pulmonary alveoli and to endow the blood with its oxygenizing properties.

ADRENOXIDASE.—As the term "oxyhæmoglobin" includes the hæmatin of the hæmoglobin molecule as well as its albuminous constituent (the oxidizing substance or oxygenized adrenal secretion), it cannot be used to denote the latter, which alone carries on all oxygenation processes. Nor is the term "oxidizing substance" applicable, since it refers to any agent capable of oxidizing. Hereafter, therefore, I will call this body "*adrenoxidase*," a term which embodies four salient features: its origin, the adrenals; its general distribution as suggested by "oxidase;" the identity of its active principle not only as a ferment, likewise suggested by "oxidase," but also as a catalytic, a property common to all oxidases.

CHAPTER XIV.

THE ADRENAL ACTIVE PRINCIPLE AS THE FERMENT OF FERMENTS.

ADRENOXIDASE AS A CONSTITUENT OF ENTEROKINASE AND OF TRYPSIN.

Notwithstanding the considerable work bestowed upon the pancreatic and other intestinal ferments, their nature has remained obscure. Moore,[1] for instance, writes: "Practically nothing is known of the enzymes of the small intestine save their action on foodstuffs, none of them have been obtained in even approximately pure condition, and the fact that they are enzymes rests on the observation (1) that the action is destroyed by boiling and (2) that it takes place under antiseptic conditions." As the oxidizing ferments alone resist heat up to the boiling point; and as Schoenbein[2] found oxidases in all secretions, the likelihood that the adrenoxidase plays an important part in intestinal digestion is very great, especially in view of the fact that all secretions obtain their fluids from the blood. This is further emphasized by the facts that the active principle of adrenoxidase is a catalytic, as we have seen, and that the ferments are known to possess this property. Thus, Moore, after defining the meaning of catalysis, states that "ferment actions are such catalytic reactions."

A zymogen or mother-substance, as is well known, is itself inactive. "The enzymes of the pancreatic secretion are derived from the granules in the cells," says Howell,[3] "but other facts show that the granules do not contain the enzymes as such, but a preparatory material or mother substance to which the name zymogen (enzyme-maker) has been given." Stewart,[4] moreover, states that the "fresh pancreas is devoid of trypsin," but that "it contains a substance which can readily be changed into trypsin; and this substance is soluble in glycerine, for the

[1] Moore: Schäfer's "T. B. of Physiol.," vol. i, p. 341, 1898.
[2] Schoenbein: Jour. f. prakt. Chemie, Bd. lxxxix, 1863.
[3] Howell: "Amer. T. B. of Physiol.," vol. i, second edition, p. 235, 1900.
[4] Stewart: "Manual of Physiol.," fourth edition, p. 324, 1900.

inert extract becomes active when it is treated with dilute acetic acid, or even when it is diluted with water and kept at the body temperature." Of the manner in which this occurs, however, nothing is known.

Recalling some of the properties of adrenal extractives, the process involved in the experiments outlined by Stewart suggests itself. In all processes attended by catalysis water is necessary. Moreover, as shown by Hoppe-Seyler,[5] Nencki[6] and others, fermentative reactions are invariably accompanied by hydration; in other words, as stated by Bunge,[7] "these processes can only take place in the presence of water." Hence, a glycerine extract of pancreatic mother-substance will not act unless water be added to it. Now, we have just seen that such an extract simply "diluted with water" becomes active. This applies equally well to the corresponding effect of the dilute acetic acid referred to by Stewart. The water of the acetic acid solution acts in the same manner, but as dilute mineral acids are even more active diluents for the adrenal extractive than water alone, the operation is facilitated, and a very active ferment is obtained.

This entails, however, the need of oxygen. Sakharoff[8] recently showed that oxygen was a *sine qua non* in the action of ferments. Starch-paste containing diastase and heated to 50° C., but covered with a thick layer of boiled oil, produced no sugar, while a similar preparation, minus oil, *i.e.*, exposed to the air, produced sugar. This portion of the fermentative process evidently begins as soon as the ferment is formed in the organ, since Barcroft and Starling[9] state, basing their conclusion on exhaustive chemical and gasometric experiments, that "the pancreatic secretion is accompanied by an increased oxygen absorption," thus confirming Sakharoff's observation.

That a direct connection between oxygen and trypsinogen in the formation of trypsin occurs, was, in fact, shown by Heidenhain over thirty years ago.[10] Indeed, Edkins[11] includes

[5] Hoppe-Seyler: Pflüger's Archiv, Bd. xii, S. 14, 1876.
[6] Nencki: Jour. f. prakt. Chemie, Bd. xvii, S. 105, 1879.
[7] Bunge: "Physiol. and Path. Chemistry," second American edition, p. 158, 1902.
[8] Sakharoff: Roussky Vratch, Apr. 24, 1904.
[9] Barcroft and Starling: Jour. of Physiol., vol. xxxi, No. 6, p. 491, 1904.
[10] Heidenhain: Archiv f. d. ges. Physiol., Bd. x, S. 557, 1875.
[11] Edkins: Schäfer's "T. B. of Physiol.," vol. i, p. 552, 1898.

among the conclusions reached by Heidenhain the following: "If an inactive glycerine extract of fresh pancreas be dissolved in sodium bicarbonate, 1 to 2 per cent. passing through it of *oxygen* will cause the same to become active." Moreover, "the converse of the change brought about by the influence of oxygen may also occur, for, through the deprivation of oxygen, activity becomes lost."

Closely related to the process is the source of the oxygen thus consumed. Barcroft and Starling found that the "increased oxidation" of trypsinogen "takes place irrespective of increased blood-flow through the organ." This confirms a corresponding observation of O. May[12] that "there is no direct relationship between the rate of secretion of pancreatic juice and the extent of the blood supply." It is evident, therefore, that inasmuch as the quantity of blood supplied to the organ is not increased while the absorption of oxygen is, the source of the excess of oxygen must have been the blood itself. "Excess," in this connection, normally entails the presence of *stored* oxygen, or rather, of stored adrenoxidase, and of storage-cells, the red corpuscles, both of which, as I have shown in the foregoing chapter, are available.

The fact that the blood-flow through the organ is not augmented appears to conflict with the memorable observation of Claude Bernard that increased activity of the submaxillary gland is attended by an increased flow of blood through the organ, but as I have shown in the first volume (page 275), the increased flow is not at the expense of the general circulation, but is due to the shifting of a portion of the blood contained in the arterioles of the organ to the capillaries of its secretory elements. The details of the mechanism will be submitted later. The result as to increased blood in the organ remains the same. Indeed, Landois[13] says, referring to the pancreas, that "during the act of secretion the blood-vessels behave like those of the salivary gland after stimulation of the facial nerve; they are dilated, the venous blood being bright red." The direct participation of oxygen in the formation of trypsin is also referred to by the same au-

[12] O. May: Jour. of Physiol., vol. xxx, p. 413, 1904.
[13] Landois: "T. B. of Human Physiol.," tenth edition, p. 307, 1905.

thor,[14] in the following words: "Trypsin results through the taking of oxygen within the pancreas, from a mother substance, zymogen, which collects in the interior of the secreting cells."

The manner in which the adrenoxidase is brought into contact with the pancreatic trypsinogen has been reviewed at length in the first volume. I may repeat, however, my eighth conclusion (page 405), that "the true secreting cells and those of the islands [of Langerhans] being in continuity and surrounding a common lumen (Opie), both bodies—(1) the zymogen, or trypsinogen-forming granules, and (2) the plasma containing the splenic ferment and the oxidizing substance [adrenoxidase]—meet in this common lumen, which connects with the terminal ramifications of the pancreatic duct." The process of trypsin formation, as I interpret it, thus involves the secretory functions of the spleen, in accord with the views of Schiff, Herzen, Pachon and Gachet and others. "Such a 'charging' of the pancreas by the spleen has been repeatedly suggested by Schiff," says Hammarsten,[15] "and his statements have not only been confirmed by these recent investigations but in part also explained." The more recent experiments of Levene and Stookey[16] afford additional evidence in the same direction.

The functional relationship between trypsin and the intestinal juices, *i.e.*, the succus entericus, includes a kindred process.

An editorial writer[17] recently remarked, referring to the succus entericus, that in the case of trypsin, "it had been found that the presence of a special body is necessary before the ferment is capable of exercising its activity." As to the nature of this agent, he says, "it has now been shown that this transformation is effected by a peculiar substance which has been termed *enterokinase* and which apparently belongs to a special class of cellular products which in themselves are inactive, but are *capable of activating certain ferments.* Of the mode of action and the chemical nature of enterokinase, we know practically nothing, and its origin even has not as yet been definitely ascertained."

Pawlow,[18] who introduced the term "enterokinase" nine

[14] Landois: *Ibid.*, p. 305.
[15] Hammarsten: "T. B. of Physiol. Chem.," fourth Amer. edition, p. 323, 1904.
[16] Levene and Stookey: Amer. Jour. of Physiol., vol. xii, p. 1, 1904.
[17] Editorial, Medical News, Dec. 31, 1904.
[18] Pawlow: "The Work of the Digestive Glands," Eng. edition, p. 159, 1902.

years ago, refers to it in the following terms: "The succus entericus undoubtedly possesses the striking capability of augmenting the activity of the *pancreatic ferments*, and more especially the proteolytic. In the case of the latter, the increase often reaches to an astonishing degree. He who has once convinced himself of this by experiment will never doubt for a moment that this *accentuating* influence is *the most important function of the succus entericus.*" He also remarks: "The application of the usual tests for ferment action—namely, *destruction by boiling*, activity in very small quantities, and so on—convinced us [Schepowalnikow, his collaborator and himself] that in this case we were dealing in point of fact with a ferment. We had, therefore, discovered a ferment, not for this or that constituent of the food, but *a ferment of other ferments.*" *

More recent work has only served to confirm Pawlow and Schepowalnikow's deductions. Thus, Bayliss and Starling[19] were led, by a study of the literature of the subject and comprehensive personal experiments, to conclude that while "trypsinogen is a stable body," . . . . "it is converted into trypsin by the action of enterokinase." They also state that "trypsin is not an expression for two bodies, enterokinase and trypsinogen acting together, but is a third substance produced as a result of the interaction of those two bodies, *i.e.*, *enterokinase* acts on trypsinogen *like a ferment* (Pawlow) *and converts it into trypsin.*" Finally they observe that enterokinase "has an extraordinary power of influencing the pancreatic juice" and that in one experiment 0.0001 cubic centimeter of an active enterokinase "was able to activate 5 cubic centimeters of pancreatic juice in three days."

That enterokinase, the identity and origin of which, we have seen, have remained unknown, contains *adrenoxidase* is shown in various ways. We have seen that while the activity of trypsin rapidly declines after 60° C.—which means that its zymogen, trypsinogen, is destroyed at that temperature—adrenoxidase is only destroyed when the boiling point is reached. Indeed, as observed by Moore and others, its active principle, that of the adrenals, requires several hours' boiling before yielding.

* The italics of the last five words are Prof. Pawlow's.—S.
[19] Bayliss and Starling: Jour. of Physiol., vol. xxx, p. 61, 1903.

Now, enterokinase presents the same peculiarities. Not only, as stated by Pawlow, must the boiling point be reached for its destruction, but as observed by Biéry and Henri,[20] heating it twenty minutes to 120° C.—20 degrees above boiling—may not entirely destroy its action. We have seen also that all adrenal extractives are extremely sensitive to the action of alkalies. "The active trypsin," says Howell,[21] *i.e.*, the trypsinogen plus enterokinase, "is very easily destroyed, especially in alkaline solutions." That enterokinase is, like adrenoxidase, an oxidizing agent, is demonstrated by the experiments of Heidenhain, Sakharoff, and Barcroft and Starling.

Other facts point in the same direction. Schoenbein[22] over forty years ago showed that the blood and the various secretions of animals, as well as the juices of the many vegetable tissues studied, produced a blue coloration with guaiac in the presence of hydrogen peroxide. He ascribed this property to the presence of soluble ferments, trypsin, pepsin, etc. As stated by Oscar Loew,[23] however, "investigations of recent years have shown that the blue guaiac reaction is due to a separate enzyme belonging to a new group of *oxidases*." In the light of the evidence adduced, the "oxidases" were obviously combined with soluble ferments referred to by Schoenbein. Finally, in the first volume (page 729), I conclude that "the oxidizing substance [now adrenoxidase] corresponds with Ehrlich's amboceptor." Benjamin Moore,[24] alluding to the investigations of Delezenne and Dastre and other French observers, says that they "regard the enterokinase 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."

Considered collectively, all these experimental facts indicate that *the substance termed by Pawlow "enterokinase" and also "a ferment of ferments" contains adrenoxidase.*

[20] Biéry and Henri: cited by Benj. Moore, Hill's "Recent Advances in Physiol. and Bio-Chemistry," p. 110, 1906.
[21] Howell: "T. B. of Physiol.," p. 704, 1905.
[22] Schoenbein: *Loc. cit.*
[23] Oscar Loew: "Catalase," U. S. Dept. of Agriculture Rep., No. 68, 1901.
[24] Benjamin Moore: Hill's "Recent Advances in Physiol. and Bio-Chemistry," p. 109, 1906.

ADRENOXIDASE AS "SECRETIN;" ADRENOXIDASE PLUS NUCLEO-PROTEID AS ENTEROKINASE, AND THE ACTIVE PRINCIPLE OF ADRENOXIDASE AS THE FERMENT OF TRYPSIN.

As is well known, the activity of ferments is dependent upon the temperature to which they are exposed. "The digestive enzymes are very sensitive to changes in temperature," writes Moore,[25] "they all act most energetically at or slightly above the body temperature." The words "slightly above the body temperature" are very suggestive when it is recalled that fever and even hyperpyrexia mean a rise of but a few degrees above the normal temperature. Hammarsten[26] states, for example, that "many circumstances exert a marked influence on the rapidity of the trypsin digestion. With an increase in the quantity of enzyme present the digestion is hastened at least to a certain point, and the same is true also of an increase in temperature at least to about 40° C. [104° F.], at which temperature the proteid is *very rapidly* dissolved by the trypsin." He also says, referring to fibrin, the proteid generally used for such experiments: "Very considerable quantities of this proteid body are dissolved by a small amount of trypsin at 37° to 40° C. [98.6° to 104° F.]." When we recall that Metchnikoff found that it was a trypsin which destroyed bacteria in his phagocytic leucocytes, we cannot but admit that there is a striking coincidence between the febrile state and the temperature at which a germ-destroying ferment is very active. This suggests that we are dealing in this connection with an important feature of pathology.

How is the rise of temperature which enhances the efficiency of ferments brought about? In the first volume I ascribed this function to the interaction of the oxidizing substance (adrenoxidase) and another familiar blood-constituent, fibrinogen, a body whose only function is now thought to be concerned with coagulation of the blood.

Fibrin—the identical fibrin obtained by whipping blood—can, as we have seen, be split into two substances, fibrin-ferment and fibrinogen. That the former is the adrenoxidase was

[25] Moore: *Loc. cit.*, p. 320.
[26] Hammarsten: *Loc. cit.*, p. 328.

shown by the facts that it gave the blue coloration with guaiac (Arthus) and that this reaction only failed to occur when it had been heated to the boiling point, *i.e.*, 100° C. (Abelous and Biarnès). Fibrinogen, on the other hand, is, according to Schäfer, probably a loose combination of three substances, one of which alone is related to the question in point, *viz.*, nucleo-proteid, and termed such because it contains nuclein. Now, "the nucleins are *rich in phosphorus,*" says Hammarsten,[27] "containing in the neighborhood of 5 per cent."

That fibrinogen and fibrin ferment (adrenoxidase) unite so readily to form the fibrin of shed blood betokens their mutual affinity. This fact is evidently of paramount importance in the vital process, for the nucleo-proteid constituent of fibrinogen is found as universally in plants and animals as is the oxidizing substance itself. Again, "the nucleo-proteids seem to be widely diffused in the animal body," says Hammarsten.[28] "They occur chiefly in the *cell-nuclei,* but they also often occur in the protoplasm." In a union between the *oxygen*-laden adrenoxidase and the *phosphorus*-laden nucleo-proteid we have a self-evident source of heat. Hence the fact that I ascribed (in the first volume) to a continuous reaction between the fibrinogen and the oxidizing substance (adrenoxidase) in the tissues and the blood-stream, not only the generation of heat in the animal organism, but also that of the heat energy to which ferments owe their exacerbations of activity. Thus a ferment is active in proportion as the relative quantity of fibrinogen and adrenoxidase present is great.

The activity of the reaction which occurs when phosphorus and oxygen are brought into contact under appropriate conditions hardly needs to be emphasized. "Ordinary phosphorus is very oxidizable," writes E. C. Hill,[29] "igniting spontaneously in air at 50° to 60° [C]. At lower temperatures it oxidizes more slowly with phosphorescence." Bunge[30] refers to oxygen in physiological functions as the "most potent source of energy."

The rôle of adrenoxidase is not only shown by the fact that as fibrin ferment it combines with the nucleo-proteid-laden

[27] Hammarsten: *Loc. cit.*, p. 125.
[28] Hammarsten: *Loc. cit.*, p. 56.
[29] E. C. Hill: "T. B. of Chemistry," Philadelphia, 1903.
[30] Bunge: *Loc. cit.*, p. 237.

fibrinogen as soon as blood is shed, but also in that this union can occur in the circulating plasma. This is well illustrated by the presence in the plasma of what have been termed blood-platelets (droplets of adrenoxidase, we have seen) *plus* nucleo-proteid. "According to the researches of Kossel and of Lilienfeld,"[31] says Hammarsten,[32] "the blood-plates consist of a chemical combination between proteid and *nuclein*, and hence they are called nuclein-plates by Lilienfeld, and are considered as derivatives of the cell nucleus." In view of the data I have adduced, they represent a combination of adrenoxidase and nuclein-laden fibrinogen, thus forming an exact counterpart, as to the bodies involved, of coagulation in shed blood. In fact, Hammarsten says in this connection, "It seems certain that the blood-plates stand in a certain relationship to the coagulation of blood." He also states[33] that "coagulation is retarded by cooling" and "by diminishing the oxygen"—additional features in which coagulation corresponds with fermentation.

The active part taken by adrenoxidase in the heat-producing process through its combination with the nucleo-proteid (shed by leucocytes as we shall see) in the blood, is illustrated by the influence of removal of the adrenals, the sources of its active principle, on temperature. That this is markedly lowered was first shown by Brown-Séquard. Schäfer, referring to the experiments of Marinesco, and Vassale and Sacchi, places "diminution of the body temperature" first among the results of bilateral extirpation. Hypothermia, as is well known, is a marked symptom of Addison's disease. Conversely, as previously stated, Oliver and Schäfer, Lépine, Morel and Reichert all observed that adrenal extracts caused a rise of temperature, "accompanied," in the case of the last named observer, "by increased metabolism." This points to the wide-spread character of the action of adrenoxidase, and to the presence in all parts of the body of the nucleo-proteid-laden fibrinogen with which it combines to liberate heat energy.

Indeed, as already stated, nucleo-proteid has been found in all parts of the organism and in enzyme-secreting elements or

[31] Lilienfeld: Dubois-Reymond's Archiv f. Physiol, 1892 u. 1893.
[32] Hammarsten: *Loc. cit.*, p. 186.
[33] Hammarsten: *Loc. cit.*, p. 189.

their products: *e.g.*, in gastric juice by Nencki and Sieber,[34] in the pancreas by Hammarsten,[35] in the liver by Halliburton,[36] in the muscles by Pekelharing,[37] in the heart-muscle by Bottazzi and Ducheschi,[38] in large quantities in the non-striated muscles by Munk and Velichi,[39] and in the brain by Levene,[40] etc. Hammarsten,[41] alluding to the presence of nucleo-proteid in the nervous system, says: "There does not seem to be any doubt that the proteids belong chiefly to the gray substance of the brain and to the axis-cylinders. The same remarks apply to the nuclein, which von Jaksch[42] found in large quantities in the gray substance."

In the intestines—as elsewhere in the body, as shown in the first volume—the nucleo-proteid is supplied by leucocytes and *is secreted by these cells as granulations.* Hardy and Wesbrook[43] state, as the result of personal experiments: "It appears to us to be clear that immigration of the oxyphile cells into the epithelium and thence into the lumen [of the intestine] is a process of constant occurrence, at times so slight as to be barely detectable, at other times so excessive that the epithelium appears to be riddled with these bodies. The most obvious change which the oxyphile cells manifest *within the epithelium* or the *lumen of the gut* is a diminution in the number of *oxyphile granules* even to the total disappearance of these structures." That the granules are *oxyphile* points to their identity as the phosphorus-laden nucleo-proteid granules (Sherrington, Milroy, and Malcolm[44]) which contribute to the formation of enterokinase by combining with adrenoxidase.

On the whole, the evidence available shows that *it is to an exacerbation of the heat liberated by a continuous reaction between the phosphorus-laden nucleo-proteid and the oxygen of the adrenoxidase that the augmentation of heat energy which endows ferments with increased activity should be ascribed.*

[34] Nencki and Sieber: Zeitsch. f. physiol. Chemie, Bd. xxxii, S. 291, 1901.
[35] Hammarsten: *Ibid.*, Bd. xix, S. 19, 1894.
[36] Halliburton: Jour. of Physiol., vol. xiii, suppl., p. 806, 1892.
[37] Pekelharing: Zeit. f. Physiol. Chemie, Bd. xxii, S. 245, 1896.
[38] Bottazzi and Ducheschi: Il Morgagni, vol. xxxix, No. 10.
[39] Velichi: Centralbl. f. Physiol., Bd. xii, S. 351, 1898.
[40] Levene: Archives of Neurol. and Psycho-Path., vol. ii, Nos. 1 and 2, p. 3, 1899.
[41] Hammarsten: *Loc. cit.*, p. 406.
[42] von Jaksch: Pflüger's Archiv, Bd. xiii, S. 469, 1876.
[43] Hardy and Wesbrook: Jour. of Physiol., vol. xviii, p. 490, 1895.
[44] Sherrington, Milroy and Malcolm: Jour. of Physiol., vol. xxv, p. 105, 1899.

Returning to the intestinal pancreatic juice, we find it to possess, in keeping with all fluids endowed with fermentative properties, its nucleo-proteid. The latter was recently found by Stassano and Billon[45] to be *a constituent of enterokinase.* In other words, *enterokinase is a compound of adrenoxidase and nucleo-proteid.*

This leaves adrenoxidase unisolated. Another substance found in the intestinal secretions, however, meets all the chemical tests of the active principle of the adrenals, *i.e.*, that of adrenoxidase.

Bayliss and Starling[46] have given the name "secretin" to "a chemical substance which is formed in the mucous membrane of the upper parts of the small intestines under the influence of acid," meaning by the latter, of course, the hydrochloric acid derived from the stomach immediately above. This was confirmed by the observations of Pawlow, Popielski, Wertheimer and Lepage and others. Although Bayliss and Starling could not "give any definite suggestion as to the chemical nature of secretin," the tests they enumerate are clearly those of the adrenal active principle. Thus, while insoluble in absolute alcohol and ether, it becomes soluble when water is added to the former; the authors state that "a short boiling does not destroy it,"—a characteristic of adrenal extractives as shown by Moore. On evaporating a solution "the activity was found to disappear," a fact which they ascribe "to slow oxidation." The characteristic reducing power is shown by the fact that "the activity of a strong solution is very readily abolished by weak potassium permanganate." Adrenalin is not an alkaloid, according to Takamine, since, among other tests, it is not precipitated by tannin. Bayliss and Starling also say, alluding to secretin: "That it is not of the nature of an alkaloid or diamino-acid is shown by the fact of its not being precipitated by tannin." It dialyses through parchment paper, so do adrenal extractives. It promptly disappears when injected into the tissues —another peculiarity of adrenal extractives, readily accounted for when we consider that it soon becomes oxygen-laden and transformed into adrenoxidase.

[45] Stassano and Billon: C. r. de la Soc. de biol., p. 623, 1902.
[46] Bayliss and Starling: Jour. of Physiol., vol. xxiv, p. 99, 1899; and vol. xxviii, p. 325, 1902.