dride and tetraethyl pyrophosphate on oxidative phosphorylation have also been investigated.

4. The inhibition of oxidative phosphorylation obtained with the six anticholinesterases is discussed in relation to the degenerative changes which certain of these compounds produce in the nervous system.

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The Respiration of Arterial Tissue

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The degenerative diseases affecting arteries have received a great deal of attention, and much work, both clinical and experimental, has been done on such problems as the pathogenesis of atherosclerosis. By comparison, little is known about the metabolism of the normal artery. The histological appearances of the artery do not suggest that it is a particularly active tissue, but the presence of *vasa vasorum* (i.e. the nutrient vessels of the artery wall) in the larger vessels does indicate that a moderate amount of respiratory exchange might be expected. This expectation has been partly confirmed for the rat, both by Lazovskaya (1943) and by Briggs, Chernick & Chaikoff (1949). As these reports, however, are somewhat conflicting, it was decided to reinvestigate the respiration of the rat aorta and to extend the work to include several other species. In addition, the respiratory activity of arterial tissue during the development of the embryo chick was studied, and the results of this work were compared with changes observed in the histological appearance of the arteries during the same period of embryological life.

MATERIALS AND METHODS

Respiration method I. The direct method of Warburg was used throughout for estimating the oxygen uptake of tissues whose dry weights were over 20 mg. Krebs solution 'Medium II' was used as the respiration medium (Krebs, 1950).

The laboratory animals were killed by stunning followed by severance of the vertebral column. A dorsal approach was used to expose the aorta, which was rapidly excised and placed in a dish containing ice-cold Krebs solution surrounded by powdered ice. The blood contained in the aorta was immediately removed by washing through with the Krebs solution by means of a syringe, and any blood and fat adhering to the outer wall were also removed. In the experiments carried out on slaughterhouse material, a portion of aorta was placed in a bottle containing the ice-cold Krebs solution, surrounded by ice in a vacuum flask and quickly transported to the laboratory. The measurement of oxygen uptake was carried out on artery rings, approx. 2 mm. in length, so that although the thickness of the artery wall varied in the different species used, the depth of tissue in the rings was relatively constant. After equilibration, the oxygen uptake was measured for 45 min., during which period it was found to be linear.

Respiration method II. The micro-respirometer devised by Cruickshank (1954) was employed in the present work for determining the oxygen consumption of tissues whose dry weights varied from 0.1 to 10 mg. It was therefore particularly useful for the work on chick-embryo arteries. The medium used by Medawar (1948) in the culturing of skin and also used by Cruickshank (1954) was chosen, but streptomycin was omitted, since the Q_{O_2} was determined over only short periods making aseptic conditions unnecessary. The medium therefore consisted of 0.5 ml. of serum, 0.3 ml. of Krebs-Ringer phosphate, 0.1 ml. of 5% glucose solution. Horse serum was used throughout, since homologous serum does not appear to be essential for *in vitro* work (Cameron, 1950).

Chick embryos ranging in age from 6 to 22 days of incubation were employed. Since the rate of growth of chicks varies considerably owing to seasonal and other factors (Hamilton, 1952), weight was used as a second criterion of development. Accordingly, each chick embryo was weighed minus its yolk sac. The truncus arteriosus was removed from the very young birds and the corresponding arteries, namely, the proximal parts of the aorta and the pulmonary arteries, were taken from the more developed specimens. The arteries were divided into fragments approximately 0.5 mm. thick.

stained by the following methods: Foot's modification of Horta's stain for reticulin, Mallory's stain and Masson's trichrome stain, both for collagen, Taenzer-Unna orcein stain for elastic fibres and Verhoeff's elastic-tissue stain. A description of these methods is given by Edwards (1950). All the tissues were given identical treatment, being fixed for the same length of time, dehydrated and embedded together, cut at the same thickness (10μ), and stained in the same jar in sets of six for each stain.

Measurement of connective tissue. The optical density of the Mallory-stained sections was determined by means of the EEL major densitometer, the mean reading for two spots being taken for each artery. Different spots on the same artery gave very similar results.

RESULTS

The accuracy of the micro-respirometer was tested by determining the Q_{O_2} values of yeast in the absence of substrate, and of rat arterial slices. The results in Table 1 show that the micro-respirometer gives values which are similar to those obtained by using much larger amounts of the same respiring material in the Warburg apparatus.

Table 1. *Comparative results using the micro-respirometer and the Warburg apparatus with air as the gas phase*

Carbon dioxide was absorbed with KOH (10%, w/v, in the Warburg apparatus and 2%, w/v, in the micro-respirometer). Values as $Q_{O_2} = \mu\text{l. O}_2/\text{mg. dry wt. tissue/hr.}$

	Fresh baker's yeast in 0.05M-KH ₂ PO ₄ at 25°			Rat-artery slices at 37°		
	No. of expts.	Range	Mean	No. of animals	Range	Mean
Micro-respirometer	3	-8.3 to -8.6	-8.4	3	-1.8 to -2.1	-1.9
Warburg apparatus	4	-8.0 to -9.5	-8.7	—	—*	—

* Not estimated in air, but values in O_2 gave a mean of -2.43 (see Table 3).

The medium (0.9 ml.) was pipetted into each glass dish. Cruickshank (1954) found that the depth of medium above the respiring tissue was of great importance in determining accurate Q_{O_2} values. This was due to dependence on oxygen diffusion, since there was no shaking mechanism. He demonstrated that the respiratory rate could be doubled by floating the respiring material. In later work (Dr C. N. D. Cruickshank, personal communication) the tissue was placed on lens paper supported by tantalum-gauze stands, so that it was barely covered with medium. This latter technique was followed in the present work. The oxygen uptake was measured for 2 hr. after equilibration and was found to be linear during this period. In all cases the oxygen uptake was calculated per mg. dry weight. The tissues were finally washed in distilled water and dried to constant weight at 100°.

Histological methods

A series of chick embryo arteries similar to those used for the respiration studies was subjected to histological examination. The ages of the embryos used for this work were 6, 7, 12, 13, 18 and 21 days of incubation. The tissues were fixed in Ringer-formol solution and paraffin sections were

Further experiments were designed to determine whether significantly larger values for the Q_{O_2} of artery slices would be obtained when using oxygen instead of air as the gas phase in the Warburg apparatus. For this purpose, the aortas from eight rabbits were used, and from the results summarized in Table 2 it can be seen that no appreciable difference was observed between the two sets of results. The remainder of the work with the Warburg apparatus was carried out with oxygen as the gas phase and the results are summarized in Table 3.

Observations on chick arterial tissue. Values for the Q_{O_2} of artery slices from twenty-seven chick embryos ranging in age from 6 to 22 days were determined by means of the micro-respirometer. The results are shown graphically in Fig. 1 in which Q_{O_2} is plotted against age, and in Fig. 2 in which Q_{O_2} is plotted against weight of chick embryos. On these same graphs are shown the

reciprocals of the densitometer readings for Mallory-stained sections of a series of similar chick-embryo arteries, plotted against age and weight as before. It will be seen that the curves for reciprocals of densitometer readings follow closely the Q_{O_2} curves in each case. The initial steepness of the curves in Fig. 2, in which the abscissae are weights of chick embryos, is due to the fact that

Table 2. *Oxygen uptake of rabbit-artery slices suspended in Krebs solution at 37° with air or oxygen as the gas phase*

Carbon dioxide was absorbed with KOH (10%, w/v). Values as $Q_{O_2} = \mu\text{l. O}_2/\text{mg. dry wt. tissue/hr.}$

Gas phase	No. of animals	Range	Mean
Air	4	-1.6 to -2.1	-1.73
Oxygen	4	-1.2 to -2.2	-1.58

Table 3. *Oxygen uptake of artery slices suspended in Krebs solution at 37° with oxygen as the gas phase*

Carbon dioxide was absorbed with KOH (10%, w/v). Values as $Q_{O_2} = \mu\text{l. O}_2/\text{mg. dry wt. tissue/hr.}$

Species	No. of animals	Range	Mean
Rat	6	-2.1 to -2.9	-2.43
Rabbit	4	-1.2 to -2.2	-1.58
Sheep	10	-0.40 to -0.68	-0.55
Pig	3	-0.34 to -0.49	-0.43
Ox	4	-0.30 to -0.46	-0.37

Fig. 1. O, Relationship between the oxygen uptake of embryo-chick arterial tissue and the incubation age of the chick. Determinations were carried out at 38° with air as the gas phase. Oxygen uptake (Q_{O_2}) expressed as $\mu\text{l. O}_2/\text{mg. dry wt. tissue/hr.}$ Mean values are given in the nine cases where two or more determinations were carried out for a particular age; x, relationship between the reciprocal of the optical density of Mallory-stained sections of embryo-chick arterial tissue (I/D) and the incubation age of the chick.

the relative increase in weight is more rapid in the younger embryos.

The other stains used for the connective tissue components were not so suitable for examination by means of the densitometer. A visual assessment of the components suggested that, like collagen, both reticulin and elastic tissue increased in amount with advancing age of the embryo. The decrease in cellularity of the arterial wall during development appeared to be due mainly to the accumulation of collagen. Reticulin and elastic tissue contributed relatively less to the extra-cellular tissue.

Fig. 2. O, Relationship between the oxygen uptake (Q_{O_2}) of embryo-chick arterial tissue and the wt. of the chick. Determinations were carried out at 38° with air as the gas phase. Oxygen uptake expressed as $\mu\text{l. O}_2/\text{mg. dry wt. tissue/hr.}$; x, relationship between the reciprocal of the optical density of Mallory-stained sections of embryo-chick arterial tissue (I/D) and the wt. of the chick.

DISCUSSION

It is evident from the results obtained with adult material that the artery is a moderately active tissue. The Q_{O_2} of -2.6 found by Briggs *et al.* (1949) for rat-aorta slices in a phosphate-buffer solution containing succinate agrees with the mean value of -2.43 obtained in the present work when the medium was Krebs solution, which contains other tricarboxylic acid cycle acids lactate, glutamate and fumarate.

The results summarized in Table 3 show that there is a definite decrease in the Q_{O_2} of aortic tissue as the body size of the animal used increases. Krebs (1950) observed a similar decrease in other tissues with increasing body size and considered that this phenomenon was due to the fact that cells with lower respiration (like those of connective tissue) are more preponderant in the larger species.

A similar decrease appears to take place during embryonic development, as shown in Figs. 1 and 2. Work on the early embryo has demonstrated neither changes during development nor local differences in the oxygen uptake if this is calculated per unit of nitrogen or dry weight (see Spratt, 1952). In the present study on older embryos, however, the reduction in Q_{O_2} with age or with increasing body weight during embryonic life was definite and characteristic. Such a reduction might conceivably be due to developmental changes in the respiratory-enzyme systems. Stained sections of embryonic arteries, however, suggested that it might be associated with an increase of relatively inert material such as reticulin, collagen and elastic tissue. The attempt to put this on a quantitative basis by means of densitometer readings gave a surprising degree of correlation between the curves for Q_{O_2} and for reciprocals of densitometer readings of Mallory-stained sections. Such reciprocals may be taken to represent the degree of cellularity of the sections. Whatever other influences may be at work, therefore, the accumulation of connective tissue is likely to be a dominant factor in determining the reduction in respiratory activity of the artery with advancing development.

SUMMARY

1. The Q_{O_2} of artery slices from the rat, rabbit, sheep, pig and ox have been determined with the Warburg manometer.

2. Similar observations on arteries derived from chick embryos ranging in age from 6 to 22 days of incubation have been made with a micro-respirometer.

3. The adult arteries were moderately active tissues with Q_{O_2} values ranging from -0.37 to -2.43 . Comparing one species with another, there was a decrease of Q_{O_2} with increase of size of the animal.

4. A regular and considerable reduction in Q_{O_2} of chick arterial tissue occurred as the age and weight of the embryo increased. This reduction was apparently associated with the development of connective tissue.

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The Isolation of a Hypertensin

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There have been a number of recent reports on the purification of the pressor substance hypertensin, or angiotonin (Bumpus & Page, 1954; Skeggs, Marsh, Kahn & Shumway, 1954a; Kuether & Haney, 1955), in which the pressor activity was associated with peptide material. The pressor activity of these recent preparations was stated to be greater than that reported by Edman (1945), but the amino acid composition of the preparations varied considerably. The isolation is now reported of an apparently homogeneous peptide containing eight

different amino acids, with a pressor activity 1.5–3 times that of (–)-noradrenaline as measured in the anaesthetized rat (Peart, 1955a). A preliminary report of this work has already appeared (Peart, 1955b).

METHODS

Materials. All solvents were redistilled and had a low peroxide content. Hyflo Supercel was obtained from Johns Manville Co., Ltd., Artillery Row, London, S.W. 1. It was suspended in 2N-HCl overnight, washed with H_2O until