

### Vitamin E Deficiency in the Suckling Rat

It has been reported previously<sup>1,2</sup> that partial deficiency of vitamin E in the doe rat leads to the production of abnormal young. This abnormality is associated with both anterior pituitary deficiency and thyroid deficiency. It is most marked when the litter is about eighteen days old and usually results in death three or four days later.

Since at birth the young, though small, appear otherwise normal, it seemed possible that the abnormality develops after birth and might be prevented if the young were fed by a normal doe. An E-deficient rat had a litter of three—two males and one female. Five days after birth, the two males were exchanged for four males from the normal breeding stock which were born on the same day. The stock mother then had her own females and the four normal males. All the young suckled by the E-deficient doe were abnormal at 16 days, and all were dead two days later. The thyroid glands were examined histologically and all showed the hypoplasia typical of this condition. All the young suckled by the normal rat grew well and showed no sign of abnormality. The experiment was repeated, with the same result, when the females from the two litters were exchanged on the fifth day, and again when the young were transferred on the day of birth.

This experiment shows that the young of vitamin E-deficient rats are born normal, but that thyroid and anterior pituitary deficiency develop as a result of the lack of some essential constituent of the mother's milk. This missing factor is almost undoubtedly vitamin E since lack of this vitamin results in similar changes in these glands in the adult rat, although the accompanying symptoms of stunted growth, uncalcified skull, clenched feet and weakness are not observed because the animal has already attained maturity.

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<sup>1</sup> Barrie, M. M. O., *NATURE*, **139**, 286 (1937).

<sup>2</sup> Barrie, M. M. O., *Lancet*, *ii*, 251 (1937).

### Vitamin P

WE have reported previously that certain flavonones (vitamin P) greatly influenced symptoms of experimental scurvy. This latter condition was interpreted as a mixed C and P avitaminosis. At our request, our experiments have been repeated by several laboratories. The results were partly corroborative and partly negative. The reason of this discrepancy is now cleared up. Vitamin P requires for its activity the presence of traces of ascorbic acid. A scurvy diet frequently contains such traces which in themselves have no influence on the development of scurvy, but enable vitamin P to act. In the entire absence of ascorbic acid, vitamin P is inactive. Quantitative data will be published elsewhere. Previous conclusions are upheld. The object of this note is to prevent superfluous discussion.

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### Choline Esterase Activity of Superior Cervical Ganglia

By a direct chemical micro method the maximum choline esterase activity of the superior cervical ganglion of the cat has been measured and found to be, on the average, equivalent to the splitting of 0.10  $\gamma$  of acetylcholine chloride per sec. per mgm. of fresh tissue. The full experimental details will be given in another publication.

This figure may be applied to the data of Brown and Feldberg<sup>1</sup>, who found that the greatest output of acetylcholine from the ganglion perfused with eserinated Locke's solution occurs in the first 5 minutes of preganglionic stimulation at 17 per sec. In this time 0.1  $\gamma$  was liberated from a ganglion weighing 12.9 mgm. Hence the time required for the splitting of the acetylcholine liberated by one nerve impulse would be

$$\frac{0.1 \gamma}{12.9 \text{ mgm.} \times (0.10 \times 10^{-3} \gamma/\text{mgm.} \sigma) \times (300 \times 17) \text{ stimulations}} \\ = 0.015 \frac{\sigma}{\text{stimulation}};$$

provided that the enzyme and substrate are localized sufficiently so as to obtain a complete combination of the two, that is, theoretical maximum velocity of hydrolysis. Actually, the velocity falls when the concentration becomes less than a certain value; hence 0.015  $\sigma$  represents a limiting least time which might be merely approached in actuality. Compared to the refractory period of the ganglion, which Brown<sup>2</sup> has found to be of the order of 2  $\sigma$ , it is apparent that the enzyme need operate only with an average rate of about 0.75 per cent of its theoretical maximum velocity in order to destroy the acetylcholine liberated by a nerve impulse within the refractory period. After the first 5 minutes, the quantity of acetylcholine liberated per impulse falls until finally only about a fifth of the initial amount is set free<sup>1</sup>; under these conditions the enzyme could hydrolyse the acetylcholine within the refractory period at about 0.15 per cent of its maximum velocity.

From the foregoing it would appear that the enzyme present is sufficient to destroy the acetylcholine liberated by a nerve impulse within the refractory period. However, it must be borne in mind that for the conditions of minimum velocity of hydrolysis, as in the case of an even distribution of enzyme and substrate throughout the tissue, the reaction velocity would be very far indeed from the maximum one, because of the low substrate concentration and affinity for the enzyme. In this case the time ( $t_s$ ) for splitting 99 per cent of the substrate is given by:

$$t_s = \frac{1}{V_{\max.}} \int_{S_1}^{S_2} \frac{K_s + S}{S} dS,$$

where  $V_{\max.}$  represents maximum velocity of hydrolysis,  $K_s$  the Michaelis constant for the affinity between enzyme and substrate,  $S$  the substrate concentration at any moment,  $S_2$  the original substrate concentration, and  $S_1$  1 per cent of  $S_2$ . It follows that:

$$t_s = \frac{1}{V_{\max.}} (K_s \ln \frac{S_2}{S_1} + S_2 - S_1) = \frac{4.6K_s + 0.99S_2}{V_{\max.}}.$$

Since the value of  $K_s$ , which I am now investigating, is unknown for the enzyme from the source in question, it is sufficient for a rough calculation to assume it to be of the same order of magnitude as the  $K_s$  already found in the case of human serum<sup>3</sup>, namely, 0.001, from which  $t_s$  becomes about 8 sec.