Where the oxygen supply becomes inadequate the cytochromes become more reduced but maintain their usual relative positions in regard to oxidation reduction potential, i.e. cytochrome a is more reduced than cytochrome a3 in hypoxia as in normoxia (Holton, 1964). The citric acid cycle functions more slowly or ceases altogether for the reasons given in section II (iii) of the previous paper. Reduced NAD produced by glycolysis (and by such continuing activity of the citric acid cycle as may persist) accumulates and a greater proportion of pyruvate produced by glycolysis is reduced to lactate and accumulates as such. The failure of electron transport leads to the accumulation of hydrogen ions in the tissue thus producing a tissue acidosis. Much of the energy derived from oxygen consumption is used to generate the high energy phosphate bonds in ATP, and so in hypoxia these bonds are not regenerated and the tissue level of ATP should fall while the level of ADP should rise (but see previous paper, section III (iv); Henderson, 1969).

In addition to these basic biochemical effects of hypoxia one should also remember that secondary biochemical changes arise in the whole organism due to the failure of specialized organ function, for example, failure of renal and/or hepatic function produces secondary biochemical disturbances discussed elsewhere in this symposium. The whole animal response to hypoxia also affects the situation, e.g. adrenaline release leads to the production of glucose from glycogen stores in the liver.

Restoration of oxygenation following a hypoxic incident leads to return to normal oxidation reduction states of the electron transport system (ETS) and of NAD. High energy phosphate bonds have to be reconstituted mainly by energy derived from metabolism of lactate in the citric acid cycle after reduction to pyruvate. Circulating excess blood lactate is taken up by the liver and kidney cortex and built up into glycogen by gluconeogenesis, the energy for this being derived from the oxidation of fats and amino acids via the high energy intermediaries ATP and GTP.

The Relationship of Biochemical Changes to Oxygen Tension

It would seem almost self-evident that the most direct measurement of hypoxia would be the determination of oxygen tension or concentration in the tissues. Unfortunately the levels of arterial Po2 at which true tissue hypoxia appears are uncertain because so many other factors may compensate for a low arterial Po2. For example, polycythaemia may be present, or the cardiac output may be increased and regional flow to organs with high metabolic requirements may be increased, e.g. brain and heart.

On the other hand, there must be some range of arterial Po2 values which do imply hypoxia because at very low arterial oxygen tensions hypoxia is invariably present. Huckabee (1958a) using his excess lactate measurement (see below) found that tissue hypoxia was present when the arterial Po2 fell to 26-32 mm Hg. These measurements were, however, made in healthy volunteers and it is certain that in ill patients Huckabee’s criteria of hypoxia would be met at considerably higher arterial Po2 values (cf. Howell, 1966).

The trouble then with arterial Po2, as a measure of hypoxia, is that the level at which tissue hypoxia appears is very variable. Furthermore, a normal or even high PaO2 does not exclude the presence of tissue hypoxia (e.g. due to anaemia or ischaemia). The relationship between arterial Po2 and tissue hypoxia can only be determined by either biochemical assessment or measurement of functional activity. Even so, any relationship so
derived can hold true only for a particular set of circumstances, with respect to cardiac output, peripheral circulation, haemoglobin level and tissue oxygen demands.

The $P_{O_2}$ of mixed venous blood is not much help either since mixed venous blood has drained from so many organs, only some of which may be hypoxic. For example, in extreme hypotension produced by ganglion blockade, the brain may be impaired by hypoxia while the mixed venous blood has a near normal oxygen saturation, partly because of the dilated peripheral circulation. As is suggested by these comments, much more valid information can be gained from measurements of the oxygen tension of venous blood draining from particular organs. However, once again these measurements cannot themselves prove hypoxia which can only be rigorously defined by direct measurement of tissue metabolism or tissue function. By measuring venous $P_{O_2}$ in relation to tissue metabolism and function it is possible, in some organs, to determine rather precisely at what level of venous $P_{O_2}$ hypoxic dysfunction appears. For example, in the case of the brain, consciousness is lost when the cerebral venous $P_{O_2}$ is reduced to 17-19 mm Hg (Ernsting, 1966). It follows, therefore, that if the jugular bulb $P_{O_2}$ is less than 17 mm Hg the brain must be hypoxic. However, areas of brain may well be hypoxic when blood in the jugular bulb has a normal or even super-normal $P_{O_2}$ because the $P_{O_2}$ of well oxygenated parts of the brain may conceal the low $P_{O_2}$ of the hypoxic region.

At this stage in the discussion one might bemoan the fact that measurement of tissue $P_{O_2}$ is so difficult. However, it is very doubtful if tissue $P_{O_2}$ measurement would be of great value either, because tissue $P_{O_2}$ varies so widely within any one organ from one point to another (Mcdowall, 1966). A more fundamental difficulty is that some cells exist normally at very low $P_{O_2}$ values of the order of 5 mm Hg or less (Silver, 1966), but do not appear to suffer hypoxic malfunction. Indeed mitochondrial suspensions show high metabolic activity down to an environmental $P_{O_2}$ of 0.5 mm Hg (Jobsis, 1964). To obtain evidence of tissue hypoxia from tissue $P_{O_2}$ measurement it would, therefore, be necessary to place the electrode in the cells with the lowest initial $P_{O_2}$ and this electrode would then have to detect changes of 1 mm Hg or less. Even so, the actual evidence of hypoxia would still have to come from collateral studies of metabolic or functional derangement.

**THE ELECTRON TRANSPORT SYSTEM AND NAD IN HYPOXIA**

In hypoxia the components of the electron transport system move to more reduced levels and as they become reduced their absorption spectra change. Thus if one exposes the tissue to light in the wave band 400-450 m$\mu$ and then measures the absorption spectrum by the technique of double beam spectrophotometry a definite change in the spectrum occurs as the cytochromes are reduced. Unfortunately, the practicable applicability of this method of detecting hypoxia is severely limited by the fact that the absorption spectrum of haemoglobin overlaps that of the cytochromes so that the cytochrome reading is submerged. This means that detection of cytochrome oxidation reduction states is impossible in any tissue containing blood. In experimental work this can sometimes be circumvented by using artificial perfusion media, or, of course, by studying tissue slices.

In the same way, as NAD moves to a more reduced level, its absorption spectrum changes but again this change is obscured if haemoglobin is present.

Chance, Schoener and Schindler (1964) have recently described a technique which allows measurement of NAD/NADH ratio despite the presence of blood. In this technique ultraviolet light, in the range 310-370 m$\mu$ is shone upon the tissue which then fluoresces (at 450 m$\mu$). This fluorescence is due to the presence of reduced NAD and therefore the amount of fluorescence increases in proportion to the severity of hypoxia. Changes in haemoglobin saturation have little effect on the fluorescence at these wavelengths. Using this technique, Chance and his colleagues have been able to show, in the intact animal, that 50 per cent reduction of NAD in the brain does not occur in hypoxic hypoxia until the arterial $P_{O_2}$ is as low as 12 mm Hg and the tissue $P_{O_2}$ is 0.2 mm Hg. They have further found that cessation of e.g. activity does not occur in the brain with normal energy reserves until 12 seconds after the full reduction of NAD. This final reserve
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of energy in hypoxia is presumably supplied by ATP and other high-energy phosphates.

These techniques for the measurement of oxidation reduction state of NAD/NADH and of the cytochromes are clearly still in the developmental stage and applicable only to laboratory investigation. However, since they provide the only true biochemical evidence of the presence and degree of hypoxia they are of interest to clinicians. It is likely that these techniques will be adapted for clinical application at least in the field of clinical investigation, although there are still formidable difficulties to be overcome (Chance, Schoener and Schindler, 1964).

LACTATE AND PYRUVATE MEASUREMENT

In hypoxia, because of the inhibition of the citric acid cycle, pyruvate derived from glycolysis accumulates and is converted to lactate (fig. 1).

The regeneration of NAD by this conversion of pyruvate to lactate provides a hydrogen acceptor for the continuance of glycolysis. In this manner, energy production by glycolysis can continue during anoxia and “the importance of this cannot be over-estimated” since this “is the only pathway which can do so” (Jobsis, 1964). As already discussed, the energy produced by glycolysis is used to regenerate ATP from ADP although the mechanism is rather an inefficient one, i.e. 1 mole of glucose-6-phosphate metabolized to pyruvate produces only 2 moles of ATP while the subsequent metabolism of pyruvate in the citric acid cycle under aerobic conditions yields 36 moles of ATP (White, Handler and Smith, 1964).

Even under normal aerobic conditions there is always some lactate formed from pyruvate and there is, therefore, a normal lactate/pyruvate ratio which is in the range 10–14 (Huckabee, 1958b). Under hypoxic conditions both pyruvate and lactate increase in concentration but the equilibrium constant shifts so that the increase in lactate outstrips the increase in pyruvate. The ratio of lactate to pyruvate therefore increases and this ratio has been proposed as a mechanism for assessing hypoxia.

Another way of assessing changes in lactate concentration is to express the changes in terms of excess lactate. This term was introduced by Huckabee (1958b) in an attempt to quantitate the degree of hypoxia present. Excess lactate is calculated as follows:

Excess lactate = (Ln—Lo)—(Pn—Po)/Lo/Po,

where Lo and Po represent the initial values for lactate and pyruvate concentration and Ln and Pn the subsequent or experimental values. Calculation of excess lactate in this way yields an answer in terms of millimols of excess lactate per litre of blood; plus values indicating a positive excess lactate (i.e. one implying a hypoxic increase in lactate) and negative values indicating the opposite. Excess lactate is therefore most easily visualized by comparison with the more familiar term, base excess, and the reason for the use of the term is similar. Unfortunately the quantitative validity of this calculation is in doubt for a number of reasons but principally because the value obtained for excess lactate is dependent upon the numerical value of Pn, which is not in itself a measure of hypoxia (Chamberlain and Lis, 1968). In these circumstances calculation of lactate/pyruvate ratio and comparison with previous lactate/pyruvate ratios in the same patient may provide a more valid guide to hypoxic changes in lactate metabolism.

Lactate and pyruvate concentration can be measured either at the tissue level or in blood, but to make tissue measurements it is necessary to perform a biopsy. The exact technique of biopsy is important so that anaerobic metabolism does not occur in the tissue during the process of obtaining the specimen. The method most com-
monly used for this is freezing in situ. The biopsy
is then homogenized and determination of lactate
and pyruvate concentrations performed enzymati-
cally.

Lactate and pyruvate concentrations can also
be measured in blood but such measurements are
less direct and less definitive than tissue deter-
minations although, of course, much more
practical clinically. Indeed determination of lactate
and pyruvate levels in blood is the only bio-
chemical measure of hypoxia which is already in
fairly general clinical use. The difficulties in
interpretation of blood values are, however,
numerous. Lactate and pyruvate are, of course,
principally produced in the cytosol of the cell and
have then to pass across the cell membrane to the
extracellular space. However, the permeability of
the cell membrane to lactate and pyruvate is high.
The second step from the extracellular space to
the blood is, for many organs, not a difficult one
and in such organs venous blood lactate and
pyruvate, sampled from the appropriate vein,
provide valid measurements of tissue levels. This
is, however, not the case for the brain where
transfer of lactate across the blood brain barrier
is slow (Posner and Plum, 1966). In this partic-
ular organ, however, one can sample extra-
cellular fluid directly in the form of cerebrospinal
fluid and cerebrospinal fluid lactate and pyruvate
levels correlate closely with tissue levels (Plum
and Posner, 1967) except that there is a time lag
after acute changes in the tissue values. Lack of
equilibration between tissue and blood values of
lactate also occurs in the heart (Glaviano, 1965).

In many clinical situations one cannot sample
the venous drainage of individual organs and one
then has to use either arterial blood or arterialized
venous blood or mixed venous blood. The lactate
and pyruvate levels in such blood are even less
direct indicators of tissue conditions because the
venous drainage from all tissues is mixed together
and one gets mean body lactate and pyruvate
concentrations which include the factor of hepatic
lactate utilization. It is important that if venous
blood is used, it should be arterialized venous
blood or mixed venous blood because venous
drainage from the muscles of the limb may have
quite atypical values of lactate and pyruvate.

The lactate/pyruvate ratio has been considered
to be an unsatisfactory measure of hypoxia for
the following reasons: (1) the ratio varies not
only with the tissue state of oxygenation but with
pH changes in a rather unpredictable fashion
(Tobin, 1964); (2) there appears to be a lag be-
tween the occurrence of cellular hypoxia as
measured by NADH/NAD ratio and the appear-
ance of lactate (Jobsis, 1964); and (3) the relation-
ship between lactate formation and the electron
transport system is only an indirect one. Lactate
is formed by glycolysis in the cytosol while the
ETS functions entirely within the mitochondria.
The link between the two is completed by a
hydrogen shuttle system (see previous paper, sec-
tion I (i, c)) and this shuttle may be ineffective as
in most malignant tumour cells (Lehninger, 1965),
or may be overwhelmed as during the dehydro-
genation of certain substances (e.g. alcohol)
(Jobsis, 1964). Under such circumstances lactate
may accumulate in fully aerobic conditions.

Despite these objections, it is likely that lactate
and pyruvate measurements are helpful in assess-
ing clinical situations of suspected hypoxia and
that such measurements will become more com-
mon in clinical practice. Assessments of the
clinical value of lactate/pyruvate measurement
have been reviewed by Howell (1966) and by
Geddes (1967).

**MEASUREMENT OF HIGH ENERGY PHOSPHATE**

Fifty per cent or more of the oxygen required
in tissue metabolism is used for the generation
of high energy phosphate bonds by the process
of oxidative phosphorylation (Lehninger, 1965). It
follows, therefore, that the levels of high energy
phosphate compounds such as adenosine triphos-
phate and creatine phosphate should be reduced
in hypoxia. Such reductions do, in fact, occur
though it takes fairly severe hypoxia to lead to
changes in creatine phosphate/creatine ratio, and
very severe changes to effect ATP/ADP ratio; for
example, Schmahl and associates (1966) found
that there was no change in high energy phos-
phates of the brain when inspired oxygen was
reduced to as little as 4–5 per cent oxygen. There
was a fall in creatine phosphate/creatine ratio
when the inspired gas was 2–3 per cent oxygen
(cortical Po
_2
= 0.2 mm Hg) which occurred at the
time of the cessation of e.e.g. activity. Even at
this extreme level of hypoxia there was no signi-
ficant change in ATP/ADP ratio. Indeed it is
believed that ATP/ADP ratio is not affected by hypoxia until almost all the creatine phosphate has been dephosphorylated. The reader will recall that in section II (i) of the preceding paper a fall in ATP level was proposed as a factor in the control of glycolysis during anoxia. The present findings are not in disagreement with this mechanism for they relate to steady state conditions; transient fluctuations in ATP/ADP such as are involved in glycolytic control are not excluded by the findings quoted here.

As evidence of hypoxia in the tissues, therefore, the ratio of creatine phosphate/creatine is of value only in severe hypoxia while the ratio of ATP/ADP is so well maintained as to be of little value at least in the case of the brain. Tissue lactate/pyruvate measurements provide an earlier indication of cerebral tissue hypoxia than do determinations of creatine phosphate/creatine or ATP/ADP (Schmahl et al., 1968).

MEASUREMENTS OF ARTERIAL VENOUS DIFFERENCES

Severe hypoxia actually depresses aerobic metabolism so that measurement of oxygen uptake may be used to provide evidence of hypoxia. Such measurements are obtained as the product of the tissue blood flow and the tissue arteriovenous difference for oxygen. For example, the oxygen consumption of the brain begins to fall when the cerebral venous PO$_2$ is reduced below approximately 19 mm Hg (Schneider, 1963).

Of course, any reduction in aerobic metabolism may be due to a reduction in total metabolism or it may be due to a shift from aerobic to anaerobic pathways. If the latter is the case then a corresponding increase in lactate production by the organ should occur.

In the case of the brain which mainly uses glucose for metabolism (but see previous paper, section I (v, a)) the glucose uptake of the organ is approximately equal to the total metabolism and this can be divided into aerobic and anaerobic percentages of total metabolism by relating glucose consumption to oxygen uptake for the aerobic component of metabolism and glucose consumption to lactate production for anaerobic metabolism (Cohen et al., 1964; Alexander et al., 1965). Care is, however, necessary in interpreting such calculations because of the poor equilibrium between brain and blood lactate already referred to and also because of the storage of glucose by the brain which means that in the short term the uptake of glucose does not necessarily equal the glucose consumption of the brain.

CONCLUSION

In hypoxia electron transport along the cytochrome chain is reduced and therefore NADH concentration and the NADH/NAD ratio increase first in the mitochondria and then in the cytosol. The tricarboxylic acid cycle becomes ineffective so that pyruvate is diverted to lactate. This reaction results in the conversion of some of the accumulated NADH to NAD and this source of supply of NAD allows glycolysis to continue for a time. In this way ATP levels are maintained until the final stages of anoxia.

These biochemical changes in hypoxia can be followed and quantitated by measuring: (a) blood or tissue PO$_2$; (b) the increased fluorescence of the tissue consequent upon accumulation of NADH; (c) the lactate/pyruvate ratio in tissue or blood; (d) the creatine phosphate/creatine ratio, and in extreme hypoxia, the ATP/ADP ratio; and (e) the tissue uptake of oxygen or the tissue output of lactate.

FOR FURTHER READING


REFERENCES


THE UNIVERSITY OF LEEDS

Department of Anaesthesia

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