The remarkable survival of the respiratory mechanism in young animals is obviously not unique; other physiological processes survive similarly.

In early age groups the survival of trunk reflexes is about two-thirds that of gasping; in the older age groups there is no clear difference. The survival of pupillary responses in the 7-day-old rat is about 3 times that of the adult. Heart action for all species studied is approximately 7 times longer in 1-day-old animals than in adults.

13340

Body Size and Metabolism of Liver Slices *in vitro*.

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Grafe, Reinwein and Singer[1] concluded from micro respiration trials that the metabolic rate of animal tissues *in vivo* was restricted by a central regulator to a fraction of the rate of oxidation of the tissues *in vitro*. The summated *in vitro* metabolism† of the rat according to Grafe is twice as great as the metabolic rate *in vivo* and the tissues of a steer respire *in vitro* 20 times as fast as they do in the living animal. Based on similar work Terroine and Roche[2] derived their rule: The metabolic rate *in vitro* per unit weight of homologous tissues of large and small animals is the same even though the metabolic rate per unit weight of the tissues in the living animals decreases systematically with increasing body size.

In contrast to Grafe's conclusion, Field, Belding and Martin[3] demonstrated that the summated metabolic rate of rat tissues *in vitro* amounted to 89% of the basal metabolic rate of the living rats. Since the ratio of *in vitro* to *in vivo* metabolic rate according to

* The author acknowledges the help of several colleagues at Davis, especially his assistant, Arthur H. Smith.

† Summated *in vitro* metabolism = metabolic rate per unit weight *in vitro* multiplied by the weight of each tissue in the living animal and summated over all its tissues.

1 Grafe, E., Reinwein, H., and Singer, *Biochem. Z.*, 1925, **165**, 102.

2 Terroine, E. F., and Roche, J., *Compt. Rend. Acad. Sci.*, 1925, **180**, 225.

3 Field, J., 2nd, Belding, H. S., and Martin, A. W., *J. Cell. Comp. Physiol.*, 1939, **143**, 14.

Grafe should be greater the larger the animal it is desirable to test his theory on larger animals. Martin has started such a test on dogs.[4]

The experiments reported in this paper were designed to test whether or not the metabolic rate *in vitro* of homologous tissues of different animals is related to the body size of these animals (Terroine's rule).

Method. Livers were secured from 7 rats killed after 12 hours' fast, 9 rabbits killed aftter 24 hours' fast, 5 sheep fasted 48 hours and 2 sheep fasted 14 hours before killing. The liver of one horse and one cow killed after 12 hours' fast are included in this investigation, though the data thus obtained are not included in the calculation of the final result. All the animals were mature.

The oxygen consumption of the hand cut liver slices was measured in Warburg micro respiration apparatuses using as a medium a balanced ion solution with glucose and phosphate buffered at pH 7.4.[5] The liver slices were in the apparatus 15 to 30 minutes after the animal was killed. The readings were started 10 to 15 minutes after the installation of the manometers in the bath, which was maintained at a temperature of 37.0°C. The mean respiratory rate during the first half hour was used for the calculation of the results. The amounts of tissue used for the trials were measured by dry matter and micro-kjeldahl determinations. In some cases the N content alone was measured and the dry matter was calculated using $\frac{\text{dry matter}}{\text{N}}$ ratios that were determined in extra liver samples. These ratios ranged from 7 to 9. The results could thus be expressed in cubic millimeters of oxygen consumed per hour per milligram of dry tissue. (Q_{O_2}).

Results. Column 5 of Table I shows that the Q_{O_2} of the liver slices *in vitro* decreases systematically with increasing size of the animal. The only exception to this rule is the result on the cow

TABLE I.
Oxidation Rate of Liver Slices *in Vitro*.

Animal	No. of animals	No. of measurements of Q_{O_2}	Mean body wt, kg	Mean Q_{O_2}, mm^3 O_2 per mg dry tissue per hr
Rats	7	20	.245	7.25 ± 0.43
Rabbits	9	54	3.3	4.17 ± 0.17
Sheep	7	38	18	2.54 ± 0.18
Horse	1	4	182	2.1
Cow	1	3	410	2.6

[4] Martin, A. W., personal communication.

[5] Kleiber, M., and Cole, H. H., *Am. J. Physiol.*, 1939, **125**, 747.

whose liver slices respired with the same intensity as those of the sheep. This latter result would be in agreement with Terroine's rule; it is, however, not conclusive since it was obtained from a single cow that was lactating and had fasted only for 12 hours.

The difference between the Q_{O_2} of the rat and rabbit liver slices and also that between the rabbit and sheep slices are statistically highly significant. In order to derive a quantitative relation between the metabolic rate of liver slices *in vitro* and the body weight of the animals the logarithm of Q_{O_2} is plotted against the logarithm of body weight as shown in Fig. 1. The Q_{O_2}'s as function of body weights of rats, rabbits and sheep can be represented by a straight line—calculated by the method of least squares—which is summarized by the equation—

$$\log Q_{O_2} = 0.721 - 0.24 \log W$$

or

$$Q_{O_2} = 5.26\, W^{0.24}$$

where Q_{O_2} = mm³ oxygen consumed per mg dry tissue per hour
W = body weight of animal in kilograms.

The metabolic rate per unit weight of tissue is thus inversely proportional to $W^{0.24}$ which is practically the same as the fourth root of body weight (see dotted line in Fig. 1). This is the same function

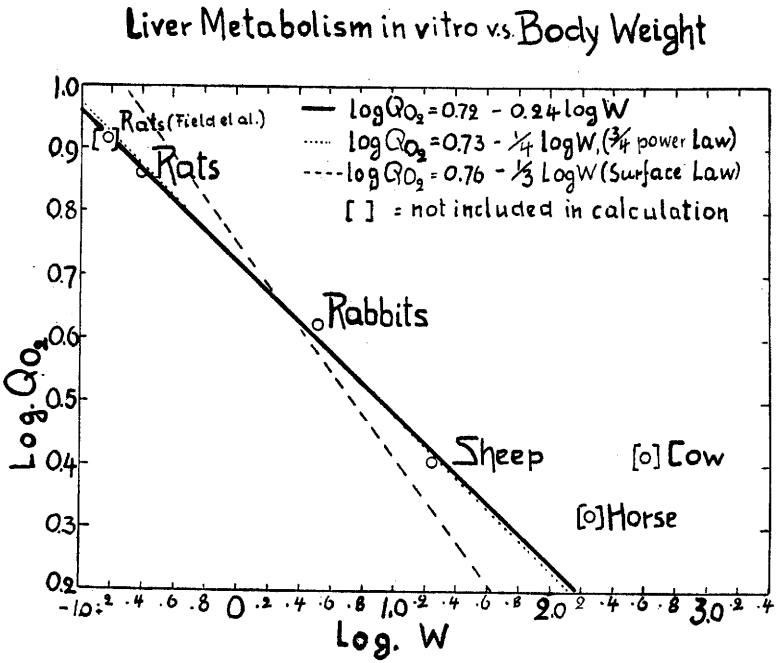

FIG. 1.

of body weight to which the basal metabolism per unit weight of warm blooded animals from rat to steer is most closely related. The metabolic rate of these animals is proportional to the ¾ power of their body weight.[6] The metabolic rate per unit weight is therefore proportional to $W^{¾}/W$ or to $W^{-¼}$. Fig. 1 shows that tissue metabolism follows the ¾ power rule more closely than the surface law which is indicated by the broken line. More measurements on cows and horses are necessary in order to answer the question whether or not the metabolism of their tissues *in vitro* deviates significantly from the rule which is valid for liver slices of rats, rabbits, and sheep. Fig. 1 indicates that the results of Field, Belding and Martin[3] on rats smaller than those used in our investigation fit very closely the function of body size and *in vitro* metabolism derived from our data.

Discussion. The results of this investigation indicate either that the metabolism of an animal is essentially determined peripherally by a condition of its tissues—often called, somewhat mysteriously, their oxygen requirement or energy requirement—or that the factors by which a central regulator (such as the nervous or the endocrine system) controls the metabolic rate of the tissues *in vivo* are still effective *in vitro,* a conclusion reached in earlier work on growth hormone rats.[5] Oxygen supply does not seem to be such a determining factor since the respiratory rates of tissue slices *in vitro,* with an equal abundance of oxygen for all, differ so greatly. The same reasoning rules out glucose or phosphate supply as major regulating factors. It can be assumed that a chemical or physical condition such as concentration of respiratory enzymes or the structural relation of cell components becomes established in the cell under the influence of a central regulator or one may assume that only cells with a metabolic condition that makes them fit into the body as a whole can survive in an intact organism and that these cells then retain this metabolic condition even when they are cut out of the organism and therefore retain a similar metabolic rate *in vitro* as they had *in vivo.*

Summary. The metabolic rates of liver slices from rats, rabbits, sheep, one horse and one cow have been measured *in vitro.* These metabolic rates per unit tissue weight for rat, rabbit and sheep liver decrease systematically and significantly with increasing body size. The metabolic rate per unit tissue weight is inversely proportional to the 4th root of body weight. Body size affects thus the metabolic

[6] Kleiber, M., *Hilgardia,* 1932, **6**, 315.

rate *in vitro* in the same way as it affects the metabolic rate of the intact animal and the factors which determine the metabolic level *in vivo* seem still to be present in the surviving tissues cut out of the organism.

13341 P

Effect of *Triturus* Toxin on Respiration of Rat Tissues *in vitro*.

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Twitty and coworkers[1] have shown that a very active toxin is present in the yolk of *Triturus* ovarian eggs and embryos. Some of the chemical properties and physiological actions of this substance have been described by Horsburgh, Tatum and Hall.[2] Among other actions the toxin paralyzes motor and sensory nerve endings and evokes central respiratory paralysis in the anesthetized cat.

Since it appeared possible that the effects on nervous tissue might be attributable, in part at least, to depression of oxidative metabolism, we have investigated the effects of graded concentrations of the toxin on the respiration of rat brain (cerebral cortex) slices. For comparison, similar experiments were carried out on kidney cortex and liver slices from the same animals. Methods of preparation of tissue slices differed somewhat from those used previously[3] and will be described elsewhere. Oxygen consumption was measured in cmm, N.P.T., per mg wet weight per hour, by the Warburg manometric method. A relatively pure preparation of the toxin was kindly furnished us by Drs. van Wagtendonk and Tatum.

Dried powder containing the toxin was taken up in Ringer's-phosphate-glucose[3] and brought to pH 7.2. The final concentrations in the respirometer vessels ranged from 0.43 mouse units (M.U.) per ml[2] to 850 M.U. per ml. Eight adult male rats were used, and at least 2 determinations were made at each dosage level. The lowest

1 Twitty, V. C., and Elliott, H. A., *J. Exp. Zool.*, 1934, **68**, 247; Twitty, V. C., and Johnson, H. H., *Science*, 1934, **80**, 78; Twitty, V. C., *J. Exp. Zool.*, 1937, **76**, 67.

2 Horsburgh, D. B., Tatum, E. L., and Hall, V. E., *J. Pharm. and Exp. Therap.*, 1940, **68**, 284.

3 Crismon, J. M., and Field, John, 2d, *Am. J. Physiol.*, 1940, **130**, 231.