Bayliss and Starling found, moreover, that secretin preparations made from the duodenum of the cat, rabbit, monkey and *man*, "were all active as regards the pancreatic secretion of the dog;" while on that of the rabbit and monkey they tested the secretin of the dog, rabbit, monkey and man, also with positive results. They concluded, therefore, that "the secretin of all these animals is one and the same body." These investigations have, as to their general features, been confirmed by Camus, Gley, Herzen and others. (Hammarsten.[47]) The conclusion of Bayliss and Starling that secretin is the specific chemical excitant of the pancreatic secretion is also sustained in the light of my views, since, as we have seen, it is adrenoxidase which endows trypsinogen with its activity in the pancreas.

On the whole, the following conclusions seem warranted: (1) *the composition of the pancreatic juice is as follows: the normal product of the pancreas, the zymogen or mother substance trypsinogen; adrenoxidase—a substance now known as "secretin;" and nucleo-proteid;* (2) *trypsin is formed when trypsinogen combines with enterokinase, a body composed of adrenoxidase and nucleo-proteid;* (3) *trypsin causes hydrolytic cleavage of proteids, and its proteolytic activity is due to the intrinsic heat-energy its oxygen-laden adrenoxidase and its phosphorus-laden nucleo-proteid liberate when combined.*

An additional conclusion warranted by the evidence submitted is that *trypsin owes its activity as a ferment to adrenoxidase, since it is the only one of its three constituents that is endowed with the properties of a ferment, i.e., of provoking catalysis.* The bearing of this fact becomes self-evident in view of Moore's[48] statement that "ferment actions are such catalytic reactions."

THE ACTIVE PRINCIPLE OF ADRENOXIDASE AS THE FERMENT OF PTYALIN, AMYLOPSIN, LIPASE, AND MALTASE, AND OF THE DIASTASE WHICH CONVERTS GLYCOGEN INTO SUGAR.

The fact that, as stated by Hammarsten,[49] the pancreatic juice (dog) contains "amylopsin, trypsin, steapsin and rennin,"

[47] Hammarsten: *Loc. cit.*, p. 322.
[48] Moore: Schäfer's "T. B. of Physiol.," vol. i, p. 317, 1898.
[49] Hammarsten: *Loc. cit.*, p. 324.

suggests that these ferments are all produced under similar conditions and that their zymogens owe their activity to adrenoxidase and nucleo-proteid. That such is actually the case, *i.e.*, that the process through which trypsin becomes a proteolytic ferment exemplifies that which prevails in the case of all pancreatic ferments, is sustained by considerable direct and indirect evidence.

The *starch-splitting ferment* of the saliva, ptyalin, is now considered by most authorities to be the same as that of the pancreas, amylopsin. As stated by Moore:[50] "In their behavior to change of temperature and reaction the two enzymes are identical; the rate of conversion of starch into other substances depends on the concentration of the enzymes in the solution"—a fact which plainly suggests that the differences observed can be "entirely produced by differences in concentration." The two ferments will, therefore, be considered together.

The first question to suggest itself is whether we are dealing with a ferment, since, as is well known, starch may be converted into sugar by ordinary chemical procedures.

That a ferment is the active agent in the physiological process is shown by the fact that Roberts[51] found that amylopsin could convert 40,000 times its weight of starch—obviously as the result of a fermentation. As the adrenal active principle of adrenoxidase is essentially a ferment—the *deus ex machina* of enterokinase—it meets the need in this particular. Again, we have seen that secretin strikingly corresponds as to its chemical reactions with the adrenal principle. Ptyalin, which is now considered identical with amylopsin, shows a similar correspondence with the same active principle, *i.e.*, adrenalin. It is precipitated by absolute alcohol, but as water is added to the latter its solubility increases; it acts best in a faintly acid solution. A pure ptyalin—which gave none of the proteid reactions—isolated by Cohnheim,[52] presented, moreover, the characteristic test of adrenalin: it resisted the boiling temperature. That the active ferment of ptyalin is that present in adrenoxidase is further shown by the fact that, as we have seen,

[50] Moore: *Loc. cit.*, p. 328.
[51] Roberts: Lumleian Lectures, London, 1891.
[52] Cohnheim: Virchow's Archiv, Bd. xxviii, S. 241, 1863.

Schoenbein[53] produced a blue coloration of saliva and secretion of mucous membranes with tincture of guaiac in the presence of hydrogen peroxide.

As to the presence of nucleo-proteid in both amylopsin and ptyalin, the evidence is also direct. Not only have we seen that the pancreas is supplied with this body available for all its zymogens, including, therefore, amylopsin, but Hammarsten[54] includes among the constituents of saliva, both nuclein and nucleo-proteid.

All this is further sustained by the fact that the needs of the catalytic process which the presence of adrenoxidase entails are met by a very marked oxygen absorption, as shown in the following lines by Bunge:[55] "That oxygen passes through the capillary wall in the salivary glands is apparent, for the simple reason that the saliva contains free oxygen. So large an amount of oxygen passes out of the blood, therefore, that the cells of the glandular tissue cannot consume it, and the excess escapes into the secretion." What this illustrates, of course, is the marked vigor of the catalytic action sustained by the adrenoxidase. Again, Pflüger[56] ascertained the presence of absorbed oxygen in the submaxillary secretion with the aid of the gas-pump; he found that it amounted to from 0.4 to 0.6 per cent. of the volume of the saliva. This fact was confirmed by Hoppe-Seyler, who "found that the secretions of both the submaxillary and of the parotid contained oxygen."

That the starch-splitting ferment is, like trypsin, activated by adrenoxidase and nucleo-proteid, appears to me self-evident.

As to the *fat-splitting ferment* lipase (pialyn, steapsin), the data available are very scant. According to Moore,[57] "very little is known of the fat-splitting enzyme, pialyn, of the pancreatic juice. That the action is due to an enzyme, however, is shown by the following experimental observations: (*a*) The action is destroyed when the pancreatic juice or active pancreatic extracts are *boiled;* (*b*) it takes place in the presence of antiseptics, and hence cannot be due to bacteria"

[53] Schoenbein: *Loc. cit.*
[54] Hammarsten: *Loc. cit.*, p. 286.
[55] Bunge: *Loc. cit.*, p. 246.
[56] Pflüger: Pflüger's Archiv, Bd. i, S. 686, 1868.
[57] Moore: *Loc. cit.*, p. 339.
[58] Nencki: Arch. f. exp. Path. u. Pharm., Bd. xx, S. 367, 1886.

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[58] Nencki: Arch. f. exp. Path. u. Pharm., Bd. xx, S. 367, 1886.

(Nencki[58]). That oxygen takes part in the process is suggested by the fact that, as stated by Landois,[59] its action on fatty acids is attended by the production of CO_2 and H in the absence of micro-organisms. Again, we have seen that Sakharoff found that ferments remained inactive in the absence of oxygen.

The rôle of the intestinal juice in the process is emphasized by the observations of Bunge,[60] that even after removal of the pancreas, the greater part of fat administered to dogs continued to be split into fatty acids and glycerin—a process which corresponds with that ascribed to enterokinase by Pawlow. The participation of the oxygen and nucleo-proteid of enterokinase in the cleavage of fat is also shown by the fact that, as stated by Moore,[61] the activity of lipase "is greatly increased by the presence of bile." Now, Glaessner[62] showed recently that this applied to diastatic ferments as well as to fat-splitting ferments—a fact which indicates that the latter must be activated by the same agents that activate ptyalin, amylopsin, etc. The identity of the agent which in bile produces this action is suggested by Moore's statement that "Paijkull[63] has proved that the mucin-like substance which gives bile its viscidity really belongs to the nucleo-proteids." As nucleo-proteid is necessarily secreted into the intestine, it plays in the fat-splitting process precisely the rôle it fulfills in conjunction with adrenoxidase in other fermentative processes reviewed. It is evidently, as in the case of trypsin, ptyalin and amylopsin, one of hydrolytic cleavage, for Claude Bernard[64] showed that the fat-molecule took up three molecules of water and split into glycerin and three molecules of fatty acid.

Although the evidence in the case of the fat-splitting ferment is partly inferential, owing to the paucity of experimental data, the temperature at which it is destroyed, its kinship to amylopsin and therefore ptyalin which colors guaiac blue; the facts that it is produced by the same organ as trypsin and that its activity is enhanced by contact with bile, a body rich in

[59] Landois: *Loc. cit.*, p. 306.
[60] Bunge: *Loc. cit.*, p. 165.
[61] Moore: *Loc. cit.*, p. 339.
[62] Glaessner: Zeit. f. physiol. Chemie, Bd. xl, S. 465, 1904.
[63] Paijkull: *Ibid.*, Bd. xii, S. 196, 1888.
[64] Claude Bernard: Ann. de chim. et de phys., Sér. iii, p. 474, 1849.

nucleo-proteid, indicate that it differs in no way from trypsin as to the manner in which it is physiologically activated.

As stated by Landois,[65] "the pancreas also prepares a *sugar-splitting ferment*. If a solution of sugar is digested with an aqueous or glycerin extract of pancreas, the amount of sugar diminishes." This ferment is maltase, which converts maltose into dextrose, the sugar found in the urine in glycosuria. Maltase is also present in the saliva, which, we have seen, colors guaiac blue and contains nucleo-proteid. Wherever found, therefore, it is accompanied by adrenoxidase and nucleo-proteid, since these bodies are also present in pancreatic juice. Now, Cohnheim[66] was recently led, after a series of experimental investigations, to compare the formation of the pancreatic sugar-splitting ferment to that of trypsin as interpreted by Pawlow, *i.e.*, with enterokinase (which, we have seen, contains both adrenoxidase and nucleo-proteid) as the activating agent of the trypsinogen. A subsequent series of researches by the same investigator,[67] having for its purpose to identify the "glycolytic body" as he terms it, showed that it *withstood boiling*, and that it was soluble in water and *96 per cent.* alcohol. Both these tests are characteristic of the adrenal active principle, since, as we have seen, it is soluble in alcohol containing water, *i.e.*, 96 per cent. alcohol. Cohnheim mentions another test which applies to the adrenal principle, *viz.*, that it is insoluble in ether. Finally, as to the nature of the glycolytic body, he concludes that it is not a ferment—such as pepsin, trypsin, etc.—but a body closely allied as to its characteristics to the constituents of various internal secretions, among which he mentions *adrenalin* and *secretin*—the identical bodies which I have identified as the active principle of adrenoxidase as to the adrenalin, and as adrenoxidase as to secretin. Cohnheim was thus brought back to a conclusion which I had reached one year before him (see page 411 in the first volume), *viz.*, that "Lépine's glycolytic ferment is the oxidizing substance"—now the adrenoxidase.

In the light of the foregoing facts, however, the adrenoxidase is not itself the glycolytic agent, but it endows the

[65] Landois: *Loc. cit.*, p. 307.
[66] Cohnheim: Zeit. f. physiol. Chemie, Bd. xxxix, S. 336, 1903.
[67] Cohnheim: *Ibid.*, Bd. xlii, S. 401, 1904.

latter, *i.e.*, the sugar-splitting ferment maltase, with its property as such, this ferment being composed of the pancreatic zymogen, adrenoxidase and nucleo-proteid. As such, it is an exact counterpart of trypsin, but acting on sugars instead of proteids.

Closely allied to this process is that through which *glycogen* is converted into sugar. When, as shown by Minkowski, von Mering, Dominicis, von Noorden and others, the pancreas is removed from animals, glycosuria appears. This is due to the absorption into the blood of sugars ingested as such or derived from starches converted into sugar in the alimentary tract and eliminated by the urine. Thus Minkowski found, and his results were confirmed by Hédon,[68] that a fixed quantity of sugar, administered to animals deprived of their pancreas, caused an increase of sugar in the urine precisely equal to the quantity given. In normal animals, however, this morbid phenomenon does not occur. The several varieties of sugar and their anhydrides, dextrine and starches, contribute to the formation of glycogen, which is stored, as is well known, in the liver, to be, as first suggested by Claude Bernard, converted into sugar for use in the organism at large; the muscles in particular. Bernard concluded that this is effected by a diastatic ferment—a view which, according to Hammarsten, "is accepted by most investigators." I may recall in this connection that I pointed out in the first volume (page 404) the source of his ferment, *viz.*, the pancreas, the pancreatic juice reaching the liver by way of the splenic and portal veins, as an internal secretion. Indeed, J. Rose Bradford[69] states that "pancreatic diabetes may be produced not only by removal of the pancreas, but also by ligature of the pancreatic veins," a fact which indicates that obliteration of the vessels which carry, as interpreted from my standpoint, the pancreatic products to the splenic vein annuls the organ's functions as to its relations with the liver. This not only sustains Bernard's conception as to the intervention of a diastatic ferment, but it assimilates the latter to the ferment by which the conversion of starches into sugar is brought about in the intestine, *i.e.*, amylopsin and its homologue in the saliva, ptyalin,

[68] Hédon: Archives de physiol., Jan., p. 154, 1893.
[69] J. Rose Bradford: Practitioner, Aug., 1900.

which we have seen contains both adrenoxidase and nucleo-proteid.

Diabetes—a form of this disease as least—should under these conditions be caused by an excessive production of amylopsin by the pancreas. Again, the overproduction of this ferment should be caused by an excess of adrenoxidase in the blood, since this body supplies the body at large with oxygen, and is the promotor of tissue metabolism. Briefly we should have (1) an increase of pancreatic activity entailing overproduction of amylopsinogen, (2) leucocytogenesis, which entails, as we will see, an increased production of nucleo-proteid, and (3) the active principle in adrenoxidase,—the three agents which jointly form the amylolytic or diastatic ferment to which the conversion of glycogen into sugar is due. That such is the case is shown by the fact that the injection of the adrenal principle into the blood, adding thereby to its normal content in adrenoxidase, gives rise to glycosuria.

We have seen, in the first volume, that Blum, Croftan, Herter and others caused glycosuria by injecting adrenal extractives. This has been confirmed by a number of investigators. Herter and Richards not only produced glycosuria in animals, whether the adrenal active principle was introduced endovenously, subcutaneously or endoperitoneally, but Metzger[70] found that when adrenal extract was given to dogs there was hyperglycæmia, *i.e.*, an actual increase of sugar in the blood. Finally, Herter and Wakeman[71] found, moreover, that compression of the adrenal glands, thus causing hypersecretion, caused glycosuria, and that removal of these organs or ligation of their vessels caused a "considerable fall of the sugar-content of the blood."

Considered collectively, all this evidence seems to me to show (1) *that the mode of formation of trypsin and the manner in which it is activated and endowed with its properties as a ferment, is common to all pancreatic ferments;* (2) *that all the pancreatic zymogens are converted into ferments by combining with adrenoxidase and nucleo-proteid;* (3) *that they are all activated by the joint action of adrenoxidase and nucleo-proteid;*

[70] Metzger: Münch. med. Woch., Mar. 25, 1902.
[71] Herter and Wakeman: Amer. Jour. Med. Sci., Jan. 1903.

and (4) *that they all acquire their property as ferments from the active principle of the adrenoxidase they contain.*

THE ACTIVE PRINCIPLE OF ADRENOXIDASE AS THE FERMENT OF THE COAGULATION FERMENT, AND RENNIN AS "FIBRINOGEN PROPER." THE ZYMOGEN OF FIBRINOGEN PROPER.

A prominent gastric ferment is the familiar milk-curdling rennin or lab discovered by Hammarsten in 1872. The process of coagulation which rennin provokes in milk was regarded by this distinguished chemist as analogous to that of blood-coagulation, but he ascribed it to the specific ferment named. Analysis of the question in the light of my views suggests, however, that milk coagulation is an artificial process, *i.e.*, one which has no physiological application, and that it is caused incidentally when the ferment which causes blood coagulation is added to milk. This entails the conclusion also that such a ferment as "rennin" does not exist as a separate entity.

A curious feature of the milk-curdling ferment is that it is found in many plants, in the blood of invertebrates, and also in that of vertebrates which do not secrete milk. "The presence of rennin in the stomach of birds and fishes is very remarkable," says Moore,[72] "and points to some wider function at present unknown to us, since it cannot be supposed that in such animals the ferment plays any part in connection with the clotting of milk." Again, while rennin is found in the stomach, where it is supposed to carry on its function, a substance capable of producing similar effects also exists in other parts of the body. Thus, extracts of testes, liver, lung, kidney, muscle, spleen, thymus, thyroid, brain, small intestine, ovary and blood were all found by Edmunds[73] to induce the formation of casein when added to milk. Moreover, Moro and Hamburger[74] found that a drop of hydrocele fluid from an infant, to which a drop of human milk was added, clotted almost immediately.

Again, although thirty years have elapsed since rennin was discovered, the actual need or usefulness of a milk-curdling ferment in the stomach has not, as yet, been shown. Howell,[75]

[72] Moore: *Loc. cit.*, p. 334.
[73] Edmunds: Jour. of Physiol., vol. xix, p. 466, 1896.
[74] Moro and Hamburger: Wiener klin. Wochen., Jan. 30, 1902.
[75] Howell: "Amer. T. B. of Physiol.," vol. i, p. 296, 1900.