EXPERIMENTAL STUDIES ON THE CIRCULATORY SYSTEM OF THE LATE CHICK EMBRYO

BY PETER T. WHITE*

Department of Biology, Guy's Hospital Medical School, London SE1 9RT

(Received 16 April 1974)

SUMMARY

A method of preparing the 15/17-day chick embryo for physiological studies is described and the results of an investigation of flow patterns in the circulatory system using oxygen determinations and dye injections is reported. A considerable separation of deoxygenated and oxygenated blood streams occurs in the incompletely divided heart such that a large part of the oxygenated stream flows through the perforated interatrial septum to the left heart for distribution to head, neck, thorax and heart. A mechanism also exists whereby the gut and yolk sac receive blood at an oxygen saturation greater than the remainder of the posterior body. Some quantitative estimates of blood and oxygen flows are made and the circulatory system of the late chick embryo is compared with that of the foetal mammal.

INTRODUCTION

The function of the cardiovascular system of the foetal mammal has been a subject for intensive study by physiologists since the early years of the present century (Barclay, Franklin & Pritchard, 1944; Barcroft, 1946). Detailed information is now available concerning the flow patterns of oxygenated and deoxygenated blood streams in the incompletely divided heart of the foetus and of their subsequent distribution to various parts of the circulatory system (Dawes, Mott & Widdicombe, 1954; Rudolph & Heymann, 1967; Assali, Bekey & Morrison, 1968). It has generally been assumed (Hamilton, 1952) that the circulatory system in the late bird embryo functions in a similar way to that of the mammal; although experimental evidence for this hypothesis, which was based upon anatomical studies, has not been available. Reasons for this are not difficult to find, for quite apart from the impetus provided for mammalian foetal research by its medical implications, the late bird embryo represents an unusually difficult object for physiological investigations.

The most readily available form of late bird embryo (15/17-day chick) is of very small size: the total blood volume in a 16-day embryo being about 2 ml (Barnes & Jensen, 1959). Moreover, the embryo is completely surrounded by its highly vascular respiratory membrane, the chorioallantois, which is in intimate contact with the shell. Any attempt to remove the embryo from the egg may result in severe haemorrhage or contraction of the umbilical and vitelline blood vessels, which are sensitive to mechanical stimuli. Should it prove possible to remove the embryo and its membranes

* Present address: Department of Zoology, Makerere University, P.O. Box 7062, Kampala, Uganda.
from the egg without undue trauma, immersion of the preparation in physiological saline may result in anoxia, for the respiratory membrane is denied direct access to atmospheric oxygen. Factors such as these can lead to irregularities of the heart beat which it is essential to avoid if meaningful results are to be obtained.

Some aspects of the anatomy of the circulatory system in the late chick embryo are reviewed by Hamilton (1952) and Romanoff (1960), although a comprehensive account of the system is not available. The heart rate (Cohn & Wile, 1925; Bogue, 1932; Romanoff & Sochen, 1936), the circulating blood volume (Yosphe-Purer, Fendrich & Davies, 1953; Rychter, Kopecky & Lemez, 1955; Barnes & Jensen, 1959) and the arterial blood pressure (Hughes 1942; Van Mierop & Bertuch, 1967) have been measured in the chick embryo at almost all stages of development. Bartels, Hiller & Reinhardt (1966) measured the oxygen affinity of the blood of the 17-day embryo and Dawes & Simkiss (1969) have investigated the acid base status of the blood throughout development.

Hughes (1949) measured the cardiac output of the chick embryo from 3 to 17 days incubation using a photographic method. All the older embryos were removed from the egg and laid in a dish prior to filming and Hughes noted the deleterious effect that this operation exerted upon the heart rate. Similar methods of preparation were used by Hait & Licata (1967) to investigate the sequence of atrial depolarization in the late embryo.

The present paper describes a method of preparing the 16-day chick embryo, for physiological studies, which allows the respiratory membrane to function normally and which exerts no obviously deleterious effect upon the heart rate. Results of an investigation into the course of circulation, made by tracing the passage of dyes through the heart and by measuring the oxygen content of blood samples taken from various points in the circulatory system, are reported.

**METHODS**

The embryos used in this study were obtained by incubating fertile white leghorn eggs for 16 days ± 1 day in a commercial still air incubator using standard procedures. They varied in developmental age between stages 40 and 43 of Hamburger & Hamilton (1951).

**Preparation of the embryo**

The extent of the air space was determined by candling, and the overlying shell was removed. The shell membrane was moistened with Locke's Ringer and peeled carefully away from the chorioallantois with fine forceps. A cut about 2 cm long was then made in the chorioallantois by means of a thermal cautery, the line of incision being chosen so as to avoid major blood vessels. Any haemorrhage which did occur at this stage was rapidly stopped by reapplication of the cautery.

The allantoic fluid was then poured off, exposing the embryo inside its amnion. By cutting the amnion and gripping the embryo's beak it was possible to draw it out through the incision in the chorioallantois and lay it on its back in a cork-lined dish containing Locke's Ringer maintained at 38–39 °C. The yolk sac was tipped out with the embryo, whilst the egg shell and its contained chorioallantois was supported in air at the side of the dish on a block of plasticine.
Circulatory system of the late chick embryo

Fig. 1. 16/17-day embryo prepared for physiological studies. U.B.V., umbilical blood vessels; V.B.V., vitelline blood vessels.

The completed preparation (Fig. 1) consisted of the embryo submerged in warm saline, attached by its vitelline blood vessels to the yolk sac, also in saline, and by its umbilical vessels to the chorioallantois which was undisturbed and occupied its normal position lining the shell. The only major difference from being inside the egg was that air was now present on both sides of the chorioallantois whereas formerly it was present only on the shell side.

The heart was exposed when necessary by making an incision in the midline, commencing in the abdomen and proceeding cephalad until the sternum was divided. The two sides of the thorax could then be drawn apart to expose the heart. Any minor haemorrhage, usually from the pectoral muscles, was stopped by a cautery.

In successful preparations of this kind the heart rate was always within the normal range of 200–240 beats/min (Romanoff, 1960) and would remain so for periods of at least 30 min and often for as long as 2 h. Blood flow through the chorioallantois was rapid, as seen through a shell window, and further evidence that the respiratory membrane was functioning normally was adduced from the fact that the blood returning to the embryo in the umbilical vein was bright red whereas that in the umbilical artery was much darker in colour. Similarly, the vitelline artery was usually redder than the vitelline vein and if the shell was experimentally submerged in saline these colour differences were rapidly abolished, the blood in both umbilical and vitelline vessels becoming uniformly dark in colour. It may be noted that although the egg shell and chorioallantois were maintained at room temperature (25 °C), the heart rate was within the normal range in spite of the cooling of the blood which must have occurred during passage through the respiratory membrane. It seems probable that the blood returning to the embryo from the chorioallantois was reheated to 38 °C during its passage through the 3–4 cm of umbilical vein connecting the embryo body to the shell (Fig. 1).

No anaesthetic was used in making these preparations, embryos incubated up to stage 43 (Hamburger & Hamilton, 1951) showing little or no reflex reaction in response to the operative procedures involved. This was not the case with older embryos and was one of the reasons which militated against their use, another being that the umbilical and vitelline blood vessels of such embryos tended to rupture during removal from the egg.
Only those embryos which had been ‘delivered’ without significant haemorrhage and in which the heart rate was within the normal range were used in the experiments to be described below.

**Anatomical studies**

Embryos prepared as above were perfused with a 1% solution of the dye Alcian Blue in Locke’s Ringer. This dye was found particularly useful as it did not stop the heart or extravasate, and moreover it stained permanently and intensely the walls of the blood vessels into which it passed. Sufficient dye was injected into the umbilical vein to permeate and slightly distend the vascular system, after which the umbilical and vitelline blood vessels were ligated and cut, and the embryo hardened in 10% formalin for some days prior to dissection under a binocular microscope. Selected portions of the vascular system were removed and cleared in glycerol for further examination and photography.

**Dye injections and cinematography**

In order to study the pattern of blood flow within the heart, embryos were injected with small volumes of a 1% solution of the dye Evan’s Blue in Locke’s Ringer and the heart filmed. The apparatus used to make injections was substantially similar to that of Simon’s (1956) and consisted of a micrometer syringe (Burrough’s Wellcome Ltd) holding the dye, connected by a length of rigid walled polythene cannula (0.5 mm bore) to the shaft of a 30-gauge hypodermic needle. The needle was fixed in a glass tube and held in a micromanipulator so that it could be inserted into a blood vessel and left in position. The volume of the dye bolus lay between 5.0 and 10.0 µl (0.25–0.5% blood volume), but in some experiments as many as six consecutive 10 µl injections were made into a single embryo. Injections were made into the umbilical vein at a point close to its entry into the embryo body or into the right external jugular vein in the neck (Fig. 2).

A Beaulieu R 16 cine camera fitted with a 50 mm lens and extension tubes was used to record the passage of dye through the heart. Lighting was provided by two Beck ‘Tenslites’ focused to give an area of intense illumination over the heart and the film was Kodak High Speed Ektachrome reversal colour film exposed at 48 frames per second with a lens aperture of f11. Processed films were examined with a Specto Analytic Projector and still photographs made from selected frames.

**Oxygen determinations**

The oxygen content of small (12 µl) blood samples, taken from two different vessels in a single embryo, was determined using a modified version of the method of Scholander, Flemister & Irving (1949); full details are given elsewhere (White, 1968). The following combinations of sampling points were chosen (Figs. 2, 6).

1. Umbilical vein–left ventricle
2. Umbilical vein–umbilical artery
3. Umbilical vein–jugular vein
4. Umbilical vein–vitelline vein
5. Vitelline artery–vitelline vein
6. Vitelline artery–left ventricle
7. Vitelline artery–umbilical artery
8. Jugular vein–vitelline vein
9. Right ventricle–left ventricle
10. Right ventricle–umbilical artery
11. Right ventricle–jugular vein
Circulatory system of the late chick embryo

The umbilical and vitelline blood vessels were sampled close to the point where they emerge from the body wall. Jugular samples were taken from the right external jugular vein in the neck. Right ventricular blood was obtained from the pulmonary arch before its division, and left ventricular blood from the aorta at a point below the origin of the brachiocephalic arteries. The combined volume of samples, about 30 μl, represented approximately 1% of the total blood volume. After paired samples had been obtained, a larger sample of some 0.5 ml was taken from the umbilical artery or the pulmonary arch for determination of oxygen capacity.

Sampling technique

The shaft of a 30-gauge hypodermic needle fixed into the end of a length of rigid walled nylon cannula (0.25 mm bore) was arranged so that it protruded from the end of a tapered glass tube, where it was fixed in position with sealing wax. The needle and cannula were flushed out with a 10% solution of sodium fluoride containing 1000 i.u. heparin/ml, after which the needle and the first few centimetres of cannula were filled with clean mercury. Samples were obtained by inserting the needle into the appropriate blood vessel under a binocular microscope and sucking blood up to a point on the cannula representing a volume of 15 μl. The needle was then removed from the vessel and a further volume of clean mercury drawn up to seal the sample from the atmosphere. Samples were stored in iced water prior to analysis and blood was transferred directly from the cannula to the Scholander syringe analyser.

Nylon is slightly permeable to oxygen, but the amount of oxygen which could enter a blood sample under experimental conditions was calculated from the permeability data for the type of nylon used and found to be negligible. Moreover, unoxidized pyrogallol stored in such cannulae was not visibly darkened after 1 h in air, a period of exposure far greater than that given to any blood sample.

In taking paired samples from a single embryo one needle would be inserted using a micromanipulator and left in position whilst the second was inserted by hand. By sucking both cannulae together it was sometimes possible to obtain simultaneous samples, but more often samples were taken consecutively within 10 sec of each other. In the latter procedure the sampling order was always reversed in successive experiments. The larger (0.5 ml) sample of blood was taken into a tapered glass tube fitted with a 20-gauge hypodermic needle and shaken with air for 5 min to saturate it with oxygen. A 12 μl aliquot was then used for determination of oxygen capacity.

The wet weight, and beak and toe length, of all experimental embryos was recorded and they were preserved in formalin for reference purposes.

RESULTS

Anatomy

A more complete account of the anatomical findings has been given elsewhere (White, 1968) and only sufficient detail will be given here to permit an adequate understanding of the experimental findings.
Fig. 2. (A) Arterial system of 16-day chick embryo. (B) Venous system of 16-day chick embryo. Abbreviations: A.A., aortic arch; C.A., coronary artery; C.L.A., coeliac artery; C.M.V., coccygeomesenteric vein; C.V., coronary veins; D.A., dorsal aorta; I.A.S., interatrial septum; J.V.S., interventricular septum; J.V., jugular vein; L.A., left atrium; L.A.V.C., left anterior vena cava; L.B.C.A., left brachiocephalic artery; L.C.C.A., left common carotid artery; L.D.A.R., left ductus arteriosus; L.E.J.V., left external jugular vein; L.H.V., left hepatic vein; L.I.A., left iliac artery; L.I.V., left iliac vein; L.P.A., left pulmonary artery; L.R.A., left renal artery; L.R.V., left renal portal vein; L.S.A., left sciatic artery; L.S.V., left sciatic vein; L.S.C.A., left subclavian artery; L.S.C.V., left subclavian vein; L.U.V., left umbilical vein; L.V., left ventricle; M.V., mesenteric vein; O.M.A., omphalomesenteric artery; O.M.V., omphalomesenteric vein; P.A., pulmonary arch; P.B.B., posterior body blood; P.V., pulmonary vein; P.V.C., posterior vena cava; P.V.CH., posterior venous channel; R.A., right atrium; R.A.V.C., right anterior vena cava; R.B.C.A., right brachiocephalic artery; R.C.C.A., right common carotid artery; R.D.A.R., right ductus arteriosus; R.E.J.V., right external jugular vein; R.H.V., right hepatic vein; R.I.A., right iliac artery; R.I.V., right iliac vein; R.P.A., right pulmonary artery; R.R.A., right renal artery; R.R.V., right renal portal vein; R.S.A., right sciatic artery; R.S.V., right sciatic vein; R.S.C.A., right subclavian artery; R.S.C.V., right subclavian vein; R.U.A., right umbilical artery; R.V., right ventricle; U.V., umbilical vein; V., ventricles; V.A., vitelline artery; V.V., vitelline vein.
(1) **Arterial system**

The arterial system of the 16-day embryo (Fig. 2) differs from that of the adult in two major respects.

Firstly, the sciatic arteries of the embryo give rise to the umbilical arteries which carry deoxygenated blood to the respiratory membrane. The left umbilical artery is far larger than the right and undoubtedly carries most of the chorioallantoic blood.

Secondly, the pulmonary arches communicate with the aortic arch of the embryo by the pair of ductus arteriosi. The junction of the ductus with the aortic arch marks the beginning of the dorsal aorta, and the origin of the coeliac and omphalomesenteric arteries in relation to this junction is of interest in the present study. These arteries (Fig. 3; Fig. 8, Plate 1) arise from the right dorsolateral side of the newly formed dorsal aorta whilst the ductus join the aortic arch ventrally and on its left side: assuming laminar flow to occur, the coeliac and omphalomesenteric arteries could not fail to receive the greater part of their blood direct from the left ventricle by way of the aortic arch.

The coronary arteries which arise directly from the aortic arch are conspicuous on the surface of the ventricles in the living heart and proved to be useful indicators of the presence of dye in the aorta in dye injection experiments (Fig. 8 D, Plate 1).
(2) **Venous system**

Except for the presence of the umbilical and vitelline veins the venous system (Fig. 2) at this stage is identical to that of the adult.

The umbilical vein is a large vessel carrying oxygenated blood from the chorioallantois to the embryo. It does not supply the liver substance with venous blood as in the mammalian foetus but enters the left hepatic vein, a short vessel with direct access to the *sinus venosus* of the heart.

The liver receives its venous supply from the omphalomesenteric vein, which is formed by the union of mesenteric and vitelline veins. The latter vessel carries blood laden with predigested food material from the yolk sac to the embryo.

(3) **The heart**

The ventricles are completely separated at the 16-day stage, but the atria communicate by way of a number of perforations occupying the central area of the interatrial septum (Fig. 4).

As noted by Lillie (1908), the internal configuration of the right atrium (Fig. 4) is such as to prevent much mixing of the anterior venous (de-oxygenated) blood and the posterior venous (oxygen and food-laden) blood. The orifice of the posterior venous channel is directed towards the interatrial septum through which a large part of its blood might be expected to flow to the left atrium. The orifices of the anterior *venae cavae* and of the coronary veins are directed towards the right atrioventricular canal.

**Dye injections**

The results of these experiments are summarized in Table 1.

(1) **Dye in anterior vena cava**

In 16 out of 17 experiments in which dye was injected into the right anterior *vena cava* the result was as follows:

The dye was seen to enter the ventrolateral part of the right atrium and then appear in the pulmonary arch (Fig. 8C, Plate 1). No trace was seen in the left atrium, the aorta or the coronary arteries. This result indicates that the blood entering the heart in the right anterior *vena cava* is largely restricted to the right side and does not normally gain access to the left atrium. The same might be expected to apply to blood from the left anterior *vena cava* and coronary vessels by virtue of the position of their openings into the right atrium (Fig. 4), although this probability was not tested experimentally.

In Expt 6 anterior caval blood must have passed across the interatrial septum and entered the left atrium, for dye was observed in the aorta and coronary arteries. Subsequent dissection of this embryo revealed no abnormalities of heart structure which might explain the result.

(2) **Dye in the posterior venous channel**

In 14 out of 20 experiments in which dye was injected into the umbilical vein the passage of dye through the heart was as follows.

The dye passed into both atria and into the pulmonary and aortic arches, appearing almost immediately in the right circumflex coronary artery on the ventral surface of
Fig. 4. The internal configuration of the heart of the 16-day chick embryo showing the probable flow patterns. The figures represent the percentage saturations with oxygen recorded in the different chambers and vessels (the query indicates an estimated figure). For key to abbreviations see Fig. 2.

The heart (Fig. 8 D, Plate 1). The dye trace in the right atrium was most conspicuous in the medial part of that chamber, and was less pronounced here and in the pulmonary arch, than in the left atrium and aortic arch. In the remaining six experiments no dye was seen in the pulmonary arch although a clear trace appeared in the aortic arch and coronary arteries.

These results indicate that a large proportion of the blood entering the heart in the posterior venous channel flows through the interatrial septum to the left atrium and thence to the left ventricle and aortic arch. This separation of anterior and posterior venous blood streams is not, however, always complete, for in 70% of the experiments some dye entered the right ventricle and pulmonary arch.

Oxygen determinations

(a) Oxygen capacity

The 94 embryos used in this study showed considerable variation in developmental age as measured by toe/beak length (Hamburger & Hamilton, 1951) or by wet weight (Romanoff, 1960). A positive correlation was found to exist between wet weight and oxygen capacity of the blood (Fig. 5), which is no doubt due to the increase in blood haemoglobin which occurs during development (Barnes & Jensen, 1959). The oxygen capacity of a 17-day embryo whose wet weight could be 18.31 g according to Romanoff (1960), is calculated from the regression equation as 10.51 vols. %, a value which is similar to that of 10.34 vols. % obtained by Bartels et al. (1966) for 17-day embryos of the Heisdorf, Nelson, Lohmann breed.
Table 1. Results of dye injection experiments

(In Expts 22–29 consecutive injections were made into each site in a single embryo. For key to abbreviations of blood vessel names see Fig. 2.)

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(b) Oxygen saturation of paired samples

The complete results for the analyses of oxygen content in paired blood samples from 94 embryos have been recorded elsewhere (White, 1968) and in this paper the more meaningful parameter of % saturation with oxygen (100 x oxygen content/ oxygen capacity) will be used in presenting the results. All figures for oxygen saturation are rounded off to the nearest whole number in the text.

Table 2 shows the mean values for the oxygen saturation of the eleven combinations of vessel pairs that were sampled, as well as the overall mean saturation for the seven blood vessels involved in the different pairs. Table 3 shows the mean difference in oxygen saturation between the various vessel pairs, calculated from the paired result and also from the means of all results. A plan of the circulatory system with the mean figures for oxygen saturation indicated (Fig. 6) will be found helpful in considering what follows.

The blood returning from the respiratory membrane to the embryo in the umbilical vein was the most highly saturated with oxygen (66 %), whilst that in the right external jugular vein was the least saturated (22 %). These figures probably represent the two extremes of oxygenation of the blood to be found in the system.
The blood in the left ventricle was more highly saturated with oxygen (50%) than that in the right ventricle (32%), indicating that a large part of the more highly oxygenated posterior venous blood stream flows through the perforated interatrial septum to enter the left atrium. It will be recalled from the dye injection experiments that little or no anterior venous blood entered the left atrium; if it is assumed that the pulmonary return to that chamber is small at this stage, then the saturation of the blood in the left ventricle (aortic arch) would be about equal to that of the posterior venous stream. Admixture of the relatively highly saturated umbilical vein blood (66%) with the less saturated blood from the yolk sac (31%) and from the posterior body (saturation not determined but = anterior caval blood 22% saturated) must produce the resultant saturation of 50% in the posterior venous channel. The probable flow patterns in the heart with added oxygen saturation values are shown in Fig. 4.

The oxygen saturation of blood in the vitelline artery (45%) was greater than that of the vitelline vein (31%) indicating a considerable oxygen consumption in the yolk sac. Moreover, oxygen saturation in the vitelline artery (45%) was above that of the umbilical artery (32%). This clearly demonstrates that a separation of blood streams occurs at the junction of the ductus arteriosi and the aortic arch; and that the omphalo-mesenteric artery, and by inference the coeliac artery, receive most of their blood from the left ventricle through the aortic arch (Fig. 3). The reduction in mean saturation from 50% in the left ventricle to 45% in the vitelline artery does, however,
Table 2. Results of 94 oxygen determination experiments expressed as % saturations

(Each entry is a mean value followed by a standard deviation and, in parentheses, the number of observations.
For key to abbreviations of vessels names see Fig. 2)

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<th>Vessel</th>
<th>U.A.</th>
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<td>Means of all results</td>
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<td>66.08, 10.44 (29)</td>
<td>45.22, 9.45 (31)</td>
<td>30.85, 6.64 (24)</td>
<td>31.72, 6.98 (22)</td>
<td>49.59, 8.35 (26)</td>
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<td>10</td>
<td>32.79, 0.60 (7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>11</td>
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</tr>
</tbody>
</table>

P. T. WHITE
Table 3. Mean difference in % oxygen saturation of the various vessel pairs sampled

(Each figure is a mean value followed by a standard error of difference and, in parentheses, the number of observations. For key to abbreviations of vessel names see Fig. 2.)

<table>
<thead>
<tr>
<th>Vessel pair</th>
<th>Mean difference of paired results</th>
<th>Mean difference of all results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Umbilical vein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle</td>
<td>15.56, 3.42 (7)</td>
<td>16.49, 2.63 (29 U.V., 26 L.V.)</td>
</tr>
<tr>
<td>2. Umbilical vein</td>
<td>32.39, 4.04 (7)</td>
<td>34.26, 2.19 (29 U.V., 29 U.A.)</td>
</tr>
<tr>
<td>Umbilical artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Umbilical vein</td>
<td>44.98, 2.94 (9)</td>
<td>42.74, 2.22 (29 U.V., 27 J.V.)</td>
</tr>
<tr>
<td>Jugular vein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Umbilical vein</td>
<td>36.85, 5.12 (6)</td>
<td>35.23, 2.42 (29 U.V., 24 V.V.)</td>
</tr>
<tr>
<td>Vitelline vein</td>
<td>14.22, 1.84 (6)</td>
<td>14.37, 2.17 (31 V.A., 24 V.V.)</td>
</tr>
<tr>
<td>5. Vitelline artery</td>
<td>4.36, 1.35 (10)</td>
<td>4.37, 2.40 (31 V.A., 26 L.V.)</td>
</tr>
<tr>
<td>Vitelline vein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle</td>
<td>14.33, 1.93 (15)</td>
<td>13.40, 1.90 (31 V.A., 29 U.A.)</td>
</tr>
<tr>
<td>7. Vitelline artery</td>
<td>7.77, 1.68 (12)</td>
<td>7.51, 1.64 (24 V.V., 27 J.V.)</td>
</tr>
<tr>
<td>Umbilical artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Vitelline vein</td>
<td>14.68, 1.63 (9)</td>
<td>17.80, 2.31 (22 R.V., 26 L.V.)</td>
</tr>
<tr>
<td>Jugular vein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Right ventricle</td>
<td>4.43, 0.92 (7)</td>
<td>0.10, 1.78 (22 R.V., 29 U.A.)</td>
</tr>
<tr>
<td>Left ventricle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Right ventricle</td>
<td>7.79, 1.81 (6)</td>
<td>8.38, 1.82 (22 R.V., 27 J.V.)</td>
</tr>
<tr>
<td>Umbilical artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Right ventricle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jugular vein</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

indicate that some blood from the right ventricle may enter the omphalomesenteric artery. In five out of the ten experiments in which the left ventricle and vitelline artery were sampled simultaneously the oxygen saturations were equal; in the remainder the oxygen saturation of the blood in the omphalomesenteric artery was lower than that in the left ventricle by a mean value of 9%.

The blood in the umbilical artery (dorsal aorta) was only slightly more saturated with oxygen than that in the right ventricle (mean increase 4%), indicating that the amount of left ventricular blood entering the dorsal aorta is small. It seems likely that most of the blood from the left ventricle is distributed to the head, neck, thorax and heart through the brachiocephalic and coronary arteries, and to the gut and yolk sac through the coeliac and omphalomesenteric arteries.

The small amount of left ventricular blood remaining would then mix with blood flowing from the right ventricle in the ductus arteriosi to constitute the blood in the dorsal aorta. It is unlikely that the non-functional lungs receive much blood at this stage as the pulmonary arteries are of small size compared to the ductus and most of the right ventricular output must be shunted into the dorsal aorta.
Quantitative estimation of blood flows and cardiac output

By making certain assumptions it is possible to calculate the cardiac output and the magnitude of blood flows through the circulatory system from the oxygen determination results. In making these calculations the mean values for oxygen saturation in the various vessel-pairs are used and, although not absolutely accurate, the results obtained do give some quantitative idea of the circulatory patterns in an average 16-day embryo. Similar methods were used by Dawes et al. (1954) to estimate blood flows in the foetal lamb.

Consider first the situation at the respiratory membrane. From Table 2 the mean oxygen saturations of the blood entering and leaving the chorioallantois might be: entering in umbilical artery, 33%; leaving in umbilical vein, 66%.

Taking the oxygen capacity of a 16-day embryo as 10.3 vols. % (Fig. 5), the actual oxygen content of blood in these vessels would be: umbilical artery, 3.4 vols. %; umbilical vein, 6.8 vols. %. The arteriovenous difference is then 3.4 vols. %; that is, for every 100 ml of blood flowing through the chorioallantois, 3.4 ml of oxygen are absorbed. If we know the amount of oxygen taken in through the shell in unit time we can calculate the blood flow through the respiratory organ (Fick principle).

According to Romanoff (1967), the oxygen uptake of a 16-day White Leghorn embryo is 397.7 ml/24 h; 15.5 ml of this oxygen is used by the chorioallantois for its own respiration so we may assume that in 24 h, 385.2 ml of oxygen enters the blood flowing through the respiratory membrane. The blood flow through that organ must therefore be:

\[
\frac{385.2 \times 100}{3.4 \times 24} = 472 \text{ ml/h. (1)}
\]

Consider now the situation at the heart (Fig. 4). We know from the paired results (Table 2) that the oxygen saturation of blood in the left ventricle might be 50% and we assume that the blood in this chamber is derived exclusively from the posterior venous channel by flow through the interatrial foramina (pulmonary inflow to the left atrium is ignored). If the further assumption is made that the oxygen saturation of blood in the posterior vena cava and the hepatic veins is low, and equal to that in the anterior vena cava, i.e. 22%, then we can calculate the relative contributions of umbilical vein blood and blood from the posterior body and yolk sac to the posterior venous blood stream in the following manner.

When \( X \) ml of blood which is \( A \) % saturated with oxygen mixes with \( Y \) ml of blood which is \( B \) % saturated to form \( Z \) ml of blood which is \( C \) % saturated, then if we know the values of \( A, B \) and \( C \), we can calculate the values of \( X \) and \( Y \) by assuming \( Z \) to be 100 ml:

\[
Y = \frac{100(A-C)}{(A-B)} \quad \text{and} \quad X = 100 - Y. \quad (2)
\]

Applying this formula to the above situation we have:

\[
A = 66\% = X \text{ ml umbilical vein blood}, \\
B = 22\% = Y \text{ ml posterior body and yolk sac blood}, \\
C = 50\% = Z \text{ ml left ventricular blood}.
\]

Then \( X = 64 \text{ ml, \quad Y = 36 \text{ ml.} \)
that is, 64% of the blood in the posterior venous stream comes from the umbilical
vein and 36% from the yolk sac and posterior body. From (1) umbilical venous flow
is 472 ml/h, so 64% of the posterior venous flow is equal to 472 ml and the total
flow must therefore be 737 ml/h, 265 ml being derived from posterior body and yolk
sac:

\[
\text{posterior venous inflow to heart} = 737 \text{ ml/h.} \tag{3}
\]

Now consider the heart again (Fig. 4), this time with respect to the relative contribu-
tions of posterior venous blood and anterior caval and coronary blood to the blood
in the right ventricle. It is assumed that blood in the left anterior \textit{vena cava} and the
coronary veins has a saturation equal to that of the right anterior \textit{vena cava} (22%).
The dye injection experiments and the anatomical findings indicate that anterior caval
and coronary blood enters only the right ventricle, so that the level of oxygen satura-
tion recorded in that chamber (32%) must be the result of mixing of anterior caval
blood (22%) with posterior venous blood (50%). Again, using equation (2) we can
calculate the relative contributions of these two streams to the blood in the right
ventricle, and we find that 64% comes from the anterior caval and coronary veins
and 36% from the posterior venous channel:

\[
\text{right ventricular blood} = 64\text{% from anterior caval and coronary veins} + 36\text{% from posterior venous blood.} \tag{4}
\]

If we assume that the output of the right ventricle is equal to that of the left and is
\(C\) ml/h, and if we also assume (as previously) that the left ventricle receives all its
blood from the posterior venous channel (pulmonary flow ignored), then the amount
of blood entering the right ventricle in 1 h must equal the total posterior venous inflow
to the heart minus \(C\) ml, i.e. \(737 - C\) ml/h.

But we know from (4) that the volume of posterior venous blood entering the right
ventricle in 1 h is equal to 36% of that chamber’s output so that:

\[
737 - C = 36\% \times C, \\
C = 542 \text{ ml/h,}
\]

\[
\text{cardiac output} = 542 \text{ ml/h or 9 ml/min.} \tag{5}
\]

Hughes (1949) using a photographic method calculated the cardiac output of the
16-day chick embryo to be 6 ml/min.

From (4), 64% of the blood entering the right ventricle comes from the anterior
\textit{vena cavae} and coronary veins. In 1 h this would represent a flow back to the heart
from the head, neck, thorax and heart of 64% of the cardiac output (542 ml/h (5)).
Thus:

\[
\text{flow to/from head, neck, thorax and heart} = 347 \text{ ml/h.} \tag{6}
\]

We know from (4) that 36% of the right ventricular blood is derived from the posterior
venous channel, so the posterior venous inflow to right ventricle must be 36% of the
cardiac output (5), i.e. 195 ml/h. This represents 26.5% of the total posterior venous
inflow to the heart (737 ml/h (3)):

\[
\text{of total posterior venous inflow to heart,} \\
26.5\% \text{ enters right ventricle, and} \\
73.5\% \text{ passes via interatrial septum to left atrium.} \tag{7}
\]
Table 4. Comparison of the distribution of cardiac output and oxygen intake in the late chick embryo and sheep foetus

<table>
<thead>
<tr>
<th>Animal</th>
<th>Head, neck, thorax, heart</th>
<th>Lungs, gut, posterior body, yolk sac</th>
<th>Chorioallantois or placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick (this study)</td>
<td>32/59</td>
<td>25/38</td>
<td>44/3</td>
</tr>
<tr>
<td>Sheep (Dawes et al. 1954)</td>
<td>15/45</td>
<td>30/55</td>
<td>55/—</td>
</tr>
<tr>
<td>Sheep (Rudolph &amp; Heyman, 1967), oxygen flows not measured</td>
<td>24/—</td>
<td>35/—</td>
<td>41/—</td>
</tr>
</tbody>
</table>

From the left ventricle 347 ml blood/h must flow to the head, neck, thorax and heart (7) through the brachiocephalic and coronary arteries, leaving 195 ml/h (cardiac output minus 347 ml) to flow to the gut, yolk sac and posterior body:

left ventricular flow to gut, yolk sac, posterior body and chorioallantois = 195 ml/h. (8)

Total blood flow to lungs, gut, yolk sac, posterior body and chorioallantois must equal 195 ml + 542 ml (output of right ventricle) = 737 ml/h. (9)

But we know from (1) that flow to the chorioallantois is 472 ml/h so that:

total flow to lungs, gut, yolk sac and posterior body must be 265 ml/h. (10)

The contributions of blood from the left and right ventricles to the blood in the dorsal aorta can be calculated from equation (2) using the oxygen saturation figures (Table 2):

Blood in dorsal aorta (saturation 33 %)
  = 77 % from right ventricle (saturation 28 %)
  = 23 % from left ventricle (saturation 50 %). (11)

It is now possible to express the estimated flows to different parts of the circulatory system as a percentage of the combined cardiac output.

Combined cardiac output = 1084 ml/h,
  flow to head, neck, thorax and heart = 347 ml/h = 32 %,
  flow to lungs, gut, posterior body and yolk sac = 265 ml/h = 24.5 %,
  flow to chorioallantois = 472 ml/h = 43.5 %.

The distribution of 397.7 ml of oxygen taken in by the 16-day embryo per 24 h (Romanoff, 1967) can also be calculated using the oxygen saturation results and assuming an oxygen capacity of 10.3 volumes %.

Total oxygen intake per 24 h = 397.7 ml (Romanoff, 1967),
  oxygen to chorioallantois (Romanoff, 1967) = 12.5 ml = 3.1 %,
  oxygen to head, neck, thorax and heart (calculated) = 233 ml = 58.6 %,
  oxygen to lungs, gut, posterior body and yolk sac (by difference) = 152.2 ml = 38.3%,
Circulatory system of the late chick embryo

The distribution of the combined cardiac output and of the oxygen intake of the chick embryo is compared with that of the sheep foetus in Table 4.

DISCUSSION

It is now possible to make some comparisons between the circulatory systems of the late bird embryo and the foetal mammal (Figs. 6, 7). All figures quoted for the mammal in this discussion are taken from the work of Dawes et al. (1954) on the sheep foetus.

In both systems the blood returning from the respiratory membrane is the most highly saturated with oxygen. In the late chick embryo, however, a lower mean saturation was recorded in the umbilical vein (66%) than in the sheep foetus (80%). This relatively low saturation level may be explicable by the build-up of carbon dioxide inside the egg and its effect upon the hatching process.

Kuo & Shen (1937) observed that the blood in the chorioallantoic vessels became noticeably darker after the 15th day of development; and Dawes & Simkiss (1969) have shown a quantitative increase in the carbon dioxide tension of the blood of the chick embryo from the 11th day until the 19th day of development, when pulmonary ventilation is initiated. It is known that the chorioallantois achieves its maximum growth around the 15th day of development (Windle & Barcroft, 1938), whereas the blood volume increases up to the time of hatching (Barnes & Jensen, 1959). It may therefore be that in the later stages of development the surface area of the chorioallantois is not great enough to maintain a complete saturation of the umbilical blood or to eliminate the increasingly large amounts of carbon dioxide produced by the growing embryo. Oxygen saturation levels in the blood returning to the embryo from its respiratory membrane might therefore be expected to fall as development proceeds with a concomitant increase in the carbon dioxide content. The increased carbon dioxide levels inside the egg eventually initiate reflex respiratory movements, which bring about the piercing of the air space, pipping and finally hatching (Windle & Barcroft, 1938). So a steady build up of anoxia during development, far from being detrimental to the embryo, is of definite adaptive value; it ensures the onset of the violent respiratory movements which lead to escape from the egg at the correct time. Eleven of the embryos used in this study were noted at the time as looking 'anoxic', the blood in the umbilical and vitelline vessels appearing darker than normal. Such anoxic embryos showed no return to a more normal blood coloration after preparation in the manner described previously, thus providing circumstantial evidence that the unnatural presence of air on both sides of the respiratory membrane has no effect upon its efficiency in gas uptake.

In the chick embryo, unlike the mammal, the umbilical blood flows directly to the left hepatic vein without entering the liver substance. The embryo derives its food through the vitelline vein which carries digested yolk material to the liver. The omphalomesenteric vein (formed by union of vitelline and mesenteric veins) breaks up and ramifies in the liver substance and all food containing blood must therefore pass through the liver before it can reach the heart. A ductus venosus such as exists in some mammals and which may act as a channel for shunting umbilical blood past the liver (Rudolph & Heymann, 1967) is not present in the late chick embryo.

A possible liver shunt channel does, however, exist, in the form of the coccygeo-
mesenteric vein which connects hepatic and renal portal systems (Fig. 2); it has been shown that this vessel is able to carry blood in either direction in the adult fowl (Akester, 1967) and the same possibility must be presumed to exist in the embryo.

In both chick and mammal the oxygenated blood from the respiratory membrane is diluted with blood of much lower oxygen content from the posterior body (and from the yolk sac in the chick) to give a blood of reasonably high oxygen saturation which enters the heart. In both animals a large proportion of the composite posterior venous blood stream flows to the left side of the heart through the perforated interatrial septum; 61% in the mammal, 73.5% in the chick. In both forms some posterior venous blood enters the right ventricle to increase the overall oxygen content of the anterior caval and coronary blood which makes up the major part of that chamber’s inflow.

In both animals a considerable part of the output of the right ventricle flows through the ductus, thus by-passing the non-functional lungs, although the contributions of right and left ventricular blood to the blood in the dorsal aorta seems to differ in the two animals. In the chick 82% comes from the right ventricle and only 18% from th
left ventricle, whereas in the sheep foetus the relative contributions are 40% right ventricle and 60% left ventricle. This difference is due to the greater blood and oxygen flows to the head, neck, thorax and heart in the chick embryo (Table 4).

These greater flows in the chick may be related to anatomical factors such as the elongation of the neck and the development of the pectoral musculature, but it is possible that this situation is normal in small embryos and foetuses which have relatively greater cardiac outputs and oxygen requirements. Unfortunately no-quantitative studies have been performed on mammalian foetuses comparable in size to the chick embryo.

As a consequence of the high blood flow from the left ventricle to the head, neck, thorax and heart in the chick, little of the left ventricular blood enters the dorsal aorta to return to the posterior body and chorioallantois. This is not the case in the sheep foetus and it is for this reason that the oxygen saturation of blood flowing back to the placenta in the sheep foetus (58%) is considerably higher than that which flows back to the chorioallantois in the chick embryo (30%). The chick seems to gain relatively more oxygen at its chorioallantois than the sheep foetus does at its placenta, mean saturation being increased by a factor of 34% in the chick and 22% in the sheep.
This might possibly reflect the fact that the chick derives its oxygen directly from the atmosphere whereas the sheep foetus must derive it from maternal blood flowing through the placenta. In both sheep and chick a large proportion of the combined cardiac output flows to the respiratory organ (Table 4).

The arrangement in the sheep foetus, whereby a relatively large part of the left ventricular output flows down the dorsal aorta, ensures that the gut and posterior body receive blood which is not very much lower in oxygen saturation and food content than that received by the head, neck, thorax and heart; 58% compared to 62%. In the chick embryo the posterior body receives blood at a much lower oxygen saturation, about 32%, but here an arrangement exists whereby the gut and yolk sac receive the greater part of their blood supply direct from the left ventricle at a saturation of about 45%.

This is entirely due to the peculiar mode of origin of the coeliac and omphalomesenteric arteries in relation to the junction of the aortic arch and the paired ductus (Fig. 8, Plate 1; Fig. 4). In describing the anatomy of this junction it was stated that, if laminar flow occurred, the coeliac and omphalomesenteric arteries could not fail to receive a large part of their blood directly from the left ventricle by way of the aortic arch. The oxygen determination results confirmed this prediction, but indicated some individual variation in the amount of mixing of the various blood streams at this junction. By using the flow rates derived in the previous section it is possible to calculate the Reynold's number of the vessels concerned and these are found to be low: ductus 37, systemic aorta 23 and dorsal aortal 72.

According to McDonald (1960), the critical Reynold's number for blood is 2000. This means that in vessels with a Reynold's number less than 2000 laminar flow occurs, whereas in vessels with Reynold's number greater than 2000 flow becomes turbulent and mixing of blood streams may ensue. It would seem therefore that flow at the junction of the ductus arteriosi with the aorta would almost certainly be laminar. However, this is not to say that no mixing of the bloodstreams occurs as laminar flow is easily upset. Side branches, such as the coeliac and omphalomesenteric arteries, can behave in two ways. If small they will 'milk' off the laminar flow from the side of the vessel, but larger side branches can cause turbulence. Moreover, the diameter of the vitelline artery is small, and in withdrawing a blood sample it is possible that the insertion of the hypodermic needle would almost occlude the lumen and upset the normal flow patterns.

Clearly a complex haemodynamic situation exists which may lend itself to some individual differences in the amount of mixing of the various bloodstreams; it does seem, however, both from considerations of the anatomy and from the experimental evidence, that the gut and yolk sac would normally receive most of their blood at a relatively high oxygen content from the left ventricle by way of the aortic arch.

When considering blood flow in the omphalomesenteric artery of the chick embryo the work of Hammett & Zoll (1928) is of interest. These authors found that the yolk-sac blood vessels in the chick embryo were sensitive to carbon dioxide concentration and responded to local increases in the level of this gas by constriction. They postulated that this reaction might form the basis of a self-regulating system for controlling the rate of development. For instance, if the incubation temperature rose, increased respiration would result; followed by increased use of food, increased growth and
Increased carbon dioxide production. The subsequent increase of carbon dioxide in the blood would tend to reduce flow through the yolk sac whose blood vessels would be stimulated to constrict; this would lead to lowered food return and consequent reduction in the growth rate. Unfortunately this work was only carried out on 4-day chick embryos where most of the material absorbed by the yolk sac is used for growth of the membrane itself (Needham, 1933). If the yolk-sac blood vessels in the 16-day embryo are similarly sensitive to carbon dioxide concentration then there would seem to be good reason for the yolk sac receiving blood with a relatively high oxygen concentration.

A final point of interest in connexion with the origin of the coeliac and omphalomesenteric arteries from the dorsal aorta (Fig. 8, Plate 1) is that these vessels originate in the mid-ventral line and only move to their definitive position of the right dorso-lateral wall of the dorsal aorta between the fourth and fifth days of development (Bremer, 1925). Bremer believed that the shift in position of these vessels was caused by traction exerted by sympathetic nerve fibres. If this is so, it is easy to visualize the selective advantage such a chance event of embryological mechanics might confer, if, as in this instance, it resulted in the yolk sac receiving more highly oxygenated blood; this could allow more efficient digestion of yolk and might result in faster growth and a shorter period of development.

It would be interesting to know if any modifications which result in the yolk sac receiving blood of a high oxygen content are present in other vertebrates having cleidoic eggs. Further discussion of this point is made elsewhere (White, 1968).

I wish to thank Professor G. E. H. Foxon, Department of Biology, Guy's Hospital Medical School, who supervised this work, and also the Science Research Council, who provided financial support.

REFERENCES


EXPLANATION OF PLATE

Fig. 8 (A) Ventral view of the junction of the ductus arteriosi with the aortic arch showing the origin of the coeliac and omphalomesenteric arteries. Dissected from a perfused embryo and cleared in glycerol.

(B) Lateral view of junction.

(C) Ventral view of heart of embryo following dye injection into right anterior vena cava. Dye trace visible in the right atrium and pulmonary arch only. Black and white photograph taken from 16 mm colour film and processed for maximum contrast.

(D) Ventral view of heart of embryo following dye injection into the umbilical vein. Dye trace clearly visible in left atrium and in right circumflex coronary artery.

For key to abbreviations used see Fig. 2.