BODY SIZE AND TISSUE RESPIRATION

by

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It has long been known that in homoiothermic animals the basal metabolic rate, per unit of body weight, decreases with the size of the animal, and the question has often been discussed whether the respiration of individual tissues of animals of different size shows the same differences as the intact organisms. TERROINE AND ROCHE[1] and GRAFE, REINWEIN, AND SINGER[2] measured the respiration of various tissues *in vitro* and came to the conclusion that homologous tissues of different animals respire *in vitro* at about the same rate, irrespective of the size of the animal. They ascribed the differences found in the intact animal to the regulatory influences of the nervous system and of hormones. KLEIBER[3, 4] on the other hand, reported that the rate of respiration of liver slices of rats, rabbits, sheep, horses and cows, per unit of weight, decreased with increasing size of the animal. The decrease observed was of the same order as the decrease of the basal metabolism of the living animal.

This lack of agreement is not due to discrepancies in experimental observations but arises from difficulties of procedure and interpretation. Whilst the measurement of the basal metabolic rate is a standardized technique, no accepted standards exist for the measurement of the oxygen uptake of isolated tissues *in vitro*. It has often been demonstrated that the oxygen uptake of tissues *in vitro* is not a constant value. Specimens of the same tissue can show wide and reproducible variations, depending on the conditions under which the measurements are made. Among the factors responsible for these variations two are of special importance: the composition of the medium in which the tissue is suspended and the physical treatment of the material. As the part played by these factors was not fully appreciated in previous investigations it was thought that new measurements of the rate of respiration of isolated tissues under standard conditions are needed. As a preliminary it was necessary to define standard conditions which would resemble as closely as possible the state of the tissues in the intact, possibly resting, animal, and which would yield a "standard rate" of tissue respiration.

A. GENERAL CONSIDERATIONS CONCERNING THE MEASUREMENT OF THE "STANDARD RATE" OF TISSUE RESPIRATION

1. *Treatment of tissue*

In order to measure the rates of metabolic processes in isolated tissues it is, as a rule, unavoidable to subject the tissues to procedures like slicing, mincing or homogenizing, so that the cells can be satisfactorily supplied with oxygen and substrates.

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These procedures affect different tissues in different ways. In the case of kidney cortex, liver, striated, and cardiac muscle, mince and homogenates show higher initial rates of respiration than sliced material when phosphate saline without a combustible substrate is used as the medium. If the medium contains substrates which stimulate respiration of slices, such as lactate, pyruvate, fumarate, and glutamate, homogenates, mince and slices give approximately the same rates of respiration[5, 6, 7]. In these tissues minced or homogenized materials give the maximum rate of respiration. In other tissues, *e.g.*, spleen[8], and lung[9], minced and homogenized material gives consistently lower rates of respiration than sliced material. The low values have been attributed to the hydrolysis of coenzymes by nucleosidases released on the destruction of the tissue[9].

It is reasonable to assume that slicing leaves the tissue nearer to the 'natural' state than mincing or homogenizing, because the number of physically damaged cells is bound to be much smaller in slices than in mince or homogenates. Slicing is therefore suggested as the procedure of choice for the measurement of the standard rate of metabolism.

2. *Choice of medium*

In this section 5 different media are considered for the measurement of a standard rate of respiration. They are:

Serum
Supplemented serum
Saline serum substitute (later referred to as 'medium I')
Phosphate saline without Ca, low in bicarbonate and CO_2 (later referred to as 'medium II')
Saline low in phosphate, bicarbonate, and CO_2 (later referred to as 'medium III').

Serum. Plasma or serum, being the natural environment of animal tissues, suggest themselves as the most physiological standard media. Plasma requires the addition of an anticoagulant and several of these, *e.g.*, sodium fluoride and sodium oxalate, are unsuitable as they inhibit metabolic processes. Among the remaining substances heparine is least likely to affect tissue metabolism, but relatively large amounts are required to prevent coagulation in the presence of tissues. In general serum is preferable to plasma because the absence of fibrinogen from the medium is less likely to affect the activities of the tissue than the addition of an anticoagulant.

Supplemented serum. Although serum resembles the physiological environment more closely than any other medium it is by no means a perfect medium for *in vitro* experiments. A tissue suspended in plasma or serum may, by its metabolism, soon cause major changes in the concentration of important constituents, such as glucose, pyruvate, lactate, and the acids of the tricarboxylic cycle, and also of bicarbonate. In the intact body the balance of activities of all organs maintains a relative constancy of the concentration of serum constituents; thus, glucose used up by some tissues, is replenished from liver stores and by the absorption from the gut. But *in vitro* the metabolic activity of a single tissue can rapidly convert serum into an 'unphysiological' medium by exhausting the available substrates.

Another factor to be taken into consideration is the circumstance that in the intact organ the path of diffusion is much shorter than *in vitro*, the average distance between capillary wall and tissue cell being much shorter than the average distance between the

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surface and the centre of the slice. Hence a concentration gradient and a rate of diffusion which might be sufficient to saturate the cells *in vivo* may become a limiting factor *in vitro*.

Both difficulties—rapid exhaustion and slow diffusion—can be overcome by increasing the concentration of the 'relevant' metabolites in the medium. This consideration raises the question of what are 'relevant' substrates. Among the very large number of organic substances known to occur in plasma and serum (listed in Table I) only a few have been found to influence the oxygen uptake *in vitro*. They are glucose, lactate, pyruvate, the acids of the tricarboxylic cycle, and glutamate (or glutamine), and some closely related substances such as phosphorylated intermediates of glycolysis which need not be considered separately. A few special amino acids (*e.g.*, tyrosine, phenylalanine, proline) can increase the respiration of liver, kidney, and spermatozoa[61, 62, 63, 64], but although these effects may be of importance in relation to the

TABLE I

COMPOSITION OF HUMAN BLOOD PLASMA

Substance	mg/100 ml		References
	Average or representative value	Range or standard deviation	
Nitrogenous substances			
Protein (total)	6720		10
Albumin	4040	S.D. 270	10
α_1-Globulin	310	S.D. 51	10
α_2-Globulin	480	S.D. 83	10
β-Globulin	810	S.D. 126	10
γ-Globulin	740	S.D. 151	10
Fibrinogen	340	S.D. 59	10
Non-protein nitrogen (total)	25	18–30	11
Amino-N (as N, ninhydrin method)	4.1	3.4–5.5	12, 13
Amino-N (as N, nitrous acid method)	4.4	3.7–5.9	12
Alanine	3.97	S.D. 0.70	14. 15
Arginine	2.34	S.D. 0.62	16, 17, 18
Citrulline	0.50	0.38–0.59	19
Glutamic acid	3.41	S.D. 1.39	20
Glutamine	5.78	S.D. 1.55	20
Glycine	1.77	S.D. 0.26	14
Histidine	1.42	S.D. 0.18	16, 18
Iso-leucine	1.60	S.D. 0.31	16, 18
Leucine	1.91	S.D. 0.34	16, 18
Lysine	2.95	S.D. 0.42	16, 18
Methionine	0.85	0.46–1.48	21, 18
Phenylalanine	1.38	S.D. 0.32	16
Threonine	2.02	S.D. 6.45	16, 18
Tryptophane	1.08	S.D. 0.21	16, 22
Tyrosine	1.48	S.D. 0.37	16
Valine	2.83	S.D. 0.34	16, 18
Ammonia (as N, whole blood)	below 0.05		23, 24
Creatine	0.9	0.62–1.02	25
Creatinine	0.4	0.28–0.62	25
Glycocyamine	0.26	0.24–0.28	17
Urea (as N)	12	10–17	11, 26
Uric acid	4	2–6	27
Allantoin		0.3–0.6	19
Allantoin (dog)		1.1–3.0	19

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TABLE I (continued)

Substance	mg/100 ml		References
	Average or representative value	Range or standard deviation	
Carbohydrate and related substances			
Glucose, fasting, venous blood	83	S.D. 4	28
Glucose, fasting, capillary blood	93	S.D. 3	28
Total reducing substances (as glucose)		90–120	29
Lactic acid (resting)		8–17	30
Pyruvic acid	1.0	0.77–1.23	31, 32
Citric acid	2.5	1.9–2.8	33, 34
α-Ketoglutaric acid	0.8		35, 36
Succinic acid	0.5		36, 37
Fat and related substances			
Fat (total)	570	360–820	11
Fatty acids (total), as stearic acid	340	200–800	11
Phospho-Lipids (total)	215	123–293	38
Lecithin		50–200	39, compare 38 and 40
Cephalin		50–130	39, compare 38 and 40
Sphingomyelin		15–35	39, compare 38 and 40
Lipid-P	9.2	6.1–14.5	11
Cholesterol, total	194	107–320	11, 41
Cholesterol, free	69	26–106	11, 41
Ketone bodies (as β-hydroxybutyric acid)		0.33–0.87	42
Bile acids (as cholic acid)		0.2–3.0	43
Vitamins			
Vitamin A	0.019–0.036	0.025	44
Carotene (total carotenoids)	0.06–0.18	0.09	44
Ascorbic acid		0.1–0.70	45
Inositol		0.42–0.76	46
Folic acid	$1.75 \cdot 10^{-3}$	$1.62–1.95 \cdot 10^{-3}$	22
Biotin	$1.27 \cdot 10^{-3}$	$0.95–1.66 \cdot 10^{-3}$	22
Pantothenic acid	$12 \cdot 10^{-3}$	$6–22 \cdot 10^{-3}$	22
Mineral constituents			
Na	309	300–330	47, 48, 49
K	18	12–29	47, 48
Ca	10	8.2–11.6	47
Mg	2.0	1.6–2.7	47
Fe, men	0.0945	S.D. 0.0295	50
Fe, women	0.0895	S.D. 0.0269	50
Cu	0.09	0.07–0.12	51
Mn (whole blood)		0.005–0.020	52
Zn	0.30	S.D. 0.16	53, 54
Cl	366	350–375	47, see also 49
I (total)		0.006–0.008	55
I (protein bound)	0.007	0.006–0.008	56
F (whole blood)		0.04–0.15	57
HCO_3^- (as vol. % CO_2)	61	55–75	29, 47, 49
Phosphate, inorganic (as P)	3.7	2.9–4.3	47
Phosphate, lipid (as P)	9.2	6.1–14.5	11
SO_4 (as S)	1.57	1.00–1.85	58, 59, 60

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specific dynamic action, they are insignificant for the conditions of basal metabolism because the concentration of these substances in plasma is too low except during the period of absorption from the intestine.

The above list of 'relevant' substances can be simplified because lactate and pyruvate have very similar effects which are not additive, and only one of the two therefore needs to be added. Of the two, pyruvate has the advantage over L-lactate of being more readily available. Furthermore, all the acids of the tricarboxylic acid cycle have very similar effects[65], as may be expected from their interconvertibility. Thus the addition of one of the acids should be sufficient. As for the choice, only three of the eight main acids of the cycle are readily available: citrate, succinate, and fumarate. Citrate has the disadvantage that it forms complexes with calcium and magnesium ions and thereby upsets the ionic balance of the medium. Succinate occupies a rather special position in that the first stage of its oxidation, the formation of fumarate, may proceed much more rapidly than the other stages of the cycle[66]; it may cause a brief period of rapid oxygen consumption followed by a steady rate at a lower level. There remains fumarate as the most suitable representative of the cycle.

From the point of view of tissue respiration the list of relevant metabolites can thus be reduced to four: glucose, pyruvate or lactate, fumarate, glutamate. As regards the concentrations to be used, experiments on kidney and brain cortex show that increasing the concentrations of pyruvate, lactate, fumarate or glutamate above 0.005 M makes no difference to the rate of respiration, except in very prolonged experiments. Glucose is usually not a limiting factor when its concentration is above 0.2%.

It is therefore suggested that serum be supplemented by adding isotonic substrate solutions in the following proportions:

100 parts of serum
3 parts of 0.16 M Na-pyruvate (or Na-L-lactate)
6 parts of 0.1 M Na-fumarate
3 parts of 0.16 M Na-L-glutamate
5 parts of 0.3 M glucose

The mixture must be in equilibrium with a gas mixture containing about 5% CO_2. The additions cause a dilution of the serum of about 15%. It is not possible when making additions to maintain both isotonicity and concentrations, and preference is given to the former.

The blood from which the serum is prepared should be cooled immediately after collection, otherwise the glycolytic activity of the blood cells will reduce the concentrations of glucose and bicarbonate and increase that of lactate. The bicarbonate content of the serum should be determined and if below 0.025 M it should be adjusted to that level by the addition of 1.3% $NaHCO_3$ solution. It is advisable to sterilize the medium by passing it through a Seitz-filter.

Saline serum substitute (*Medium I*). Serum contains unknown and variable, and thus uncontrolled, constituents. It is furthermore difficult to obtain in sufficient quantities in the case of small animals, and heterologous serum may contain inhibitory antibodies. There is therefore a case for a serum substitute which can be easily prepared and whose composition is exactly known.

As a rule serum does not preserve the metabolic activities of isolated tissues more effectively than do saline media supplemented with substrates. The rates of the metabo-

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lic processes in isolated material which have so far been studied have usually been found to be of the same order in serum and in suitable saline media, at least for the usual experimental periods of under two hours. But some tissues, in particular brain, retina, choroid plexus, and foetal membranes, assume an opaque appearance on incubation in saline and tend to break up into fragments whilst appearance and texture remain unchanged in serum. The use of serum may therefore be advantageous in some investigations.

The earlier serum substitutes, such as RINGER's solution, were designed on an empirical basis. RINGER[67] tested the effect of various saline media on the beat of the isolated frog heart, and found that solutions containing certain quantities of Ca and K ions, in addition to NaCl maintained the beat for longer periods than NaCl solutions. Later, when precise data on the chemical composition of blood serum became available, saline media were modelled on these data[68, 69, 70, 71]. It has been found repeatedly that the closer the medium resembles serum the better does it maintain tissue activities *in vitro*. The previous attempts to copy the composition of serum, however, considered only the inorganic constituents and glucose.

The saline medium of KREBS AND HENSELEIT[71] closely reproduces the inorganic constituents of mammalian serum except that the concentration of Cl is about 20% higher. A discrepancy of this kind is unavoidable in a purely inorganic medium because in serum a fraction of the anions, amounting to about 22 milliequivalents, consists of organic substances. Replacement of part of the NaCl by the Na salts of pyruvic (or L-lactic), fumaric and glutamic acids and addition of glucose eliminates the discrepancy in the chloride concentration and introduces the 'relevant' metabolites. The following composition is suggested for the saline serum substitute. Mix

1. 80 parts of 0.9% NaCl (0.154 M)
2. 4 parts of 1.15% KCl (0.154 M)
3. 3 parts of 0.11 M $CaCl_2$
4. 1 part of 2.11% KH_2PO_4 (0.154 M)
5. 1 part of 3.82% $MgSO_4.7H_2O$
6. 21 parts of 1.3% $NaHCO_3$ (0.154 M); treated with CO_2 until p_H is 7.4
7. 4 parts of 0.16 M Na-pyruvate (or L-lactate)
8. 7 parts of 0.1 M Na-fumarate
9. 4 parts of 0.16 M Na-L-glutamate
10. 5 parts of 0.3 M (5.4%) glucose

Prepared by neutralizing a solution of the acids with M $NaHCO_3$ solution

The mixture must be saturated with a gas mixture containing about 5% CO_2. The stock solutions are approximately isotonic.

Solutions 7 to 10, unless sterilized, cannot be kept at room temperature. In the refrigerator they keep for about a week if gross bacterial infections are avoided.

Solutions 1 to 6 are mixed in the same proportion as the medium of KREBS AND HENSELEIT[71], except that 80 parts NaCl solution instead of 100 parts are used. The difference of 20 ml is made up by the solutions 7 to 10. The concentrations of the constituents of this medium are shown in Table II. For comparison, data for human and rat sera are also given and it will be seen that the concentration of the electrolytes in the sera and the 'serum substitute' are very similar.

Sera of different mammalian species show relatively small variations except in the case of inorganic sulphate. Normal human serum is reported to contain 1 to 1.5 mg SO_4

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TABLE II

COMPARISON OF THE COMPOSITION OF SERUM AND SERUM SUBSTITUTE

Substance	Concentration in medium (milliequivalent/litre)	Concentration in serum (milliequivalent/litre)	
		Human[75]	Rat[76, 77]
Na	141.0	142	134
K	5.93	5	5.1
Ca	5.08	5	6.05
Mg	2.36	3	2.57
Cl	104.8	103	102
Phosphate* (inorganic)	2.22	2	4.3
Sulphate (inorganic)	2.36	1	
HCO_3	24.9	27	22
CO_2 (at 40°)	1.0		
Pyruvate	4.9		
Glutamate	4.9		
Fumarate	5.4		
Total organic anions	20.7	22	
Glucose	9.2		

* In accordance with common usage one P is taken as 1.8 equivalent.

(expressed as S) per 100 ml = 0.7 to 1.0 milliequivalent per litre[58, 60]; somewhat higher figures are given by GUILLAUMIN[72]. For dog, ox, goat, and horse figures between 3 and 4 mg S per 100 ml are reported[73, 74]. The serum substitute, being primarily intended for use with animal tissues, copies the sulphate concentration of animal serum. If a substitute for human serum is required half of the $MgSO_4$ should be replaced by an equivalent amount of $MgCl_2$ solution.

Owing to the danger of bacterial infection the solutions containing organic substances should be freshly prepared before use. A composite stock solution containing solutions 1–5 in the proportion stated and 3 parts of solution 6 is stable; the use of this mixture shortens the procedure for preparing the full medium.

Phosphate saline without Ca, and low in bicarbonate and CO_2 (Medium II). Serum and the saline serum substitute may be inconvenient in the manometric measurement of respiration because they must be kept in equilibrium with gas mixtures containing about 5% CO_2. The measurement of the oxygen uptake is simpler and more accurate if the CO_2 pressure of the gas phase can be kept near zero by absorbing the gas with alkali. A reduction of the CO_2 pressure necessitates an equivalent reduction in the bicarbonate concentration if p_H is to remain within the physiological range. The following two types of media with low bicarbonate and CO_2 concentrations have been in use:

Type A. The greater part of the bicarbonate-CO_2 buffer system is replaced by a phosphate buffer of the same p_H and approximately equivalent concentration. As a high concentration of phosphate is incompatible with the physiological concentration of calcium ions the latter are usually omitted from such media. Ca-free phosphate salines are especially valuble as a medium for minced tissues and homogenates, as they give higher and steadier rates than calcium containing media[78, 79, 5, 80, 81, 82].

Type B. The bicarbonate content is reduced to about one-tenth of the physiological value, with no change in the other constituents[83, 84]. Such a medium has the advantage of having a physiological concentration of calcium, but its buffering capacity is much below that of the media of Type A. The p_H is not precisely defined but indicator tests

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show that if the medium is shaken with respiring tissues which produce CO_2 continuously p_H remains about 7.3. When the medium is allowed to stand for long periods or shaken without tissues p_H rises.

Comparative measurements have shown in many cases[85, 86] that tissues kept in these types of media respire at about the same rate as serum or saline serum substitutes containing Ca and bicarbonate in physiological concentrations.

A medium of the type A is prepared by omitting $CaCl_2$ from medium I and replacing 18 parts of the $NaHCO_3$ solution by an isotonic phosphate buffer. Mix

83 parts of 0.9% NaCl
4 parts of 1.15% KCl
1 part of 2.11% KH_2PO_4
1 part of 3.82% $MgSO_4.7H_2O$
3 parts of 1.3% $NaHCO_3$
18 parts of Na-phosphate buffer (100 parts of 0.1 M Na_2HPO_4 (1.78% $Na_2HPO_4.2H_2O$) and 25 parts of 0.1 M NaH_2PO_4 (1.38% $NaH_2PO_4.H_2O$))
4 parts of 0.16 M Na-pyruvate (or L-lactate)
7 parts of 0.1 M Na-fumarate
4 parts of 0.16 M Na-L-glutamate
5 parts of 0.3 M (5.4%) glucose

In this calcium-free medium the concentrations of Na, K, Mg, Cl and SO_4 approximate to those of serum; the concentration of phosphate is about 20 times higher, and that of HCO_3 about 10 times lower, than the physiological values.

Saline low in phosphate, bicarbonate, and CO_2 (Medium III). Many previous observations indicate that calcium ions can influence the rate of respiration[87, 88, 89, 90]. It is therefore useful to have a medium which, like the synthetic serum substance, contains Ca in physiological concentrations but can, at the same time, be used in manometric experiments where CO_2 is being absorbed by alkali. The medium suggested differs from medium II, apart from the inclusion of Ca, by a lower phosphate concentration and therefore lowering buffering capacity. These differences are necessitated by the limited solubility of Ca-phosphates. Mix

95 parts of 0.9% NaCl
4 parts of 1.15% KCl
3 parts of 0.11 M $CaCl_2$
1 part of 2.11% KH_2PO_4
1 part of 3.82% $MgSO_4.7H_2O$
3 parts of 1.3% $NaHCO_3$
3 parts of Na-phosphate buffer (as described for medium II)
4 parts of 0.16 M Na-pyruvate
7 parts of 0.1 M Na-fumarate
4 parts of 0.16 M Na-L-glutamate
5 parts of 0.3 M (5.4%) glucose

O_2 pressure

In order to safeguard saturation of tissue slices with O_2 it is generally necessary to have an O_2 pressure of one atmosphere in the cup. It is known[91, 92, 93] that O_2 of this pressure has a poisoning effect on some of the oxidative enzymes. As these effects are

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small when the medium contains Mg ions and the period of observation is below 2 hours[92] they may be neglected in many cases.

B. MEASUREMENT OF Q_{O_2} OF FIVE MAMMALIAN TISSUES

1. *Procedure*

At the start of this investigation it was decided to use medium II for the main measurements in preference to medium I because the absorption of CO_2, permissible in the case of medium II, simplifies the manometric technique. It was expected, on the basis of the results of previous investigators on similar media[86, 85], that the three media would all give approximately the same Q_{O_2} values, but later comparative measurements of Q_{O_2} in the three different salines gave consistent differences in the case of some tissues, especially brain.

The measurements of the O_2 uptake were carried out on sliced material in conical Warburg flasks of 20 to 26 ml capacity, provided with a centre well. The main compartment contained 4 ml medium, the centre well 0.3 ml 2 N NaOH, the gas space O_2. The temperature was 40°. All measurements were done in duplicate.

Five tissues, brain cortex, kidney cortex, liver, spleen, and lung, were examined. They were removed from the fasting animal as soon as possible after death and placed in ice-cold saline (medium III, in which the organic substrate solutions were replaced by an equal volume 0.9% NaCl). Slices were made free-hand or by the method of DEUTSCH[94]. During the slicing operation the tissue and razor blades were bathed in the modified medium III. Readings began after an equilibration period of 15 min and were continued at 5 or 10 min intervals for 45 min, so that the total period of incubation was 60 min. Q_{O_2} was calculated from the pressure change observed during the 45 min period of recording.

Abattoir material was collected in Dewar vessels containing 250 ml water, 250 g ice, 3.5 g NaCl, 15 ml 1.15% KCl and 12 ml 0.11 M $CaCl_2$. On addition of the tissue most of the ice melted and the resulting solution contained Na, K, Ca and Cl in approximately physiological concentrations. The material usually reached the laboratory within about one hour after killing. To test to what extent this treatment affected the rate of respiration samples of guinea pig and rat tissue were sliced immediately after death and another portion of the organ was subjected to storage in iced saline in the same way in the abattoir material, except that the period of storage was 4 hours. The results are shown in Table III. It will be seen that small losses of activity exceeding the limits of error occurred in storing guinea pig liver and guinea pig lung. As the delay in the examination of abattoir material was usually only one quarter of the time allowed for storing in this experiment it may be assumed that the losses in activity due to storage were negligible. If losses actually occurred the value given for abattoir material would be too low. Prolonged storage in iced saline caused considerable losses of activity. In an experiment in which guinea pig tissue was examined after a storage period of 24 hours Q_{O_2} of brain cortex fell 37%, of kidney cortex 11%, of liver 77%, of spleen 43%, and of lung 29%.

2. Q_{O_2} *in phosphate saline without calcium (medium II)*

Data obtained on 9 different mammalian species are given in Table IV. Of each tissue 6 specimens were examined in the case of the rat, guinea pig, rabbit, sheep, cattle

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and horse, 7 in the case of the mouse, 5 in the case of the dog and 2 in the case of the cat. The mean Q_{O_2} values for each tissue are given in Table V, together with mean values of heat production, for animals of the same average weight. The heat values are taken from BENEDICT[95].

TABLE III

EFFECT OF STORAGE OF TISSUES ON Q_{O_2}

Tissue	Q_{O_2} (average of duplicate)		Change in Q_{O_2} due to storing (%)
	Sliced immediately	Stored 4 hours in iced saline	
Brain cortex, guinea pig	— 25.1	— 23.9	— 4.7
Kidney cortex, guinea pig	— 32.9	— 34.8	+ 5.7
Liver, guinea pig	— 13.7	— 11.7	— 14.5
Lung, guinea pig	— 9.1	— 8.0	— 12.1
Liver, rat	— 19.7	— 19.2	— 2.5

TABLE IV

Q_{O_2} OF 5 TISSUES OF 9 MAMMALIAN SPECIES

Slices suspended in medium II (phosphate buffered, no calcium); the data are the averages of duplicate determinations)

No	Species	Breed	Sex	Age		Weight (kg)	Q_{O_2}				
				years	months		Brain cortex	Kidney cortex	Liver	Spleen	Lung
1	Mouse	Albino	m			0.035	— 32.2	— 50.3	— 22.2	— 20.3	— 13.5
2	,,	,,	m			0.034	— 30.2	— 35.5	— 20.2	— 15.3	— 10.6
3	,,	,,	m			0.012	— 30.0	— 53.4	— 21.9	— 17.1	— 10.4
4	,,	,,	m			0.028	— 35.6	— 48.1	— 23.7	— 14.8	— 11.6
5	,,	,,	m			0.015	— 30.2	— 41.5	— 20.9	— 16.3	— 9.2
6	,,	,,	m			0.009	— 33.1	— 37.0	— 25.6	— 17.1	— 15.4
7	,,	,,	m			0.031	— 39.6	— 56.8	— 27.5	— 19.0	— 13.6
1	Rat	Albino	m			0.22	— 24.8	— 33.8	— 15.7	— 11.9	— 9.0
2	,,	,,	m			0.22	— 25.6	— 26.8	— 13.9	— 12.3	— 9.6
3	,,	,,	m			0.20	— 21.5	— 41.6	— 19.7	— 12.2	— 9.0
4	,,	,,	f			0.19	— 35.8	— 43.1	— 16.5	— 12.0	— 8.0
5	,,	,,	f			0.24	— 20.2	— 38.5	— 14.7	— 12.8	— 7.0
6	,,	,,	m			0.18	— 30.0	— 45.3	— 22.6	— 14.7	— 9.1
1	Guinea pig		m			0.74	— 28.7	— 33.5	— 13.6	— 9.9	— 8.5
2	,,		f			0.54	— 27.8	— 31.3	— 13.7	— 13.5	— 7.6
3	,,		m			0.42	— 27.3	— 27.5	— 12.6	— 12.2	— 9.2
4	,,		m			0.40	— 22.5	— 31.0	— 11.0	— 11.7	— 7.4
5	,,		m			0.49	— 25.1	— 32.9	— 13.5	— 10.9	— 9.1
6	,,		m			0.41	— 32.1	— 34.3	— 13.6	— 11.6	— 9.4
1	Rabbit	Chinchilla	f			1.03	— 26.6	— 31.4		— 15.5	— 7.5
2	,,	,,	m			1.34	— 30.6	— 33.0	— 12.7	— 16.4	— 7.9
3	,,	,,	m			1.31	— 33.2	— 36.7	— 9.6	— 19.1	— 9.1
4	,,	,,	f			1.23	— 27.0	— 40.0	— 10.9	— 12.5	— 8.6
5	,,	,,	f			1.36	— 28.2	— 31.0	— 13.1	— 12.6	— 8.2
6	,,	,,	f			1.11	— 23.3	— 34.8	— 11.8	— 9.3	— 6.5
1	Cat		m			3.11	— 29.4	— 21.0	— 13.3	— 7.3	— 3.1
2	,,		m			2.39	— 24.3	— 24.4	— 13.0	— 9.5	— 4.7

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TABLE IV (continued)

No	Species	Breed	Sex	Age		Weight (kg)	Q_{O_2}				
				years	months		Brain cortex	Kidney cortex	Liver	Spleen	Lung
1	Dog	Mongrel	m			12.1	—20.7	—24.7	—12.0	—7.2	—4.5
2	,,	,,	m			12.5	—19.9	—24.5	—12.2	—6.2	—4.9
3	,,	,,	f			18.2	—18.3	—25.3	—12.7	—6.2	—3.9
4	,,	,,	m			22.5	—22.4	—32.0	—10.5	—6.5	—4.6
5	,,	,,	f			14.1	—24.5	—28.7	—11.1	—7.1	—6.4
1	Sheep		f	2	6	72	—19.3	—26.9	—9.3	—7.2	—7.0
2	,,		m		6	36	—18.6	—31.3	—8.3	—10.5	—5.1
3	,,	Cheviot	f	4	0	63	—19.6	—27.1	—7.8	—6.8	—4.7
4	,,	Scotch	m		8	36	—22.4	—26.1	—9.2	—5.5	—5.2
5	,,	Massam	f		7	41	—20.2	—29.9	—9.6	—6.5	—5.8
6	,,	Cheviot	f	1	6	45	—18.3	—23.6	—6.6	—4.8	—4.7
1	Cattle	Cross	f	3	6	320	—17.9	—22.8	—8.2	—4.2	—4.9
2	,,	Short horn	f	4	6	380	—20.1	—22.0	—7.9	—4.2	—3.9
3	,,	,,	f	4	6	510	—16.5	—23.6	—8.0	—4.2	—3.9
4	,,	,,	m	4	0	440	—18.1	—21.9	—7.3	—4.9	—3.3
5	,,	Aberdeen angus	m	2	0	570	—13.4	—30.3	—9.6	—4.4	—4.8
6	,,	Short horn	f	3	0	320	—17.3	—19.2	—8.1	—4.7	—4.2
1	Horse	Shire	f	25	0	610	—16.4	—18.2	—6.1	—4.4	—5.3
2	,,	Cross	f	15	0	610	—17.6	—21.0	—5.7	—3.8	—4.6
3	,,	Shire	m	10	0	790	—17.4	—23.5	—6.1	—4.9	—4.1
4	,,	,,	m	6	0	790	—12.0	—22.6	—4.0	—4.4	—4.0
5	,,	,,	f	7	0	760	—16.5	—19.1	—5.9	—4.1	—4.6
6	,,	,,	m	18	0	790	—14.1	—24.5	—4.5	—3.8	—4.1

TABLE V

AVERAGE Q_{O_2} OF 5 TISSUES OF 9 MAMMALIAN SPECIES COMPARED WITH AVERAGE BASAL HEAT PRODUCTION (Q_{O_2} MEASURED IN MEDIUM II)

(The average Q_{O_2} values are computed from Table IV. The data on average basal heat production per kg bodyweight are taken from BENEDICT's graphs[95]. The heat data refer to animals of the average body weight given in the third vertical column).

Species	Bodyweight (kg)		Q_{O_2}					Basal heat production/kg bodyweight in 24 hours (Cal)
	Range	Mean	Brain cortex	Kidney cortex	Liver	Spleen	Lung	
Mouse	0.012–0.035	0.021	—32.9	—46.1	—23.1	—16.9	—12.0	158
Rat	0.18–0.22	0.21	—26.3	—38.2	—17.2	—12.7	—8.6	100
Guinea pig	0.42–0.74	0.51	—27.3	—31.8	—13.0	—11.6	—8.5	82
Rabbit	1.03–1.36	1.05	—28.2	—34.5	—11.6	—14.2	—8.0	60
Cat	2.39–3.11	2.75	—26.9	—22.7	—13.2	—8.4	—3.9	50
Dog	12.1–22.5	15.9	—21.2	—27.0	—11.7	—6.6	—4.9	34
Sheep	36–72	49	—19.7	—27.5	—8.5	—6.9	—5.4	25
Cattle	320–570	420	—17.2	—23.5	—8.2	—4.4	—4.3	20
Horse	610–790	725	—15.7	—21.5	—5.4	—4.2	—4.4	17

As already stated the values for Q_{O_2} were calculated from the pressure changes recorded between 15 and 60 min after the start of the incubation period. The rate of oxygen uptake often showed a progressive fall during the 45 min of observation, and

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Q_{O_2} values calculated for the period of incubation between 20 to 40 min were therefore as a rule somewhat higher than those given in the Table. In the case of brain and kidney the difference was no greater than 5%. In the case of the other three tissues it was of the order of 10%.

3. *Q_{O_2} in saline containing calcium and low in phosphate and bicarbonate (medium III)*

On each of the 9 species 1 or 2 experiments were carried out in which Q_{O_2} was measured at the same time in media II and III. These experiments showed that in general the Q_{O_2} values calculated from the early readings (20 to 40 min) tended to be somewhat lower in medium III, but the progressive fall with time was less in this medium, and the Q_{O_2} values calculated for the 15 to 60 min period were within 10% the same in the case of kidney cortex, lung and spleen in all 9 species. On the other hand Q_{O_2} for brain, and some species of liver, was considerably lower in medium III, and of these 2 tissues further specimens were examined. The results are given in Tables VI and VII.

TABLE VI

Q_{O_2} OF BRAIN CORTEX AND LIVER OF 9 MAMMALIAN SPECIES

(Slices suspended in medium III (low in bicarbonate; containing calcium); the data are the averages of duplicate determinations).

No	Species	Breed	Sex	Age		Weight (kg)	Q_{O_2}	
				years	months		Brain cortex	Liver
1	Mouse	Albino	m			0.045	—19.9	—15.6
2	,,	,,	m			0.044	—22.9	
3	,,	,,	m			0.031	—24.4	
4	,,	,,	m			0.040	—23.2	—20.2
5	,,	,,	f			0.009	—24.3	—22.3
6	,,	Black	m			0.020		—18.3
7	,,	,,	m			0.013		—20.3
1	Rat	Albino	m			0.27	—20.6	—12.3
2	,,	,,	m			0.18	—20.8	—17.4
3	,,	,,	m			0.37	—17.9	—13.9
4	,,	,,	m			0.24	—18.3	—14.0
5	,,	,,	m			0.25	—18.5	—15.5
1	Guinea pig		m			0.58	—18.5	—6.07
2	,,		m			0.58	—20.0	—6.60
3	,,		m			0.41	—17.4	—9.50
4	,,		m			0.28	—16.4	—11.6
5	,,		m			0.50	—15.8	—9.95
1	Rabbit	Chinchilla	f			1.11	—15.3	—7.6
2	,,	,,	f			0.93	—15.0	—8.1
3	,,	,,	f			1.12	—15.6	—7.5
4	,,	,,	f			1.53	—15.6	—7.8
5	,,	,,	f			1.35	—14.2	—6.9
1	Cat		m			3.11	—14.9	—9.4
2	,,		m			2.39	—16.1	—11.0
1	Dog	Mongrel	f			18.2	—16.0	—12.9
2	,,	,,	m			22.5	—13.8	—9.5
3	,,	,,	f			14.1	—14.5	—9.9

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TABLE VI (continued)

No.	Species	Breed	Sex	Age		Weight (kg)	Q_{O_2}	
				years	months		Brain cortex	Liver
1	Sheep	Scotch	m	0	9	27	—12.4	—7.2
2	,,	,,	f	2	6	36	—10.0	—8.6
3	,,	Cheviot	f	1	6	45	—10.0	—6.7
4	,,	Sussex	f	0	8	41	—10.1	—3.5
5	,,	Lincolnshire crossbred	m	0	8	27	—14.1	—5.2
1	Cattle	Shorthorn	m	4	6	280		—2.6
2	,,	Shorthorn crossbred	m	3	0	290	—12.3	—4.3
3	,,	Shorthorn	f	3	0	320	—15.4	—3.8
4	,,	,,	m	2	6	380	—10.8	—4.0
5	,,	,,	f	4	6	320	—10.6	—2.2
6	,,	,,	f	3	6	290	—11.4	—3.5
1	Horse	Shire	m	18	0	790	—10.0	—1.8
2	,,	,,	f	7	0	760	—8.64	—2.4
3	,,	Belgian	m	15	0	710	—13.7	—2.5
4	,,	Shire	f	10	0	760	—11.5	—3.2
5	,,	,,	m	13	0	760	—8.78	—2.9

TABLE VII

AVERAGE Q_{O_2} OF BRAIN CORTEX AND LIVER OF 9 MAMMALIAN SPECIES COMPARED WITH AVERAGE BASAL HEAT PRODUCTION (Q_{O_2} MEASURED IN MEDIUM III)

(The average Q_{O_2} values are computed from Table VI. The average basal heat production is taken from BENEDICT'S[95] graphs

Species	Body weight (kg)		Q_{O_2}		Basal heat production/kg body-weight in 24 hours
	Range	Mean	Brain cortex	Liver	
Mouse	0.009–0.045	0.038	—22.9		145
	0.009–0.045	0.026		—19.3	152
Rat	0.176–0.365	0.26	—19.2	—14.6	92
Guinea pig	0.279–0.58	0.44	—17.4	—9.5	85
Rabbit	0.93–1.53	1.21	—15.1	—7.6	57
Cat	2.39–3.11	2.75	—15.5	—10.2	50
Dog	14.1–22.5	18.3	—14.8	—10.8	31
Sheep	27–45	35	—11.3	—6.2	27
Cattle	280–380	320	—12.1	—3.6	21
Horse	710–790	760	—10.5	—2.6	17

4. Q_{O_2} *in saline serum substitute (medium I)*

In order to decide whether the difference between the Q_{O_2} values obtained for brain and liver in media II and III were due to the differences in the calcium content, or in the bicarbonate and phosphate content, comparative measurements were made on the same tissue sample in media I, II and III. The 'indirect' method of WARBURG[96] was used for the measurements in medium I, in preference to those of DICKENS AND SIMER[97] or DIXON AND KEILIN[98], because with this method it is possible to follow the time course of the oxygen uptake. Duplicate sets of vessels were used in each measurement. Q_{O_2} was again calculated for the 15 to 60 min period of incubation. The results of the comparative measurements are given in Table VIII.

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TABLE VIII

COMPARATIVE MEASUREMENTS OF Q_{O_2} IN 3 DIFFERENT SALINE MEDIA

Tissue	Species	Q_{O_2}		
		Medium I (Containing physiological concentrations of HCO_3' and CO_2)	Medium II (Phosphate buffered, no calcium)	Medium III (Low in bicarbonate; containing calcium)
Brain cortex	Guinea pig	— 18.6	— 34.2	— 16.4
„ „	Rabbit	— 17.5	— 23.9	— 15.6
„ „	Sheep	— 13.5	— 17.6	— 12.4
„ „	Cattle	— 9.9	— 15.9	— 10.8
„ „	Horse	— 13.7	— 16.5	— 13.7
Liver	Mouse	— 19.6	— 18.6	— 20.2
„	Guinea pig	— 10.8	— 12.2	— 11.6
„	Rabbit	— 10.3	— 9.9	— 8.1
„	Sheep	— 5.2	— 6.0	— 5.1
„	Cattle	— 3.6	— 4.7	— 3.5
„	Horse	— 2.7	— 3.2	— 2.9

C. DISCUSSION OF RESULTS

1. *Comparison of the Q_{O_2} values obtained in the 3 media*

Kidney cortex, spleen and liver gave about the same Q_{O_2} in all three media, but differences exceeding 10% were found in brain cortex and in liver. A comparison of the data from Tables V and VII (see Table IX) shows that the average Q_{O_2} values for brain cortex in medium II were between 37 and 87% higher than those obtained in medium III. In the case of the liver the differences were smaller; they are of doubtful significance in the small animals (mouse, rat) and increase approximately (though not strictly) parallel with the body weight of the species, being greatest in cattle and horse.

TABLE IX

DIFFERENCES IN THE AVERAGE Q_{O_2} VALUES IN MEDIA II (CONTAINING NO Ca) AND III (CONTAINING Ca)

(The figures show the level of $Q_{O_2}^{\text{Medium II}}$ expressed as per cent of $Q_{O_2}^{\text{Medium III}}$, calculated from the data in Tables V and VII).

Species	$\frac{Q_{O_2}^{\text{Medium II}}}{Q_{O_2}^{\text{Medium III}}} \cdot 100$	
	Brain cortex	Liver
Mouse	144	120
Rat	137	118
Guinea pig	157	137
Rabbit	187	153
Cat	174	129
Dog	143	108
Sheep	174	137
Cattle	142	227
Horse	150	208

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According to Table VIII, media I and III give approximately the same Q_{O_2} values. The considerable differences in the concentration of bicarbonate, CO_2 and phosphate in these two media have thus no major effect on the Q_{O_2} under the conditions tested. Since medium I resembles the physiological environment more closely than the other media, Q_{O_2} values obtained with this medium might be regarded as approximating more closely to the physiological value than higher values found for brain, and the liver of the larger animals in medium II. The latter are not likely to be standard Q_{O_2} values but no definite statement can be made on this point because reliable data on the O_2 consumption of tissue *in vivo* are too scanty. In experiments of NOELL, AND SCHNEIDER[99] the O_2 consumption of dog brain cortex *in vivo* was 4.5 ml per minute per 100 g fresh weight, and on the assumption that the dry weight of dog brain cortex is 21% of the fresh weight[100] Q_{O_2} *in vivo* was −12.9. This value is in good agreement with the figure of −14.8 found in medium III (Table VII) and favours the view that the values found for brain in the Ca-free medium II are abnormally high.

Effects of calcium in the Q_{O_2} of slices and homogenates have been described before and have recently been reviewed by CUTTING AND MCCANCE[90]. ELLIOTT AND LIBET[6] found that Ca depresses the initial rate of respiration of brain homogenates, but delays the falling off at the later stages of incubation, thus steadying the rate of respiration. It does not seem to have been noted before that the effect of Ca on tissue slices is greater in brain than in other tissues.

Whilst there is some uncertainty as to which of the values obtained in the different media constitute the 'basal' Q_{O_2}, it should be stated that the conclusions drawn in the following sections are not affected by this uncertainty.

2. *Absolute level of* Q_{O_2}

The Q_{O_2} values in all 3 media tend to be considerably higher than the values reported in the literature for saline media[101], especially in the case of brain, liver and kidney. However, no strict comparison is possible because different substrates were used in previous measurements. The combination of substrates added in the present experiments give, in general, higher values than the substrates added in most previous work (glucose or lactate). The Q_{O_2} values observed in the new media are of the same order as the highest values recorded for serum. Thus the intention to include in the new media the substances in serum which stimulate respiration[85] seems to have been accomplished.

3. Q_{O_2} *and body size*

General survey. The data given in Tables IV, V and VII show that the Q_{O_2} values of the tissues of the larger species are, in general, somewhat lower than the homologous values of the smaller species. But there are many exceptions to this general rule. No strict parallelism exists between the Q_{O_2} values of the homologous tissues and the basal heat production per unit body weight of the intact animal. The Q_{O_2} values for brain, kidney, spleen, and lung change much less, and those for liver slightly less, with the body weight than the rate of basal heat production. Neither is there a simple correlation between body size and Q_{O_2} within the same species. The body weights of the 7 mice listed in Table IV varied between 9 and 35 g and that of the 5 mice listed in Table VI between 9 and 45 g. There were variations between 36 and 72 kg in the body weight of the 6 sheep of the first series. These differences of the body weight within one species are not reflected by differences in the Q_{O_2} values, with the doubtful exception of the

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values for brain in Table VI, where the brains of the 2 smaller sheep show higher values than the 3 brains from the larger animals.

Brain cortex. In the largest species (horse) the average Q_{O_2} of brain cortex was about half the average Q_{O_2} value of the smallest species (mouse) namely 48% for the measurements in medium II, and 46% for the measurements in medium III. In contrast, the basal heat production per kg bodyweight of the horse is only 11% and 12% respectively of that of the mouse.

Kidney cortex. The changes of the Q_{O_2} values from species to species in this tissue were similar to those of brain cortex. The average Q_{O_2} value of horse kidney cortex was 47% of that of mouse kidney. The average Q_{O_2} value for sheep kidney was only 14% below that for guinea pig kidney, whilst the basal heat production per kg. bodyweight of the sheep is only 37% of the guinea pig. Thus the decrease of the Q_{O_2} values with body size was again much smaller than the decrease in the rate of the basal heat production.

Spleen, lung. For the horse the Q_{O_2} value of spleen tissue was about a quarter, and for lung about one third, of the corresponding values for the mouse. In these two tissues the discrepancies between the changes in Q_{O_2} and the changes in basal heat production in relation to body size are thus not as great as in brain and kidney, but they are still considerable.

Liver. Liver shows greater Q_{O_2} changes with body weight than any other tissue tested, especially in medium III (Tables V and VII). In medium II Q_{O_2} of horse liver was 23%, and in medium III it was 13.5% of that of mouse liver. Thus, when comparing the Q_{O_2} values obtained in medium II for these two species, about the same percentage change is found as for the basal rate of heat production. But the parallelism over the whole series of species is very imperfect. For example, the Q_{O_2} values for guinea pig, cat and dog are about the same (−9.5; −10.2; −10.8), whilst the basal rate of heat production shows a progressive fall with body weight (85; 50; 31).

The changes of Q_{O_2} of liver with body weight reported in this paper are similar to those found by KLEIBER[3], but owing to the differences in the media used the present Q_{O_2} values are all higher than those reported by KLEIBER.

4. *Rôle of muscle tissue in chemical temperature control*

As the rate of respiration of a number of homologous tissues of animals of different sizes fails to show a strict parallelism with the basal rate of heat production of the intact body, it remains to be explained how the characteristic differences in the basal rates of heat production of animals of different sizes arise. One kind of explanation is contained in various publications by KESTNER[102, 103] and BLANK[104], who stated that the proportion of highly active organs is somewhat greater in the body of small animals than in that of large animals. He expressed the view that the "relative size of the brain and the large glands can give a complete explanation of the different heights of metabolism in different animals[103]". This view is not substantiated by quantitative measurements and such data as are available cannot be reconciled with KESTNER's hypothesis (see KLEIBER[4]).

An alternative explanation is offered by the conception that the relation between Q_{O_2} and body weight found for the 5 tissues tested does not hold for every tissue; that there is at least one major tissue whose "basal" Q_{O_2} changes with the body weight approximately parallel with the basal heat production; that this organ is the striated musculature.

The substance of this conception is, of course, not new in that it is commonly

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accepted that the muscles play a leading part in the regulation of heat production. Evidence in support of this conception is the increased muscular activity on exposure to cold, manifesting itself by increased tension and shivering, and the failure of the curarized animal to maintain the physiological temperature level on exposure to cold. It has not been directly demonstrated that the basal respiration of the musculature varies with body size in the postulated fashion, and no satisfactory experimental procedure has as yet been devised to carry out the necessary measurements. Data obtained on isolated muscles bear no simple relation to the basal respiratory rate of the muscle *in situ* because the Q_{O_2} of muscle depends more than that of any other tissue on the state of activity of the tissue. Activity may cause a thirty-fold rise of the resting rate of respiration (BARCROFT[105], MEYERHOF[106]). As the state of activity is controlled by the higher nervous centres detachment from the nervous system is bound to affect the rate of respiration.

5. *Factors determining the level of tissue respiration*

If body size is not a major factor determining the Q_{O_2} of the 5 tissues tested it remains to be examined which other factors control the level of respiration of these tissues. As the respiration of living tissues primarily serves to supply energy, the level of tissue respiration is expected to be determined by the energy requirements. A variety of factors contribute to the requirements. They may be classed in three groups:

1. Energy is required when tissues perform mechanical, osmotic, chemical, or other kinds of external work.

2. Energy is required to maintain structures which are thermodynamically unstable. An example is the maintenance of concentration gradients between tissue and blood plasma of readily diffusable substances, such as inorganic ions, amino acids, coenzymes.

3. Energy is required to maintain the body temperature.

Energy generated for the first two purposes always yields heat as a by-product and in homeotherms this heat is partly, or wholly, utilised to maintain the body temperature. In an organism performing some physical exercise, and living at a temperature not far removed from that of the body temperature, the heat arising as a by-product may be enough for the upkeep of the body temperature. In a cold environment the heat arising as a by-product in a resting organism may no longer be sufficient to maintain the body temperature, and extra heat has to be formed. It is reasonable to assume that the highly differentiated cells whose task it is to carry out specialised functions, as do those of brain or the glands, are designed to deal solely with these specialized functions rather than to act as heat generators in the case of exceptional loss of heat. The extra source of heat might be expected to be the muscle tissue which, for other reasons, has the capacity of varying the rate of heat production. If this assumption is correct, in other words, if the level of respiration of highly specialized tissues is determined by the energy requirement falling under categories (1) and (2), it is to be expected that the rate of energy production of the highly differentiated cells is not dependent on the size of the animal, because the energy needed for the performance of a given piece of work is independent of the size of the body.

However, somewhat different from the question of energy requirements of the highly differentiated *cells* is the problem of the energy requirements of organs as a whole. Homologous organs of different species have by no means identical structures. For

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example, in a larger species, tissue structures accessory to the main functional cells are bound to constitute a relatively larger portion of the organ than in the homologous tissue of a smaller animal. Such accessory structures are, among others, blood vessels, glandular ducts, connective tissues.

Thus some changes of the Q_{O_2} values with body size may be expected in homologous *tissues* even if the Q_{O_2} of homologous *cells* is the same. In general the change will be a decrease with body size because cells with lower respiration, like those of connective tissue, blood vessels and ducts, are bound to became more preponderant in the larger species. The changes in the Q_{O_2} with body size, seen in Tables V and VII, may in part be due to this factor.

SUMMARY

The factors affecting the rate of respiration in isolated tissues are discussed with reference to the measurement of a "standard rate" of metabolic processes *in vitro*. Media for the suspension of tissues are devised; their composition is essentially based on the available analytical data for blood plasma.

Q_{O_2} of liver, brain cortex, kidney cortex, spleen, and lung was measured for 9 mammalian species of different body size (mouse, rat, guinea-pig, rabbit, cat, dog, sheep, cattle, horse). Three different media were used ("phosphate saline without Ca", "saline low in phosphate, bicarbonate and CO_2" and "saline serum substitute" containing physiological concentrations of inorganic ions in addition to organic substrates). Kidney cortex, spleen, and liver gave about the same Q_{O_2} values in all three media. Q_{O_2} for brain cortex was for all species higher in the medium containing no Ca, the average level being 37–87% higher. Q_{O_2} for liver was also higher in the absence of Ca, especially in the larger species.

Q_{O_2} values of the tissues of larger animals were in general somewhat lower than the homologous values of the smaller species but no strict parallelism between Q_{O_2} values and basal heat production of the intact animal was found. The Q_{O_2} values for most tissues changed much less with the body weight than the rate of basal heat production.

The absolute level of Q_{O_2} in the new media (which apart from glucose contain pyruvate, fumarate and L-glutamate) was higher than the values reported in the literature for saline media. They are of the same order as the highest values recorded for serum.

The characteristic differences in the basal rate of heat production in animals of different size are to be attributed mainly to variation in the Q_{O_2} of the musculature. It is suggested that the Q_{O_2} of tissues other than muscle is in the first place governed by the specific energy requirements of the tissues, and not by the heat requirements of the whole body.

RÉSUMÉ

Les facteurs qui influencent la vitesse de la respiration dans les tissus isolés sont discutés par rapport aux mesures d'une "vitesse standard" des processus métaboliques *in vitro*. L'auteur décrit des milieux de suspension de tissus; leur composition se base essentiellement sur les données analytiques connues pour le plasma sanguin.

Le Q_{O_2} a été déterminé pour le foie, le cortex du cerveau et du rhein, la rate et le poumon de 9 espèces de mammifères de taille différente (souris, rat, cobaye, lapin, chat, chien, mouton, bétail, cheval). Trois milieux différents ont été employés, le "phosphate salin sans Ca", "la solution saline faible en phosphate, bicarbonate et CO_2 et "la solution saline, remplaçant le sérum" qui contient des concentration physiologiques d'ions inorganiques en plus du substrat organique. Dans les trois milieux le cortex rhénal, la rate et le foie donnèrent environ les mêmes valeurs de Q_{O_2}. Pour le cortex cervical ce facteur était plus élevé pour toutes les espèces animales examinées dans les milieux exempts de Ca. En moyenne les valeurs étaient de 37 à 87% supérieures. Pour le foie le Q_{O_2} était aussi supérieur en absence de Ca, surtout dans les espèces plus grandes.

En général les valeurs de Q_{O_2} étaient plus basses pour les tissus des animaux plus grands que les valeurs homologues pour les animaux plus petits. Cependant nous n'avons pas trouvé un parallèlisme stricte entre les valeurs de Q_{O_2} et la production de chaleur des animaux intacts.

Dans les nouveaux milieux (qui, à part le glucose, contiennent du pyruvate, du fumarate et du L-glutamate) le niveau absolu du Q_{O_2} était plus élevé que les valeurs rapportées dans la littérature pour une solution saline. Elles sont du même ordre que les valeurs les plus élevées rapportées dans la littérature pour le sérum.

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Les différences caractéristiques dans la vitesse de base de la production de chaleur des animaux de differente taille doivent être attributées surtout à la variation du Q_{O_2} de la musculature. L'auteur suggère l'idée que le Q_{O_2} des tissus autres que le muscle serait gouverné en premier lieu par les besoins spécifiques d'énergie des tissus et non par les besoins de chaleur du corps entier.

ZUSAMMENFASSUNG

Die Faktoren, welche die Geschwindigkeit der Atmung in isolierten Geweben beeinflussen, werden diskutiert und zwar mit Rücksicht auf die Messungen einer "Standardgeschwindigkeit" metabolischer Prozesse *in vitro*. Medien für Gewebesuspensionen werden vorgeschlagen, deren Zusammensetzung sich hauptsächlich auf die für Blutplasma bekannten analytischen Werte gründet.

Der Faktor Q_{O_2} von Leber, Gehirnrinde, Nierenrinde, Milz, und Lunge wurde für 9 Säugetierarten verschiedener Körpergrosse (Maus, Ratte, Meerschweinchen, Kaninchen, Katze, Hund, Schaf, Vieh, Pferd) bestimmt. Drei verschiedene Medien wurden verwendet, nämlich "Phosphat-Salz-Lösung ohne Ca", "Salzlösung mit geringem Gehalt an Phosphat, Bicarbonat und CO_2" und "Salzlösung-Serumersatz", welche ausser anorganischen Ionen in physiologischen Konzentrationen organische Substrate enthält. Nierenrinde, Milz und Leber gaben ungefähr dieselben Q_{O_2}-Werte in allen drei Medien. Der Q_{O_2} der Gehirnrinde war für alle Arten in dem Ca-freien Medium höher und zwar betrug der Unterschied durchschnittlich 37 bis 87%. Auch für die Leber lagen die Werte höher in Abwesenheit von Ca und zwar insbesondere in den grösseren Arten.

Im Allgemeinen lagen die Q_{O_2}-Werte der Gewebe grösserer Tiere etwas niedriger als die homologen Werte kleinerer Arten; es konnte aber kein strenger Parallelismus zwischen den Q_{O_2}-Werten und der Wärmebildung unverletzter Tiere gefunden werden. Die Q_{O_2}-Werte der meisten Gewebe varieren viel weniger mit dem Körpergewicht als die Geschwindigkeit der Wärmebildung.

Die absolute Lage der O_{O_2}-Werte war in den neuen Medien, die ausser Glucose noch Pyruvat, Fumarat und L-Glutamat enthalten, höher als die in der Literatur für Salzlösungen beschriebenen Werte. Sie sind von der gleichen Grössenordnung wie die höchsten in der Literatur für Serum angeführten Werte.

Die charakteristischen Unterschiede in der Geschwindigkeit der Wärmebildung von Tieren verschiedener Körpergrösse müssen hauptsächlich auf die Änderungen des Q_{O_2} in der Muskulatur zurückgeführt werden. Die Ansicht wird ausgesprochen, dass der Q_{O_2} von anderen Geweben als Muskeln an erster Stelle durch die Energiebedürfnisse der Gewebe und nicht durch den Wärmebedarf des ganzen Körpers bedingt wird.

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