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.

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## 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.

yellow oxidation-enzyme. The formation of the enzyme is supposed to occur by combination of the flavine with a colloidal-carrier. In this connection it is very remarkable that we have observed an excellent increase in growth of animals deficient in vitamin-$B_2$, which have been given well dialyzed preparations of the yellow enzyme.[8] Therefore there exist not only free vitamin-$B_2$, which dialyzes easily, but also vitamin-$B_2$ attached to carriers of high molecular weight, which can not be dialyzed—just as in the case of the flavines. The relationship, which has been established, can be seen from the following table:

| | Soluble in | | | Activity as | |
|---|---|---|---|---|---|
| | Dialysis | Water | Chloroform | Vitamin | Enzyme |
| 1. Flavo-proteins | | | | | |
| Flavo-polysaccharides | − | + | − | + | + |
| 2. Flavines | + | + | − | + | − |
| 3. Irradiated flavines | + | + | + | − | − |

Following a discussion of flavines as biological hydrogen acceptors, Kuhn concludes:

. . . the flavines are not only of importance as prosthetic groups and precursors of the yellow enzyme, but they can themselves act as hydrogen acceptors, in other words, as intermediate substances in cell respiration. . . . One may be justified in calling the flavines the "methylene blue" of living cells.

Enzymic activity might also appear or become accentuated by the mere aggregation of specific molecules into groups sufficiently large to establish a favorable reduction in the kinetic activity of over-active units. Nor can we overlook the fact that electronic surface areas may arise from such aggregation and also that changes in the milieu (pH, salts, etc.) may exert an effect. Thus The Svedberg[9] has demonstrated the effect of changing pH in forming and in breaking up groups of ultramicroscopic protein units, and Richard Willstätter[10] has shown how adsorption and desorption (*elution*) of enzymes depends upon the milieu, as well as on the physicochemical nature of the adsorbent. The inquiry naturally suggests itself as to whether co-enzymes may not function, in some cases at least, by dispersing or else aggregating the enzyme particles (which may be molecules) to the colloidal state favorable to activity. Co-enzymes may also represent particles (they may be molecules), which, on adsorption, complete an otherwise imperfect prosthetic group. Similarly, anti-enzymes may produce an unfavorable degree of dispersion or may mask or "poison" satisfactory prosthetic areas.

The immense effectiveness of minute quantities of vitamins, of hormones, of antigens and of certain potent substances such as histamine and acetylcholine becomes comprehensible when we realize that one single molecule of a specific substance might, under suitable conditions, form, activate or modify a cellular catalyst, and thus suddenly alter the whole internal economy of a cell.[11] The action of specific drugs (including narcotics), and the ultimate nature of pathological intracellular changes (including such as may be associated with insanity and drug addiction), must, in many cases, be considered in the light of possible interference with or alteration of the normal catalytic syndrome of a few or of many cells, wherein *chemical specificity* and *optimum dispersity* are mutually cooperative factors. At the lower range of the colloidal zone, we have a reconciliation between the "chemical" and "colloid" aspects of living matter.

The importance of the time factor in biological reactions is obvious. The influence of the zone of maximum colloidality in controlling the velocity of chemical reactions has been stated thus:[12]

A most striking example of optimum dispersion is found in living matter. Figuratively speaking, if all the chemical substances comprising our organism were in true or crystalloid dispersion, reactions would proceed so rapidly that we would, so to say, live ten years in ten minutes. On the other hand, if coarse dispersion prevailed, it would take ten years to live ten minutes. Every organism is dependent upon the coordination of its chemical reactions *in point of time*, and this leisurely procedure depends largely on *degree of dispersion*, which keeps chemical reaction velocities within certain speed limits through its regulation of free surface and kinetic activity. Life lies between lysis and coagulation. The colloidal zone is, as it were, a vital metronome tolling off the tempo of life.

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[8] R. Kuhn, *Nachr. Kaiser Wilhelm-Ges.*, 2: 13, 1933; *Beilage, Naturwiss.*, 21.

[9] The Svedberg, *Jour. Am. Chem. Soc.*, 1924 *et seq.*; SCIENCE, 79: 327–332, 1934.

[10] R. Willstätter, Alexander's "Colloid Chemistry," Vol. II, pp. 361–66, 1928.

[11] J. Alexander, *Protoplasma*, 14: 296–306, 1931; *Scientia*, October, 1933; *Arch. di Sci. Biol.*, 19: 409–413, 1934.

[12] J. Alexander, Alexander's "Colloid Chemistry," Vol. II, p. 27, 1928.

## BOOKS RECEIVED

FELDMAN, W. T. *The Philosophy of John Dewey.* Pp. vii + 126. Johns Hopkins Press. $1.75.

PEARL, RAYMOND and RUTH DEWITT PEARL. *The Ancestry of the Long-lived.* Pp. xiii + 168. Illustrated. Johns Hopkins Press. $3.00.

ROEWER, C. FR. *Dr. H. G. Bronn's Klassen und Ordnungen des Tierreichs.* Pp. 481–608. Illustrated. Akademische Verlagsgesellschaft, Leipzig.