinjection—Microscopical Technique, 1929. R. Chambers, edited by McClung, Paul Hoeber, N. Y., pp. 39-73.

[3] Cohen, B., Chambers, R., and Reznikoff, P., *J. Gen. Phys.*, 1928, **11**, 585.

a negative maximum. There is then a slight positively directed rise before the initiation of the next beat. When activity ceases, the potential of the final beat apparently follows the same course except that it returns to the resting potential after this small positive rise (or decrease in negativity) instead of going into the next contraction. This gives rise to a characteristic final or "after-potential" as shown in Figs. 1a and 1b.

On one occasion in a slowly beating culture—60 per min.—somewhat similar after-potentials were noted following each response

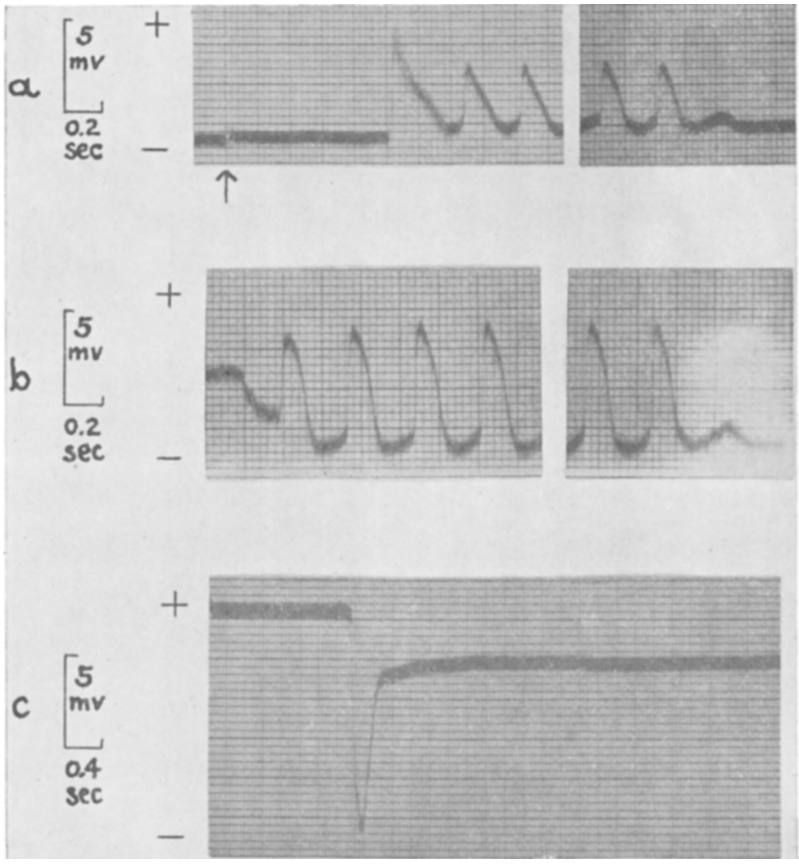

FIG. 1a. Potential difference between exploring electrode near negative center and ground electrode in culture medium when rhythmic contraction was initiated by tapping the microscope and ceased spontaneously.

FIG. 1b. Same as Fig. 1a, except that activity was initiated by moving exploring electrode nearer negative center.

FIG. 1c. P. D. between exploring electrode and ground electrode in medium when the former was forced through the surface of a single cell of a resting culture. A rapid darkening of the cell indicated its death.

which seemed to be coincident with mechanical relaxation or with the reflected wave seen in the slow-motion pictures of contracting cultures.[4] Close examination of the records would seem to suggest that such after-potentials occur with each beat in the more quickly beating cultures (150 per min.) but are incorporated in the record of the following beat. Faster galvanometer and film speeds would be necessary to demonstrate this.

Efforts to obtain diphasic responses were unsuccessful when the electrodes were placed one to 2 cell lengths apart along a 2 to 3 cell strand of contracting muscle. The only diphasic record was obtained by putting one electrode near each of 2 negative centers which were slightly out of phase.

With the unaided eye it is impossible to say whether the electrical centers serve also as centers for the spread of mechanical contraction or not. No apparent difference was found in the action potentials of non-striated cultures and those in which striation was almost complete.

An attempt was made to register the potential changes across the membrane of a single contracting cell, but failed because the excessive vibration of the inserted electrode caused almost immediate death of the cell as judged by refractive properties. The rather characteristic "death wave", Fig. 1c, shows a sudden large negative rise (which may be 20 millivolts) followed by an almost equally rapid fall to about half this value or less. Small variations on some of these records were attributed to contractions of neighboring cells.

7656 P

Note on the Metabolism of Copper in Splenectomized Rabbits.

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In previous studies[1] it was found that removal of the spleen in albino rats free from *Bartonella muris* infection is followed by an increased elimination of copper, which produces a negative copper balance. In a study of the metabolism of rabbits before and after splenectomy we found that removal of the spleen causes an increase in copper excretion in this species as well.

[4] Goss, C. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 292.

[5] Goss, C. M., *Arch. f. Exp. Zellforsch.*, 1933, **14**, 175.

[1] Sandberg, M., and Perla, D., *J. Exp. Med.*, 1934, **60**, 395.