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THE CONSTRICTION OF THE DUCTUS ARTERIOSUS CAUSED BY OXYGEN AND BY ASPHYXIA IN NEWBORN LAMBS

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Since the beginning of this century it has been recognized that closure of the ductus arteriosus at birth occurs in two stages, a rapid constriction of the vessel which is later followed by gradual anatomical obliteration of its lumen over a period of weeks (Gérard, 1900*a*, *b*; Wells, 1908). Recently it has been shown that, in the newborn lamb, the constriction which normally occurs within 5–20 min of birth does not at once lead to complete obliteration of the lumen. Blood continues to flow through the constricted lumen at high velocity, and this flow gives rise to the characteristic murmur and thrill over the junction of ductus arteriosus and pulmonary trunk (Dawes, Mott & Widdicombe, 1955*a*, *b*). The appearance of this murmur in unanaesthetized, spontaneously breathing lambs is therefore a useful indication of constriction of the ductus arteriosus. In anaesthetized lambs in which the chest has been opened, changes in the external diameter of the ductus arteriosus may be measured directly under a variety of experimental conditions. The appearance of the murmur and direct measurement of the ductus have been used in this paper to test the various conflicting theories of the mechanism whereby the ductus constricts shortly after birth: a preliminary account of the observations has already been published (Born, Dawes, Mott & Rennick, 1955).

METHODS

Ewes of the Clun–Hampshire cross-breed were mostly used, together with a few Kerry Hill, Suffolk and Welsh sheep. The gestation age was estimated from tupping dates and was checked by radiography, which showed the number as well as the approximate size of the foetuses, before anaesthesia.

The ewe was placed on a large operating table, the top of which sloped towards a gutter at one side, to catch the amniotic and allantoic fluids. General anaesthesia was induced by intrajugular injection of sodium pentobarbitone, and was maintained by a slow drip of a dilute solution into

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a cannula tied into the left external jugular vein (10–20 mg/kg/hr). The ewe's trachea was cannulated and her blood pressure was recorded from a carotid artery with a mercury manometer. She was laid on her right side, and the uterus was approached through a mid-line incision orientated so as to avoid the large superficial abdominal veins when possible. The foetus was delivered by Caesarean section and laid on a small heated table which was attached to the main operating table, with its surface 10 cm above the latter; the abdomen of the ewe was closely pressed against the lateral border of the small table, and a metal apron prevented the abdominal viscera from spilling out. In this way the position of the foetus was readily adjusted so that it was no longer in direct contact with the mother, and hence undisturbed by her respiratory movements; yet there was no tension on the umbilical cord, which was covered with gauze soaked in warm saline. The top of the foetal table was slotted to hold metal uprights, which were used to support manometers. On delivery the head of the lamb was often covered with a rubber bag filled with warm saline in order to prevent inhalation of air. The trachea was then cannulated as soon as possible, using a glass cannula filled with saline and with obstructed outlets.

Blood pressure was recorded from the femoral or carotid arteries by mercury or condenser manometers. The condenser manometers were attached by a short length of polythene tubing; the overall natural frequency of the manometer, gauge head and tubing normally exceeded 150 c/s. Pulmonary pressure was recorded by direct puncture of the pulmonary trunk with a short wide-bore needle attached to a condenser manometer, giving an overall frequency response of up to 250 c/s. The pulses recorded from condenser manometers were displayed on oscilloscopes and photographed; the mean pressures were displayed on meters and plotted at $\frac{1}{2}$ min intervals. The heart rate was either calculated from the oscilloscope record or it was displayed on a kymograph. For this purpose the voltage change recorded by a condenser manometer with each pulse wave was amplified and used to operate an impulse counter (Thorp, 1948). The pressure in the left atrium was recorded with a saline manometer containing heparin to prevent clotting.

Blood samples were withdrawn for the analysis of O_2 and CO_2 content and O_2 capacity from a catheter in the left carotid artery in most experiments. In some lambs the brachiocephalic artery was tied, and then samples were taken elsewhere, usually from a femoral artery. The samples were mixed with a trace of heparin (Boots Pure Drug Co.) and sodium fluoride in 1 ml. syringes, and stored in ice-cold water in vacuum flasks. Most of them were analysed by a modification of the Barcroft–Haldane method for use with Warburg manometers (Born, Dawes & Mott, 1955), but a few were analysed for O_2 content by the method of Roughton & Scholander (1943). The pH was measured either by a glass electrode and pH meter (Electronic Instruments Co. Ltd., 23 A) or by a phenol red colorimetric method (Hawkins, 1923) and the p_{CO_2} was calculated from the Henderson–Hasselbalch equation. Varying concentrations of O_2, N_2 and CO_2 were mixed after passage through calibrated rotameters and delivered to the ewe or the lamb. The lamb was ventilated by positive pressure from a Starling Ideal pump, at a rate of about 24 c/min, and the intratracheal pressure was recorded by a critically damped mercury manometer.

In order to measure the external diameter of the ductus arteriosus the chest was opened on the left side and two or three ribs were removed. The left upper lobe of the lung was retracted downward, and in some experiments the pericardium overlying the vessel was removed. The external diameter of the ductus was measured with a pair of calipers. Murmurs were detected in the great vessels by applying a conventional stethoscope with a small head. They were recorded on paper by an Elmquist phonocardiograph, which has a linear response up to 600 c/s.

Bilateral thoracic sympathectomy was performed after a mid-line incision and splitting the sternum. The upper five or six ganglia were removed on either side. In other experiments the brain and spinal cord were destroyed. The chest was first opened on the left side and the brachiocephalic artery tied. The lamb was then turned on its right side, in such a way that the umbilical cord was not at any time occluded, and the neck was twisted to allow the operator access to the back. The muscles and bone were removed from the upper part of the spinal cord, which was divided at C 1–2, and a blunt rod was then inserted to destroy first the brain and then the spinal cord. There was little haemorrhage and that was readily controlled by inserting plasticine and

cork plugs. The edges of the wound were drawn together and the lamb was laid on its back once more.

In some lambs the blood pressure was stabilized in the following way. Heparin was administered, and either the brachiocephalic or a femoral artery was attached by a wide-bore tube to a reservoir which contained about 250 ml. of dextran solution (Glaxo) or of heparinized maternal blood. This reservoir was kept under a constant pressure, slightly in excess of the normal foetal blood pressure, by connexion to a 10 l. flask containing air under pressure. In other lambs both systemic and pulmonary arterial blood pressures were stabilized. The chest was opened in the mid-line to give access to a large branch of the right pulmonary artery, which was also connected to the stabilizer. Thus the pressures in the pulmonary and systemic circulation were kept equal, even when the ductus arteriosus constricted.

Cross-perfusion with the lungs of a twin lamb. The object of this preparation was to replace the placenta by the lungs of a twin lamb. The first lamb (*A*) to be delivered was used for some other purpose, at the end of which its lungs were ventilated with O_2 and the umbilical cord was tied and cut. It was then moved to the far end of the operating table and the second lamb (*B*) was delivered. A cannula filled with saline was tied into the trachea of lamb *B*. The chest was opened to the left of the mid-line and three or four ribs were removed. A short length of the descending aorta below its junction with the ductus was prepared and ligatures were placed around it. The left brachial artery was also prepared. The abdomen was opened to the left of the umbilicus, and a length of inferior vena cava was freed from the surrounding tissue, below the entry of the left renal vein. Attention was then turned once more to lamb *A*, which was heparinized and bled out. Artificial ventilation was temporarily stopped. The front part of the thoracic cage was removed as rapidly as possible, and cannulae were tied into the pulmonary trunk and left atrium. The ductus arteriosus, descending aorta, brachiocephalic artery, superior and inferior venae cavae were tied to prevent loss of blood from the pulmonary circulation of lamb *A* into the systemic circulation. The lungs of lamb *A* were then washed out with dextran solution. Lamb *B* was heparinized and its left brachial artery was joined, through a Starling resistance, to the pulmonary trunk of lamb *A*. Finally the left atrium of lamb *A* was joined through a bubble-trap to the inferior vena cava of lamb *B*, and inflation of the lungs of lamb *A* was begun again.

At this point, then, the lungs of lamb *A* were introduced in parallel with the placental circulation of lamb *B* (Fig. 1), flow through which was not so far interrupted. The descending aorta was now tied below the entry of the ductus and the blood pressure rose because of the increase of systemic arterial resistance. Even so, not enough blood passed from lamb *B* through the lungs of lamb *A* to increase the arterial O_2 saturation sufficiently, because of the resistance to blood flow offered by the small left brachial artery. The descending aorta of lamb *B* was therefore cannulated, and the tube connecting lamb *B* to the pulmonary trunk of lamb *A* rapidly moved from the left brachial artery to the descending aorta (Fig. 1). The blood pressure now fell to the level determined by the Starling resistance, which was set at the normal foetal pressure. This completed the preparation, during the course of which the arterial pressure did not fall unduly, and the lamb was not asphyxiated. Since the lungs of lamb *A* were washed out, little foreign blood was introduced into lamb *B*. The communicating tubes were all filled with warm dextran solution before connexion was made. The exposure of the left side of the chest of lamb *B* also provided adequate room in which to measure the external diameter of the ductus directly. The immediate neighbourhood of the vessel was not disturbed during the preparation. It will be noted that tying the descending aorta interrupts blood flow through the lower part of the body, including the liver and other abdominal viscera.

The isolated heart–ductus arteriosus–artificial lung preparation. This preparation was devised to isolate the living heart and great vessels from the rest of the body. A foetal lamb was delivered by Caesarean section as usual, and its trachea tied off. The chest was opened widely down the mid-line. The branches of the right pulmonary artery, pulmonary veins and bronchi were ligated and the whole of the right lung was removed piecemeal. The left pulmonary artery was tied where it passed through the pericardium. The descending aorta was explored and its branches immedi-

ately below the junction with the ductus were tied. A short length of the brachiocephalic artery and superior vena cava were then prepared and ligatures placed in position.

The lamb was heparinized and the brachiocephalic artery joined through a Starling resistance to an artificial lung, the outflow of which was connected to the superior vena cava. The pressure of the Starling resistance was set just above the normal foetal blood pressure. All connecting tubes were filled with warm dextran solution. The descending aorta was then tied below its junction with the ductus arteriosus and the consequent rise in pressure started blood circulating through the artificial lung at the moment when blood flow to the placenta was stopped (Fig. 2). The inferior vena cava was tied in one lamb; in the remainder it was left intact. Blood samples were withdrawn from the brachiocephalic artery for O_2 analysis.

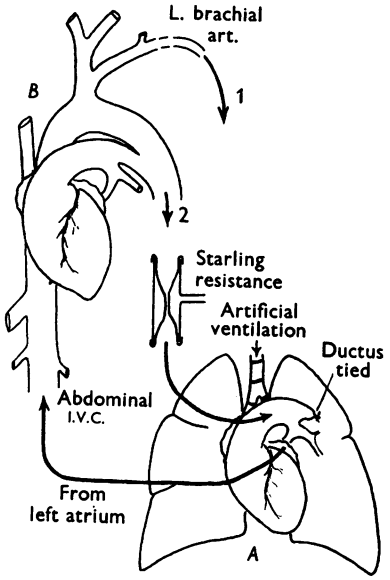

Fig. 1.

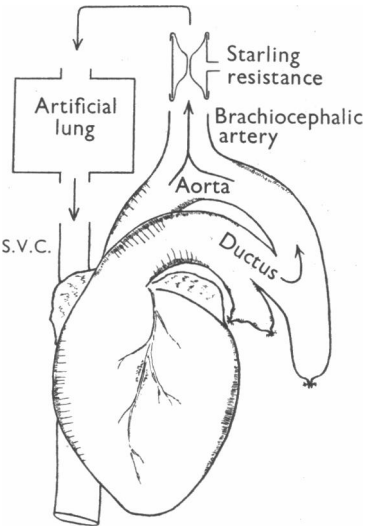

Fig. 2.

Fig. 1. Diagram of cross-perfusion preparation in which the placenta of a foetal lamb *B* is replaced by the lungs of a twin lamb *A*. The left brachial artery of *B* is first joined (1), through a Starling resistance, to the pulmonary trunk of *A*; the left atrium of *A* is joined to the lower abdominal inferior vena cava of *B*. The descending aorta of *B* is then tied and cannulated at leisure, and is finally (2) joined to the Starling resistance and pulmonary trunk of *A*, in place of the left brachial artery.

Fig. 2. Diagram of heart–ductus arteriosus–artificial lung preparation. The right lung is removed and the left pulmonary artery tied. The brachiocephalic artery is joined through a Starling resistance to the artificial lung, whence blood is returned to the superior vena cava (s.v.c.). The descending aorta is then tied.

The artificial lung was constructed so as to offer a surface area of 350 cm^2 for gaseous exchange to the circulating blood. It was based upon the design used by Melrose (1953) and consisted of twenty Perspex plates, separated by spacing rings and bolted together to form a cylinder. This cylinder was rotated at about 1 rev/sec by a velodyne motor (Willlams & Uttley, 1946) so that the blood covered the upper surface of every plate and fell through the cylinder in a series of cascades. The apparatus was tilted at an angle of about 15° to the horizontal, and was held 35 cm above the top of the operating table. The foetal arterial pressure was therefore adequate to raise the blood up to

the cylinder, and there was sufficient room beneath the latter for a collecting funnel. A stream of O_2 or N_2 was passed through the cylinder at a rate of 1–2 l./min. Heat loss from the apparatus was rapid, so that by the end of an experiment the temperature of the circulating blood had fallen to less than 30° C. In one experiment, therefore, the blood was returned to the heart through a glass coil around which warm water was circulated. This artificial lung worked well for its limited purpose, and fairly rapid changes in arterial O_2 saturation were obtained (Fig. 8).

Lambs breathing spontaneously. Lambs which were to breathe spontaneously were delivered under local anaesthesia as described previously (Dawes *et al.* 1955*a*). They were prevented from breathing on delivery by pulling a saline-filled rubber bag over the head. A carotid catheter was inserted for withdrawal of blood samples. Blood pressure and heart rate were measured by a condenser manometer attached to a catheter in a carotid or femoral artery.

In some experiments tidal air was measured by the method described by Widdicombe (1954) after cannulating the trachea, and intrapleural pressure was recorded by a condenser manometer, from a catheter in the pleural cavity. The necessary operative procedures were all performed under local anaesthesia, and without causing the lamb any distress. The rubber bag was then removed from the lamb's head (if the trachea was not already cannulated), the umbilical cord tied and severed, and the lamb then began to breathe.

The effect of CO_2 tension on the O_2 saturation of blood from foetal and newborn lambs. Heparinized blood obtained from foetal and newborn lambs was stored overnight in the refrigerator at 2–4° C. 4 ml. samples were equilibrated with CO_2 and O_2 by standard tonometric procedures. The calculated gas tensions at which the degree of saturation with O_2 was determined were, for O_2, 20 and 30 mm Hg, and for CO_2, 35, 50 and 65 mm Hg. The actual tensions of the gases with which each blood sample was equilibrated were determined with the gas analysis apparatus of Haldane. The O_2 saturation of the blood was measured by the method described by Born, Dawes & Mott (1955), in triplicate.

RESULTS

Constriction of the ductus arteriosus in response to O_2

Neonatal lambs: constriction of the ductus on ventilation of the lungs. In mature foetal lambs the ductus arteriosus, when fully dilated, is some 8–10 mm in external diameter and 15–20 mm long; it has, therefore, almost the same diameter as the pulmonary trunk and aortic arch. Indeed, when the chest is opened down the mid-line the aortic arch is almost entirely concealed from view by the ductus, which lies anterior to it. It is covered by pericardium, across which run the left phrenic and vagus nerves. In our first experiments the pericardium was cut and retracted, and the nerves were displaced laterally, in order to facilitate measurement of the external diameter of the ductus. While this procedure did not cause any noticeable change in diameter there was a tendency for small haemorrhages to occur after a time in the outer coat of the ductus, followed by oedema in the surrounding tissue which made it impossible to define the margins of the vessel. These haemorrhages were caused by the cut edges of the pericardium rubbing against the vessel, and were difficult to avoid because of the large size of the vessel and its rapid movements. The ductus, if the pericardium over it was kept intact, remained in good condition for many hours and repeated estimates of its external diameter by the same observer did not vary by more than 0·3–0·4 mm. The diameter often varied considerably along the vessel; measurements were

therefore taken at the same point, usually between the middle and the aortic end.

In order to measure the external diameter of the ductus arteriosus directly, the left side of the chest must be opened, and this might in itself have caused some alteration in size. However, we think this is improbable. Provided the arterial O_2 saturation and the level of anaesthesia were kept constant, the diameter of the ductus did not alter significantly for a period of an hour or more after exposure. In some lambs very considerable operative procedures were undertaken, involving dissection of the thoracic descending aorta, ligature of the left pulmonary and brachiocephalic arteries, or bilateral thoracic sympathectomy and vagotomy, without any alteration of the ductus diameter.

When the lungs were adequately ventilated, so that the systemic arterial O_2 saturation rose to 90% or more, the ductus arteriosus constricted until its external diameter was reduced to about 5 mm. The process of constriction was slow; it usually occupied 5 min or more and was accompanied by considerable shortening of the vessel. After a quarter of an hour, because of this shortening, the angle between the ductus and the aorta became more acute, and the vessel gradually adopted the position seen in 1- to 2-day-old lambs. The alteration in the appearance of the ductus during constriction was dramatic. When fully dilated it occupied the whole of the space between the pulmonary trunk and left pulmonary artery on the one hand, and the arch of the aorta on the other hand; when it had become constricted and shortened, a gap appeared so that the aortic arch was readily visible. Thus, although the absolute change in the external diameter of the ductus was not great, the alteration in its appearance was so obvious that there was never any doubt about its constriction.

Certain other observations also have helped in many experiments to reinforce this conclusion. Thus the appearance of a murmur and thrill in the pulmonary trunk at its junction with the ductus was good evidence that blood was flowing at high velocity through a constricted ductus from the aorta to the pulmonary arteries. Similarly, the development of a large pressure difference between the aorta and pulmonary trunk was indicative of partial constriction of the ductus. These points are illustrated in Fig. 3.

Neonatal lambs: inflation with O_2 *and* N_2 *and destruction of the central nervous system.* In a previous paper we observed that the ductus arteriosus constricted on ventilation with O_2 in six newborn lambs ranging from 109 to 139 days gestation age (term 147 days), but that it dilated again when the systemic arterial O_2 saturation was reduced by replacing the O_2 with air (Born, Dawes & Mott, 1955). The stroke of the respiration pump was not altered during these manoeuvres. This observation, which has been repeated many times since (Fig. 7), suggested that constriction of the ductus resulted from an increase

in the arterial O_2 tension, rather than from mere inflation of the lungs. This conclusion has been reinforced by the observation in three mature lambs that inflation of the lungs with nitrogen for 12–14 min, with the placental circulation intact, did not cause the ductus to constrict. Similarly, in other lambs, if inflation of the lungs was begun with air, and the pump stroke was adjusted so that the arterial O_2 saturation did not change from the foetal level for up to 30 min, the ductus did not constrict. It constricted, however, when the air was replaced by O_2, which caused a rise in the arterial O_2 saturation.

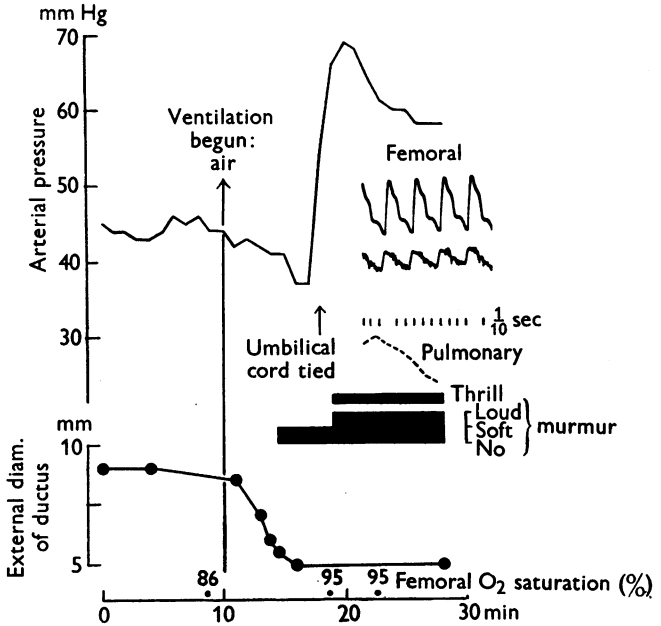

Fig. 3. Lamb 429 B, 136 days gestation age, under barbiturate anaesthesia. Spinal cord and brain destroyed. Ventilation causes an increase of arterial O_2 saturation, constriction of the ductus arteriosus, wide separation of pulmonary and femoral arterial pressures, and the development of a murmur and thrill at the pulmonary end of the ductus. Condenser manometer records (inset) show the consequent rapid vibrations on the lower pulmonary record only.

It was still not clear whether the increased O_2 tension was acting directly upon the ductus or through the central nervous system. However, the same phenomenon was observed in lambs after bilateral sympathectomy and vagotomy, and also in five lambs in which the brachiocephalic artery was tied and the brain and spinal cord were destroyed. In three of the latter, inflation of the lungs was adjusted so that the arterial O_2 saturation rose rapidly, and the ductus constricted within a few minutes of beginning ventilation (Fig. 3). The other two lambs were underventilated for 40 min or more after the umbilical cord was tied; the ductus did not constrict until the stroke of the respiration pump was increased and 100 % O_2 was administered. Under these

conditions, then, closure is independent of the integrity of the central nervous system or of any structure supplied by the brachiocephalic artery (that is, the upper part of the body, since the left subclavian artery is derived from the brachiocephalic in sheep).

Foetal lambs: effect of increase in arterial O_2 *saturation.* If an increase in arterial O_2 saturation causes constriction of the ductus arteriosus in newborn lambs, it might also be expected to have the same effect under foetal conditions. The only simple way in which to increase the foetal arterial O_2 saturation without gross operative interference is to administer 100% O_2 to the ewe.

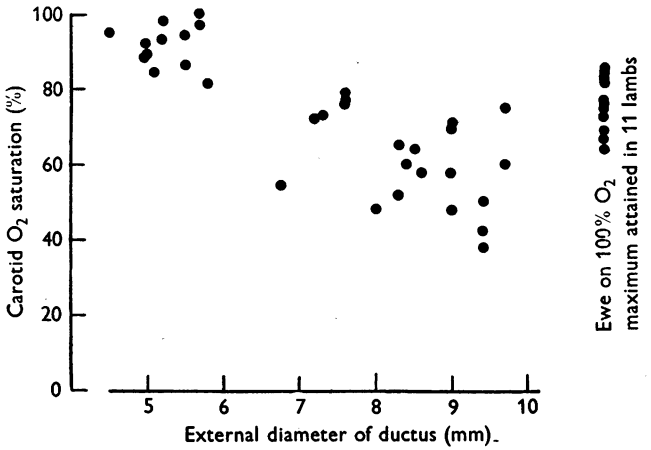

Fig. 4. The data in the graph were obtained from ten lambs of 131–141 days gestation age. The O_2 saturation has been plotted against the external diameter of the ductus arteriosus in the foetal condition and after ventilation with different N_2:O_2 mixtures. The points on the right-hand side of the graph record the maximum O_2 saturations observed in eleven foetal lambs of similar gestation age during ventilation of their mothers with 100% O_2.

However, this only raised the foetal carotid O_2 saturation above 80% in four out of eleven lambs of similar age and weight (right-hand part of Fig. 4). The arterial O_2 saturation required to cause constriction is high. Thus in only five out of eighteen lambs ventilated with air or O_2, in which the ductus shrank in external diameter to 5·5 mm or less, was the carotid arterial O_2 saturation less than 90%, and in each of these five it exceeded 80%. The left-hand part of Fig. 4 shows the relation between carotid O_2 saturation and ductus diameter in ten of these lambs, which were of comparable weight and of gestation age 131–141 days. One cannot therefore expect dramatic changes in the diameter of the ductus on giving 100% O_2 to the ewe. Nevertheless, in four out of six lambs the administration of 100% O_2 to the ewe did cause a small but significant decrease in ductus diameter associated with the rise in carotid O_2 saturation. The clearest example of this is shown in Fig. 5. A rise in carotid O_2 saturation to 69% was associated with a decrease in the external diameter of

the ductus from 9·9 to 7·2 mm. There was no change in blood pressure or heart rate such as might have been expected if this had been associated with release of sympathetic amines, which subsequent experiments have shown to cause constriction of the ductus (see below). When the same lamb was later ventilated with air the carotid O_2 saturation rose to 94%, and the ductus then constricted still further to 5·4 mm. If the ductus is constricted in the foetal condition, by drawing a tape around it, a systolic murmur appears at the aortic end of the ductus, and this murmur radiates in the direction of flow, down the aorta. In only one of these four lambs did such a murmur appear, when the carotid arterial O_2 saturation had been raised from 63 to 81%, and the diameter of the ductus had fallen from 9·0 to 7·8 mm. The murmur was soft and could only be heard by applying the stethoscope bell directly to the vessel wall;

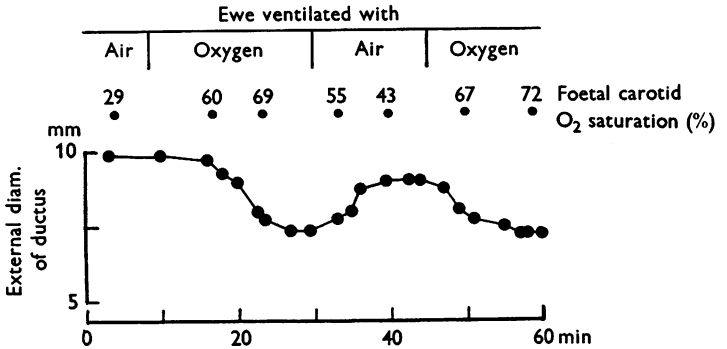

Fig. 5. Lamb 520A, 139 days gestation, under barbiturate anaesthesia; foetal condition with intact umbilical cord and placenta. Ventilation of the ewe with 100% O_2 raised the foetal carotid O_2 saturation and caused constriction of the ductus arteriosus.

there was no thrill. These experiments demonstrate two points: first, that it is possible to cause a small change in ductus diameter by altering the arterial O_2 saturation in the foetal condition; and secondly, that it is unlikely that under normal circumstances in the foetus the ductus will constrict to a significant degree as a result of high arterial O_2 tension.

Next it seemed desirable to determine whether a still larger increase in arterial O_2 saturation would cause even more constriction of the ductus arteriosus, while the circulation was still substantially in the foetal condition, at least in so far as the direction of blood flow through the ductus was from pulmonary trunk to aorta. Since the placenta was clearly inadequate as an organ of gaseous exchange for this particular purpose, it was replaced with the lungs of another lamb. Twin lambs were used, and in the completed preparation blood flowed from the thoracic descending aorta (below its junction with the ductus) of one lamb through the isolated lungs of the second lamb (which were ventilated with 100% O_2), and back from the left atrium

into the abdominal inferior vena cava of the first lamb (Fig. 1). In three such preparations the systemic arterial O_2 saturation rose considerably—in the experiment illustrated in Fig. 6 to 92%, and in another experiment to 95%. In each experiment the ductus constricted to less than 5·5 mm diameter and a murmur appeared over the ductus and radiated down the aorta. These changes were reversed when the lungs were inflated with nitrogen. Thus it is possible to produce a considerable constriction of the ductus while the circulation through the heart and the great vessels is substantially in the foetal condition, provided that the arterial O_2 saturation is raised sufficiently.

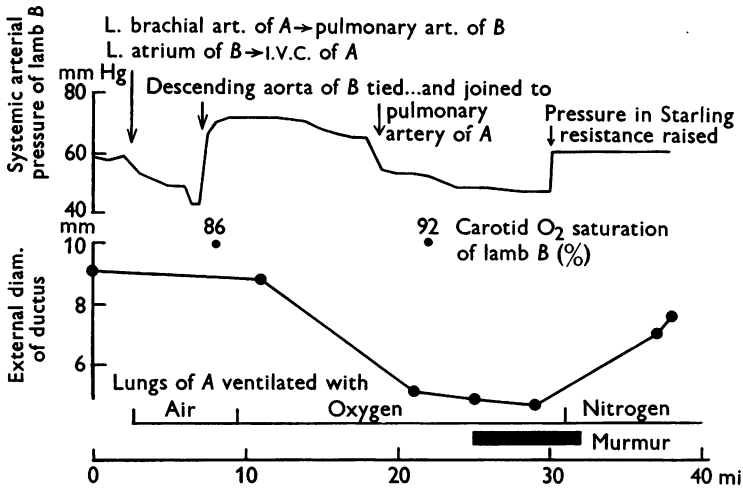

Fig. 6. Lambs 574 A and B, 134 days gestation, under barbiturate anaesthesia. Cross-perfusion preparation in which the placenta of lamb B was replaced by the lungs of lamb A (see Fig. 1). A rise in carotid O_2 saturation caused constriction of the ductus arteriosus and the development of a loud murmur over the vessel.

Arterial blood pressure and placental flow. The pulmonary and systemic blood pressures normally fall when a mature foetal lamb is well ventilated because of the increase in pulmonary vascular conductance. Fig. 7 shows that constriction of the ductus as a result of ventilation occurs even when the fall of systemic arterial pressure is prevented by means of a stabilizer attached to a femoral artery. Five such experiments were performed in all, and in two of them the pulmonary arterial pressure was also stabilized; the ductus still became constricted when the arterial O_2 saturation rose on ventilation.

It was evident from the experiments in foetal and newborn lambs that the ductus could become constricted whether or not the placental circulation was intact. In seven lambs the umbilical cord had been left untied when ventilation was begun, and the ductus became constricted in the usual manner.

Fig. 3 illustrates this point; in this lamb a large placental flow continued, as shown by the rise of systemic blood pressure due to the increase in peripheral resistance when the cord was tied. In another lamb placental flow was measured directly by a flowmeter in the abdominal umbilical vein, and the systemic arterial pressure was maintained by a pressure stabilizer attached to a femoral artery. On ventilation, the carotid O_2 saturation rose to 102%, the ductus became constricted and a murmur appeared in the pulmonary trunk;

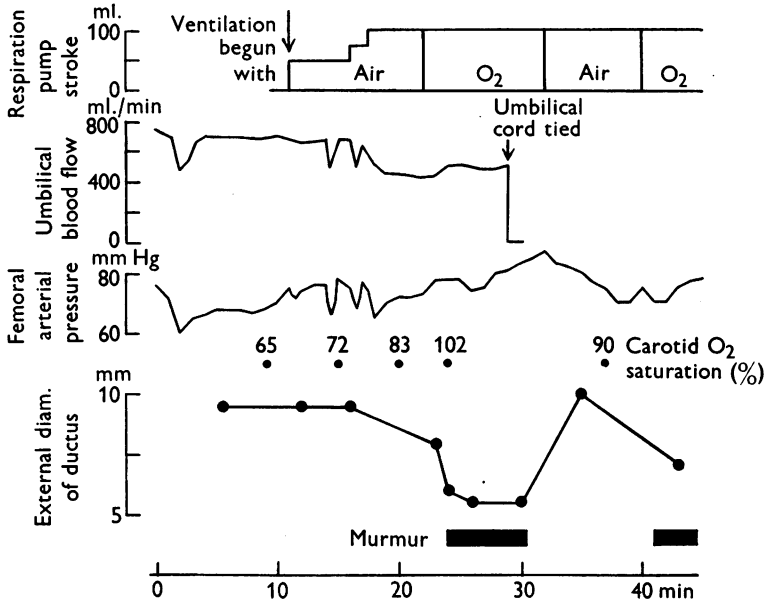

Fig. 7. Lamb 512B, 139 days gestation age, under barbiturate anaesthesia. The average level of the blood pressure was slightly raised by the adjustment of a stabilizer attached to the femoral artery. Ventilation caused a rise in carotid O_2 saturation, a small reduction in umbilical blood flow and constriction of the ductus although the blood pressure did not fall.

during this period placental blood flow fell by more than 30% (Fig. 7). It may therefore be that a rise in arterial O_2 saturation can also cause constriction of placental blood vessels. While this hypothesis clearly needs more thorough investigation, it would be consistent with the observation that inflation of the lungs and the subsequent rise in arterial O_2 saturation is often associated with a diminution in the external diameter of the umbilical arteries, irrespective of changes in blood pressure.

Isolated heart–ductus arteriosus preparation. The experiments described above made it probable that the increase in arterial O_2 saturation on ventilation of the lungs acted directly on the ductus to cause it to become constricted. Such constriction had been observed in the absence of the central nervous system, the upper part of the body, and the placenta. It had also proved possible to

bring about constriction of the ductus by raising the arterial O_2 saturation without inflating the lamb's own lungs. However, it might be argued that a metabolite was formed in another organ, the lungs for instance, which was carried by the blood stream to the ductus. In order to test this hypothesis a heart–ductus arteriosus–artificial lung preparation was used.

When completed, the preparation included only the heart, the great vessels, and an artificial lung which presented a large surface area for gaseous exchange between the blood and the external atmosphere. The advantage of retaining the heart was that it served to pump the blood through the artificial lung and it was then possible to complete the preparation without any substantial change in blood pressure, and without interrupting the flow of blood through the great vessels. In all four such preparations the ductus became constricted when the

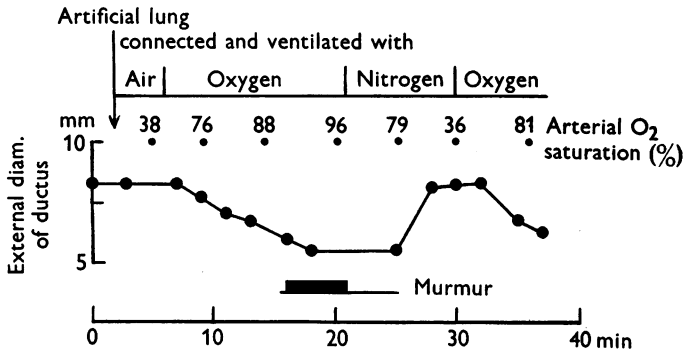

Fig. 8. Lamb 517A, 141 days gestation age, under barbiturate anaesthesia. Heart–ductus arteriosus–artificial lung preparation (see Fig. 2). Ventilation of the artificial lung with oxygen causes a rise in arterial O_2 saturation, constriction of the ductus and development of a murmur over the vessel.

arterial O_2 saturation was raised by exposing the blood to an atmosphere of 100% O_2 (Fig. 8). Also a murmur was heard over the constricted ductus radiating along the aorta. When the arterial O_2 saturation was reduced by exposing the blood to an atmosphere of nitrogen, the ductus dilated and the murmur disappeared. This excluded the possibility that constriction was caused by some substance which was formed as a result of the trauma involved in the preparation or in the passage of blood through the artificial lung. Though the heart has not yet been excluded, these experiments reinforce the conclusion that O_2 causes the ductus to become constricted by a direct action.

Constriction of the ductus arteriosus in response to asphyxia

Observations on newly delivered lambs breathing spontaneously. If the ductus arteriosus becomes constricted at birth only as a result of the rise in arterial O_2 tension, then the murmur which is heard over the ductus in newborn lambs

should not appear until the lamb is well oxygenated. We had previously observed that the murmur usually appeared within 5–15 min after tying the umbilical cord (Dawes *et al.* 1955*a*) and also that the rate of rise of arterial O_2 saturation was often slow (Dawes *et al.* 1955*b*). In twelve lambs delivered under local anaesthesia, a catheter was inserted into a carotid artery, also under local anaesthesia. Blood samples were withdrawn at varying intervals after tying the umbilical cord, as shown in Fig. 9. Table 1 records the O_2 saturation of those samples which were obtained before and after the appearance of a

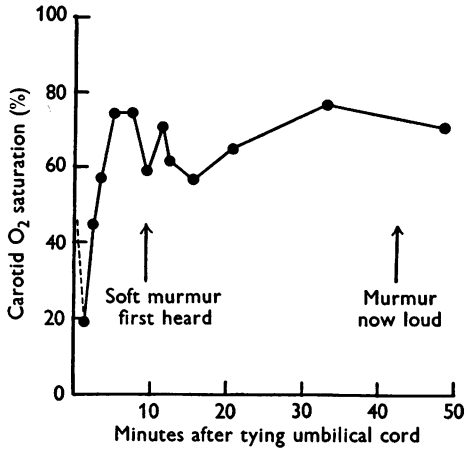

Fig. 9. Lamb 455, 141 days gestation age, delivered under local anaesthesia. The umbilical cord was tied at zero time and the lamb began to breathe. A murmur was first heard over the ductus arteriosus at the end of 10 min, when the carotid O_2 saturation was still within the normal foetal range.

TABLE 1. The O_2 saturation of carotid blood at the time of appearance of the ductus arteriosus murmur in mature lambs delivered under local anaesthesia and breathing spontaneously

			Carotid O_2 saturation			
			Before appearance of murmur		After appearance of murmur	
Lamb no.	Breed	Gestation age (days)	%	min from tying cord	%	min from tying cord
516A	H	139	42	18	43	23
326B	H	143	52	6	49	57
319	H	143	43	13	57	27
350	W	142	61	4½	61	6
317	H	145	24	8	66	63
355	W	141	59	9½	71	11
559A	K	141	45	8	71	12
318	H	146	40	7	74	39
357	W	141	68	8	77	11
340	W	139	64	−4	86	9
356	W	141	99	11	96	16
358	W	—	100	4½	100	6

H, Hampshire; W, Welsh; K, Kerry Hill.

murmur. Since the presence of a murmur is good evidence of constriction, it was concluded that the ductus became constricted while the O_2 content of the systemic arterial blood was surprisingly low, in nine lambs less than 80% and in two less than 50%. The carotid O_2 saturation in mature foetal lambs normally ranges from 40% to more than 70% (Dawes, Mott & Widdicombe, 1954). It therefore appeared that some factor other than oxygen was causing the ductus to become constricted in asphyxia.

A few more physiological observations on newborn spontaneously breathing lambs must now be described. In some newborn lambs the O_2 saturation of the arterial blood rises smoothly when breathing begins on tying the cord, without any period of asphyxia (Barcroft, 1938; Barcroft, Barron, Kramer & Millikan, 1938; Dawes *et al.* 1955*b*). In others breathing begins with difficulty and there are periods of cardiac irregularity. In both groups the blood pressure

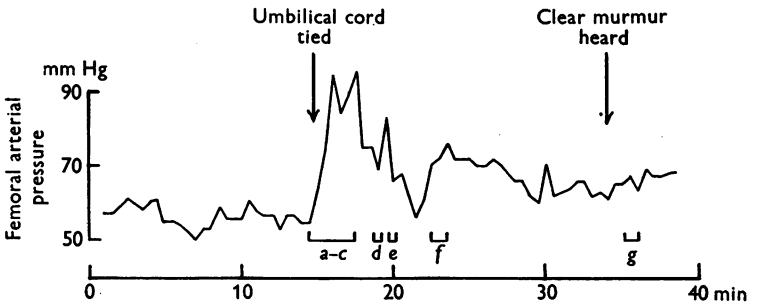

Fig. 10. Lamb 405A, 143 days gestation age, delivered under local anaesthesia. Mean arterial pressure was measured by a condenser manometer from a catheter in a femoral artery (local anaesthesia). The letters *a–g* indicate the times at which the records shown in Fig. 11 were taken.

rises. The course of this rise in blood pressure is particularly interesting in those lambs which are partly asphyxiated. Observations have been made on five such lambs, in three of which intrapleural pressure and tidal air were also measured. Fig. 10 shows the changes in mean blood pressure, recorded at half-minute intervals, and indicates the points at which the records shown in Fig. 11 were taken. In Fig. 11*a* it can be seen that tying the umbilical cord caused a small primary rise of blood pressure due to the increase of peripheral vascular resistance. Respiratory movements did not yet begin, the heart slowed, and then, more than half a minute after tying the cord, a secondary rise of blood pressure was observed. Eventually, after two ineffectual gasps, the lamb took a deep breath, and 4–5 sec later the blood pressure rose even higher and the heart rate increased considerably (Fig. 11*b*). After one further breath the blood pressure began to fall and the heart slowed until another pair of large respiratory efforts caused the same response as before (Fig. 11). This

cycle of events was repeated again and again over the next few minutes until gradually the intervals between breaths decreased, and although breathing was still periodic, the fluctuations in blood pressure and heart rate gradually became smaller (Fig. 11*f*, *g*). Both blood pressure and heart rate were now higher than they were before the cord was tied, and a clear murmur was heard over the ductus arteriosus.

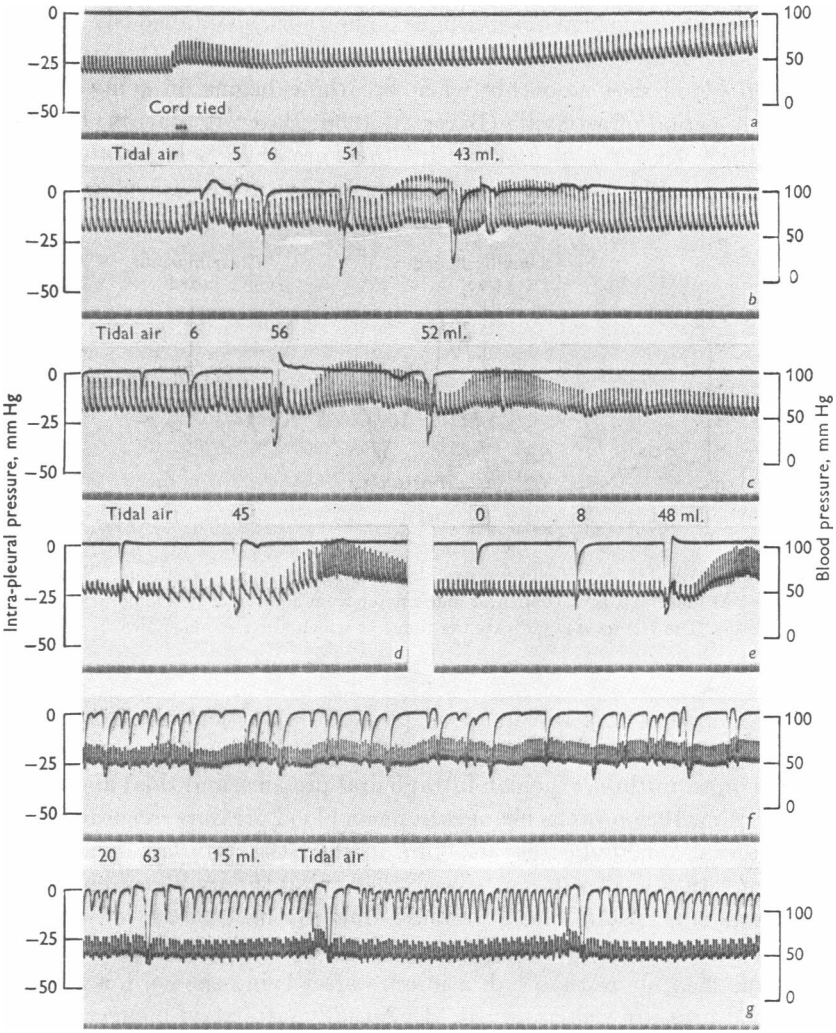

Fig. 11. As Fig. 10. Condenser manometer records of femoral arterial pressure and of intrapleura pressure (from a catheter in the intrapleural space under local anaesthesia). Tidal air was measured from a tracheal cannula, inserted under local anaesthesia. Time marks $\frac{1}{10}$ sec, interrupted every sec.

It seems probable that the secondary rise of blood pressure after tying the cord was due to asphyxia, which caused liberation of sympathetic amines and peripheral vasoconstriction. Thereafter the heart slowed as the asphyxia became more severe. The subsequent rises of blood pressure and heart rate when an effective breath was taken can be attributed to a transient increase in the O_2 content of the arterial blood. Barcroft (1938, pp. 56–64) also considered the possibility that respiratory movements may have increased venous return under like circumstances.

There are a few other points which deserve attention in Fig. 11. Inspiratory efforts caused very large changes in intrapleural pressure, which was reduced during gasps to as low as −50 mm Hg on occasion in mature lambs. It can also be seen that these strong inspiratory efforts are accompanied by a small fall in blood pressure, which may have been due to reduced cardiac output. The effectiveness of respiratory efforts was dependent not only on the size of the fall in intrapleural pressure, but also on its duration. Thus in Fig. 11*d* and *e* a number of inspirations can be observed. Only those which are maintained for half a second or more lead to the intake of any substantial volume of tidal air, and hence are followed by a rise in blood pressure and heart rate.

The effect of underventilation in newly delivered anaesthetized lambs. The experiments described above showed that in newborn lambs which were breathing spontaneously, and which were not under *general* anaesthesia, both blood pressure and heart rate were raised and a murmur appeared in spite of a relatively low arterial O_2 saturation. In previous experiments on lambs under general anaesthesia we had always been concerned to ensure effective positive pressure ventilation with a pump. These lambs were never asphyxiated and pulmonary vascular conductance was much increased by thorough and rapid expansion of the lungs, so that the pulmonary and systemic arterial pressures fell at once. Both fell because the ductus arteriosus was still open. The ductus arteriosus became constricted only when the arterial O_2 saturation rose considerably. In the next series of experiments, therefore, lambs under general anaesthesia were deliberately underventilated after tying the umbilical cord, in order to see whether the ductus arteriosus would become constricted during partial asphyxia. In assessing the degree of ventilation to which the lamb should be subjected we were guided by the level of the arterial blood pressure and also by inspection of the colour of the blood in the carotid arterial catheter. Samples were withdrawn for analysis at less frequent intervals. With practice it was found possible to regulate the degree of asphyxia satisfactorily. The cord was tied and ventilation was not begun for 1–2 min thereafter, by which time the arterial blood was dark in colour and the lamb had begun to make respiratory efforts. Then the respiration pump was started and the pump stroke raised at once to about 30 ml., giving a peak intratracheal pressure of 20–25 mm Hg, which gradually declined as the

distensibility of the lungs increased over the next few minutes. The pump stroke was then gradually increased in steps of 5–10 ml. at a time at varying intervals, so that the carotid O_2 saturation rose slowly over a period of many minutes. This procedure is illustrated in Fig. 12, which shows that, at the time the ductus

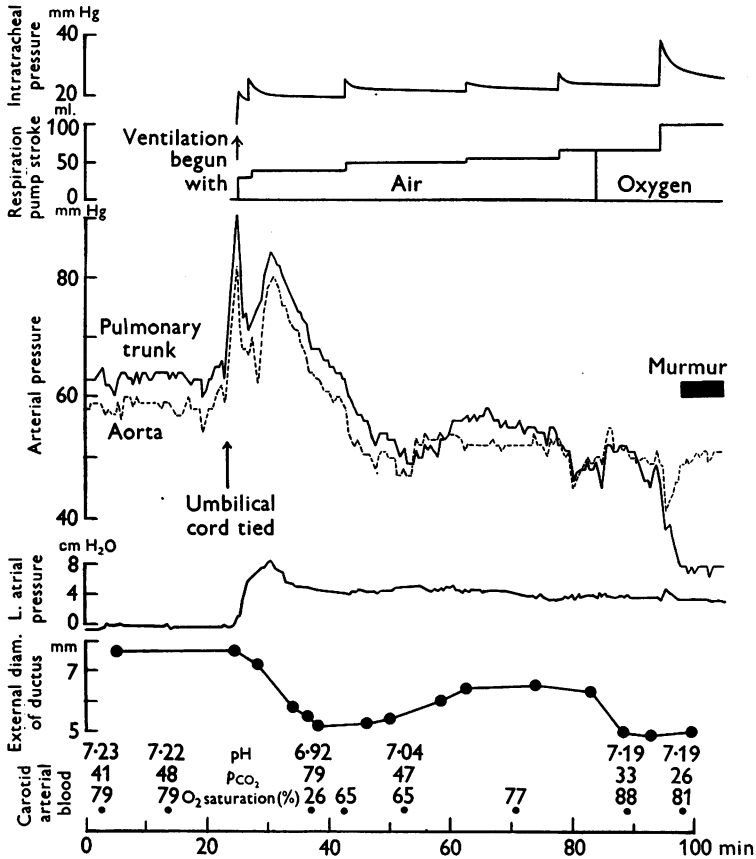

Fig. 12. Lamb 536A, >135 days gestation age, under barbiturate anaesthesia. The umbilical cord was tied and the lamb underventilated; the ductus became constricted at a low carotid O_2 saturation. Ventilation was then gradually increased, the carotid O_2 saturation rose to rather less than the foetal value and the ductus dilated. Ventilation with oxygen caused a further rise in carotid O_2 saturation and constriction of the ductus. A murmur was not heard over the ductus until pulmonary ventilation was still further increased, when a large pressure difference developed between aorta and pulmonary trunk.

had become constricted to less than 5·5 mm external diameter 13 min after tying the cord, the arterial O_2 saturation had only risen to 26%. Observations of this type have been made on a total of twelve lambs, in all of which during underventilation the ductus became constricted to varying degrees. The

relevant results are assembled in Table 2, in order of ascending carotid O_2 saturation at which the greatest constriction was observed. In five lambs a large reduction in the external diameter of the ductus was seen at an arterial O_2 saturation of less than 30%. In some lambs (e.g. 527 A, 583 A, 513 A) it was clear that the vessel was not wholly constricted, since subsequent ventilation with O_2 caused a considerable further reduction in external diameter when the carotid O_2% saturation increased sufficiently. In other lambs the degree of constriction approximated closely to that attained when the lamb was ventilated with oxygen (Table 2 and Fig. 12).

TABLE 2. Effect of underventilation, after tying the umbilical cord, on the diameter of the ductus arteriosus of lambs delivered by Caesarean section

		After tying umbilical cord and underventilation with air			After subsequent ventilation with 100% O_2	
Lamb no.	Foetal condition. Initial ductus diameter (mm)	Minimum ductus diameter (mm)	Min from tying cord	Carotid O_2 saturation (%)	Minimum ductus diameter (mm)	Carotid O_2 saturation (%)
522 A	9·4	5·7	8	9	—	—
533 A	8·8	4·9	24	13	—	—
527 A	8·3	6·4	22	14	5·1	96
583 A	9·0	7·1	37	18	5·0	87
536 A	7·6	5·2	13	26	4·9	88
536 B	8·0	5·0	10	32	4·9	91
441 A	8·5	5·8	18	46	5·4	92
516 B	8·0	5·6	30	55	—	—
513 A	9·0	7·0	36	55	6·0	85
430 A	9·0	6·0	8	57	5·5	94
568	9·2	4·6	20	62	4·4	85
501	10·0	5·7	50	70	5·4	89

Breed, Hampshire or Kerry Hill. Gestation age, 132–141 days. Anaesthetic, pentobarbitone. Chest open to measure ductus diameter directly; positive pressure ventilation.

In lambs which were well ventilated with air or O_2, and in which the ductus became constricted at the same time as the rise in arterial O_2 saturation, a loud murmur was always heard at the junction of pulmonary trunk and ductus, and a thrill was often present. By contrast, in underventilated lambs, in which the ductus became constricted during asphyxia, a murmur was not always heard over the ductus, and when heard it was sometimes of a wholly different character. In four of the lambs in Table 2 a murmur was not heard when the ductus constricted; lamb 536 A (Fig. 12) was one of these. In four of the remainder a faint murmur was heard on one occasion only at the pulmonary end of the ductus, and in a further two lambs there was a clear or loud murmur at the same place. A thrill in the pulmonary trunk was felt in one lamb only. Finally, in lamb 583 A a clear systolic murmur was heard at the *aortic* end of the ductus, radiating down the aorta, when the ductus diameter was reduced from 9·0 to 7·1 mm, 27 min after tying the umbilical cord.

The inference was drawn that in these underventilated lambs the pulmonary arterial pressure did not fall so much as in well-ventilated lambs, and simultaneous measurements of pulmonary arterial and aortic pressure confirmed this hypothesis. Fig. 12 shows such an experiment, in which pulmonary arterial and aortic pressures did not differ by more than some 5–6 mm Hg, even though the ductus arteriosus became constricted during asphyxia 13 min after tying the cord, dilated again as the arterial O_2 saturation gradually rose, and once more became constricted when the O_2 saturation was increased to 88% by ventilation with 100% O_2. There was a small rise of left atrial pressure on ventilation. No murmur was heard during this period at either end of the ductus arteriosus. Only when the lungs were still further expanded, by raising the pump stroke from 65 to 100 ml., did the pulmonary arterial pressure fall considerably (although the external diameter of the ductus was unchanged) and a loud murmur appear at the pulmonary end of the ductus. Incidentally, this Fig. 12 also shows that on every occasion on which the pump stroke was increased, there was an immediate small fall of pulmonary arterial pressure, usually accompanied by a simultaneous fall in aortic pressure. In five other lambs which were underventilated after tying the umbilical cord, the pulmonary arterial pressure was measured and found to be very high. In lamb 527 A (Fig. 13) the ductus had become partly constricted in response to asphyxia and then dilated again. At the beginning of the graph the pump stroke was raised from 40 to 60 ml. and then ventilation with O_2 was begun in place of air. Pulmonary arterial pressure was still considerably higher than aortic, and when the arterial O_2 saturation rose and the ductus again became constricted, a soft murmur appeared at the *aortic* end of the ductus. The pump stroke was now raised to 100 ml., pulmonary arterial pressure fell, the murmur disappeared and a stronger murmur appeared at the *pulmonary* end of the ductus. When a murmur has been heard at the aortic end of the ductus it has usually been difficult to hear, and invariably much softer than that normally heard at the pulmonary end. The obvious explanation is that the pressure difference between the two ends of the vessel is usually far less when blood flows from pulmonary trunk to aorta, than when it flows in the reverse direction after full inflation of the lungs.

Fig. 13 illustrates another point. It can be seen that administration of O_2 to the foetus caused a fall in pulmonary arterial pressure, and return to air a rise. This had often been noticed in previous experiments, but as it was usually accompanied by a change in the diameter of the ductus arteriosus, which became constricted on administration of O_2, it was not possible to say for certain whether the phenomenon was due to an alteration of the volume of blood flow through the ductus, or to a change in pulmonary vascular resistance or cardiac output. However, since the change in pulmonary arterial pressure always occurred rapidly, and the ductus reacted comparatively slowly, it

seemed probable that a change in the volume of blood flow through the vessel was not of crucial importance. In order to test this the ductus was tied in lamb 527 A (Fig. 13). The pulmonary arterial pressure fell immediately and appeared to fall still further on exposure to 100% O_2 and to rise on return to ventilation with air. The implications of this observation need further examination.

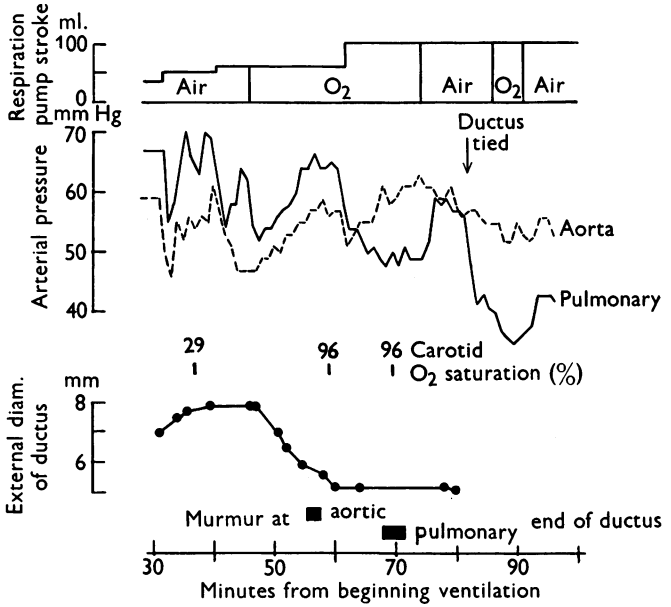

Fig. 13. Lamb 527 A, 139 days gestation age, under barbiturate anaesthesia. This lamb had been underventilated after tying the umbilical cord and the ductus had become constricted from 8·5 to 6·4 mm at 14% O_2 saturation. Progressive small increases in ventilation now caused the ductus to dilate. Subsequent ventilation with O_2 caused constriction, with the development of a murmur at the *aortic* end. An increase in respiration pump stroke, with further expansion of the lungs, caused a temporary disappearance of the murmur, which was next heard at the *pulmonary* end of the ductus. When the ductus was tied, ventilation with O caused a fall in pulmonary arterial pressure.

Underventilation after the umbilical cord was tied was always associated with a large rise of blood pressure. The pressure rose in two stages as with lambs breathing spontaneously, and it was easy to see on kymograph records of the femoral arterial pressure (Fig. 14) the preliminary rise due to an increase of systemic vascular resistance when the cord was tied, followed by a slower and more prolonged rise due to progressive asphyxia. (The same change was seen in lamb 536 A but the time scale of Fig. 12 is too small to show it.) The ductus also often became constricted while the pressure was still higher than under foetal conditions (Fig. 12), as in lambs which breathed spontaneously and

which were not under general anaesthesia (Figs. 10, 17). The heart rate was normally increased in the latter after a period of irregularity. In lambs which were deliberately underventilated the same cardiac irregularities were seen during severe asphyxia (Fig. 14), and thereafter the heart accelerated, though it did not necessarily exceed the initial foetal heart rate if this was unusually high. Thus the principal physiological changes observed in spontaneously breathing newborn lambs could be reproduced by underventilation of anaesthetized lambs. The changes in blood pressure and heart rate would be consistent with liberation of sympathetic amines during asphyxia, and it therefore seemed desirable to find out whether these would cause constriction of the ductus.

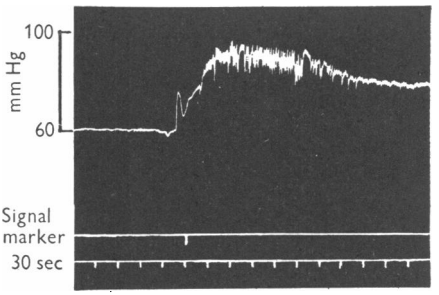

Fig. 14. Lamb 553 A, 142 days gestation age, under barbiturate anaesthesia. The umbilical cord was tied at the signal mark and inadequate ventilation was begun. The blood pressure rose at once when the placenta was cut off, fell and then rose again higher during the consequent anoxaemia.

Infusion of sympathetic amines into foetal lambs. Infusion of adrenaline (B.D.H.) or noradrenaline (Bayer) into four foetal lambs brought about constriction of the ductus arteriosus. The doses used varied from 1·7 to 4·9 μg/kg/min over 6–16 min. In a fifth lamb infusion of adrenaline 1·2 μg/kg/min for 5 min had no effect on the ductus. The larger doses which caused constriction of the ductus led to a considerable rise of blood pressure and to a small though not invariable reduction in carotid % O_2 saturation (Fig. 15). There was no increase in the latter sufficient to account for the constriction. When the ductus became constricted a clear murmur was heard which radiated down the descending aorta. Fig. 16 shows a phonocardiograph record taken, during an infusion of noradrenaline, from the descending aorta just below the entry of the ductus. The murmur appeared towards the middle of systole, coinciding almost exactly with the arrival of the pulse wave in the aorta. It was sometimes almost musical in character and disappeared just before the second heart sound. The mean aortic and pulmonary arterial pressures were not very different, but the pulmonary pulse wave preceded the aortic at the ductus, as has also been shown previously without an infusion of sympathetic amines

(Dawes *et al.* 1955*a*). The increased pressure difference across the ductus at this phase of the cardiac cycle would therefore account for the appearance of the murmur at mid-systole.

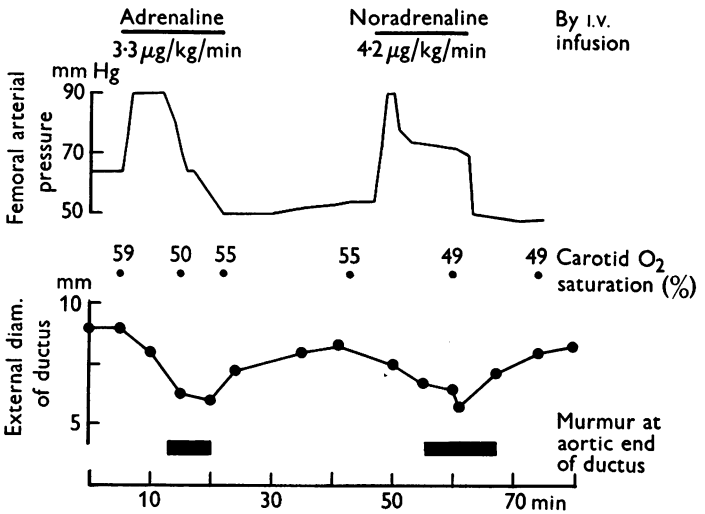

Fig. 15. Lamb 527 B, 139 days gestation age, barbiturate anaesthesia; foetal condition with intact umbilical cord and placenta. Infusion of adrenaline and noradrenaline into a femoral vein caused constriction of the ductus arteriosus and development of a soft systolic murmur (see Fig. 16) at its aortic end, with no great change in carotid % O_2 saturation.

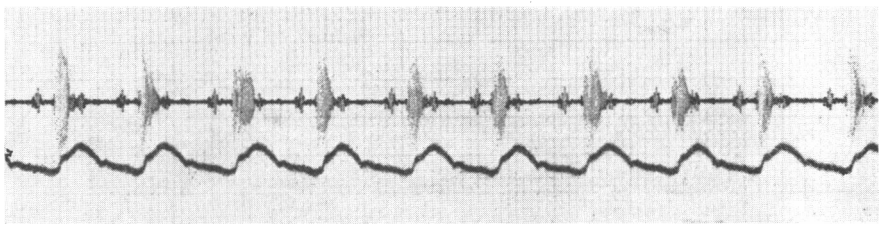

Fig. 16. Lamb 536 B, 140 days gestation age, barbiturate anaesthesia; foetal condition, with intact umbilical cord and placenta, during noradrenaline infusion. Phonocardiograph record from aortic end of ductus arteriosus (above) and condenser manometer record of aortic pressure (below). A systolic murmur is present between the first and second heart sounds.

Observations on carbon dioxide and pH

Changes in CO_2 *tension at birth.* The CO_2 tension in the carotid arterial blood of seven mature foetal lambs ranged from 30 to 50 mm Hg (Table 3). Two of these lambs had been delivered under local anaesthesia, and they breathed spontaneously when the umbilical cord was tied. In both, the CO_2 tension rose and the pH fell. In one of these, lamb 516 A, whose respiratory efforts were ineffective for some minutes, the CO_2 tension rose from 44 mm Hg to a

maximum of 68 mm Hg (Fig. 17). This was a lamb of 139 days gestation age which barely survived and in which the murmur over the ductus arteriosus appeared at an exceptionally low arterial O_2 saturation. It thus provided some indication of the maximal increase in CO_2 tension which might be expected under asphyxial conditions after birth. In the other lamb which breathed spontaneously the CO_2 tension rose from 29 to 42 mm Hg.

TABLE 3. CO_2 tension in the carotid blood of foetal sheep

Lamb no.	Breed	Anaesthetic	Gestation age (days)	p_{CO_2} (mm Hg)
583A	K	Pentobarbitone	133	50·5
533A	H	Pentobarbitone	135	45·7
527	H	Pentobarbitone	139	40·4
516A	H	Local	139	44·5
516B	H	Pentobarbitone	139	39·1
522	H	Pentobarbitone	141	31·5
559A	K	Local	141	29·4

K, Kerry Hill. H, Hampshire.

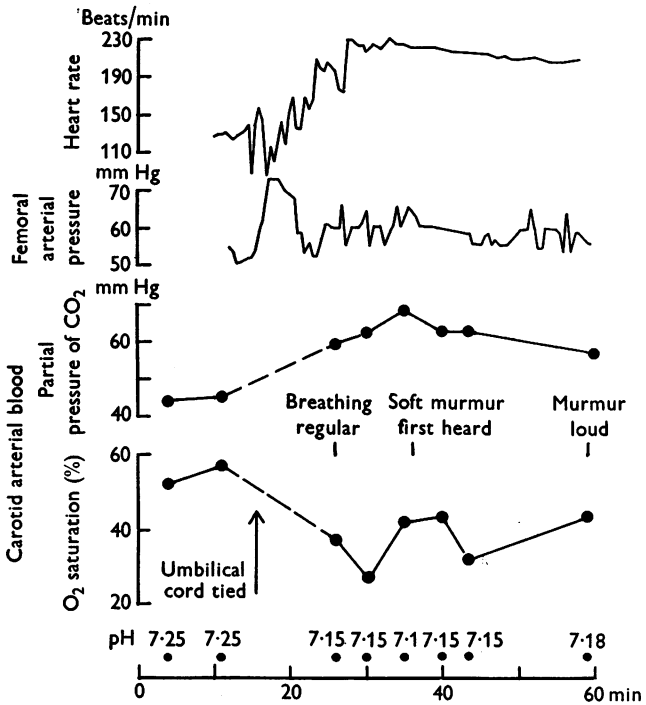

Fig. 17. Lamb 516A, 139 days gestation age, delivered under local anaesthesia. Blood samples were withdrawn from a catheter in the left carotid artery under local anaesthesia. When the umbilical cord was tied breathing started but was irregular for the first few minutes. A murmur was heard through the chest wall, over the ductus arteriosus, within 20 min of tying the cord and was still present some hours later. There was a rise of blood pressure and heart rate and a fall in blood pH during asphyxia.

Similar changes were observed in six lambs under general anaesthesia which were deliberately underventilated after the umbilical cord had been tied. The CO_2 tension rose during asphyxia and the pH fell (Fig. 12). As ventilation was gradually increased they returned towards the previous levels. These changes are so large that it was thought they might influence the ductus arteriosus. They might do this indirectly through the Bohr effect, the shift to the right of the O_2 dissociation curve with increased CO_2 tension, or directly. Some experiments to examine these possibilities were therefore performed.

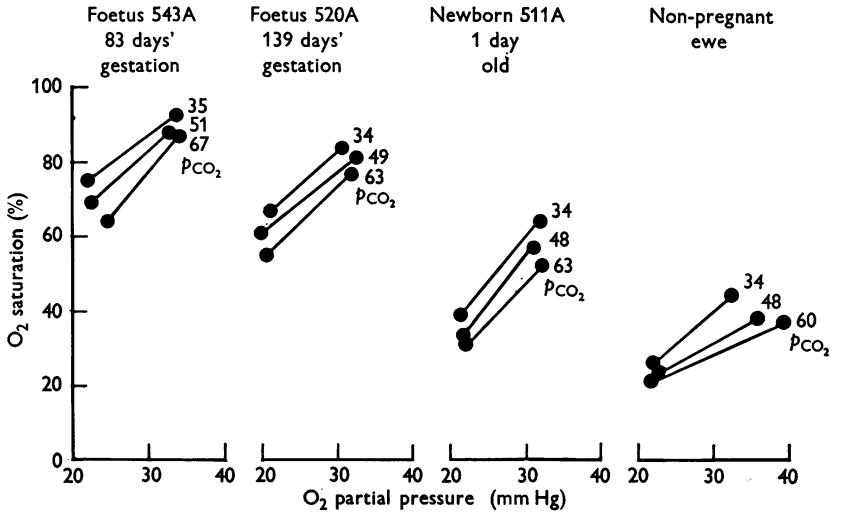

Fig. 18. Sample records of the effect of changes in the CO_2 partial pressure on the O_2 dissociation curves of blood from foetal and newborn lambs and an adult ewe. The partial pressures of CO_2 are recorded in mm Hg alongside each set of observations.

The Bohr effect. The CO_2 tensions used in the tonometers were adjusted to approximately 35, 50 and 65 mm Hg in order to cover the range which was most likely to be found in practice. The O_2 tensions were 20 or 30 mm Hg, which gave a saturation of 20–80% in experiments with blood from sheep of different ages. Observations were carried out on blood from six foetal lambs, two newborn lambs and two adult ewes. Representative records are shown in Fig. 18. The progressive fall in the regression lines with increasing age is attributable to the well-known fact that the O_2 dissociation curve of foetal blood lies to the left of that for maternal blood (Barcroft, 1946). Raising the CO_2 tension by about 30 mm Hg decreased the O_2 saturation by 5–12% at a given O_2 tension. In other words, in the middle range of the O_2 dissociation curve of the foetal or newborn lamb (20–40 mm Hg O_2 tension), an increase of CO_2 tension of 30 mm Hg increased the O_2 tension (at equal O_2 saturations) by only about 5 mm Hg. This is not large enough to account for the appearance

of a cardiac murmur at low O_2 saturations after birth, if an increase in arterial O_2 tension was the only cause of constriction of the ductus arteriosus. There was no indication from this small group of observations that the size of the Bohr effect differed with gestation age over the range of ages examined, although the proportion of foetal to adult haemoglobin must have differed considerably (Karvonen, 1949).

CO_2 *and the ductus arteriosus.* While no experiments have yet been carried out in which the CO_2 tension has been varied independently of changes in the O_2 tension, a number of observations have been made which are relevant to any future consideration of the part played by CO_2 in the constriction of the ductus arteriosus. Thus when lambs were fully ventilated under positive pressure with O_2, the CO_2 content of the blood fell as the O_2 saturation rose and the ductus became constricted. It seems unlikely that this reduction in CO_2 tension should have caused the plain muscle of the ductus to contract; nevertheless, a few experiments were done to test this possibility. Positive pressure ventilation of one lamb with 10% CO_2 and 90% O_2, and of two lambs with 7% CO_2 and 93% O_2, caused constriction of the ductus arteriosus within a few minutes, as the arterial O_2 saturation rose to 90% or above. Thus administration of CO_2 with O_2 did not in these experiments prevent constriction.

In one further lamb, ventilation with 10% CO_2 and 90% O_2 caused the ductus to become constricted from 10 mm external diameter to only 8·4 mm, even though the carotid O_2 saturation rose to 100%. Subsequent ventilation with air or 100% O_2 for 35 min caused no further change in the ductus. The lamb was in excellent condition, and post-mortem examination revealed no abnormality of the heart or great vessels. A twin of this lamb was then delivered and the ductus became constricted normally, from 10·2 to 5·7 mm, on ventilation with air. There was no difference between specimens of the ductus from these two lambs, when examined histologically. We are at a loss to suggest why the ductus should not have become fully constricted in the first lamb, but only in this one out of some fifty lambs has it failed to do so.

Returning to CO_2, there is equally no positive evidence that it plays a part in the constriction of the ductus during asphyxia. Although Figs. 13 and 18 show that severe asphyxia was associated with large rises in CO_2 tension, yet in one lamb the ductus was observed to become constricted slowly during a mild degree of asphyxia extending over some 30 min, with no significant change in CO_2 tension. While these scattered observations do not exclude the possibility that CO_2 may have a direct effect upon the ductus arteriosus, they do not suggest that it is of crucial importance.

Dilatation of the ductus arteriosus after previous constriction

When the ductus had become constricted for a short while, a return to the conditions which obtained before constriction led to a rapid dilatation to approximately the initial diameter. This was true under foetal conditions whether the constriction was induced by a rise in the arterial O_2 saturation (Figs. 6, 7) or by infusion of adrenaline. It was also true after tying the cord and beginning adequate ventilation, provided that the period during which the constriction lasted was not too long. Thus, in the experiment illustrated in Fig. 9, the initial diameter of 9·5 mm was reduced on ventilation with O_2 to 5·5 mm for about 7 min and increased to 10 mm on ventilation with air. During asphyxia, in the experiment recorded in Fig. 13, the ductus became constricted from 7·6 mm to less than 5·5 mm, and on return to an intermediate arterial O_2 saturation of 65–77 % dilated again to 6·5 mm. If the ductus was allowed to remain constricted for a longer period of time it did not dilate so readily. Thus in one lamb the maximum diameter was 9·9 mm, which was reduced rapidly on ventilation to 5·4 mm and an hour later was 4·8 mm; a period of moderate asphyxia, lasting 20 min, only caused a dilatation to 5·7 mm. The reaction of the ductus to moderate asphyxia was also examined in four lambs which had been born spontaneously and which were 5–10 hr old. These lambs were anaesthetized and the external diameter of the ductus was measured directly, as in the previous experiments. In two of these lambs a short period of asphyxia, 10–20 min, failed to cause any change in the ductus; in the other two, partial asphyxia for up to 1 hr. caused a small dilatation, from 4·6 to 5·8 mm, and from 5·4 to 6·7 mm respectively.

These observations suggest that during a few hours after birth some further change occurs in the ductus arteriosus which makes it no longer so sensitive to the changes in the O_2 tension of its environment. It seems possible that this may be related to the processes which eventually lead to total closure of the vessel and to the anatomical obliteration of its lumen.

DISCUSSION

Many hypotheses of the mechanism of closure of the ductus arteriosus have been proposed in the past, though few of them have been based on experiments on living animals. In the nineteenth century it was at first a matter of dispute as to whether obliteration occurred by 'inflammation' (*sic*) or intimal proliferation alone, or whether this was preceded by some mechanical process at birth. The idea that thrombosis was the immediate cause of closure was disproved by von Rauchfuss (1859) who found thrombi in the ductus of only four out of 1400 newborn infants on post-mortem examination. His observations have been confirmed by many subsequent workers (Mulherin & Krafka, 1936). Virchow (1856) suggested that the smooth muscle of the vessel wall

might contract sufficiently to block the lumen, and Schulze (1871) proposed that the ductus shut by mechanical means until obliteration by growth of the tissues was complete. Thoma (1883) also thought that constriction of the smooth muscle of the media preceded intimal proliferation. Finally, Gérard (1900*a*, *b*) and Wells (1908) clearly distinguished two phases: primary functional closure of the vessel by some mechanical process within a few minutes of birth, followed by secondary anatomical obliteration of the lumen. Since that time the nature of this mechanical process has been the subject of abundant speculation, though Patten (1930) continued to hold the view that a gradual process of closure, dependent on changes resembling those of endarteritis obliterans, was responsible.

Three principal mechanical hypotheses have been proposed. In the first hypothesis, which has been put forward in a number of different forms, it is suggested that the vessel is compressed or constricted by external forces. Walkhoff (1869) thought that the change in position of the ductus after birth led to kinking, interruption of blood flow and hence to coagulation of the blood. Schanz (1889) stated that the aortic end of the ductus was fixed, and that when breathing began the ductus was stretched by the pericardium and pulmonary arteries attached to its other end, leading to mechanical obliteration of the lumen. Stienon (1912) discussed dilatation of the aortic isthmus and turgescence of the left pulmonary artery as well as dislocation of the thoracic organs as possible factors in closure, and Melka (1926) concluded that the aorta and pulmonary artery compressed the ductus between them, since the ligamentum arteriosum was flattened from side to side. Some have thought that dilatation of the left bronchus (when breathing begins) compressed the ductus, or that tension on the recurrent laryngeal nerve was responsible, while other authors, such as Linzenmeier (1914), have included kinking as a principal cause of closure, but have added subsidiary factors. All these speculations were based on post-mortem material. It is undoubtedly true that the position and appearance of the ductus does alter after birth. Barcroft (1946) noted in the lamb that it shortened, and the angles of junction with the aorta and pulmonary trunk gradually became more obtuse. But nobody who has actually watched this process in the newborn living animal, in the guinea-pig (Kennedy & Clark, 1942), lamb or puppy with the chest opened, could believe that either kinking, traction or compression by external forces plays any part in the mechanism of closure. The vessel is too large to be kinked, and it visibly shortens as it becomes constricted.

The second hypothesis was proposed by Strassmann (1894), who thought that the tongue of tissue at the acute angle formed by the junction of ductus and aorta (the crista reuniens of Barclay, Franklin & Prichard, 1944) could act as a valve. When breathing began, pressure rose in the left heart and fell in the right heart, the aorta pressed on the anterior wall of the ductus, and

mechanical closure resulted. Strassmann's opinion has been supported by Roeder (1902), Kirstein (1910), Fromberg (1914) and by Hamilton, Woodbury & Woods (1937) in various species. This hypothesis has been vigorously attacked by a number of authors, on the grounds that the crista reuniens is too thick to act as a valve, and that the experiments which purported to show that blood cannot pass from the aorta through the ductus to the lungs were crude and carried out under unphysiological conditions (Haberda, 1886; Scharfe, 1900; Gräper 1921; Swennson, 1939; Barclay *et al.* 1944). In the lamb the crista reuniens certainly does not act as a valve and blood readily passes from aorta to the lungs when the aortic pressure exceeds the pulmonary (Barclay, Barcroft, Barron & Franklin, 1939; Dawes, Mott, Widdicombe & Wyatt, 1953). The same is true in the rabbit and dog (Handler, unpublished), and in the human baby (Lind & Wegelius, 1954).

The third hypothesis (Virchow, 1856) is that primary functional closure of the ductus arteriosus is effected by contraction of the longitudinal and circular muscle in the media of the vessel. As long ago as 1857 Langer (1857) recognized that the structure of the ductus was different from that of the pulmonary trunk and aorta. This view has received abundant confirmation, by Gérard (1900*a*, *b*), Schaeffer (1914), Gräper (1921), von Hayek (1935), Swennson (1939), Kennedy & Clark (1941), Boyd (1941), Jager & Wollenman (1942), and Danesino, Reynolds & Rehman (1955). The ductus has a looser structure than that of the aorta or pulmonary vessels, has less elastic tissue and no external elastic lamina. The hypothesis has therefore a good anatomical basis, but it leaves the problem of the stimulus to contraction at birth unsolved.

The nature of the effective stimulus to constriction of the ductus arteriosus in well-ventilated animals

Barcroft, Kennedy & Mason (1938) and Kennedy & Clark (1941, 1942) described the first systematic experimental studies on the stimulus to constriction of the ductus in foetal guinea-pigs. A wide variety of procedures was effective, including normal breathing, inflation of the lungs with a gas mixture containing oxygen, intravenous injection of oxygen, mechanical or electrical stimulation of the ductus, injection of adrenaline, haemorrhage, and stimulation of various nerves. From the evidence presented it is impossible to decide which stimuli, alone or in combination, were effective under normal physiological circumstances. Changes in blood pressure and arterial O_2 saturation were not recorded in these small foetuses, and although the vessel was observed directly through an opening in the chest wall, changes in diameter were not measured. There is one curious point about these experiments, that introduction of bubbles of O_2 into the umbilical vein of the foetus was found to lead to constriction of the ductus, and this was attributed to a rise in arterial O_2 content. Many investigators have in the past attempted to raise the O_2 content

of circulating blood in this way, but have failed to produce a substantial increase because of the slow uptake of O_2 by the blood from the small surface area presented by circulating bubbles. Gas embolism is an obvious hazard. We have repeated these experiments in mature foetal guinea-pigs. They have sometimes led to constriction of the ductus, but this has always been accompanied by visible gas embolism in various vessels, including the umbilical arteries, and by large changes in blood pressure and heart rate; the systemic arterial blood turned dark blue except in the immediate vicinity of the embolic bubbles. The interpretation of these observations is therefore a matter for speculation. Nevertheless, the other experiments reported by Kennedy & Clark suggested that arterial O_2 tension was one of the operative factors, and that it might be effective in the absence of the central nervous system.

Record & McKeown (1953) observed that in human patients with persistent patency of the ductus arteriosus there was a much higher incidence of a history of foetal distress at birth than in the ordinary population. Alzamora *et al.* (1953) also concluded that persistent patency of the ductus might be more common in children born at high altitudes. Record & McKeown (1955*a*, *b*) therefore exposed guinea-pigs to a reduced O_2 content in the inspired air for 24 hr after birth, and found at post-mortem examination that the diameter of the ductus arteriosus was wider than in control animals.

The experiments reported in the present paper give direct support to the hypothesis that an increase in arterial O_2 saturation causes constriction of the ductus, and suggest that O_2 acts directly on the smooth muscle of the ductus, in a preparation in which it is isolated except for the heart and adjoining vessels. One of the curious features about this phenomenon is the fact that the ductus takes some 5–10 min to become constricted and to attain a new equilibrium. Most other smooth muscles react to stimuli much more rapidly. This slow response to a change in the environment may explain why Barron (1944) failed to observe constriction of the vessel on perfusing it with well-oxygenated blood.

Barcroft (1946), writing of Kennedy & Clark's work, said that 'it is well known that the coronary vessels will relax in default of oxygen, but the conception of oxygen as a stimulus to unstriped muscle seems to constitute a challenge and demands further exploration'. Since then Ashton, Ward & Serpell (1953, 1954) and Ashton & Cook (1954) have demonstrated an analogous effect of oxygen in causing the retinal vessels of newborn kittens to constrict, indicating that excessive administration of oxygen to premature infants is a cause of retrolental fibroplasia. It is not clear whether oxygen acts directly upon the retinal vessels under these conditions, but it seems possible. There may be other vessels whose smooth muscle reacts to changes in O_2 tension under comparable conditions, such as the umbilical and cerebral arteries. The evidence for this is not conclusive. Lack of oxygen also usually causes a rapid

fall in the tone of excised surviving visceral muscle (Garry, 1928; Prasad, 1935; West, Hadden & Farah, 1951). There is therefore evidence from several different sources which suggests that the supply of oxygen to smooth muscle may be a critical factor in determining tone, under appropriate conditions.

The effect of asphyxia on the ductus arteriosus

Turning now to lambs which have been delivered under local anaesthesia, it is evident that many suffer from asphyxia after birth, and yet the ductus still becomes constricted within a short time. Similarly, in lambs under general anaesthesia which are deliberately underventilated after tying the umbilical cord, the ductus becomes constricted. This asphyxial constriction may explain a single observation made by Ardran, Dawes, Prichard, Reynolds & Wyatt (1952), in which the umbilical cord of a lamb was tied before ventilation of the lungs, and cineangiographic records showed no evidence of contrast medium passing through the ductus 64 sec later. Reynolds (1954*a*) also has reported that asphyxia causes constriction of the ductus under foetal conditions. This may be explained by the liberation of sympathetic amines, since both adrenaline and noradrenaline have been shown to cause constriction of the ductus when infused into the foetus. It has not yet been established that the concentration of these amines in the blood rises during asphyxiation of the newborn lamb, but in newborn lambs, which are normally subjected to some degree of asphyxia, the blood pressure and heart rate increase. The spleen also contracts (Taylor & Gotsev, 1938). Also, in one foetal lamb Reynolds (1954*b*) found that the increase in heart rate on asphyxia, which persisted after removing both stellate ganglia and cutting the vagi, disappeared on bilateral adrenalectomy.

The nerve supply of the ductus is not known with certainty. Takino & Watanabe (1937), Goormaghtigh & Pannier (1939) and Kennedy & Clark (1941) described nerve-endings in the ductus. Boyd (1941) described structures in close relationship to smooth muscle fibres which had the appearance of motor nerve-endings in various species, but their connexion with the autonomic nervous system was not established. Barron (1944) failed to find nerve fibres 'penetrating to the muscle layer or terminating in motor type endings' in sheep foetuses. Nevertheless, it is possible that stimulation of, say, a sympathetic nerve supply to the ductus might likewise cause constriction. So far, no such effect has been demonstrated. Kennedy & Clark (1942) stimulated various nerves in the foetal guinea-pig, including the left vagus and left stellate ganglion, without any effect on the ductus. Further work is therefore required to establish the details of the mechanism by which asphyxia causes constriction of the ductus.

Other authors have attributed closure of the ductus to changes in the pressures on its walls, the determinant factors being: an increase of blood flow

through the lungs (Kilian, 1826), the cessation of blood flow through the ductus arteriosus (because aortic and pulmonary arterial pressures become equal), and a fall in intravascular or transmural pressure on ventilation (Schulze, 1871; Thoma 1883; Ziegenspeck, 1885, 1905; Strassmann, 1894; Jores, 1924; Barron, 1944; Reynolds, 1953, 1954*a*). The experiments described in the present paper do not support these hypotheses. Pulmonary blood flow may increase without constriction of the ductus arteriosus. Both asphyxia and a substantial rise in arterial O_2 saturation (consequent on ventilation with O_2) have led to constriction, while flow through the ductus continued in either direction. Moreover, in lambs born under natural conditions, constriction of the vessel is accompanied by an audible murmur which is attributed to the continuance of blood flow through a narrow lumen. Similarly, both asphyxia and excess O_2 have led to constriction of the ductus, in different experiments, whether the blood pressure was raised above or lowered below the foetal value; variations of about 20 mm Hg above and below the normal foetal pressure did not lead to constriction in the absence of any change in arterial O_2 content. The diameter of the vessel must be determined by the transmural pressure on the one hand and the elastic and muscular tensions of the vessel wall on the other hand: but small changes in transmural pressure, such as occur in the newly delivered lamb, do not by themselves lead to irreversible constriction.

When the ductus has remained in the constricted state for a certain length of time, a return of the O_2 saturation of the arterial blood to the foetal level is no longer followed by full dilatation of the vessel to the foetal diameter. This observation suggests that some progressive change in the ductus has begun as a result of constriction. The nature of this process is not known. It is, however, appropriate to draw attention to the physical consequences of severe constriction of the vessel. Burton (1951, 1952) and Nichol, Girling, Jerrard, Claxton & Burton (1951), by applying the law of Laplace to blood vessels, predicted that there should be a critical closing pressure below which the lumen of a blood vessel would be entirely obliterated. If this hypothesis is correct, one may infer that, as complete obliteration is approached, even in the presence of a very small muscular tone, a very great transmural pressure is required to cause an increase in diameter. It would therefore follow that, once the ductus is constricted, it may be kept constricted by very little muscular effort.

The effects of underventilation on the pulmonary pressure in newborn lambs

In previous experiments it was observed that positive pressure ventilation of the lungs of mature foetal lambs, delivered by Caesarean section under general anaesthesia, caused a fall in systemic and pulmonary arterial blood pressure (Ardran *et al.* 1952). In these experiments the stroke of the respiration pump was so adjusted that the lungs were rapidly and fully expanded, as

judged by direct observation in the open chest. It was later found that the peak intratracheal pressures required were very considerable, about 25–35 mm Hg (Dawes *et al.* 1953). Lambs which are delivered under local anaesthesia, and which breathe spontaneously, can achieve intrapleural pressures as low as −50 mm Hg, but in spite of this they are often underventilated (Dawes *et al.* 1955*b*). In many lambs the systemic arterial O_2 saturation only rises slowly to 90% after birth, and the blood pressure rises on tying the cord and does not fall when breathing begins. These phenomena can be reproduced in lambs under general anaesthesia by underventilation.

In lambs delivered under local anaesthesia there appears, within 5–20 min of tying the umbilical cord and the onset of breathing, a murmur which is characteristic of blood flow through a constricted ductus arteriosus from aorta to lungs. This is attributed to a fall of the blood pressure in the pulmonary trunk below that in the aorta (Dawes *et al.* 1955*a*). But in lambs delivered under general (barbiturate) anaesthesia, underventilation does not usually lead to a comparable fall in pulmonary arterial pressure (relative to aortic pressure), and the murmur characteristic of flow from aorta to lungs may not develop. There is a possible explanation for the difference produced by barbiturates. They are well known to cause peripheral vasodilatation, and may reduce the pressor response to anoxaemia. Injection of sodium pentobarbitone into a foetal lamb causes a fall of blood pressure. It would not be surprising, therefore, if there were a relative reduction in systemic as compared with pulmonary arterial resistance in newborn lambs under barbiturate anaesthesia. This point will have to be considered further in future work for two reasons. First, we have no information as to the effect of a barbiturate on the ductus arteriosus itself. It does not prevent the ductus becoming constricted in response to asphyxia, to sympathetic amines or to a rise in the arterial O_2 tension, but it might modify its response. Secondly, and equally important, there might possibly be factors in the cardiovascular changes at birth, which could be modified considerably by barbiturate anaesthesia. We have considered above the possibility that the smooth muscle of various blood vessels may become constricted in response to an increase in arterial O_2 tension. After birth there is a large increase in arterial O_2 tension which has been shown to affect the calibre of the ductus arteriosus, and which might also alter the vascular tone of systemic or indeed pulmonary arterioles. Further experiments will be required to investigate this point.

The changes in venous pressures in underventilated newborn lambs are also of interest in determining whether or not the valve of the foramen ovale is closed throughout the cardiac cycle, and hence whether the cyanosis observed is in part due to a right-left shunt. The left atrial pressure rises in these lambs (Fig. 15), as it does in well-ventilated lambs (Dawes, Mott & Widdicombe 1955*c*). The immediate rise might be partly caused by cardiac distress during

the initial period of profound asphyxia. There is no doubt that pulmonary blood flow also increases, both as a result of the rise of blood pressure and because of the gradual expansion of the lungs. But at what stage these changes cause final closure of the valve of the foramen ovale in underventilated lambs is not known.

Cardiac murmurs in newborn lambs and babies

The cardiac murmur which is heard shortly after birth in newborn lambs is characteristic of blood flow from aorta to the lungs, first because it is a continuous murmur, similar to that described by Gibson (1898, 1900) in children or adults with a patent ductus; secondly, because it radiates medially from the point of maximum intensity; and thirdly, because when the chest is opened, the pressure in the aorta is found to exceed that in the pulmonary trunk (Dawes *et al.* 1955*a*). It is unlikely to be confused with the murmur which is due to flow from pulmonary trunk to aorta in the lamb, because the latter is very much softer, not continuous and radiates down the descending aorta.

In some newborn babies cardiac murmurs are heard after birth, though comparatively infrequently (2–5%) (Lyon, Rauh & Stirling, 1940; Taylor, 1953; Richards, Merritt, Samuels & Langmann, 1955; Eldridge, Hultgren & Wigmore, 1955). Some of these investigators listened to the chest on one occasion during the first 24 hr after birth, others listened more often. Many of the murmurs which were then heard disappeared subsequently, and no entirely satisfactory explanation for them has been offered, though it has been suggested that they were due to the continuation of flow through foetal channels. A few murmurs persisted, and were later associated with congenital heart disease of various kinds. Owen & Kingsbury (1924) and others since then have stated that they had never heard 'the classical murmur of a pathologically patent ductus within the first few days of life'. Various explanations for the difference between babies and lambs will therefore have to be considered. If it is conceded that the physical circumstances which lead to the production of the murmur in lambs and babies are the same, then in the latter:

(1) The ductus may not become constricted at birth. This seems unlikely in view of the cineangiographic observations of Lind & Wegelius (1949, 1954) and Keith & Forsyth (1950). This hypothesis would also require the postulation of an entirely different mechanism of constriction from that which obtains in the lamb and guinea-pig.

(2) The ductus may become completely constricted at birth.

(3) The ductus may be only partly constricted and pulmonary arterial pressure may not fall below aortic pressure so rapidly as in the lamb.

Eldridge *et al.* (1955) observed that, in some infants during the first 3 days after birth, the oxygen content of blood withdrawn from the foot was less than

that withdrawn from the hand. They deduced that there was in these infants a right-left shunt, probably through a patent ductus arteriosus. Prec & Cassels (1955) found that a large proportion of infants less than 15 hr old showed abnormal dye dilution curves after injection of T-1824 into the umbilical vein. These were attributed to persistent patency of the ductus arteriosus. The post-mortem observations of Gérard (1900*a*, *b*), Scammon & Morris (1918), Christie (1930), Leech (1935) and Jager & Wollenman (1942) on newborn infants also suggest that the ductus does not always close completely for some weeks after birth. Perhaps no one of these observations may be regarded as conclusive by itself. The interpretation of dye dilution curves is hazardous, and other workers (Barron, 1944; Barcroft, 1946) have hesitated to draw conclusions from post-mortem material. Yet it now seems probable that in newborn infants, as in lambs, the ductus arteriosus does not always close completely within a few minutes of birth. In such cases, if no murmur is to be heard, we must suppose, with Eldridge *et al.* (1955), that the pressure in the pulmonary artery is still high. Yet it seems likely that the pulmonary arterial pressure must fall soon after birth, since the weight of the right ventricle becomes considerably less than that of the left within a month (Keen, 1955). In children who are later found to have a patent ductus arteriosus the cardiac murmur is often atypical during early infancy. There may be no murmur, or a systolic murmur only. By the age of 3–4 years the continuous murmur is usually present, and its appearance has been attributed by Gross & Longino (1951) to an increase in the pressure difference across the ductus. However, if the ductus is widely open a murmur is not to be heard, and so in these particular infants appearance of the murmur might actually have been due to progressive constriction of the ductus at this age. Clearly more information is needed as to the course of events in the newborn baby.

Constriction of the ductus arteriosus in a *foetal* lamb, in which the direction of blood flow is from pulmonary trunk to aorta, is associated with the development of a soft systolic murmur. A number of authors have reported observations on human patients suffering from pulmonary hypertension and persistent patency of the ductus, in which the direction of blood flow from pulmonary trunk to aorta was confirmed by measuring the O_2 content of blood samples (Table 4). In one of these nine patients, no murmur was heard; the remainder all had a systolic murmur to the left of the sternal border, and two had, in addition, a diastolic murmur (Dammam, Berthrong & Bing, 1953; patients 2 and 4). In a tenth patient, Johnson, Werner, Kuschner & Cournand (1950) found intermittent flow in either direction through the ductus; this patient had both systolic and diastolic murmurs. Although it would be hazardous to make any generalization from these few instances, it seems probable that in man, as in sheep, unidirectional flow through a constricted ductus from pulmonary trunk to aorta may normally give rise to a systolic murmur. Blood

flowing in the reverse direction may also very rarely give rise to a systolic murmur (Gross & Longino, 1951; Bonham Carter & Lovel, 1953, case 3).

TABLE 4. Murmurs recorded in patients with blood flow through a patent ductus arteriosus from pulmonary trunk to aorta, confirmed by analysis of blood samples for O_2 content

Author	Patient no.	Age (years)	Murmur
Cosh (1953)	1	27	Faint systolic at pulmonary area
	2	9	Loud systolic over precordium
Hultgren *et al.* (1953)	2	34	No murmur
	3	31	Systolic left sternal border, 4th interspace
	4	21	Systolic left sternal border, 5th interspace
Dammam *et al.* (1953)	2	29	Systolic + thrill, 2nd left interspace; diastolic to left of sternum
	3	20	Systolic left sternal border
	4	30	Systolic at base to left of sternum, + diastolic 2nd left interspace
Smith (1954)	—	19	Systolic left sternal border, 3rd interspace

SUMMARY

1. In lambs delivered by Caesarean section the ductus arteriosus becomes constricted when the arterial O_2 saturation is raised by ventilating the lungs, and dilates when it is lowered by reducing the O_2 content of the inspired air. Inflation of the lungs with nitrogen (the placental circulation remaining intact) does not cause constriction of the ductus. The constriction still occurs after destruction of the brain and spinal cord, and when the systemic and pulmonary arterial pressures are stabilized.

2. In unventilated foetal lambs the ductus arteriosus also becomes constricted when the arterial O_2 saturation is raised, either by giving the ewe 100% O_2 to breathe, or when the placenta is replaced by the lungs of a twin lamb.

3. In an isolated heart–ductus–artificial lung preparation the ductus arteriosus becomes constricted when the arterial O_2 saturation is raised, and dilates when it is lowered.

4. In spontaneously breathing lambs delivered by Caesarean section under local anaesthesia, the ductus arteriosus often becomes constricted, as indicated by the development of a characteristic murmur, at a low arterial O_2 saturation. These lambs are partially asphyxiated at birth; there is a large fall of blood pH and a rise of CO_2 partial pressure. There is also a large increase in blood pressure and heart rate.

5. In lambs delivered by Caesarean section under general anaesthesia, deliberate underventilation and consequent asphyxia causes constriction of the ductus arteriosus. In these underventilated lambs the pulmonary arterial pressure does not fall, as it does in well-ventilated lambs, when the ductus becomes constricted.

6. Infusion of adrenaline and noradrenaline into foetal lambs causes constriction of the ductus arteriosus.

7. It was concluded that either a large increase in arterial O_2 tension, or the release of sympathetic amines by asphyxia, may cause constriction of the ductus arteriosus at birth.

8. The changing character of the cardiac murmur with the direction of blood flow through the ductus arteriosus was discussed, together with the possible reasons for its failure to appear in newborn babies.

9. The shift of the O_2 dissociation curve with change in CO_2 partial pressure (Bohr effect) was measured in foetal and newborn lambs. It was not sufficient to explain the constriction of the ductus arteriosus observed at low arterial O_2 saturations.

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REFERENCES

ALZAMORA, V., ROTTA, A., BATTILANA, G., ABUGATTAS, R., RUBIO, C., BOURONCLE, J., ZAPATA, C., SANTA-MARIÁ, E., BINDER, T., SUBIRIA, R., PAREDES, D., PANDO, B. & GRAHAM, G. G. (1953). On the possible influence of great altitude on the determination of certain cardiovascular anomalies. *Pediatrics*, **12**, 259–262.

ARDRAN, G. M., DAWES, G. S., PRICHARD, M. M. L., REYNOLDS, S. R. M. & WYATT, D. G. (1952). The effect of ventilation of the foetal lungs upon the pulmonary circulation. *J. Physiol.* **118**, 12–22.

ASHTON, N. & COOK, C. (1954). Direct observation of the effect of oxygen on developing vessels. *Brit. J. Ophthal.* **38**, 430–433.

ASHTON, N., WARD, B. & SERPELL, G. (1953). Role of oxygen in the genesis of retrolental fibroplasia. *Brit. J. Ophthal.* **37**, 513–520.

ASHTON, N., WARD, B. & SERPELL, G. (1954). Effect of oxygen on developing retinal vessels with particular reference to the problem of retrolental fibroplasia. *Brit. J. Ophthal.* **38**, 397–432.

BARCLAY, A. E., BARCROFT, J., BARRON, D. H. & FRANKLIN, K. J. (1939). A radiographic demonstration of the circulation through the heart in the adult and in the foetus, and the identification of the ductus arteriosus. *Brit. J. Radiol.* **12**, 505–517.

BARCLAY, A. E., FRANKLIN, K. J. & PRICHARD, M. M. L. (1944). *The Foetal Circulation.* Oxford: Blackwell Scientific Publications.

BARCROFT, J. (1938). *The Brain and its Environment.* London: Humphrey Milford, Oxford University Press.

BARCROFT, J. (1946). *Researches on Prenatal life.* Oxford: Blackwell Scientific Publications.

BARCROFT, J., BARRON, D. H., KRAMER, K. & MILLIKAN, G. A. (1938). Factors which influence the oxygen supply of the brain at birth. *J. Physiol. U.S.S.R.* **24**, 43–50.

BARCROFT, J., KENNEDY, J. A. & MASON, M. F. (1938). The relation of the vagus nerve to the ductus arteriosus in the guinea-pig. *J. Physiol.* **92**, 1–2*P*.

BARRON, D. H. (1944). The changes in the fetal circulation at birth. *Physiol. Rev.* **24**, 277–295.

BONHAM CARTER, R. E. & LOVEL, K. W. (1953). Atypical murmurs in patent ductus arteriosus. *Great Ormond Street J.* **6**, 93–101.

BORN, G. V. R., DAWES, G. S. & MOTT, J. C. (1955). The viability of premature lambs. *J. Physiol.* **130**, 191–212.

BORN, G. V. R., DAWES, G. S., MOTT, J. C. & RENNICK, B. R. (1955). The mechanism of constriction of the ductus arteriosus in the newborn lamb. *J. Physiol.* **128**, 28–29*P*.

BOYD, J. D. (1941). The nerve supply of the mammalian ductus arteriosus. *J. Anat., Lond.*, **72**, 146–147.

BURTON, A. C. (1951). On the physical equilibrium of small blood vessels. *Amer. J. Physiol.* **164**, 319–329.

BURTON, A. C. (1952). Laws of physics and flow in blood vessels. *Ciba Foundation Symposium: Visceral Circulation*, pp. 70–84.

CHRISTIE, A. (1930). Normal closing times of the foramen ovale and the ductus arteriosus. *Amer. J. Dis. Child.* **40**, 323–326.

COSH, J. A. (1953). Patent ductus arteriosus with pulmonary hypertension. *Brit. Heart J.* **15**, 423–429.

DAMMAM, J. F., BERTHRONG, M. & BING, R. J. (1953). Reverse ductus. A presentation of the syndrome of patency of the ductus arteriosus with pulmonary hypertension and a shunting of blood flow from pulmonary artery to aorta. *Johns Hopk. Hosp. Bull.* **92**, 128–150.

DANESINO, V. L., REYNOLDS, S. R. M. & REHMAN, I. H. (1955). Comparative histological structure of the human ductus arteriosus according to typography, age and degree of constriction. *Anat. Rec.* **121**, 801–829.

DAWES, G. S., MOTT, J. C. & WIDDICOMBE, J. G. (1954). The foetal circulation in the lamb. *J. Physiol.* **126**, 563–587.

DAWES, G. S., MOTT, J. C. & WIDDICOMBE, J. G. (1955*a*). The cardiac murmur from the patent ductus arteriosus in newborn lambs. *J. Physiol.* **128**, 344–360.

DAWES, G. S., MOTT, J. C. & WIDDICOMBE, J. G. (1955*b*). The patency of the ductus arteriosus in newborn lambs and its physiological consequences. *J. Physiol.* **128**, 361–383.

DAWES, G. S., MOTT, J. C. & WIDDICOMBE, J. G. (1955*c*). Closure of the foramen ovale in newborn lambs. *J. Physiol.* **128**, 384–395.

DAWES, G. S., MOTT, J. C., WIDDICOMBE, J. G. & WYATT, D. G. (1953). Changes in the lungs of the newborn lamb. *J. Physiol.* **121**, 141–162.

ELDRIDGE, F. L., HULTGREN, H. N. & WIGMORE, M. E. (1955). The physiologic closure of the ductus arteriosus in newborn infants. *J. clin. Invest.* **34**, 987–996.

FROMBERG, C. (1914). Studien über den Ductus arteriosus. *Arb. path. Anat. Bakt.* **9**, 198–224.

GARRY, R. C. (1928). The effect of oxygen lack on surviving smooth muscle. *J. Physiol.* **66**, 235–248.

GÉRARD, G. (1900*a*). Le canal artériel. *J. Anat., Paris*, **36**, 1–21.

GÉRARD, G. (1900*b*). De l'oblitération du canal artériel, les théories et les faits. *J. Anat., Paris*, **36**, 323–357.

GIBSON, G. A. (1898). *Diseases of the Heart and Aorta*, pp. 61, 303, 310–312. Edinburgh: Young J. Pentland.

GIBSON, G. A. (1900). Clinical lectures on circulatory affections—Lecture I. Persistence of the arterial duct and its diagnosis. *Edinb. med. J.* (N.S.), **8**, 1–10.

GOORMAGHTIGH, N. & PANNIER, R. (1939). Les paraganglions du cœur et des zones vasosensibles carotidienne et cardio-aortique chez le chat adulte. *Arch. Biol., Paris*, **50**, 455–526.

GRÄPER, L. (1921). Die anatomischen Veränderungen kurz nach der Geburt. III. Ductus Botalli. *Z. ges. Anat.* 1. *Z. anat. EntwGesch.* **61**, 312–329.

GROSS, R. E. & LONGINO, L. A. (1951). The patent ductus arteriosus. *Circulation*, **3**, 128–137.

HABERDA, A. (1886). *Die fötalen Kreislaufsorgane des Neugeborenen und ihre Veränderungen nach der Geburt.* Wien: J. Safář.

HAMILTON, W. F., WOODBURY, R. A. & WOODS, E. B. (1937). The relation between systemic and pulmonary blood pressures in the foetus. *Amer. J. Physiol.* **119**, 206–212.

HAWKINS, J. A. (1923). A micromethod for the determination of the hydrogen ion concentration of whole blood. *J. biol. Chem.* **57**, 493–495.

HULTGREN, H., SELZER, A., PURDY, A., HOLMAN, E. & GERBODE, F. (1953). The syndrome of patent ductus arteriosus with pulmonary hypertension. *Circulation*, **8**, 15–35.

JAGER, B. V. & WOLLENMAN, O. J. (1942). An anatomical study of the closure of the ductus arteriosus. *Amer. J. Path.* **18**, 595–613.

JOHNSON, R. E., WERNER, P., KUSCHNER, M. & COURNAND, A. (1950). Intermittent reversal of flow in a case of patent ductus arteriosus. *Circulation*, **1**, 1293–1301.

JORES, L. (1924). Offener Ductus arteriosus Botalli; in Henke E. & Lubarsch O., *Handbuch der speziellen pathologischen Anatomie und Histologie:* Herz und Gefässe, pp. 614–616. Berlin: Springer.

KARVONEN, M. J. (1949). In Roughton, F. J. W. & KENDREW, J. C., *Haemoglobin*, p. 279. Barcroft Memorial Conference.

KEEN, E. N. (1955). The postnatal development of the human cardiac ventricles. *J. Anat., Lond.*, **89**, 484–502.

KEITH, J. D. & FORSYTH, C. (1950). Aortography in infants. *Circulation*, **2**, 907–914.

KENNEDY, J. A. & CLARK, S. L. (1941). Observations on the ductus arteriosus of the guinea-pig in relation to its method of closure. *Anat. Rec.* **79**, 349–371.

KENNEDY, J. A. & CLARK, S. L. (1942). Observations on the physiological reactions of the ductus arteriosus. *Amer. J. Physiol.* **136**, 140–147.

KILIAN, H. F. (1826). *Ueber den Kreislauf des Blutes im Kinde, welches noch nicht geathmet hat.* Karlsruhe: Verlag der Chr. Fr. Müllerschen Hofbuchhandlung.

KIRSTEIN, F. (1910). Der Verschluss des Ductus arteriosus (Botalli). *Arch. Gynaek.* **90**, 303–334.

LANGER, C. (1857). Zur Anatomie der fötalen Kreislaufsorgane. *Z. Ges. Aerzte Wien*, **36**, 329–339.

LEECH, C. B. (1935). Congenital heart disease. *J. Pediatrics*, **7**, 802–839.

LIND, J. & WEGELIUS, C. (1949). Angiocardiographic studies on the human foetal circulation. *Pediatrics*, **4**, 391–400.

LIND, J. & WEGELIUS, C. (1954). Human fetal circulation: changes in the cardiovascular system at birth and disturbances in the post-natal closure of the foramen ovale and ductus arteriosus. *Cold Spr. Harb. Symp. quant. Biol.* **19**, 109–125.

LINZENMEIER, G. (1914). Der Verschluss des Ductus arteriosus Botalli nach der Geburt des Kindes. *Z. Geburtsh. Gynäk.* **76**, 217–253.

LYON, R. A., RAUH, L. W. & STIRLING, J. W. (1940). Heart murmurs in newborn infants. *J. Pediatrics*, **16**, 310–317.

MELKA, J. (1926). Beitrag zur Kenntnis der Morphologie und Obliteration des Ductus arteriosus Botalli. *Anat. Anz.* **61**, 348–361.

MELROSE, D. G. (1953). A mechanical heart lung for use in man. *Brit. med. J.* **2**, 57–62.

MULHERIN, W. A. & KRAFKA, J. (1936). Intravascular clotting in abandoned fetal channels in the newborn. *J. Pediatrics*, **9**, 318–322.

NICHOL, J., GIRLING, F., JERRARD, W., CLAXTON, E. B. & BURTON, A. C. (1951). Fundamental instability of the small blood vessels and critical closing pressures in vascular beds. *Amer. J. Physiol.* **164**, 330–344.

OWEN, S. A. & KINGSBURY, A. N. (1924). The diagnosis of congenital heart disease in the newborn including a record of cases of foetal endocarditis and maldevelopment. *Brit. J. Child. Dis.* **21**, 161–172.

PATTEN, B. M. (1930). The changes in circulation following birth. *Amer. Heart J.* **6**, 192–205.

PRASAD, B. N. (1935). The mechanical activity of gut muscle under anaerobic conditions. *J. Physiol.* **85**, 249–266.

PREC, K. J. & CASSELS, D. E. (1955). Dye dilution curves and cardiac output in newborn infants. *Circulation*, **11**, 789–798.

RECORD, R. G. & MCKEOWN, T. (1953). Observations relating to the aetiology of patent ductus arteriosus. *Brit. Heart J.* **15**, 376–386.

RECORD, R. G. & MCKEOWN, T. (1955*a*). Anatomical closure of the ductus arteriosus in the guinea-pig. *Clin. Sci.* **14**, 213–224.

RECORD, R. G. & MCKEOWN, T. (1955*b*). The effect of reduced atmospheric pressure on closure of the ductus arteriosus in the guinea-pig. *Clin. Sci.* **14**, 225–233.

REYNOLDS, S. R. M. (1953). Circulatory adaptations to birth. *Sci. Mon. N.Y.* **77**, 205–213.

REYNOLDS, S. R. M. (1954*a*). Hemodynamic characteristics of the fetal circulation. *Amer. J. Obstet. Gynec.* **68**, 69–80.

REYNOLDS, S. R. M. (1954*b*). Homeostatic regulation of resting heart rate in fetal lambs. *Amer. J. Physiol.* **176**, 162–168.

RICHARDS, M. R., MERRITT, K. K., SAMUELS, M. H. & LANGMANN, A. G. (1955). Frequency and significance of cardiac murmurs in the first year of life. *Pediatrics*, **15**, 169–179.

ROEDER, H. (1902). Die Histogenese des arteriellen Ganges. *Arch. Kinderheilk.* **33**, 147–161.

ROUGHTON, F. J. W. & SCHOLANDER, P. F. (1943). Micro-gasometric estimation of the blood gases. I. Oxygen. *J. biol. Chem.* **148**, 541–550.

SCAMMON, R. E. & MORRIS, E. H. (1918). On the time of the post-natal obliteration of the fetal blood-passages (foramen ovale, ductus arteriosus, ductus venosus). *Anat. Rec.* **15**, 165–180.

SCHAEFFER, J. P. (1914). The behaviour of elastic tissue in the post-fetal occlusion and obliteration of the ductus arteriosus (Botalli) in *Sus scrofa*. *J. exp. Med.* **19**, 129–143.

SCHANZ, F. (1889). Ueber den mechanischen Verschluss des Ductus arteriosus. *Pflüg. Arch. ges. Physiol.* **44**, 239–269.

SCHARFE, H. (1900). Der Ductus Botalli. Beiträge zur Physiologie und Pathologie des Verschlusses. *Beitr. Geburtsh. Gynäk.* **3**, 368–381.

SCHULZE, B. S. (1871). *Der Scheintod Neugeborener.* Jena.

SMITH, G. (1954). Patent ductus arteriosus with pulmonary hypertension and reversed shunt. *Brit. Heart J.* **16**, 233–240.

STIENON, L. (1912). Sur la fermeture du canal de Botallo. *Arch. Biol., Paris*, **97**, 801–813.

STRASSMANN, P. (1894). Anatomische und physiologische Untersuchungen über den Blutkreislauf beim Neugeborenen. *Arch. Gynäk.* **45**, 393–445.

SWENNSON, Å. (1939). Beitrag zur Kenntnis von dem histologischen Bau und dem postembryonalen Verschluss des Ductus arteriosus Botalli. *Z. mikr.-anat. Forsch.* **46**, 275–298.

TAKINO, M. & WATANABE, S. (1937). Über die Bedeutung des Ligamentum arteriosum bzw. des Ductus Botalli und der Ansatzstelle desselben an der Pulmonalwand (A. Pulm.) als Blutdruckzügler bei verschiedenen Tierarten. *Arch. Kreislaufforsch.* **2**, 18–27.

TAYLOR & GOTSEV (1938). Cited by Barcroft, J., in *The Brain and its Environment*, p. 56. New Haven: Yale University Press; London: Oxford University Press.

TAYLOR, W. C. (1953). The incidence and significance of systolic cardiac murmurs in infants. *Arch. Dis. Child.* **28**, 52–54.

THOMA, R. (1883). Die Rückwirkung des Verschlusses der Nabelarterien und des arteriösen Ganges auf die Structur der Aortenwand. *Virchows Arch.* **93**, 443–505.

THORP, R. H. (1948). A simple recording impulse counter. *Brit. J. Pharmacol.* **3**, 271–272.

VIRCHOW, R. (1856). Die Thrombosen der Neugebornen, pp. 591–597, in *Gesammelte Abhandlungen zur wissenschaftlichen Medicin.* Frankfurt A. M.: Verlag von Meidinger Sohn & Comp.

VON HAYEK, H. (1935). Der funktionelle Bau der Nabelarterien und des Ductus Botalli. *Z. ges. Anat.* **1**. *Z. Anat. EntwGesch.* **105**, 15–24.

VON RAUCHFUSS, C. (1859). Ueber Thrombose des Ductus arteriosus Botalli. *Virchows Arch.* **17**, 376–397.

WALKHOFF, F. (1869). Das Gewebe des Ductus arteriosus und die Obliteration desselben. *Z. rat. Med.* **36**, 109–131.

WELLS, H. G. (1908). Persistent patency of the ductus arteriosus. *Amer. J. med. Sci.* **136**, 381–400.

WEST, T. C., HADDEN, G. & FARAH, A. (1951). Effect of anoxia on response of the isolated intestine to various drugs and enzyme inhibitors. *Amer. J. Physiol.* **164**, 565–572.

WIDDICOMBE, J. G. (1954). Respiratory reflexes from the trachea and bronchi of the cat. *J. Physiol.* **123**, 55–70.

WILLIAMS, F. C. & UTTLEY, A. M. (1946). The Velodyne. *J. Instn elect. Engrs.* **93**, III A, 1256–1274.

ZIEGENSPECK, R. (1885). In W. Preyer's *Specielle Physiologie des Embryo*, pp. 596–607. Leipzig: Th. Grieben (L. Fernau).

ZIEGENSPECK, R. (1905). *Samml. klin. Vortr.* (neue Folge), **401**, Gynäk 134–164, 405–430.