STUDIES ON THE BLOOD VESSELS IN THE MEMBRANES OF CHICK EMBRYOS

PART IV. MODIFICATION OF IRRITABILITY OF THE BLOOD VESSELS

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We studied the reactions of the blood vessels in the vascular membranes of chick embryos in Part II of this series. There we described changes which took place quantitatively and qualitatively in response to stimuli. It was our care in the earlier experiments to maintain the environment constant. In the present experiments we intended conversely to keep the stimulus constant but to change the environmental conditions. The question we have asked ourselves is, of course, whether with the same stimulus a difference in the irritability of the blood vessels can be brought about by altering the environment.

To obviate the danger of drying, due to the increased length of time required by these experiments, a substitute procedure was devised. We placed the eggs in Ringer's solution through which oxygen was bubbled. It was, of course, necessary first to be certain that this arrangement did not interfere with the viability of the embryos.

Experiment 1.—Eggs were opened on the second day of incubation, the albumen for the most part was drained off, and the yolk was laid in the Ringer's solution through which, as has been said, oxygen was continuously bubbled. At the beginning of the experiment the embryo with its membranes was 11 mm. in diameter, the blood vessels were not yet developed, and the embryo itself white in color. 24 hours after the beginning of the experiment the embryo and its membranes measured 14 mm., the heart was red and contracted 120 times per minute. In the blood vessels there was flow of red blood. At the end of 48 hours the embryo and its membranes measured 16 mm., the heart beat at the rate of 120, and there was

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vigorous flow in the vessels. On the 3rd day, the rhythm of contraction of the heart was irregular.

It is clear from these observations that the embryo including the circulatory apparatus grows normally for 2 days in this solution.

The demonstration of the viability of embryos and their membranes was sufficient to warrant the performance of experiments, the duration of which in no case exceeded 1 hour. We needed, of course, to be certain that oxygen in the concentration in which it was used exercised no effect on the irritability of the blood vessels of the embryonic membranes.

Experiment 2.—This was of a preliminary nature and was repeated in each case before proceeding to further tests. After opening the egg a branch of an artery was stimulated. Stasis occurred after the stimulus had been applied 3 times for 10 seconds. Then the preparation was irrigated with Ringer's solution through which oxygen was bubbled for 5 minutes. The vessel was again stimulated and stasis occurred again after 3 successive periods of stimulation each 10 seconds long.

We concluded that Ringer's solution through which oxygen was passed did not alter the irritability of the blood vessels.

We used carbon dioxide of known concentration in our experiments. It appears from the experiments of Hammett and Zoll (1) that saturated solutions of carbon dioxide bring about constriction of the vessels. Contraction takes place if the solution saturated with gas is injected under the membranes, as in the experiments of Hammett and Zoll, as well as when the surface is bathed with Ringer's solution which during this process is exposed to carbon dioxide in the usual fashion by bubbling the gas through the solution. These results we were able to confirm. We reduced, therefore, the concentration of carbon dioxide in Ringer's solution to a point at which it had no longer an effect upon the blood vessels.

Experiment 3.—Ringer's solution was placed in a glass burette having a capacity of 50 cc. Through this, air containing 5 per cent of carbon dioxide was bubbled so that 60 bubbles passed through in a minute. The egg after it had been opened was bathed with this solution. At the end of an hour the contractions of the heart remained unchanged, the rate being 128 per minute. No change took place in the diameter of the vessels, nor in the flow of blood through them.

We concluded that bathing the membranes with Ringer's solution containing carbon dioxide, the hydrogen ion concentration being between 7 and 6, would occasion no change in the rate of the heart or in the diameter of the vessels.

In view of the preliminary experiments which have just been described we adopted the following plan of operation.

A burette was provided with: (1) a tube through which oxygen was introduced, (2) a similar one for the delivery of carbon dioxide, and (3) a funnel through which salt solutions could be delivered. A tube led from the burette to a small glass cannula from which the solution designed to bathe the egg was permitted to drop. The perfusion stream was directed so that it fell not upon the embryo itself nor upon its membranes but on the surface at a distance from them, yet in such a way that the stream passed over the vascular layer. Direct impact of the fluid would have occasioned a mild stimulus the effect of which would have been characterized as we now know by dilatation of the vessels and a rapid rate of flow, the "state of fluxion."

Stimulation was supplied by means of a faradic current, the source being an Edison storage battery. To be certain that the current was constant, its voltage and amperage were continuously observed. The electrodes consisted of two copper wires a millimeter in diameter, placed a millimeter apart. With the exception of their poles they were carefully insulated. Observations were made with a binocular binobjective Zeiss microscope.

We measured the duration from the beginning of stimulation to the time of occurrence of stasis. To recognize this stage presented no difficulty because of the magnitude of the effect. Nor did this observation lead to error for the effect did not disappear and itself, therefore, served to control the observation. The experiments were all undertaken in a constant temperature room. Since the solutions and the gases were also kept here, the possibility of error due to alterations in temperature was excluded.

These arrangements permitted observations in which the vessels were stimulated alternately in milieux containing carbon dioxide and in others free of this gas.

After opening the egg: (1) Stimulation with a faradic current for 20 seconds was undertaken. The result was *stasis* of the blood stream. (2) When irrigation with Ringer's solution, *free* of carbon dioxide had been carried on for 2 minutes, stimulation with the faradic current was again applied for 20 seconds. *Stasis* again occurred. (3) When irrigation with Ringer's solution containing *carbon dioxide* had gone on for 3 minutes followed by stimulation with the faradic current lasting

150 seconds, *no stasis* appeared. (4) Next we irrigated the membrane with Ringer's solution which contained *oxygen* for 5 minutes and stimulated the vessels for 30 seconds. *Stasis* again appeared. (5) We irrigated again for 3 minutes with Ringer's solution containing carbon dioxide. This was followed by stimulation with a faradic current for 200 seconds, but *no stasis* occurred. Finally, we irrigated the preparation with Ringer's solution containing oxygen for 5 minutes and now stimulation with faradic current for 30 seconds only, was followed by the occurrence of *stasis*. Forty experiments like this were performed.

DISCUSSION AND SUMMARY

When faradic stimulation was undertaken of vessels irrigated with Ringer's solution, which alternately contained and was free from carbon dioxide, it was observed that the reaction was far less when the solution contained carbon dioxide. A reversal of the effect could be obtained many times. It appears, therefore, that when Ringer's solution contained carbon dioxide in the concentration described, the irritability of the vessels to electrical stimuli decreased, although carbon dioxide by itself and in the absence of the application of the stimuli, appeared to be void of effect upon the vessels. The rare, divergent results were traced to technical errors.

We attempted to discover whether the observed decrease in irritability of the vessels might not be due to the absence of oxygen. For this purpose we irrigated the vessels with Ringer's solution alternately containing nitrogen and oxygen. When nitrogen caused any change this was due to an influence on the rate of the heart and not on the irritability or reactivity of the arteries. In whatever way we tried we were unable to bring about a change in reactivity of the arteries by creating a condition of oxygen lack independently of a change in the rate of the heart beat.

We attempted to study also the effect of other acids beside carbon dioxide on the changed reactivity of the arteries. Irrigation with various concentrations of lactic acid was without result. We also employed solutions buffered with potassium and sodium phosphate. When the irrigation was undertaken with these solutions having a pH range varying from 7.7 to 5.9 we observed neither a direct action nor

one which modified the preparation in such a way as to change its susceptibility to faradic stimulation.

Important investigations have been published recently by Atzler and Lehmann (2) on the direct influence of the hydrogen ion concentration on the behavior of blood vessels. Hammett and Zoll believed that, as the result of their experiments in which they attempted to bring about stimulation with solutions of concentrated carbon dioxide, they were able to exclude the possibility of action due to acid alone and therefore ascribed to carbon dioxide a specific effect. In our own experiments the method of irrigation does not permit an inference whether, or how far, an acid effect plays a rôle in the carbon dioxide experiments. For beside the question of hydrogen ion concentration and of buffering, the question of the penetration of substances from the surface to the contractile elements of the wall of the vessels requires to be considered. Carbon dioxide has an ability, beyond that of all other substances, to penetrate through tissues (3). It may be owing to this property that we could influence the reactivity of the blood vessels with it and it alone.

This possibility must be further investigated. In these experiments, however, it was our object to show only that it was possible to influence the irritability of blood vessels experimentally. The conclusion is justified by our experiments that carbon dioxide in small concentrations reduces the threshold of irritability for electrical stimuli of the blood vessels of the embryonic membrane.

REFERENCES

1. Hammett, F. S., and Zoll, D., Studies of the embryonic circulatory system. I. The influence of H-Ion concentration and CO_2 on the yolk-sac blood vessels of the chick embryo, *Amer. Jour. Physiol.*, 1928, **86**, 520–527.
2. Atzler, E., and Lehmann, G., Reaktionen der Gefässe auf direkte Reize, Handbuch der normalen und pathologischen Physiologie, 1927, **7**, pt. 2, 963–997.
3. Jacobs, M. H., Permeability of the cell to diffusing substances, General Cytology, Section III, Chicago, 1924, 99–164 (see page 129).