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A COMPARATIVE STUDY OF FOUR MEANS OF EXPRESSING THE METABOLIC RATE OF RATS

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The oxygen consumption of rats is frequently used as an indication of heat production. Before we can draw conclusions regarding the effects of any procedure on the heat production of the animals it is necessary to eliminate differences arising from age, sex and weight. By using the animals as their own controls or by using control groups of the same age and sex differences due to these factors are removed. The procedure to be tested may, however, lead to changes in weight or in growth rate of the test animals. These changes may be sufficient to result in differences in weights of the test and control groups. Hence it is not often possible to follow the advice of Benedict (1938) to compare the metabolism of animals having the same weight. The usual practice has heen to refer the absolute oxygen consumption to some unit which supposedly corrects for body weight or size. While the situation has improved somewhat since Kleiber (1944) first expressed alarm, four reference units are still in general use. These are: body weight (kg), 'metabolic size' ($kg^{\frac{2}{3}}$) or ($kg^{\frac{3}{4}}$) and surface area (m^2 calculated from body weight). Since there can be only one correct way of expressing oxygen consumption and since conclusions often depend upon the assumption that the reference unit used is the proper one, it is surprising that no comparative study of the four means of expressing heat production has been made. This paper reports the results of such a study.

METHODS

Male albino rats were used for all experiments. They were kept in individual metal cages and fed on Purina Laboratory Chow and water *ad libitum.*

Oxygen consumption was recorded for 1 hr by a closed-circuit method previously described (Hsieh & Chiu, 1959). Six units were used, making possible twelve tests each morning. During the tests the air surrounding the animals was maintained at about 25° C.

In one series of experiments the ages of the rats were ignored. Ninety animals of different weights were obtained from the animal house and kept at 28° C for about 5 days. Oxygen consumption was determined after this period. This experiment was designed to imitate the usual procedure, in which rats of different ages and weights are used.

To test the effects of age on oxygen consumption and to obtain rats of the same age but with different weights the following experiments were performed. Thirty-three rats about 7 weeks old were divided into three groups of eleven each. One group (*WN*) was kept at

28° C. The second group (*WT*) was kept at 28° C and deprived of both food and water one day a week. The third group (*C*) was kept at about 6° C.

To produce short-term changes in weight a group of six rats was deprived of both food and water for 2 days. Another group of six rats was given a single subcutaneous injection of 0·5 mg reserpine (Serpasil, Ciba). Rats of these groups were maintained at 28° C.

Surface area was calculated from body weight (g) by the formula: Surface area (m^2) = 0·0011 ($g^{0\cdot63}$) (Kibler & Brody, 1942). For convenience we have expressed surface area in units of 0·1 m^2.

RESULTS

The absolute rates of oxygen consumption of ninety rats are plotted against their body weights in Fig. 1. If we assume linear regression, basing absolute oxygen consumption on surface area yielded the highest correlation coefficient (Table 1). However, the line best fitting the results is of the type: $Y = a + bX + cX^2$ (second degree polynomial), where X represents body weight in kg. The correlation coefficient of this line is significantly higher than that of the others. When the oxygen consumption of rats weighing over 200 g is expressed in terms of body weight, 'metabolic size' or surface area there is a significant negative regression with body weight (Fig. 2). The average hourly oxygen consumption of the rats weighing over 200 g is shown in Table 2.

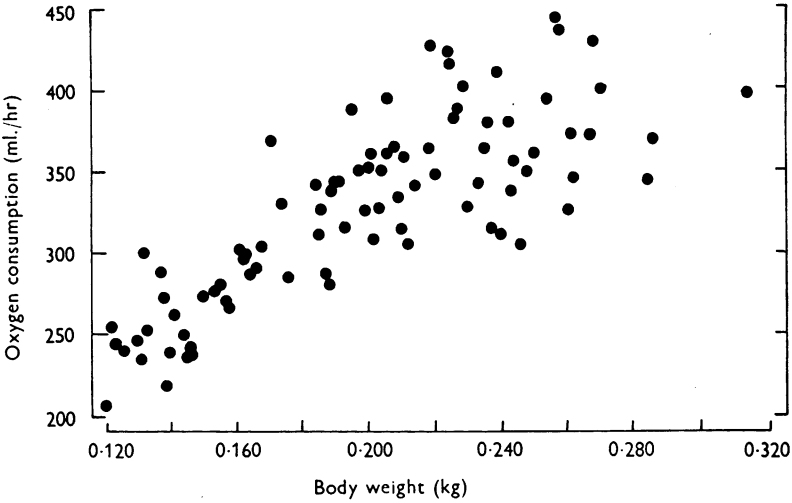

Fig. 1. Oxygen consumption of ninety rats plotted against their body weights. The rats were kept at about 28° C and oxygen consumption measured for 1 hr at about 25° C.

The comparative rates of growth of the *WN*, *WT* and *C* rats are shown in Fig. 3. The rats which fasted recovered their weights on the day after the fast and then grew at about the same rate as the group which did not fast. Thus the *WT* group lost about 2 days of growth per week and at the age of 19 weeks, after 11 days of weekly fasting, they weighed the same as the *WN* rats at 15 weeks of age. At 19 weeks the group kept at 6° C weighed the same as the *WN* group at 12 weeks of age.

The change in metabolic rate with age of the *WN* group is shown in Fig. 4. There was a gradual decrease in metabolic rate with increasing age. A statistical analysis of the data indicates, however, that the change

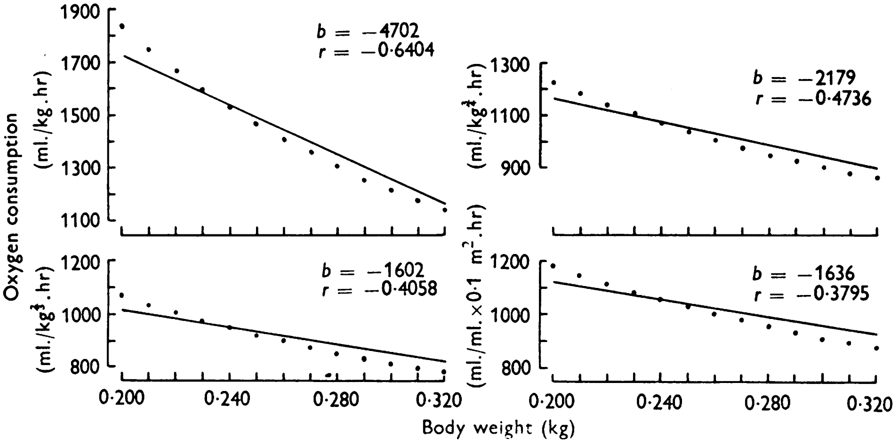

Fig. 2. Regression of metabolic rates, expressed in various units, of forty-six rats. To simplify calculations linear regression was assumed. The negative slopes, *b*, are all significant at the 2 % level. The dots indicate rates calculated by assuming a fixed consumption of 368 ml./hr. Expressing the oxygen consumption of rats weighing more than 200 g in terms of their body weight, 'metabolic size' or surface area does not correct for differences in body weight.

TABLE 1. Regression equations of oxygen consumption of rats on their body weights (kg), 'metabolic sizes' (kg¾ and kg⅔) and surface areas (0·1 m²)

Reference unit (X)	Experimental range of X	Regression equation Y = O_2 consumption (ml./hr)	S.E. of estimate	Correlation coefficient
kg	0·120–0·314	$Y = 3341X - 5899X^2 - 92{\cdot}4$	30·8	0·8344
kg	0·120–0·314	$Y = 133 + 972{\cdot}4X$	33·3	0·8001
kg¾	0·204–0·419	$Y = 68{\cdot}4 + 872{\cdot}9X$	32·8	0·8076
kg⅔	0·243–0·462	$Y = 36{\cdot}4 + 856{\cdot}8X$	32·7	0·8084
0·1m²	0·225–0·413	$Y = 20{\cdot}1 + 999{\cdot}1X$	32·6	0·8103
	$n = 90$			

TABLE 2. The oxygen consumption of a group of forty-six rats with average weight 236 g (S.D. 25·7 g)

Hourly rate of oxygen consumption	S.E.	Coefficient of variation (%)
367·7 ml.	4·94	9·2
1558 ml./kg	27·8	12·1
1082 ml./kg¾	17·4	10·9
958·3 ml./kg⅔	15·0	10·6
1064 ml./0·1 m²	16·4	10·4

is due to increasing weight and not to age. This fact is evident from inspection of Fig. 5.

The relative rates of increase in body weight and absolute oxygen consumption of the *WN*, *WT* and C rats are shown in Fig. 6. To enable comparison these are expressed as percentages, with body weight and oxygen consumption at 7 weeks taken as 100%. The relative rates of increase in body weight and oxygen consumption are different in the different groups.

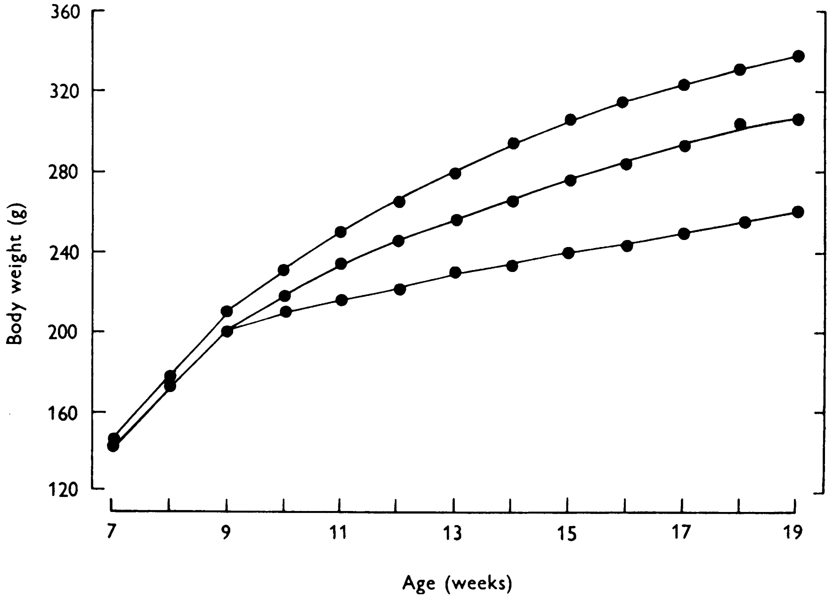

Fig. 3. The growth rates of rats from 7 to 19 weeks of age. Upper curve, rats kept at 28° C (*WN*). Middle curve, rats kept at 28° C and deprived of food for 1 day a week beginning with the eighth week (*WT*). Lower curve, rats kept at 6° C (*C*). Each group consisted of eleven rats.

The oxygen consumption of the C rats unfortunately was not determined between the eighth and thirteenth week. The results for the last 5 weeks of the experiment suggest that the absolute oxygen consumption of this group may have increased at a more rapid rate than those of the other two groups. At 19 weeks of age no significant difference existed in the oxygen consumption of the three groups. There was a difference in their metabolic rates (Table 3). However, the mean metabolic rates of the three groups, adjusted for differences in weight, were not significantly different.

The effects of a two-day fast and of reserpine on the body weight and oxygen consumption are shown in Figs. 7 and 8. On the second day of the fast body weights had fallen by about 39 g and oxygen consumption by about 89 ml./hr. These represent reductions of about 18 and 26 %, respectively. The reduction in metabolic rates depends on the unit used, being about 11 % when based on body weight and 17 % when based on surface area. The injection of reserpine was followed by a reduction in oxygen consumption which persisted for about 3 days. On the first day after the injection of reserpine there were small, but significant, increases in metabolic rates.

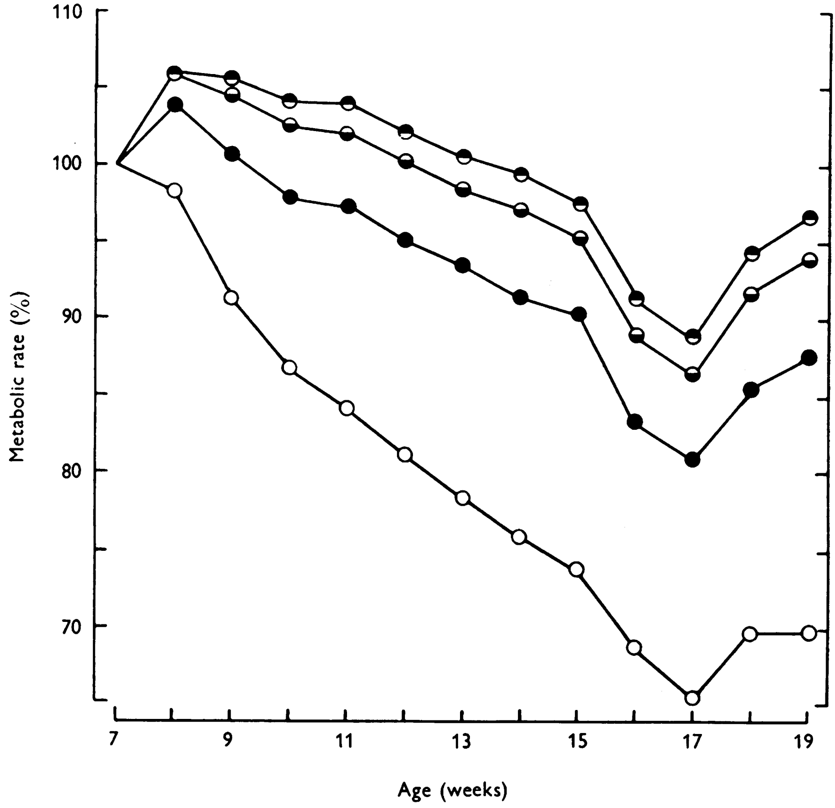

Fig. 4. Metabolic rates of *WN* rats, expressed as percentages, plotted against their ages in weeks. The metabolic rate at 7 weeks was taken as 100 %. ○, rate in ml./kg.hr; ●, rate in ml./$kg^{3/4}$.hr; ◒, rate in ml./$kg^{2/3}$.hr; ◓, rate in ml./0·1 m^2.hr.

DISCUSSION

The term 'metabolic rate' is poorly defined. Kleiber (1956) uses it to mean either the absolute rate of heat production or the rate of heat production per unit of body size. We feel that a distinction between these two meanings is necessary. In experiments on fasting rats, Rixon & Stevenson (1957) observed that metabolic rate based on absolute weight showed better correlation to weight loss during fasting than when based on $\mathrm{kg}^{\frac{3}{4}}$ units or on surface area. However, the linear regression of pre-starvation metabolic rate on the rate of weight loss during fasting was obtained by plotting oxygen consumption, in ml./min/100 g pre-fasting body weight, against loss of weight in g/day/100 g pre-fasting body weight. Hence the absolute rate of oxygen consumption was plotted against the absolute rate of weight loss. These authors also obtained a linear regression when they plotted the ratio, total weight loss (g/100 g pre-fasting weight): pre-fasting oxygen consumption (ml./100 g pre-fasting weight), against survival time. Here again absolute values were employed. Indeed, if $\mathrm{kg}^{\frac{3}{4}}$ units, or any other unit, had been used to express both rates of oxygen consumption and weight loss similar results would have been obtained. These facts are pointed out not to criticize the conclusions of Rixon & Stevenson (1957) but to show that the free use of the term 'metabolic rate' can be misleading. In the present discussion 'oxygen consumption' is used to indicate the rate of oxygen consumption per rat and 'metabolic rate' is limited to those observations in which the absolute rates have been based on some unit of size.

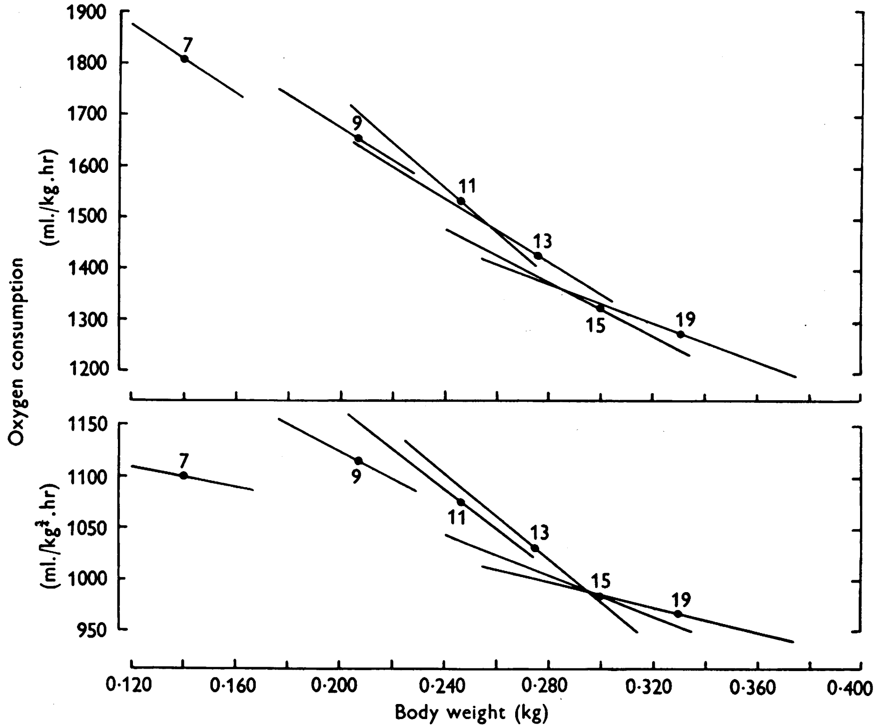

Fig. 5. The regression of metabolic rate on body weight of *WN* rats. Upper, rate in ml./kg.hr; lower, rate in ml./$\mathrm{kg}^{\frac{3}{4}}$.hr. The dots represent mean values and the nearby numbers the ages in weeks of the rats.

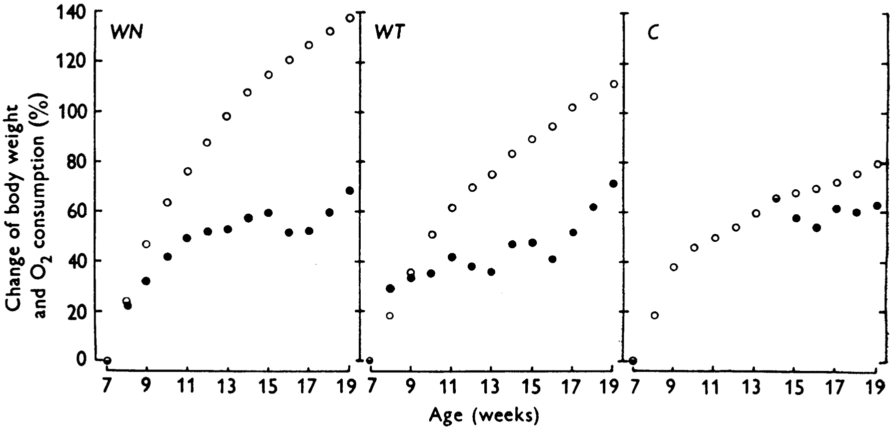

Fig. 6. The relative rates of increase in body weight and oxygen consumption of *WN*, *WT* and *C* rats. Body weight and oxygen consumption at 7 weeks of age are taken as 100%. ○, body weight; ●, oxygen consumption.

TABLE 3. Oxygen consumption of warm-adapted rats (*WN*, *WT*) and cold-adapted rats (*C*) 19 weeks of age

Group	No.	Average weight (g)	Average hourly oxygen consumption ml.	Average hourly oxygen consumption ml./kg
WN	11	346 ± 4·8 %	434 ± 7·7 %	1254 ± 6·3 %
		$P < 0.001$	N.S.	$P < 0.001$
WT	11	299 ± 7·2 %	428 ± 6·0 %	1436 ± 7·2 %
		$P < 0.001$	N.S.	$P < 0.01$
C	11	260 ± 5·7 %	412 ± 6·6 %	1580 ± 6·0 %
WN + *WT*	22	322 ± 9·5 %	431 ± 6·8 %	1345 ± 9·6 %
WN + *WT* + *C*	33	301 ± 13·1 %	425 ± 7·1 %	1423 ± 11·4 %

		Average hourly oxygen consumption ml./$kg^{3/4}$	Average hourly oxygen consumption ml./$kg^{2/3}$	Average hourly oxygen consumption ml./0·1 m^2
WN	11	962 ± 6·3 %	880 ± 6·4 %	992 ± 6·4 %
		$P < 0.01$	$P < 0.01$	$P < 0.01$
WT	11	1063 ± 6·0 %	960 ± 5·8 %	1074 ± 5·8 %
		$P < 0.05$	$P < 0.05$	$P < 0.05$
C	11	1130 ± 5·6 %	1040 ± 5·5 %	1130 ± 5·5 %
WN + *WT*	22	1012 ± 7·9 %	920 ± 7·4 %	1033 ± 7·2 %
WN + *WT* + *C*	33	1051 ± 8·8 %	960 ± 8·2 %	1065 ± 7·9 %

± = coefficient of variation

Horst, Mendel & Benedict (1934) noted that the metabolic rates of their rats were not in accord with an earlier study. As the weights of the two groups of rats differed widely these authors suggested that the lack of agreement was due to the fact that heat production expressed per unit of weight or per unit of surface area did not rule out the difference in body weight. Our results clearly show that there is a negative regression of metabolic rate on body weight (Figs. 2 and 5). Therefore, when comparing the metabolic rates of groups of animals with different average body weights, it is necessary to perform an analysis of co-variance and to seek differences in the adjusted mean metabolic rates. If these steps are not taken, conclusions will depend upon the degree of difference in body weight and on the reference unit used, since the regression coefficients are different for the different reference units. Benedict & Sherman (1937) have pointed out that the metabolic rate of old rats is higher than middle-aged ones when based on body weight but not when based on surface area. Black & Murlin (1938) observed that the metabolic rate of rats falls gradually

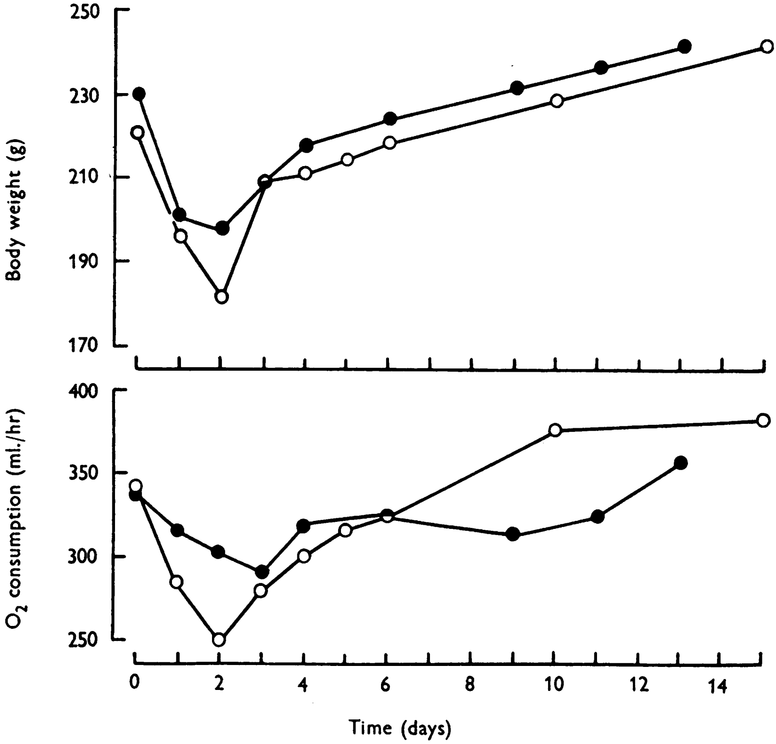

Fig. 7. The effects of a two-day fast, ○, and of a single subcutaneous injection of 0·5 mg reserpine, ●, on the body weight and oxygen consumption of rats. Each point represents the average from six rats.

during the first 6 months of life. Figure 4 shows that our results indicate the same relationship. If it is assumed that corrections for changes due to weight have been made by using metabolic rate then one may conclude that the gradual reduction of metabolic rate is due to age. But age does not affect the metabolic rate (Fig. 5). Another example of erroneous conclusions arising from the assumption that metabolic rates correct for weight is shown in Table 3. The metabolic rates of the moderately fasted group (*WT*) are significantly higher than those of the normal controls (*WN*). In the present experiments one might conclude that this was an effect of moderate fasting. Without knowing that the *WT* group had been deprived of food one might conclude, of course, that the rats in the *WT* group were thinner because of their higher metabolic rate. There is, how-

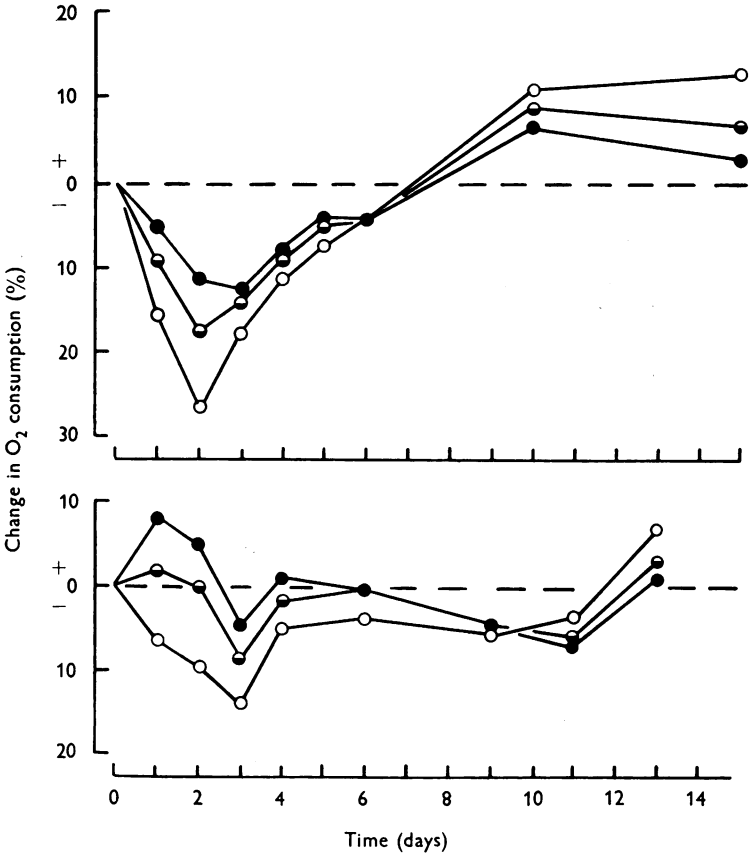

Fig. 8. Upper, change in oxygen consumption of rats deprived of food for 2 days; lower, after receiving a single subcutaneous injection of 0·5 mg reserpine. Oxygen consumption is expressed in ml./hr, ○; ml./kg.hr, ●; ml./0·1 m^2.hr, ◓. Each point represents the average from six rats.

ever, no difference in the metabolic rates of the two groups when these are adjusted for body weight.

One of the reasons for basing oxygen consumption on body size is the desire to reduce the coefficient of variation of the results. When the range of body weights of the animals is small not much difference is found between the coefficients of variation of oxygen consumption and metabolic rates. However, when the range of body weights is large, expressing oxygen consumption in terms of a unit of size increases the coefficient of variation (Table 2 and 3). From this it follows that if an analysis of co-variance is not performed on the results, comparing the absolute oxygen consumption of rats of the same age but of different weights will introduce less error.

Another reason advanced for using metabolic rates is the desire to predict absolute heat production from body size (Kleiber, 1947). Kleiber (1956) offers the formula: $M = 3$(body weight in $\text{kg}^{\frac{3}{4}}$); where M is the heat production in kcal/hr. This formula is only an approximation and Kleiber suggests that $M = k$(body weight in $\text{kg}^{\frac{3}{4}}$) be used for more accurate work. Since k has to be calculated from the absolute heat production and is different for different body weights we fail to see the usefulness of this formula.

Kleiber (1947) points out that considerations of heat transfer do not support the hypothesis that the heat production of homoeotherms should be strictly proportional to surface area. He does not say in what way these considerations support his hypothesis that heat production should be proportional to the body weight in $\text{kg}^{\frac{3}{4}}$ units. The $\text{kg}^{\frac{3}{4}}$ unit was chosen in order to make the metabolic rates of homoeotherms, from mice to elephants and whales, approximately the same. In studies on temperature regulation the reference of heat loss to surface area is theoretically correct. It should be noted, however, that the surface area of rats, and of other laboratory animals, is really an expression of weight, since it is calculated from body weight and the constant is merely for the sake of units.

The oxygen consumption of an animal at rest and at its 'critical' environmental temperature increases with age, just as the actual body weight increases with age, but the relative rates of increase of the two are different (Fig. 6). This can be explained by assuming that the oxygen consumption depends upon the 'activity' and amount of metabolically active tissue while the actual body weight depends upon the amount of metabolically active tissue plus inert material (e.g. fat and water). It is conceivable that during different stages of development the 'activity' and the rate of production of metabolic material are different. In addition, the rate of production of inert material may also vary. These considerations make it illogical to expect a constant relationship between oxygen consumption and body weight, or any power of body weight, throughout the life of an animal. The relative rates of increase of oxygen consumption and of total body weight may also depend upon environmental (temperature) conditions and the nutritional (quantity and quality of food available) and hormonal status of the animal. For these reasons one cannot expect the ratio oxygen consumption: body size to be the same in normal animals and, say, in animals kept in a cold environment. The proper unit for comparing the metabolic activity of the tissues of animals is the ratio oxygen consumption: size of tissues involved. The use of lean body mass as an indication of metabolic size has been recommended (Keys & Brožek, 1953). But the estimation of lean body mass of rats is often not practical, as all procedures, with the exception of the one proposed by Lesser, Blumberg & Steele (1952), involve the death of the animals. With small animals the use of specific gravity as an indication of fat content results in large errors of determination which cancel the theoretical advantages from the use of this unit. Da Costa & Clayton (1950) found good correlation ($r = -0{\cdot}63$) between body density and fat content in rats. However, Keys & Brožek (1953) have pointed out that the correlation coefficient was not high enough to permit precise individual predictions. The calculation of body fat content from specific gravity data is based on the principle of Archimedes and requires prior knowledge of the densities of the 'fat' and the 'lean' portions of the animal. At present the conversion of specific gravity to percentage of fat is based almost entirely on data obtained from the guinea-pig (Rathbun & Pace, 1945; Pace & Rathbun, 1945).

Our results suggest that it is safe to assume that mature rats of the same age have the same amounts of metabolically active tissue. Hence it is possible simply to use oxygen consumption as a direct indication of tissue activity. The results, however, hold only for rats that weigh between 250 and 350 g at 19 weeks of age. With greater discrepancies in mean body weights between two groups direct comparison may lead to errors and should not be undertaken. In such cases it is necessary to use both absolute oxygen consumption and oxygen consumption adjusted for weight for comparisons, assuming a specific action on energy metabolism to have occurred when both have changes in the same direction. The basis for this type of comparison is given by Heroux & Gridgeman (1958).

In experiments of a few days duration and where the animals are used as their own controls the problem of determining the effects of a procedure on energy metabolism is simpler. Here it can be assumed that changes with time in the amount of metabolically active tissue are small; thus any alteration in oxygen consumption may be ascribed to changes in the activity of the cells. Carr & Beck (1937), basing heat production on body weight, noted that there was no change in the metabolic rate of rats which fasted for 48 hr. Our results are not comparable since the starved rats of the present experiments suffered both inanition and dehydration. However, the use of absolute oxygen consumption as an indication of tissue activity suggests that the effects of fasting are greater than have been assumed.

The results obtained 24 and 48 hr after the injection of reserpine suggest that this drug depresses the heat production of rats. A single large dose of reserpine in rats results in diarrhoea and marked loss of weight for about 2 days. This may be due to increased peristaltic activity (Bülbring & Crema, 1959). Hoffman (1958), basing oxygen consumption on body weight, obtained a 25–30 % increase in metabolic rate of rats 1–6 hr after the injection of reserpine. Only part of this increase can be ascribed to a decrease in weight of the animals. It would seem therefore that the short-term effects of reserpine lead to an increase in heat production, possibly due to vasodilatation and increased heat loss.

SUMMARY

1. A comparative study has been made of four means of expressing the metabolic rate of rats by determining their absolute oxygen consumptions and basing results on body weight (kg), 'metabolic size' ($kg^{\frac{2}{3}}$ and $kg^{\frac{3}{4}}$) and surface area (m^2) calculated from body weight.

2. Expressing the oxygen consumption of rats weighing more than 200 g in the above terms does not correct for differences that are caused by differences in body weight.

3. There is a gradual fall in metabolic rate of rats with increasing age from 7 to 19 weeks. This is due to increasing weight of the animals.

4. After 11 weeks of moderate fasting (removal of food and water one day each week) rats have higher metabolic rates than normal controls. This is due to the lower body weights of the fasted group.

5. Rats kept in a cold environment (6° C) have a higher metabolic rate than controls kept at 28° C. This is due to the lower body weights of the former group.

6. The shortcomings of the term 'metabolic rate' are discussed and reasons advanced why one cannot expect a constant relationship between oxygen consumption and body weight or any power of body weight.

7. It is suggested that, where discrepancies in the body weights of two groups are small, comparing absolute oxygen consumption will yield more accurate results. Where the difference in weight between two groups is greater than 100 g (the limits of the present experiments) it will be necessary to compare both absolute oxygen consumption and oxygen consumption adjusted for weight, regarding a specific action on energy metabolism as having taken place when both have changed in the same direction.

8. By the above method it is concluded that acute fasting (removal of food and water for 48 hr) has a greater depressing effect on energy metabolism than can be presumed from the calculated metabolic rate. This method also indicates that reserpine has a depressing effect on heat production.

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REFERENCES

BENEDICT, F. G. (1938). *Vital Energetics*, pp. 39–56. Washington: Carnegie Institute.

BENEDICT, F. G. & SHERMAN, H. C. (1937). Basal metabolism of rats in relation to old age and exercise during old age. *J. Nutr.* **14**, 179–198.

BLACK, A. & MURLIN, J. R. (1938). Total energy of rats at various ages as affected by protein and exercise. *J. Nut*. **15**, 13.

BÜLBRING, E. & CREMA, A. (1959). The action of 5-hydroxytryptamine, 5-hydroxytryptophan and reserpine on intestinal peristalsis in anaesthetized guinea-pigs. *J. Physiol.* **146**, 29–53.

CARR, C. J. & BECK, F. F. (1937). The metabolism of adrenalectomized rats. *Amer. J. Physiol.* **119**, 589–592.

DA COSTA, E. & CLAYTON, R. (1950). Studies of dietary restriction and rehabilitation. II. Interrelationships among the fat, water content and specific gravity of the total carcass of the albino rat. *J. Nutr.* **41**, 597–606.

HEROUX, O. & GRIDGEMAN, N. T. (1958). The effect of cold acclimation on the size of organs and tissues of the rat, with special reference to modes of expression of results. *Canad. J. Biochem. Physiol.* **36**, 209–216.

HOFFMAN, R. A. (1958). Temperature response of the rat to action and interaction of chlorpromazine, reserpine and serotonin. *Amer. J. Physiol.* **195**, 755–758.

HORST, K., MENDEL, L. B. & BENEDICT, F. G. (1934). The influence of previous diet, growth and age upon the basal metabolism of the rat. *J. Nutr.* **8**, 139–162.

HSIEH, A. C. L. & CHIU, C. C. (1959). The effects of sodium salicylate on the oxygen consumption of rats. *Brit. J. Pharmacol.* **14**, 219–221.

KEYS, A. & BROŽEK, J. (1953). Body fat in adult man. *Physiol. Rev.* **33**, 245–325.

KIBLER, H. H. & BRODY, S. (1942). Metabolism and growth rate of rats. *J. Nutr.* **24**, 461–468.

KLEIBER, M. (1944). Energy metabolism. *Annu. Rev. Physiol.* **6**, 123–154.

KLEIBER, M. (1947). Body size and metabolic rate. *Physiol Rev.* **27**, 511–541.

KLEIBER, M. (1956). Energy metabolism. *Annu. Rev. Physiol.* **18**, 35–52.

LESSER, G. T., BLUMBERG, A. G. & STEELE, J. M. (1952). Measurement of total body fat in living rats by absorption of cyclopropane. *Amer. J. Physiol.* **169**, 545–553.

PACE, N. & RATHBUN, E. N. (1945). Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. *J. Biol. Chem.* **158**, 685–691.

RATHBUN, E. N. & PACE, N. (1945). Studies on body composition. I. The determination of total body fat by means of the body specific gravity. *J. Biol. Chem.* **158**, 667–676.

RIXON, R. H. & STEVENSON, J. A. F. (1957). Factors influencing survival of rats in fasting, metabolic rate and body weight loss. *Amer. J. Physiol.* **188**, 332–336.