References and Notes

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7. Although the two enzyme systems which have been described [by C. Mitoma, *Arch. Biochem. Biophys.* 60, 476 (1956), and S. Kaufman, *J. Biol. Chem.* 226, 511 (1957)] which are capable of catalyzing the conversion of phenylalanine to tyrosine are similar, they do differ in several respects. The first appears to be specific for DPNH, while in the second, TPNH is more active. More pertinent to the present discussion are differences in nomenclature. The two enzymes in the system described by Kaufman were purified from different sources, one from rat liver and the other from sheep-liver, extracts. Throughout the text of this report these are referred to as the rat and sheep enzymes, respectively. In all probability, the purified rat enzyme of Kaufman is equivalent to Mitoma's rat fraction I (the labile enzyme), while the enzyme purified from sheep-liver extracts by Kaufman is equivalent to Mitoma's rat fraction II (the stable enzyme).
8. The following abbreviations are used in this report: DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide, respectively; TPN and TPNH, oxidized and reduced triphosphopyridine nucleotide, respectively.
9. S. Kaufman, *J. Biol. Chem.* 230, 931 (1958).
10. ———, *Biochim. et Biophys. Acta* 27, 428 (1958).
11. ———, in preparation.

12. I would like to thank Dr. C. Everett Koop of the Children's Hospital of Philadelphia for the liver biopsy samples which made this study possible.

13. Although this is not shown in Table 1, in a few experiments the addition of THF did not restore the activity when added to the phenylketonuric liver homogenates.
14. It may be noted that the group of phenylketonurics used in this study is younger than the group of normals. Supplementary, unpublished data indicate that the age of the subjects (within the limits described in Tables 1 and 2) has no effect on the results which have been obtained.

28 July 1958

A Consideration of the Metabolic Rates of Some Shrew Tissues

Abstract. Metabolic rates of certain tissues of a shrew, *Cryptotis,* were lower than had been expected in view of the high total metabolism characteristic of shrews. Similar trends were shown in the rodent *Reithrodontomys.* The depression of metabolism of some tissues that was observed in these very small mammals may aid them in conserving energy during periods of inactivity.

The general inverse correlation between metabolic rate of an intact animal and body weight has been well demonstrated (*1*). Among the mammals whose respiratory rates have been measured, shrews of the family Soricidae appear to occupy a unique position (*2*). The asymptotic nature of the body-weight–metabolic-rate curve calculated for shrews suggests that the smallest species measured lie close to the theoretical lowest limit of adult mammalian size. Even the larger species exhibit a metabolic rate well above that of rodents of equal weight (*2*). In view of these findings, the relationship between the total metabolic rates and those of isolated tissues in shrews is of interest.

This report is concerned with presentation of some preliminary data on O_2 consumption of isolated liver, diaphragm, kidney, and lung tissues of the shrew *Cryptotis parva,* together with comparative information on similar tissues from the harvest mouse (*Reithrodontomys humulis*), white mouse, mole (*Scalopus aquaticus*), white rat, and domestic rabbit. Determinations were made on the tissues of seven shrews of both sexes, weighing from 4.1 to 5.0 g. The specimens were wild-caught and were maintained from 1 to 6 wk in the laboratory before sacrifice. The animals were killed by crushing the cervical vertebrae; the organs were immediately removed and placed in cold Ringer's phosphate-glucose (0.1*M*) solution (*3*). The tissues were prepared by the hand-slicing technique (*3*). Respiratory rates were measured by the direct Warburg method at a temperature of 37° ± 0.03°C, air being utilized as the gas phase. Three milliliters of Ringer's phosphate-glucose solution constituted the vessel medium. Flasks were shaken at 120 cy/min, and 15 minutes of equilibration time were allowed, after which readings were taken at 15-minute intervals for 1 hour.

The results are presented in Fig. 1. Although differing absolutely to a considerable extent, the relative metabolic rates of the various tissues of white mouse, white rat, and rabbit appear to present the same qualitative relationship to body weight as does metabolism of the intact animal. A similar trend was obtained by Kleiber (*4*) for liver slices over a size range of larger species, although his values for rat and rabbit liver tissue were higher than those obtained in the present study. With the exception of the value for kidney, the values for shrew tissues fall below the extrapolated curve of the three species mentioned above. This effect is most marked in the case of liver. In view of the small size and high metabolic rate of the intact shrew, this departure is rather striking and would seem to indicate that the high respiratory rates exhibited by shrews must be due to "extrinsic" factors such as nervous stimulation, hormone levels, or concentrations of metabolites in blood or tissue fluids rather than to generally higher "inherent" rates of tissue metabolism. Because of the significantly greater metabolic rates for shrews as compared with those

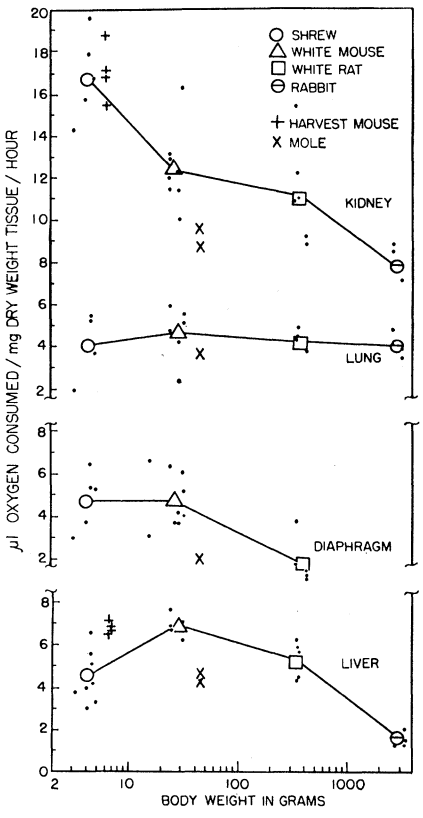

Fig. 1. Tissue metabolic rates of several small mammals.

for other mammals of similar size, the preceding statement would be valid even if the observed rates for shrew tissues fell in the position on the curve that would be predicted on the basis of size alone. The fact that they actually lie below this curve is even more unexpected.

These observations suggest two alternative explanations. Both are highly speculative in view of the limited data available for shrew and other mammalian tissues and for total respiratory rates measured under comparable conditions. On the one hand, the observed rates for lung, diaphragm, and liver tissues of the shrew may be a reflection of the relatively primitive status of the insectivores among mammals, the high metabolic rate exhibited by the intact shrew representing an adaptation of controlling mechanisms to elevate metabolic processes in order to compensate for heat loss or other factors in the physiology of these small creatures. Alternatively, the relatively low respiratory rates exhibited by three of the four tissues measured may in themselves be an adaptation from a "primitive" condition in which higher rates existed. In any case, the functional significance of this situation would seem to lie in a possible marked lowering of metabolism when the animals are inactive, thus resulting in a considerable

conservation of energy by the individual.

Observations indicate that shrews of the genera *Cryptotis* and *Blarina* often sleep very soundly and may awaken slowly, making feeble or trembling, uncoordinated movements before becoming fully active (*5*). This behavior is reminiscent of that of a bat emerging from a torpid state and may similarly indicate a reduction in metabolic rate in the inactive condition. We have made measurements which indicate that the shrew, unlike the bat, shows no marked reduction in body temperature during sleep. Determinations of respiratory rates of sleeping or lightly anesthetized shrews would be highly instructive in this connection.

The data for liver and kidney tissues of *Reithrodontomys,* a mammal which lies in the same weight range as *Cryptotis,* indicate trends similar to those for the latter. The metabolic rates for kidney are quite comparable, while the rates for liver show a similar, but less pronounced, departure from the expected. This suggests that the depression of certain tissue rates may, at least in part, be a general characteristic of small mammals and cuts across phylogenetic lines. Kleiber noted a tapering off and slight reversal of rates in liver slices of larger mammals (horse and cow). The present data suggest a similar phenomenon at the "small-sized" end of the curve. The values for diaphragm, kidney, and liver tissues from a single mole fall noticeably below the general curve. This may indicate that insectivores in general have inherently low metabolic rates for tissue, and this, in turn, may be a physiological indication of their primitive nature.

The correspondence of the high metabolic rates for kidney of shrew and harvest mouse with their expected position on the general curve is not understood. The explanation that the discrepancy is a result of diet, with consequent differences in the level of nitrogenous excretion, is made unlikely by the fact that the essentially carnivorous shrew and the herbivorous harvest mouse exhibit similar trends.

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9 July 1958

Inhibition of Enzymatic Synthesis of Pantothenate by 2,3-Dichloroisobutyrate

Abstract. The investigations reported here have shown that 2,3-dichloroisobutyrate is uncompetitive with β-alanine and competitive with pantoate for a site on the enzyme of pantothenate synthesis. The enzyme dissociation constant of the inhibitor was comparable to that of the competitive substrate.

Evidence implicating pantothenate synthesis as a metabolic pathway involved in the herbicidal action of several chloro-substituted aliphatic acids was recently obtained from yeast growth experiments (*1*). One of these compounds, 2,3-dichloroisobutyrate, prevents pollen development without causing female sterility when applied to plants at low concentrations (*2*). This "gametocidal" property of the chemical has been evaluated for use in production of hybrid cotton seeds on male-sterile parent plants. A knowledge of biochemical mechanisms inhibited by dichloroisobutyrate could facilitate further development of the "gametocide" principle. The experiments reported here were initiated to determine the effect of 2,3-dichloroisobutyrate on the enzymatic synthesis of pantothenate.

The pantothenate-synthesizing enzyme was prepared from *Escherichia coli* (*3*), and its activity was determined manometrically at 30°C by following the rate of acid liberation of CO_2 from bicarbonate buffer (*p*H 8) in Warburg vessels containing a 5-percent CO_2 atmosphere. The reaction mixture was adjusted to a total volume of 3.0 ml containing 0.1*M* KCl, 0.01*M* $MgSO_4$, 0.02*M* β-alanine, 0.02*M* pantoate, 0.01*M* adenosine triphosphate, 0.066*M* $KHCO_3$, and sufficient enzyme to give the activity desired. The adenosine triphosphate was placed in a side arm during the equilibration period and tilted into the body of the flask to initiate the reaction.

Initial rates of CO_2 liberation were proportional to enzyme concentrations up to rates of 350 μl of CO_2 per hour. The enzyme concentration was adjusted to give rates of approximately 250 μl/hr, and readings were taken at 5-minute intervals for a 1-hour period. An additional 20 to 40 μl of CO_2 per hour was released by the adenosine triphosphatase, which contaminated each of the enzyme preparations. This CO_2 production was not inhibited by 2,3-dichloroisobutyrate. Appropriate corrections were made for adenosine triphosphatase activity in all tests.

Inhibition of the pantothenate-synthesizing enzyme by 2,3-dichloroisobutyrate (*4*) was tested under conditions in which one substrate (β-alanine or pantoate) concentration was held constant at 0.02*M* and the other varied over a range of 0.00167 to 0.02*M*. The data presented in Fig. 1 were obtained from three independent determinations and combined for analysis by the method of Lineweaver and Burk (*5*). The family of parallel lines obtained when β-alanine was considered as substrate is generally known as "coupling inhibition" or "uncompetitive inhibition" and indicates that the inhibitor couples with the enzyme-substrate complex rather than with the free enzyme. Therefore, the inhibitor-enzyme complex must have occurred at a site independent of β-alanine. This site was evidently the point at which pantoate combines with the enzyme, since a typical competitive inhibition test was obtained when pantoate was considered as substrate for the reaction. This is apparently the first instance in which these two types of inhibition have been demonstrated in one enzymatic reaction by a single inhibitor.

The values obtained for the enzyme dissociation constant for this inhibitor (K_i), when three independent preparations of the enzyme were used, were 0.0014, 0.0019 and 0.0064*M*, respectively. The corresponding values for the dissociation constant for pantoate (K_m) were 0.0025, 0.0032 and 0.0060*M*, respectively. The variability of the latter values was in agreement with the values reported previously (*3*). A comparison of the K_i and K_m values obtained with the individual enzyme preparations

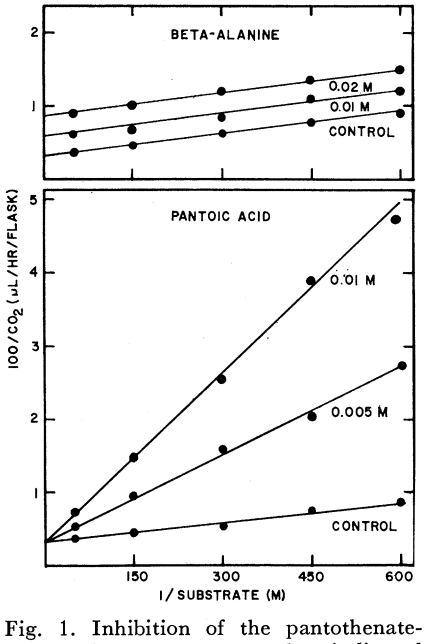

Fig. 1. Inhibition of the pantothenate-synthesizing enzyme at the indicated concentrations of 2,3-dichloroisobutyrate shows the inhibitor to be uncompetitive with β-alanine and competitive with pantoate.