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

Table	1.	Varia	ubility	of	mai	ndibula	r to	ooth
format	ion	(in	mont	hs)	as	found	in	the
present	t s	tudy,	and	as	con	nmonly	gi	ven.

	Г	Kron-			
Tooth	NT.	Perc	entile	feld	
	No.	5th	95th	"Range"*	
	Begin	nning ca	lcificati	on	
$\overline{\mathbf{P}}_{1}$	164	19	36	21-24	
$\overline{\mathbf{P}}_{2}$	179	32	56	27-30	
$\overline{\mathbf{M}}_{1}$	157	1	3	birth	
$\overline{\mathbf{M}}_{2}$	196	34	58	30- 36	
$\overline{\mathbf{M}}_{3}$	135	90	131	96-120	
Cro	wn com	pletion-	root fa	ormation	
$\overline{\mathbf{P}}_{1}$	172	72	97	60- 72	
$\overline{\mathbf{P}}_{2}$	166	80	112	72-84	
$\overline{\mathbf{M}}_{1}$	175	37	58	30- 36	
$\overline{\mathbf{M}}_{2}$	177	88	122	84-96	
$\overline{\mathbf{M}}_{3}$	53	143	2 <b>05</b>	144-192	
Ra	oot com	pletion-	-apical	closure	
$\overline{P}_1$	40	134	168	144-156	
$\overline{\mathbf{P}_{2}}$	32	145	184	156-168	
$\overline{\mathbf{M}}_{1}$	87	105	139	108-120	
M.	37	154	211	168-180	

\* Identical ranges given in Kronfeld (4) and Wil-kins (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

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 toothstage 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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# 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 vac-uum 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  $3.0 \times$ 10<sup>20</sup> 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  $4.0 \times 10^{21}$  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<sup>-4</sup>; 2,2,4-trimethylpentane,  $1 \times 10^{-4}$ . These values are cal-

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Table 1. Product radicals (detected as RC<sup>14</sup>OONa) (percentage of total activity)

Parent hydrocarbon	Octyl	Heptyl	Hexyl	Pentyl	Butyl	Propyl	Ethyl	Methyl	н
n-Pentane n-Hexane n-Heptane 2-Methylpentane	0.5	2.0 0.6	3.0 0.5 10.0	2.0 1.0 2.0 1.5	6.0 0.6 1.0	2.8 0.4 1.0	2.0 2.0 8.5 4.0	20 41.0 37.0 5.0	65 55 36 86
2,2,4-Trimethylpentane		14.0	6.0	6.0	5.0	17.0	8.0	15.0	23

culated from the known specific activity of the C<sup>14</sup>O<sub>2</sub> used and from reasonable assumptions about counting efficiency, self absorption, geometry, and other factors. Energy absorption is based on the ferrous sulfate dosimeter yield of 15.6 ferric ions per 100 ev absorbed, with necessary corrections for differences in absorption coefficients. These calculations are based on the energy absorbed in the liquid hydrocarbon only.

The 1000-fold difference in total free radicals originating from the straightchain hydrocarbons captured by sodium as compared with the branched compounds is possibly in part due to the presence, in the latter compounds, of a tertiary hydrogen atom which would be especially susceptible to attack by the free radicals produced in the bulk of the liquid. This is substantiated by the observation that in the case of both branched compounds the percentage of methyl radicals captured is appreciably lower, and the larger radicals higher, than in the case of the straight-chain compounds, while the over-all yield remains low.

That diffusion rates of the free radicals may also play a role can be inferred from the relative yields (Table 1). In all cases, except 2,2,4-trimethylpentane, the H-atom and methyl yields are much greater than those of any of the larger species produced by radiolysis.

For the highly branched compound, random carbon-carbon scission seems to predominate, while in the case of the straight-chain compounds, the order of susceptibility to breakage is  $C_1 - C_2 > C_2$ - $C_3 > C_3 - C_4$ —that is, a terminal methyl group is most readily removed. This generalization must be tempered, of course, with the observation that the methyl radical may simply have a better chance of being captured and that it thus gives the appearance of being produced in greater abundance. The observation is, however, in general agreement with the results obtained by Gevantman and Williams (4), who used iodine as a free radical trap. These workers found that in the case of ethane, propane, n-butane, and n-pentane, the radicals corresponding to the parent hydrocarbon were not predominant and that methyl radicals represented 25 to 50 percent of the total radical yield, with other small fragments making up the bulk of the remaining products of the radiolysis.

It is not clear from this study whether or not the reactive species captured are produced on the monolayer of hydrocarbon immediately adjacent to the sodium surface or in the bulk of the system.

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## **Prolonged Natural Deferment of** Hatching in Killifish

Abstract. The drying up of flooded lowlands stranded eggs of Fundulus confluentus, which were unhatched and viable after as much as 3 months' exposure. The eggs were found in the moist plant debris on the soil surface. When ovigerous sods were immersed in tap water, normal fish hatched within 15 to 30 minutes.

Under natural conditions, delay in hatching greatly exceeding the minimal incubation period is recorded only of the California grunion (Atherinidae) and certain of the Cyprinodontidae in South America, Africa, and India. The eggs of these fishes lie buried in sand (1) or mud (2) or immersed in stagnant water (3), as the case may be, pending onset either of a rainy season or of spring tides, when they hatch. Fundamentals of the hatching mechanism have been worked out experimentally for the Nearctic cyprinodont, Fundulus heteroclitus (4), and its hatching has been indefinitely postponed artificially (5). The hatching physiology of the Japanese cyprinodont, Oryzias latipes, has been clarified by a series of experiments (6). Since neither species is at present known to undergo a significant delay in hatching in a state of nature, the experimental results have not been examined in an ecological context.

In the autumn of 1957, in Vero Beach,

Florida, eggs of marsh killifish, Fundulus confluentus Goode and Bean, were found to remain viable out of water for long intervals, when stranded on the ground surface among plant litter after accumulations of rainfall runoff had dried up. Sods cut during the period 9-18 December from a swale bottom from which the water had disappeared 2 months or more earlier yielded hatchlings 15 to 30 minutes after the sods were immersed in pans of tap water. At the time of hatching, the yolk sac is obsolescent, and the larval fish start feeding as soon as they escape from the chorion. A sod cut from an exposed lowland bottom on 19 September was kept out of water in the shade for 3 months and 5 days and lightly sprinkled once a week. After being immersed in a pan of water on 23 December, it yielded a hatchling fish.

All oviposition sites located by sampling were at the margins of formerly flooded areas, where the eggs would be left stranded by the first contraction of the perimeter of the standing water. Oviposition had been confined to accumulations of fresh water from rainfall runoff, which may have devious temporary connections with nearby brackish waters during the August-September rainy season. However, F. confluentus is euryhaline, with a preference for brackish water, and is abundant in regions without access to fresh water, so that it probably oviposits also at the fluctuating margins of brackish tidal waters.

The hardiness of the stranded eggs exposed to the atmosphere is evident from the fact that fry were obtained from the swale on 29 January, after it had been reflooded by heavy rainfall at the beginning of January, following the most sustained cold weather in Florida in over 15 years. These ranged from roughly 2-week to 4-week size, as measured by those reared from the egg in the laboratory. In both field and laboratory, the hatchlings developed normally and rapidly in fresh water (7).

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