sistently present, and if so whether they indicate important peculiarities in the sexual biochemistry of the animals concerned.

SUMMARY

1. A 7-dehydrosterol, presumably 7-dehydrocholesterol, was detected spectrophotometrically in high concentrations in the caput epididymis, corpus epididymis and preputial glands of male rats, and in the bulbi vestibuli of female rats. It could not be detected in other parts of the male or female genital systems.

2. The concentration of 7-dehydrosterol in its sites of deposition was not influenced by substitut-

ing a stock diet for an experimental diet, nor by varying the intake of vitamin A. About the same amounts of 7-dehydrosterol were found in wild brown rats as in tame piebald rats. No 7-dehydrosterol was detected in the rudimentary epididymides of sexually immature rats.

3. An irradiated extract from preputial glands had strong antirachitic activity.

4. No evidence of similar concentrations of 7-dehydrosterol was found in the male sex organs of the limited number of other animals investigated.

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The Activity of the Cytochrome System in Muscle and its Relation to Myoglobin

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Data secured during an investigation of factors affecting the percentage of myoglobin in muscle (Lawrie, 1950) had confirmed that a high concentration of this pigment is usually found in muscles of high physiological activity. This is of particular interest in view of the strong affinity of myoglobin for oxygen which enables it to act as an intramuscular oxygen store for the tissue's principal oxidizing catalysts, the cytochromes (Hill, 1936; Millikan, 1937). Keilin (1925) had early pointed out that all aerobic cells contain cytochromes, and that the concentration of cytochromes, as determined spectroscopically, is highest in the most actively respiring cells. Nevertheless, the concentration of cytochromes has been reported both as being higher (Fujita, Hata, Numata & Ajisaka, 1939) and lower (Hill, 1936) in myoglobin-rich or 'red' muscles than in the 'white' variety. It therefore appeared desirable to study the distribution of certain components of the cytochrome system in

muscle and their relation to the content of myoglobin. A preliminary account of this work has already been published (Lawrie, 1952).

EXPERIMENTAL

An assessment of the cytochromes in muscle involves consideration of a series of components. Of these, only cytochrome *c* can be estimated quantitatively as protein. The determination of cytochrome *c* itself affords to a certain extent a measure of the cytochrome system as a whole, since its concentration in various tissues has been shown to follow closely the rate of O_2 uptake (Junowicz-Kocholaty & Hogness, 1939). However, it was the immediate relation of the cytochrome system to O_2 which was of interest in the present study. It was thus considered more appropriate to determine the system's power of catalysing the uptake of O_2 by assessing the activity of cytochrome oxidase (Keilin & Hartree, 1939; Slater, 1949*a*), which can be estimated by the rate of oxidation of ascorbic acid or *p*-phenylenediamine in the presence of excess cytochrome *c*. For the determination, the procedure of Slater (1949*b*) has been followed, as

described below. In addition, estimations were made of enzymic activity at succinate level. As a measure of succinic dehydrogenase, the rate of reduction of methylene blue in the presence of excess sodium succinate was employed. As a measure of succinic oxidase, the rate of O_2 uptake during the oxidation of sodium succinate in the presence of excess cytochrome *c* was employed. The heart-muscle preparation of Keilin & Hartree (1938, 1947) was used since it contains the complete cytochrome system including succinic dehydrogenase. It has been necessary to assume that strict adherence to their procedure would ensure the production of comparable enzyme-bearing particles in each preparation. Such an assumption seems reasonable in view of the similarity in activity found by Slater (1949c) in a large number of preparations of this kind. To relate enzyme activity in muscle preparations to the basis of whole muscle, it is necessary to assume further that high activity per unit weight of enzyme-bearing particles is reflected by a correspondingly high 'density' of such particles in the whole tissue. There is tentative support for this in the present work.

Preparation of enzyme suspensions from muscle. The method of Keilin & Hartree (1947) for heart-muscle preparations was employed for all the muscles examined. It was scaled down to handle relatively small amounts of muscle mince and was strictly standardized to allow comparison of the data secured. 80 g. mince, 25 g. acid-washed sand, and 160 ml. 0.02M-phosphate buffer, pH 7.3, were ground together for 2 hr. The mixture was then diluted with a further 65 ml. of 0.02M-phosphate buffer and centrifuged for 20 min. at 2000 rev./min. Thereafter the method and quantities followed the original. The activity of the resultant enzyme preparations was always measured within 20–36 hr. to minimize the effect of possible deterioration.

Estimation of the activities of cytochrome oxidase, the succinic-oxidase system and succinic dehydrogenase. Cytochrome oxidase was measured in Barcroft manometers, at 38–39°, by determining the rates of oxidation of neutralized ascorbic acid in 0.1M-phosphate buffer, pH 7.3, and in the presence of known amounts of added cytochrome *c* (Slater, 1949b). By extrapolation the activity of cytochrome oxidase at infinite cytochrome *c* concentration could be calculated: this gave the maximum rate of O_2 uptake which the enzyme could catalyse under optimum conditions. The succinic-oxidase system was measured in Barcroft manometers, at 38–39°, by determining the rate of oxidation of sodium succinate in 0.18M-phosphate buffer, pH 7.3, and in the presence of excess cytochrome *c* (Slater, 1949c). Succinic dehydrogenase was measured anaerobically at 37° using the Thunberg technique, by determining the time for 90% reduction of 0.00043M-methylene blue in 0.18M-phosphate buffer, at pH 7.3, and in the presence of excess sodium succinate (cf. Tsou, 1951).

The activity of these three enzyme systems was expressed as μ l. O_2 taken up/mg. dry, fat-free weight of enzyme solids/hr. The Q_{O_2} values for succinic dehydrogenase were measured at suboptimal levels of methylene blue, since this dye poisons the enzyme in high concentrations (Slater, 1949c).

The dry, fat-free weight of the enzyme preparations was determined as described by Slater (1949c). Cytochrome *c* (containing 0.34% iron) and iron were determined by the methods of Keilin & Hartree (1945). Myoglobin was determined by a method previously described by the author (Lawrie, 1950). It was prepared for manometric work as

metmyoglobin (Lawrie, 1951), since the ferrous forms of the pigment readily autoxidize.

Phosphate buffers were prepared from A.R. Na_2HPO_4 and $NaH_2PO_4 \cdot 2H_2O$.

L-Ascorbic acid, B.P., and sodium succinate, laboratory reagent grade, were used; distilled water was employed for all solutions.

RESULTS

Variation of enzymic activities with age of animals

The general form of Figs. 1–3 shows that up to an age of 2 yr., the enzymic activities of cytochrome oxidase, the succinic-oxidase system and succinic

Fig. 1. The effect of the age of the animal on the cytochrome-oxidase activity of enzyme preparations from various horse muscles. G.P. = gestation period; CD = linear regression, 0–2 yr.; DE = linear regression, 2–12 yr. Cytochrome oxidase - Q_{O_2} max. = μ l. O_2 uptake/mg. fat-free dry enzyme solids/hr., at infinite cytochrome-*c* concentration. Experimental conditions (Slater, 1949b): enzyme preparation diluted tenfold with 0.10M-phosphate buffer, pH 7.3, 0.2 ml.; ascorbic acid (50 mg./ml., neutral), 0.3 ml.; cytochrome *c*, 6.7×10^{-4} M in 0.5% NaCl, 0.1–0.3 ml.; phosphate buffer, 0.10M, pH 7.3, 2 ml.; water, 0.5 ml.; total vol., 3.3 ml.

dehydrogenase, respectively, increase swiftly in the following muscles of horse: psoas, diaphragm, heart and longissimus dorsi (l. dorsi); thereafter in mature animals, all three enzymic activities appear to change relatively little. The data have been treated statistically in Table 1, from which it may be seen that the initial rates of increase of the three enzymic activities in preparations from the four muscles represent significant correlations with age up to 2 yr.: subsequent changes are insignificant.

Another interesting feature of Figs. 1–3 is the fact that early in the gestation period when no enzymic activity is exhibited in psoas, l. dorsi and

Fig. 2.

Fig. 3.

Fig. 2. The effect of the age of the animal on the activity of the succinic-oxidase system in enzyme preparations from various horse muscles. G.P.=gestation period; *CD*=linear regression, 0-2 yr.; *DE*=linear regression, 2-12 yr. Q_{O_2} = μ l. O_2 taken up/mg. fat-free dry enzyme solids/hr. Experimental conditions (Slater, 1949c): enzyme preparation diluted fivefold with 0.18M-phosphate buffer, pH 7.3, 0.2 ml.; 0.4M-sodium succinate, 0.2 ml.; cytochrome c, 6.7×10^{-4} M in 0.5% NaCl, 0.2 ml.; phosphate buffer, 0.18M, pH 7.3, 2.7 ml.; total vol, 3.3 ml.

Fig. 3. The effect of the age of the animal on succinic-dehydrogenase activity in enzyme preparations from various horse muscles. G.P.=gestation period; *CD*=linear regression, 0-2 yr.; *DE*=linear regression, 2-12 yr. Q_{O_2} = μ l. O_2 taken up/mg. fat-free dry enzyme solids/hr. Experimental conditions (cf. Tsou, 1951): enzyme preparation diluted fivefold with 0.18M-phosphate buffer, pH 7.3, 0.5 ml.; 0.4M-sodium succinate, 0.2 ml.; 0.005M-methylene blue, 0.3 ml.; phosphate buffer, 0.18M, pH 7.3, 2.5 ml.; total vol., 3.5 ml. Time for 90% reduction measured.

Table 1. *Statistical analysis of data in Figs. 1-3*

CD=linear regression 0-2 yr. *DE*=linear regression 2-12 yr.)

Linear regression coefficients				
Muscle	<i>CD</i>	Significance	<i>DE</i>	Significance
Cytochrome oxidase (Fig. 1)				
Heart	+ 1001.8957	+	- 55.6962	Nil
L. dorsi	+ 360.5463	+	- 30.0633	
Diaphragm	+ 651.8285	+	+ 11.0759	
Psoas	+ 605.9563	+	+ 9.1772	
Succinic-oxidase system (Fig. 2)				
Heart	+ 218.0095	+	- 4.2722	Nil
L. dorsi	+ 57.3399	+	- 4.9684	
Diaphragm	+ 117.0629	+	- 6.2975	
Psoas	+ 94.7237	+	+ 0.2532	
Succinic dehydrogenase (Fig. 3)				
Heart	+ 90.6398	+	- 3.4494	Nil
L. dorsi	+ 14.5645	+	- 0.1741	
Diaphragm	+ 21.1007	+	+ 0.6329	
Psoas	+ 20.8713	+	+ 2.1835	

+, Significant at 5% probability level.

Table 2. *Enzymic activities in preparations from the muscles of adult horses*

(Q_{O_2} = μ l. O_2 /mg. dry, fat-free wt. enzyme solids/hr.)

Muscle	(a) Succinic- oxidase system	(b) Cytochrome oxidase	(c) Succinic dehydrogenase	Ratios	
				<i>b/a</i>	<i>c/a</i>
Heart	510	2700	190	5.3	0.37
Diaphragm	260	1700	60	6.5	0.23
Psoas	240	1600	60	6.6	0.25
L. dorsi	130	900	30	6.9	0.23

diaphragm, the activities of the succinic-oxidase system, succinic dehydrogenase, and especially cytochrome oxidase, are appreciable in the heart. In Table 2 are given values for the activities of the three enzyme systems in preparations from the four muscles studied, averaged over the period 2-12 yr.,

Table 3. *Enzymic activities in preparations from horse heart during early phase of growth, as % average adult value*

Age	Cytochrome oxidase	Succinic-oxidase system	Succinic dehydrogenase
5 months before birth	10	3	3
6 weeks before birth	56	31	29
1 yr. after birth	96	86	79

i.e. in the adult animal. It is obvious that the three enzymic activities are considerably higher in the heart than in the skeletal muscles. While the ratios of the average activities of cytochrome oxidase, succinic oxidase, and succinic dehydrogenase, are of the same order in the two skeletal muscles and the diaphragm, they are different in the heart. Generally, the average values for heart are lower than those obtained by Slater (1949c), who has reported a Q_{O_2} of 3200, 625 and 240 for cytochrome oxidase, the succinic-oxidase system and succinic dehydrogenase respectively. Values of this order have been, however, observed in individual cases.

When the enzymic activities for horse heart obtained from animals less than 2 yr. old are expressed as a percentage of the corresponding average values for preparations from adult animals (cf. Table 3), it may be seen that cytochrome-oxidase activity increases initially at a markedly higher rate than succinic dehydrogenase and the

succinic-oxidase system. This difference becomes less marked after 1 yr. from birth. The data in Table 1 (column *CD*) show that the rates of increase in the activity of the three enzyme systems are highest in heart, intermediate in diaphragm and psoas, and least in l. dorsi. When, however, these rates are expressed not in terms of Q_{O_2} units but as percentages of the final adult values, a high degree of similarity is revealed. This has been done in Table 4 which also gives the corresponding values for myoglobin, calculated from data given in a previous paper (Lawrie, 1950). It can be seen from this table that the high rates of increase of enzymic activities in the period 0-2 yr., and the very slow rates of change in the period 2-12 yr., are not significantly different from the corresponding changes in the percentage of myoglobin.

Species variations

In Table 5 the Q_{O_2} values are given for cytochrome oxidase, the succinic-oxidase system and succinic dehydrogenase in muscle preparations from a variety of species. For comparative purposes the corresponding data for the percentage of myoglobin in whole muscle have been included. In general, the activities of the three enzyme systems decrease together, but succinic dehydrogenase does so to a proportionately greater extent. It may be noticed, also, that myoglobin concentrations in the whole muscle tend to follow cytochrome-oxidase activities in the enzyme preparations. In this respect, however, whale-psoas, horse-heart and pigeon-pectoral muscles are striking exceptions. In Fig. 4, the percentages of myoglobin in the psoas muscles of different species have been plotted against cytochrome-oxidase activities in the corresponding enzyme preparations. The values, except those for elephant and blue whale, lie on a sigmoidal curve.

Table 4. *Yearly increase in enzymic activities and myoglobin concentration of muscles in young horses expressed as percentage of the average values in adult animal*

Muscle	Cytochrome oxidase	Succinic-oxidase system	Succinic dehydrogenase	Myoglobin
(a) 0-2 yr.				
Heart	+37.0	+42.7	+47.7	+38.9
Diaphragm	+38.4	+45.0	+35.0	+40.1
Psoas	+37.9	+40.0	+34.7	+42.8
L. dorsi	+40.0	+44.1	+46.7	+37.3
Mean	+38.3	+42.9	+41.0	+39.8
S.E.	± 1.8	± 2.2	± 7.1	± 2.4
(b) 2-12 yr.				
Heart	- 2.1	- 0.8	- 1.8	+ 0.7
Diaphragm	+ 0.7	- 2.4	+ 1.1	+ 3.6
Psoas	+ 0.6	+ 0.1	+ 3.6	+ 2.1
L. dorsi	- 3.3	- 3.8	- 0.6	+ 1.5
Mean	- 1.0	- 1.7	+ 0.6	+ 2.0
S.E.	± 1.8	± 1.7	± 2.7	± 1.2

Table 5. *Enzymic activities in muscle preparations from various species and myoglobin concentrations in corresponding whole muscle*

Species	Muscle	Q_{O_2}			Myoglobin (% wet wt.)
		Cytochrome oxidase	Succinic- oxidase system	Succinic dehydrogenase	
Horse	Heart	2700	510	190	0.33
Pigeon	Pectoral	2300	260	50	0.22
Manx shearwater	Pectoral	1800	230	50	0.69
Horse	Diaphragm	1700	260	60	0.61
Horse	Psoas	1600	240	60	0.71
Ox	Psoas	1200	160	40	0.60
Pig	Psoas	1000	130	20	0.43
Sheep	Psoas	950	180	30	0.35
Horse	L. dorsi	900	130	30	0.47
Elephant	Psoas	800	140	30	0.46
Hare	Psoas	650	110	30	0.16
Blue whale	Psoas	600	80	20	0.84
Rabbit	Psoas	250	60	10	0.02

*Anomalous enzymic activity in avian
breast muscle preparations*

The content of cytochrome *c* in pigeon-breast muscle is about twice as high as in horse heart (Junowicz-Kocholaty & Hogness, 1939). In view of the relationship between the content of cytochrome *c* and the rate of oxygen uptake by the tissues, it

Fig. 4. The relation between myoglobin concentration in psoas muscle from the adults of various animals and cytochrome-oxidase activity (Q_{O_2}) in the corresponding enzyme preparations. Q_{O_2} = calculated μ l. O_2 uptake/mg. fat-free dry enzyme solids/hr., at infinite cytochrome-*c* concentration (Slater, 1949b). Experimental conditions as in Fig. 1.

might have been expected that the enzymic activity of the avian pectoral muscle should also be high. Spectroscopic examination of the breast muscles from willow warblers killed immediately after their migratory flight from southern latitudes and from Manx shearwaters (birds which fly for great distances without rest) certainly revealed a concentration of cytochrome *c* which was high even in comparison

with pigeon. However, as can be seen from Table 5, the enzymic activities of preparations from pigeon and Manx shearwater pectoral muscles are lower than those from horse heart. This was even more surprising in view of the well-known capacity of minced pigeon-breast muscle to sustain a rate of oxygen uptake considerably higher than that of horse heart (cf. Krebs & Johnson, 1948). A more detailed comparison of enzymic preparation from horse heart and from pigeon breast was therefore made.

80 g. mince were used for each preparation. A summary of the data obtained is given in Table 6. The term 'intermediate layers' (I.L.) refers to gel-like material which came down in the centrifuge at 2000 rev./min. It was clearly quite distinct from the underlying fibrous mass of sand and connective tissue, etc., which is normally obtained at this stage, and from the enzyme colloid, which remained in suspension.

It is evident that there is a profound difference between preparations from the horse-heart and pigeon-breast muscle. The concentration of I.L. is 10 times higher in pigeon-breast muscle than in an equivalent weight of horse heart. As a result, although the I.L. has low enzymic activity, it accounts for a considerable proportion of the total oxygen uptake in the pigeon-breast muscle. Indeed, when its contribution is added to that of the corresponding normal preparation, the oxygen uptake of pigeon breast (40 900) is seen to exceed that of horse heart (30 700) by 30 %.

The possibility was investigated that the I.L. added to a succinic-oxidase preparation may exert a specific effect on the enzymic activity. However, as can be seen from Table 7, both in the absence and in the presence of cytochrome *c*, the I.L. has only an additive effect; even when incubated with pigeon-breast muscle preparation, it does not enhance the activity.

Table 6. *Comparative data on cytochrome-oxidase activity in muscle preparations from horse heart and pigeon breast*

Preparation	Cytochrome oxidase Q_{O_2}	Vol. prep. (ml.)	Mg. dry fat-free wt./ml.	Calculated O_2 uptake in whole tissue (μ l./g./hr.)
Horse heart:				
Normal	2500	30	30	28 100
Intermediate layer	700	10	30	2 600
Pigeon breast:				
Normal	2300	20	28	16 100
Intermediate layer	640	100	31	24 800

Table 7. *Succinic-oxidase activity of pigeon-breast muscle (PBMP)*

Contents of right-hand flasks of manometers					
Volume (ml.)					
PBMP	Cytochrome (6×10^{-4} M)	Sodium succinate, 0.4 M	Phosphate buffer, 0.18 M	PBMP I.L.	O_2 uptake (μ l./hr.)
0.2	0.2	0.2	2.7	—	480
—	0.2	0.2	2.7	0.2	160
0.2	0.2	0.2	2.5	0.2	600
0.2	—	0.2	2.7	—	370
—	—	0.2	2.7	0.2	110
0.2	—	0.2	2.5	0.2	470
0.2*	0.2	0.2	2.7	—	290
0.1†	0.2	0.2	2.7	0.1†	150
—	0.2	0.2	2.7	0.2*	65

* Incubated at 37° for 2 hr.

† Equal vols. I.L. and PBMP incubated together at 37° for 2 hr.; 0.2 ml. mixture used.

DISCUSSION

Before considering the principal results of this investigation, it is first desirable to assess the implications regarding technique arising from the discovery of a large quantity of I.L. gel in pigeon-breast muscle. With enzyme preparations from all sources, as has been mentioned above, procedures were identical. It is therefore most unlikely that activities differed merely through casual admixture of more or less I.L. gel material having low Q_{O_2} and arising from curtailment or prolongation of centrifuging or grinding operations outside normal limits. Had low enzymic activities in the muscle preparations been due to incomplete centrifugal removal of such gel material, the corresponding content of 'dry, fat-free enzyme solids', calculated per gram of whole tissue, would have been relatively high. In fact, low enzymic activity in preparations corresponded to a low 'density' of enzyme solids in the whole tissue and vice versa (Table 8).

The present study shows that variations in the activities of the cytochrome system are directly paralleled by corresponding variations in the percentages of myoglobin in the whole tissue. As may be seen from Tables 4 and 5, this parallelism extends over muscles differing in age, type and species. At the beginning of the century, Batelli & Stern (1907,

1912) had shown that, in general, the overall oxygen uptake and, in particular, the oxidation of *p*-phenylenediamine and succinate by washed

Table 8. *Relation of cytochrome-oxidase activity to calculated 'density' of enzyme solids in whole tissue*

Muscle preparation	Q_{O_2} (μ l./mg. dry, fat-free wt./hr.)	Mg. fat-free dry enzyme solids/g. wet tissue
Horse heart	2700	12
Pigeon pectoral	2300	11
Horse psoas	1600	8
Horse diaphragm	1700	7
Horse l. dorsi	900	3
Whale psoas	600	1
Rabbit psoas	250	1

muscle, was greater in 'red' than in the 'white' variety. Later Ahlgren (1921), besides confirming this work, showed that 'red' muscle had a greater capacity for reducing methylene blue. Nevertheless, there has been hitherto no attempt to carry out a co-ordinated study on myoglobin and the cytochrome system.

A particularly interesting example of the cytochrome-myoglobin relationship is shown by the data for the closely related species rabbit and hare. Low myoglobin levels in the muscles of the former

have been attributed to infrequent muscle usage (Hammond, 1932). It may now be seen that the activity of the cytochrome system is also low in rabbit psoas, whereas in that of the hare, an animal capable of enduring prolonged muscular exertion, both myoglobin and the activities of the cytochrome enzymes are high.

Striking non-conformity to the sigmoid myoglobin-cytochrome oxidase relationship (Fig. 4) is shown by the blue whale psoas muscle. The content of myoglobin in this muscle is approximately the same as that of the horse psoas, yet enzyme preparations made from it are only one-third as active. Moreover, from the data in Table 8, it can be calculated that on a whole tissue basis the difference is even greater: thus the store of oxygen represented by 0.7–0.8% oxygenated myoglobin will last for about 20 times as long in whale psoas as in the corresponding muscle of the horse under conditions of maximum activity. Although 48 hr. elapsed between harpooning (off the Island of Harris) and the experimental determinations, the muscle samples were kept in an insulated container at a temperature just above the freezing point during the greater part of this time, and it is considered unlikely that any major deterioration of enzymic activity occurred. In any case the enzyme solids were found to be very low, which is in line with the low activity (Table 8). It may be pointed out that the normal duration of the dive in the sperm whale is about four times as long as that in the blue whale (Irving, 1939); and its muscles have a myoglobin content 5 times as great as those of the latter (Case, 1953). The other exception to the relationship shown in Fig. 4 is the elephant. However, the animal from which the samples were obtained had been in captivity for 10 yr., and the enforced inactivity may have had an adverse effect on its musculature. Such conditions certainly alter normal myoglobin levels (Lawrie, 1953). According to Krogh (1922) active muscles have a greater degree of vascularization, and thus a more plentiful blood supply, than those used less frequently. Differences of vascularity may exist between the psoas muscles of the different animals, and may account for the sigmoidal shape of the curve relating myoglobin to cytochrome-oxidase activity.

The importance of muscle function, in the widest sense, in determining the activity of the oxygen-utilizing enzyme systems is emphasized by the elaboration of the latter in heart muscle early in gestation when this organ probably represents the only muscle in action (Figs. 1–3). In adult life (Table 2) the heart is still by far the most continuously used muscle. This is reflected by a higher enzymic activity in heart than in skeletal muscles. Of the muscles studied the psoas and diaphragm are next in functional activity to the heart and the

enzymic activities are found to be correspondingly intermediate between those of heart and l. dorsi.

The low cytochrome-oxidase activity of enzyme preparations from pigeon-breast muscle in relation to those from horse heart (Table 5) was at first difficult to reconcile with reports that minced horse heart had a considerably lower capacity for oxygen uptake than minced pigeon-breast muscle. The contribution of the large volume of I.L. gel obtained from the latter to the overall oxygen uptake of this tissue (calculated on a cytochrome oxidase basis) appears largely to explain the discrepancy. If the existence of a large volume of I.L. material in pigeon-breast muscle be accepted as a genuine phenomenon, one might regard the gel as an aggregate of tissue particles considerably larger in size than those of the highly colloidal suspension normally employed in Q_{O_2} studies. Although exhibiting little activity itself, and apparently ineffective in enhancing the oxygen uptake of the colloidal suspension *in vitro*, it may well represent a tissue structure which is especially necessary *in vivo* for maximum enzymic activity in the unusually vigorous pigeon-breast muscle.

Although, in general, the activity of the succinic dehydrogenase-cytochrome system in enzyme preparations has been shown to increase as the percentage of myoglobin in the whole muscle increases, the quantity of myoglobin in heart is disproportionately low as judged by this criterion. This would follow from the possession by this organ of a blood supply capable of constantly providing oxygen in amounts almost sufficient to meet the needs of its oxidase enzymes, high though these demands might be. Even so, it is particularly noteworthy that such myoglobin as the heart does possess varies directly with the cytochrome enzymes in the developing animal in precisely the same way as in skeletal muscle (Table 4). This not only reconciles the divergent views in the literature, but also provides strong evidence that myoglobin does indeed function as an oxygen store for the tissue-oxidizing enzymes.

SUMMARY

1. The activities of succinic dehydrogenase, the succinic-oxidase system and cytochrome oxidase were determined in enzyme preparations from a variety of muscles: the effects of age, type of muscle and species, were considered.

2. In preparations from heart, longissimus dorsi, diaphragm and psoas muscles of the horse the three enzymic activities increased rapidly up to the age of 2 yr. and thereafter were relatively constant.

3. In the adult animal the order of increasing enzymic activity was given by: longissimus dorsi, psoas, diaphragm and heart.

4. In enzyme preparations from the foetal horse heart the activity of cytochrome oxidase was appreciable when that of succinic dehydrogenase and the succinic-oxidase system was very low.

5. In muscle preparations having relatively high cytochrome-oxidase activity, and especially in those from horse heart, the activities of succinic dehydrogenase and of the succinic-oxidase system were proportionately greater than in those with low cytochrome-oxidase activity.

6. During the preparation of enzyme suspensions from pigeon-breast muscle, a large quantity of gel material was always obtained. This had low enzymic activity: its significance is discussed.

7. A significant direct correlation was found between the percentage of myoglobin in muscle and the activity of the corresponding enzyme preparation.

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The Relation of Energy-rich Phosphate in Muscle to Myoglobin and to Cytochrome-oxidase Activity

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In a previous communication (Lawrie, 1953) it has been shown that a statistically significant correlation exists in muscle between the percentage of myoglobin and the activity of the succinic dehydrogenase-cytochrome system. Myoglobin is the oxygen-storing pigment principally responsible for red coloration in muscle, the succinic dehydrogenase-cytochrome system catalyses intracellular oxygen

uptake for energy production by the oxidation of metabolites. It is generally agreed (cf. Needham, 1942) that the main form in which energy can be used by muscle for mechanical work is as it is stored in the terminal energy-rich phosphate ($\sim P$: Lipmann, 1941) of adenosine triphosphate (ATP). It seemed reasonable to anticipate that the production of $\sim P$ might be related to the activity of the succinic de-