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The Respiration of Cardiovascular Tissues of the Developing Chick and Adult Fowl

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Little information is available on the oxygen uptake of cardiac tissue, one probable reason for this being the technical difficulty of making accurate observations on such tissue owing to the rapidity with which it deteriorates after removal from the experimental animal (Bernheim & Bernheim, 1944; Webb, Saunders & Thienes, 1949*a*). Values have been reported for the mouse heart by Pearce (1936), for the rat heart by Bertalanffy & Pirozynski (1951), Webb *et al.* (1949*a*) and Nowy & Helmreich (1950), but only a few observations have been made on the chick-embryo heart (Warburg & Kubowitz, 1927).

The present study mainly deals with the oxygen uptake of cardiac tissue in the chicken (*Gallus domesticus*) during incubation, after hatching and in adult cockerels. The results are compared with histological changes observed in the heart during the various stages of development. In addition, further observations were made on the aorta to supplement the previously reported work of Henderson & MacDougall (1956).

MATERIALS AND METHODS

Determination of oxygen uptake

The differential microrespirometer described by Cruickshank (1954) was used for determining the oxygen uptake of tissue slices. Chick embryos ranging in age from 5 to 22 days of incubation, chickens aged 1–16 days after hatching and mature cockerels provided the tissues. In addition to the incubation age, the weight of each embryo minus its yolk sac was recorded. Observations on the external form of the embryo as described by Hamilton (1952) for determining the stage of development were made, but were not so useful for the present purpose as age and weight.

The method was essentially the same as that used by Henderson & MacDougall (1956) in the study of the respiration of chick-embryo arteries. Immediately after removal from the bird, the heart was placed in ice-cold Krebs-

Ringer phosphate and cut by free-hand slicing into 0.5 mm. thick fragments. Preliminary experiments showed that slices which were much thicker or thinner than 0.5 mm. gave less than optimum Q_{O_2} values. The speed of transfer of the tissue from the animal, the temperature of the Krebs-Ringer phosphate and the thickness of the slices proved to be factors more critical than in the previous work on arteries.

The medium was that used by Cruickshank (1954), but without streptomycin since prolonged observation was not required. It consisted of 0.5 ml. of horse serum, 0.3 ml. of Krebs-Ringer phosphate and 0.1 ml. of 5% (w/v) glucose solution. The Krebs-Ringer phosphate solution was composed of (as parts by vol.) 0.154 M-NaCl (100); 0.154 M-KCl (4); 0.11 M-CaCl₂ (3); 0.154 M-MgSO₄·7H₂O (1). To this was added phosphate buffer (21 parts) prepared by mixing 40 ml. of 0.25 M-Na₂HPO₄ with 2 ml. of N-HCl and diluting this mixture to 100 ml. The tissue slices were placed on lens paper and held floating on the surface of the medium on a platform of tantalum gauze. The gas phase was air, and carbon dioxide was absorbed by filter paper soaked in KOH (2%, w/v). It is possible that higher values for oxygen uptake might have been obtained with oxygen as the gas phase, but this was not used as it was desired to compare the results of the present investigation with those of Henderson & MacDougall (1956). After thermal equilibration for $\frac{1}{2}$ hr. in a water bath at 38°, readings were taken every 10 min. for periods of up to 2 hr., during which time the oxygen uptake was found to be linear for at least the first hour in all cases. After the tissue slices had been dried to constant weight at 100° the uptake of oxygen was calculated as $Q_{O_2} = \mu\text{l. of } O_2/\text{mg. dry wt. of tissue/hr.}$

Histological methods

Portions of the atrium, apex of the ventricle and aorta adjacent to those used for the respiration studies were examined by a variety of histological methods. Immediately after the death of the animal the tissues were placed in one or more of the following fixatives: Ringer-formol, formol corrosive and Zenker's fluid. After identical processing, paraffin sections (10 μ) were stained by standard methods for connective tissues. To demonstrate collagenous fibres Van Gieson's stain, Mallory's aniline blue and Masson's trichrome (light-green) stain were used. For

elastic tissue, the stains employed were Weigert's, Verhoeff's and Taenzer-Unna's orcein. Two silver-impregnation methods were used for reticulin fibres: Foot's modification of Hortega's silver carbonate method (Edwards, 1950) and Lendrum's (1951) rapid method. Every precaution was taken to ensure that each series of sections received as nearly identical treatment as possible.

Measurement of connective tissue

The optical density of a series of sections of the embryonic ventricle treated by Foot's modification of Hortega's method without counterstain was determined in an EEL Major densitometer (Evans Electroselenium Ltd.). The mean reading for several spots was taken for each section. Owing to the lack of adequate colour in silver-impregnated sections of very young embryos it was impossible to centre such sections accurately over the aperture in the densitometer. Values for the younger embryos therefore could not be obtained.

RESULTS

Oxygen uptake of the ventricle in chick embryos

The Q_{O_2} of the apices of the ventricles from 41 chick embryos was determined in the micro-respirometer. Values were obtained for each day of incubation from 5 to 22 days, and the Q_{O_2} was found to fall from -24.3 at 5 days to -4.1 at 22 days. Mean values for each day of incubation are shown in Fig. 1, in which Q_{O_2} is related to age. It will be noted that apart from the initial steepness of the curve between 5 and 9 days, the fall in Q_{O_2} is almost linear. When the Q_{O_2} is related to the weights of the chick embryos as in Fig. 2, the

initial steepness of the curve becomes more pronounced owing to the relatively greater rate of growth in the earlier stages of incubation.

Oxygen uptake of the ventricle in chicks after hatching and in adult cockerels

A similar series of observations was made on the oxygen uptake of the apices of the ventricles of 30 chicks ranging in age from the newly hatched to 15 days after hatching. The results are summarized in Fig. 3, from which it will be seen, by comparison with Fig. 1, that there is an initial rise in the Q_{O_2} , becoming evident on the 1st day after hatching, and falling by the third day to the value found on the last day of incubation before hatching. Thereafter there is a continued fall to a value of -1.7 by the fifteenth day.

Estimations were also made on the oxygen uptake of corresponding portions of the ventricle from 10 cockerels varying in age from 6 months to 3 years. The results, also shown in Fig. 3, show that there is a further reduction of Q_{O_2} with increasing age, to a final value of -0.5 at 3 years.

Comparison between the oxygen uptake of atrium and ventricle

A similar series of observations to those on the ventricle was carried out on the atria of young hatched chicks and older cockerels. The results, shown in Fig. 3, indicate that the oxygen uptake of atrial tissue is consistently higher than that of the ventricle, but that a similar continuing fall in values occurs as age advances.

Fig. 1. Relationship between the oxygen uptake of chick-embryo ventricle and the incubation age of the chick. Determinations were carried out at 38° with air as the gas phase. Mean values of two or more determinations are given in all cases, and the range of individual values was within $\pm 5\%$ of the mean value. Although the normal incubation period is 21 days, occasionally an embryo takes longer to hatch. Oxygen uptake (Q_{O_2}) is expressed as $\mu\text{l. of } O_2/\text{mg. dry wt. of tissue/hr.}$

Fig. 2. O, Relationship between individual values of the oxygen uptake (Q_{O_2}) of chick-embryo ventricle and the weight of the chick. Determinations were carried out at 38° with air as the gas phase. Oxygen uptake is expressed as $\mu\text{l. of } O_2/\text{mg. dry wt. of tissue/hr.}$ ▲, Relationship between the reciprocal of the optical density of silver-impregnated sections of chick embryo ventricle ($1/E$) and the weight of the chick.

Oxygen uptake of the aorta

Values were also obtained for the Q_{O_2} of the aorta in young hatched chicks and in cockerels, and were found to be less than the corresponding values for the ventricle. They followed the same general pattern of a gradual fall throughout life, as can be seen in Fig. 3.

Histological observations

The methods used for collagen, elastic tissue and reticulin showed that all three of these fibrous components of the cardiovascular tissues increased with the age of the animal. There were differences between the tissues themselves, however. In the ventricle there is a certain amount of elastic and collagenous material in the subendocardial tissue. Elastic tissue is also present in the walls of the blood vessels found throughout the myocardium. The most striking change with increasing age, however, was found in the proportion of reticular fibres, which were more numerous, thicker and more tortuous in the older hearts.

An increase of elastic tissue with age was more obvious in the atrium than in the ventricle. The development of this tissue appeared to be accelerated in later years.

In the aorta also an increase in the amount of all three connective-tissue fibres was apparent with increasing age. The collagenous and elastic fibres were more affected than the reticular tissue,

becoming progressively thicker, longer and more wavy throughout life.

The difference between the fibrous content of any of these tissues in the embryo and in the adult bird was obvious at a glance. The progressive increase began during embryonic life, however, and an attempt to demonstrate this in a quantitative way was made by measuring the optical density of silver-impregnated sections of the chick-embryo ventricle without counterstain, as already described. The values obtained are shown in Fig. 2 as reciprocal readings to facilitate comparison between the results and those of the Q_{O_2} determinations. The similarity in the curves can be seen.

DISCUSSION

As shown by Bernheim & Bernheim (1944) and Webb *et al.* (1949a) the extreme sensitivity of the heart to anoxia leads to irreversible changes if any delay occurs between the death of the animal and transfer of tissue to the respirometer. In addition, the large size of cardiac-muscle cells (Shorr, 1942) results in a larger proportion of damaged cells. In the present work, consistent results were obtained by rapid manipulation, and careful slicing of the tissue to the optimum thickness while maintaining it at low temperatures. The inclusion of glucose in the medium, as was shown by Webb, Saunders & Thienes (1949b), allowed steady rates of respiration to be maintained for 1–2 hr.

The general pattern of the values for the oxygen uptake of chick-embryo ventricle was similar to that found by Henderson & MacDougall (1956) in chick-embryo arteries and by Maier & Haimovici (1957), who demonstrated a decrease in the oxidative capacity of human aortic tissue with ageing. If the results of the present investigation are compared with those of Henderson & MacDougall (1956), it will be seen that in the younger embryos arterial tissue respire at a slightly higher rate, but that from about the thirteenth day of incubation onwards cardiac tissue is more active, this difference being maintained in the adult bird, whereas the oxygen uptake of atrium, ventricle and aorta continues to decline. The figures for the oxygen uptake of cardiovascular tissue therefore are in agreement with those reported for the intact chick embryo by Philips (1941), and for its homogenate by Albaum, Novikoff & Ogur (1946), and parallel the declining basal metabolism of the fowl described by Mitchell, Card & Haines (1927).

The curious temporary rise in the oxygen uptake of ventricular tissue soon after hatching appears to be an expression of the bird's attempts to adjust itself to a new environment. It was shown by Pembry, Gordon & Warren (1894) that during its life within the egg, the chick is poikilothermic,

Fig. 3. Relationship between the oxygen uptake (Q_{O_2}) of cardiovascular tissue, in the hatched chick and fowl (inset graph), and the age of the bird (●, Q_{O_2} of atrium; ○, Q_{O_2} of ventricle; △, Q_{O_2} of aorta). Determinations were carried out at 38° with air as the gas phase. Each value represents the mean of two determinations. Oxygen uptake is expressed as $\mu\text{l. of O}_2/\text{mg. dry wt. of tissue/hr.}$

except for a few hours preceding hatching, when the transformation to the homoiothermic state occurs. In its changing response to environmental temperatures before and after hatching, the embryo may be compared with the hibernating animal. During hibernation, the response of the animal is typically cold-blooded, but when the animal wakes there is an outburst of metabolic activity and a rapid increase in heat production (Pembry, 1901, 1903). A similar but smaller outburst of metabolic activity occurs in the hatching chick. Apart from this minor fluctuation, more evident in the ventricle than in the aorta, the fall in Q_{O_2} continues and can be demonstrated in the adult cockerel.

The higher values found in the atrium as compared with the ventricle confirm similar results obtained in the rat by Nowy & Helmreich (1950).

As with chick-arterial tissue, the fall in the oxygen uptake of the ventricle with increasing age or weight of the animal appears to be related to an increase in the amount of connective-tissue fibres. In the atrium, as already mentioned, the development of elastic fibres appears to be accelerated in later years, and as can be seen from Fig. 3, the absolute fall in Q_{O_2} of the atrium is greater between 18 months and 3 years than it is with either the ventricle or the aorta.

The available information in regard to other animals is as follows. Lowry, Hastings, Hull & Brown (1942) found that in the rat there was a considerable rise in both the collagen and elastin content of cardiac muscle with increase in age. Bacon (1948) showed that in the myocardium of the mouse the number and thickness of reticular fibres increase with age. Dogliotti (1931) could find no difference in reticular fibres between young and senile human hearts, but Ehrenberg, Winnecken & Biebricher (1954), using quantitative methods, showed a continual increase in the amount of connective tissue in the human heart throughout life.

It is not clear, however, whether the increase of these relatively inert fibres is sufficient to explain the whole of the considerable reduction in the oxygen uptake which was observed. In further work, to be published later, it has been confirmed by chemical and histochemical methods that the increase of these fibres in the cardiovascular tissue of the fowl continues after embryonic life in both heart and aorta, just as the respiration rate falls.

SUMMARY

1. The Q_{O_2} of cardiac- and aortic-tissue slices from chick embryos, hatched chicks and adult cockerels of various ages was determined by means of a microrespirometer.

2. The embryonic ventricles showed Q_{O_2} values ranging from -24.3 at 5 days to -4.1 at 22 days of incubation.

3. After hatching there was a further fall in the oxygen uptake of the ventricle, apart from an initial temporary rise. The fall continued into adult life, reaching a Q_{O_2} value of -0.5 at the age of 3 years.

4. The atria of hatched chicks and adult cockerels showed higher values for Q_{O_2} than the corresponding ventricles. The ventricles in turn gave higher values than the aorta, except in the earlier phases of embryonic life.

5. The continual fall in oxygen uptake of atrium, ventricle and aorta, throughout the life of the animal, appeared to be associated with a gradual increase in the amount of connective-tissue fibres present in the tissue.

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