Following the injection of kaolin into the cistern the blood pressure, as a rule, remained within normal limits. Three readings were obtained ranging between 155 and 165 mm on different animals, but this trifling hypertension had returned to normal by the next week in 2 animals, while the third died before a subsequent measurement had been made.

The cerebrospinal pressure was definitely increased, being 140, 158, and 175 mm of water in 3 animals with consistently normal blood pressures. All animals autopsied showed marked internal hydrocephalus, this being present 4 or more weeks after the injection of kaolin.

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Enzymes in Orthopteran Ontogenesis. VI. Autocatalytic Nature of *in vivo* Formation of Protyrosinase.*

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Northrop[1] considered the autocatalytic formation of pepsin and trypsin from their zymogens as being an instance of protein synthesis. Therefore, since Wrinch's[2] theory described strata of two-dimensional, cyclol-structured lamina, Langmuir and Schaefer[3] point out that protein growth, as with crystal formation, is a determined one and should reasonably be autocatalytic. In the light of these observations, it should be of some general interest to describe the *in vivo* formation of an enzyme precursor. The present paper is concerned with the reporting of the autocatalytic nature of the formation of protyrosinase within eggs of the grasshopper, *Melanoplus differentialis*.

The preparation and activation of grasshopper egg protyrosinase has been dealt with in a series of papers (Bodine and Boell,[4] Bodine, Allen, and Boell,[5] Bodine and Allen[6, 7]) so that here a brief statement

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1 Northrop, J. H., *Physiol. Rev.*, 1937, **17**, 144.

2 Wrinch, D. M., *Roy. Soc.*, 1937, A **160**, 59.

3 Langmuir, I., and Schaefer, U. J., *J. Am. Chem. Soc.*, 1938, **60**, 1351.

4 Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1935, **6**, 263.

5 Bodine, J. H., Allen, T. H., and Boell, E. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 450.

6 Bodine, J. H., and Allen, T. H., *J. Cell. and Comp. Physiol.*, 1938a, **11**, 409.

7 Bodine, J. H., and Allen, T. H., *J. Cell. and Comp. Physiol.*, 1938b, **12**, 71.

of the essential procedures should suffice. Centrifugation of an egg brei prepared in a physiological normal, phosphate buffered (pH 6.8) sodium chloride solution results in the formation of 3 layers, termed A, B, and C respectively, from the centripetal to the centrifugal poles. The lipoidal layer A contains a naturally occurring activator of the protyrosinase which occurs entirely in the cell-free, comparatively voluminous B layer. Layer C consists of the inert egg membranes and structural elements. The brei was always diluted to such a volume that this precursor or inactive form of tyrosinase present in 1.0 cc of fraction B was that derived from 20 eggs irrespective of their particular age.

A solution of tyramine hydrochloride was used for substrate in such an amount that during the tyrosinase-catalyzed oxidation to the end product, melanin, 388 mm^3 of oxygen was consumed. The rate of this oxygen uptake was determined in the standard Warburg manometer at 25°C and was expressed as the reciprocal of the time necessary for half completion of the reaction.

As previously pointed out, sodium oleate appears to be an "ideal" activator in the sense that once superoptimal amounts of this compound are present, all the protyrosinase presumably becomes tyrosinase (Bodine and Allen[7]). In many instances it is a general rule that the rate of enzymatically catalyzed reactions varies directly with the amount of enzyme (Tauber[8]). Such has been found to be true (Bodine and Allen[9]) for the amounts of tyrosinase used in the present experiment. Therefore, it seems proper to consider the rate of oxidation of tyramine hydrochloride as a measure of the quantity of tyrosinase. In turn, these rates may actually be interpreted as a comparison of the various amounts of protyrosinase, since as mentioned above this inactive form is converted quantitatively to the active one by the sodium oleate.

A convenient form of the simple autocatalytic reaction (Herriott[10]) may be stated

$$+\frac{dA}{dt} = KA(A_e - A)$$

which after integration is

$$K = \frac{2.303}{A_e t} \log \frac{A}{A_e - A} \frac{A_e - A_o}{A_o}$$

8 Tauber, H., *Enzyme Chemistry*, 1937, John Wiley and Sons.

9 Bodine, J. H., and Allen, T. H., unpublished observations, 1937.

10 Herriott, R. M., *J. Gen. Physiol.*, 1938, **21**, 501.

where A is the rate at time t and A_e is the equilibrium and A_o the initial value of A. In Table I, K is calculated as derived by the equation from the mean value for the amount of protyrosinase obtained from 20 eggs of t days of age. Actually the eggs must be 8 days of age before an initial determination of protyrosinase may be made (Bodine, Allen, and Boell[5]). Therefore, t + 7 days of chronological age, *i. e.*, days' development at 25°C, is t days of the equation. The mean value of K, 0.00138, has been used in the construction of the theoretical curve (Fig. 1). Inspection reveals that this falls reasonably close to the probable errors of the means (Table I).

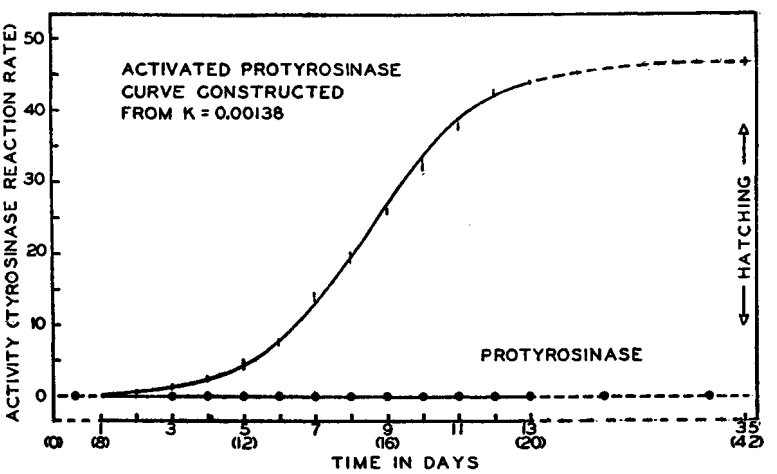

FIG. 1.

Formation of protyrosinase during development of the grasshopper egg. Ordinate denotes reciprocal of half oxidation period of tyramine hydrochloride $\times 10^3$. First abscissa gives days of apparent increase in protyrosinase, that is, t of the autocatalytic equation. Second abscissa (in parentheses) states days of development at 25°C minus the diapause period. The vertical lines of the theoretical curve represent the probable error of the means for determinations of protyrosinase activated by a hypercritical amount of sodium oleate. The solid circles show the inactivity of protyrosinase, *i. e.*, protyrosinase in the absence of sodium oleate. For further description see text.

In the absence of any data from experimental manipulation, such as variation of the ratio of end product to reactant concentrations, it seems best to merely examine the significance of the autocatalytic equation. This equation may be derived from either of 2 assumptions as regards the introduction of an equation of constraint. Either the constraint may be introduced as a function of the concentration of an intermediate acting as a catalyst in the formation of the end product, or the catalytic influence may be attributed to the operation

TABLE I.

Days development (25°C)	t (days) of equation	$A_e = 46.5$ $A_o = 0.2$ K	Rate of tyramine hydrochloride oxidation catalyzed by activated protyrosinase	
			Theoretical	Experimental
8	1	—	—	*0.2 ± .18
9	2	.00136	0.7	*0.7 ± .14
10	3	.00137	1.3	*1.3 ± .45
11	4	.00139	2.5	2.5 ± .51
12	5	.00138	4.5	4.5 ± .68
13	6	.00138	7.8	7.8 ± .51
14	7	.00142	12.8	14.2 ± .71
15	8	.00141	19.5	19.6 ± .71
16	9	.00136	26.8	26.1 ± .60
17	10	.00134	33.6	32.1 ± .77
18	11	.00134	38.6	37.4 ± .28
19	12	.00139	42.0	42.4 ± .24
20	13	.00137	43.9	44.0 ± .21
42	35	—	—	46.5 ± .22

*Rate determined by extrapolation; all other rates by interpolation.

of the end product itself. The resulting forms of the final differential equations are identical.†

The very nature of autocatalytic reactions (Northrop[1]) intimates that at least one unit of the catalyst must be present among the reactants for the reaction to take place. Perhaps, in the way of speculation, the action of some gene mechanism may not be adverse to these observations (Gulick[11]).

It seems logical to conclude that within the grasshopper egg a precursor of tyrosinase is formed by autocatalysis.

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Variation in Distribution of Type and Group Substances in Smooth-Phase Cultures of Group A Beta Hemolytic Streptococci.

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For a decade it has been recognized that the amount of type-substance in different cultures of the same serological type of Group

† For these suggestions the authors are indebted to Dr. Gordon Marsh of this department.

11 Gulick, A., *Quart. Rev. Biol.*, 1938, **13**, 140.