Correlations between Components of the Glycolytic Pathway

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1. The contents of dihydroxyacetone phosphate, fructose diphosphate, pyruvate and lactate and the activities of aldolase and lactate dehydrogenase in the liver, kidney, testis, skeletal muscle, blood cells, sarcoma and hepatoma of rats were measured. 2. Correlations were established between components of the glycolytic pathway as follows: activities of aldolase and lactate dehydrogenase; contents of fructose diphosphate and pyruvate; activity of aldolase and content of fructose diphosphate; activity of lactate dehydrogenase and contents of fructose diphosphate and of pyruvate.

The components of the glycolytic pathway occurring in animal tissues are well known, but investigation of their quantitative relations in tissue extracts was started only recently (Hohorst, Kreutz & Bücher, 1959; Pette, Luh & Bücher, 1962a). In the present work correlations between the amounts of aldolase and lactate dehydrogenase and their respective substrates, fructose diphosphate and pyruvate, in extracts of tissue of the rat have been investigated.

MATERIALS AND METHODS

Rats

Black-and-white hooded male rats weighing 100–250 g. were used for most experiments, but 40 g. rats were used for freezing whole. The animals were fed on Medical Research Council diet no. 41b, which contains approx. 58% of carbohydrate, 16% of protein, 2.5% of oil, 4.7% of ash and 11.8% of water. Sarcomata (RD₃) were provided by Dr R. H. Thomlinson of the Medical Research Council Experimental Radiopathology Unit. Hepatomata were given by Dr E. Reid of the Chester Beatty Research Institute.

Ether anaesthesia

Rapid anaesthesia associated with minimal disturbance of the rat was achieved as follows. A layer of cotton-wool about 2 cm. thick was placed inside a 1.5 l. desiccator at 23–25°, and a wire gauze was superimposed. Ether was poured on to the gauze and cotton-wool until the latter was saturated. The rat was then put on to the gauze and the lid of the desiccator was adjusted so that there was about 2 mm. overlap through which vapour could escape. Air entered through a perforated rubber bung that replaced the stopcock. After inhaling the mixture of ether and air for 60–75 sec. the animal became unconscious. It was then

transferred in the desiccator to a room maintained at 4°, and after a total time of anaesthesia of 75–120 sec. it was removed and the appropriate tissue was excised (see below). About 70% of the rats appeared undisturbed during the induction of anaesthesia, 25% moved about more than usual and 5–10% struggled violently. This technique was used to obtain tissues for the assay of substrates and enzymes but with enzymes the procedure was carried out at about 20°.

Removal of tissue

Exposure. The liver was exposed by opening the rat's belly in the midline and along the costal margins. The kidney was approached by extending the incision around the left costal margin as far as the sacrospinalis muscle; the lower pole was partially freed from the surrounding fat to provide access. The testis was approached via combined midline and suprainguinal incisions; the tunica vaginalis was incised to facilitate compression in the tongs. Abdominal muscle was sampled after first reflecting the skin and dividing the muscle in the midline. Sarcomata were divided by two parallel incisions about 5 mm. apart; samples were taken from an area free from macroscopic necrosis. The time required for these exposures and samplings varied from 15 to 30 sec. according to the tissue, that for liver being shortest and for sarcoma longest. Blood (about 1 ml.) was collected from the severed aorta and inferior vena cava directly into 5.0 ml. of 6% (v/v) perchloric acid for assay of substrates. When the content of enzymes in whole blood or of enzymes or substrates in plasma were required, 3–4 ml. of blood was allowed to flow into a mixture of 0.2 ml. of heparin plus 0.3 ml. of 0.2 M-NaCl.

Excision. Tissue used for the extraction of substrates was frozen rapidly *in situ* in the anaesthetized animal by means of metal tongs precooled in liquid O₂ to approx. –190°, excised and plunged into liquid O₂ (Hohorst *et al.* 1959). The tongs were a modification of those described by Eranko (1954). They consist of a pair of surgical forceps 25 cm. long, to each blade of which is screwed a block of aluminium (5 cm. × 2 cm. × 1 cm.); the blades are bent so that when the forceps are closed the surfaces of the blocks

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are parallel. Strips of stainless steel (1mm. thick) are screwed along the middle 3.5 cm. of each side of each block and the whole of the far end. The strips project 0.5 mm. towards the opposing block to prevent complete closure of the tongs when pressure is applied. A leaf of frozen tissue 0.5–1.5 mm. thick is obtained, the excess being squeezed through the gaps at the ends of the steel strips. A sample of tissue 0.7 mm. thick obtained in this way reaches 0° in 0.1 sec. and –80° in a further 0.1 sec. (Wollenberger, Ristau & Schoffa, 1960).

Tissues excised for the assay of enzymes were stored for 30–60 min. in test tubes standing in melting ice.

Extraction of tissue

Substrates. The frozen tissue was pulverized, extracted in 6% (v/v) HClO₄ and the pH of the extract was brought to 3.5 (glass electrode) with 10N-KOH (Hohorst *et al.* 1959). The precipitate of KClO₄ was allowed to age at 0° for 30 min. and the supernatant was removed after centrifuging at 4000g for 5 min. The instruments used for pulverization were precooled in powdered solid CO₂. The tissue was kept frozen during pulverization by frequent additions of liquid O₂ to the stainless-steel percussion mortar.

Enzymes. The tissues were disrupted in 9 vol. of 0.25M-mannitol with a Potter–Elvehjem-type homogenizer. Muscle was cut into pieces of the largest size compatible with satisfactory homogenization. Small pieces of muscle resulted in lower aldolase activity in the extract: e.g., pieces measuring about 1mm. × 1mm. × 0.5mm. gave an extract with 25% of the activity of that obtained from pieces measuring 5mm. × 2mm. × 1mm., which was the size used. The activity of lactate dehydrogenase was not affected by the size of the pieces of muscle. The supernatant obtained by centrifuging at 500g and 2° for 5 min. was used for estimating the activities. Enzymes were released from blood cells by lysis in 4 vol. of water.

Analytical methods

Sugar phosphates and pyruvate. The reagents used were as follows. Glass-distilled water was used throughout. The 0.1M-tris-HCl buffer, pH 7.6, used was 1mm with respect to EDTA. NADH₂ was obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Aldolase, glycerol phosphate dehydrogenase and lactate dehydrogenase were purchased from C. F. Boehringer und Soehne G.m.b.H., Mannheim, West Germany. Triose phosphate isomerase was prepared from rabbit muscle (Beisenherz, 1955). Dihydroxyacetone phosphate was prepared from the dimethylketal dicyclohexylamine salt monohydrate according to the instructions from the suppliers (California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.). D-Fructose 1,6-diphosphate calcium dihydrogen salt was obtained from British Drug Houses Ltd., Poole, Dorset, and sodium pyruvate from L. Light and Co. Ltd., Colnbrook, Bucks.

The method described by Hohorst *et al.* (1959) was used. The reaction mixture contained initially 2.5 ml. of extract, 2.5 ml. of tris-HCl buffer, pH 7.6, and 40 μ l. of 7.5 mM-NADH₂. Then the following enzymes, each in 10 μ l. of tris-HCl buffer, pH 7.6, were added in the order given, the decrease in extinction at 340 m μ being measured after each addition: 10 μ g. of glycerol phosphate dehydrogenase,

20 μ g. of aldolase plus 4 μ g. of triose phosphate isomerase, and 10 μ g. of lactate dehydrogenase. The concentrations of dihydroxy-acetone phosphate, fructose diphosphate and pyruvate were calculated from the relevant decreases in extinction.

Lactate. The method of Barker & Summerson (1941) was used, except that lactate was converted into acetaldehyde at 60° for 30 min. (Hullin & Noble, 1953).

Enzymes. Aldolase activity was assayed by the method of Sibley & Lehninger (1949). Lactate-dehydrogenase activity was determined by the method of Kubowitz & Ott (1943) as described by Kornberg (1955). The temperature coefficient of the lactate-dehydrogenase reaction was measured by observing the rate of reduction of pyruvate between 17° and 29°. It was then used to correct the activities observed to 25°. The activities of both enzymes are expressed as μ moles of substrate changed/min. per g. fresh wt. of tissue or per mg. of N. The substrate used for aldolase was fructose diphosphate, and that for lactate dehydrogenase was pyruvate. The activity of aldolase was measured within 1–2 hr. of preparing the extract because both freezing and thawing whole liver and storage of homogenates at either –20° or 2° overnight resulted in losses of 55–75% of the activity. The lactate-dehydrogenase activity of the extracts was estimated within 24 hr. of extraction; more prolonged storage was avoided because the activity diminished by 20% at –20° between 24 and 72 hr.

Other estimations. Total N was estimated in homogenates prepared in water or mannitol by a Kjeldahl digestion (King, 1946) and nesslerization (King & Wootton, 1956). The packed-cell volume was measured as described by Wintrobe (1951).

Effects of rate of inactivation of enzymes on amounts of substrates in extracts

The mean content of dihydroxyacetone phosphate was decreased by 25–50% of the values obtained by the procedure outlined above by varying the method in any of the following ways: homogenizing fresh liver directly in HClO₄, freezing a whole lobe in liquid O₂, applying light pressure to the precooled tongs to produce a thick leaf of tissue or omitting pulverization. Fructose diphosphate and pyruvate were also decreased significantly but lactate was not affected. When muscle was frozen directly in liquid O₂ there was an increase of about 33% in the content of lactate.

RESULTS

Content of substrates. The amounts of dihydroxyacetone phosphate, fructose diphosphate, pyruvate and lactate in liver, kidney, testis, skeletal muscle, cells and plasma of blood, sarcomata and hepatomata of rats are presented in Table 1. The values for pyruvate and lactate are corrected for the amount present in the extracellular fluid (see Table 1); no correction was needed for the sugar phosphates because they are not detectable in plasma. The mean content of dihydroxyacetone phosphate in fresh tissue varies from 52.6 μ moles/g. in liver to 12.6 μ moles/g. in testis. The range of values of fructose diphosphate is larger, the highest and

Table 1. Concentrations of metabolites in rat tissues

Dihydroxyacetone phosphate and fructose diphosphate are absent from extracellular fluid; the content in whole tissue is assumed to arise from the cells. The correction for the content of substrate in the extracellular fluid is based on estimates of the volumes of extracellular fluid obtained from the literature. These are as follows: liver, 24% (Truax, 1939); renal cortex, 20% (Robinson, 1960); testis, 21% (Cole, 1950); muscle, 21% (Flear, Crampton & Matthews, 1960). Values are not available for the tumours; the value was assumed to be similar to that of liver. The plasma concentrations of pyruvate and lactate in one sarcoma-bearing rat were 275 and 5150 μ moles/g. respectively. Corrections for the hepatoma were based on the pyruvate and lactate concentrations in normal plasma, no blood being available from the host animals. The total N content (mean $\pm$ s.e.m.) in mg./g. in each tissue was as follows: liver, 32.08 ± 0.97 ; kidney, 27.06 ± 0.30 ; muscle, 32.92 ± 0.42 ; testis, 14.80 ± 0.15 ; sarcoma, 22.36 ± 0.44 ; hepatoma, 23.8 (three samples). The content for blood cells, namely 44 mg./g., was calculated from the following information: packed-cell volume, 50%; haemoglobin, 15.6 g./100 ml. (Farries & Griffith, 1942); sp.gr. of rat blood, 1.056 (Sherrington & Copeman, 1893); sp.gr. of rat plasma, 1.026. Parentheses are used to show the number of rats where it differs from that for the remainder of group. The results are expressed as mean values; where g. fresh wt. is the index the s.e.m. is given.

Tissue	No. of rats	Substrate (μ moles)									
		Dihydroxyacetone phosphate		Fructose diphosphate		Pyruvate		Lactate		Per mg. of N	Per mg. of N
		In cells in lg. of tissue	Per mg. of N	In cells in lg. of tissue	Per mg. of N	In lg. of tissue	In cells in lg. of tissue	In lg. of tissue	In cells in lg. of tissue		
Liver	27	52.6 ± 1.7	1.72	27.4 ± 1.4	0.85	219	190 ± 11	1320 (17)	654 ± 39	5.94	21.5
Kidney	10	25.4 ± 3.2	0.94	16.8 ± 1.4	0.62	109	76.8 ± 5.8	1170	441 ± 60	2.84	16.3
Testis	6	12.6 ± 0.7	0.85	12.3 ± 1.6	0.83	66.2	40.2 ± 1.9	1150	560 ± 49	2.72	37.8
Muscle	9	36.4 ± 3.0	1.10	86.4 ± 9.0	2.62	78.3	52.3 ± 6.5	1340	752 ± 78	1.59	22.9
Blood cells	10	18.0 ± 1.7	0.39	6.0 ± 0.2	0.13	—	135 ± 8.5	—	—	2.94	—
Plasma	—	—	—	—	—	124 ± 9 (9)	—	2790 ± 30 (6)	—	—	—
Sarcoma RD ₃	7	13.6 ± 2.1	0.61	23.6 ± 3.5	1.06	255	189 ± 18	6720	5480 ± 290	8.45	244
Hepatoma DAB	3	31.7 ± 5.6	1.33	30.0 ± 6.6	1.26	182	152 ± 22	3940	3270 ± 600	6.40	137

Table 2. *Maximum activities of aldolase and lactate dehydrogenase in rat tissue*

The results are expressed as mean values $\pm$ S.E.M. Total N content is given in Table 1.

Tissue	No. of rats	Aldolase activity (μ moles of fructose diphosphate split/min. at 37°)		Lactate-dehydrogenase activity (μ moles of pyruvate reduced/min. at 25°)	
		Per g. fresh wt. of tissue	Per mg. of N	Per g. fresh wt. of tissue	Per mg. of N
Liver	12	10.9 $\pm$ 0.4	0.338	243 $\pm$ 7	7.55
Kidney	11	5.41 $\pm$ 0.30	0.200	119.0 $\pm$ 4	4.40
Muscle	11	55.7 $\pm$ 3.6	1.69	—	—
	14	—	—	249 $\pm$ 11	7.58
Testis	11	2.73 $\pm$ 0.05	0.184	36.4 $\pm$ 0.9	2.46
Sarcoma RD ₃	6	5.65 $\pm$ 0.34	0.254	134 $\pm$ 7	6.04
Hepatoma DAB	3	5.30 $\pm$ 0.57	0.223	138 $\pm$ 15.0	5.81
Blood cells	8	1.33 $\pm$ 0.04	0.029	41.60 $\pm$ 1.2	0.91

Table 3. *Correlations between components of glycolytic pathway*

The values for the contents of substrates and activities of enzymes were obtained from different lots of rats; therefore it was not possible to use paired values to calculate the correlation coefficient of these variables. Instead, the mean values for each substrate and enzyme were used and the equation giving the correlation coefficient was weighted as indicated by the factor w . Thus:

$$r = \frac{\sum w(x-\bar{x})(y-\bar{y})}{\sum w(x-\bar{x})^2 \sum w(y-\bar{y})^2}$$

where r is the correlation coefficient and w is the reciprocal of the product of the standard error of the two means. This device ensures that appropriate weight is given to the number and variance of the determinations of each variable in each tissue.

x	y	t	P
Fructose diphosphate	Pyruvate	0.46	< 0.001
Aldolase	Lactate dehydrogenase	0.90	< 0.001
Aldolase	Fructose diphosphate	0.99	< 0.001
Lactate dehydrogenase	Pyruvate	0.89	< 0.02
Lactate dehydrogenase	Fructose diphosphate	0.97	< 0.01
Aldolase	Pyruvate	0.70	< 0.1

lowest being 86.4 and 6.0 m μ moles/g. in muscle and blood cells respectively. With respect to the content of pyruvate (corrected for the extracellular contribution) the tissues are divisible into two groups: liver, blood cells and tumours, containing larger amounts (130–190 m μ moles/g.); testis, muscle and kidney, containing smaller amounts (40–80 m μ -

moles/g.). The lactate content of the normal tissues examined is between 440 and 760 m μ moles/g. The chief effect of using the total nitrogen as an index is to increase the apparent content of all of the substrates in the testis and to decrease the contents in blood cells relative to those of other tissues.

Activity of enzymes. The activities of aldolase and lactate dehydrogenase are presented in Table 2. No correction is made for the activities present in the extracellular fluids, including blood, because these were found to be negligible. The mean activities of aldolase in most tissues are in the range 1–11 units/g. fresh wt. of tissue, the value for muscle being exceptional, namely 55.7 units/g. The activities in tumours are similar to those in the liver, kidney and testis. The mean activities of lactate dehydrogenase/g. fresh wt. of tissue range from 36.4 units in the testis to 249 units in skeletal muscle.

Correlation between components of the glycolytic pathway. The significance of the correlations tested is presented in Table 3. The values for skeletal muscle are excluded because its metabolism differs from that of other tissues in being concerned largely with glycolysis and in lacking fructose 1,6-diphosphatase (Newsholme & Randle, 1962). The correlations between the amounts of the two enzymes and of two of the substrates, namely fructose diphosphate and pyruvate, were significant in five of the six combinations tested. The exception was aldolase activity and pyruvate content, which appeared to be independent of each other. The relations between the amounts of dihydroxyacetone phosphate and lactate and the other substances assayed were not examined statistically because it was evident from graphs that the values were widely scattered.

Table 4. *Comparison of results obtained by various authors*

Tissue and reference	Conditions of sampling	Metabolite (mμmoles/g. fresh wt. of tissue)			
		Dihydroxy-acetone phosphate	Fructose diphosphate	Pyruvate	Lactate
Liver					
(Hohorst <i>et al.</i> 1959)	Ether anaesthesia for 3–5 min.; frozen with tongs precooled to –180°	38	22	154	1540
(Threlfall & Stoner, 1961)	Guillotine; frozen in liquid N ₂	—	160	33	1060
(Table 1)	Ether anaesthesia for 75–120 sec.; frozen with tongs precooled to –180°	53	27	219	1320
Skeletal muscle					
(LePage, 1948, 1950)	Sodium pentothal anaesthesia; rat frozen whole in liquid air	—	70	81	1800
(Threlfall & Stoner, 1961)	Sodium pentobarbitone anaesthesia; muscle frozen in liquid N ₂	—	220	113	4710
(Hohorst, Reim & Bartels, 1963)	As for liver (Hohorst <i>et al.</i> 1959)	31	—	—	—
(Table 1)	As for liver (Table 1)	36	86	78	1340
Kidney					
(LePage, 1948, 1950)	As for skeletal muscle (LePage, 1948, 1950)	—	40	33	1550
(Table 1)	As for liver (Table 1)	25	17	109	1170
Blood plasma					
(Hohorst <i>et al.</i> 1959)	Ether anaesthesia for 3–5 min.	0	0	234	2960
(Table 1)	Ether anaesthesia for 75–120 sec.	0	0	124	2790
Blood cells					
(Hohorst <i>et al.</i> 1959)	Ether anaesthesia for 3–5 min.	55	27	135	1360
(Table 1)	Ether anaesthesia for 75–120 sec.	18	6	135	710

DISCUSSION

Substrate contents. The results described above are compared with relevant data from the literature in Table 4. Results obtained by the following techniques, which do not bear critical examination (R. A. Dale, unpublished work), are not shown: (a) sodium pentobarbital anaesthesia followed by either freezing the whole animal (liver only excluded) (LePage, 1948, 1950) or exsanguination plus freezing samples of tissues with solid carbon dioxide (Frohman, Orton & Smith, 1951); (b) anaesthesia or decapitation followed by extraction into cold perchloric acid (Holzer, Sedlmayr & Kiese, 1956; Lamprecht & Trautschold, 1958; Thorn, Heimann, Gercken & Muldener, 1958). The present results for liver differ somewhat from those obtained by Hohorst *et al.* (1959); this is probably due to the period of anaesthesia of 1–2 min. duration used in the present work compared with the period 3–5 min. used by these authors (R. A. Dale, unpublished work). The different values obtained by Threlfall & Stoner (1961) for liver and muscle may be due to the

lower specificity of the chemical methods of analysis used as compared with enzymic methods.

The absence of dihydroxyacetone phosphate and fructose diphosphate from plasma (Hohorst *et al.* 1959) was confirmed. The relatively high values obtained by these authors for pyruvate in plasma and for dihydroxyacetone phosphate and fructose diphosphate in blood cells is attributed to the longer period of anaesthesia and the delay in removing blood until after removing samples of liver.

Enzyme activities. The activities of aldolase observed are generally similar to those described by Sibley & Lehninger (1949). The chief differences are that the values obtained in liver and blood cells are 20–25 and 100% greater respectively, and in a sarcoma 55–60% lower. The results for lactate dehydrogenase in liver, skeletal muscle and sarcoma are comparable with those obtained by Delbrück, Schimassek, Bartsch & Bücher (1959), but are greater than those given by Wenner, Spirtes & Weinhouse (1952), probably because the latter authors used acetone-dried powder extracts.

Dihydroxyacetone phosphate/fructose diphosphate

and lactate/pyruvate concentration ratios. Hohorst *et al.* (1963) showed that the ratio of the concentration of substrate to the concentration of product in a number of reactions in the glycolytic pathway in rat muscle (as determined in tissue extracts) approximated to the equilibrium constant calculated from free-energy data. The use of the content of fructose diphosphate and dihydroxyacetone phosphate obtained in the present work resulted in values for ' $K_{\text{zymohexase}}$ ' varying from 10^{-4}M for liver to $8 \times 10^{-6}\text{M}$ for skeletal muscle compared with a value of $7 \times 10^{-3}\text{M}$ given by Meyerhof (1951). This difference is attributed to the necessity to refer the concentrations to g. fresh wt., and not to the volume of their solution (H. J. Hohorst, personal communication). Thus the value obtained for ' $K_{\text{zymohexase}}$ ' is diminished by the factor that relates these two parameters and that varies with the tissue.

The lactate/pyruvate ratio obtained from crude extracts has been used to estimate the NAD/NADH₂ ratio between the free thermodynamically-active

forms of NAD as they occur in tissues. Hohorst *et al.* (1959) obtained a value of approx. 2000 for the liver of the rat sampled under ether anaesthesia. The present results give a value of 5600. The difference between the two estimations is attributed to the effects of the longer period of ether anaesthesia used by Hohorst *et al.* (1959) (see above). This method of obtaining the NAD/NADH₂ ratio is not valid in tissues such as skeletal muscle where the NAD-linked redox systems are not in equilibrium (Hohorst, 1962).

Lactate dehydrogenase/aldolase activity ratio. In skeletal muscle of the rat this is low, namely 4.5; in other tissues it is higher, e.g. in liver it is 22. This low value occurs also in human skeletal muscle but not in other human or rat tissues (Table 5); in particular, it does not occur in heart muscle in man or the rat, or in smooth muscle of the stomach and uterus in man (Schmidt & Schmidt, 1960). The difference between the ratio in skeletal muscle and other tissues is possibly due to differences in the metabolic pathways. Thus in skeletal muscle, the chief function of which is glycolysis and where most of the products of the splitting of fructose diphosphate are eventually transformed into lactate, a low lactate dehydrogenase/aldolase ratio might be expected. In liver, the direction of the lactate-dehydrogenase reaction appears to be chiefly from lactate to pyruvate, and the synthetic pathway for glycogen is only one of several available to pyruvate (Haslam & Krebs, 1963). Therefore a high lactate dehydrogenase/aldolase ratio might occur.

Correlations. Correlations between the activities of the enzymes of the glycolytic pathway from triose phosphate isomerase to enolase inclusive, as measured in crude extracts of tissues, were described by Bücher and his colleagues (Pette *et al.* 1962a; Pette, Klingenberg & Bücher, 1962b). The present results indicate that the so-called 'constant proportion groups' of Bücher and co-workers can be extended to include aldolase and lactate dehydrogenase.

The association of the highest concentration of substrate with the highest enzymic activity in tissue extracts is the reverse of the situation existing *in vitro*. However, in conditions *in vitro* there is usually an excess of free substrate and generally the fraction bound to enzyme is unknown. In resting tissues, on the other hand, it has been suggested that there is little free substrate (Racker, 1954; Krebs & Kornberg, 1957) because it is transformed rapidly by the large amount of enzyme present. It is submitted that the observation made in the present investigation would be satisfactorily accounted for if the substrates were largely bound to enzyme.

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Table 5. *Lactate dehydrogenase/aldolase activity ratio in various tissues*

The activities of lactate dehydrogenase and aldolase respectively were calculated from the rates of reduction of pyruvate and of splitting of fructose diphosphate. References: (1) Holzer *et al.* (1956); (2) Trautschold (1956); (3) Delbrück *et al.* (1959); (4) Schmidt & Schmidt (1960); (5) Dale (1965); (6) Table 2.

Tissue	Species	Ratio	Reference
Liver	Rat	9.6	(1)
		38	(2)
		56	(3)
		36	(4)
		22	(6)
Skeletal muscle	Man	25	(4)
		4.5	(6)
		3.1	(4)
Heart muscle	Rat	11.3	(3)
		25	(4)
Smooth muscle			
Stomach	Man	21	(4)
Uterus		27	(4)
Kidney	Rat	22	(6)
	Man	62	(4)
Blood cells	Rat	14.5	(1)
		32	(6)
		36	(4)
Colon	Man		
Mucosa		8.1	(5)
Polyp		10.5	(5)
Carcinoma		14.4	(5)
Brain	Man	10-16	(4)
Testis	Rat	14	(6)
Sarcoma	Rat	24	(6)
Hepatoma	Rat	26	(6)

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