MOTOR INHIBITION IN AMBLYSTOMA PRODUCED BY TRITURUS TRANSPLANTS

IN a study of the growth of heteroplastically transplanted salamander eyes,[1] the surprising incidental observation was made that eyes grafted embryonically from the western newt, *Triturus torosus,* to *Amblystoma tigrinum* cause complete paralysis of the hosts, from which they do not recover until two or three days before they begin to feed. In the meantime, development is otherwise normal, neither hosts nor grafts suffering any apparent ill effects. When embryos of these two species are joined as parabiotic twins, recovery of the *tigrinum* twin from the paralysis does not occur until the *Triturus* mate, which develops much more slowly than Amblystoma, has completely resorbed its yolk. There is no reciprocal effect of the Amblystoma grafts or twins on the motility of Triturus.

The substance or substances causing this effect are perhaps associated with the embryonic food reserve of Triturus. When ovarian eggs or embryo[2] are ground with Ringer's, the filtered extract causes paralysis lasting about an hour when injected into the coelom of *A. tigrinum* larvae. Similarly prepared extracts of larvae, following resorption of the yolk, give gradually weaker and finally negative effects, as growth proceeds. The blood of adult females, but not of males, gives positive results, in about one half of the cases tested. It is suggested that the presence of the active substance in female blood can be correlated with periods during which it is being stored in the developing ova.

Oral administration of the extracts in heavy doses has no influence on the *A. tigrinum* larvae, indicating that the material is either converted in the alimentary tract, or is not absorbable by the latter. It can evidently penetrate the skin, although at a very slow rate, judging by a few preliminary tests. Concerning its chemical and physical nature, we know only that it is not present in ether extracts, whereas the residue remains potent; and that it is destroyed by boiling.

It is known that adult Triturus skin is rich in toxic secretions, which have been identified, for some amphibians at least, as alkaloidal in nature, and shown to exert a strong depressive action on the heart when injected into other species.[3] In order to test the possible relationship of these adult secretions with those responsible for the paralysis described by us, we injected aqueous extracts from minced adult Triturus skin into larvae of *A. tigrinum.* The physiological effects are quite different in the two cases. Skin extracts, while depressing heart action strikingly, paralyze the larvae for only brief periods, providing the injection is not so heavy that it causes complete and permanent stoppage of the heart beat; while embryonic extract, or adult female blood, produces prolonged paralysis without affecting the heart beat significantly. On these grounds we are inclined to believe that the substance with which we are concerned is not interpretable as the embryonic forerunner of the adult toxic secretions.

Experiments on this phenomenon of motor inhibition with other forms of the two genera show that it is not confined to *T. torosus* and *A. tigrinum,* although the effect exerted by Triturus, and the reaction exhibited by Amblystoma, may vary greatly in degree with the species. The strongest effect is produced by *T. torosus,* while *T. pyrogaster* (Japan) and *T. viridescens* produce much weaker results. In the same way, *A. tigrinum* reacts much more positively than the other species of Amblystoma, followed by *A. mexicanum* (axolotl), *A. punctatum* and *A. californiense,* in about the order given. For example, eye-vesicles of *T. pyrogaster* grafted to *A. tigrinum* do not paralyze the host embryo, the effect with this combination first becoming apparent when embryos of the two species are joined in parabiosis. Even then, the *A. tigrinum* twin does not remain immotile as long as when it is joined with *T. torosus. T. viridescens* gave much the weakest effect, paralyzing the *tigrinum* twin for only about 24 hours. *T. torosus* and *T. pyrogaster,* while both paralyzing *Amblystoma,* do not affect the motility of one another when joined in parabiosis.

An interesting effect of temperature was noted in embryos of *A. californiense* bearing grafted *T. torosus* eyes. When kept in a cool room (12 to 14 degrees C.) the hosts were motile, and it was at first assumed that this species of Amblystoma does not react as do the others. On bringing them into a room at ordinary temperature, however, they became insensitive almost immediately. This behavior can be repeated a number of times by alternately lowering and raising the temperature. It is suggested that an increase in temperature might have this effect either by increasing the amount of the paralyzing substance liberated by the cells of the graft, by increasing its rate of distribution (through effect on pulse rate) or by lowering the threshold of sensitivity of the host's nervous system to the agent.

The paralysis produced by transplanted eyes is evidently not due to the immediate connection of the grafts with the central nervous system. *A. punctatum* hosts remain insensitive for the same period of days, irrespective of whether the Triturus vesicles are grafted in the normal position or in the region of the

[1] Twitty and Elliott, in press, *Jour. Exper. Zool.,* Vol. 68.

[2] Embryos younger than "tail-bud" stage have not yet been tested.

[3] See Noble, "Biology of the Amphibia."

limb bud. Results kindly communicated by Professor Ross G. Harrison show that other embryonic tissues of Triturus in addition to the eye, namely, limb ectoderm or limb mesoderm, cause paralysis when grafted to Amblystoma. The responsible agent is probably distributed throughout the embryo, although perhaps in unequal concentrations.

We are not yet prepared to generalize concerning the extent to which other amphibians, and possibly more distantly related animals as well, may react to Triturus tissue in the manner described for Amblystoma.

V. C. TWITTY
H. H. JOHNSON

STANFORD UNIVERSITY

ENZYMES, VITAMINS AND THE ZONE OF MAXIMUM COLLOIDALITY

ARE enzymes definite chemical substances or do they consist of chemically definite active or prosthetic groups attached to a colloidal carrier? The following considerations will reconcile the apparent antithesis which this question assumes to exist.

As with other particulate or dispersed catalysts, the action of enzymes depends primarily upon the exposure to the milieu of large interfacial areas having specific electronic fields of force. But there are other factors. Too great a degree of aggregation of an otherwise suitable enzyme would mean that insufficient areas would be exposed to produce marked activity. On the other hand, too great a degree of dispersion of the enzyme might lead to a particulate kinetic activity so intense that the number of *successful* encounters[1] between enzyme and reactants would be reduced to the level of inefficiency. High efficiency for the enzyme demands, *inter alia,* an intermediate degree of dispersion, which involves a high exposure of active interfacial surface, coupled with a great, but not disruptively great, kinetic activity. These criteria seem to be met in the lower range of the ultramicroscopic field, which approximates the *zone of maximum colloidality.*[2]

It, therefore, makes no practical difference whether an enzyme consists of a large molecule or a small group of molecules or of a chemically definite active or prosthetic group fixed to a colloidal carrier; for any of these structures might yield particulate units having the essential basis of enzymic efficiency: (1) large exposure of specific active areas; (2) great, but not disruptively great, kinetic activity.

These views are confirmed by certain experimental data. Thus,[3] a dilute aqueous dispersion of egg white, heated until it becomes opalescent, showed in the ultramicroscope a field crowded with very actively moving ultramicrons. On addition of some essence of pepsin (15 per cent. alcohol), there was an immediate aggregation of the ultramicrons into coagulated, immobile masses. When dilute hydrochloric acid was allowed to diffuse in, the clumps instantly broke up, the ultramicrons resumed their active kinetic motion, and then quickly melted away like snowflakes in cold water, the whole field becoming brighter as dispersion into individually indistinguishable smaller particles proceeded.

Some of the same brand of essence of pepsin, when made more acid, showed a marked increase in Faraday-Tyndall effect, and on standing deposited a fine floc. Ultramicroscopic examination also indicated that acidification increased the number and apparent size of particles in the ultramicroscopic field. These experiments were just now made, and an attempt will be made to see what correlation, if any, exists between the acidity, degree of apparent dispersion and activity of pepsin. Changes in the milieu will, of course, affect the substrate as well as the enzyme, so that in considering the final result many factors must be allowed for.

In the case of diastase attacking ungelatinized starch granules, actively moving ultramicrons in the enzyme dispersion were seen to gather at and oscillate about the surface of the starch grains, which soon showed indented or "gnawed" margins.

Recent work of Professors Richard Kuhn, Otto Warburg and their collaborators[4] indicates that the newly isolated water-soluble lyochromes, the flavines,[5] which apparently constitute vitamin B_2, exhibit enzymic activity when brought into the colloidal state, presumably by fixation on a colloidal carrier. Kuhn states:[4]

> According to recent investigations of O. Warburg and W. Christian,[6] a yellow oxidation-enzyme occurs in yeast. This enzyme, together with a second colorless enzyme and a co-enzyme, is capable of oxidizing hexose-monophosphoric acid. By irradiation in alkaline solution O. Warburg and W. Christian[7] have obtained crystals of a chloroform-soluble pigment. This has the composition $C_{13}H_{12}N_4O_2$. It is remarkable that we have obtained apparently the same pigment by irradiation of ovoflavine. The chloroform-soluble pigment has no more vitamin activity.
>
> Our observations suggest for the first time a reversible relationship between a vitamin and an enzyme. One may imagine that vitamin-B_2 is the exogenous precursor of the

[1] J. Alexander, SCIENCE, 65: 62, 1927.
[2] J. Alexander, *Jour. Am. Chem. Soc.*, 43: 434, 1921.
[3] J. Alexander, *Jour. Am. Chem. Soc.*, 32: 680, 1910.
[4] *Chemistry and Industry*, 52: 985, 1933.
[5] About 17,000 eggs (50 kilos. dried egg albumin) yielded 50 mg ovoflavine; 3,000 liters of cows' milk gave 60 mg lactoflavine.
[6] *Naturwissensch.*, 20: 688, 1932.
[7] *Naturwissensch.*, 20: 980, 1932; *Biochem. Zeits.*, 254: 438, 1932; 257: 492, 1933.