for instance, states that "the value of the curdling action is not at once apparent," but "we may *suppose*," he adds, "that casein is more easily digested by the proteolytic enzymes after it has been brought into a solid form." Bunge[76] says that "the significance of rennet ferment and casein coagulation in the stomach is still unexplained." Stewart[77] also says that "we have no precise knowledge" as to its exact function.

Conversely we have in the similarity between the blood and milk good ground for the belief that a phenomenon provoked in the former by a given ferment will likewise induce it in the latter. Harris[78] has called attention to the many points of resemblance between milk and blood coagulation. "Both fluids coagulate by an enzyme," says this investigator, "the clot entangling the solid bodies being jelly-like, filling the dish, then contracting and expressing serum and whey respectively. Decalcification of each prevents clotting. In both heat is given out during coagulation. In both a low temperature retards coagulation." He also states, however, that while "blood has all its factors for clotting within itself" . . . . "milk has not."

It is as a nutritional agent that milk differs from blood. This is shown by the relative structure of these fluids. "Milk is a perfect food, but blood is not," continues Harris; "blood-clot has fibers in a felt-work; curd has no visible fibers, but is molecular." Though derived from the blood, in other words, and containing its main components, the structure of milk *as it is provided by Nature*, is eminently fitted for the nutrition of the suckling.

The clot formed in shed milk by rennet becomes, therefore, an adventitious product, and the presence of "rennet" in plants, in the stomachs of animals which are not milk-fed, and in the blood of organs other than the stomach, suggests that we are dealing with a constituent of the general blood-stream irrespective of any specific action of milk.

The true identity of rennin is shown by the nature of the milk clot and its mode of formation.

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76 Bunge: *Loc. cit.*, p. 109.
77 Stewart: *Loc. cit.*, p. 304.
78 Harris: Jour. Anat. and Physiol., vol. xxix, p. 188, 1895.

Referring to milk-curdling, Howell[79] says: "The whole process resembles the clotting of the blood not only in the superficial phenomena, but also in the character of the chemical changes." Granting that caseinogen, as its name implies, is a zymogen or mother substance and that it is an homologue of fibrinogen, we are brought to assimilate rennin to fibrin ferment. Yet, Moro and Hamburger have suggested, on the basis of their experiments, that milk might also contain fibrin ferment. Moreover, we have seen that the presence of oxidase —fibrin ferment—has been made evident by the guaiac test (Arthus), thus confirming Moro and Hamburger's surmise. If coagulation were ascribed to fibrin ferment, therefore, the process would have to be explained by an increase in the relative proportion of the latter in the milk—an obvious fallacy.

Fibrinogen, on the other hand, meets all the needs of the phenomena witnessed. It is not a normal constituent of milk, but a *sine qua non* of the blood coagulation. The fibrin ferment being present in milk, the addition of fibrinogen causes coagulation simply because it re-establishes conditions as they were in the blood-stream—conditions which *it is the function of the mammary glands to prevent*. This explains why "rennin" is found in the blood of some plants, fishes and birds, and in organs which should not contain it according to the prevailing doctrine, and why, also, fibrinogen, a constituent of the general blood-stream, can act as rennin.

Again, fibrinogen is no less able to provoke active fermentation than rennin. The fact observed by Hammarsten,[80] for instance, that one part of rennin can curdle 400,000 to 800,000 parts of milk, is but a parallel of the process evoked when trypsinogen and the nucleo-proteid-laden enterokinase combine to form the trypsin. The mere addition of a small amount of mother substance to a fluid containing ferment suffices to start this action, and the milk's adrenoxidase being the fibrin ferment, a small quantity of fibrinogen (plus calcium salts) suffices to start the process ascribed to rennin, *i.e.*, coagulation. And this process differs in no way from that ascribed to the latter: the caseinogen is split into two substances, the curd (casein) and a soluble proteid, by the fibrin ferment. The only differ-

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79 Howell: "Amer. T. B. of Physiol," vol. i, p. 295, 1900.
80 Hammarsten: Jahrb. u. d. Fort. d. Thier.-Chem., Bd. vii, S. 166, 1877.

ence in the clot is that, as already stated, it is not filamentous as is the fibrinous blood-clot.

This interpretation eliminates all the discrepancies which have attended rennin from the start and which have never been met.

Thus, we have seen that milk, as shown by Arnold, Spolverini, Marfan, Gillet, Wender and others, contains oxidase, *i.e.*, the fibrin ferment, but that it has not been found to contain fibrinogen. This suggests that the fluidity of the milk is a physiological necessity which the glandular elements of the mammary glands insure by preventing the access to it of this substance. Under these circumstances, it becomes evident that: (1) Rennin being fibrinogen, the addition of this body to milk causes clotting because adrenoxidase is present in the latter. (2) The clotting of human milk when hydrocele fluid is added to it (Moro and Hamburger) is due to the fact that the latter contains fibrinogen while the former contains fibrin ferment. (3) The clotting of milk by organic and blood extracts (Edmunds) is due to the fibrinogen combined with adrenoxidase which all such extracts contain. (4) The clotting of milk on adding pancreatic juice (Roberts, Edkins, Halliburton) is due to the presence in the latter of adrenoxidase and of the fibrinogen combined with it. (5) Rennin being fibrinogen, it is a normal constituent of the blood of invertebrates and of the fluids of plants.

On the whole, it seems evident that such a ferment as rennin, for which no specific function has so far been found, does not exist as a separate entity, and that, wherever it is associated with a given process, the rôle ascribed to it can be fulfilled by fibrinogen.

We must not, however, lose sight of the fact that fibrinogen contains several substances. Schäfer,[81] for instance, states that "fibrinogen is probably a mixture, or loose combination, of at least three substances: (1) *Fibrinogen proper*, coagulating at 56° C., (2) the globulin described by Hammarsten and termed *fibrino-globulin*, coagulating at 65° C., (3) a *nucleo-proteid*."

With which of these constituents does rennin correspond?

Rennin is evidently not adrenoxidase, which, we have now

[81] Schäfer: *Loc. cit.*, vol. i, p. 165.

seen repeatedly, resists all temperatures up to 100° C., the boiling point, for Hammarsten states[82] that "if an active and strong infusion of the gastric mucosa in water containing 3 per mille hydrochloric acid is heated to 37-40° C. for 48 hours, the *rennin is destroyed*, while the pepsin remains." In neutral solution, however, rennin is only destroyed at 70° C.

Is it fibrinogen proper?

While fibrin is formed, as is well known, when blood is withdrawn from the vessels, Alex. Schmidt observed that pericardial, ascitic and other fluids did not coagulate spontaneously notwithstanding the presence of fibrinogen in them, but that they did so on the addition of blood-plasma. He attributed this result to a combination of the serum-globulin and fibrin ferment. Hammarsten[83] showed, however, that serum-globulin played no rôle in fibrin formation, and that by half saturating blood-plasma with sodium chloride, a precipitate was obtained, "fibrinogen proper," which by the action of Schmidt's fibrin ferment was converted into fibrin. Fibrin ferment being, as we have seen, adrenoxidase, the formation of fibrin suggests itself as another example of the influence of the adrenoxidase on a zymogen. It becomes a question, therefore, whether fibrinogen proper actually plays the same rôle here that trypsinogen does in the building up of trypsin.

If such is the case, the third constituent of the triad we have met in all ferments so far studied should also be present, *i.e.*, nucleo-proteid. That it is present is strikingly shown by the following quotation: "Fluids which collect in the serous cavities of the body (pericardial fluid, hydrocele fluid, ascitic fluid) frequently contain no leucocytes," writes Schäfer.[84] "When this is the case, they are also *devoid of nucleo-proteid and of the property of spontaneous coagulation, although they contain fibrinogen.*" It is evident, therefore, that coagulation cannot occur without nucleo-proteid. Indeed, coagulation occurs whether this body be derived from the blood or from the cellular elements. Buchanan[85] found that extracts of lymphatic glands and various tissues caused coagulation. "But it is only quite

[82] Hammarsten: *Loc. cit.*, p. 305.
[83] Hammarsten: Arch. f. d. ges. Physiol., Bd. xix, S. 563, 1879.
[84] Schäfer: *Loc. cit.*, vol. i, p. 165.
[85] Buchanan: cited by Schäfer: "T. B. of Physiol.," vol. i, p. 168, 1898.

recently," says Schäfer, "that the active substance extracted by Buchanan has been examined and found to belong to the class of bodies known as nucleo-proteids." From this standpoint, therefore, fibrinogen proper (rennin) asserts itself as a zymogen.

This is shown, moreover, by the chemical connection between nucleo-proteid and fibrinogen proper. These two bodies are closely linked in their solutions and may be precipitated by the same agents. "The nucleo-proteid is precipitated from oxalate plasma by allowing it to stand for twenty-four hours at 0° C.", writes Schäfer. "The addition of acetic acid also throws it down but not in its pure form, for *fibrinogen is carried along down with it.*" This represents undoubtedly the compound met with in all the reactions previously considered, for the same author says, "Fibrin obtained by whipping blood leaves a considerable *phosphorus-containing residue* (*nuclein*) after subjection to peptic digestion."

Rennin, as studied from various standpoints, thus asserts itself as a zymogen *fibrinogen proper,* which becomes active when *nucleo-proteid* and *fibrin-ferment* (adrenoxidase) are present—all constituents of the blood. Hence its behavior precisely as if it were a *bona fide* enzyme such as trypsin—in the light of prevailing views. That no satisfactory function has been found for it in the stomach is also explained: the zymogen itself, *i.e.,* the fibrinogen proper, is not a usual constituent of the gastric juice, although both nucleo-proteid and, as we will see, fibrin ferment are. Indeed, this becomes evident when we take into account the mode of preparation of "rennin," viz., by infusion either of the stomach or of its mucosa, or, in other words, of *tissues* rich in blood and leucocytes, and therefore rich in fibrinogen, including its zymogen, fibrinogen proper. This does not invalidate the fact that gastric juice itself may provoke coagulation, since it contains nucleo-proteid while fibrinogen proper and fibrin ferment are ubiquitous constituents of blood from which its fluids are derived. What it does show, however, is that *the gastric juice does not include as a functional entity an autonomous ferment such as "rennin," which has no existence as such.*

Direct experimental evidence to the latter effect was recently contributed by Pawlow and Parastschuk[86] which led to the conclusion, previously formulated by Danilewsky, that the coagulation of milk is, from the standpoint of the experimenter, an *accidental* expression of a *general* reaction, and that a separate and individual milk-curdling ferment does not exist. They fail, however, to show the *cause* of the coagulation, attributing it theoretically with Danilewsky to some constituent developed during the digestion of proteids by pepsin and forming part of the latter.

My own view that *the coagulation of milk attributed to rennin is in reality due to the ferment which causes coagulation of the blood, and that "rennin" is one of the constituents of this ferment, i.e., fibrinogen proper, a zymogen,* is not only sustained, we have seen by considerable direct and indirect evidence, but the active agent it introduces is one which logically meets the needs of the process.

All this has also served to show (1) *that the zymogen of coagulation is fibrinogen proper;* (2) *that like all other ferments, the coagulating ferment is composed of this zymogen, nucleo-proteid and adrenoxidase;* (3) *that fibrinogen proper not being itself a ferment, it is activated, like the pancreatic zymogens, by the adrenal ferment in the adrenoxidase with which it is combined;* and (4) *that the coagulating ferment acquires its properties as such from the adrenal ferment of the adrenoxidase it contains.*

THE ACTIVE PRINCIPLE OF ADRENOXIDASE AS THE FERMENT OF PEPSIN.

The presence of adrenoxidase and nucleo-proteid in the gastric juice and in pepsin is shown by direct and indirect evidence.

As to adrenoxidase, Schoenbein[87] found that the *gastric juice* gives a blue coloration with guaiac. We have seen, in the case of other ferments, that fermentation in which oxidases took part was attended by an output of CO_2. Bunge[88] states that "the gases which are formed by the process of fermentation in the stomach are *carbonic acid gas,* hydrogen, and marsh gas."

[86] Pawlow and Parastschuk: Zeit. f. physiol. Chemie, Bd. xlii, S. 415, 1904.
[87] Schoenbein: *Loc. cit.*
[88] Bunge: *Loc. cit.,* p. 143.

He also refers to a case of dilatation of the stomach studied by Kuhn[89] in which "one liter of gastric contents developed four liters of gas in four hours when kept outside of the body at the body temperature." This gas was found to contain twenty per cent. of carbon dioxide.

The presence of nucleo-proteid is as evident. Thus Nencki and Sieber[90] found nucleo-proteid in the gastric juice. Moore, moreover, states that while in pure gastric juice the acidity is due chiefly to hydrochloric acid, it is also due "in part, to acid phosphates and phosphoric acid."

The following lines of Hammarsten[91] are striking in this connection, when we consider that the presence of such a substance as adrenoxidase was not suspected by the author: "As chief organic constituent, perfectly fresh gastric juice (of dogs) contains a very complex substance (or perhaps a mixture of substances) which coagulates on *boiling* and which separates on strongly cooling the juice. This substance is considered by certain experimenters (Nencki and Sieber, and Pawlow) as the conveyer of the several *ferment actions* of the gastric juice, *i.e.*, the pepsin as well as the rennin action." By "conveyor" is meant "transmitter," the characteristic property of the adrenoxidase acting as a catalytic in relation to ferments in general, its identity as such being further shown by its coagulation temperature, the boiling point. With Schoenbein's observation that the gastric juice gave the blue coloration to guaiac, the evidence that the gastric juice contains what might be termed "gastrokinase" as a counterpart of Pawlow's enterokinase, may be considered as conclusive. Its purpose is evidently the same as the latter, since, as stated by Landois,[92] "the glands themselves contain no pepsin but only a zymogen," *i.e.*, pepsinogen.

The composition of *pepsin* also coincides with that of trypsin. As to the pepsinogen, Edkins[93] states that "though the chief cells will yield pepsin, yet they do not actually contain pepsin. If the granules then are connected with pepsin, it must be in some antecedent form. The probable explanation

[89] Kuhn: Zeit. f. klin. Med., Bd. xxi, S. 584, 1892.
[90] Nencki and Sieber: Zeit. f. physiol. Chemie, Bd. xxxii, S. 291, 1901.
[91] Hammarsten: *Loc. cit.*, p. 298.
[92] Landois: *Loc. cit.*, p. 293.
[93] Edkins: Schäfer's "T. B. of Physiol.," vol. i, p. 532, 1898.

of this is that the granules of the chief cells consist wholly or in part of *pepsinogen*, the precursor of pepsin." Hammarsten[94] regards as "proved" the statement of Schiff[95] that there exists "a substance forming pepsin, a pepsinogen or propepsin."

The presence of the adrenoxidase is evident. We have already seen that Nencki and Sieber, and Pawlow, have ascribed to what they consider the chief organic constituent of fresh gastric juice, the function of a "transmitter" or catalytic, pepsin being mentioned as one of the bodies influenced. This catalytic body was said to be destroyed when boiled, thus indicating its identity as the active principle of an oxidase, *i.e.*, adrenoxidase. That the latter is intimately connected with pepsin is also shown by the fact that it meets the tests which indicate the presence of its active principle, *i.e.*, that of the adrenal secretion. Thus, Glaessner[96] found in the pyloric end of the stomach what he termed "pseudopepsin," which in turn yielded tryptophan (Neumeister). This substance is even now of unknown composition, though found in the pancreatic juice over seventy years ago by Tiedemann and Gmelin.[97] When compared with the characteristic tests of adrenal extract the correspondence is suggestive: both tryptophan and adrenalin become rose-red on the addition of chlorine water; they both react in the same manner to a bromine solution. Both are but slightly soluble in ordinary alcohol. Again, Hammarsten[98] states that pepsin "is as difficult to isolate in a pure condition as other enzymes." This accounts for the fact that all the soluble ferments, including pepsin, were found by Schoenbein, Flügge, Epstein, Jacobson and others to decompose hydrogen peroxide, and by Schoenbein and others to give the guaiac blue reaction—additional proof that the contaminating substance is adrenoxidase.

As to the nucleo-proteid, Hammarsten[99] states that while an extra-pure pepsin, *i.e.*, that of Pekelharing, "was free from phosphorus and yielded no nucleo-proteid," thus pointing to the latter as a possible contaminating body, "pepsin, according to

[94] Hammarsten: *Loc. cit.*, p. 307.
[95] Schiff: "Leçons sur la physiol. de la digestion," Florence, 1867.
[96] Glaessner: Hofmeister's Beiträge z. Chem. u. Physiol. u. Path., Bd. i, 1901-02.
[97] Tiedemann and Gmelin: "Recherches expér. physiol. et chim. sur la digestion, etc.," 1827.
[98] Hammarsten: *Loc. cit.*, p. 299.
[99] Hammarsten: *Loc. cit.*, p. 300.

Nencki and Sieber, was rich in phosphorus and contained nucleo-proteid."

In the presence of all these tests—those pertaining to pepsin confirming the data afforded by those upon the gastric juice —it is difficult to escape the conclusion that pepsin is built up in the same manner as trypsin, pepsinogen replacing trypsinogen, and that it owes its ferment to its adrenoxidase. We may conclude therefore, (1) *that the gastric zymogen, pepsinogen, is converted into pepsin by combining with adrenoxidase and nucleo-proteid;* (2) *that pepsinogen is activated by the joint action of adrenoxidase and nucleo-proteid;* and (3) *that pepsin acquires its property as a ferment from the adrenal ferment of the adrenoxidase it contains.*

THE ADRENAL ACTIVE PRINCIPLE AS THE FERMENT OF FERMENTS; AND ALL HYDROLYTIC FERMENTS AS COMPOUND BODIES CONTAINING A ZYMOGEN, NUCLEO-PROTEID AND ADRENOXIDASE.

Having ascertained the general composition of the various hydrolytic ferments, it becomes a question whether the conclusions reached are in accord with what data the direct analyses of these ferments have furnished. That they harmonize is shown in the following pages.

B. Moore,[100] in a recent work (1906), says: "Little is known regarding the chemical nature of enzymes, because all attempts to isolate them in a state of purity have hitherto failed. In fact," adds the author, "there is nothing to give certainty that at the end of any process the product in the case of such complicated substances is pure." Still, he also states that "in elementary composition the enzymes do, however, resemble the proteids more than any other class of bodies." As this statement applies to hydrolytic ferments in general, we may infer that all these ferments contain proteids—in accord with the direct evidence I have submitted in the present chapter. Again, Moore refers only to simple proteids while those we found in the various ferments were invariably the phosphorus-laden nucleo-proteid. Recent labors have shown that the proteids bound up with ferments contain phosphorus. "Ma-

[100] B. Moore: Hill's "Recent Advances in Physiol. and Bio-Chem.," p. 117, 1906.

callum has shown microchemically," says an editorial writer,[101] "that phosphorus is closely associated with the formation of zymogen granules in cells, which seem to be started in the nucleus, and there are many other observations suggesting that certain ferments are closely related to the nucleo-proteids. This is particularly true of the oxidases." We may conclude, therefore, in view of the presence of nucleo-proteid in all the ferments studied in this chapter, that the proteids present in the ferments in general, as implied by Moore, are nucleo-proteids.

The presence of zymogens in hydrolytic ferments needs hardly to be emphasized. After referring to the fact that Langley discovered pepsinogen and Heidenhain trypsinogen, Moore says, that "since then, the existence of a pro-ferment [zymogen] has been shown for most of the enzymes." This applies to all the ferments we have reviewed.

The presence of the third constituent, adrenoxidase, may be shown by tracing to its source the characteristic property of ferments, catalysis. Here, again, as we have seen, Moore[102] speaks of ferment actions as being "such catalytic reactions," thus characterizing catalysis as a property of all ferments. To which of the three constituents can we attribute this property?

That the nucleo-proteid need not be considered as a possible factor in this connection is obvious. Neither does the zymogen possess fermentative activity. Thus, Moore writes: "These zymogens, as has been stated, are inactive while in the cell and exist in granular form visible under the microscope; they are converted into the active form, either at the time of secretion or later, on coming in contact with certain substances which have been termed zymo-excitators, or, in certain cases, kinases." He then refers to Pawlow's enterokinase as an example of these agents, and to the belief of this investigator that this body is a "ferment of ferments." It is to enterokinase, therefore, that we are relegated for the agent which confers the properties of a ferment upon the zymogen.

To the questions: what is the agent *in* enterokinase which endows trypsinogen with the properties of a ferment, and what

[101] Editorial, Jour. Amer. Med. Assoc., Feb. 17, 1906.
[102] Moore: Schäfer's "T. B. of Physiol.," vol. i, p. 317, 1898.

is the source of this agent, however, no answer is vouchsafed. These features of the problem do not seem to have, so far, engaged the attention of investigators. Indeed, though Delezenne, Camus and Gley and others were led experimentally to agree with Pawlow as to the effects witnessed, some contend that they are not due to a ferment. A suggestive fact asserts itself in this connection, however, *viz.*, that the main objections that have been raised are annulled when, in accord with my own view, adrenoxidase is considered as one of the constituents of the "ferment of ferments." Thus Delezenne and Dastre[103] deny that it is a ferment, but regard it "as an 'amboceptor,' in the language of Ehrlich, which serves to link together the attacked proteid and the trypsinogen and so invokes the proteid cleavage." Now, we have seen that adrenoxidase *is* Ehrlich's amboceptor and that it becomes linked with trypsinogen to cleave the proteid. If we asked Delezenne and Dastre to point to the constituent of the "amboceptor" which brings about such a result, their answer could only be that of Ehrlich, *i.e.*, that the nature of this body is unknown. "Proteid cleavage" whenever witnessed in the organism is due to hydrolysis provoked by a ferment; can they assert that we have here an exception to this rule? Not without introducing suppositious postulates—a weak weapon to offset the mass of evidence I have adduced in this and the preceding chapter as to the presence of the adrenal active principle in enterokinase. Largnier des Barcels, Biéry and Henri[104] have also pointed out as evidence against enterokinase being an enzyme, that it is "much more slowly destroyed by heat than are most enzymes," all having found that it could resist boiling, and in some instances higher temperatures. We have seen that of all the ferments, that containing the adrenal principle is the only one which remains unaffected by heat up to the boiling point and that it can even resist the latter several hours.

That we are dealing with a ferment is emphasized, moreover, by direct experimental testimony. "Bayliss and Starling," writes Moore, "have brought forward strong evidence in favor of enterokinase being a ferment. Thus, they have shown

[103] Delezenne and Dastre: cited by Moore: Hill's "Recent Advances in Physiol. and Bio-Chemistry," p. 109, 1906.
[104] Biéry and Henri: *Ibid.*

that there is no stoichiometric relationship between the amount of trypsinogen and the amount of enterokinase necessary to activate it, as little as 0.0001 cubic centimeter of an active enterokinase being capable of activating 5 cubic centimeters of pancreatic juice provided it was allowed two or three days to act." This sustains the evidence adduced to the effect that enterokinase is activated by a ferment—that to which secretin owes its activity and the chemical properties of which are precisely those of the active principle of the adrenal secretion. The conclusion is warranted, therefore, that *enterokinase contains a ferment and that it owes its property as a "ferment of other ferments" (Pawlow) to the active principle which its adrenoxidase contains, i.e., that of the adrenal secretion.*

Such being the case, we are brought, by the evidence submitted in this and the previous chapter, to consider the adrenal active principle as the sole ferment of ferments. Not only have we seen that the character of a ferment is determined by its zymogen, but we found that in every instance it was the adrenal principle of its adrenoxidase which endowed the ferment molecule, so to say, with the characteristic of a ferment.

The ability of the adrenal principle to carry on such a process is, in the light of available evidence, beyond question. Moore,[105] we have seen, states that ferments are catalytic agents, adding, however: "But when we say that ferments act catalytically, the problem of how they act is by no means solved; we have merely found a name for it." Now, Loew[106] was led to conclude in the course of an extensive research that "there does not exist a group of organs or any organ, or even a single vegetable or animal cell that does not contain some catalase,"—a conclusion sustained by the testimony of Bertrand, Abelous and Biarnès, Phisalix, Schmiedeberg, Jaquet, Salkowski and other investigators, who found that the oxidases in all plants, invertebrates and vertebrates studied by them acted as catalytics.

A salient feature of the problem left in abeyance so far requires elucidation at this time, *viz.*, the independence of the catalytic action from the oxidizing action of oxidases observed

[105] Moore: Schäfer's "T. B. of Physiol.," vol. i, p. 317, 1898.
[106] Loew: *Loc. cit.*

by many investigators, and which led Jolles[107] to conclude that the blood contained both oxidases and catalase. The reason for this becomes plain—in the light of my views—when it is borne in mind that while the *adrenoxidase* as such is the oxidizing body, its *active principle, i.e.,* the adrenal active principle, is the catalytic. The two separate functions are thus fulfilled by the adrenoxidase, its active principle, embodied in the adrenal secretion, enabling the latter to take up the oxygen in the air of the pulmonary alveoli to transfer it via the red corpuscles and the blood-plasma to the tissues. It is strictly as an "oxygen transmitter" (using Traube's phrase) therefore, that the active principle of the adrenals behaves in the process, *i.e.,* as a pure catalytic.

Finally, Poehl[108] wrote recently, referring to the adrenal principle: "This body brings about reduction processes when present in such very small quantities that I cannot otherwise than see in adrenal (*sic*) a specifically positive katalysator of such processes. If we observe these reactions, we must conclude that we have here no ordinary reagent, that is to say, not a chemical body which enters into a change in which it is consumed itself, but on the contrary, that adrenal is a real katalysator which undergoes no change." Applying this concept to the human adrenals, the strength of my position will become apparent. Indeed, Jolles has not only ascertained, as previously stated, that the human blood is endowed with oxidizing and catalytic properties, but he observed that these properties bear a definite relation to the number of red corpuscles present, being raised in proportion as their number is increased. As I have shown that these corpuscles are storage-cells for adrenoxidase—the albuminous oxyhæmoglobin—the cause of this relationship is self-evident, provided, however, the active principle of the latter—that of the adrenals—is considered as a catalytic.

From all directions, therefore, the evidence available leads to the general conclusion *that the active principle of the adrenal secretion is the only true ferment in the organism, and that all bodies now known as hydrolytic ferments owe their activity as*

[107] Jolles: Münch. med. Woch., Nov. 22, 1904.
[108] Poehl: Indian Lancet, May 23, 1904.

such to the adrenal active principle which one of their constituents, adrenoxidase, contains.

As corollaries to this fact, additional conclusions, based on the evidence recorded in the present chapter, are now in order:

(1) *That adrenoxidase fulfills two functions simultaneously: (a) that of catalytic, carried on by the active principle of the adrenal secretion it contains, and (b) that of oxidizing substance, after the adrenal secretion has become oxygenized in the lungs, and converted into adrenoxidase.*

(2) *That hydrolytic ferments are composed of three substances: (a) a zymogen, which endows the ferment with its specific character; (b) adrenoxidase, which confers upon the zymogen its properties as a ferment and catalytic; and (c) nucleoproteid, the phosphorus of which by combining with the oxygen of the adrenoxidase liberates heat energy and thus governs the activity of the ferment.*

Concluding Remarks.—Since Schwann, in 1836, demonstrated the presence of pepsin in the gastric juice, many theories have been introduced to account for the process of fermentation. "The general point of view regarding the mode of action of enzymes that is most frequently met with to-day," wrote Howell in his text-book,[109] "is that advocated especially by Ostwald. He assumes, reviving the older view (Berzelius), that the ferment actions are similar to those of catalysis." We have seen, however, that, as stated by Moore, this affords only a name for the process. The identity of the catalytic agent, its origin in the body and the manner in which it influences the substances with which it is linked have remained unknown. These three cardinal features are pointed out in the foregoing pages.

Moore[110] has likewise expressed the view that "little is known regarding the chemical nature of enzymes, because all attempts to isolate them in a state of purity have hitherto failed." The data submitted in these pages also indicate why laboratory methods have remained sterile in this connection, *viz.,* that the bodies which have been regarded as ferments (or enzymes) are

[109] Howell: "T. B. of Physiol.," p. 658, 1905.
[110] Moore: Hill's "Recent Advances in Physiol.," &c., p. 117, 1906.

in reality not ferments at all, but compounds of several substances having marked affinity for one another, and formed adventitiously when removed from their normal environment, the living body. As will be shown in the next chapter, one of these substances, *proteid, is a passive constituent of these so-called ferments,* while the three others, the zymogen, the nuclein and the adrenoxidase, are active, the active principle of adrenoxidase remaining, as stated above, the only true ferment.

THE TRIADS.—The compounds now termed "ferments" represent, nevertheless, aggregates of bodies which jointly take part in all fermentative processes, and it is necessary, in order to interpret satisfactorily the functions in which they take part, to treat them as autonomous entities. Especially is this desirable in view of the fact that as such they retain the specific properties now attributed to them as "ferments." In succeeding chapters, however, they will not be referred to as ferments, but as "triads," each being composed of a zymogen, nuclein and adrenoxidase. To indicate the specific action of each triad, the familiar terms "proteolytic," "amylolytic," "lipolytic" and "glycolytic" will be employed as qualifying adjectives, according to whether the food-stuff hydrolyzed is proteid, starch, fat or sugar. Thus the triad which acts on proteids will be referred to as the "proteolytic triad;" that acting on sugars as the "glycolytic triad," etc.

CHAPTER XV.

THE ADRENAL ACTIVE PRINCIPLE AS THE DYNAMIC ELEMENT OF LIFE AND THE GRANULATIONS OF LEUCOCYTES AS THE LIVING SUBSTANCE.

THE LEUCOCYTES AS TISSUE BUILDERS.

Beddard, referring to the end-products of intestinal digestion in Leonard Hill's recently published (1906) treatise, writes:[1] "Our knowledge of the actual path taken by different substances is extremely meagre. We know that the products of fat digestion pass into the epithelial cells, but we know nothing of the path taken by the products of proteid and carbohydrate digestion."

In the first volume[2] I pointed out that the granulations of the leucocytes played a far more important rôle in physiological functions than was credited to them in text-books, and that in all tissues these granulations were a source of energy when brought into contact with the oxidizing substance, *i.e.*, adrenoxidase. The final conclusion submitted, after showing that the leucocytes took up the products of intestinal digestion in the alimentary canal to convert them into granulations which they deposited in the tissues, was that these cells supplied "the entire organism with the agencies which combine with the oxidizing substance to insure the continuation of life and the efficiency of all organic functions." Additional researches have only served to strengthen this conclusion.

A bar to progress in our knowledge of the function of these minute bodies is the prevailing view that "elementary granules [the granulations of leucocytes] are minute particles of proteid matter, probably arising from the *disintegration* of white corpuscles or of the blood-platelets." That leucocytes do not physiologically yield their contents by becoming decom-

[1] Beddard: Hill's "Recent Advances in Physiology and Bio-Chemistry," p. 643, 1906.

[2] *Cf.* vol. i, pp. 677 to 728 in the first three editions.