ferments, or oxidases, the origin or source of which, in animals, has *not been found*. That these are also prominent factors of the vital processes is suggested by Howell's[43] concluding remark, after reviewing briefly the most salient available data, that "such facts as these lend great probability to the belief that eventually it will be shown that the oxidations in the body are effected by the influence of oxidases or peroxidases acting singly or in combination or in sequence with the hydrolytic enzymes."

Again, Moritz Traube,[44] in 1858, called attention to the need of some substance in the blood which could act as intermediary between hæmoglobin and tissue-cells to account for various phenomena witnessed. The term "oxidase" was introduced to describe ferment-like bodies which, without themselves undergoing destruction, could so influence oxygen as to increase greatly its oxidizing activity. In 1858, Traube[45] had already emphasized the need of such a substance to explain the physiological action of the oxygen carried to the tissues by the hæmoglobin—a substance which, he thought, could take up oxygen from the oxyhæmoglobin and transfer it to the tissues, thus acting as an "oxygen transmitter." Experimental demonstration of the existence of a body endowed with such properties was not made, however, until 1876, when Schmiedeberg[46] published his first paper. His investigations, and particularly those of Jaquet and the other chemists referred to above, having shown that the blood-*serum* contained a substance which could absorb oxygen from the air and then surrender it to reducing substances, the requirements of cellular metabolism outlined by Traube were met. It could act, in keeping with the latter observer's conception, as an "oxygen transmitter."

That a ferment was the active factor of the process was demonstrated a few years later. In 1883 a Japanese chemist, Hikorokuro Yoshida,[47] found that the lacquer-forming juice of *Rhus vernicifera* underwent, while hardening, slow oxidation. He ascribed this phenomenon to a diastase which lost its power as such when heated to the boiling point. A French

[43] Howell: "Text-book of Physiol.," p. 836, 1905.
[44] Traube: "Theorie der Fermentwirkungen," Berlin, 1858.
[45] Traube: *Loc. cit.*
[46] Schmiedeberg: Archiv f. exper. Path. u. Pharm., Bd. vi, S. 233, 1876.
[47] Hikorokuro Yoshida: Jour. of the Chemical Soc., vol. xliii, p. 472.

chemist, however, G. Bertrand,[48] succeeded in isolating the ferment itself and termed it "laccase." The juice from which he isolated this body was obtained by incising the tree *Rhus succedenea*. He found that this cream-like juice could be kept in its normal state a long time in well-stoppered bottles, but that as soon as it was *exposed to the air*, it became *brownish*, and soon acquired a thin, intensely black layer, insoluble in ordinary solvents and resisting the action of liquid alkalies and acids, *i.e.*, lacquer. The process was due, as stated by Bertrand, to the very active absorption of oxygen from the air by the laccase and oxidation of the remaining bodies of the juice, collectively known as "laccol." It is plain that in order to do so, laccase acted as "oxygen transmitter."

This latter process, and the fact that oxidation actually occurred, Bertrand was able to prove experimentally. Thus, 1 gramme of hydroquinone in a one per cent. solution, shaken three hours in the presence of but 0.1 gramme of laccase and 174.9 cubic centimeters of air was found to have *absorbed* 25.4 cubic centimeters of oxygen. Even more (32 cubic centimeters) was taken up in a second experiment, in the presence of more air. In another investigation the *carbonic acid output* was also ascertained, the ratios being 23.3 cubic centimeters of O absorbed to 13.7 cubic centimeters of CO_2 output in one experiment, and 20.8 of O absorbed to 16.4 of CO_2 in the second. A large number of plants of various kinds were then analyzed by Bertrand[49] and found to contain this laccase. With Bourquelot,[50] he also found it in mushrooms, in gum arabic, etc. It is through the intermediary of laccase that, for instance, pyrogallol, gallic acid, tannic acid and other familiar substances are oxidized.

A property among others which distinguishes any vegetable or animal fluid or tissue that contains the "oxygen transmitter" is that of causing tincture of guaiac to become blue. This phenomenon was first observed in the nineteenth century by Taddey, Rudolphi, and Planche, the latter observer having found that boiling of an organic substance caused its fluids to lose this property; but it was only when Schoenbein, the discov-

[48] G. Bertrand: Archives de physiol., T. viii, p. 23, 1896.
[49] Bertrand: C. r. de l'Acad. des sci., T. cxxi, p. 166, 1895.
[50] Bourquelot: C. r. de la Soc. de biol., 2e Série, T. ii, p. 579, 1895.

erer of ozone, took up the question in 1856[51] and showed that many plants gave the guaiac blue test, that it began to receive serious attention. Since then, oxidases have been found by various investigators in so many plants that their presence may be regarded as universal. Indeed, referring to an oxidase he named "catalase"—a term which implies the function of "oxygen-transmitter" *i.e.*, *catalysis*—Oscar Loew[52] wrote after a very large number of experiments: "There does not exist a group of organisms or any organ or even a single vegetable or animal cell that does not contain some catalase, as far as the observations of the writer go. This general occurrence of catalase in the organized world cannot be accidental and must have a certain significance." The oxidizing property was combined with that of catalysis (oxygen-transmission) in all these organisms precisely as in the case of the plants studied by Bertrand—and also in that of animals, as we shall see presently.

One still meets occasionally in literature with the statement that respiration in plants is the opposite of that in animals, *i.e.*, that while the latter take up oxygen from the air, plants absorb carbonic acid. Sachs showed over thirty years ago, however, that such is not the case. The absorption of carbon dioxide and excretion of oxygen by the chlorophyll of the leaves is concerned with the nutrition of the plant only; and this occurs in the daylight, while respiration goes on both day and night. The plant takes up oxygen from the surrounding air and gives off carbon dioxide, precisely as do animals. This fact adds greatly to the interest of the experimental data just outlined. Indeed, so strikingly like the respiratory process were the gasometric results obtained by Bertrand that, although his experiments aimed only to establish the identity of the ferment which caused the oxidation of lacquer, he refers to them as being "the first example of diastatic reaction with interchange of gases." He also says that "it is all the more remarkable in that it [the interchange] resembles in a way artificial respiration, and one may at least suppose that it represents a phenomenon very nearly akin to that attending respiration in the vegetable kingdom." As interpreted from my

[51] Schoenbein: Cited by P. Sée: Arch. gén. de méd., July 14, 1906.
[52] Oscar Loew: "Catalase," U. S. Dept. of Agriculture Rep., No. 68, 1901.

standpoint, this does not exemplify *artificial* respiration, but instead, the foundation of the true respiratory process of plant-life.

The blood of many invertebrates, which contains no blood-pigment, hæmocyanin or hæmoglobin, has likewise been found to contain an oxidase, or oxidizing ferment.

Piéri and Portier[53] studied experimentally the blood of mollusks to ascertain whether it contained an oxidizing ferment. Freshly prepared tincture of guaiac had been found by Bertrand to turn blue when in contact with his laccase. The labial palps of acephalic mollusks when dipped in a few drops of water to which one or two drops of tincture of guaiac had been added, showed blue streaks; the water also soon became blue. A similar effect was produced when the juices of a palp were dropped in the same solution. That this was not due merely to oxygen liberated by the living cells is shown by the fact that when Piéri and Bertrand exposed both the palp and the liquid to a temperature of 50° to 60° C., the activity of the oxidizing substance was enhanced. Now, Salkowski[54] has shown that the oxidizing body in the plasma is only destroyed at 100° C. (212° F.), the boiling point, thus identifying it as a ferment. Important in this connection is the fact, previously referred to (page 804), that when the blood leaves the arteries, its oxygen is rapidly exhausted by a plasmatic constituent. This is the substance, as will be shown later, that is destroyed between 50° and 60° C. The conclusion of Piéri and Portier that the effects witnessed were ascribable to an *oxidizing* ferment is thus clearly sustained. The gills, treated in the same way, gave identical results; so did an emulsion of thirty-six macerated sets of gills and palps—a saturated solution of salicylic acid being used in its preparation to eliminate all possibility of bacterial intervention.

These results, as emphasized by Piéri and Portier, could not be ascribed to corpuscular, *i.e.*, iron-containing hæmoglobin. A solution of this pigment, whether prepared with distilled water or with a saturated solution of salicylic acid, turned muddy-red when tincture of guaiac was added thereto.

[53] Piéri and Portier: Archives de physiol., T. ix, p. 60, 1897.
[54] Salkowski: Archiv f. path. Anat., Bd. cxlvii, S. 1, 1897.

Heated to the boiling point a few minutes, a solution (whether prepared with salicylic acid or not) of macerated tissues such as the above no longer gave the guaiac reaction, notwithstanding prolonged shaking in the presence of air. Heated to 90° C., however, a portion of this solution gave a precipitate which, after being rapidly dried on blotting paper and dissolved in distilled water, gave the reaction. A similar precipitate allowed to dry in the laboratory air first became gray, then *black*. Placed in water in this condition, it did not dissolve, and the liquid did not give the guaiac reaction, thus showing that it had been oxidized by taking up the *oxygen of the air*. Precisely the same results had been obtained by Bertrand with laccase.

The gills and palps of mollusks were found particularly active as compared to the blood. As these organs had given a positive reaction with other reagents, guaiacol in concentrated solution in distilled water (Bourquelot[55]) was used as control. This agent gives an orange-red color to a solution containing the oxidizing ferment. The gills of sixteen *Ostrea edulis* (oyster) were left three days in a saturated solution (90 cubic centimeters) of salicylic acid, then filtered. Three cubic centimeters of this extract were then added to a guaiacol solution, and an equal quantity to distilled water. The first solution became red; the second remained clear. These tests were controlled, in turn, by means of two others, the hydroquinone and pyrogallol tests, with positive results. Hashed gills proved as active. Positive results were also obtained with blood (excepting when boiled) from other parts of the body, but Piéri and Portier specify that the *gills* and *palps* respond most actively to the reagents. I have repeated some of these experiments in the clam, oyster and sea mussel, and obtained identical results. The similarity between the chemical properties peculiar to the blood of the respiratory organs of mollusks, the oxidizing ferment and the vegetable ferment laccase, is evident.

Crustaceans were found by Abelous and Biarnès[56] to correspond with mollusks as to the presence of an oxidizing ferment. The hæmolymph of crayfish gave a positive reaction not only with the tincture of guaiac, but also with other reagents

[55] Bourquelot: C. r. de la Soc. de biol., Nov. 7, 1896.
[56] Abelous and Biarnès: Archives de physiol., T. ix, p. 277, 1897.

used. Heating to 60° C. did not prevent the reaction, but the latter no longer occurred after the boiling point had been reached. With Rohmann and Spitzer's reagent (a solution of paraphenylene-diamine), which gives a solution containing the ferment a violet color, Abelous and Biarnès obtained but a very slight reaction with generative organs and muscles, and *decoloration* of the reagent in the case of the liver—due to reduction—followed by intense violet coloration. Both actions of the ferment, reduction and oxidation, were thus manifest. The *gills* showed a marked reaction, and the violet color persisted.

In a second set of experiments, the liver again showed the two phases of action, while other organs responded only slightly or not at all to the guaiac test, but the *gills* became blue *"very rapidly* and *energetically."* The guaiacol, hydroquinone and pyrogallol tests also gave positive results. A precipitate obtained with alcohol, when dried and dissolved in distilled water, gave similar results, thus showing that the investigators were dealing with the typical oxidizing ferment. An important feature of these experiments is that when an extract of gills, a solution of pyrogallol, and water were placed in a tightly closed flask containing air, Abelous and Biarnès ascertained gasometrically not only that oxygen had been consumed, but that *carbonic acid had been evolved.*

The respiratory organs of these crustaceans clearly showed, therefore, that they contained the specific ferment. It became evident, also, that the function of these organs was to absorb oxygen and to transmit it to the elements represented by the pyrogallol in the reaction, namely, the tissue-cells.

That a close connection between the oxidizing ferment and the respiratory process actually exists is emphasized by experiments in vertebrates. In those of Schmiedeberg, Jaquet, Salkowski, Abelous and Biarnès, the tissues of higher mammals, the horse, ox, calf, etc., were used; a fact which suggests that the domain of the oxidizing ferment is limitless in organic life. In batrachians, whose adrenals consist of a narrow patch along each kidney and are connected with the main blood-vessels, arterial and venous, as in man, a direct connection between the oxidizing ferment and "the respiratory function" has been suggested by C. Phisalix[57] (though this author, of course, refers in no way to the adrenals in this connection), as Bertrand had in respect to plants.

Phisalix justly contended that if oxidizing ferments actually presided over the chemical phenomena of respiration, they should be present in tissues, such as the skin, that are capable of carrying on supplementary respiratory functions. He therefore studied the subject in *Rana esculenta* and *temporaria* and *Bufo vulgaris*. The skin of these batrachians was allowed to macerate in salt water and the solution thus obtained was placed in three tubes: the first specimen was boiled; the air in the second was removed and the tube sealed; the third was left open, and its solution exposed to the air. The contents of the first two tubes remained unchanged; that of the third became brown, the color proceeding downward from the surface. After a few days the liquid had become almost black. The connection between these and Bertrand's experiments in plants is obvious. Now, we have seen that boiling destroys the ferment, thus accounting for the first tube's unchanged state; sealing of the second deprived it of air, thus showing that *oxygen* was indispensable to the assumption of the *brown color*. Phisalix further proved the presence of the oxidizing ferment in the batrachian blood by submitting the expressed juice of frog's skins to the guaiac test. The blue color appeared here as it had in plants and invertebrates, thus showing that the ferment could not only absorb oxygen, but also surrender it to reducing agents. The skin typified the lung surface, *i.e.*, the *external* respiration, in these experiments, and the guaiac the tissue elements, *i.e.*, the *internal* respiration.

Additional evidence to the effect that the blood contains an oxidizing substance is that a similar substance is present in the liquid portion of milk, which, as stated in the first volume, corresponds with and is derived from the blood-plasma.

The first to draw attention to these reactions was Arnold,[58] who showed that "fresh cow's milk on the addition of a little tincture of guaiac, gives a *blue* color of varying intensity." He ascribed this phenomenon to the presence of ozone, but subse-

[57] C. Phisalix: Jour. of physiol., vol. xxiii, Suppl., p. 49, 1899.
[58] Arnold: Arch. d. Pharmak., Nu. 41, 1881.

quent labors showed its true identity. Dupouy,[59] Raudnitz,[60] Gillet,[61] Nobécourt and Merklen,[62] and others have also found oxidase similar to that in blood. Spolverini[63] observed that it possessed glycolytic properties identical to those of oxidase.

As we will see farther on, Abelous and Aloy[64] recently found that the *catalytic* action of the oxidizing substance was most active in the absence of air—precisely the condition that prevails in tissue respiration, *i.e.*, cellular metabolism.

Bourquelot[65] showed in 1897 that while milk could not itself oxidize directly, it could act as reducing agent and then oxidize. This action proved to be due to a substance to which he applied the term "anaeroxydase"—*i.e.*, a ferment capable of carrying on the oxidizing process in the absence of air, by means of oxygen derived from other constituents of the medium of which it is itself an occupant.

Returning to the blood of higher animals, we must not lose sight of the fact that all tests indicating the actual presence of an oxidizing substance were based on the action of tissues upon reducing substances. As this might be ascribed to an excess of oxygen in these tissues irrespective of the presence in the blood-stream of any oxidizing agent, it is necessary to show that the blood itself can respond actively to guaiac. We have but to recall that the guaiac test is, in medical jurisprudence, one of the most reliable in the detection of *human* blood-stains, even in the absence of hæmatin, the iron-containing constituent of hæmoglobin. Seifert[66] not only found that an almost colorless solution became "either at once or in the course of a minute or two, intensely blue," but that the test "frequently demonstrates the presence of blood when the result of the spectroscopic test is negative and hæmatin crystals cannot be obtained."

Finally, Jolles[67] has recently demonstrated that the human blood contained both oxidase and catalase (one and the same body, we have seen) and that the catalasic, *i.e.*, catalytic,

[59] Dupouy: Thèse de Bordeaux, 1897.
[60] Raudnitz: Centralbl. f. Physiol., Bd. xii, S. 790, 1898.
[61] Gillet: Jour. de phys. et de path. gén., T. iv, p. 439, 1902.
[62] Nobécourt and Merklen: La presse méd., Dec. 24, 1902.
[63] Spolverini: Atti del iv, Congr. Ital. de Pediat., 1901.
[64] Abelous and Aloy: C. r. de la Soc. de biol., T. lv, p. 891, 1903.
[65] Bourquelot: Jour. de pharm. et de chimie, T. v, 1897.
[66] Seifert: Vierteljahresschrift f. gerichtl. Med., Bd. xvi, H. 1, S. 1, 1898.
[67] Jolles: Münch. med. Woch., Nov. 22, 1904.

power of the blood appeared to have a definite relation to the number of red corpuscles. This suggests clearly a relationship between these bodies and respiration; indeed, Duclaux[68] has expressed the belief that "oxydases are the diastases of respiration."

On the whole, this evidence has been submitted to show (1) *that an oxidizing substance occurs in the blood of all living organisms, i.e., from plant to man;* (2) *that it is a respiratory function that it subserves in both kingdoms, not only in so far as the tissues themselves are concerned, but also in respect to the organs which serve for the absorption of oxygen from the surrounding media: the gills, skin, and lungs;* (3) *that in all organic life, plants, invertebrates and vertebrates, this oxidizing substance absorbs oxygen, and liberates it, thus acting as an "oxygen transmitter," i.e., as a catalyser.*

What is the relationship between this oxidizing substance or oxidase and hæmoglobin?

THE OXIDIZING SUBSTANCE (OXIDASE) AS THE ALBUMINOUS CONSTITUENT OF HÆMOGLOBIN.

Hæmoglobin, as we have seen, occurs only in the blood of animals already far advanced in the evolutional scale, to increase, according to zoölogists, its capacity for oxygen. As red corpuscles appear in still higher organisms, *i.e.*, only in vertebrates—a relatively small proportion of the animal kingdom—it seems evident that they, too, are tardy additions intended still further to increase the blood-plasma's efficiency as an oxygen carrier *pari passu* with the increasing needs of the higher animals. This is a necessary feature of their development, since, as shown by Claude Bernard, Magnus, Lothar Meyer, and Hoppe-Seyler, the blood "holds in solution an amount of oxygen greatly in excess of that which could exist in a state of simple solution." The history of the red corpuscle thus suggests that it acts as a storage-cell for the oxidizing substance, oxidase or catalase, which, in the light of the foregoing evidence, is required to account for tissue respiration.

With which of the known constituents of the blood-plasma or of corpuscles do these oxidizing bodies correspond?

[68] Duclaux: *Loc. cit.*

The identity of this substance suggests itself when a source of confusion is eliminated, *viz.*, the prevailing belief that tissue metabolism is accomplished by, or is due to, oxidation of tissue elements, and that the tissues are the seat of an exchange of oxygen and carbon dioxide similar to that believed to prevail in the lung.

C. R. Barnes, professor of plant physiology at the University of Chicago, wrote recently:[69] "I diligently examined the most modern and most thorough text-books on physiology," naming several familiar to us all, "but in them I found no treatment whatever, indeed no mention whatever, of the real problems of respiration, that is, of what is happening in the tissues, the processes of which these external phenomena are the sign........The respiratory ratio has proved a veritable will-o'-the wisp, leading investigators into a bog where their labors and their thinking were alike futile. For, as a sign of what is going on within, the respiratory quotient is absolutely valueless."

The line of evidence offered by Professor Barnes is particularly applicable in this connection, since it corresponds in a measure with that of Bohr, Haldane and Lorrain Smith, and others, in respect to the pulmonary process: "Von Frey and Gruber[70] showed that in a dog's muscle, with artificial circulation, contractions are accompanied by an increase in the carbon dioxide added to the blood, but they found this increase variable (46-10 per cent.) *and less than the corresponding absorption of oxygen,* so that the respiratory ratio became lowered during contraction. Tissot[71] showed that the production of carbon dioxide in excised muscles was increased if the muscle were killed by heat or were fatigued by prolonged stimulation. The output of carbon dioxide in such cases *was not related to the rate of absorption of oxygen.* Six years ago Fletcher,[72] using Blackman's apparatus, the most intricate and accurate apparatus yet devised for following gaseous exchanges, showed that the evolution of carbon dioxide from excised frog's muscles is *independent of the amount of oxygen taken up during the period.*

[69] C. R. Barnes: Science, vol. xxi, No. 529, p. 241, 1905.
[70] Von Frey and Gruber: Dubois-Reym. Arch. f. Physiol, S. 533, 1885.
[71] Tissot: Arch. de phys., T. vi, p. 838, 1894, and *Ibid.*, T. vii, p. 641, 1895.
[72] Fletcher: Jour. of Physiol., vol. xxiii, p. 10, 1898.

He distinguished, in the production of carbon dioxide, first, a short period (about six hours), which he thinks dependent upon the presence of oxygen; and second, a long-continued evolution of carbon dioxide 'due to chemical processes occurring spontaneously within the muscle, in which complex molecules are replaced by simpler ones, with the conspicuous results of the appearance of [sarcolactic] acid and of free carbon dioxide.' He adds: 'Under suitable conditions the occurrence of active contractions in an excised muscle is *not* accompanied by an increase in the rate at which carbon dioxide is yielded by the muscle,' though oxygen is abundantly supplied then by the blood."

"A great number of researches of the same tenor can be found in botanical literature," continues the author. "A single example must suffice. In an elaborate paper, Purjewicz[73] shows that the variations in the carbon dioxide produced and the oxygen absorbed during a given period under various conditions *are not parallel,* the amount of *carbon dioxide ranging within far wider limits than the oxygen.* Thus, the carbon dioxide varied from 0.14 to 120 per cent. of the average; the oxygen varied from 0 to 48 per cent. of the average. Purjewicz, indeed, expressed his conviction that the respiratory ratio has no value as indicating the actual course of respiration, and would separate the taking up of oxygen and the production of carbon dioxide as two processes indirectly related."

This evidence speaks for itself. There is no more correspondence between the oxygen intake and the CO_2 output in the tissues than there is in the pulmonary process. What is there, however, to replace oxidation?

Armand Gautier,[74] professor of physiological chemistry in the Faculty of Paris, as far back as 1881 called attention to the fact that the truly active and living portion of our cells (the nucleus and protoplasm) carried on its functions without the direct participation of free oxygen, and that it was only outside, as it were, of the protoplasm itself and at the expense of its products, that the combustion phenomena occurred. To this extraprotoplasmic combustion he also ascribed the greater

[73] Purjewicz: Jahr. wiss. Bot., Bd. xxxv, S. 573, 1900.
[74] Armand Gautier: "La chimie de la cellule vivante," Paris, 1881.

part of the body's heat and energy—the phenomena which, owing to their prominence, had alone attracted the attention of physiologists. Indeed, analogy could not but suggest, he thought, the direct participation of oxygen in the intraprotoplasmic processes. Surrounded as it was, *intus et extra,* by oxygen, the animal organism logically suggested itself as the seat of a gradual though ceaseless combustion capable of supplying its heat and power. Gautier showed, however, that the protoplasm of our tissues carries on a function similar to the respiratory process of *anaerobic* bacteria, though unlike the latter, it cannot, from simpler materials, ammoniacal salts and a few other mineral salts, elaborate albuminoid substances. But apart from the fact that it requires the latter ready-made, as it were, it is able to modify them, *build them up* in a complicated fashion and simplify them again without direct oxidation of the materials involved in the process.

Evidence to the same effect is contributed by Barnes: "Pflüger, in the early seventies," says this author, "discovered what seemed a peculiar form of respiration. He found that a frog put into a *vacuum* continued to give off carbon dioxide; and presently the same phenomenon was observed by Pfeffer and others in plants." He also remarks in the same connection: "Plainly the changes that were going on within the organism which enabled it to give off carbon dioxide when no free oxygen was to be had could only be a rearrangement of atomic groups within the molecule and the formation of products which were simpler than those from which they arose." These are adduced by the author as examples of *anaerobic* respiration. After submitting additional testimony, he states that "the analogy between anaerobic respiration and *fermentation* has been suggested early—even by Pasteur—and has thus been growing closer with each added bit of knowledge." Morat and Doyon,[75] in their recently published treatise, also state that "the view that the process which in the human organism provokes a rise of temperature involves the presence of *oxidizing ferments,* is being increasingly accepted"; the present trend being that *"fermentation is the prevailing chemical process in living beings."*

[75] Morat and Doyon: "Traité de physiologie," Paris, 1899-1904.