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BIOLOGICAL BULLETIN

RECOVERY OF THE HEART BEAT OF *FUNDULUS* EMBRYOS AFTER STOPPAGE BY POTASSIUM CHLORIDE.

MELVIN W. THORNER,

Zoölogy Laboratory, University of Pennsylvania and The Biological Laboratory, Cold Spring Harbor.

Introduction.

Loeb and Cattell (1) found that when the embryonic hearts of fertilized *Fundulus* eggs have begun to pulsate, they may be completely stopped by placing the eggs in a solution of KCl. They found further that when eggs so treated were placed again in sea water, the hearts resumed beating. These authors also pointed out that "embryos whose hearts had stopped beating under the influence of a sufficient dose of KCl did not begin to beat when put into distilled water." However, in another part of the same paper a table is given in which it is shown that three of thirty of the hearts stopped by KCl recovered in distilled water after a short period. The experiment was discontinued at the end of this time.

Although Loeb and Cattell noted these facts, they failed to extend their investigations in a quantitative manner. This paper endeavors to determine the quantitative relationships of the various factors concerned in effecting recovery of the heart-beat after stoppage by KCl. This recovery was studied in sea water, distilled water and in various salt solutions.

Material.

The material used was the developing egg of *Fundulus heteroclitus,* collected at the Biological Laboratory, Cold Spring Harbor. The eggs were "stripped" from the females within an hour of the time the fish were taken from the ocean. The eggs were then immediately fertilized by a speım suspension milked from the males. The egg stock thus prepared was kept at room temperature (22–24° C.) in sea water changed daily.

At the end of the fourth day after fertilization the heart normally begins to pulsate. All experiments were carried out at room temperature (22–24° C.). The heart was considered as "stopped" when all three chambers had ceased pulsating.

Experiments.

(*a*) *Recovery in Sea Water.*

The first set of experiments represents an attempt to determine the relationship between the time of immersion in KCl and the time of recovery in sea water. Also different concentrations of KCl were tried to see if the relationship varied for different strengths. For this purpose lots of from 40–75 eggs were chosen for each experiment. The heart of each embryo was observed to be in good physiological condition. Each lot was placed in a

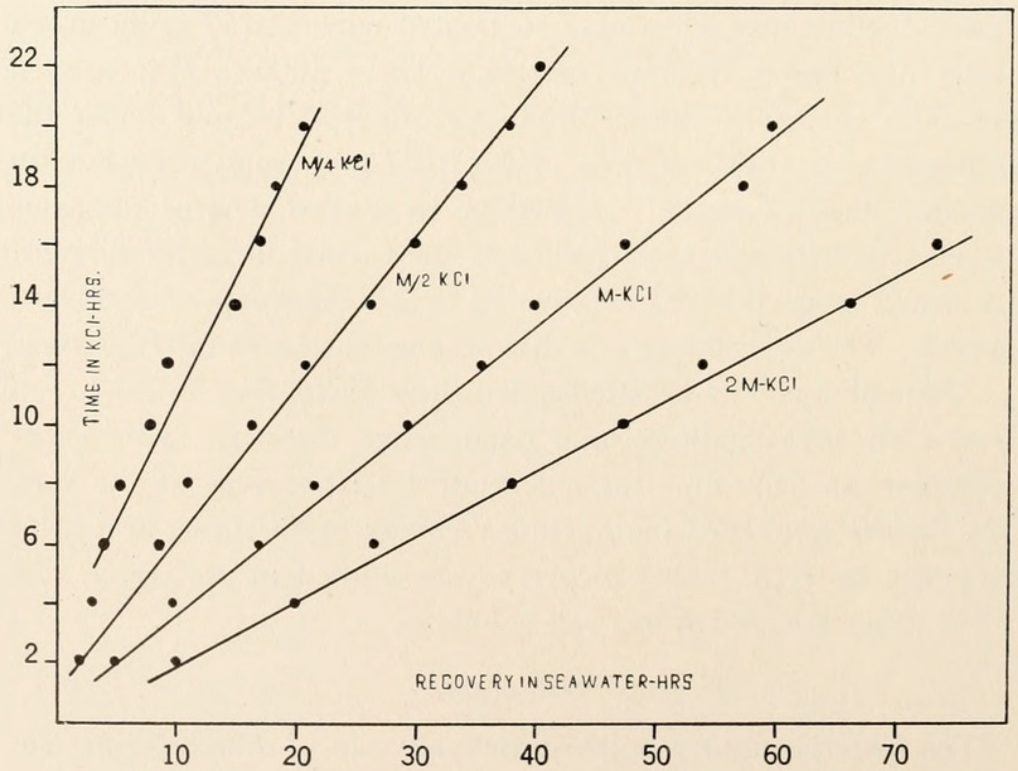

Fig. 1. Shows relation between period of recovery in sea water and period of immersion in different strengths of potassium chloride for hearts of Fundulus embryos.

KCl solution, the strength of which varied from 0.25 M to 2.00 M, for a period varying from 2 to 22 hours. The eggs were then rinsed in distilled water and placed again in sea water and observed at intervals and the time of recovery (*i.e.*, recovery of all three chambers) for each egg noted and the average time computed. By rinsing the eggs in two changes of distilled water each time they were to be placed in a new solution, the possible salt antagonism at the surface of the egg was reduced. At the same time the immersion in the distilled water was too short to cause permeability changes in the membrane. Fig. 1 shows results of such experiments.

From Fig. 1 it may be seen that the time of recovery is directly proportional to the duration of immersion in KCl solution—all other factors remaining constant. This relationship holds within the limits of 0.25 M and 2.00 M.

The next experiments to be tried were an endeavor to determine the relationship between the strength of KCl used and the period of recovery in sea water. Again lots of 40–75 eggs were selected. Each lot was placed in a KCl solution of from

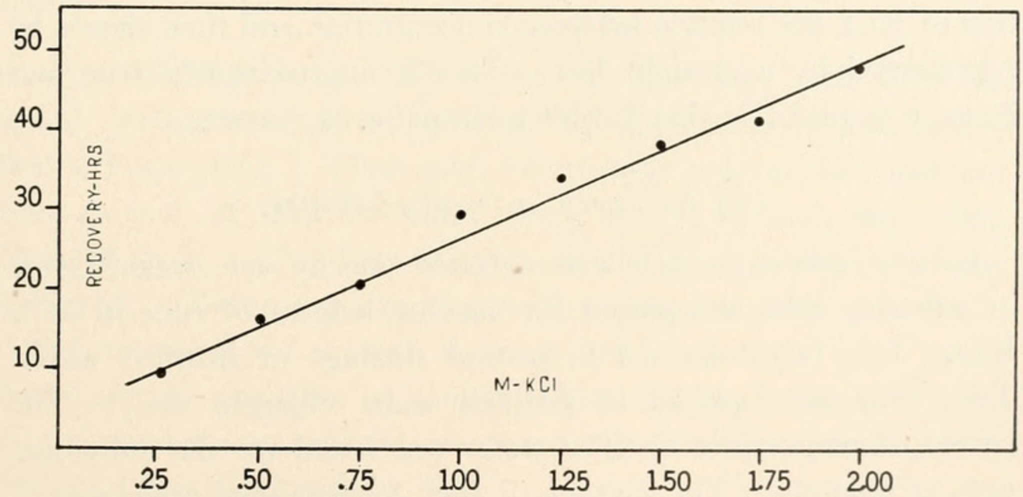

FIG. 2. Shows relationship between concentration of potassium chloride and period of recovery in sea water for hearts of Fundulus embryos after immersion in KCl for ten hours.

0.25 M to 2.00 M for ten hours. The periods of recovery were noted and averaged as before. Fig. 2 shows the results of this group of experiments.

From Fig. 2 it is evident that the duration of the period of recovery is directly proportional to the concentration of KCl used.

It was then decided to determine the relation of strength of KCl to the time required for complete heart cessation at the various strengths (Fig. 3).

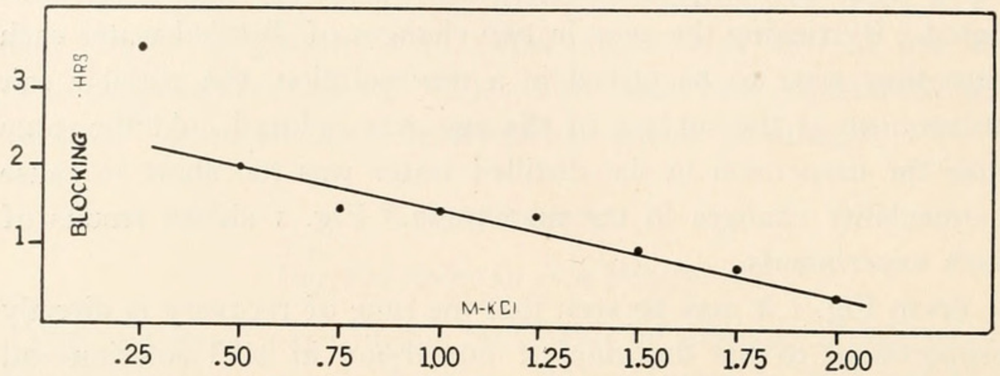

Fig. 3. Shows relationship between molar strength of potassium chloride and the time required for complete heart cessation in Fundulus embryos.

Figure 3 suggests the possibility of a direct proportionality. If the assumption of Loeb is correct, *i.e.*, that the same amount of KCl is needed each time to block completely the heart-beat and if the rate of entry of KCl is directly proportional to the concentration of KCl, the relation between concentration and time should be represented by a straight line. This is approximately true and hence it is probable that Loeb's assumption is correct.

(*b*) *Recovery in Distilled Water.*

Lots of 200 eggs each were selected one by one, washed free of adhering salts and placed for varying lengths of time in M/2 KCl. They were washed in several rinsings of distilled water. Then they were placed in distilled water changed daily. The period of recovery in distilled water was noted for the individual eggs at intervals. The average of each lot was then calculated.

From Fig. 4 it may be seen that, contrary to the opinion of Loeb, recovery of an appreciable number of eggs is effected in distilled water. Instead of merely non-recovery, coagulation of the embryo was used as a criterion of death and each egg was carried through to either coagulation or recovery. It was found that mortality increases with the length of immersion in KCl. The extreme variation from the mode also increases with the length of immersion.

In an attempt to confirm Loeb's statement (1) that "KCl cannot diffuse out of *Fundulus'* egg in distilled water" and assuming that it does diffuse in instead of remaining at the vitelline membrane, we endeavored to discover why the heart-beat recov-

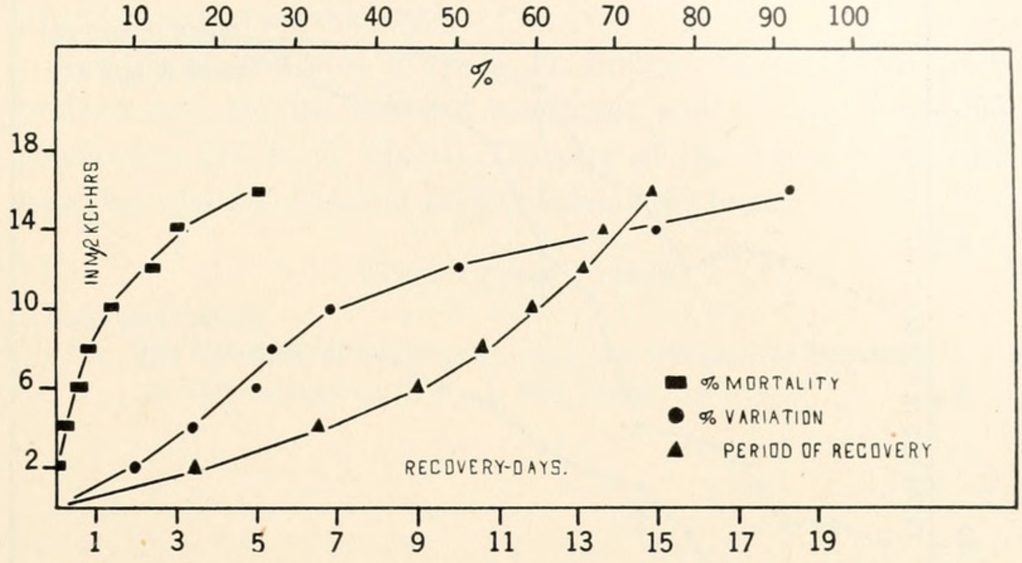

FIG. 4. Shows relationship between time for recovery in distilled water and length of exposure to M/2 KCl for hearts of Fundulus embryos. The percentage mortality and percentage variation for same eggs are also indicated.

ered in distilled water. Three lots of 200 eggs each were selected, washed and placed in M/2 KCl for six, eight and twelve hours, respectively. Then each lot of eggs was washed and put into 40 mls. of distilled water (in a 200 × 25 mm. test tube) changed daily. The daily *increase* in electrical conductivity was followed. (Before each conductivity measurement the water was boiled momentarily to free it from CO_2). Limited time made necessary the omission of a quantitative chemical analysis of the K content but conductivity measurements furnish a possible indication of the K extruded.

The graph of Fig. 5 shows that some electrolytic substance was extruded through or at least given off from the membranes of the egg. The fact that the hearts began to beat again leads one to suppose that it is at least partly KCl. With one exception there was found to be a daily increase in the rate of extrusion. The rate increased most just before recovery of the hearts began to be manifest. The amount extruded was dependent on, but not proportional to the length of immersion in M/2 KCl.

It is readily apparent that the mechanism of recovery in sea water and in distilled water is fundamentally different. That in sea water is affected by concentration and duration of immersion

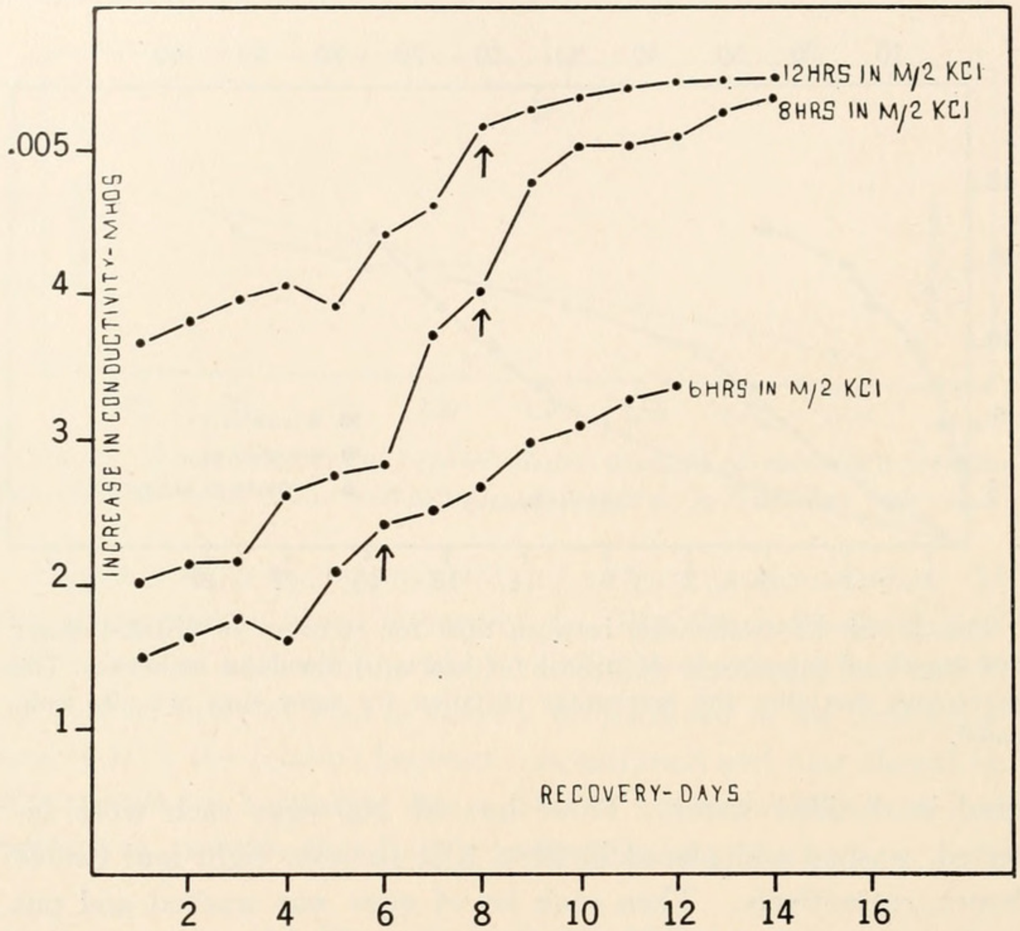

FIG. 5. Shows increase in electrical conductivity of distilled water in which eggs, immersed for different periods in M/2 KCl are placed. Arrows indicate time heart resumed beating.

in KCl in direct proportionality. The recovery in distilled water is more complicated.

Loeb (1) noted recovery with various cations and anions. The cations here tried effect a momentary recovery. This is probably not due to salt antagonism but to a direct overstimulation resulting in death of the temporarily aroused hearts.

SUMMARY.

1. The heart-beat of eggs blocked completely by KCl recover in both sea and distilled water although the two recovery processes are dissimilar.

2. In sea water the duration of the period of recovery is directly proportional to the concentration of KCl and to the time of immersion in KCl. The same amount of KCl is required to block completely the heart-beat.

3. The recovery of eggs in distilled water was studied with the aid of electrical conductivity.

I am deeply indebted to Dr. J. H. Bodine for suggestion of the problem and for his valuable assistance and advice. I am also indebted to Dr. R. G. Harris, Director of the Biological Laboratory, for making research facilities available to me.

LITERATURE CITED.

1. **Loeb and Cattell.**
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