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

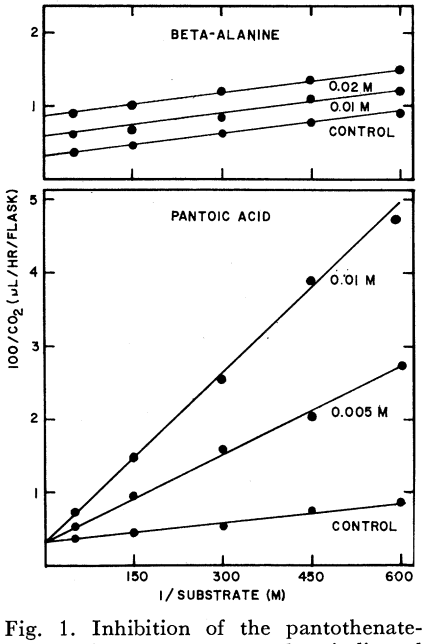

Fig. 1. Inhibition of the pantothenate-synthesizing enzyme at the indicated concentrations of 2,3-dichloroisobutyrate shows the inhibitor to be uncompetitive with β-alanine and competitive with pantoate.

shows that the inhibitor and the natural substrate have approximately the same affinity for the enzyme.

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Variability of Tooth Formation in Man

Data on the timing of tooth formation are of potential value in a wide variety of applications, ranging from the estimation of age in skeletal remains and accident victims (*1*) to the investigation of dental development in precocious puberty and endocrinopathies (*2*). However, it would appear that values for tooth formation commonly given in the literature greatly underestimate the variability that exists.

Using serial oblique-jaw x-rays of a total of 255 white Ohio-born participants in the Fels Longitudinal Studies, we determined the time of occurrence of three stages of formation in five mandibular teeth on an individual basis, after reference to each succeeding and each previous x-ray in the series (*3*). Because of skewness, percentiles were computed, rather than means and standard deviations. Combined-sex distributions were employed throughout.

The 5th and 95th percentiles from the present study were compared with the "ranges" given by Kronfeld (*4*), which are the basis for the varying values cited in abridged form by other authors (*2, 3, 5, 6*). As is shown in Table 1, the present 5th to 95th percentile ranges greatly exceed in magnitude the "ranges" previously given, for each of 14 tooth-stage comparisons. On the average, the present ranges and those published by Kronfeld differ by a factor of 3.

There are several possible explanations for the fact that variability of tooth formation as determined here is so much greater than has been accepted hitherto. These possibilities include: the inevitable differences between histological and radiographic approaches; differences in the measure of variability employed; and differences in the populations sampled. However, the most likely explanation lies in the extremely small samples previously investigated. The earlier values are based on a total of 25 to 30 cadavers, most of them from children who were debilitated at the time of death, and many of whom were developmentally abnormal (*7*). For most of the developmental stages of the teeth here compared, the ranges previously given could not have been based on more than two individuals. In contrast, the present data, though not intended for use as norms, are based on from 32 to 196 examples of each stage of each tooth considered (*8*).

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Table 1. Variability of mandibular tooth formation (in months) as found in the present study, and as commonly given.

Tooth	This study: No.	This study: Percentile 5th	This study: Percentile 95th	Kronfeld (*4*) "Range"*
Beginning calcification				
$\overline{P}_1$	164	19	36	21– 24
$\overline{P}_2$	179	32	56	27– 30
$\overline{M}_1$	157	1	3	birth
$\overline{M}_2$	196	34	58	30– 36
$\overline{M}_3$	135	90	131	96–120
Crown completion—root formation				
$\overline{P}_1$	172	72	97	60– 72
$\overline{P}_2$	166	80	112	72– 84
$\overline{M}_1$	175	37	58	30– 36
$\overline{M}_2$	177	88	122	84– 96
$\overline{M}_3$	53	143	205	144–192
Root completion—apical closure				
$\overline{P}_1$	40	134	168	144–156
$\overline{P}_2$	32	145	184	156–168
$\overline{M}_1$	87	105	139	108–120
$\overline{M}_2$	37	154	211	168–180

* Identical ranges given in Kronfeld (*4*) and Wilkins (*2*). Values given by Schour and Massler (*5*) and Arey (*6*) were obtained by combining maxillary and mandibular "ranges." $\overline{P}$, premolar; $\overline{M}$, molar.

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Formation of Metal Alkyls by Ionizing Radiation

Abstract. It has been demonstrated that liquid hydrocarbons, under the influence of gamma radiation, react with "high-surface sodium" to form metal alkyls. The nature of these metal alkyls has been determined, and possible mechanisms for their formation are discussed.

The interaction of alkyl free radicals with metals to form metal alkyls is a well-known reaction. We have found that the irradiation of liquid hydrocarbons in contact with sodium metal supported on aluminum oxide (*1*) results in the formation of low concentrations of metal alkyls and sodium hydride (*2*).

Samples of the five saturated hydrocarbons studied were prepared for irradiation by fractional distillation of the best grades of materials available with the final distillation taking place in a vacuum from "high surface sodium." The irradiation vessels were 4.0-ml Pyrex ampoules in which 0.75 to 1.0 g of the "high-surface sodium" (25 percent sodium by weight) had been loaded in a nitrogen-filled dry box. One-gram samples of the hydrocarbons were distilled into the irradiation vessels and degassed thoroughly by repeated freezing, pumping, and thawing cycles. Irradiations were carried out in a 500-c cobalt-60 source having a dose rate of about 3.0×10^{20} ev/lit. min. Carbonation of the samples with $C^{14}O_2$ following irradiation was carried out by the method of Collins (*3*). The resulting carboxyl-labeled sodium alkanoates were separated by paper chromatography, located on the paper by means of a thin-window Geiger tube, and identified by comparison with the known R_f values for these compounds.

Experimental results relating to the relative yields of the various free radicals captured by sodium are summarized in Table 1. The total dose was 4.0×10^{21} ev for each sample, based on the weight of hydrocarbon in the sample. For the lower hydrocarbons (C_5 or less) the yield of free radicals isomeric to the parent hydrocarbon was about equal to that of the parent. The chromatographic procedure employed did not separate the isomers efficiently above C_5.

Approximate values of the 100-ev yield of total free radicals captured by sodium are: *n*-hexane, 0.10; *n*-heptane, 0.16; 2-methylpentane, 4×10^{-4}; 2,2,4-trimethylpentane, 1×10^{-4}. These values are cal-