dentin apposition and of tooth eruption is, therefore, not secondary to the relatively mild retardation in body weight. That the relationship between body weight and tooth development is not a primary one is also shown by the fact that normal animals subjected to insufficient food intake and retarded gain in body weight do not show significant effects upon tooth development.

Calcification. There is a characteristic disturbance of calcification rhythm in magnesium deficiency (Fig. 2). Irving[3] has reported this to be of a 2- or 3-day rhythm on the basis of measurements and comparison with the normal 16 μ daily rhythm. It appears that since apposition is slowed the 16 μ standard cannot be used. The difference between the calcification patterns of the labial and lingual dentin is probably linked with the difference in rate of apposition.

Enamel hypoplasia is not specific for Mg deficiency but is of a severe type (Becks and Furuta[4]).

Summary and Conclusions. In magnesium deficiency the rate of eruption of the incisor is reduced to approximately one-third the normal. The outstanding effect is on dentin apposition which is progressively decelerated, the enamel-covered dentin being affected more acutely than the cementum-covered portion. Temporary local cessations of growth may occur. The formation of alveolar bone is also decelerated to one-half or less of the normal rate and the periodontal membrane is abnormally wide. The dentin of the rat incisor acts not only as a kymograph of calcium metabolism (Fig. 2), but also as a growth kymograph (Fig. 1, D.2).

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Protyrosinase and Polar-Nonpolar Cations and Anions.*

THOMAS HUNTER ALLEN AND JOSEPH HALL BODINE.

From the Zoölogical Laboratory, State University of Iowa.

The sodium alkyl sulfates, which activate protyrosinase,[1] yield polar-nonpolar anions, (RSO_4^-). Alkylamine hydrochlorides, however, give polar-nonpolar cations ($RNH_3.H^+$), and do not activate

[3] Irving, J. T., *J. Physiol.*, 1940, **99**, 8.

[4] Becks, H., and Furuta, W. J., *J. A. D. A.*, 1941, **28**, 1083.

* Aided by a grant from the Rockefeller Foundation for research in cellular physiology.

[1] Allen, T. H., and Bodine, J. H., *Proc. Nat. Acad. Sc.*, 1941, **27**, 269.

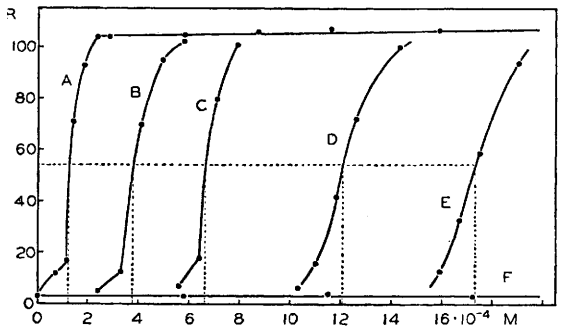

FIG. 1.

The effect at 24.9° of dodecylamine hydrochloride upon the activation of a constant amount of protyrosinase by sodium dodecyl sulfate. Ordinate: 1000 × the reciprocal of the time for the uptake of 100 μl of oxygen. Abscissa: molarity of sodium dodecyl sulfate for A, B, C, D, and E; molarity of dodecylamine HCl for F. Curve A, sodium dodecyl sulfate.

B, " " " plus 0.96 × 10⁻⁴ M dodecylamine HCl.
C, " " " " 1.91 × 10⁻⁴ M " "
D, " " " " 3.82 × 10⁻⁴ M " "
E, " " " " 5.73 × 10⁻⁴ M " "
F, dodecylamine HCl.

protyrosinase.† This distinction may be of value in the search for the kind of chemical group or part of protyrosinase which is involved in its activation.

Three similar preparations of protyrosinase were extracted from grasshopper eggs *(Melanoplus differentialis)* by a method involving trituration, fractional precipitation, and dialysis.[1] Activities, measured in a Warburg apparatus, were compared by the reciprocal—multiplied by 1000—of the time in minutes for the first 100 μl of oxygen uptake with tyramine hydrochloride as substrate.

The ascending limb of curve A (Fig. 1) shows the activation of protyrosinase that occurs in the presence of sodium dodecyl sulfate. With dodecylamine hydrochloride (Curve F, Fig. 1) there seems to be no conversion of protyrosinase into tyrosinase. When solutions of alkyl sulfates and alkylamines are mixed, a precipitate forms. Thus, from activation experiments performed by adding a constant amount of protyrosinase to such mixtures (Curves B, C, D, E, Fig. 1), it was found at half activation that 1.00 mole of either decyl- or dodecylamine hydrochloride removes 0.36, 1.01, and 2.79 moles of sodium octyl, decyl, and dodecyl sulfates (Fig. 2). These values may well be a function of the dissociation constants of the compounds formed by cations and anions.

The effects of variation in the order of mixing of reagents is

† The sodium alkyl sulfates were a gift from E. I. du Pont de Nemours; the alkylamine hydrochlorides were generously supplied by Dr. G. E. Boyd of the Department of Chemistry of the University of Chicago.

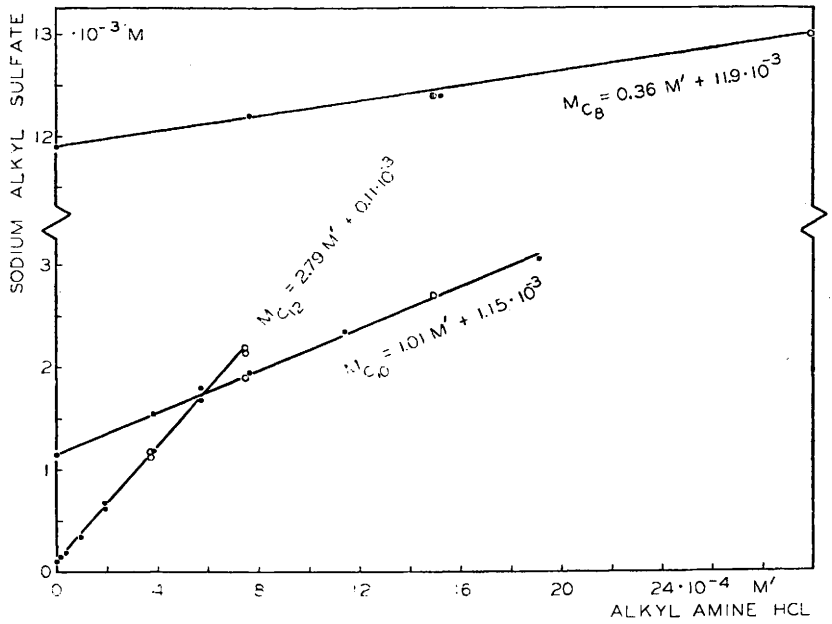

FIG. 2.

The effect of alkylamine hydrochlorides upon the activation of protyrosinase by sodium alkyl sulfates. Ordinate: molarity of alkyl sulfate needed for half activation. Abscissa: molarity of alkylamine hydrochloride mixed with alkyl sulfate at least 4 hours before adding protyrosinase. $M_{C_8} = 0.36M' + 11.9 \times 10^{-4}$ is the equation for the least squares line representing the effect of alkylamine hydrochlorides upon the activation of protyrosinase by sodium octyl sulfate. M_{C_8}, $M_{C_{10}}$, $M_{C_{12}}$ are the molarities of sodium octyl, decyl, and dodecyl sulfates. Open circles, decylamine HCl; closed circles, dodecylamine HCl. pH = 6.8, 0.033M phosphate buffer; temp. = 24.9° C. Reaction fluid volume = 3.0 ml.

graphically illustrated in Fig. 3. The upper curve (A) shows only a slight decrease in tyrosinase when excess dodecylamine is added *after* the mixing of protyrosinase and dodecyl sulfate. Curve B describes the effect, previously noted, of mixing dodecyl sulfate with dodecylamine and, *after* equilibration, adding the protyrosinase. Curve C refers to the results of mixing protyrosinase and dodecylamine and *then* adding the dodecyl sulfate. The difference between Curves A and B suggests that the activation of protyrosinase is irreversible, while the similarity in curves B and C would show that the dodecylamine cation not only fails to form a stable complex with protyrosinase but also reacts more readily with the alkyl sulfate anion than does protyrosinase.

In order to account for the drop of curve A (Fig. 3) a protyrosinase was heat-treated (5 min. at 70°).[2] The resulting tyrosinase, in the presence of increasing dodecylamine hydrochloride, showed a

[2] Bodine, J. H., and Allen, T. H., *J. Cell. and Comp. Physiol.*, 1938, **12**, 71.

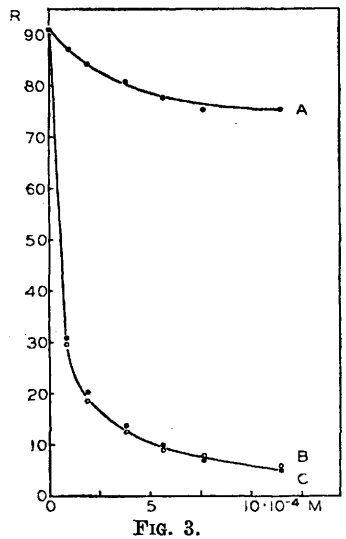

FIG. 3.

Variation in the order of mixing 1.59×10^{-4}M sodium dodecyl sulfate, various amounts of dodecylamine HCl, and a constant amount of protyrosinase. Ordinate: specific activity (see Fig. 1). Abscissa: molarity of dodecylamine HCl. For description of Curves A, B, C see text.

similar drop in activity. Since this inhibition is independent of the method of activation, it seems that only *free* alkylamine cations are poisons for this tyrosinase. Yet, the alkylamine did not form a stable complex with protyrosinase (Curve C. Fig. 3). Apparently the alkylamine cation will react with tyrosinase but not with protyrosinase. If an ammonio-copper complex were formed between the copper[3] of this tyrosinase and the polar group of the alkylamine, it seems of interest to note that such a complex is apparently not formed with the copper[4] of protyrosinase.

It is evident that the polar groups of alkyl sulfates and alkylamines will combine with each other (Fig. 2). This fact in itself offers a suggestion as to the locus of activation. Perhaps an alkyl sulfate ion reacts with the amino group of a diamino acid belonging to a protyrosinase molecule. Since the intercept constants (Fig. 2) decrease with increasing size of the alkyl group thus introduced, it seems necessary to conclude that the efficiency[1] of activation is markedly increased by the addition of carbon atoms to the nonpolar group.

[3] Bodine, J. H., and Allen, T. H., *J. Cell. and Comp. Physiol.*, 1941, **18**, 151.

[4] Allen, T. H., and Bodine, J. H., *Science*, 1941, **94**, 443.