NOTE ON HYDROLYSIS OF VEGETABLE OILS BY EMULSION OF *RICINUS COMMUNIS*

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In 1902 Connstein Hoyer and Wartenberg described a series of experiments carried out at Charlottenburg, by which it was sought to determine the *modus operandi* of fat-splitting in vegetable oils by an emulsion of the castor bean. Hoyer continued the work in 1904 and made an attempt to isolate the enzyme. He used as activators of his enzyme acetic acid and sulphate of manganese.

In 1906 Nicloux, after several years' work on the fat-splitting properties of the castor oil bean, came to the conclusion that the cytoplasm of the seed contains the active agent, and that this substance acts in every way as an enzyme, but is not an enzyme.

It is customary to-day to assume that hydrolysis is preceded by a combination of the hydrolyte with the enzyme—Emil Fischer's lock and key relationship between bodies of asymmetric configuration.

It is known that esters of the lower terms are more difficult to hydrolyse than those of the higher; in other words those esters most readily soluble in water are most difficult of hydrolysis. Is this due to a previous hydrolysis of some portion of the hydrolyte, or to a previous hydrolysis of some portion of the so-called enzyme?

In reviewing the experiments of Nicloux and Hoyer I worked with the castor oil bean acting on its own oil and on cottonseed oil. The results were equally good in the two cases, so that no specificity can be claimed for the enzyme of the castor seed towards its own oil.

I prepared an emulsion of ground castor beans and incubated it at 25° C. until hydrolysis of the oil had been definitely established. A measured quantity of the incubated emulsion was then added to a mixture of measured quantities of oil (castor or cotton, as the case might be) and water. The mixture was shaken for a few minutes at intervals of a quarter of an hour for some hours and then left. The percentage of free fatty acids formed in three days at laboratory temperature ranged from 80 to 85.

On repeating Hoyer's experiments, in which he used acetic acid and manganese sulphate, I did not find that the addition of these bodies produced any increase in the amount of free fatty acids liberated.

I noticed early in the experiments that if hydrolysis (fatty acid liberation) were not properly established in the emulsion (enzyme) the yield of free fatty acids was correspondingly low. Success in the experiment largely depends on the intimate mixture of oil and enzyme. The preparation of the enzyme demands the use of a quantity of water from which the enzyme can later be separated. This water when applied to oil is wholly inactive. Further, the active enzyme, when mixed with two or three volumes of water, alcohol, or acetone, rapidly loses its activity; whereas when mixed with two or three volumes of ether or benzene no loss of activity occurs. On the lock and key hypothesis the meaning of these contrasts is difficult to seek.

Nicloux described his enzyme as refractory to heat when protected by oil; my emulsion, when heated to 60° C., rapidly loses its activity. But when the bean from which the emulsion is prepared is heated to 100° C. for twenty-four hours in a dry bath little or none of its activity is lost.

Pancreatic lipase has been separated into two portions by ordinary filtration, and it has been found that the activity of the filtrate is not diminished by boiling, whereas heating the residue destroys its potential energy. I have not been able to effect any such separation in the castor bean enzyme.

That the activity is related to nitrogen-containing matter can be demonstrated; but whether the nitrogen is active or not it is impossible to say. The total organic nitrogen of my active emulsions, as estimated by Kjeldahl's method, always amounted to at least 0.2 per cent.

Cotton oil and castor oil, when carefully neutralised and freed from proteins fails to undergo hydrolysis. If to this oil an enzymic emulsion, in which hydrolysis of fat has not yet commenced, be added, nothing results. But let hydrolysis be established in the emulsion, and hydrolysis proceeds in the added oil, irrespective of whether this oil be neutral or contain within wide limits (25 per cent.) free fatty acids.

Perhaps the most striking of all these features is the failure of the prolonged heating at 100° C.—a temperature which kills practically all proteins—to interfere with the activities of the bean. Is it that in the case of the dry bean, when heated, hydrolysis fails to commence, whereas in the mixture of enzymes and water hydrolysis rapidly exhausts itself?