The principal feature, however, is the high stress intensity in the tyre, which, over a spoke, has a maximum intensity at its outer edge, and a minimum at the inner edge, as shown in Fig. 2, *b*, while midway between the spokes this state of stress is reversed, as we then find a maximum stress at the inner edge with a corresponding minimum at the outer circumference.

In railway work it is the usual practice to force the axle into the wheel centre first, and then shrink on the tyre. When this is accomplished, a stress distribution is found of the kind shown in the photograph (Fig. 1, *c*), in which both the hub and the tyre are highly stressed, while the spokes are still only moderately loaded. As will be noticed here, the two outer groups of stress difference zeros are still present, although in slightly different positions radially. This is seen more clearly in Fig. 2, *c*, where the measured principal stresses P and Q are shown with reference to a radial plane through the centre of a spoke.

These distribution curves also show that the combined effect of both axle and tyre is to increase the radial stress on the axle greatly. In the tyre, increased stress is also apparent, although it remains of the type already described with reference to Figs. 1, *b*, and 2, *b*. This latter distribution is more particularly interesting in its application to much larger wheels, such as the driving wheels of locomotives, where, on account of their size and the necessity of supporting the tyre at short intervals, the number of spokes is much increased, and since, unlike wagon wheels, they are required to exert great tractive force at the rails, it is found necessary to fasten the tyres more securely to the rim of the wheel centre by additional devices, in order to prevent circumferential slipping.

Formerly it was the practice to screw radial studs D (Fig. 2, *c*) through the rim of the wheel centre and into the tyre at places midway between the spokes, as indicated here, thereby making holes in the tyre at places where the stress is already intense, and therefore adding to it very greatly, owing partly to the lessened cross-section of the tyre, and also to the effect of the discontinuity, necessitating changes of direction of the tensional stress in passing round the hole so formed. This practice has now been given up in favour of transverse rivets E inserted in holes made in the wheel centre rim, very close to the stress difference zeros C, and passing through a flange formed on the tyre, which is now constructed to lap over the rim, as indicated by a dotted line in Fig. 2, *c*.

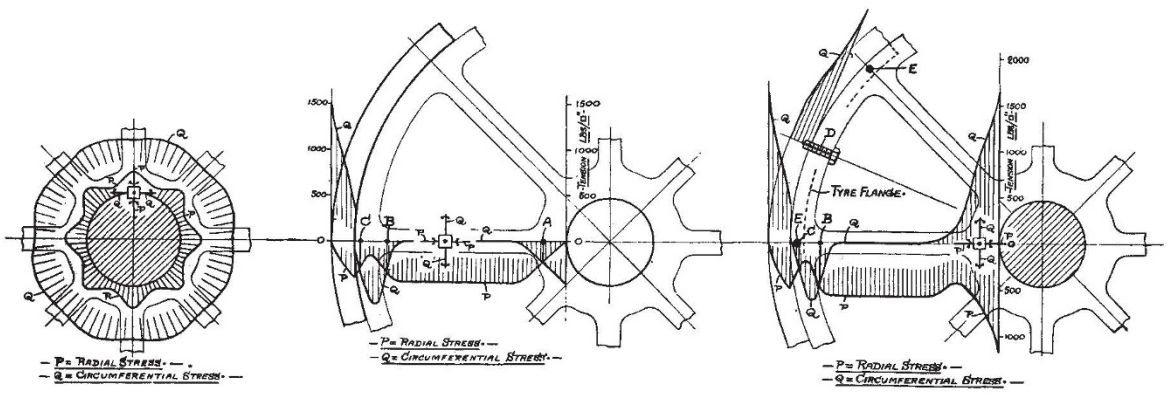

FIG. 2.—*a*: Stress distribution in the hub of a wheel at the axle, when the latter is forced on. *b*: Stress distribution in a wheel, along the line of a spoke, when a tyre is forced on to a wheel centre. *c*: Stress distribution in a wheel when the axle has been forced into the wheel centre and the tyre shrunk on.

The photoelastic effect shows clearly the advantage of this method of fastening, for the discontinuity now produced by boring a hole at a place near the position of the stress difference zero C, where the principal stresses P and Q are both small, only causes a very moderate increase in both stresses, while no inroad is made into the main cross-section of the tyre. Practical experience has shown that tyres fastened in this way are much stronger and safer than those secured to the wheel centre by the older method, and the advantages of this new form of fastening have secured its very general adoption.

Oxidation by Living Cells.*

By Prof. J. B. S. HALDANE.

UNTIL recently our knowledge of the chemistry of respiration stopped abruptly at the boundary of the cell. We knew how the oxygen was carried to it in vertebrate blood, and the carbon dioxide carried away. We also knew that the rate of oxygen consumption by the body as a whole, and by certain organs, was a function of numerous variables, such as temperature, hydrogen ion concentration, nervous stimulation, and so on. A certain number of partially oxidised metabolites, such as β-hydroxybutyric acid, had been isolated. But such quantitative knowledge as existed with regard to the details of oxidation was mainly confined to reactions in which coloured molecules were

* Substance of lectures delivered at the Royal Institution on Feb. 5, 12, and 19.

involved: for example, the reduction of methylene blue to a colourless substance, or the oxidation of *p*-phenylene-diamine to a coloured one.

The modern period began with the work of Batelli and Stern, and of Bach and Chodat, in Geneva, and since the War the most important centres of research have been the laboratories of Thunberg in Sweden, of Warburg and Wieland in Germany, and of Hopkins in England. This work has led to the recognition of a number of distinct catalysts, each responsible for a different part in the process of respiration. Inorganic catalysts of oxidation may activate the oxidant, the reducer, or both. Thus, Langmuir concluded that when a hot platinum surface catalyses the union of hydrogen and oxygen, a layer of adsorbed O_2 molecules is so activated as to unite with H_2 striking them; but adsorbed H_2 does not unite with bombarding O_2. On the other hand, when the same reaction is catalysed by porcelain, both molecular species must be adsorbed side by side before they react.

We need not, therefore, be surprised to find in the cell catalysts which activate O_2, alongside of activators of reducers such as the lactate ion, and shall be prepared to steer a course between the unitary theories of Warburg [1] and Wieland,[2] who respectively regard the activation of oxygen and of reducers as the fundamental feature of respiration.

When an enzyme catalyses the reaction between two molecular types, one very restricted, the other very general, we describe it as specific for the former. Thus Dixon [3] and Coombs [4] found that xanthine dehydrogenase catalyses the reaction $AH_2 + B = A + BH_2$, where A must be one of a small number of purine bases (it is possible that the same enzyme also activates aldehydes; if so, they are oxidised at about one per cent of the rate of the purines). But B may be oxygen, iodine, nitrate, permanganate, or any of a large number of dyes, such as methylene blue. These latter are perhaps all held on the enzyme surface near the former, but it is difficult to imagine that there is a single molecular grouping responsible for activating all of them. For reaction it is not sufficient that a molecule should be united with the enzyme; it must be activated as well. Thus, uric acid unites with xanthine dehydrogenase at the same spot as xanthine, thus inhibiting its oxidation, but is not oxidised, though another enzyme can accomplish this process.

A large number of dehydrogenases are known which act in a similar manner, each causing the activation of one or more organic substrates. Thus, lactic dehydrogenase, which can be obtained in solution from a number of sources, activates several α-hydroxyacids; succinic dehydrogenase, another enzyme easily obtained in solution, activates succinic and methyl-succinic acids; and so on. The activity of these enzymes is generally measured by the rate at which they catalyse the reduction of methylene blue by their substrates. They usually have a wide range of optimal *p*H from about 7 to 10, instead of a small range like hydrolytic enzymes, and a fairly constant Q_{10} in the neighbourhood of 2, that is, a critical increment of about 12,000 calories. They are not inhibited by small concentrations of cyanide or sulphide, but are so by the usual enzyme poisons, such as heavy metals and nitrites, and oxidising agents. The formic dehydrogenase of *Bacillus coli* appears to be a copper compound, but there is no evidence that most dehydrogenases contain metals. Quastel [5] and his colleagues have made a very thorough study of the dehydrogenases on the surface of *Bacillus coli*. There are probably at least seven different ones, and possibly many more. In this case they can readily be shown to be concerned in oxygen uptakes. In certain conditions malonic acid inhibits methylene blue reduction by succinic acid, both uniting with the enzyme at the same point; and in high concentrations of both, the rate of reduction of methylene blue depends on the ratio of the two. Cook [6] found that oxygen is reduced at nearly the same rate as methylene blue, and malonic acid inhibits its reduction to about the same extent.

When a dehydrogenase and its specific substrate act directly on O_2 it is reduced to H_2O_2. Some anaerobic bacteria act in this way. Bertho and Glück [7] found that at least 90 per cent of the O_2 consumed by *Bacillus acidophilus* is converted into H_2O_2, which damages the bacteria. But some dehydrogenases when separated will not reduce O_2, and a separate activator is required. We know rather less about the activation of O_2 than of H_2O_2. This latter can be activated by two different enzymes, catalase and peroxidase, and by heat-stable peroxidase-like substances such as cytochrome and hæmatins.

Catalase catalyses the reaction $2H_2O_2 = 2H_2O + O_2$. Zeile and Hellström [8] have shown that it is a derivative of hæmatin with a definite spectrum, and convertible into a hæmochromogen, or into protophyrin. It unites with HCN to give an inactive compound. Under suitable conditions a catalase molecule can destroy more than 10^5 H_2O_2 molecules per second. Peroxidase catalyses the reactions $H_2O_2 + X = H_2O + XO$, or $nH_2O_2 + nX = nH_2O + nXO$, where X may be a large variety of molecules, generally aromatic, but including nitrite and HI. It can be very highly concentrated, and appears to be a coloured iron compound. Its extreme sensitivity to cyanide suggests that it is of a similar nature to catalase.

The oxygen activators (oxygenases or *Atmungsferment*) have been specially studied by Warburg.[1] They unite not only with O_2 but also with CO, for which they have a rather smaller affinity. Like CO-hæmoglobins, the CO-oxygenases are generally sensitive to light. Thus the oxygen uptake of yeast in presence of glucose or alcohol is reduced to about 50 per cent, in a mixture containing ten parts of CO to one of O_2, in the dark. In strong light it returns to almost normal values. By studying the relative efficiencies of different monochromatic lights, Warburg and Negelein [9] found that its spectrum is very similar to that of alkaline hæmatin, and still closer to that of iron-phæophorbide-*b*.

Cook, Haldane, and Mapson [10] worked with *B.*

coli in toluene-saturated buffer solutions. Under these conditions, succinic, lactic, and formic acids each lose two hydrogen atoms and no more. It is thus possible to study reactions much simpler than the complete oxidation of a substance such as glucose. They found that CO and HCN, which in moderate amounts do not prevent oxidations by methylene blue, inhibit oxygen reduction. In both cases the oxidation of lactate is more sensitive than that of formate, while that of succinate is intermediate. Hence there appear to be three oxygenases with specific relative affinities for CO and O_2, like those of the hæmoglobins, and also with different affinities for HCN. Each dehydrogenase is associated with a particular set of oxygenase molecules, for oxygen uptakes in presence of formate and lactate are strictly additive, even when oxygenase activity has been reduced by HCN. If the various dehydrogenases could draw on the same common stock of oxygenase molecules for activated oxygen, this would not be so. This rather rigid organisation is probably exceptional, for oxidations by *B. coli* are largely carried out on its surface, instead of internally, and it does not contain all the three cytochromes. In the absence of toluene, similar but not quite so clear-cut results are obtained.

Cytochrome is the name for a group of metal-porphyrin compounds, the metal being probably iron, which are found in almost all cells, and have been studied by Keilin.[11] When the cell runs short of oxygen, through asphyxia, intense metabolism, or cyanide poisoning, a strong spectrum of cytochrome, resembling that of a mixture of hæmochromogens, appears. If the supply of oxygen becomes adequate, the characteristic bands disappear, being replaced by a fainter spectrum of the alkaline hæmatin type. Cytochrome is not oxygenase, as it does not combine readily with CO or HCN. One of the three components of cytochrome, cytochrome *c*, has been obtained in fairly strong solution. It is a red substance, only slowly oxidised by molecular oxygen, readily by mild oxidising agents. It can be reduced by reducing agents or living tissues, and is an iron-porphyrin compound. Keilin found that the oxygen uptake of a system composed of oxygenase from heart muscle, cytochrome, and cysteine behaves like that of a tissue to cyanide and CO. With this system he was able to show that oxygenase is heat-labile like an enzyme, which cytochrome is not. In plants, a particular type of oxygenase, which Keilin calls catechol oxidase, and the CO compound of which, where investigated, has been found to be insensitive to light, yields H_2O_2 when oxidising catechol and its derivatives, as shown by Onslow.[12]

We can thus give a scheme (Fig. 1) which probably covers most of the oxidation process in the average cell. In anaerobes one or more of the catalysts is absent. Oxygen is activated by oxygenase, which is reduced by cytochrome, and the latter is reduced in turn by dehydrogenases of the common or anaerobic type, that is, those which cannot reduce O_2 directly. This process is occasionally simplified, as in *B. coli*, where cytochrome does not seem to intervene in certain oxidations. Oxygen can also be reduced to H_2O_2, either by catechol oxidase or by an aerobic dehydrogenase. This H_2O_2 is used for further oxidation with peroxidase, or is destroyed by catalase. The latter can act as a safety-valve owing to its low affinity. Whereas peroxidase acts most rapidly in a concentration of H_2O_2 which may be as low as 10^{-6} *M.*, catalase has an optimum H_2O_2 concentration of

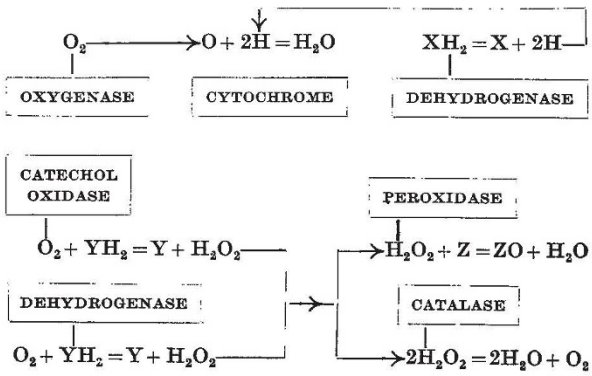

FIG. 1.—The names of the catalysts are given in rectangles, the molecular species activated by each being indicated. The molecule YH_2 is catechol or a derivative in the case of catechol oxidase (Onslow's oxygenase) and is a purine base in the case of xanthine oxidase acting as a reducer of O_2. X and Z may be very varied.

about 0·2 *M.*, and at 10^{-6} *M.* is working at only about 0·0004 of its maximum rate. Other substances may act as intermediates. Glutathione appears to remove hydrogen from certain groupings in proteins, the reduced glutathione being later re-oxidised. St. György's[13] hexuronic acid is apparently reduced by dehydrogenases and oxidised by peroxidase. Doubtless many more similar substances will be discovered in future.

We note the great importance of metal-porphyrin compounds (Table I.). It is fairly clear that their catalytic function is primitive. They have afterwards been modified to act as stores or carriers of oxygen in higher animals. Except in chlorophyll, a magnesium compound, the metal united with the porphyrin is usually, if not always, iron. As oxygenase, catalase, and cytochrome are almost universally distributed, we need not be surprised that hæmoglobin and related pigments such as chlorocruorin have often been independently evolved, a suitable protein being combined with iron-porphyrin residue.

TABLE I.—METAL-PORPHYRIN IN COMPOUNDS FOUND IN CELLS.

1. Catalysts. Chlorophyll *a* and *b*. Oxygenases. Catalases. Probably peroxidases. Cytochrome *a*, *b*, and *c*.
2. Mainly concerned in oxygen storage. *Arenicola* hæmoglobin.
3. Mainly concerned in oxygen carriage. Vertebrate hæmoglobins.
4. Uncertain whether in group 2 or 3. Many invertebrate hæmoglobins. Chlorocruorin. Helicorubin, etc.

We know little as to the immediate source of CO_2. Two obvious processes are available, the dehydrogenation of formic acid, and the decarboxylation

of pyruvic acid and related compounds by the enzyme carboxylase according to the equation: $R-CO-COOH=R-COH+CO_2$. The enzymes concerned in both processes have a wide distribution.

Still less is known of how in detail the energy made available in oxidation is passed on, or of how the rate of oxidation is controlled, though both processes are evident enough in the whole organism. The control is largely exercised on the fuel supply. Thus a rise of blood sugar in man causes an increased oxygen consumption. Hormones such as thyroxin are also concerned, but their mode of action is not understood. The energy is not generally liberated directly, but is largely employed in building up compounds of high chemical potential. These may either form new tissue or be available for rapid energy production. Thus the oxidation of sugar or lactic acid in muscle provides energy for the resynthesis of glycogen from lactic acid, and of phosphagen from creatine and phosphate.

If these processes are to be efficient, two conditions must be fulfilled. The energy of oxidation must be made available in quanta somewhat, but not greatly, larger than those required for synthesis; and the molecules undergoing oxidation and synthesis must be united with the same catalyst, which must thus have a double specificity. The significance of the complicated oxidising systems here described will remain obscure until we know what syntheses are correlated with each of them. A beginning of such an analysis has been made by Wurmser.[14] But an indispensable preliminary is a study of the total and free energy changes in the various reactions which are linked. This is still in its very early stages.

But even when we know the stages in the oxidation of different substances, and the use to which the energy thus made available is put, we shall be faced with the problem of regulation. In the living cell the activity of peroxidase or lactic dehydrogenase is doubtless governed by laws as definite as those which govern that of the heart in the living organism, laws which can be stated both in terms of chemistry and of biological function. The question of whether these two types of explanation can be reconciled, or whether one of them is superfluous, will then have to be fought out. However, that day is far distant; meanwhile the biochemist can continue to accumulate knowledge without committing himself on philosophical questions.

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[11] Keilin, *Proc. Roy. Soc.*, B, **98**, p. 312; 1925: **104**, p. 236; 1929.
[12] Onslow and Robinson, *Biochem. Jour.*, **20**, p. 1138; 1926.
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[14] Wurmser, "Oxidations et réductions", 1930.

Obituary.

Lieut.-Col. Sir Charles Bedford.

THE death of Lieut.-Col. Sir Charles Bedford, which occurred on July 8, terminates a career of scientific activity. Born in 1866, the youngest son of Dr. F. W. Bedford, headmaster of George Heriot's Hospital, Edinburgh, Bedford graduated in science and medicine at the Universty of Edinburgh and entered the Indian Medical Service in 1889. His special aptitude for work of an investigatory character was soon recognised in his appointment by the Punjab Government to be chemical examiner and professor of chemistry at Lahore Medical College. In 1897 he transferred to similar posts in Bengal. Whilst holding these appointments, Bedford rendered valuable assistance to the Indian Excise authorities, particularly in connexion with alcoholic liquors. He became scientific and technical adviser to a committee of investigation into excise questions, and later was appointed director of the Central Excise Laboratory, which was formed on the recommendation of that committee.

It was whilst holding these positions that Bedford performed his most valuable work. Under his direction an extensive investigation was made of all alcoholic liquors produced in or imported into India, their composition and physiological effects, with those of the by-products of manufacture. In 1907 he made a report upon the determination of alcoholic strengths. He submitted new tables intended for use with a glass Sikes's hydrometer in place of those which for upwards of a century had formed the basis of revenue charge. The old tables which were legalised in England in 1816 were compiled for use with a brass hydrometer and covered a range of temperature from 30° to 80° F. For use in warmer climates the tables were extrapolated to 100° F. Strengths determined under these conditions could be regarded as little better than approximations, and Bedford demonstrated that the inevitable errors operated for the most part against the revenue. The new tables were officially adopted in 1908, and remained in use until replaced in 1922 by others calculated from data which formed the basis of the British tables legalised in 1915.

Bedford was also responsible for the methods officially adopted in India for rendering unpotable, alcohol intended for commercial and industrial purposes.

After his retirement from the Indian Service, when he was rewarded with a knighthood, Bedford continued to render valuable service in various directions, being a member of numerous committees and occupying advisory positions at home and in connexion with the Dominions. Bedford also found time in the course of a busy life to indulge in considerable literary activity. One of his first publications was a memoir of his father, followed by a history of George Heriot's Hospital. His later