There is nothing contradictory in the idea that one and the same gland cell in one segment of the digestive tract may contain two kinds of chemical messengers for the succeeding segment of the digestive apparatus. One kind stimulates secretion in the following segment and a second kind inhibits or arrests it.

Starling ("Recent Advances in the Physiology of Digestion," p. 90) speaks only of *hormones* (from *ορμάω*, to excite, arouse or stimulate). But on reflection it must be evident that for the normal regulation of life processes, it may, under certain conditions, be equally important that any process of secretion or vascular tonus should be capable of inhibition by chemical messengers. Two such diagonally opposed chemical substances which are concerned in coagulation have been isolated from lymphocytes by Lilienfeld and Delezenne, one of which *leuconuclein* favors coagualtion and a second *histon* which inhibits it. The leuconuclein corresponds to the *hormones* but the *histon* is an inhibitor. For such chemic bodies — physiologic arresters like *histon* — I would suggest the name *koliones* from the Greek *χωλύων*, to inhibit, to prevent, arrest or check.

16 (354)

A critical study of the conditions under which zymase and its associated co-enzyme bring about alcoholic fermentation.

By **GEORGE H. A. CLOWES.**

[*From the Agricultural Chemical Laboratory of Professor Buchner in Berlin, and the New York State Laboratory, Buffalo.*]

Zymase, the enzyme of yeast discovered in 1896 has since been proved by Harden and Young to consist of two parts, (1) zymase proper, an enzyme-like body possessed of high molecular complexity, non-diffusible and thermo-labile, and (2) a readily diffusible, thermo-stabile, relatively simple, chemical complex, which, for lack of a better term, has been designated as the co-enzyme of zymase.

Harden and Young separated the bodies in question by diffusion, but owing to the paucity of their materials and the destructive effect exerted by secondary causes during the lengthy process involved, it was found impossible to obtain any clear insight into the conditions of physico-chemical equilibrium obtained in this reaction. Buchner's *dauerhefe*, that is to say, a preparation of pressed yeast precipitated by an excess of acetone or alcohol ether, can be prepared in large quantities and exhibits a high degree of resistance to the action of destructive enzymes. We therefore directed our attention to the preparation of *dauerhefe* containing as large a zymase content and as small a co-enzyme content as possible, our object being to study the effect exerted by a preparation of this nature upon fermentable sugars, when used in conjunction with varying proportions of a boiled yeast extract containing co-enzyme. It was found possible to produce a preparation of acetone *dauerhefe* which in itself alone possessed no fermentative activity whatsoever, but which when used in conjunction with a suitable quantity of boiled yeast extract, exhibited an unusually active fermentation, 2 grams mixed with 6 grams of sugar and 20 c.c. of extract producing from 1 to 2 grams of CO_2 in the course of 8 to 10 days.

Having thus demonstrated that it is possible to obtain a stable preparation containing relatively large quantities of zymase, and also to prepare a relatively stable boiled extract of yeast containing co-enzyme, a series of experiments was commenced, the object of which was to determine the effect of varying proportions of co-enzyme used in conjunction with a constant amount of zymase, and vice versa. In several series of experiments in which a constant amount of zymase (2 grams *dauerhefe*), was used in conjunction with 6 grams of sugar and from 1 to 50 units of co-enzyme, it was found that the velocities of reaction and the fermentation end results were directly proportional to the number of units of co-enzyme employed up to an optimum concentration, after which a fall in the value of both these quantities was to be observed. The same phenomenon exhibited itself when varying proportions of *dauerhefe* were employed with a constant amount of co-enzyme, other conditions being constant.

The velocity of reaction k, is calculated from the formula

$$K = ln \frac{A}{A-X} \cdot \frac{1}{T},$$

where A represents the relative molecular concentration of the sugar and $A - X$ the concentration at any given time T. The progress of the reaction is readily followed by estimating the loss in weight of fermentation tubes due to the evolution of CO_2. In all cases in which no disturbing influence has been allowed to exert an effect, this velocity of reaction is found to be constant for a period of three or four days immediately following the establishment of active fermentation. Provided all experiments are carried out at a constant temperature and that other conditions are maintained on a uniform basis, the value of K is found to be directly proportional to the product of the concentrations of the zymase and its co-enzyme, according to the formula

$$\frac{K}{K_1} = \frac{ZC}{Z_1C_1},$$

where K and K_1 represent velocities of reaction and Z and Z_1 concentrations of zymase and C and C_1 concentrations of co-enzyme in comparative series. The accuracy of this formula over a comparatively wide range was demonstrated by means of tables, in which the observed and calculated values of K were compared for a series of tubes in which zymase (*dauerhefe*) and co-enzyme (boiled yeast extract) were employed in varying proportions.

Herzog, from experiments carried out previous to the discovery of the heat-resistance component, came to the conclusion that the fermentation process was to be represented by the formula

$$\frac{K}{K_1} = \left(\frac{C}{C_1}\right)^x,$$

where C and C_1 represent comparative concentrations of zymase and x has any value from 1 to 2. Such a formula would obviously only hold in those cases in which the ratios between zymase and co-enzyme are maintained on a constant basis. The formula which we have developed above gives results closely agreeing with theoretical conclusions in all cases in which sources of experimental error, such as the action of outside enzymes, are eliminated.