TISSUE RESPIRATION, GROWTH, AND BASAL METABOLISM

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It is a phenomenon general in mammals and other classes of the animal kingdom that metabolic rate per unit weight decreases with increasing body size. This is expressed in the surface rule of Rubner who stated that metabolic rate decreases per unit weight, but is constant per unit surface. More recent investigations (Brody, 1945; Kleiber, 1947) indicate that interspecifically, *i.e.*, comparing mature animals of different species, basal metabolic rate in mammals is proportional rather to a ¾ power of weight than to surface or the ⅔ power of weight. Intraspecifically, *i.e.*, comparing animals of different body size within the same species, the surface rule applies to the general trend of the size-metabolism relation in rats, although qualifications have to be made in detail (Bertalanffy, Müller and Racine, unpublished data). These complications, however, do not alter the fundamental fact of the decrease in weight-specific metabolic rate with increasing body size. However, we do not have a satisfactory explanation for this phenomenon.

The basic alternative seems to be whether the dependence of metabolism on body size is based upon *cellular* or *organismic* factors. It may be due to intrinsic differences in the metabolism of the cells of smaller and larger individuals which will show up also in isolated tissues; or it may be due to regulative factors lying in the organism as a whole. There may be also a combination of both.

Earlier work on the relation between tissue metabolism and body size (reviewed by Kleiber, 1947) is contradictory. Terroine and Roche (1925), and, independently, Grafe (1925; Grafe, Reinwein and Singer, 1925), stated that the metabolic rate per unit weight of homologous tissues *in vitro* is essentially the same for small and large animals, although basal metabolic rate per unit weight *in vivo* decreases systematically with increasing body size. Grafe assumed that the metabolic rate of tissue *in situ* is checked by central regulators, mainly the nervous and endocrine system. On the other hand, LeBreton and Kayser (1926; Kayser, LeBreton and Schaeffer, 1925), and Borger and Groll (1926) reported variation of the respiration rate of tissues with increasing body size, in individuals of the same species as well as in different species. This earlier work is open to criticism, and Grafe *et al.'s* calculations, in particular, were based upon erroneous assumptions (*cf.* Field *et al.*, 1939).

More recent results, however, are also contradictory. According to Field *et al.* (1939) the summated tissue respiration (*i.e.*, metabolic rate *in vitro* per unit fresh weight, multiplied by the weight of the respective organ, and summated over 20 main organs) amounts to 66% of the basal metabolic rate of the rat, and, if allowance is made for minimal functional activity (muscle tone, cardiac, respiratory, smooth muscle, secretory function), even for 89% of the respiration of the intact animal. The authors conclude that basal metabolism is the arithmetic sum

of tissue respiration as measured by the Warburg technique, and that there is no reason to assume that the level of tissue respiration *in situ* is determined by organismic factors which are not operative *in vitro*. Similar experiments were made by Martin and Fuhrmann (1941) on the dog, where summated tissue respiration gives 79% of the resting metabolism. It is concluded, therefore, that resting metabolism can be accounted for by summated tissue respiration and minimal functional activity.

However, it is well-known that the respiration rates of tissues show considerable variation, depending on the medium used. In serum, or in media containing the relevant metabolites (Krebs, 1950), they are often two or three times the values obtained in saline. It appears, therefore, that it is hard to decide which values should be considered to be the "true" metabolic rates of tissues corresponding to those *in situ,* so that these values, multiplied by organ weight, would give the total respiration of the organ in question. If, instead of saline, the previously mentioned authors had used serum, they would probably have obtained not 66%, but more than 100% of basal metabolic rate as summated tissue respiration.

Kleiber (1941; Weymouth, Field and Kleiber, 1942) investigated tissue respiration of liver from rats, rabbits, sheep, horse and cow, and found that Q_{O_2} decreases to the same extent (namely, the $-\frac{1}{4}$ power) as does the basal metabolic rate per unit weight, according to the $\frac{3}{4}$ power rule. He concludes (1941; p. 422), therefore, that "the factors which determine the metabolic level *in vivo* seem still to be present in the surviving tissue cut out of the organism." Similarly, Weymouth *et al.* (1944) studied the Q_{O_2} of the midgut gland of individuals of different size in the kelp crab *Pugettia*. They also found a systematic decrease of the Q_{O_2} values with increasing size, paralleling the decrease in weight-specific basal metabolic rates of the intact animals. Krebs (1950) compared the Q_{O_2} values of 5 tissues of 9 mammalian species. He found that, in general, the Q_{O_2} values of the larger species are somewhat lower than the homologous values of small species; but there is no parallel decrease in the different tissues, nor a consistent relation to the decrease of weight-specific basal metabolic rate with increasing size. The greatest decrease of Q_{O_2} in species of larger size is found in the liver (comparable to Kleiber's results though with some exceptions); but in the other tissues the decrease in Q_{O_2} is much smaller than the decrease in basal metabolism.

There seems to be, as yet, no systematic investigation on the intraspecific size dependence of the Q_{O_2} of different tissues. The present work (*cf.* Bertalanffy and Pirozynski, 1951; Pirozynski and Bertalanffy, 1952) was started before Krebs' investigation came to our attention.

The preliminary report of our results (Bertalanffy and Pirozynski, 1951) has been followed by an interesting investigation of tissue respiration of kidney and liver in growing chicken (Crandall and Smith, 1952) which confirms the results and conclusions of the present work. Three groups of chickens (body weight 66, 178, and 2350 gm.) were investigated. Apart from an early minimum of total metabolism as well as of liver Q_{O_2} after hatching, characteristic of chicken and not present in the rat, the relations found correspond to our results: No correlation between body size and Q_{O_2} in the kidney, a slight decrease of Q_{O_2} ($a \sim 0.1$) with increasing body size in liver slices.

A remark seems to be appropriate as to the relative value of interspecific and intraspecific comparisons. The first has, of course, the advantage of allowing a much greater range of body sizes to be compared; further, adult animals are compared so that developmental differences cancel out. On the other hand, although the physiological differences between newborn and adults are great, the same is true for the anatomical, physiological, biochemical, ecological, etc. differences even between related species, not to speak of comparisons "from the mouse to the elephant." The startling fact is that in spite of this, simple quantitative relations in basal metabolism can be established, intraspecifically as well as interspecifically.

MATERIAL AND METHODS

In our experiments male and female albino rats (Wistar strain) were used, representing a continuous series from newborn animals of 9 gm. body weight to adults of 392 gm. The animals, except for the newborn, were kept for 24 hours in individual cages prior to the experiment. All rats were fed Purina Fox Chow and tap water *ad libitum,* before the actual experiment took place. The diet was restricted to tap water only for 12 to 18 hours (basal metabolism regime). All animals were killed by breaking the cervical vertebra.

The organs to be investigated were carefully removed immediately after death and placed in ice-cold saline. The pieces of whole organs were sliced by free hand according to the method introduced by Deutsch and Raper (1936). Tissues were sliced up to the thickness of 0.4 mm. except the diaphragm, which was wholly removed from the animal by means of sharp scissors or a razor blade, and cut into two or three pieces. The muscular parts were then carefully separated from the adjacent connective tissue and placed in the flasks. Before transferring the slices to the Warburg flasks, they were gently dried by touching with a piece of hard filter paper. The slices of organs from newborn rats were generally prepared in the same manner except the brain, which was cut in half and minced by means of a forceps before being placed in the flask. A few embryonic tissues were obtained from pregnant rats. The smaller embryos of 25–35 mgm. body weight were transferred into the flask after removing the fetal membranes; the larger specimens were sliced using the same technique as with the organs of adult animals.

The oxygen consumption was determined by the direct method of Warburg using the technique described by Umbreit *et al.* (1949). The oxygen uptake in Krebs-Ringer-phosphate solution of pH 7.4 was measured in pure oxygen with carbon dioxide absorbed by alkali-soaked filter paper in the center well. The flasks attached to the manometers were placed in a constant temperature bath of 37.0° C. (± 0.1) and shaken at the rate of 124 oscillations per minute. The equilibration period in the water bath was approximately 20 minutes. The standard experimental period was 60 minutes with readings taken every 10 minutes. The dry weight of the investigated tissue was determined on an analytical balance after drying the slices for at least two hours at 105–110° C.

Higher Q_{O_2} values can be obtained in other media. However, in phosphate Ringer we found the Q_{O_2} values to remain approximately constant during the experimental period, except in the case of brain (Fig. 1), while if glucose or other metabolites are added, the Q_{O_2} values often decrease. Since our study amounts

to a comparison under standard conditions, it was preferable to use a medium where there is no decrease of Qo_2 during the experiment, and therefore no need to make extrapolations.

The order of the differences to be expected if the decrease of basal metabolic rate is based upon differences in tissue respiration can be estimated as follows. Basal metabolism is generally proportional to a power of the weight:

$$M = bW^\alpha, \tag{1}$$

where M is the rate of basal metabolism, W the body weight, the exponent α indicates the slope of the regression line in log-log plot, and b is a constant, indicating the extrapolated value of M for $W = 1$. The dependence of metabolism on body size is a special case of the general law of allometric growth (cf. Bertalanffy,
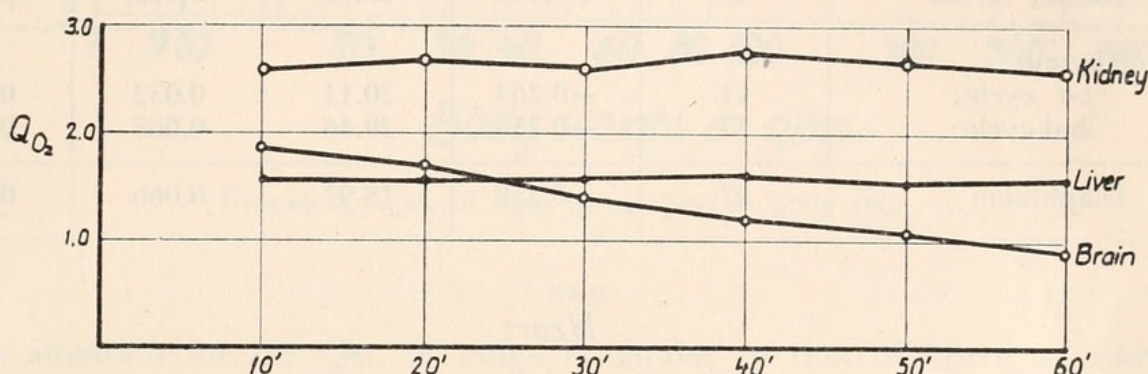

FIGURE 1. Qo_2 as a function of time. Typical experiments.

1951a). In the case of the surface rule, $\alpha = \frac{2}{3}$. The surface rule holds, although with qualifications, for intraspecific comparison in the rat (Bertalanffy, Mueller and Racine, unpublished); this is also stated by Kleiber (1947), according to Benedict's data (1938). Therefore,

$$M/W = bW^{-1/3} \tag{2}$$

applies for metabolic rate per unit weight. Thus, the exponent α should be $-.33$ in the case of the surface rule (or $-.25$ for the $\frac{3}{4}$ power rule). If, for example, the weights compared are $1:2:4:8:16$, metabolic rates per unit weight (a measure of which is Qo_2) should decrease in the ratio $1:.79:.63:.50:.4$, in the case of the surface rule. Differences of this order should be readily detected by the Warburg method.

The statistical evaluation of the data, and calculation of α, b, $S_{(\log y \cdot \log x)}$ (standard error), and ρ (coefficient of correlation) were made according to the method indicated by Brody (1945, pp. 398 ff.). In Figures 2–8, the central line gives the regression of Qo_2, and the two parallel lines give the standard error in per cent, including $\frac{2}{3}$ of the cases.

RESULTS

The results are indicated in Table I and Figures 2–8. As to the individual organs, the following remarks can be made.

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TABLE I

Statistical evaluation of the relation of Q_{O_2} to body size in organs of the rat

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Organ	N	α	b	$S_{(\log y \cdot \log x)}$	ρ
Heart	27	−0.050	10.83	0.102	0.229
Lungs	30	−0.085	10.33	0.073	0.501
Liver					
1st cycle:	30	−0.116	13.65	0.063	0.497
2nd cycle:	15	−0.018	8.59	0.074	0.107
Brain cortex	30	+0.047	6.89	0.064	0.263
Kidney cortex	43	+0.030	13.91	0.060	0.170
Thymus					
1st cycle:	11	−0.263	20.11	0.032	0.740
2nd cycle:	17	−0.253	29.46	0.068	0.514
Diaphragm	27	−0.258	18.92	0.066	0.929

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Heart

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The Q_{O_2} values are presented in Figure 2. There is a slight decrease with increasing body size, but the correlation coefficient is low, as can be seen from Table I.
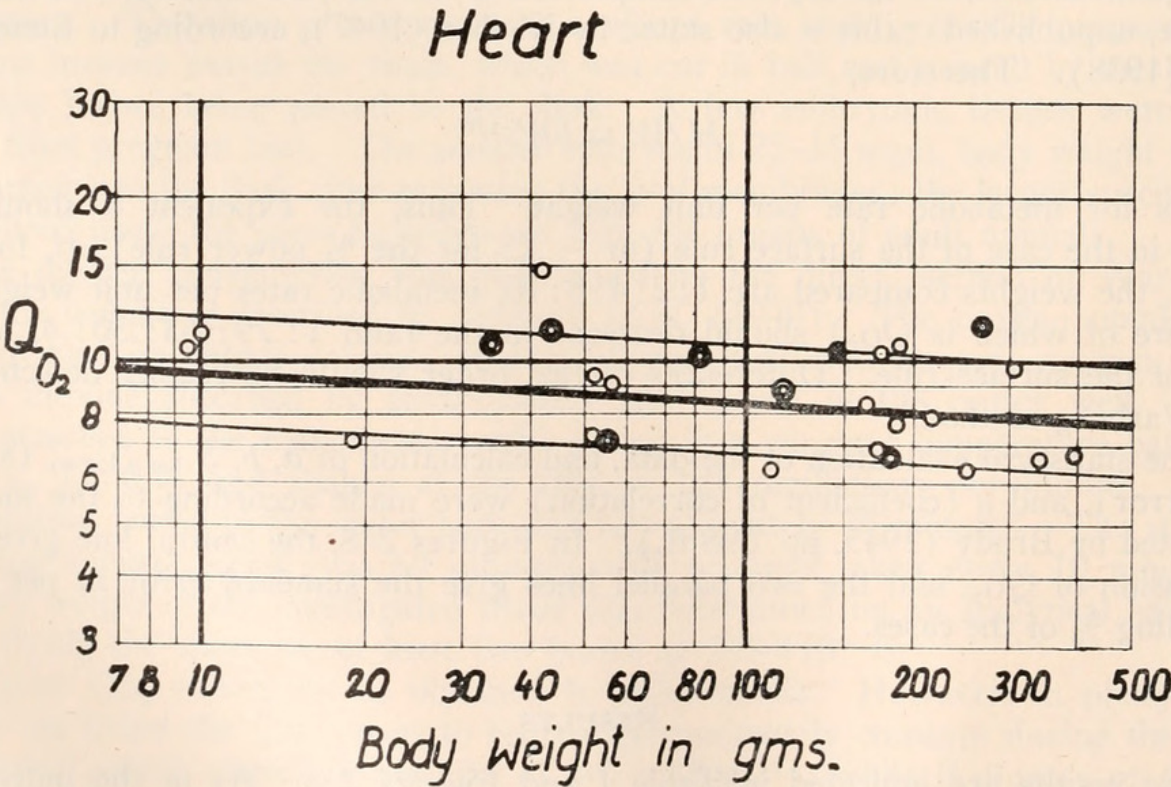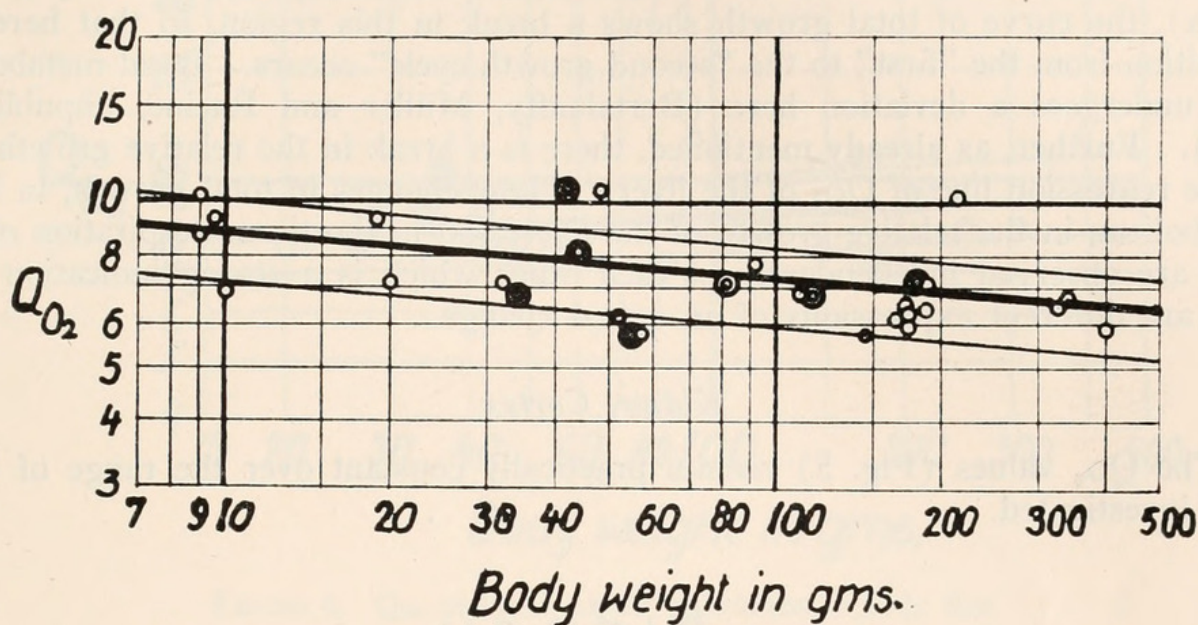

FIGURE 2. Q_{O_2} of heart in relation to body size. o = males, ◎ = females in Figures 2–8.

Lungs

FIGURE 3. Q_{O_2} of lung in relation to body size.

Lung

The situation for the Q_{O_2} of lungs is similar to that of heart, as shown in Figure 3.

Liver

It appears that a break can be assumed in the allometric line of the Q_{O_2}, somewhere in the region of 100 gms. body weight (Fig. 4). This break would correspond to a break in the curve of the relative growth of this organ (Bertalanffy and Pirozynski, 1952). It seems that at this body weight and in this time which

Liver
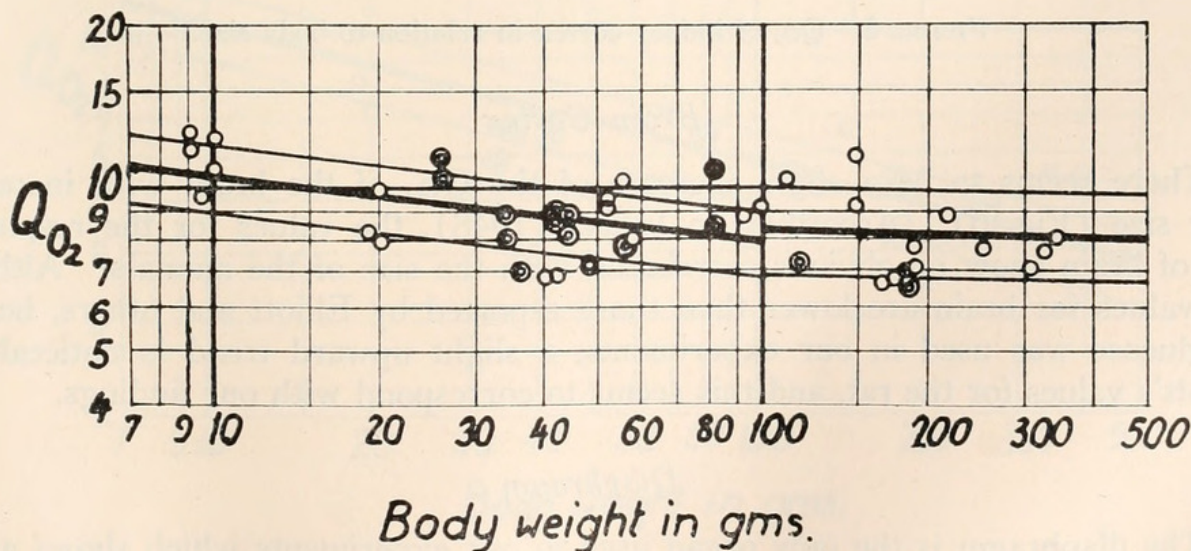
FIGURE 4. Q_{O_2} of liver in relation to body size.

corresponds to the start of puberty, a deep-reaching physiological change takes place which can be observed in different ways. According to Bertalanffy (1938, 1951a), the curve of total growth shows a break in this region, so that here the transition from the "first" to the "second growth cycle" occurs. Basal metabolism also undergoes a deviation here (Bertalanffy, Müller and Racine, unpublished data). Further, as already mentioned, there is a break in the relative growth and in the regression line of Q_{O_2} of the liver. These changes in total growth, in basal metabolism, in the relative growth of the liver, and in the tissue respiration of the liver are observed independently of each other which is a strong indication that they are different expressions of an actual change.

Kidney Cortex

The Q_{O_2} values (Fig. 5) remain practically constant over the range of body sizes investigated.
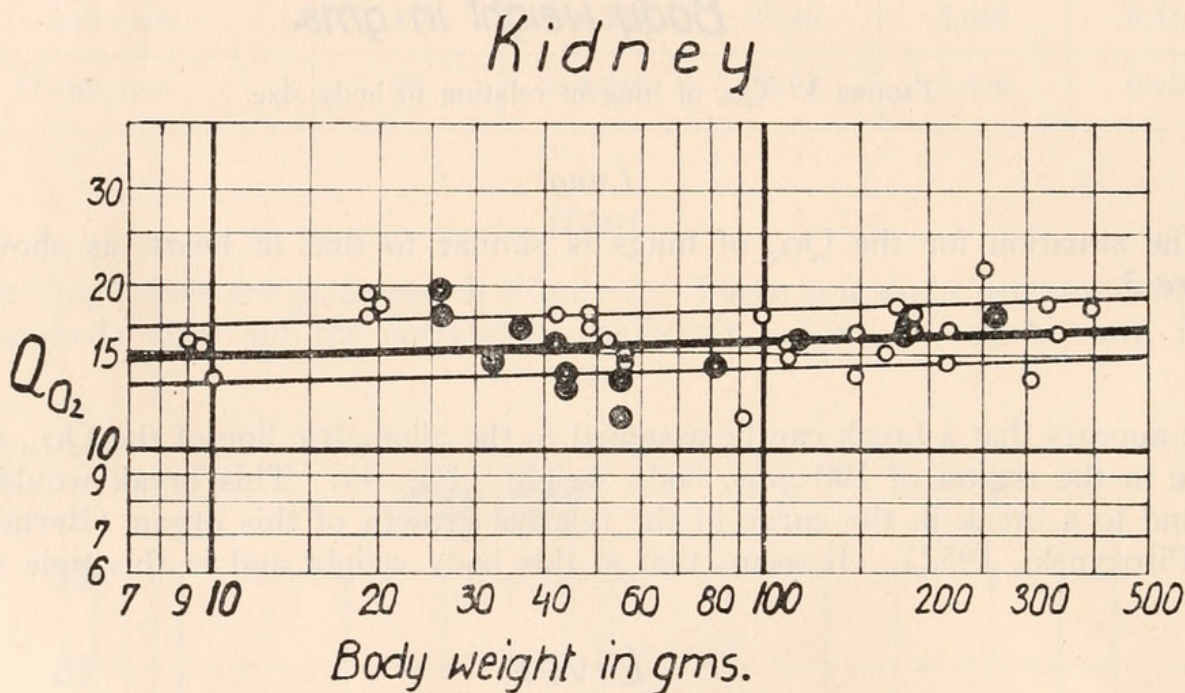

FIGURE 5. Q_{O_2} of kidney cortex in relation to body size.

Brain Cortex

There seems to be a slight increase of the Q_{O_2} of the brain with increasing body size (Fig. 6). According to Elliott (1948), the values for the respiration rate of brain show no obvious correlation with the size of the animals. Although our values for brain are lower than those reported by Elliott and others, because no glucose was used in our experiments, a slight upward trend is noticeable in Elliott's values for the rat, and this seems to correspond with our findings.

Diaphragm

The diaphragm is the only organ used in our experiments which shows a definite correlation of Q_{O_2} with body size, giving an exponent $\alpha = -.26$, and a high
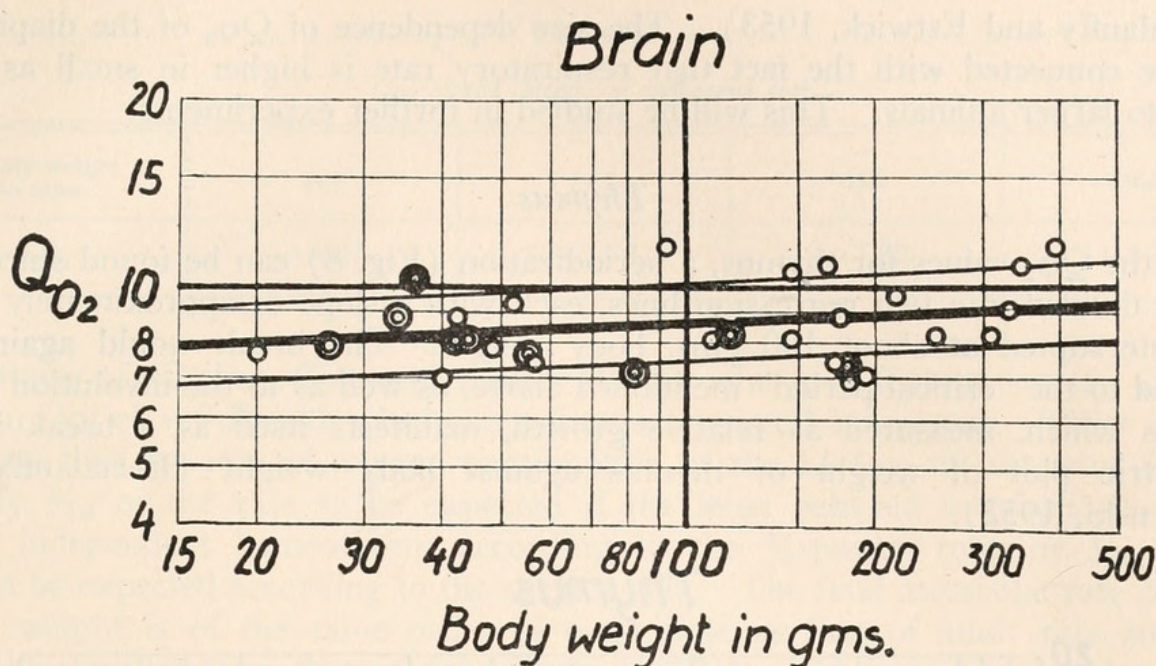
Brain

FIGURE 6. Q_{O_2} of brain cortex in relation to body size.

correlation coefficient, $\rho = .93$ (Fig. 7). It is to be noted that the diaphragm was not sliced as were the other tissues.

The marked decline of Q_{O_2} of the diaphragm with increasing body size may be connected with the continuous activity of this organ in respiration, since it serves oxygen uptake which, in total metabolic rate, shows a similar dependence on body weight. Also in interspecific comparison of adult rats and mice, there is a similar correlation of the Q_{O_2} of diaphragm to body size as is found intraspecifically
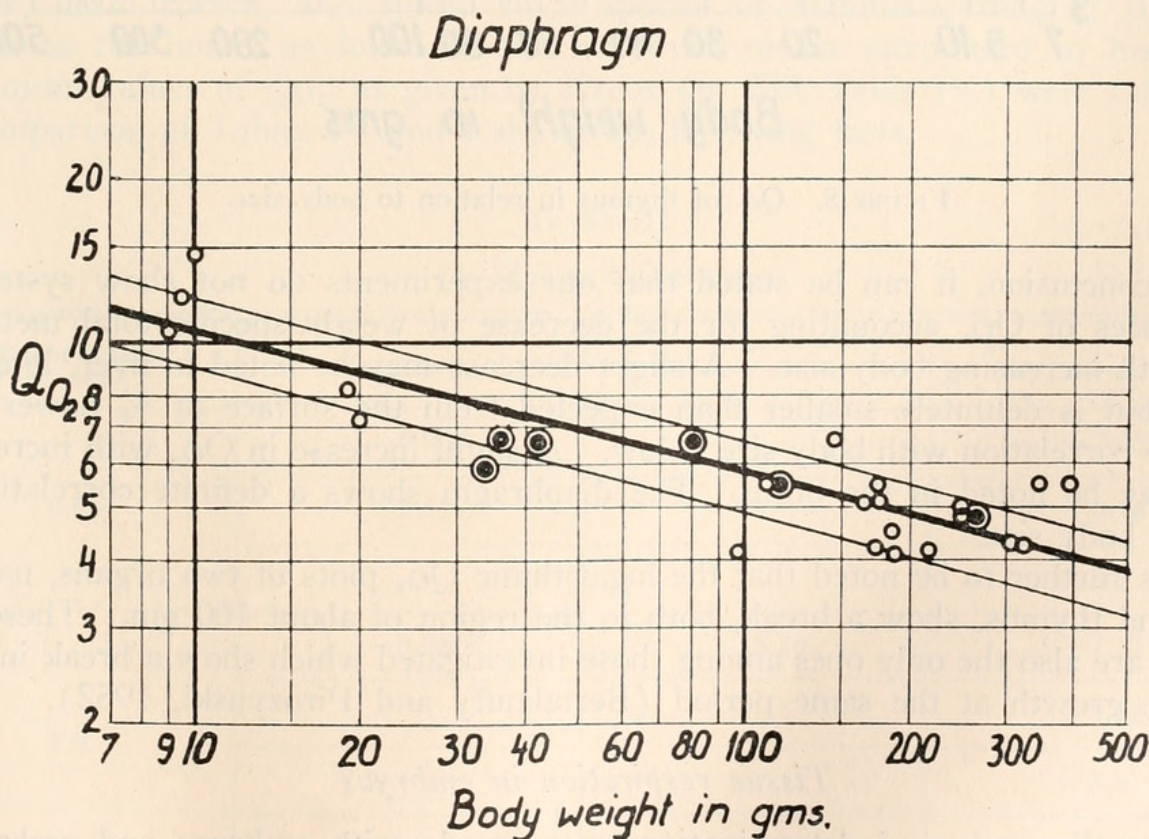
Diaphragm

FIGURE 7. Q_{O_2} of diaphragm in relation to body size.

(Bertalanffy and Estwick, 1953). The size dependence of Q_{O_2} of the diaphragm may be connected with the fact that respiratory rate is higher in small as compared to larger animals. This will be studied in further experiments.

Thymus

In the Q_{O_2} values for thymus, a periodization (Fig. 8) can be found since they can be divided into two regression lines, each with a slope of approximately $- \frac{1}{4}$, and interrupted at about 100 gms. body weight. The break would again correspond to the "critical period" mentioned above, as well as to the involution of the thymus which, measured as relative growth, manifests itself as a break in the allometric plot of weight of thymus against body weight (Bertalanffy and Pirozynski, 1952).
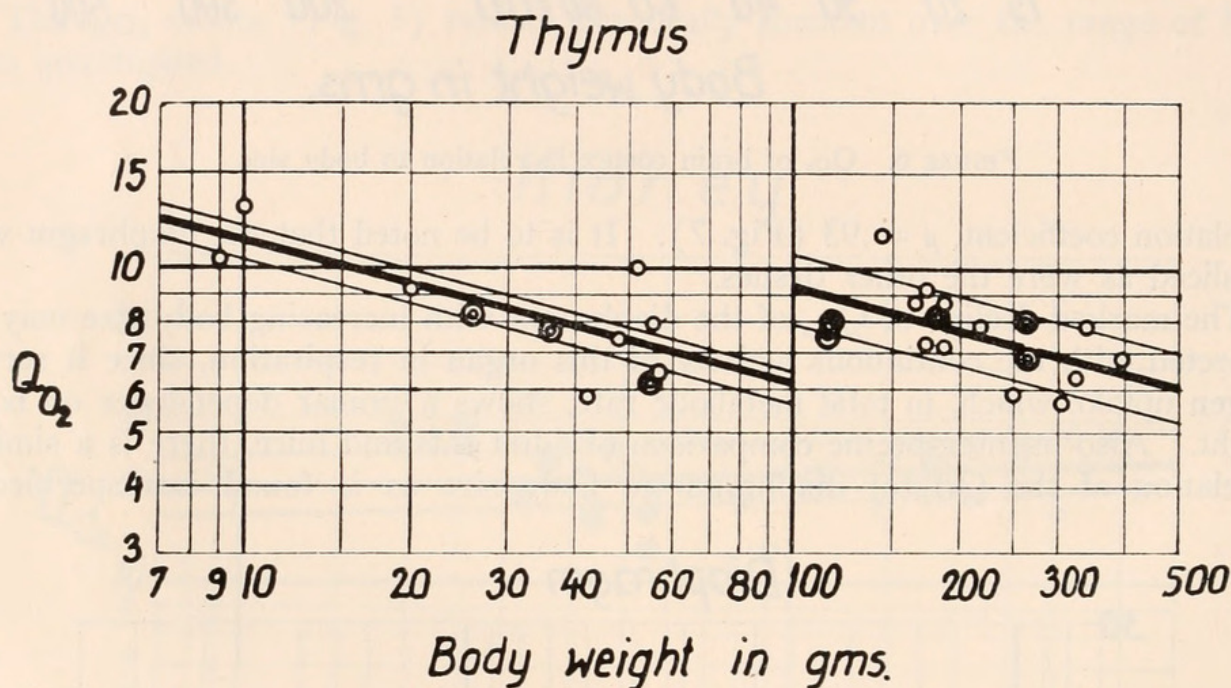

FIGURE 8. Q_{O_2} of thymus in relation to body size.

In conclusion, it can be stated that our experiments do not show systematic differences of Q_{O_2} accounting for the decrease of weight-specific total metabolic rate with increasing body size. A slight decrease may be noted in liver, lung and heart, but is definitely smaller than expected from the surface or $\frac{3}{4}$ power rule, and the correlation with body size is low. A slight increase in Q_{O_2} with increasing size may be noted in the brain. The diaphragm shows a definite correlation of Q_{O_2} to body size.

It is further to be noted that the logarithmic Q_{O_2} plots of two organs, namely, liver and thymus, show a break, both in the region of about 100 gm. These two organs are also the only ones among those investigated which show a break in their relative growth at the same period (Bertalanffy and Pirozynski, 1952).

Tissue respiration in embryos

A small number of determinations were made with embryos and embryonic tissues. Our values for naked fetuses are given in Table II. These results con-

TABLE II

Q_{O_2} of rat fetuses of different size

Body weight in mgm.	24.1	27.2	33.0	650.0
Q_{O_2}	−6.24	−7.38	−7.82	−6.12

firm those of Kleiber, Cole and Smith (1943) who give for rat fetuses (naked) a mean Q_{O_2} of − 7.2, with which our values are in good agreement. These authors estimate that the rate of oxygen consumption of the 13-day old rat fetus *in vitro* is only ⅒ of the rate to be expected if the fetus behaved metabolically like a small independent homeotherm according to the ¾-power rule, or 1/20 of the rate to be expected according to the surface rule. The fetal metabolic rate per unit moist weight is of the same order of magnitude as that of adult rats and considerably smaller than that of newly born to 12-day old rats. A few determinations of fetal organs are given in Table III. It appears that the Q_{O_2} of fetal liver is characteristically smaller than that of newborns.

DISCUSSION

1. Interspecific and intraspecific comparison

In order to compare intraspecific and interspecific size dependence of tissue metabolism, the data of Krebs (1950) were given a statistical treatment identical to that used for our own experiments. Krebs gives the Q_{O_2} for lung, liver, kidney cortex, brain cortex, and spleen in 9 species of mammals, ranging from the mouse to the horse, as found in the improved media introduced in his paper. The mean values of Q_{O_2}, as given by Krebs (p. 259, Table IV) were calculated. A comparison of Tables IV and I shows the following facts.

TABLE III

Q_{O_2} of some fetal organs

Weight of fetus in grams	Brain cortex	Kidney cortex	Liver
	−6.99		−7.45
0.65	−7.52		−9.18
			−8.8
	−8.08	−15.24	−7.13
			−9.38
2.9			−8.83
			−8.14

1) The decrease in Q_{O_2}, as measured by the exponent α, is, in all tissues studied by Krebs, considerably smaller than would correspond to the ¾-power rule (in the order of $\alpha = - .06$ to $- .14$, as compared to $- .25$, demanded by this rule). Therefore, interspecific comparison shows in the same way as does intraspecific comparison, that variations in tissue respiration, measured by the Warburg technique, cannot be the decisive factor for the decrease of weight-specific basal metabolic rate. Insofar as the brain is concerned, Krebs' determinations correspond well with those given by Elliott (1948). Elliott gives respiration per unit fresh weight in mice, rats, guinea pigs, rabbits, dogs, man and beef. He finds that respiration of cortex slices decreases inversely with body size, the exponent α being $- .1$. Since Elliott's regression line is drawn by estimate and without statistical calculation, this value is practically identical with the value obtained by statistical evaluation of Krebs' data $(- .07)$.

2) The interspecific and intraspecific α-values are partly similar (liver, lung), partly they are significantly different (brain, kidney). This is an expression of a fact that is found also in morphological phenomena (Bertalanffy and Pirozynski, 1952), namely, that intraspecific and interspecific allometry (a special case of which are the relations between metabolic phenomena and body size) need not necessarily be identical.

TABLE IV

Statistical evaluation of Krebs' data on the relation of Q_{O_2} to body size in nine mammalian species

Organ	α	b	$S_{(\log y \cdot \log x)}$	ρ
Brain cortex	−0.069	35.69	0.029	0.969
Kidney cortex	−0.064	43.79	0.057	0.882
Liver	−0.115	23.43	0.063	0.949
Spleen	−0.139	20.21	0.059	0.968
Lung	−0.095	11.18	0.098	0.846

3) The values of the integration constant b, which indicates the extrapolated value of Q_{O_2} for a weight $W = 1$ gm., are much higher in Krebs' than in our data. This is a consequence of the fact that the media applied by Krebs give higher absolute Q_{O_2} values than those obtained in Ringer-phosphate, as used in our experiments.

4) The values of the standard errors $S_{(\log y \cdot \log x)}$, as given for our own and Krebs' data, are not directly comparable. Table I gives the standard errors, calculated for our individual determinations, while Table IV is calculated, as a matter of convenience, for the mean values obtained from a larger number of determinations. Nevertheless, the comparison is rather favorable for our results because the standard errors for our individual determinations are not larger than those for the average values given by Krebs.

5) The correlation between body size and tissue metabolism, as expressed by ρ, is much higher in interspecific than in intraspecific comparison. In other words, the factor of body size plays a greater role in determining the rate of tissue metabolism within different species of different size, than it does in determining tissue metabolism of individuals of different size within the same species.

2. *The explanation of the relation between metabolism and body size*

Our results lead to a number of conclusions with respect to possible explanations of the rule that weight-specific metabolic rate decreases with increasing body size.

1) A first explanation is that this decrease, as expressed in the surface or ¾-power rule, is due to intracellular factors, *i.e.*, to a corresponding decrease of the Q_{O_2} of the tissues the sum of which is the organism. Krüger (1940) suggested that the surface rule is based upon the principle of chemical allometry (Needham, 1934), and possibly upon the decrease in concentration of respiratory enzymes with increasing body size. Such decrease is actually found with respect to some systems taking part in respiration. According to Rosenthal and Drabkin (1943), the cytochrome *c* concentration decreases in the series mouse, rat, rabbit, dog, pig, man, and horse, with the −.278 power of body weight, *i.e.*, approximately according to the ¾-power rule. A decrease in glutathione with increasing body size was found by Gregory and Goss (1933) and Patrušev (1937). A similar concept was advanced by Weymouth *et al.* (1944). The coincidence of the values for total and liver respiration in rats, rabbits, and sheep, and equally for total respiration (interspecific and intraspecific comparison) and respiration of the midgut gland in crustaceans, leads these authors to the conclusion that "the regressions of the weight-specific rates for the different tissues apparently form, in the log-log plot, a family of parallel lines, some high and some lower, corresponding to the intensity of respiration, but all showing the same slope as the regression of the weight-specific rate of the entire animal" (p. 68).

There are two objections to be made against this argument. As far as crustaceans are concerned, total metabolism in *Pugettia* follows, according to Weymouth *et al.* (1944), the ¾-power rule. Investigations with other crustaceans (isopods: Müller, 1943b; Bertalanffy, 1951b; *Daphnia:* Jančařik, 1948; *Artemia:* Bertalanffy and Krywienczyk, 1953) show, however, that here the surface rule applies.

With respect to the general viewpoint, our results show that the extrapolation to all organs from one organ investigated—the liver in the rat, the midgut gland in crabs—is not justified. Our results, obtained in intraspecific comparison, correspond in this respect with the interspecific comparison, as carried through by Krebs: The regressions of Q_{O_2} with respect to body size are different for the various organs.

2) Krebs (1950), not finding a systematic decrease of the Q_{O_2} values investigated which would correspond to the decrease of weight-specific total metabolic rate, concludes (p. 266) that "the characteristic differences in the basal rate of heat production in animals of different size are to be attributed mainly to variations in the Q_{O_2} of the musculature." Krebs did not investigate the Q_{O_2} of muscles in animals of different size. His conclusion is based upon the fact that the muscles play a leading part in thermoregulation which, according to the classical explanation given by Rubner, is at the basis of the surface law. Krebs offers this explanation for interspecific comparison of metabolic rates in the series of mammals, but naturally it could be applied also to intraspecific comparison.

Two viewpoints are to be distinguished in this hypothesis. *a*) Insofar as muscular activity is a means of thermoregulation, this cannot be the basic principle in the decline of weight-specific metabolic rate with increasing body size. For the

latter is a phenomenon in no way specific of, or limited to homeotherms, but uni-versal in most animal phyla (Bertalanffy, 1951b). The surface rule applies, even more unequivocally than in mammals, to cold-blooded vertebrates and many inverte-brate classes. It was found to apply in roundworms (*Ascaris:* Krüger, 1940) ; in certain molluscs (*Lamellibranchiata:* Weinland, 1919; Ludwig and Krywienczyk, 1950; *Prosobranchia:* Krywienczyk, 1952a) ; in crustaceans (*Branchiopoda:* Jančařik, 1948; Bertalanffy and Krywienczyk, 1953; *Isopoda:* Müller, 1943b; Will, 1952) ; in fish (Bertalanffy and Müller, 1943) and reptils (Kramer, 1934). A decline of metabolic rates corresponding to the ¾-power rule is found in turbel-larians (Bertalanffy and Müller, 1943) and in pond snails (*Limnaeidae:* Bertalanffy and Müller, 1943; Füsser and Krüger, 1951; Krywienczyk, 1952b). The only groups where no decline of weight-specific metabolic rates is found, *i.e.,* where total metabolism is directly proportional to weight, are land snails (*Helicidae:* Liebsch, 1929; Bertalanffy and Müller, 1943) and insects (Kittel, 1941; Bertalanffy and Müller, 1943; Müller, 1943a; Will, 1952)—possible due to pecularities of their respiratory mechanisms. Thus, homeothermy and muscular activity in its service is certainly not the basic principle in the size dependence of metabolic rate.

In mammals, the condition of thermoneutrality of environment, as applied in basal metabolism determination, amounts to minimize the energy expense for thermoregulation. The environmental temperature is so adjusted as to keep the body temperature normal without regulation, the heat arising as a by-product of the reactions in metabolism being sufficient to maintain body temperature. Only in conditions of non-thermoneutrality, muscular activity in the form of increased tension, shivering, etc. comes into play. This represents an excess superimposed over basal metabolic rate which is considerable indeed even in rather slight devia-tions from thermoneutrality, and which is measurable in appropriate experiments. This, however, does not concern basal respiration. Considering the fact that the decline of metabolic rate with increasing body size is a general phenomenon found also in animals without thermoregulation, it may be safely concluded that varia-tions in the Q_{O_2} of musculature due to thermoregulation are not the factor responsi-ble for the phenomenon in question.

b) It remains to be seen whether variations in the tissue respiration of skeletal muscle can account for the decline of weight-specific metabolic rate. Assuming a decrease of Q_{O_2} of musculature according to the surface or ¾-power rule, and considering the percentage of musculature in relation to total weight in small and large rats, according to Donaldson (1924, Table 124, p. 184), a simple estimate shows that the decline in muscular Q_{O_2} would not be sufficient to account for the decline in weight-specific basal metabolic rate of the entire animal.

The relation of Q_{O_2} of skeletal muscle to body size was recently studied by Bertalanffy and Estwick (1953). The Q_{O_2} of leg muscles of rats decreases, with increasing body size, with $\alpha = -.07$, *i.e.,* much less than would correspond to the surface or ¾-power rule. Interspecifically, the Q_{O_2} of skeletal muscle of adult mice (25–30 gm.) is similar to that of adult rats (over 300 gm.).

Thus there seems to be no indication that the decrease of weight-specific metabolic rate is to be explained by variations of the Q_{O_2} of musculature.

3) A third hypothesis often offered is that the mass of "metabolically active" organs decreases relatively with increasing body size. The high metabolism of small and young animals, as compared to the adult animals, would be based on the

fact that the inner organs which have a high oxygen consumption, are comparatively larger in the first (Kestner, 1934; Blank, 1934). Blank comes to the conclusion that the relatively larger size especially of the heart, kidney, intestinal tract and nervous system in smaller animals is responsible for their high oxygen consumption. However, already in 1942 (Bertalanffy, p. 198 ff.; 1951a, p. 249 ff.) we had shown by an analysis of the relative growth of the organs concerned that the latter is much too involved and too varying from one organ to the other, intraspecifically as well as interspecifically, that its combined effect is likely to result in a phenomenon so universal as the systematic decrease of weight-specific metabolic rate according to the simple power formula (on relative growth of inner organs of the rat; cf. Bertalanffy and Pirozynski, 1952).

A quantitative estimate may show that this factor cannot be the decisive one in the relative decrease in basal metabolism. As mentioned above, Field et al. (1939) have calculated summated tissue respiration for the mature rat. We have made a similar estimate for the 10-gm. rat, using Field's values for oxygen consumption per unit fresh weight for the individual organs, the wet weights of organs according to Donaldson (1924), and assuming a regression for Q_{O_2} of skeletal muscle, liver, lung, and heart as found in the present experiments. As calculated by Field et al., summated tissue respiration gives 110 cc. O_2/hr. for the 150-gm. rat. The calculation carried through as mentioned above, gives a summated tissue respiration of ca. 8.7 cc. O_2/hr. for the 10-gm. rat. Since the basal metabolic rate of an animal of 150 gm. is ca. 165 cc. O_2/hr., that of an animal of 10 gm. ca. 25 cc. O_2/hr., summated tissue respiration as based upon Q_{O_2}-measurements in saline, accounts for 66% of basal metabolism in the mature rat, but only for 35% in the 10-gm. rat. Although such estimate is, of course, crude and over-simplified, it shows that a) the different proportion of inner organs in small and large animals does not account for the differences in their basal metabolic rates; and b) that respiration in a young animal must be considerably higher than the values obtained in saline.

4) Another possible correlation is that between tissue respiration and age. According to Hawkins (1928) who studied liver slices of three groups of rats, aged 3–21 days, one year, and 22 months, respectively, and Pearce (1936) who used liver, heart and kidney of two groups (4–9 and 50–60 weeks) of mice, Q_{O_2} values decrease with age. Although our experiments show a decrease of Q_{O_2} with increasing body size in some organs, they do not show a definite relation between Q_{O_2} and age. Our determinations are not in contradiction with those of the authors mentioned. However, one would expect that the organs which show the most conspicuous histological changes (deposits of lipofuscins) and are particularly involved in the process of aging, namely, brain and heart, should also show definite changes in respiration with increasing age. This is not the case.

5) Our experiments seem, therefore, to contradict explanations of the decline in basal metabolic rate with increasing size which are based upon factors lying in the tissues themselves, and active, therefore, also in tissue respiration as observed with the Warburg technique. It appears that the decline in basal metabolic rate depends upon regulative factors lying in the organism as a whole. Many such factors can be taken into account: hormonal and neural regulators, supply of oxygen and metabolites especially of the Krebs cycle, etc. Variations in the energy expense for minimal functional activity (heart, lungs, kidney, etc.) are naturally also to be taken into account.

Our conclusion that respiration as determined *in vitro* does not necessarily correspond to respiration *in situ,* is derived from a study of normal animals and tissues. It may be mentioned that similar conclusions result from the study of malignant tissues. Potter (1951), investigating the Krebs cycle in tumors, states (p. 569) that "experiments with whole animals using fluoroacetate to block citrate oxidation *in situ* suggest that the enzyme activity of tissues *in situ* may be different than either the homogenate or slice would indicate." It may be hoped, therefore, that further study of the factors modifying tissue respiration can throw light on the problem of regulation of metabolism under conditions of normal as well as pathological growth.

Taking, as a starting point, the metabolic level in a young animal which perhaps roughly corresponds to the metabolic rate as observed in media containing all necessary metabolites, such as serum or the solutions indicated by Krebs (1950), it would seem that tissue respiration is damped in a larger animal, to a level roughly corresponding to that obtained in saline. If this is the case, it is suggestive to remark that also the growth of organs is limited by organismic factors. The "growth potency" of tissues is not limited by factors lying in those tissues themselves. This is shown by the fact that tissues which would not grow within the organism, do so if removed from the organism and cultivated *in vitro;* similarly, if the steady state and the "balance of organs" are disturbed, as in regeneration and compensatory hypertrophy; and finally under pathological conditions, in malignant growth. It may be that the metabolic activity of organs is bridled, as it were, within the organism, so that the systemic decline of weight-specific metabolic rate with increasing size takes place; that the same is true for their growth potency; and that both factors are connected. Investigation of these factors may perhaps lead into a deeper insight on growth, normal as well as malignant.

Summary

1. The relation between tissue respiration and body size was investigated in the rat. Determinations of Q_{O_2} were made on heart, lung, liver, kidney cortex, brain cortex, diaphragm, and thymus of animals ranging from 9 gm. to 392 gm. body weight, including some determinations on fetuses and fetal tissues. A statistical evaluation of *ca.* 230 experiments is given.

2. The diaphragm is the only organ investigated to show a definite and significant correlation between rate of tissue respiration and body size. Liver and thymus show a break in the regression line which corresponds to a number of other characteristic changes in metabolism and growth.

3. The experiments do not show systemic differences in tissue respiration accounting for the decrease of total metabolic rate with increasing body size.

4. A comparison between intraspecific and interspecific size-dependence of tissue metabolism is made.

5. The current theories on the systematic decrease of weight-specific metabolic rate, as expressed in the surface or ¾ power rule, are discussed in the light of the experiments presented. It is shown that none of the explanations proposed (decline of total metabolic rate as based upon decrease of the rate of tissue respiration, upon thermoregulation, upon decrease of Q_{O_2} of musculature, upon the relative decrease of "metabolically active" organs, upon age) in consistent. It appears

that the decline in basal metabolic rate depends on regulative factors lying in the organism as a whole.

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