CI. OXIDATIONS IN CENTRAL AND PERIPHERAL NERVOUS TISSUE.

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Introduction.

The experiments described in this paper were undertaken in the hope of throwing some light on the question of the oxidation mechanisms of nerve and of brain tissue. The data at present available show that the rate of oxygen consumption by the two tissues is vastly different. There can be no doubt that the brain as a whole uses oxygen at a very much greater rate than does peripheral nerve. The literature bearing on this point has been briefly discussed in previous communications [Holmes and Gerard, 1929; Holmes, 1929, 1] and will not again be referred to here. How far these data can be accepted in a strictly quantitative sense is, of course, open to question. All the experiments on the intact brain have necessarily involved the use of anaesthesia at some stage in the proceedings, while there is no means of judging how far isolated portions of brain may be expected to behave as they would *in situ*. In the experiments on nerve, the tissue has always been isolated. Still, isolated nerves can be proved to be functionally active, and it seems reasonable to assume that the oxygen which they use can be taken as a fair measure of that demanded by them while *in situ* in the living animal. On the whole, therefore, brain is likely to be more adversely affected by experimental conditions than is nerve, and probably experiments have under-rated, rather than over-rated, the true difference between the tissues.

In the present experiments, isolated rabbit or guinea-pig nerves have been employed, and their oxygen consumption has been determined by the use of a Barcroft's differential manometer. The brain tissue was that of cats, rabbits, and mice. Sometimes chopped whole brain was used; sometimes slices of cortex were employed; the oxygen uptake was observed in the same way as was that of the nerves.

1. Oxygen uptake of untreated tissue.

In the first place, the oxygen uptake of cortex slices, chopped whole brain (mixed grey and white matter), pure white matter from the spinal cord and peripheral nerve, was compared (Fig. 1). In these, and in subsequent experiments mentioned in this paper, the tissue was put up in a fluid having the

following composition: Ringer's solution 80 parts, $M/2$ acid potassium phosphate 20 parts, NaOH, sufficient to bring the p_H to 7·6.

It will be seen that the most rapid uptake is that of the cortex (it has been found that it matters little whether it is chopped or sliced) and that the uptake of whole, chopped brain is less. In the case of white matter from the spinal cord (obtained by exposing the cord and cutting off strips of the posterior columns with sharp scissors) affairs are very different. While rabbit cortex takes up about 1200 mm.^{3} of O_2 per g. of tissue per hour, white matter takes up only from 200 to 300 mm.^{3}, an amount not very much greater than that used by peripheral nerve. This is hardly a matter for surprise, since one has no reason, at present, to suppose that the conducting elements inside the

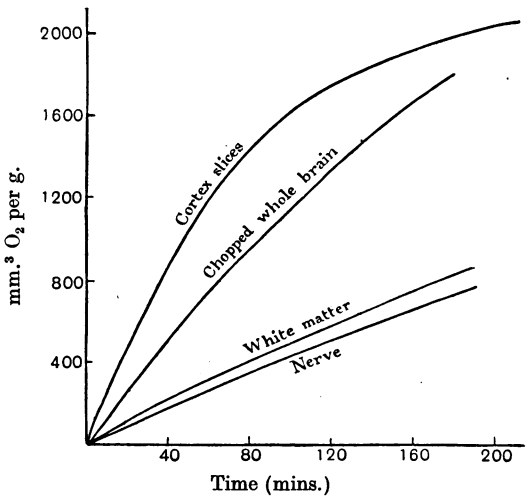

Fig. 1. Rabbit.

central nervous system differ in any essential way (except in power of regeneration) from those which carry impulses to and from the periphery. With regard to the uptake of oxygen by cortex, it must be remembered that the brain tissue was bathed only in Ringer's solution. If 0·25 % glucose is added to the fluid, the uptake in 3 hours is about doubled. In life, the cells are bathed in a fluid containing glucose, and in all probability therefore, from this cause alone, their uptake *in vivo* is much greater than these experiments indicate.

The results obtained with grey matter agree on the whole with those of Loebel [1925] (allowing for the fact that he used rat-cortex, and worked at 40°), while those for nerve tally well with Gerard's results [Holmes and Gerard, 1929] and with those of Sherif [1929]. It has been found that there is a very marked difference in the rate of oxygen consumption of brain tissue of different species, in the sense that smaller animals give tissue with a larger uptake. Loebel's values for rat-brain are rather higher than those here reported for rabbit (even allowing for the fact that he worked at a higher

temperature). Experiment shows, on the other hand, that chopped mouse-brain (whole) takes up oxygen more quickly even than rat-cortex (*e.g.* over 1600 mm.^{3} per hour). Dixon and Elliott [1929] on the other hand, report much lower values for ox-brain.

2. Oxidation mechanisms.

Attempts to investigate some of the oxidation mechanisms involved have naturally followed the lines laid down by the work of Keilin [1929][1].

A series of preliminary experiments with "Nadi" reagent left no doubt that grey matter gave a much more active indophenol oxidase reaction than did either white matter or peripheral nerve. Vernon [1911, 1912] had previously investigated the indophenol oxidase activity of various tissues, among them brain. He made his experiments quantitative by comparing the intensity of colour formation by various tissues after a given time. He observed that the intensity of the reaction varied inversely with the size of the animal. He does not seem to have employed nerve, nor to have differentiated in the brain between grey matter and white. In all cases he found that the most active reaction was given by heart muscle, and that cerebral cortex occupied a high place on the list.

Work on quantitative lines was undertaken in the present circumstances as follows. The oxygen uptake of the washed tissue, ground and suspended in buffered Ringer's solution, to which *p*-phenylenediamine was added, was observed in a Barcroft's apparatus. Without added *p*-phenylenediamine, the O_2 consumption of the suspensions was almost nil. Rabbit, mouse, ox and cat tissues were used at different times, and all gave similar results.

Cat tissues were the most convenient, since from these animals fresh tissue can be obtained in adequate amounts; they have been used to construct the curves in Fig. 2. Keilin's [1929] instructions were followed in preparing the tissue emulsions, the volumes of fluid being proportionately reduced to suit the smaller amounts of tissue. In each case, to provide a basis for comparison, 1 cc. of tissue suspension was pipetted into the cup of the Barcroft's apparatus, and a similar amount, measured with the same pipette, was introduced into a crucible, and dried at 100°. The crucible was weighed, ignited, and re-weighed, the difference giving the amount of organic matter present. The results are expressed as mm.^{3} of oxygen per 100 mg. of organic matter.

This experiment leaves no doubt that the indophenol oxidase of grey matter is much more active than that of white matter, or of peripheral nerve. The ratio of the total uptakes in this particular case is grey: white: nerve = 26·2: 4·3: 1. The difference is very much greater than that shown by the total oxygen uptakes of the three tissues, though the latter is obviously in the same direction. Since, according to Keilin, the function of indophenol oxidase is to oxidise cytochrome reduced by the tissue dehydrases, it seemed possible that the distribution of the pigment in the three tissues might follow similar lines. To

[1] See also review by Dixon [1929].

investigate this, a rabbit was anaesthetised with ether, the chest opened, and cannulae tied into the aorta and right ventricle. The pulmonary artery was clamped, and the animal perfused through the aorta with Ringer's solution kept in a reservoir at 37°, the fluid escaping through the cannula in the right ventricle. (The purpose of the perfusion is, of course, to remove haemoglobin.) When about 4 litres of fluid had been run through, the perfusion was stopped, and the brain, portions of white matter from the spinal cord (posterior and lateral columns), and the sciatic nerves were removed. The three tissues were examined for cytochrome by means of a microspectroscope by Mr R. Hill. He reported that, in the cortex, there was about half as much cytochrome as in yeast; that is, much more than in skeletal muscle, but less than in heart

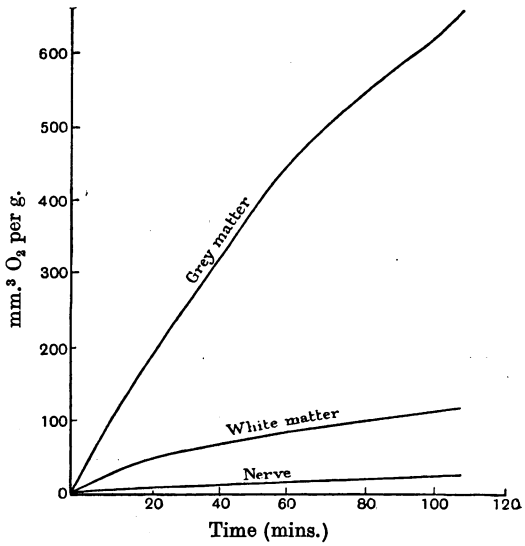

Fig. 2. Cat.

muscle. White matter from the cord contained $\frac{1}{4}$ to $\frac{1}{5}$ as much as cortex; he could not state with certainty that any of the pigment was present in the nerves. The comparison is necessarily rough, and is based on measurement of the thickness of the slices of tissue which give absorption spectra of equal intensity. It is interesting that the amounts of cytochrome in cortex and white matter are approximately in the same ratios as the intensity of the indophenol oxidase activity—about 4 to 1 in the one case and 6 to 1 in the other.

Since nerve is relatively so deficient in indophenol oxidase and cytochrome, it would be of interest to see if this fact could be correlated with its incapacity to oxidise lactic acid, a feature of its metabolism which has been previously reported [Holmes and Gerard, 1929]. Thunberg states that lactic acid will donate hydrogen to methylene blue in the presence of nerve under anaerobic conditions; it seemed, therefore, possible that methylene blue might act as a carrier of hydrogen, in the presence of oxygen, and enable lactic acid to

be oxidised, thus replacing the absent oxidase system. Nerve was therefore put up in a Barcroft's apparatus with 0·5 % lithium lactate, in buffered Ringer-phosphate (p_H 7·6) containing methylene blue $M/2500$. Some experiments showed no increase in oxygen uptake in the presence of methylene blue and lactate, others an increase so slight that it cannot be accepted as significant.

3. Dehydrase systems.

The fact that nerve tissue will reduce methylene blue in the absence of oxygen [Thunberg, 1923; Sherif, 1929] shows that it possesses dehydrase systems, and Thunberg reports that certain substances—glutamic acid, ketoglutaric acid, succinic, fumaric, and lactic acids—are capable of acting as hydrogen donators to methylene blue. Sherif finds that neither the presence of glucose nor of galactose effects any decrease in the reduction time.

Herter [1905] showed that methylene blue was reduced by brain tissue *in vivo*, and was re-oxidised again if the brain was exposed to the air after the death of the animal. Szent-Gyorgyi [1924] found that succinic acid acted as a hydrogen donator to brain tissue *in vitro*. He was able to show that, in the case of brain (unlike that of other tissues), the rate of oxidation of succinic acid was not limited by the rate of activation of oxygen. It has already been remarked that, in the present experiments, methylene blue was found to have no effect on the oxygen uptake of nerve. Table I shows some results obtained during the course of the present work with grey and white matter of ox-brain.

Table I.

	Reduction times in mins.	
	Grey matter	White matter
Control	13½	70
$M/50$ Lithium lactate	7	24
$M/100$ Glucose	6	48
$M/100$ Galactose	10	70

It is quite evident, from these figures, that the dehydrase mechanisms of grey matter, like the indophenol oxidase, are far more active than are those of white matter.

Since grey matter consumes oxygen so readily, and since its oxidation mechanisms seem to act so much more intensely than those of white matter or nerve, it is of interest to see what information is available as to the substrates which the three tissues are able to use.

It is well known that cortex, or whole brain, causes the rapid disappearance of lactic acid, provided oxygen is available [Warburg *et al.*, 1924; Holmes and Holmes, 1925]. It is also known that the tissue can convert glucose to lactic acid with great rapidity. From Table I it will be seen that the reduction time of methylene blue by ox-cortex is about equally rapid in the presence of glucose, as it is in that of lactic acid. To decide whether glucose was oxidised as such, or was first converted to lactic acid, advantage was taken of the fact that

fluoride would prevent the formation of lactic acid by brain tissue [see Ashford and Holmes, 1929].

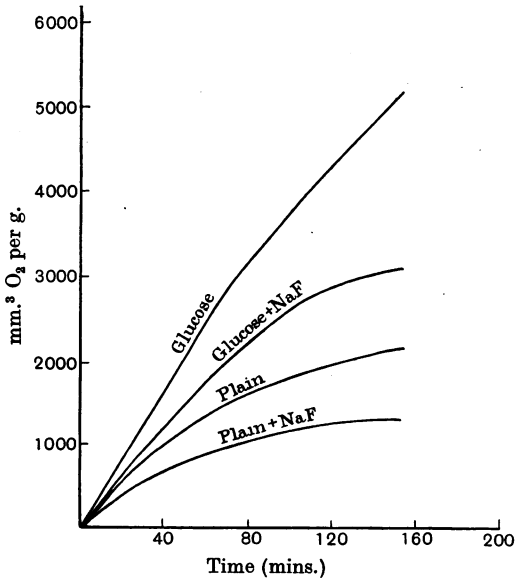

Fig. 3. Mouse.

The present experiment was arranged as follows. Equal amounts of chopped brain tissue (rabbit and mouse) were put up in each of four Barcroft's apparatus[1]. The cups of the first contained plain Ringer's solution, buffered with phosphate to p_H 7·6. The second contained a similar solution plus 0·25 % glucose, the third, as well as the glucose, 0·01 *M* sodium fluoride, and the fourth 0·01 *M* sodium fluoride, but no glucose. The curves of oxygen uptake are given

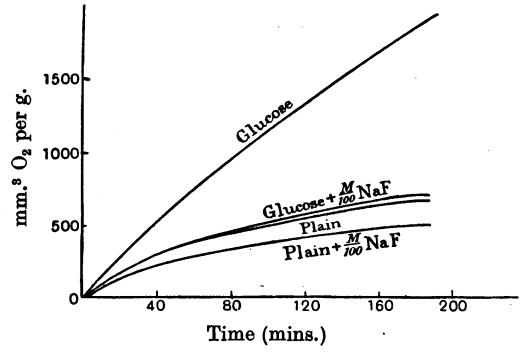

Fig. 3 *a*. Washed mouse brain.

in Fig. 3. It will be seen that the extra oxygen uptake due to the glucose is very greatly inhibited by the fluoride. There is also some inhibition of uptake

[1] I have to thank Mr C. A. Ashford for assistance with these experiments.

by the fluoride in the case of the tissue to which no glucose was added. This presumably indicates the inhibition of lactic acid formation from some precursor already present in the tissue. To test this point, the experiment was repeated with washed tissue. Washing very much decreases the oxygen uptake of chopped brain, and the uptake is restored by adding glucose. Fig. 3 *a* shows that this restoration is largely prevented by 0·01 *M* NaF. At the same time, fluoride still has some effect on the reduced uptake which goes on in the absence of glucose, suggesting that perhaps here it exerts an effect on the metabolism of other substances besides lactic acid—a very probable happening, in view of the findings of other workers.

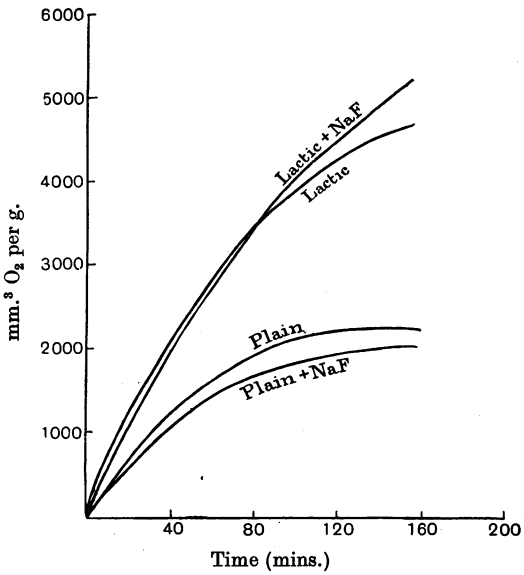

Fig. 4. Mouse.

It may be remarked that from previous experience [Ashford and Holmes, 1929] a complete inhibition of lactic acid formation by 0·01 *M* NaF was not to be anticipated; the figures previously reported indicated an inhibition of about 80 %.

If now, instead of glucose, lithium lactate be used in the above experiment, there is no inhibition of oxygen consumption: the "lactate" and "lactate and NaF" curves are almost identical (Fig. 4). It seems safe, therefore, to conclude that glucose must be converted into lactic acid before it can be oxidised by the grey matter.

As has previously been reported [Sherif and Holmes, 1930] the only effect of the addition of glucose to peripheral nerve is to prolong the period during which a nerve is able to take up oxygen at an approximately linear rate. Even this effect is usually only discernible during the course of an experiment deliberately prolonged for many hours. The same appears to be true of white

matter from the cord, though the point has not yet been very fully investigated; it can, however, be said with certainty that in the case of such white matter there is no marked increase in oxygen uptake in the presence of glucose, such as is invariably obtained with grey matter in similar circumstances. It has been shown [Holmes and Gerard, 1929] that there is no disappearance of lactic acid from peripheral nerve when the tissue is kept in oxygen. It is, therefore, to be expected on all these grounds that fluoride has no influence on the oxygen uptake of nerve. Fig. 5 shows that this expectation is realised.

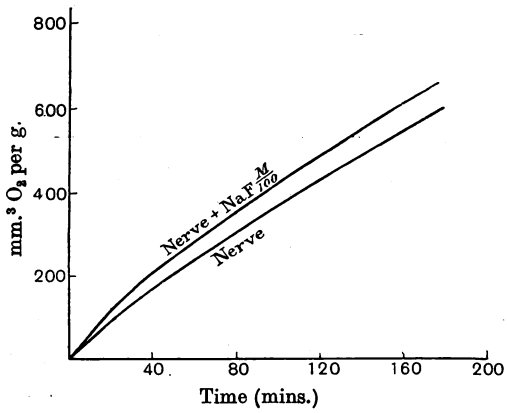

Fig. 5. Guinea-pig nerve.

4. Oxidation of lactic acid by "central" white matter.

Most of the facts so far elicited seem to suggest that white matter from the central nervous system behaves very much like peripheral nerve, a finding which at least fits in with anatomical and histological conceptions. It is, therefore, disconcerting to find that white matter incubated aerobically seems to possess some power of causing the disappearance of lactic acid. Its performance in this respect is a very poor one compared with that of grey matter; it seems, however, that it must be accepted as real.

The results of a number of experiments are given in Table II. The amounts of tissue available when rabbits or cats were employed was small, and the experimental error correspondingly large (in the neighbourhood of 10 %). The final experiment, however, in which ox-brain was used and ample tissue was available, seems to put the matter beyond doubt.

The white matter, in most of these experiments, was placed in a cup of a Barcroft's apparatus and shaken in a thermostat at 37° for 3 hours, the oxygen uptake being measured at the same time. The tissue is much more friable than is peripheral nerve, and at the end of the experiment is, for the most part, broken up; nerve, of course, in similar circumstances, remains intact. An experiment with finely chopped nerve did not, however, show any fall in lactic acid, so that apparently the phenomenon is not dependent upon the mechanical disintegration of the tissue.

Table II.

	Lactic acid: mg. per 100 g. fresh tissue			O_2 absorbed (mm.^{3})	Duration of exp. (hours)	Temp. 0°	Tissue
	Initial	Final	Change				
1*	108	88	− 20	—	2½	37	Cat's cord
2†	102	40	− 62	885	3	37	Rabbit's cord
3	93	109	+ 16	781	3	37	,,
4	107	115	+ 8	845	3	37	,,
5	119	81	− 38	751	3	37	,,
6	134	102	− 32	830	3	37	,,
7	83	84	− 1	566	3	37	,,
8	118	110	− 8	794	3	37	,,
9	116	109	− 7	575	3	37	Cat's cord
10	102	72	− 30	—	3	37	Rabbit's cord
11‡	153 (*a*)	120	− 33	—	3	37	Ox-brain
	154 (*b*)	121	− 33	—	3	37	,,
Average	116	96	− 20	753	—	—	

* Tissue kept in a tube in O_2. All remainder shaken in air.

† Extraction with trichloroacetic acid used for estimation. All remainder worked up by technique described by Holmes and Gerard.

‡ "*a*" and "*b*" duplicate samples, each of 3 g.

5. Effect of washing nerve and brain tissue.

Whatever the materials may be which are responsible for the oxygen consumption of nerve, they are not easily washed away. There is very little difference between the oxygen uptake of nerve that has been soaked in Ringer's solution for 4 hours and one kept in a moist chamber for the same period (Fig. 6). The observations of Meyerhof and Schmidt [1929] on the R.Q. of resting nerve suggest that fats are being oxidised, and this, perhaps, fits in with the foregoing observations.

In contrast to this, soaking the chopped brain tissue for 4 hours in Ringer's solution reduces the oxygen uptake enormously (Fig. 6): in contrast to nerve, therefore, either the brain depends chiefly on freely diffusible substrates, or its oxidising mechanisms are damaged by this treatment. To try to decide between these possibilities, two mice were killed, their brains chopped and sampled, and the chopped tissue soaked for 4 hours in Ringer's solution. The oxygen uptake of the washed tissue was very small, but was very much increased by the presence of 0·25 % glucose in the fluid (compare Fig. 3 *a*). In another experiment, it was found that 0·5 % lithium lactate had an even more pronounced effect. Clearly, in brain as in many other tissues, washing removes substrates, but leaves oxidising mechanisms intact.

That lactic acid is not the only substance oxidised by brain tissue is clear from a consideration of the following experiments.

(*a*) 2 g. of chopped rabbit brain were shaken in a Barcroft's apparatus[1] at 37° in air for 276 minutes. During this time, they consumed 5393 mm.^{3} of oxygen. Another 2 g. sample of the same lot of tissue gave an initial value for

[1] Apparatus specially constructed to deal with large amounts of tissue, to be described later in reporting other work.

lactic acid of 2·53 mg. The oxygenated sample contained at the end of the experiment 0·41 mg. 2·13 mg. had therefore disappeared.

Supposing this to have been oxidised, it would have required 1555 mm.^{3} of oxygen: 3838 mm.^{3} of oxygen are therefore "surplus" and must have been used for oxidising other substances. It may be objected that there is no proof that lactic acid was not formed and oxidised during the course of the experiment, so that, in reality more disappeared than is indicated by these figures. We have previously shown, however, that the anaerobic increase in lactic acid is small [Holmes and Holmes, 1925; Ashford and Holmes, 1929]: there is

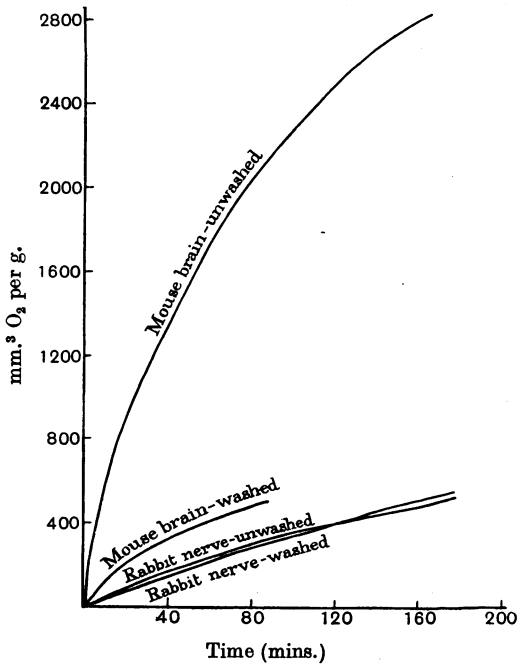

Fig. 6. Mouse brain and rabbit nerve: effect of washing.

very little precursor available in the tissue, and 2·53 mg. is nearly, if not quite, a maximum value. At the most, an extra 0·5 mg. of lactic acid might have appeared.

(*b*) Six mice were starved for 12 hours. Three of them were then given 4 units each of insulin (Burroughs Wellcome) subcutaneously. After 1¾ hours, these three all showed severe hypoglycaemic symptoms. Both groups of mice were then killed, their brains excised, and the two groups of brains separately chopped and sampled. 100 mg. of tissue were then taken from each group, and the oxygen uptake observed (Fig. 7). The remainder of the tissue was used for lactic acid determinations, which gave the following values: normals 1·148 mg. per g.; insulin 0·560 mg. per g.[1]

[1] I have to thank Mr C. A. Ashford for performing these estimations.

It is obvious that the oxygen consumed by the brains of the insulin-treated animals is less than that consumed by the normals. It is also plain that there is a very definite oxygen consumption still taking place even though there is very little lactic acid available.

It would seem, reviewing the evidence that has so far been brought forward, that we have to do with two very different types of metabolism in the brain and in the purely conducting parts of the nervous system. Such a conclusion only bears out many already well-known facts of physiology, such as the great sensitivity of the brain, and the relative insensitiveness of nerves to oxygen lack. We must assume for the present that these differences are characteristic of the nerve cell, or perhaps of the synapse on the one hand, and of the conducting elements on the other. The rate of oxygen consumption of conducting elements is of the same order, whether they be central or peripheral. Nerve cannot oxidise lactic acid, either at rest or during activity

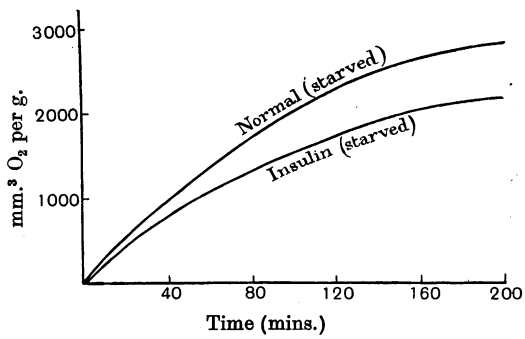

Fig. 7. Mouse.

[Holmes, Gerard and Solomons, 1930]. "Central" white matter can do so, but only to a limited extent. Carbohydrate disappears from resting nerve if oxygen is available, but, even if it is oxidised, it can account for only a part of the observed oxygen consumption [Holmes and Gerard, 1929].

In grey matter, replacing, or perhaps superimposed upon, a metabolism of this type, there appears to be another of a more intense kind. Oxygen is used far more freely, and the substance oxidised is certainly, in great part, lactic acid, though there is clear evidence that other substrates contribute a very appreciable quota.

The tissues dealt with in these experiments have been medullated nerve and nerve cells from the mammalian central nervous system. It is worth recalling that both the heat production [Hill, 1929] and the oxygen consumption [Meyerhof and Schultz, 1929] of non-medullated nerve are far greater than those of medullated nerve, and that the carbohydrate content [Holmes, 1929, 2] of the former is many times greater than that of the latter. No cytochrome could be detected by the writer in non-medullated nerve ganglion.

SUMMARY.

1. It has been shown that the various types of tissue that compose the central nervous system in mammals have different rates of oxygen consumption, and that the activity of grey matter is in this respect far greater than that of white matter, whether the latter forms part of the central system or is taken from a peripheral nerve. The term grey matter necessarily includes without discrimination both nerve cells and synaptic junctions.

2. The distributions of indophenol oxidase and of cytochrome run (roughly) parallel both to each other and to the rate of oxygen consumption of the different types of tissue.

3. The greatly increased oxygen uptake which grey matter displays in the presence of glucose is dependent upon the conversion of the glucose to lactic acid, and disappears if this conversion is prevented by fluoride.

4. Besides lactic acid, there are certainly other substrates responsible for some of the oxygen uptake by brain tissue; and as these substrates are not easily removed by washing, they are probably not carbohydrate in nature.

5. Carbohydrate is probably not responsible for any considerable part of the oxygen uptake of nerve, but this statement must carry the reservation that little or nothing is known of the part played by the carbohydrate fraction of the cerebrosides.

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