TISSUE RESPIRATION

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A DISCUSSION on tissue respiration was organized for August 31 jointly between Sections B and I (Chemistry and Physiology) of the British Association at Dundee. Owing to the imminence of war several of the proposed speakers were unable to attend, namely, the organizer and opener Prof. R. A. Peters, Dr. Malcolm Dixon, Prof. H. Theorell, Dr. D. E. Green and Dr. T. Mann. Prof. D. Keilin also did not attend. Prof. H. S. Raper, who was intending in any event to give a contribution towards the end of the discussion, kindly offered to introduce the subject and ably substituted for the absent speakers, so that the comparatively large attendance was not in vain. In order to give a more comprehensive survey of the subject, it has been thought advisable to follow in this account the lines of the addresses as originally planned.

To make a coherent account of tissue respiration for the benefit of those unfamiliar with it, the subject was divided into the parts concerned with the organized tissue cell and those relating to the isolated tissue component. Starting with the present position of knowledge of the isolated components, the discussion worked through to the respiration of the more highly organized tissue slice, and ultimately to the chemical control of the whole system.

The address of the opener was intended to deal briefly with the historical background, pointing out that the subject had roots in the distant past, but had developed rapidly only in the most recent years. In a short time it was clearly impossible to do justice to the work of the pioneers; but the following must be especially mentioned: the importance of Wieland's theories of hydrogen activation, of Hopkins' isolation of glutathione, of Thunberg's methylene blue technique, of Keilin's cytochrome, and of the various contributions from Warburg and his co-workers and from the Stockholm school under von Euler. Much advance in knowledge had come from the use of the *in vitro* study; to allay any doubts as to the value of the isolated tissue preparation, it must be emphasized that in studies at Oxford there has been found to be a remarkable parallelism between the improvement of respiration in the avitaminous pigeon's brain by the addition of vitamin B_1 *in vitro*, and the similar action of the latter in curing symptoms of deficiency *in vivo*. Such studies form a valuable bridge for the interpretation of *in vivo* happenings by the use of *in vitro* preparations. Owing to the short time available, some important aspects of the subject had to be pruned, among them being glutathione, vitamin C, oxidation-reduction potential in relation to tissue respiration processes, adequate discussion of the Szent-Györgyi catalytic system of C_4 acids, also the plant oxidase reactions, which, though not strictly tissue respiration in the animal in this sense, have been so valuable a feature of contributions from the laboratory of Prof. Raper.

Dr. Malcolm Dixon's introductory address (Cambridge) dealt with the general subject "Catalysis in Respiration". Defining tissue respiration as the utilization of molecular oxygen for the oxidation of organic substances in tissues, Dr. Dixon stressed the fact that tissue respiration is essentially a problem of catalysis, and dealt with the questions: How do the catalysts work? What is their chemical nature? And how do they co-operate in respiration? Owing to recent work, the main features of this mechanism are now fairly clear. We have the two types of respiratory catalysts, the *activators* and the *carriers* respectively. "The *activators* are enzymes, very highly specific in many cases, which combine in a loose but highly specific way with their substrates, so that the combined substrate is reactive". The *carriers* work differently; though mostly not enzymes, they enable two compounds which we may designate AH_2 and B, incapable of reacting with one another directly, to react through their agency along the lines of equations 1 and 2:

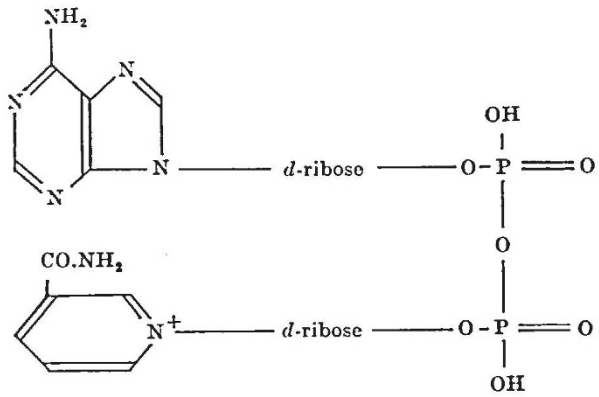
$$AH_2 + C = A + CH_2 \quad (1)$$

$$CH_2 + B = C + BH_2, \quad (2)$$

in which 2H is so transferred from A to B. Since the organic 'substrates of respiration' are oxidized in an ordered series of successive reactions (steps of 2H at a time), a large number of activating catalysts is involved; about twenty-five distinct dehydrogenases (enzymes concerned in H transfer) are known. There is the further complication that activated substrates require further carriers to bring them into relation with molecular oxygen. As an example,

$$\underset{\text{(Triosephosphate D)}}{\underset{(1)}{\text{Triosephosphate}} \rightarrow \underset{(2)}{\text{CoZ}} \rightarrow \underset{(3)}{\text{FP}} \rightarrow \underset{\text{(Cytochrome oxidase)}}{\underset{(4)}{\text{Cytochrome}} \rightarrow \underset{(5)}{O_2}}} \quad (3)$$

FP=Flavoprotein D=dehydrogenase



COZYMASE

Fig. 1

In such a scheme H is transferred in the direction of the arrows. The carrier cozymase (CoZ) (Fig. 1) is very important. It is a compound of adenylic acid and nicotinic amide-ribose-phosphate, in which the essential group is the nicotinic amide; one double bond of this is capable of reversible reduction. The cozymase like the triosephosphate combines loosely with the dehydrogenase; the enzyme then catalyses the bimolecular reaction which results in the oxidation of the triosephosphate and reduction of the cozymase. The latter then combines loosely with the flavoprotein and becomes re-oxidized. In its turn the reduced flavoprotein reduces Keilin's cytochrome *C*, a specialized hæmochromogen, the latter being oxidized by oxygen in presence of the enzyme cytochrome oxidase. This means that the real function of respired oxygen is to keep the tissue cytochrome in an oxidized state; and that the oxidations of tissue constituents are really a succession of H transfers from them to the oxidized cytochrome, sometimes interpolated with reactions involving the addition of the elements of water.*

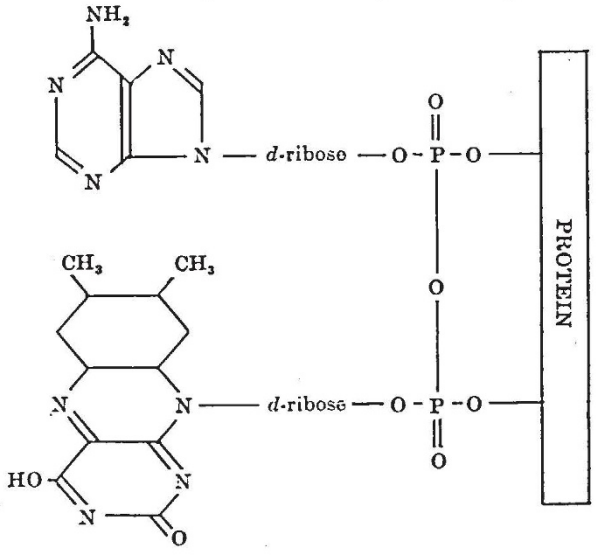
It is important to realize that these enzymes are quite general for both yeast and animal tissues. Parts 2, 3, 4 and 5 of equation 3 are common for the oxidation of many different substances; only the dehydrogenases are different; the reactions in which cozymase plays a part are reversible and in the absence of oxygen the dehydrogenase systems may react with one another through cozymase, producing fermentations. Dr. Dixon concluded by reference to known variations upon the main theme. In some cases there is a substitution of coenzyme II (triphosphopyridine nucleotide) for cozymase; there exists a small group of dehydrogenases which react direct with cytochrome; Szent-Györgyi has produced evidence that in total respiration a definite part is played by the C_4 dicarboxylic acids (fumaric, etc.) which bring about the oxidation of reduced cozymase by cytochrome in an indirect way. Dixon further pointed out that most links in the chain can be represented artificially with other substitutes such as dyes. The use of methylene blue instead of molecular oxygen plus the cytochrome system is the basis of the well-known Thunberg method of investigating dehydrogenases.

Dr. Dixon's succinct but comprehensive paper was to be followed by Dr. Theorell (Sweden), dealing with the developments in our knowledge of the flavoproteins (Fig. 2). These are compounds of proteins with the component of the vitamin B_2 complex, which is known as riboflavin, di-methyl-alloxazine-ribose-phosphate. The old yellow ferment of Warburg and Christian, which was finally obtained in quite pure condition by Theorell himself, and which consisted only of riboflavin-protein, is probably an artefact. In its place now six new flavoproteins have been described. All of these have as their prosthetic group riboflavin and adenylic acid, called after Warburg alloxazine-adenine-dinucleotide; the protein component differs.

FLAVOPROTEINS

No.	Transfers H between		Workers	Source
1.	O_2; di-H_2-pyridine		Warburg and Christian	Yeast
2.	O_2; amino-acids	Krebs' *d*-amino-acid oxidase	Das, Straub, Warburg and Christian	Kidney
3.	Meth. blue; di-H_2-pyridine		Haas	Yeast
4.	O_2; xanthine	Xanthine oxidase	Ball, Green	Milk
5.	Cytochrome; di-H_2-pyridine		Corran and Green	Milk
6.	Cytochrome; di-H_2-pyridine	Diaphorase	Green, von Euler, Straub	Heart muscle

With the exception of No. 1, all have protein



FLAVOPROTEIN (STRAUB)

Fig. 2

* Such a view of the function of respiratory oxygen is revolutionary as compared with views of, say, ten years ago.

components different from the original Warburg and Christian 'yellow ferment'.

The contribution given by Dr. F. Dickens (Newcastle) formed the first bridge between the properties of the isolated systems and those systems organized in the tissue cells themselves. Directing attention to the tissue slice technique of Warburg for studying respiration, he considered that this proved in practice a fruitful compromise between the intact and macerated state, though minced tissue 'brei' where cell boundaries were destroyed was valuable for substances to which intact cells were impermeable. Tissue slices must be of appropriate thickness to allow of proper gas and substrate exchange. Under good conditions, slices of tissue in glucose solutions (Ringer bicarbonate or phosphate) will maintain remarkably constant rates of respiration, whereas with succinate as substrate this is not so; useful information as to the type of substance undergoing change can also be obtained by an estimation of the respiratory quotient, CO_2/O_2, as also by employing poisons which inhibit certain parts of the respiratory system. Dr. Dickens pointed out in a striking way the essential difference between the views of Szent-Györgyi and Krebs and Johnson as to the effect of the C_4 dicarboxylic acids. In the former case, catalysis is considered to be due to a series of reversible reactions, whereas in the latter the reactions proceed only in *one* direction, citric acid being supposed to form part of the reaction chain. In a discussion of this, Dr. H. A. Krebs (Sheffield) considered the dismutation of keto acids in bacteria and animal tissue, along the lines indicated by the following equations.

Anaerobic:

$$2COOH.CH_2CH_2.CO.COOH + NH_3 = COOH.CH_2CH_2CHNH_2.COOH + COOH.CH_2CH.COOH + CO_2 \quad (4)$$

Aerobic (with O_2 present):

$$COOH.CH_2CH_2.CHNH_2.COOH + O \rightarrow COOH.CH_2CH_2.CO_2.COOH + NH_3 \quad (5)$$

Glutamic acid can here act as a hydrogen carrier. This is a special modern example of the so-called Krebs' dismutations of α-keto acids in which carbon dioxide arises by decarboxylation of one molecule with simultaneous formation of the corresponding hydroxy acid from another molecule. In this paper we get the first introduction to one mechanism for the formation of carbon dioxide, probably a subsidiary one. In other cases in bacteria Dr. Krebs alluded to the use of succinic acid as a hydrogen carrier in the oxidation of acetic acid, part of the C_4 catalytic system being so employed. Dr. Mann's contribution should have dealt not only with the processes of H transfer involved in fermentation and respiration, but also with that of phosphate transfer; attention was directed to the coupling of oxidations with phosphorylations recently shown by Meyerhof, Needham and Lipmann, and to the fact that these reactions can be studied with advantage in tissue brei in which the fundamental cell organization has been destroyed.

With the contribution of Dr. J. H. Quastel (Cardiff), we turn to the reactions of the organized tissue respiration system (as found in the brain-slice) to the action of narcotics. Though it has been long considered that narcosis was due to diminished oxidations, it has only recently been emphasized by his school at Cardiff that narcotics have specific effects on tissue oxidizing systems; at low concentrations they greatly inhibit the oxidation by brain tissue of glucose, lactate and pyruvate, but not that of succinate or α-glycerophosphate, leaving cytochrome oxidase unaffected. Their inhibiting effects on brain respiration in the presence of glucose are definite at concentrations of narcotic which would be narcotizing *in vivo*; and with several narcotics the effects even on the tissue slice can be proved to be reversible. Though anaerobically there is no inhibition of pyruvate dehydrogenation by low concentrations of narcotics, in presence of oxygen there is marked inhibition; this may be related to the cocarboxylase (or ? diaphorase). These experiments were carried out with pyocyanin and ferricyanide. In a new point, they had found that suspensions of bacteria grown in media deficient in vitamin B_1, and suspended in pyruvate, showed a stronger inhibition of oxygen uptake by narcotics in absence of added vitamin B_1.

While Dr. Quastel's paper showed how tissue respiration could be altered by the action of drugs, Prof. Raper dealt with the equally fundamental and fresh point of how *in vivo* the cell controlled the rate of its respiration; How is the increased respiration consequent upon tissue activity regulated? He described experiments with slices of cat's submaxillary gland; the oxygen uptake is markedly stimulated with acetylcholine, this stimulation being inhibited by atropine. This important observation brings the whole question into relation with modern work upon the nervous system in its relation to these two substances, and forms a further valuable and much needed 'bridge' study. Acetylcholine did not affect anaerobic glycolysis though it slightly increased aerobic glycolysis. The effect of atropine suggests that acetylcholine does not act by increasing the available substrates, but rather by changing the availability of the catalysts concerned. (I think that this may have interesting relations to the address by Prof. E. K. Rideal upon surface film action.)

The question of carbon dioxide production through one of the main channels for this in

tissue respiration was dealt with by Dr. S. Ochoa (Oxford), namely, the oxidation of pyruvic acid ($CH_3.CO.COOH$). There is a strong probability of a common path of carbohydrate breakdown to the pyruvic acid stage; the phosphorylated triose is oxidized anaerobically by dismutation, aerobically by giving up hydrogen to the cytochrome system through coenzyme I, flavoprotein and C_4 dicarboxylic acids. The phosphorylated oxidation product (phosphoglyceric acid) yields pyruvic acid which anaerobically is reduced to lactic acid, but aerobically is decarboxylated and oxidized; this gives, so far as is known, the main reaction in which carbon dioxide is liberated in the cell, and so forms the main source of respiratory carbon dioxide. It is catalysed, as has been shown for brain in Oxford, by cocarboxylase (vitamin B_1 pyrophosphate). (1 mol. cocarboxylase catalyses optimally the uptake of 1,500 mol. oxygen, producing 2,000 mol. carbon dioxide per min.) For complete oxidation inorganic phosphate, fumarate, adenylic acid and coenzyme I are required. During oxidative decarboxylation in brain brei, there appears to be an unstable C_2 intermediate which may appear as acetic acid in the absence of the rest of the enzyme system. There is some indication of a possible cycle of phosphorylation of pyruvate beyond this stage. Recent available evidence suggests that when dialysed brain preparations are incubated aerobically with fluoride, some inorganic phosphate disappears and a phosphate ester (not phosphopyruvate) accumulates in equivalent amounts only if pyruvate is present.

Striking a much more practical note, Dr. E. P. Poulton dealt with the question of local tissue anoxia in arterial disease and its treatment with oxygen, by the use of an oxygen tent. The improvement in the oxygen saturation of the tissues *in vivo* was of the greatest value in conditions such as rheumatism, a man being kept in the tent for days on end. That this is related to the oxidation changes in the tissues is shown by the fact that a high level of blood lactic acid, which would be caused by tissue anaerobiosis, is much reduced.

To summarize, it may be said that, with the exceptions mentioned in the opening address, the whole field of tissue respiration was well covered in summary form as it stands at the present time. As compared with twenty or even five years ago, the advance is truly marvellous and forms a fitting further chapter to the accurate knowledge of the carriage of oxygen and carbon dioxide in the blood, which has formed until now a main line of physiological research and thought upon respiration. From the purely chemical as distinct from the biochemical point of view, the strange medley of substances assembled by the cell to form the complex of catalytic systems necessary for the combustions in the tissue must always appear without logic and in strong contrast to the ordered schemes of pure organic chemistry. They will only receive a chemical meaning when we know more about the details of electron activation and exchange. Then we may be able to understand better why catalyses requiring low conditions of temperature and comparatively neutral reactions necessitate the compounds which we find. Until then, with biochemists, we can merely continue to record and wonder, and to enjoy the opportunities of intellectual interest to scientific workers of varied outlook which are presented by a study of this subject; actually it lies at the basis even of the material foundation of thought.

NOTE.—I am grateful to Dr. M. Dixon for access to the manuscript of his proposed address, and also to Dr. S. Ochoa for valuable notes of the proceedings at the meeting.

TWO BRITISH EXPEDITIONS TO UBEKENDT ISLAND, WEST GREENLAND

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THE motive directly responsible for the organization of two British expeditions, the Cambridge West Greenland Expedition, 1938, and the St. Andrews University West Greenland Expedition, 1939, to West Greenland, was the inviting geological problems of Ubekendt Island (see Fig. 1) first encountered in 1937[1]. It was clear to me that apart from geological research, other work could also be undertaken in the same area, and that a party with a varied programme would offer, among other things, the further intrinsic interest of an 'expedition', the successful organization and conduction of which is in direct relation to the best working conditions and congenial co-operation. It is, in itself, a real problem in a non-scientific sphere.

Apart from the detailed geological examination of Ubekendt Island and similar work in parts of Upernivik Island, psychological, ethnological, archæological and botanical work was carried out, and