

Summary. Experiments on dogs linked in cross-circulation, by connecting the central end of the aorta of one dog to the peripheral end of the aorta of the other and vice versa, have confirmed earlier results in this laboratory indicating that a hypertensive substance is liberated on stimulation of the central end of the cut vagus nerve. Comparison of the results of the vagus stimulation and noradrenaline injection have provided further evidence for the assumption that the hypertensive substance is probably liberated from the ar-

terial walls themselves.

1. Jiménez Diaz, C., de la Barreda, P., Souto, J., and Alcalá, R., *Bull. Inst. Med. Res. Univ. Madrid*, 1950, v3, 131.
2. Jiménez Diaz, C., de la Barreda, P., Molina, A. F., and Alcalá, R., *Ibid.*, 1951, v4, 167.
3. de la Barreda, P., Molina, A. F., Alcalá, R., and Jiménez Diaz, C., *Ibid.*, 1952, v5, 65.
4. Taylor, R. D., Page, I. H., and Corcoran, A. F., *Arch. Int. Med.*, 1951, v88, 1.

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Effects of Ouabain on Beat and Oxygen Consumption of Embryonic Chick Hearts.* (21221)

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Friedman and Bine(1) demonstrated that the embryonic duck heart is very sensitive to digitalis preparations. Preliminary experiments showed us that embryonic chick hearts, although not as sensitive to digitalis as embryonic duck hearts, are, nevertheless, satisfactory in this respect, easier to obtain, and less expensive. The cartesian diver technic is admirable for measuring metabolism of minute tissues. Further preliminary experiments showed that it could be adapted to measuring oxygen consumption of relatively large embryonic chick hearts weighing as much as 400 μ g, dry weight, after 6 days of incubation. In another communication(2) we describe the necessary basic modifications for measuring oxygen consumption of embryonic chick heart with the cartesian diver.

Methods. The cartesian diver with a shaker and divers with caps and mixers(2) was employed. Fertile chicken eggs were incubated 5 days, producing embryos with hearts of maximum size (80 to 300 μ g, dry weight), which would beat regularly and reliably. These hearts were dissected into Ringer or

other suitable solution until needed. The solutions were kept at 37°C. A heart was placed in the bulb of a diver, 10 mm³ of Ringer or other suitable solution was added. Air was replaced by 100% oxygen. Exactly one mm³ of same solution (control) or of a solution containing ouabain was deposited on surface of the neck, and a magnetic mixer placed in this droplet. After reoxygenating, adding the KOH seal, and placing a buoyed cap on the diver, it was immersed in the flotation medium in a diver holder. About 10 minutes was allowed for temperature equilibration at 37.3°C. Observations on rate and regularity of the beat were made on the odd numbered minutes, and measurements of suspension pressures made on even numbered minutes for 42 minutes, then at less frequent and less regular intervals up to 4 hours. Mixing was performed at 11 minutes as described elsewhere(2). Changes in suspension pressures were corrected for changes in barometric pressure and were multiplied by the diver constant to give oxygen consumption. At the close of each experiment the hearts were weighed on a quartz fiber balance; all oxygen consumption figures were converted to mm³/mg of dry weight. The results of comparable experiments were averaged.

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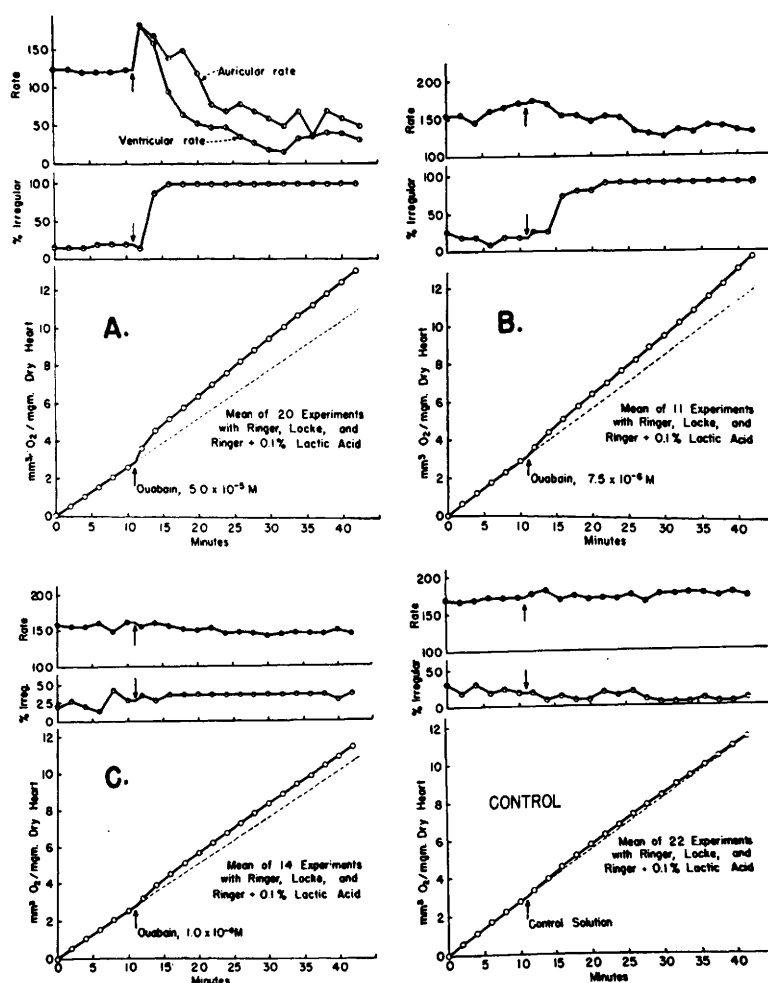


FIG. 1. Effects of ouabain on beat and oxygen consumption of embryonic chick hearts.

Results. A total of 108 experiments were performed. The following concentrations of ouabain were employed in Ringer, Locke, and Ringer plus 0.1% lactic acid at pH 7.2: 5.0×10^{-5} M, 2.5×10^{-5} M, 1×10^{-5} M, 7.5×10^{-6} M, 5×10^{-6} M, 2.5×10^{-6} M, and 1×10^{-6} M.

The results obtained with Ringer, with Locke, and with Ringer plus lactic acid were identical, and therefore, averaged; the results are shown in Fig. 1. For the sake of brevity only 3 concentrations are graphed; intermediate concentrations gave intermediate results.

Fig. 1A shows that when ouabain, at a concentration of 5.0×10^{-5} M, was added to the

hearts, there was an immediate increase in oxygen consumption that persisted throughout the period of observation. In addition, ouabain caused an immediate increase in heart rate, followed, after about 3 minutes, by the appearance of irregularities, and, simultaneously, a decrease in both auricular and ventricular rates, particularly the latter.

Figs. 1B and 1C show that when lower concentrations of ouabain were used, the increase in oxygen consumption persisted, although of smaller magnitude, but changes in the rate and production of irregularities diminished to zero.

Fig. 1 Control shows that oxygen consumption, heart rate, and incidence of irregularities

was not altered when control solution was mixed with control solution.

In some experiments the hearts were dissected directly into Locke solution containing one mg % prostigmine and 0.5 mg % acetylcholine, or Locke solution containing 2 mg % atropine. These drugs did not alter the heart rates nor the incidence of irregularities. After 30 minutes in these solutions, observations on rates, regularity, and oxygen consumption were begun. Ouabain, 5×10^{-5} M, was added at 11 minutes. In such experiments the increase in oxygen consumption produced by ouabain was not altered, but the effect on rate and incidence of irregularities produced by ouabain was exaggerated by prostigmine and acetylcholine and were reduced by atropine.

In many experiments, observations were continued over periods up to 4 hours. In all, including both control and ouabain experiments, there was a gradual decrease in oxygen consumption; the decrease could not be correlated with any previous treatment.

In several experiments, the hearts were kept at 37°C for one or 2 hours before being placed in divers. Deterioration, as indicated by a diminishing oxygen consumption, occurred, but the response to ouabain was not altered.

Discussion. That ouabain causes an increased oxygen consumption agrees with many previous observations(3). That ouabain causes irregularities is in accord with our predecessors.

Investigators(4,5) have reported an increase in oxygen consumption after digitalis in the preparations, such as heart slices, in which there is no beat nor visible muscular contraction. In such preparations the change in oxygen consumption can not be due to alterations in heart rate nor heart beat. Our results show that the time of onset of irregularities in a heart preparation that is capable of supporting a beat differs from the time of onset of increased oxygen consumption; furthermore, the minimum concentration of ouabain that produces irregularities differs from the minimum that causes increased oxygen consumption.

In addition, in a few experiments with prostigmine plus acetylcholine and with atropine, the severity of the irregularities may be modified without essentially altering the increase in oxygen consumption.

These results may be interpreted to mean that the increase in oxygen consumption after ouabain is not directly related to changes produced in the beat of the heart.

Summary and conclusions. 1. Ouabain, in concentrations from 1×10^{-6} M to 5×10^{-5} M, was added to embryonic chick hearts whose oxygen consumption was being measured in the cartesian diver. Controls, in which Ringer, Locke, etc., was mixed with Ringer, Locke, etc., respectively, were also employed. 2. Ouabain, in concentrations from 1×10^{-6} M to 5×10^{-5} M, inclusive, caused a prompt increase in oxygen consumption, roughly proportional to concentration. 3. Ouabain, at a concentration of 5×10^{-5} M, caused an immediate increase in heart rate, and, after about 3 minutes, irregularities in the beat of the heart. 4. Ouabain, in a concentration of 1×10^{-6} M, did not produce irregularities and did not alter the heart rate. 5. Oxygen consumption, heart rate, and incidence of irregularities were not altered by mixing control solutions with control solutions. 6. Acetylcholine plus prostigmine increased, whereas atropine decreased the severity of the irregularities produced by ouabain, without appreciably altering the increase in oxygen consumption caused by ouabain. 7. The increase in oxygen consumption after ouabain is not directly related to changes in the beat of the heart.

1. Friedman, M., and Bine, R., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1947, v64, 162.

2. Smith, J. A., Glassman, M., Lind, A. H., Sohn, H., and Warren, S., in preparation.

3. Wollenberger, A., *Pharmacol. Rev.*, 1949, v1, 311.

4. ———, *J. Pharmacol. and Exp. Therap.*, 1947, v91, 39.

5. Burdette, W. J., *J. Lab. and Clin. Med.*, 1952, v40, 867.