PHYSIOLOGICAL ONTOGENY.

A. CHICKEN EMBRYOS.

XIII. THE TEMPERATURE CHARACTERISTIC FOR THE CONTRACTION RATE OF THE WHOLE HEART.

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In a former paper in this series (1), Murray reported a study of the temperature characteristic of explants from auricles and ventricles of chicken embryos which was undertaken with a view to ascertaining whether a relation exists between the age of the embryo and this measurement. In the case of the explants he failed to find the relation. Fragments from hearts of embryos of the same age yielded different values and those of different ages, values which did not lend themselves to a characteristic arrangement. Murray suggested the possibility that if the observations were extended to a study of the whole embryo, in which the activities of all the structures were interrelated, greater uniformity of behavior might be detected. Crozier and Stier (2) have since reviewed Murray’s conclusions. They doubt the probability of obtaining consistent values for the temperature characteristic $\mu$ in preparations such as Murray used in which the absence of a controlling focus permits the independent and alternating activity of a number of non-related pace makers, that correspond neither in their rates nor in their internal metabolic activities. They think significant results are less likely to be encountered in studying organs of intact animals, but “suggest their probable occurrence in the heart rhythms of embryos.” They suspect that this is the state of affairs because in developing Limulus there is “different chemical control of heart pulsation in embryo and in adult Limulus,” and because of “the relative diversity of pace-making control in the developing embryos.”
Meanwhile experiments have been in progress designed to discover whether such changes exist in developing chicken embryos and to test the utilizability of the temperature characteristic in describing these changes in activity with age. This report has been delayed on account of difficulties encountered first in changing the temperature of the preparation uniformly and second in obtaining its measurement.

Technique.

The embryos were grown and their ages ascertained according to methods described in earlier papers in this series. To count the pulsation of the heart,

**TABLE I.**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>No. experiments</th>
<th>Average rate of contractions</th>
<th>Standard deviation</th>
<th>Average temperature characteristic</th>
<th>Standard deviation</th>
<th>Average temperature coefficient</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7</td>
<td>182</td>
<td>19.87</td>
<td>12,400</td>
<td>1,100</td>
<td>2.1</td>
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<td>4</td>
<td>11</td>
<td>191</td>
<td>21.10</td>
<td>12,700</td>
<td>1,970</td>
<td>2.3</td>
<td>0.2</td>
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<tr>
<td>5</td>
<td>6</td>
<td>210</td>
<td>52.60</td>
<td>12,900</td>
<td>1,590</td>
<td>2.1</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>221</td>
<td>11.70</td>
<td>10,800</td>
<td>1,640</td>
<td>1.8</td>
<td>0.15</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>220</td>
<td>12.49</td>
<td>8,700</td>
<td>270</td>
<td>1.5</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>239</td>
<td>9.64</td>
<td>8,300</td>
<td>1,500</td>
<td>1.5</td>
<td>0.1</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>213</td>
<td>5.47</td>
<td>7,400</td>
<td>1,000</td>
<td>1.5</td>
<td>0.7</td>
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<tr>
<td>12</td>
<td>4</td>
<td>224</td>
<td>10.29</td>
<td>8,600</td>
<td>1,400</td>
<td>1.6</td>
<td>0.1</td>
</tr>
<tr>
<td>13</td>
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<td>245</td>
<td>7,600</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>15</td>
<td>5</td>
<td>242</td>
<td>7.48</td>
<td>6,300</td>
<td>200</td>
<td>1.3</td>
<td>0.04</td>
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<tr>
<td>18</td>
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<td>200</td>
<td>7,300</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* At 38°C.

small windows were cut in the shell, care being taken to avoid hemorrhage; if visible hemorrhage occurred the embryo was discarded. The egg was placed on the stage of a microscope and the whole enclosed in a small thermostat, the temperature of which was altered by the opening and shutting of one or more of several doors. Petri dishes full of water were kept in the chamber. The eggs lost no weight in the operation. The site at which pulsations either of the heart itself or of a prominent vessel, were counted was illuminated by a beam of light brought to a focus at that point. The light passed through a chamber in which water was kept flowing, to absorb heat.

The temperature to which the embryo and the heart itself were exposed was...
measured with a thermocouple and galvanometer devised by Clark (3). By the use of this device the accuracy is correct to 0.04°C.; the temperature could be taken at a distance only 1 to 2 mm. removed from the heart. The lag often encountered in experiments in which the sensitiveness of the heart to change in temperature can be detected by a change in its rate before the mercury thermometer registers the new level, is avoided. Counts of the heart rate were made several times both with rising and falling temperature for half minutes, the intervals being sounded by an electric buzzer in series with a chronograph. Cases in which there was conspicuous lack of uniformity of behavior were omitted.

![Graph showing the relation of contraction rate to temperature for embryos of different ages.](image)

**Fig. 1.** The relation of the contraction rate to the temperature is presented for all the experiments performed in embryos 3, 4, 10 and 15 days old.
from the series. On account of the rapidity of the temperature adjustment the duration of the observation could be correspondingly brief. To avoid injury the temperature range was confined to limits between 33° and 39°C. This range is short, but to exceed it might subject the hearts to injury and as a result interfere with repeating the observations.

![Graph showing the relation of the contraction rate to temperature](image)

**Fig. 2.** The relation of the contraction rate to the temperature is shown for one experiment each in embryos 3, 4, 10 and 15 days old.

For the calculation of the temperature characteristic the equation of Arrhenius was used in the form given by Murray (1):

$$\mu = 4.61 \frac{\log K_2 - \log K_1}{\frac{1}{T_1} - \frac{1}{T_2}}$$
Observations.

Observations were made in embryos 3, 4, 5, 6, 8, 10, 11, 12, 13, 15 and 18 days old (Table I). The results obtained at ages of 3, 4, 10 and 15 days are reproduced graphically in Fig. 1. Except at the age of 4 the curves at each age present a fair degree of similarity. The details of a single experiment at each of these ages is given in Fig. 2. As in the case of Murray's experiments there was at a given age no striking uniformity; in two cases the fluctuations were as wide (8,300 to 15,900 \( \mu \)) as that which he found in fragments of 8 day auricles. In general there appears to be a reduction in the temperature characteristic, whether the highest, lowest or the average values for each age are compared (Fig. 1). Instances in which \( \mu = 14,000 \) were found in the case of 3 and 4 day embryos only, but in four only of the eighteen experiments.

The rate of the heart at 38°C. was taken in each case; the averages are given in Table I. There appears to be a slight change with time. The results exhibit a considerable difference between these and those found in a former series (4). The difference may be due to three factors, at least. In the earlier experiments, temperature regulation was in the first place less satisfactory than in the present series, the thermometer was read at a distance farther removed from the heart and there was a certain amount of evaporation of water from the egg in the course of the observation. These errors were all avoided in the present series. When the curves for heart rate seen in these experiments were extended upward to 40°C. and downward to 30°C., theoretical contraction rates were obtained so that the temperature coefficient \( Q_{10} \) might be calculated. In this there was a slight fall though somewhat irregular from 2.1 at 3 days to 1.5 at 18 days. Obviously the hearts exhibited much more uniform behavior than did the auricular fragments (1).

Discussion.

The curves which serve as a basis for calculating the temperature characteristics in intact chicken embryos approach more closely to the form usually found in such calculations than do those taken from fragments of the auricles and ventricles. Curves drawn through the
marginal rates (2) are parallel with the best average curve. Although there is less variation at a given age in the undisturbed hearts than in fragments taken from them, a certain amount of variation nevertheless exists. Whether this variation is due, as Crozier and Stier (2) suggest, to "diverse pace-making processes in the hearts of different embryos," cannot of course yet be known. Their mention of the possibility occurs in connection with isolated heart preparations. These experiments indicate that at a given age, even when the rates at 38°C. are similar, there may be variations in the temperature characteristic. That the fluctuations are not great in most instances is apparent. A striking point consists in the manner in which a change with age takes place (Fig. 3). There is perhaps too great a range of values at any age to permit the data to do more than suggest this possibility. But that this result might be anticipated arises from considering the changes in rate which occur and from a knowledge, though still incomplete and inexact, of the organization of heart muscle tissue which is synchronously taking place. The results obtained are otherwise not inconsistent with those collected by Crozier (5).

Fig. 3. A curve indicating the alteration of the temperature characteristic, \( \mu \), with time is shown. It represents averages of all the data at each age. It exhibits a systematic change although at a given age the range of individual observations is large.
The relation of heart rate frequencies to temperature in intact chicken embryos has been studied and the temperature characteristics calculated for each of a number of ages. These have been found to vary from 14,000 or better 12,000 \( \mu \) for embryos 3 days old, to about 6,000 \( \mu \) for others of 15 days. There appears to be a systematic change with time. If this inference is correct, important correlation with other properties of the pace-making function in the intact heart should become possible.

BIBLIOGRAPHY.