The Effect of Epinephrine on the Myocardium of the Embryonic Chick

By Alexander Barry, Ph.D.

The myocardium of the embryonic chick heart, before it has been innervated, responds to concentrations of epinephrine as dilute as 1 part in 20,000,000 by an abrupt acceleration in its inherent rate of pulsation. The more slowly the heart beats initially, the more marked is the accelerating effect of epinephrine. Usually the initial acceleration is followed by a period during which the heart slows to a rate intermediate between the initial and the maximal. This deceleration is not due to a decrease in the stimulating effect of the drug applied.

The following report is concerned with the moot question as to whether or not the sympathomimetic substance, epinephrine, is effective in accelerating the rate of pulsation of the myocardium of the chick embryo prior to the establishment of its innervation (i.e., before the fifth day of incubation, according to His). In 1927, Fujii reported that in chick embryos up to the third day of incubation, adrenalin had no effect on the rate of cardiac pulsation except in "large doses," and that the first characteristic response was obtained in chicks during the fourth day of incubation. In 1931, Nordmann and Rüther stated that the rate of pulsation of the pacemaking centers in explants of the myocardium of chick embryos of various ages was not influenced by adrenalin. In the same year, Markowitz examined the effect of epinephrine on explanted cardiac tissue from chick embryos. This work assumed that no change in rate less than 25 per cent was significant. Using this criterion, it was found that out of 38 tested, ten explants from chick embryos of 2 to 3 days of age reacted to epinephrine; and that explants from older embryos yielded a higher percentage of positive reactions. All explants showed "typical physiologic reactions" when they were taken from embryos older than 6 days of incubation. It was assumed from the foregoing observation that a substance within the myocardium was responsible for the "typical response" of cardiac tissue to epinephrine. This substance appeared to be absent from most 72-hour embryos, but was present in increasing concentrations in older embryos.

Two years later Hsu reported that epinephrine was effective in accelerating the rate of intact embryonic chick hearts between the ages of 53 and 103 hours, and of fragments from hearts between the ages of 37 and 480 hours of incubation. The greatest acceleration obtained for any fragment of the heart was 115 per cent, and Hsu stated that there was a great variation in the sensitivity of the individual hearts which was not correlated with differences in age, concentration of drug, or other known environmental factors. It was because of the disagreements in the above reports, that the present investigation was undertaken.

Methods

Chick embryos of Rhode Island Red stock were removed from the egg and placed in sugar-free oxygenated Ringer-Locke solution. The body of the embryo was transected immediately cephalic to the aortic arches and caudal to the sinus venous. The parietal pericardium and attached body wall were removed to allow free access of the oxygenated saline to the epicardium. The resulting block of tissue was placed in 20 cc. of oxygenated Ringer-Locke solution at 38 C. The desired segment of the cardiac tube was excised and mounted in the recording apparatus. When the rate of the entire heart or of the sinus segment was studied, the caudal end of the heart was left attached in its normal relationship to the axial portion of the body.

The recording apparatus, illustrated in figure 1, can for brevity be called a micromyograph. It consists of a small glass hook attached to an adjustable glass arm, which can be raised or lowered by means of a fine screw adjustment. The piece of tissue to be examined is impaled by one end to this hook. The other end of the segment of tissue is attached to the hooked end of a thin glass filament. The upper end...
of this filament hooks over a short side-arm which is so cemented to a rubber band that a vertical movement imparted to it will twist the band. A mirror 0.015 by 0.030 inches in size (GE #NP-61770) is segment was slightly influenced temporarily by sudden increases in the tension to which it was subjected. Thus, if the saline medium surrounding the tissue was unduly agitated, the resulting eddy cur-

fixed to the rubber band so that it deflects a beam of light as the rubber band is twisted. The movements of this optical lever are recorded on the moving film of a slit camera. The rate of pulsation of the heart segment can thus be determined by measuring the distance between deflections on the photographic record. When such precision is not needed, the rate of pulsation is measured with a stopwatch.

It was found that the rate of pulsation of the heart rents tended to stretch the tissue, and its rate of pulsation increased slightly. In no case was this increase in rate found to exceed 10 per cent. Nevertheless, to control this slight source of variability, after a given segment of heart was placed in the apparatus it was allowed to remain untouched until its rate of pulsation reached a steady state (usually about ten minutes). Then, while its pulsations were being photographically recorded, 0.2 cc. of Ringer-Locke

**FIG. 1.—Diagram showing the construction of the micromyograph used to record length changes of small segments of embryonic myocardium.**
solution was added to the saline bath. The small resultant alterations in rate, if present, served as a control for evaluating the effect of epinephrine which was subsequently added in precisely the same manner.

A, 0.2 cc. of Ringer-Locke solution was added to the saline bath. The resultant increase in rate was minimal. After one minute's pause, 0.2 cc. of epinephrine at a concentration of 1 part in 200,000 was added (point B). This resulted in a concentration of 1 part in 20,000,000 in the saline solution surrounding the cardiac tissue. As can be seen from figure 2, the pulsation frequency had increased to 150 beats per minute by the time the last of the dose of epinephrine had been added (i.e., within five seconds). The pulsations were recorded for a full minute. After one minute's pause the same

Results

The photographic records from a typical experiment are shown in figure 2. Figure 3 presents the results from the same experiment, plotted as rate against time. The ventriculoconus segment of the heart of a 70-hour chick was placed in the micromyograph. After ten minutes its rate of pulsation was reasonably steady at about 55 beats per minute. At point
dosage of epinephrine was repeated, bringing its concentration to 1 part in 10,000,000 (point C). There was no further appreciable increase in rate. After another minute’s pause more epinephrine was added, bringing its concentration to 1 part in 500,000 (point D). Again, this higher concentration caused no further increase in the pulsation frequency of the heart segment.

Segments from the atria, ventricles and conus showed the expected lower inherent rate of pulsation.2,7 The results from tests on 55 hearts and segments from hearts of chick embryos of between 40 and 170 hours of incubation are shown in fig. 4. It can be seen that the degree of maximum acceleration depends upon the initial rate.

![Graph showing the effect of epinephrine on the pulsation rate of segments of embryonic chick myocardium.](http://circ.ahajournals.org/)

**Fig. 4.**—Graph showing the effect of epinephrine on the pulsation rate of segments of embryonic chick myocardium. The ordinate shows the increase in rate as per cent of the initial rate, which is plotted in beats per minute along the abscissa.

From a series of such experiments it was found that concentrations of epinephrine varying between 1 part in 20,000,000 and 1 part in 20,000 would produce maximal effects on the rate of beating of the cardiac segments. However, these should not be considered limiting values since no attempt was made to establish threshold concentrations.

Segments of various regions of the heart at different ages were tested as described above (figs. 2 and 3). It was found that, under the experimental conditions described, all but a few segments from the sinus venosus of hearts at any stage after this chamber had been formed beat consistently faster than 160 beats per minute before the addition of epinephrine. Segments from the atria, ventricles and conus showed the expected lower inherent rate of pulsation.2,7

If the tissue was beating at a rate faster than 180 beats a minute, the acceleration induced by epinephrine was less than 10 per cent, and was not considered significant. Tissues with an initial rate slower than 180 beats per minute were accelerated by the addition of the drug; the more slowly the tissue beat initially, the more marked was its acceleration.

In some cases the initial acceleration induced by epinephrine was followed by a more gradual deceleration which usually reached a plateau at a rate between the initial and maximum rates of pulsation. There was a general trend among all the cardiac segments tested for the more slowly pulsating segments to show...
this secondary phase of deceleration more markedly than those segments whose initial rate was more rapid. In other words, segments with a lower inherent rhythmicity tended to respond to epinephrine by a more transient phase of acceleration. However, there were many exceptions to this general rule, which could not be correlated with any of the variables measured, such as age, initial rate, and location within the heart.

It was not thought that this deceleration was due to a decrease in the concentration of epinephrine surrounding the tissue to a level giving less than the maximal response, since in most tests a concentration of epinephrine of 1 part in 100,000 was employed. At this strength, 99 per cent of the epinephrine could be destroyed or inactivated, and still leave a concentration (1 part in 10,000,000) which had been previously shown to elicit a maximal response from the cardiac tissue. There was no indication that under the conditions of the experiment any significant degree of oxidation of the drug took place. Nevertheless, a test was made to determine whether the epinephrine might have been inactivated in some unsuspected manner during the period of observation. The heart from a chick embryo of 72 hours' incubation age was tested as described above with a concentration of 1/100,000 epinephrine. This heart showed the usual acceleration, increasing its rate of pulsation by 50 per cent. After ten minutes the heart was discarded, but the solution was saved and kept at room temperature. A second heart of the same age as the first was mounted for testing in 20 cc. of Ringer-Locke's solution. After it had attained a steady rate of pulsation at 73 beats per minute, 20 cc. of the 1/100,000 solution used in the previous experiment was added. The rate of pulsation promptly accelerated to 118 beats per minute (62 per cent). Thus it is evident that the solution of epinephrine at the end of the first experiment was sufficiently effective so that even when diluted to half strength, it still elicited a typical response from a second heart. This seemed to give adequate evidence that the deceleration following the initial acceleration of the pulsation rate was not due to a decrease in the concentration of the epinephrine in the environment of the heart segment. Neither was it due to alterations in temperature nor in tension.

**Discussion**

The sinus venosus of the embryonic chick heart has been formed and has assumed its role of pacemaker at about 22 to 25 somites (46 to 48th hour of incubation, according to the age scale of Patten10). At this time the rate of the excised heart or of the sinus venosus is about 160 to 180 beats per minute at 38 C.1 Thus it might be predicted that the rates of entire hearts, or segments of the heart whose pace is set by the sinus venosus, (i.e. from embryos older than 46 to 48 hours, incubation age), will not be accelerated by epinephrine. This was found to be the case. However, in a few instances it was found that a segment of the sino-atrial region of the heart pulsed initially at a rate less than 160 beats per minute. In these cases epinephrine was effective in accelerating its rate of pulsation. It is possible that in these segments the pacemaker was actually atrial in position, either because the sinus was not pulsating, or because there was a sino-atrial conduction block. Either of these hypotheses would account for the unexpectedly low inherent rate of pulsation of the segment.

It is interesting to note that the response of the embryonic myocardium of the chick to epinephrine is essentially the same as that of the ventricle of the adult human heart in cases of complete heart block. Concerning this condition, Goodman and Gilman4 state that there is a direct correspondence between the drug induced acceleration of the ventricle and the initial rate, such that the slower the original rate, the more pronounced is the accelerating effect of epinephrine. When the initial rate is high, epinephrine has little further action on it. This behavior is not found in the case of the atrial contractions of a heart in complete heart block. It is perhaps significant that the rate of the atrial contractions is set by the rate of the sino-atrial node, a region morphologically and physiologically distinct from the rest of the cardiac muscle, and derived embryologically.
from the right horn of the sinus venosus. This region, moreover, even in a case of complete heart block, would still be under the influence of nervous regulation.

Thus the myocardium, even during its first hours of pulsation, long before it receives its autonomic innervation, is capable of responding to this sympathomimetic drug in a manner quite comparable to that of the adult myocardium. It seems unnecessary to assume any qualitative changes in the myocardium in this respect between the time of its first pulsation and the time of its innervation. The characteristic myocardial response to epinephrine is present in essentially its adult condition in the epimyocardium of the young embryo during its prenervous phase of pulsation.

It is obvious that the conclusions to be drawn from this study with regard to the effectiveness of epinephrine in stimulating the embryonic myocardium differ markedly from the results of the work of Fuji, Markowitz and Nordmann and Rüther, and agree, at least qualitatively, with those obtained by Hsu. The work of Nordmann and Rüther and that of Markowitz was done on explanted fragments of embryonic myocardium in tissue culture. The present tests were carried out in sugar-free Ringer-Locke solution to avoid the unmeasurable effects of the tissue extracts used in culture media, and to eliminate the necessity of evaluating the effects of possible metabolic changes which might be undergone by the myocardial cells as they proliferate in tissue culture.

Hsu determined the effect of epinephrine by transferring the cardiac tissue to be observed between the test concentration of the drug and a control saline environment. It was to avoid this mechanical stimulation as a possible source of error that the tissues in the present experiments were subjected to as little mechanical disturbance as possible during the tests. The photographic recording of the contractions of the cardiac segments in the present series of experiments also allowed one to measure the time between each pulsation of the tissue before, during, and after the addition of the epinephrine, thus allowing one to observe and measure even a transitory phase of acceleration, which might otherwise have been overlooked.

**Summary**

Epinephrine in concentrations as dilute as 1 part in 10,000,000 in Ringer-Locke’s solution was applied to entire hearts and segments of hearts of chick embryos between the ages of 40 hours and 7 days of incubation. It was found that epinephrine caused an abrupt acceleration of slowly beating tissues, reaching a maximum rate within a few beats. Usually within thirty seconds this rapid rate began to decrease until in ten to fifteen minutes it had leveled off to a rate intermediate between the initial and the maximal rate. This deceleration was not due to a decrease in the stimulating effect of the drug applied.

The degree of the first acceleration depended upon the initial rate of pulsation before the drug was added. The slower the initial rate, the greater was the per cent acceleration. When the initial rate was faster than 180 beats per minute, the addition of epinephrine produced no significant acceleration. This relationship held true for entire hearts and segments of hearts regardless of the age of the embryo from which they were taken.

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Circulation. 1950;1:1362-1368
doi: 10.1161/01.CIR.1.6.1362
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/1/6/1362

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