Several epidemiological studies have shown a positive correlation between viral diseases early in pregnancy and congenital heart disease in human beings. Since viral diseases are accompanied commonly by fever, it was considered important to investigate the effect of high temperature alone on the development of the heart in chick embryos. It was also thought that incubation at low temperature might delay the development of certain embryonic tissues, particularly the heart. In the very few experiments in which the effect of low temperature on development has been studied, congenital heart defects were not mentioned. The results described here indicate that high temperature does not induce cardiac abnormalities in chicks but that low temperature does.

Methods

Eggs were obtained from healthy young white leghorn hens that were fed a balanced diet and had been immunized against Newcastle disease (dead virus preparation) and fowl-pox.

Incubation of Control Group

A control group of 200 eggs was incubated at 37.65°C and constant humidity of 86 to 87%. The eggs were rolled over once every two hours. After 18 days of incubation the air vents of the incubator were opened, but both temperature and humidity were still kept constant.

Incubation at Different Temperatures

A batch of 200 eggs was incubated at 39.44°C and a second batch of 100 eggs was incubated at 35.83°C. These temperatures were selected because they are still compatible with the development of chick embryos and because they differ from normal by the same value. Apart from this difference of temperature all the conditions of incubation were identical to those of the control group.

All incubated eggs, control and experimental, were observed once every two days and each time only the dead embryos were reviewed. Ages of embryos were calculated according to the classification of Hamburger and Hamilton. Those with a morphological age under stage 22 were block-stained in hematoxylin. The external features of the embryos beyond stage 22 were observed and their hearts were microdissected. The atrial and ventricular cavities, the valves, the great vessels, and the interventricular septum were studied.

Chicks hatched from the eggs used in these experiments were sacrificed periodically and their hearts were observed and dissected with study of the same structures as in the embryos.

Results

Control Group

This group of embryos incubated under optimal conditions, i.e., 37.65°C, provided a base line for the interpretation of findings in the other groups, and served as a method of determining whether the white leghorn hens, whose eggs were used, carried any genetic factor capable of inducing congenital heart defects. In this control group 187 of the 200 eggs were fertile; 26 embryos died during incubation; 161 chicks were hatched (tables 1 and 2); and the mortality distribution was normal (fig. 1). The frequency of congenital heart defects among the dead embryos was 3.84%. The heart malformation found in one embryo was a lack of fusion of the primitive cardiac tubes, and not a septal defect. The frequency of malformations in other structures and systems was 15.38%.
(table 3). The first of these figures rules out the presence of genetically borne congenital heart defects. In the 161 chicks hatched from this group of eggs, no heart defects were found (table 2).

GROUP INCUBATED AT 39.44°C

Among the 200 eggs incubated at 39.44°C, 179 were fertile; 177 embryos died during incubation; and two chicks were hatched (tables 1 and 2). The mortality distribution was very abnormal, both the early and the late normal mortality peaks were extremely high (fig. 1). The mortality rate was significantly greater than normal \( (P < 0.0001) \), whereas the frequency of congenital heart defects, 2.82% in this group, was not significantly different from that of the control group \( (P = 0.80) \). Malformations in other structures and systems are listed in table 3. The table shows that the only significant difference was in the incidence of ventricular septal defect, which was much higher in the 39.44°C group than in the control group.

### TABLE 1

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Fertile eggs</th>
<th>Dead embryos</th>
<th>Per cent mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal, 37.4°C</td>
<td>187</td>
<td>26</td>
<td>13.9</td>
</tr>
<tr>
<td>High temperature, 39.4°C</td>
<td>179</td>
<td>177</td>
<td>98.8</td>
</tr>
<tr>
<td>Low temperature, 35.83°C</td>
<td>89</td>
<td>57</td>
<td>64.04</td>
</tr>
</tbody>
</table>

*Apart from temperature, incubation conditions were identical to those used for the control group.

### TABLE 2

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Number of chicks</th>
<th>Number with congenital heart defects</th>
<th>Number with malformation of other structures and systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ventricular septal defect</td>
<td>Other heart defects</td>
</tr>
<tr>
<td>Optimal conditions, 37.65°C</td>
<td>161</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High temperature, 39.4°C</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Low temperature, 35.83°C</td>
<td>32</td>
<td>6 (18.75%)</td>
<td>1 (3.12%)</td>
</tr>
</tbody>
</table>

*Apart from temperature, incubation conditions were identical to those used in the control group.

### TABLE 3

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Number of embryos</th>
<th>Number with congenital heart defects</th>
<th>Number with malformation of other structures and systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ventricular septal defect</td>
<td>Other heart defects</td>
</tr>
<tr>
<td>Optimal conditions, 37.65°C</td>
<td>26</td>
<td>—</td>
<td>1 (3.84%)</td>
</tr>
<tr>
<td>High temperature, 39.4°C</td>
<td>177</td>
<td>—</td>
<td>5 (2.82%)</td>
</tr>
<tr>
<td>Low temperature, 35.83°C</td>
<td>57</td>
<td>14 (24.5%)</td>
<td>6 (10.5%)</td>
</tr>
</tbody>
</table>

*Apart from temperature, incubation conditions were identical to those used in the control group.

†Embryos incubated at 39.44°C died at stages of development when this lesion would have been detected easily.
systems were found in 11.86% of the embryos and this value was not significantly different from normal \((P = 0.67)\). These data can be seen in table 3.

In contrast to the normal incidence of cardiac defects, abnormalities of the nervous system and of the eyes, as well as abnormal torsion of the longitudinal axis of the body, were found in 11.29% of the dead embryos in this group (fig. 2). These findings are similar to those produced by certain viruses.\(^{10-13}\) No such malformations were seen in embryos of the control group or in those incubated at 35.83°C.

The two chicks hatched from this group were normal (table 2).

**GROUP INCUBATED AT 35.83°C**

Eighty-nine of the 100 eggs in this group were fertile; 57 embryos died during incubation; and 32 chicks were hatched (tables 1 and 2).
The second mortality peak, near the end of incubation (fig. 1), was much increased and produced a significantly increased mortality rate ($P < 0.0001$). The frequency of congenital heart defects in this group was 35%, which is also significantly different ($P = 0.002$) from the control group (table 3). Interventricular septal defect was present in 24.5% of the embryos in this group. The significance of this figure is obvious, because this malformation was never found in the control group (table 3). The difference between the frequency of interventricular septal defects and that of other cardiac malformations within this group was also significant ($P = 0.04$).

The frequency of malformations in other structures and systems, which was 12.3% in this group, is not significantly different from that in the control group ($P = 0.70$). In particular, no abnormalities of the nervous system or of body axis torsion were found (table 3).

The 32 chicks hatched from eggs in this group were hatched two days after the expected date (fig. 1). Most of them were sacrificed after reaching adulthood. Six (18.75%) had an interventricular septal defect. Again, the significance of this figure is obvious because no such lesion was found among the chicks hatched from the eggs in the control group (table 2). As in the case of the embryos, the frequency of interventricular septal defect differed significantly ($P = 0.05$) from that of other heart malformations.

The adult roosters and hens with interventricular septal defects always had a prolapse of one aortic sigmoid cusp into the septal defect (fig. 3A), whereas this prolapse was not seen in the embryos and newborn chicks with septal defects (fig. 3B).

**Discussion**

Elevation of temperature to 39.4°C was not teratogenic with respect to the heart. The conclusion that no congenital heart lesions were found is definite because the mortality peak for this group appeared after the sixth day of incubation. At that time the development of the heart and great vessels is complete and any abnormality would have been detected easily.

Incubation at 35.8°C was followed very frequently by the presence of a high interventricular septal defect. Since this defect is due to arrested development, it might be inferred that incubation at a reduced tempera-
ture leads to a delay of those growth processes that are involved in the closure of the basal part of the interventricular septum. Other investigators, working at temperatures below 35.83°C, have, in fact, observed a considerable delay in the appearance of other embryonic structures, such as the primitive streak and Hensen’s node. It is possible that agents capable of producing a delay in the development of mammalian embryos might produce the same heart malformations found among the chick embryos incubated at 35.83°C. In fact, Ingalls et al. found interventricular septal defects in mouse embryos when they were submitted to five hours of anoxia daily, from 1.5 to 16.5 days of pregnancy. In their experiments anoxia, through a delay in development, could be responsible both for the septal defect and for the other anomalies he observed, such as anencephaly, hemivertebra, fused ribs, cryptorchidism, cleft palate, open eye and spina bifida.

Rychter et al. and Siller found “spontaneous” interventricular septal defects among brown leghorn chicks, and they proved that this defect was genetically transmitted. The absence of this malformation in our control group rules out this possibility for the embryos and chicks we studied which were of the white leghorn strain.

Finally, the adult roosters and hens that had an interventricular septal defect presented also a prolapse of one aortic sigmoid cusp through the septal defect, whereas this abnormality was never seen in embryos or newborn chicks with the septal defect. This malformation is apparently acquired and is secondary to the sustained pressure to which the aortic cusps are subjected during diastole and to the lack of support at the site of the septal defect.

The high mortality rate near the end of the incubation at 39.44°C could be due to dehydration, because humidity was maintained at the same level as when incubating at 37.65°C, instead of being increased in proportion to the elevation of temperature. Dehydration is probably most harmful just prior to hatching, when the extra-embryonic fluids have been reabsorbed. In fact, the embryos dying at this late stage of incubation were normal, so their death should be attributed to dehydration rather than to any malformation produced by the high temperature. On the other hand, the embryos with malformations of the central nervous system all died before the fifth day of incubation, with a normal amount of extra-embryonic fluid. These lesions are consequently attributed to the high temperature, rather than to dehydration. These malformations are similar to those produced experimentally by influenza A virus and by Newcastle disease virus. This agrees with the conclusions of Stockard and Wilson that different teratogenic agents acting at the same stage of development can lead to similar patterns of malformation.

Summary

The effect of incubator temperature on mortality rates and on appearance of congenital malformations in white leghorn chicks and embryos was studied. The elevation of incubation temperature to 39.44°C led to a high mortality and to malformations in the eyes and the nervous system, as well as to abnormal torsion of the body axis. It did not produce any cardiac abnormalities, which would have been detected easily because the mortality peak occurred after the development of the heart was complete. Decreasing temperature of incubation to 35.83°C produced a high mortality rate and, very frequently, interventricular septal defects. These results and the possible mechanisms by which changes of temperature exert teratogenic effects are discussed.

References


Congenital Heart Defects in Chick Embryos Subjected to Temperature Variations
María V. de la Cruz, Carlos Campillo-Sáinz, Simón Muñoz-Armas, Guillermna Pallares and Rafaela Zamudio Bandera

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