TABLE I.
Energy and Protein Utilization of Flavin-Deficient Chicks and Pairfed Flavin Supplied Controls.

| Flavin in food | Body wt, g | Total efficiency* of utilization of | | Mean partial efficiency† of utilization of | |
|---|---|---|---|---|---|
| | | Energy % | Protein % | Energy % | Protein % |
| Deficient | 121 ± 5 | 4.8 ± 1.1 | 9.8 ± 1.6 | 44.7 ± 1.0 | 20.1 ± 1.5 |
| Supplied | 128 ± 6 | 5.1 ± 1.0 | 24.8 ± 2.0 | 45.7 ± 0.8 | 35.8 ± 2.0 |

*Total efficiency $= \frac{\text{gain}}{\text{food}} \times 100.$

†Mean partial efficiency $= \frac{\text{gain} + \text{basal loss}}{\text{food}} \times 100.$

Basal metabolism measured in previous trials.
Basal N loss assumed to be 2 mg N per kcal. basal metabolism.
[Smuts, D. B., *J. Nutr.*, 1935, **9** (4), p. 427.]

were nearly equalized and since the fasting heat production is not affected by flavin deficiency, it is to be expected that the influence of maintenance requirements on total efficiency does not affect the comparisons. Conclusions based on total efficiency should therefore parallel those based on mean partial efficiency which is essentially independent of maintenance requirement. Table I confirms this expectation. Flavin deficiency had no effect on the utilization of food energy but lowered significantly the utilization of nitrogen. For every 100 kcal gain in energy the flavin-deficient chicks gained 2.3 ± 0.7 g nitrogen; their flavin-supplied controls on equal food intake, 5.1 ± 1.4 g. These controls gained protein while losing body fat.

*Summary.* Respiration trials with groups of flavin-deficient and flavin-supplied chicks as well as pair trials with carcass analysis indicated that flavin deficiency decreased the utilization of protein but did not affect the utilization of food energy beyond the effect of greatly decreased appetite.

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## Cholinesterase in Developing Amblystoma.

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The physiological significance of cholinesterase (ChE) in neuromuscular activity and its importance in the development of behavior

has recently been reviewed by Nachmansohn.[1] He has shown that ChE first appears in appreciable quantities with the beginning of motility in the chick embryo. The concentration of the enzyme at nerve endings is considered high enough to hydrolyze and thereby inactivate, within the refractory period of the contracting muscles, the acetylcholine (ACh) concerned with the neuromuscular transmission. Since the development of behavior pattern in Amblystoma has been so completely elaborated by Coghill,[2] this form seems ideal material on which to study the relationship between enzyme development and behavior. Studies revealing a sharp rise in the esterase content of whole Amblystoma larvae at the time when rapid movements first occur, have already appeared.[3, 4]

The results reported in the present paper represent an extension of this work and an attempt to localize the enzyme throughout ontogeny, *i. e.*, to determine what element or elements in the embryonic neuromuscular apparatus are most active in its production. Direct estimations of enzyme activity were made by means of a modification of Glick's microtitration procedure.[5]

Small amounts of the esterase are present in the premotile embryo where it is already more highly concentrated in nerve and muscle than in other tissues. With the onset of non-tetanic S-flexure responses in the embryo, the concentration of ChE increases to a level more than twice that for the earlier stages. Fig. 1 shows the developmental curves of the esterase in nervous tissue and innervated muscle throughout ontogeny and in nerveless muscle during the prefeeding stages. Both the nerve and innervated muscle esterase curves reach a peak during the early feeding stages and decline through metamorphosis. The innervated muscle curve is similar to that found in the chick by Nachmansohn, who attributes the peak to the higher relative volume of nerve endings during early development. However, Nachmansohn finds no corresponding decline in the chick nerve esterase curve, the adult level being the highest attained. The decline noted in the present work may possibly be caused by the increasing medullation of nerve fibers since myelin contains little ChE. It is significant that the speed of motility and sensitivity to reflex stimulation appear to be greatest during early feeding when ChE content is maximal, and that the marked retarda-

1 Nachmansohn, D., *Yale J. Biol. and Med.*, 1940, **12**, 565.

2 Coghill, G. E., *Anatomy and the Problem of Behavior*, 1929, Cambridge.

3 Youngstrom, K., *J. Neurophysiol.*, 1938, **1**, 357.

4 Sawyer, C. H., *Anat. Rec.*, 1940, **78**, Supp. 57.

5 Glick, D., *J. Gen. Physiol.*, 1938, **21**, 289.

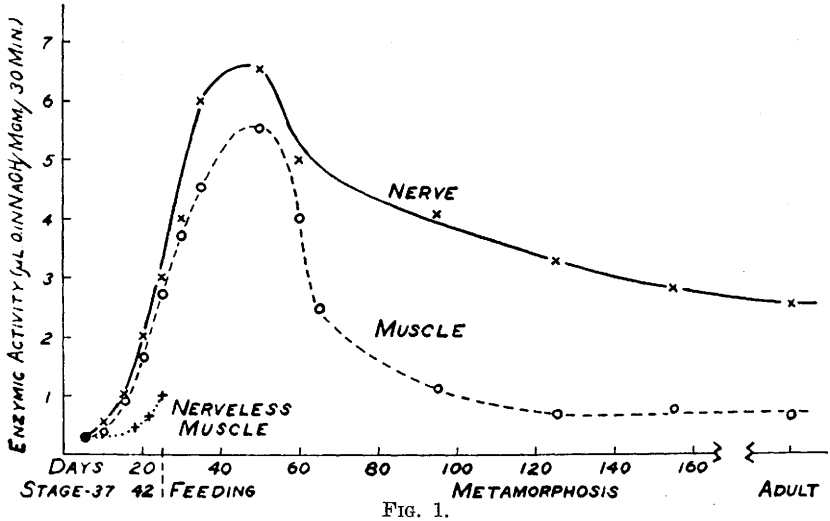


FIG. 1.

Development of cholinesterase in nervous tissue, innervated muscle, and nerveless muscle of *Amblystoma punctatum*. Each point represents the average of several determinations.

tion of movement and increased threshold to stimuli during metamorphosis can be correlated with decreased enzyme content.

The peaks in nerve and muscle esterase curves occur at the period when, according to Wills,[6] the respiratory rate of developing Amblystoma is highest. This relationship may well be causal; increased ChE permitting more rapid movements which in turn lead to a higher metabolic rate.

There is disagreement as to the effect of denervation on the content of muscle esterase. Martini and Torda[7] find a considerable decline of esterase activity in denervated muscle of rats and dogs, but Couteaux and Nachmansohn[8] report that the concentrations of enzyme ($Q_{ChE}$) are equal in denervated and innervated guinea pig gastrocnemius. To determine the part played by nerve endings in the development of ChE in embryonic muscle, nerveless muscle has been produced by removing the spinal cord from embryos at an early premotile stage. The resulting muscle differs from ordinary denervated muscle in that it has never been under the influence of a functional nervous system. It is at first responsive to direct stimuli but later fails to respond to direct induction coil shocks. Enzyme determinations (Fig. 1) indicate that the amount of esterase in this

[6] Wills, I. A., *J. Exp. Zool.*, 1936, **73**, 481.

[7] Martini, E., and Torda, C., *Klin. Wochensch.*, 1938, **17**, 97.

[8] Couteaux, R., and Nachmansohn, D., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 177.

nerveless muscle is about a third of that in muscle with nerves. Thus it appears that the bulk of ChE production can be ascribed to nerve endings. These findings, in harmony with those of Martini and Torda, are further links in the chain of evidence coupling cholinesterase and neural activity.

To summarize, cholinesterase in the embryo appears to be concentrated at nerve endings and to be directly related to speed of motility and to metabolic rate. It seems clear that cholinesterase content is a biochemical criterion of functional capacity in the developing neuromuscular apparatus.

13456

## Phosphate Equilibrium Between Plasma and Saliva.*

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Incidental to a trial of radioactive phosphorus in the therapy of carcinomatous metastases to bone we have been able, through the courtesy of Dr. George E. Fahr, to make some observations as to the secretion of radiophosphorus in the saliva. The results have some bearing on the interpretation of the studies by Sognnaes and Volker[1] and Barnum and Armstrong[2] dealing with the uptake of radioactive phosphorus by dental enamel from the saliva. The subject was a woman, aged 52, with advanced carcinoma of the breast, and the observations were made 19 days before death when the patient was not cachectic. Four grams of sodium phosphate, containing 42,000,000 Geiger-Mueller counts of radioactive phosphorus, dissolved in 100 cc of water were administered by stomach tube.

One hour following the administration of the radioactive salt a sample of saliva, stimulated by the chewing of paraffin, was collected over a 26-minute period. A second saliva sample was collected 3 hours after administration of the salt and, midway in this collection, a sample of venous blood was obtained. The final saliva sample

* This work was carried out with the aid of a grant from the Carnegie Corporation. The radiophosphorus used in this work was kindly supplied by Professor S. K. Allison of the University of Chicago.

1 Sognnaes, R. F., and Volker, J. F., *Am. J. Physiol.*, 1941, **133**, 112.

2 Barnum, C. P., and Armstrong, W. D., *Am. J. Physiol.*, in press.