The morphologic expression of each cardiac primordium in the chick embryo

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SUMMARY

The cardia bifida in amphibians has the bulboventricular loop of the two hemi-hearts convex towards the mid-line. In birds the two loops have been reported to be convex towards the outside because all the experimental methods of producing cardia bifida in birds, unlike amphibians, impede the closure of the foregut.

The morphologic expression of each cardiac primordium was studied without altering endodermal tubulation or altering it only to the least possible degree. It has been demonstrated that the cardia bifida in birds has the two bulboventricular loops convex towards the mid-line and that the heart loops formed by only one primordium also tend to be convex towards the mid-line. Previous studies have been confirmed in the sense that the caudal portion of the left hemi-heart is the more prominent than the right one and that the left hemi-heart has faster beating rate than the right one until stage 12.

When the caudal portion of the left cardiac primordium was removed at stage 9 of Hamburger & Hamilton, in all cases a loop convex to the right was formed. This fact excludes that this portion of the left primordium is the origin of the right bending of the cardiac tube.

INTRODUCTION

The first appearance of asymmetry in the embryo is the bending of the bulboventricular loop. For a long time the causes of this bending have interested researchers (Hensen, 1876; Patten, 1922; Davis, 1927), but up to now a completely satisfactory explanation of this has not been found (Stalsberg, 1970).

It has been known since Hensen (1876) that the heart develops from two primordia which fuse at the mid-line to form a single ventral heart. In birds the cardiac primordia have been studied extensively (Yoshida, 1932; Rawles, 1943; Orts Llorca & Ruano Gil, 1967), being mapped by chorioallantoic grafts (Rawles, 1943) and autoradiography (Rosenquist & DeHaan, 1966; Stalsberg & DeHaan, 1969), and identified biochemically by Ebert (1953). An attempt has been made, by means of these maps, to explain the contribution of each primordium in the formation of the normal bulboventricular loop (Stalsberg, 1969).

The morphogenetic capacity of each primordium has been studied in amphibians

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(Spemann & Falkenberg, 1919; Ekman, 1925; Copenhaver, 1926; Bacon, 1945; Fales, 1946), in birds (Gräper, 1907; DeHaan, 1958a, b, 1959; DeHaan & Carter, 1959; Orts Llorca, 1964; Van Praagh & DeHaan, 1967; Lepori, 1967) and in mammals (Goss, 1935; Dwinnell, 1939), but the results obtained, especially in birds, have been confusing because of the experimental models utilized. In amphibians the fusion of both cardiac primordia at the mid-line can be prevented without damaging the foregut and without affecting endodermal tubulation, but in birds all of the experimental models used impede the closure of the foregut either mechanically, by microsurgery (Gräper, 1907; DeHaan, 1959; DeHaan & Carter, 1959; Van Praagh & DeHaan, 1967; Lepori, 1967), or chemically (DeHaan, 1958a, b). The latter cause great alteration of morphogenesis of the embryo, leading more to formation of pulsing vesicles than to true hemi-hearts, having good morphologic differentiation. When the closure of the foregut is impeded by mechanical methods the great alteration that the embryo undergoes when endodermal tubulation is not produced introduces new factors which make the interpretation of the results difficult.

In amphibians the loop of each of the hemi-hearts is convex toward the mid-line, as a result of which it has been said that it is the left primordium which develops a normal loop (convex to the right) and the right one an inverted loop (convex to the left) (Copenhaver, 1955). On the other hand, in birds both loops are convex outward and concave toward the mid-line (DeHaan, 1959; Van Praagh & DeHaan, 1967; Lepori, 1967), and therefore the normal loop would be the right one. Nevertheless, in many publications on normal bulboventricular loop in birds, it is said that it is the left primordium which produces the normal loop, without noting the morphological differences between cardia bifida in birds and in amphibians (DeHaan, 1959; Stalsberg, 1969).

Recently a theory has been proposed on the bending of the normal and inverted bulboventricular loop, based in part on the morphological aspects of the cardia bifida (Lepori, 1967). This theory, together with the morphological differences between cardia bifida in birds and in amphibians reported to date, led us to plan an experiment in which the morphological expression of each of the cardiac primordia could be studied without altering endodermal tubulation or altering it only to the least possible degree. In addition, the time of the beginning of the beats and the heart rate of each of the hemi-hearts of most of the operated embryos were recorded.

**MATERIALS AND METHODS**

Fertile Rhode Island hen eggs were used, incubated at 37.5 °C, humidity 86–87 %, until they reached stage 8 of the Hamburger & Hamilton classification (1951). The embryos were explanted and cultivated by New's technique (1955), endoderm up, and were reincubated in a Hot Pack incubator at 37.5 °C, 5 % CO₂ pressure and humidity 100 %. When they reached stage 9 they were experi-
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mented on and then reincubated up to stage 12, after which they were fixed and stained with light green.

The experiments were divided into five groups (Fig. 1): Group I: control group. In 25 stage-9 embryos the heart tube was exposed by dissecting the yolk-sac membrane with fine glass needles, making longitudinal cuts on both sides of the embryo and joining them at the level of the subcephalic fold. Then the membrane was folded toward the caudal part and was allowed to develop until stage 12.

Group II: cutting of the foregut in stage 9. In 100 stage-9 embryos, after performing the same dissection as in the control group, the foregut was slit to the level of the subcephalic fold by means of a fine glass needle according to DeHaan’s technique (1959) and then the embryos were reincubated to stage 12.

Group III: removal of the left primordium in stage 9. In 60 stage-9 embryos the fusion site of both cardiac primordia was exposed following the previously mentioned technique, then the left primordium was removed at its fused part as well as a large part of the left cardiac mesoderm which had not yet fused, by means of fine dissection with a glass needle. In stage 10 or 11—most of the embryos had to be re-operated upon to remove the most caudal part of the left cardiac primordium which sometimes remained after the first operation, before this part could fuse with the contralateral primordium. The dissection of the fused part was performed by separating both primordia at the level of the fusion line and by cutting the primordium at the level of the floor of the foregut, without injuring it.

Group IV: removal of the right primordium in stage 9. The same operation as in the previous group was performed on 42 embryos, removing the right primordium. They were also operated upon in stage 10 or 11— to remove the caudal part of this primordium which may have remained after the first operation.

Group V: removal of the caudal part of the left primordium in stages 9, 9+, and 10−. Twenty-five embryos of each of these stages were operated in the same way as in the previous groups, but only the unfused caudal part of the left primordium was removed. They were re-operated at stage 11— to remove the part of this primordium which may have remained after the first operation.

Serial histological sections were made in some embryos of each of the five groups in order to study the location and relative morphology of each of the hemi-hearts.

In most of the operated embryos the initiation time of the contractions of each or both primordia was recorded and the heart rate was counted before fixing. In the instance of the cardia bifida, it was observed in which of the two components the contraction began and if both hemi-hearts beat synchronously or not.

Drawings and photographs were made of the embryos to study the morphology and relative size of both hemi-hearts, especially in the cephalic and caudal parts.
RESULTS

GROUP I. The 25 embryos in which the yolk-sac membrane was dissected to expose the heart tube developed normally to stage 12 from the morphological as well as the functional points of view, since they began to beat at stage 10 and the heart rate and contraction were normal.

GROUP II. Of the 100 embryos operated upon 82 developed to stage 12, forming a cardia bifida, that is, two hemi-hearts which were joined by the cephalic
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Fig. 2. Cardia bifida at stage 12. It is evident that both hemi-hearts are convex to the mid-line.

end in the zone corresponding to the truncus. In most of the cases (93 %) the left hemi-heart began to beat first. In stage 12 before fixing, the right hemi-heart had a more rapid rate than the left in 18 %, while it was more rapid in the left in 64 %. In 18 % both hemi-hearts beat synchronously, although in one case contraction began in the caudal part of the right hemi-heart and in the rest (17 %) in the caudal part of the left hemi-heart. In these stages the slowness of propagation of the contraction wave made it relatively easy to know the approximate site of the pacemaker without need of electrophysiological studies.

In 82 embryos (100 %) both loops were convex toward the mid-line (Figs. 1, 2). In those cases in which the foregut was very open and the caudal part of both loops very laterally placed, growth of the loop created a very marked curvature toward the caudal part, but even in these cases it could be recognized that they were convex toward the mid-line and that distortion of the shape was due to the large opening of the foregut. In many cases both hemi-hearts had a normal myocardial mantle over all of their surface, but in others it was thinner in the mid part, especially in the region corresponding to the primitive ventricle, possibly as a consequence of the slit performed at stage 9.
Fig. 3. Embryo of group III at stage 12, in which the left cardiac primordium was removed at stage 9. The cardiac loop is concave to the left and the caudal portion is not prominent.

In 78·5 % the caudal part of the left hemi-heart was more prominent than the caudal part of the right hemi-heart; in 15·5 % both caudal parts were almost equal and in 6 % the caudal part of the right hemi-heart was more prominent.

The cephalic part was difficult to analyse because both hemi-hearts were joined in the part corresponding to the truncus, but in 27 % of the cases the part of the bulbus cordis of the right hemi-heart was more prominent.

In the serial histological sections it was observed that both hemi-hearts were located in the ventral part of the embryo and in the caudal part it was evident that the left hemi-heart was more voluminous.

Group III. Of the 60 embryos in which the left cardiac primordium was removed in stage 9 without injuring the foregut, only 35 developed to stage 12. In these embryos, the initiation time of the contractions was very irregular, varying between stage 10+ and 11+. The heart rate varied around 65/min. Thirty-two embryos (91 %) developed a bulboventricular loop toward the left (inverted loop), one formed a very abnormal loop (loop with an undefined curvature), and two formed a loop toward the right. In all the cases the morphology of the curvature was very similar to that of the inverted loops, although
the size of the loop was less than in the control hearts, especially in the caudal part, since the part corresponding to the primitive ventricle was not very prominent (Figs. 1, 3).

The serial histological sections showed that the foregut was intact, that the heart occupied a ventral position and that in most of the cases the left edge of the myocardial mantle has gaps that were filled by endocardium covered by cardiac jelly.

**GROUP IV.** Of the 42 embryos in which the right cardiac primordium was removed, 35 reached stage 12. In those embryos the beginning of the heart contraction was also very irregular, varying between stage 10+ and 11+. The average heart rate on reaching stage 12 was 63/min. All 35 embryos (100 %) developed a loop convex to the right (normal loop). The morphology of the curve was very similar to that of the bulboventricular loop of the control embryos and the caudal part, corresponding to the primitive ventricle, was well developed (Figs. 1, 4). In the histological sections the heart occupied a ventral position and in some of them the same gaps in the myocardial mantle were seen, this time located in the right edge of it and also occupied by the endocardium covered by cardiac jelly.
The cephalic parts of the hearts of groups III and IV in most cases were the same or had minimal differences, but in a small group the cephalic part of group III was greater.

GROUP V. Of the 75 operated-upon embryos, 68 reached stage 12. All developed a convex bulboventricular loop to the right, but with the caudal part less prominent than in the control embryos (Fig. 1).

DISCUSSION

The following conclusions were drawn from the results of the experiments reported in this study:

1. The dissection of the yolk-sac membrane (splachnopleure) did not affect the embryonic development, at least up to stage 12.
2. It is possible to produce cardia bifida in birds by mechanical means up to relatively advanced stages of cardiac development, such as stage 9.
3. The hemi-hearts developed a convex bulboventricular loop towards the mid-line and not toward the outer part as had been stated.
4. The more open the foregut is, the more the morphology of the loops is distorted and the more convex the loops become towards the caudal part.
5. Despite the differences in the direction of the loop, the cephalic part of the right hemi-heart and the caudal part of the left hemi-heart are more prominent than those of the contralateral hemi-heart, as has already been reported by other authors (Van Praagh & DeHaan, 1967).
6. In the first phases of cardiac development, the automatism of the left hemi-heart predominates, and diminishes progressively. This fact was evident in cardia bifida, but could not be proved in the series of embryos with a single primordium, perhaps because the operating shock was greater in these embryos.
7. The direction of the curve and the morphological aspect of the cardia bifida were corroborated in the embryos in which one of the cardiac primordia was removed without injuring the floor of the foregut. When the left cardiac primordium was removed, the right primordium formed a bulboventricular loop directed toward the left in 81 %, which was smaller than the control hearts and its caudal part was not very prominent (corresponding to the primitive ventricle). When the right primordium was removed, the loops which formed were directed to the right and had a prominent caudal part in 100 % of the cases.

Despite the fact that the first cardia bifida was obtained in chick embryos by Gräper in 1907, most of the studies on the morphological and functional expression of each separate cardiac primordium has been carried out on amphibians, because from a technical point of view, it is relatively easy to prevent fusion of both cardiac primordia by mechanical means, without injuring the foregut. On the other hand, in bird embryos cultivated in vitro the two methods that have been used to prevent fusion of both cardiac primordia have been:

1. Treatment by chemical means (DeHaan, 1958a, b) which damaged the
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Fig. 5. A shows the morphology of the cardia bifida in the chick embryo reported up to now. The arrows indicate the supposed movement of the hemi-hearts to obtain the morphology indicated in B. In A the embryo is flat and in B it has an almost normal tubular morphology.

endoderm and prevented the closure of the intestine, produces great disturbances in the morphologic as well as functional development of the hemi-hearts.

(2) Microsurgery by cutting the foregut of Hamburger & Hamilton stage 8—and 8 (DeHaan, 1959; Van Praagh & DeHaan, 1967; Lepori, 1967). In these cases development of the nervous system, notochord and somites continues rather normally, although somewhat delayed regarding control embryos, but when the endodermal tubulation is prevented, a great alteration in the space relationships of the developing organs is produced. The parts of the embryo which should lie in the ventral mid-line are located in the lateral edges of the embryonic body, with their dorsal faces lying ventrally. In this way, all the cases of cardia bifida which have been obtained in the chick embryo have the convexity of the bulboventricular loops directed outward, exactly opposite what happens in cardia bifida of amphibians which have their loops convex toward the mid-line. If we suppose that these chick embryos in which cardia bifida has been induced by opening the foregut had undergone a normal endodermal tubulation, it is evident that both lateral edges of the embryonic body would have united at the ventral mid-line and then both bulboventricular loops would have been convex toward the mid-line with their ventral faces located dorsally (Fig. 5). This hypothesis is plainly confirmed by the present results since when the foregut is opened in a more advanced stage of development, endodermal tubulation has already progressed enough so that despite the operation, the embryo keeps its tubular shape. Thus the heart lies ventrally and maintains its space relationships. Since the caudal part is more separated from the mid-line than in normal hearts, the loops tend to become somewhat horizontal and the morphology obtained is not exactly the same as in the amphibian, because in our experimental model, devices created by the opening of the foregut still persist. In any case, each of the primordia tends to form a convex loop toward the mid-line, as has also been demonstrated when one of them is removed without injuring the foregut. Therefore, the morphology of cardia bifida and hemi-hearts formed by each primordium in birds and amphibians is identical and not opposite as
had been reported up to now, and in both species it is the left primordium which forms a bulboventricular loop convex toward the right.

Although the morphology obtained in our experiments is opposite to what had been reported up to now, the findings of Van Praagh & DeHaan (1967) have been confirmed in the sense that the caudal part of the left hemi-heart and the cephalic part of the hemi-heart are more prominent, which agrees with the fact that in the normal loop, the contribution of the right primordium is predominant in the cephalic part, and the left primordium in the caudal part (Stalsberg & DeHaan, 1969; Castro-Quezada, Nadal-Ginard & de la Cruz, 1972). It has also been confirmed that the automatism of the left primordium is greater and appears earlier, as had been reported by DeHaan (1959).

Lepori (1967), recently has put forward a theory to explain normal and inverted bending of the bulboventricular loop, in part based on the fact that both hemi-hearts have convexity toward the external part and convacity toward the mid-line. For this author, curvature into a C-shape of the two primordia occurs asynchronously, with the curvature of the left primordium more rapid, which leads to insertion of its posterior end inside the concavity of the right primordium this being 'the primary event accounting for right-side bending of the cardiac tube'. In the case of inverted loops it is the right primordium which curves inward more rapidly and its caudal part is placed inside the concavity of the left primordium. From the results obtained in our experiments, it is impossible that the caudal part of either of the two primordia enter inside the concavity of the other, because even supposing that both primordia begin to curve inward before fusing at the ventral mid-line, the tendency of each of them is to form a convexity toward the fusion line.

The innermost cause of bending of each of the primordia and that this is done with the convex part toward the mid-line is unknown and the intrinsic factors such as cell multiplication rate in the different regions of the heart must be studied.

From the results of these experiments it is difficult to come to any conclusion regarding the contribution of each of the two primordia to the formation of the normal bulboventricular loop since although the caudal part of the left primordium seems to be very important in the accentuation of the curvature, it is evident that it is not the cause of it because when it is removed as in group V experiments it continues forming a convex loop to the right. The cephalic part of both primordia, either through factors such as the different cell multiplication rates and differential cellular adhesiveness (etc.), plays an important role in bending which has not been clarified in this study.
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REFERENCES


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