This coincides with Howell's reference to the great probability 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." If, however, we eliminate therefrom the word "singly" which implies direct oxidation—a fallacy we have just seen—and the words "in sequence," which can only be applicable to catabolism, leaving only "*in combination*," we will be brought within the precincts of demonstrated facts. Indeed, as will be shown in succeeding chapters, there is abundant evidence to the effect that in the tissues as well as in the alimentary canal, the oxidizing substance, or oxidase, acts in combination with hydrolytic enzymes. We have already seen, in the preceding section, that even in invertebrates there are two different substances in the body fluids which respond to the guaiac test: the oxidase, which remained active up to the boiling point, and another which was invariably destroyed "between 50° and 60° C." The identity of the latter suggests itself when we recall that while trypsin is found in all cells, its activity, as stated by Moore,[76] "increases, according to Roberts, with rising temperature, until 60° C. is reached, and then rapidly falls."

In what form does the oxidizing substance take part in this combination? We are no longer dealing with oxygen simply liberated by the red corpuscles, as taught in text-books, but with a substance dissolved in the blood—that which reacts to the guaiac test. As shown by Jolles,[77] the oxidase-catalases (found by Loew, we have seen, in the fluids of all animals and plants studied) are colloid, and are in solution in the blood. Jolles noted, moreover, that they showed, as previously stated, a striking characteristic, *viz.*, that their activity was in definite relation to the number of red corpuscles present. Now, in the first volume (page 715), I had previously shown that droplets derived from the red corpuscles, traced by Hirschfeld from the interior of these cells to the periphery and, through a minute aperture in the latter, to the surrounding plasma, were minute drops of *oxidizing substance*—the so-called blood-plates, platelets, or

[76] Moore: Schäfer's "T. B. of Physiol.," vol. i, p. 337, 1898.
[77] Jolles: *Loc. cit.*

hæmatoblasts of as many authors, who had not discovered their true identity. Jolles thus not only contributes additional testimony to the presence of an oxidizing substance in the plasma, but he emphasizes a fact I had previously pointed out, viz., that it was through the red corpuscles that the blood-plasma was kept supplied, not with oxygen as now taught, but with its oxidizing substance or oxidase.

With what substance known to be present in the red corpuscles does this oxidizing colloid correspond? Gamgee, we have seen in the preceding section, states that "it may be assumed that hæmoglobin exists in the blood corpuscles in the form of a compound with a yet unknown constituent of the corpuscle." Elsewhere, however, he also writes that under the influence of various chemical agents, hæmoglobin "undergoes a decomposition of which the chief products are an *albuminous substance* or substances, *and* a *coloring matter* which contains the whole of the iron originally present in the oxyhæmoglobin or hæmoglobin decomposed." The albuminous substance is evidently the "unknown constituent" referred to above, since he likewise remarks: "As to the true nature of the albuminous residue, we have very little knowledge."

It becomes a question, however, as to which of the two bodies mentioned, the albuminous substance or the coloring matter, is the one capable of leaving the corpuscle, since "coloring matter" suggests that it might be the oxidizing substance. But Gamgee[78] says in this connection: "The coloring matter of the red corpuscles is not extracted from them by the *plasma*;" Schäfer, moreover, states that "it is indiffusible through the unaltered envelope of the corpuscle." It is self-evident, therefore, that it is the albuminous constituent of hæmoglobin that is secreted in droplets by the red corpuscles.

Finally, is the albuminous constituent of hæmoglobin secreted by the red corpuscle the oxidizing substance? Were hæmatin to leave the corpuscles at all, it could not fulfill the rôle of oxidizing substance, for as shown by Hoppe-Seyler (cited by Gamgee[79]), "perfectly pure solutions of hæmatin are quite unaffected by reducing agents." Nor is hæmoglobin, *i.e.*,

[78] Gamgee: *Loc. cit.*, p. 189.
[79] Gamgee: *Loc. cit.*, p. 252.

hæmatin and its albuminous moiety conjoined, endowed with the properties of the oxidizing substance, since, as shown by Piéri and Portier,[80] a solution of this pigment, when tincture of guaiac is added thereto, turns a muddy-red. The oxidizing substance can only be, therefore, the albuminous moiety which is extracted from the corpuscles by the plasma. We have seen that, as shown by Jolles, the activity of the oxidase-catalase is marked in proportion as the number of these cells is great; and also that as stated by Seifert,[81] a solution of human blood becomes in the course of a minute or two, intensely blue, and that this test prevails even when hæmin crystals cannot be obtained.

Summarizing this evidence, and pending additional testimony, we may conclude: (1) *that an exchange of gases does not occur in the tissues any more than in the pulmonary alveoli;* (2) *that tissue metabolism or "respiration" is not due to combustion or oxidation of the tissues;* (3) *that tissue metabolism is due to a process of fermentation in which the oxidizing substance (oxidase, catalase, etc.) takes part;* (4) *that the oxidizing substance required for this function is supplied in the form of droplets (the so-called "blood-platelets" or "hæmatoblasts") by the red corpuscles;* (5) *that the oxidizing substance is the albuminous constituent of hæmoglobin.*

THE RED CORPUSCLES AS STORAGE-CELLS FOR THE OXIDIZING SUBSTANCE.

How is the oxidizing substance held within the red corpuscles pending its gradual distribution? This question brings to light another obscure feature of the problem. Howell,[82] for instance, says: "The point that remains uncertain is the condition in which the hæmoglobin exists within the corpuscle. It is evidently not in solution, since the amount present is too great to be held in solution in the corpuscle, and moreover, even a thin layer of corpuscles is far from being transparent." The answer to this is embodied to a great extent in the data submitted in the foregoing pages: hæmatin is a permanent resident of the red corpuscles; the oxidizing substance only remains

[80] Piéri and Portier: *Loc. cit.*
[81] Seifert: *Loc. cit.*
[82] Howell: *Loc. cit.*, p. 385.

within its precincts until a substance having a very marked affinity for its oxygen "extracts" it, in the form of droplets—the minute "blood-platelets." These, as emphasized in the preceding section, are in reality droplets of oxidizing substance, *i.e.*, of oxygen-laden adrenal secretion. As the latter leaves the adrenals in the form of colloid granules and is absorbed by the red corpuscles, the hæmoglobin in the cells meets the conditions defined by Howell: the 94 per cent. of oxygen-laden adrenal secretion they contain besides their hæmatin (which gives the blood its red color) is not in "solution;" it is composed of hyaline masses sufficiently viscid when secreted by the adrenals to have suggested the term "protoplasmic masses" to Gottschau.[83] Indeed, as stated by Landois,[84] hæmoglobin "is a colloidal substance."

Again, text-books of physiology teach that it is the iron of the hæmoglobin that takes up the oxygen of the air. We have seen, however, that in all the higher animals the substance which fulfills this function is the albuminous portion of hæmoglobin, that which contains *no* iron. This is further shown by the fact that it is this albuminous body which in the blood fulfills the rôle of catalytic or "oxygen transmitter," a rôle as clearly carried on in plants and invertebrates, in which no hæmatin is present, as it is in animals supplied with this iron pigment. We have seen, also, that it is the substance which is *outside* the red corpuscles, this same albuminous substance, which turns guaiac blue; it is obviously not the hæmatin, since this pigment remains *within* the corpuscles. Finally, if the iron-laden hæmatin were the substance which absorbs the oxygen of the air to supply the tissues, it should also be capable of oxidizing guaiac, *i.e.*, of turning it blue; but it does not; even when mixed with the albuminous substance it turns guaiac a muddy-red. Finally, the albuminous constituent is evidently the familiar carrier of oxygen, oxyhæmoglobin, for as stated by Hammarsten,[85] the substance has "a direct action upon tincture of guaiacum."

This apparently leaves the hæmatin without function, since

[83] Gottschau: *Loc. cit.*
[84] Landois: *Loc. cit*, p. 51.
[85] Hammarsten: "Text-book of Physiol. Chemistry," fourth edition, p. 169, 1904.

the only rôle ascribed to it at present is that of taking up the oxygen of the air. Its history suggests, however, that it is endowed with an important though different rôle. Indeed, we have seen that hæmoglobin occurs in relatively few invertebrates, whereas it is present in practically all vertebrates, *i.e.*, at a stage of the animal scale where the blood's volumetric capacity must be greatly increased to satisfy the needs of greater aggregates of cell colonies such as those of which the higher animals are composed. In the preceding section I termed the red corpuscles "storage-cells." Indeed, their advent coincides with a time when, although hæmoglobin is present, its mere dissolution in the blood fluids—as it is in some Annelides—would fail to supply enough oxygen to sustain all the vital functions of the organism. Griffiths,[86] for instance, says: "In the higher animals the corpuscles are of two kinds, red and colorless; but in the Invertebrata there are, as a rule, only colorless corpuscles." A rôle such as that I ascribe to the red cells, therefore, is a logical feature of animal evolution, since by storing a large quantity of the oxygen-laden adrenal secretion or oxidizing substance, these cells meet the needs of advanced development. This involves, however, the presence, in the corpuscle, of a body capable of anchoring the oxidizing substance. Hæmatin is not only endowed with the properties required to fulfill this rôle, but there is in the red corpuscle no other substance to assume it.

That hæmatin has considerable affinity for oxygen is sufficiently emphasized by the fact that physiologists have long held and now teach, that its iron (Howell[87] states that hæmatin "contains all the iron of the original hæmoglobin molecule") combines with the oxygen of the air, to carry it to the tissues. Given, therefore, a substance such as the *oxygen-laden* adrenal secretion in the blood-stream, and coincidently red corpuscles containing little else than hæmatin, after circulating in the whole body this hæmatin should reduce the oxygen-laden adrenal secretion—provided the hold of the latter upon its own oxygen is sufficiently loose. We have seen, however, that such is not the case, and that the affinity of the adrenal secretion for oxygen

[86] Griffiths: *Loc. cit.*, p. 125.
[87] Howell: *Loc. cit.*, p. 390, 1905.

is very marked. Hæmatin does not deprive the adrenal secretion of its oxygen, but through its own affinity for the latter draws *within the corpuscle* both this gas and the adrenal secretion. The latter, after its exposure to the air of the alveoli being, as we have seen, the albuminous constituent of hæmoglobin, it serves, on entering the corpuscle and combining with the hæmatin, to build up the hæmoglobin molecule. The hæmatin is evidently capable of thus causing an enormous proportion of the oxygenized adrenal secretion to enter the cell, for the albuminous portion of hæmoglobin represents 94 per cent. (Gamgee) of the whole. This clearly points to hæmatin as the agent which causes accumulation of the oxidizing substance in the red corpuscle and to the latter as a storage-cell.

Evidence to this effect is also afforded by the fact that such a function accounts for the existence of the albuminous component of hæmoglobin. We have seen that Gamgee characterized this body as the "unknown constituent of the hæmoglobin molecule." The iron of the hæmatin being solely credited with the rôle in respiration ascribed to hæmoglobin as a whole, this "unknown constituent," though it represents *94 per cent.* of the entire molecule is devoid, according to present teachings, of all function! Again, as Hammarsten[88] says, hæmoglobin "occurs only in very small quantities in arterial blood, in larger quantities in venous blood." Why should such be the case if the iron of the hæmatin alone carries on the respiratory process? The excess of hæmoglobin in the venous blood—which necessarily applies to the albuminous portion, since the iron does not leave the corpuscles—is another feature left in abeyance by the prevailing teachings. With the red corpuscles as storage-cells, however, the reason for this becomes self-evident: In arterial blood the albuminous portion of the hæmoglobin, *i.e.*, the oxygenized adrenal secretion, is stored in the corpuscles; gradually, as it leaves these cells in droplets, we have seen, to circulate in the *minute capillary networks of the cellular elements into which the red corpuscles do not penetrate,* it passes on, as a worn-out substance, to the veins. Briefly, in the arterial blood, it is stored in the corpuscles, while in the venous blood it is free—though deprived of oxygen.

[88] Hammarsten: *Loc. cit.*, p. 170.

It becomes a question now as to where the red corpuscles are charged, as it were, with their oxygen-laden albuminous constituent, *i.e.*, their oxyhæmoglobin. In the first volume (page 145), I submitted the following conclusions: "(1) When the secretion of the adrenals reaches the pulmonary alveoli, it absorbs oxygen from the air and forms with the latter a compound or 'oxidizing substance.' (2) A part of this oxidizing substance is absorbed by the hæmoglobin of the corpuscles and the balance remains in the blood-plasma." All the evidence collected since has only served to confirm these conclusions.

The course of the adrenal secretion from the adrenals to the lungs having been given in detail in the second section of this chapter (page 806), the changes it undergoes when it reaches the alveoli need alone be reviewed in the present connection.

The need of a secretion to account for the respiratory process pointed out by Bohr and subsequently defended by his collaborator, Henriques, Haldane and Lorrain Smith, and Harley, has steadily gained ground of late. Pembrey, in a very recent (1906) publication,[89] after a careful review of the respiratory process, concludes: "The body of evidence has thus been steadily increasing in favor of the secretory theory, especially as regards the absorption of oxygen." We have seen, however, that for want of a known secretion to account for the phenomena witnessed, Bohr assumed that the lungs themselves supplied the secretion—a view sustained in no way by experimental facts. We have seen, on the other hand, that my own view that the secretion of the adrenals fulfills this function is backed by very strong testimony from whatever direction the question as a whole is considered.

Once in the close capillary network of the alveoli, the adrenal secretion is only separated from the air they contain by an extremely thin layer composed of delicate alveolar membrane and its alveolar epithelium, which together are barely 0.001 millimeter thick. Indeed, according to Böhm, Davidoff and Huber,[90] the capillary network, "which is extremely fine," is "sunken into the epithelium." Now it is *in these extremely fine capillaries that the red corpuscles containing the hæmatin meet the adrenal secretion.* Both being reducing agents, they would simultaneously combine with the oxygen of the alveolar air and remain apart were it not for an important fact, *viz.*, that the adrenal secretion, besides being free in the blood, is much more energetic as a reducing agent than the hæmatin within the red corpuscles. As a result, the secretion alone absorbs the oxygen of the air through the delicate alveolar membrane, and the blood in the capillary network is thus constantly saturated, so to say, with oxygen-laden adrenal secretion. It is at this stage that the corpuscular hæmatin comes into play. The red corpuscles—each of which, as stated by Howell, "forms a meshwork or spongy mass"—being surrounded by this blood, their hæmatin absorbs oxygenized secretion until replete, and ready, therefore, to carry on their active function as constituents of the arterial blood, laden as they are with oxyhæmoglobin.

[89] Pembrey: Hill's "Recent Advances in Physiol. and Bio-Chemistry," p. 549, 1906.

[90] Böhm, Davidoff and Huber: "Text-book of Histology," second edition, p. 316, 1905.

That a reducing agent actually exists in the lungs was emphasized by the labors of Bohr, Haldane and Smith, and others. Garnier,[91] twenty years ago, showed that a solution of ultramarine blue was decolorized when sprayed into the lungs. This can only be produced by a powerful reducing agent. Carbonic acid and taurin, the only two possible components of the alveolar fluids which might act as such, failed invariably to produce such an effect on the solution. Pembrey[92] also writes: "A still further piece of evidence in favor of the secretory theory is the great capacity of the pulmonary tissue to *reduce* alizarin-blue when, as in Ehrlich's experiments, it is injected into the living body, and air is still passing in and out of the lungs."

That this reducing agent is the adrenal secretion is shown in various ways. Oxyhæmoglobin is "readily decomposed," as stated by Hammarsten,[93] by alkalies. Moore and Purinton[94] and others emphasize the fact that the active substance of the adrenals is rapidly destroyed by alkalies. Hammarsten also says that "oxyhæmoglobin is insoluble in ether, chloroform, benzene and carbon disulphide." Vulpian[95] found the expressed juice of the adrenals insoluble in ether and benzene.

[91] Garnier: C. r. de l'Acad. d. sci. de Paris, July 26, 1886.

[92] Pembrey: *Loc. cit.*, p. 549.

[93] Hammarsten: *Loc. cit.*, p. 169.

[94] Moore and Purinton: Amer. Jour. of Physiol., vol. iii, p. xv, 1900.

[95] Vulpian: C. r. de l'Acad. d. sci. de Paris, Sept. 29, p. 663, 1856.

Moore[96] states that the adrenal active agent is insoluble in ether, chloroform and carbon disulphide. Throughout the various stages of organic life, we have seen, the oxidizing substance, *i.e.*, the oxyhæmoglobin, resists all temperatures up to the boiling point. Cybulski,[97] Moore, and others observed that boiling alone annulled the activity of the adrenal extractives. In other words, the chemical properties of the adrenal secretion are similar to those of free oxyhæmoglobin, the product it becomes, as I pointed out in the first volume, after traversing the alveolar capillaries.

Griffiths states that in *Annelida* the hæmoglobin is dissolved in the fluid Huxley called "respiratory blood," and "does not belong to the corpuscles." Nor does it in man, in whom these corpuscles act only as storage-cells.

This evidence seems to me to warrant the following conclusions: (1) *that it is not the iron of the hæmoglobin as now taught which combines loosely with the oxygen of the air to carry it to the tissues;* (2) *that this function is fulfilled by the oxidizing substance alone;* (3) *that the red corpuscles are storage cells for the oxidizing substance, i.e., the oxygenized adrenal secretion;* (4) *that the corpuscular hæmatin, owing mainly to its iron, is the substance which in the corpuscles acts as storage material;* (5) *that the affinity of its iron for oxygen causes it to combine loosely with the oxygen of the oxidizing substance, as fast as the adrenal secretion is being converted into the latter in the alveolar capillaries;* (6) *that this process entails the absorption into the red corpuscles of all the oxidizing substance they can accommodate;* (7) *that the red corpuscles do not, as now taught, supply free oxygen to the tissues through the intermediary of the plasma;* (8) *that droplets of oxidizing substance are abstracted from the red corpuscles, when any substance having greater affinity for its oxygen than the hæmatin appears in the blood-stream;* (9) *that it is the albuminous oxidizing substance* (*oxyhæmoglobin*) *itself which is absorbed by the tissue-cells.*

To sustain adequately these conclusions, however, it is necessary to show that the oxidizing substance is present in all parts of the organism and that its chemical properties coincide wherever found with those of the adrenal secretion.

[96] Moore: Jour. of Physiol., vol. xvii, p. xiv, 1894-95.
[97] Cybulski: *Loc. cit.*

THE ADRENAL SECRETION AS THE CONSTITUENT OF HÆMOGLOBIN WHICH, WHEN OXIDIZED, PRODUCES BRONZING.

As the albuminous constituent of hæmoglobin, the secretion of the adrenals must necessarily invade all tissues. We have additional evidence to this effect in the pathology of Addison's disease, and particularly in that of its characteristic symptom, bronzing, which, as is well known, may invade the entire surface of the body and all exposed mucous membranes. We are again, however, brought face to face with an unknown factor in this connection, *viz.*, the true identity of the bronze pigment and its origin. Indeed, Hammarsten[98] says that "so little is known about the structural products of melanins or melanoids that it is impossible to give the origin of these bodies." In a special study of these pigments, Walter Jones[99] also states that "they have been the subject of a number of researches of chemical nature, yet for some reason these researches have been so fruitless that at the present time we are not in a position even to define a melanin in a chemical sense; in fact, we are not all agreed as to what chemical elements are necessary constituents of the melanin molecules."

Alezais and Arnaud,[100] Marino-Zucco,[101] and Boinet,[102] found dark pigment in various organs after injuring or removing the adrenals. In Boinet's experiments this pigment proved to be identical with "bronze" pigment obtained from the skin, mucous membranes and other structures derived from two fatal cases of Addison's disease. As I pointed out in the first volume, bronzing occurs in this affection only when the lesion of the adrenals is *far advanced*, each of these organs (an indication of their important physiological function) being supplied, as shown by Langlois, Gourfein and others, with ten or eleven times the quantity of medulla required to sustain life. Lesions may thus be found *post-mortem* in the adrenals and no bronzing occur during life, merely because the local changes are not sufficiently advanced, while, conversely, a lesion of the nervous or

[98] Hammarsten: *Loc. cit.*, p. 592.
[99] Walter Jones: Amer. Jour. of Physiol., vol. ii, p. 380, 1899.
[100] Brown-Séquard: C. r. de la Soc. de biol., 1892.
[101] Marino-Zucco: Arch. Ital. de Biol., vol. i, 1888; and Riforma Medica, vol i, p. 759, 1892.
[102] Boinet: Marseille méd., Apr. 15, 1896.

vascular supply of the adrenals may so inhibit their functions, without entailing discernible local changes, as to cause marked bronzing. Under these conditions, any advanced lesion of the adrenals should give rise to bronzing irrespective of Addison's disease. Several such instances have been reported by E. Sergent and Leon Bernard[103] and others, besides those referred to in the first volume of this work.

In the first volume, I also stated (page 93) that bronzing was attended with "hæmoglobin disintegration." The pigment found by Boinet in his decapsulated rats, and corresponding to the "bronzing" pigment, presented microscopically all the characters of hæmatoidin, a substance also found in old blood extravasations and in apoplectic clots, and which, as stated by Gamgee, is "certainly derived from *hæmoglobin*." Melanin "may be uniformly regarded," according to Charles,[104] "as a derivative of the blood pigment." In a recent work, E. C. Hill[105] also states that "it is derived from the blood pigment."

Chittenden and Albro,[106] however, state that the presence of *sulphur* "in very appreciable amounts constitutes one of the reasons for the belief that these substances have their origin in some proteid antecedent, while the *absence of iron,* in most cases, *excludes* the view that they originate from the blood pigment." That such is not the case, however, may be shown by the evidence these investigators adduce to support their conclusion. Hæmoglobin, we have seen, may be split into hæmatin, which contains all the iron, and the albuminous body, *i.e.*, the oxidizing substance. Since, as shown, all the iron remains in the corpuscles, it is self-evident that melanin may not contain iron and still form part of the hæmoglobin molecule. As stated by Chittenden and Albro, melanin contains sulphur. This necessarily refers it to the *albuminous* constituent of hæmoglobin also, since Gamgee states that "the *sulphur* belongs to the albuminous part of the molecule." Again, the presence of this element indicates that "hæmoglobin belongs to the *proteid* compounds" (Halliburton[107]), and inasmuch as it is the albuminous body which contains the sulphur, it is this body

[103] Sergent and Bernard: Arch. gén. de méd., p. 27, July, 1899.
[104] Charles: "Elements of Physiol. and Path. Chemistry," p. 284, 1884.
[105] Hill: "T. B. of Chemistry," p. 374, 1903.
[106] Chittenden and Albro: Amer. Jour. of Physiol., vol. ii, p. 291, 1899.
[107] Halliburton: Schäfer's "T. B. of Physiol.," vol. i, p. 27, 1898.

which is of proteid nature. It is evident, therefore, that Chittenden and Albro's conclusion that melanin does not originate from blood-pigment, cannot hold.

Melanin remaining, therefore, a constituent of hæmoglobin, *i.e.*, the albuminous constituent I have identified as the oxidizing substance, there is good ground for the belief that it contains adrenal secretion. This postulate acquires additional strength in view of the cardinal rôle I have ascribed to this secretion in tissue metabolism, and the fact that the skin-fluids—of batrachians at least—show by their positive reaction to the guaiac test that the oxidizing substance is present in them.

Points of analogy between hæmoglobin and melanin on the one hand, and the oxidizing substance considered as active through its adrenal secretion, on the other, are discernible in various directions. Much of the chemical work done on the melanins, however, is misleading in that possible sources of error were not taken into account. "If we consider for a moment the *methods* which have been employed for the isolation and purification of the pigment," says Walter Jones, "and at the same time grant that these substances may be *very sensitive* to the action of chemical reagents, and that it is also within the bounds of possibility that the composition of the pigment, like that of hæmoglobin, is different for different animal species, we will then be in a position to appreciate just such a discordance of analytical results as that which actually exists." This remark applies forcibly to the question in point, for if melanin should prove to be the catalytic body it now appears to be, the promiscuous use of reducing and oxidizing agents cannot but have introduced contradictory results at every turn.

The marked sensitiveness to the action of reagents to which Walter Jones alludes is confirmed by Abel and Davis,[108] who found that the composition of melanins isolated by them was "easily subject to change by subsequent treatment of the material with alkalies." This same sensitiveness attends the albuminous constituent of hæmoglobin; thus Gamgee refers to it as being "characterized by remarkable instability." Strong alkalies that cause the hæmoglobin molecule to break down also annul the activity of adrenal extractives: "The use of alka-

[108] Abel and Davis: Jour. of Exper. Med., vol. i, p. 381, 1896.