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THE INFLUENCE OF THE REACTION OF BLOOD PLASMA ON OXYGEN CONSUMPTION IN RELATION TO THE LAW OF ISODYNAMIC EQUIVALENCE

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THAT animal metabolism is organized to yield, under standard conditions, a constant expenditure of energy, is a conception which goes back to Liebig, but was first put to the test by Rubner [1883] in experiments mainly designed to demonstrate that when fat is replaced in metabolism by other food substances, the substituted food is burnt in a quantity yielding the same amount of energy. Though Rubner's experiments came, with progress in accuracy, to have only historical value [Tigerstedt, 1909], the isodynamic law is held to be approximately true for carbohydrate and fat over a wide range, chiefly as a result of the investigations of Atwater. The precision with which it is followed has not been established, and the issue is confused by the appearance of so-called specific dynamic effects. Such an effect is so prominent with protein [Rubner, 1902] that it is scarcely possible to speak of an isodynamic law in connexion with it, and the following is limited to a consideration of substitution between carbohydrate and fat.

The isodynamic equivalence might be imagined as resulting from a control of the total heat production in connexion with the regulation of temperature, but as the same body temperature may be maintained under identical external conditions at very different metabolic levels, in myxœdema and in thyrotoxicosis, for example, there is no inextricable association between body temperature and energy production. The isodynamic law might also be conceived as a consequence of the energy expenditure of every organ being fixed by the demands of functional activity. The function, it might be thought, comes first, the energy required for that function is then supplied from whatever sources are possible for the body; and this is the arrangement suggested by the

physiology of active muscle. But even in such a case the energy is eventually supplied by oxidation, and the isodynamic law (in so far as it prevails) implies a control of the oxidation so as to yield the required energy. That the metabolism of all organs is similar in spite of great differences in their function, appears clear from the parallelism of the complex action of temperature on the individual organs with its action on the oxygen consumption of the whole animal. It has been shown that the excitability of the cold end organs of the skin [O'Connor, 1935*a*, 1936*b*], the resting oxygen consumption of muscle [O'Connor, 1936*b*], the functional activity of the kidney [Conway *et al.* 1937] are influenced by temperature in a manner closely parallel with the influence of temperature on oxygen consumption of the body as whole; and the probability of analogous effects on the function of other organs has been pointed out [O'Connor, 1935*b*]. It seems difficult to imagine that this close parallelism could depend on an identical influence of temperature on the diverse functional activities of the separate organs. Rather does it seem likely that the rate of oxidation controls or limits the functional activity, and that the oxidation is itself governed by a system identical in all the cells of the body. The consumption of oxygen depends on and must be largely governed by the nature and concentration of the oxidative catalysts.

The enzymatic processes resulting in oxidation may be summarily described as consisting of a system of dehydrases activating the hydrogen of the different substrates at various stages of metabolic decomposition, together with a system of oxidizing ferments activating or transferring oxygen to the activated hydrogen [see Franke, 1934]. It is through the oxygen activators that the different substrates are united into a common metabolism. There is reason to believe that the oxygen activators rather than the dehydrases control the rate at which the breakdown occurs [Szent-Györgyi, 1924]. If they do in fact control oxidation, they must also control the parallel changes in function, and it is through them that we might most easily conceive a mechanism resulting in the isodynamic equivalence.

In a preliminary communication [Kane & O'Connor, 1936] attention was directed to the possibility that such a regulation might occur through changes in acidity. When carbohydrate is substituted for fat in isodynamic equivalence, there is a fall of about 7 p.c. in the oxygen consumption, but an increase of 33 p.c. in the carbon-dioxide production. This increase of CO_2 production will tend to cause an increase in the hydrogen-ion concentrations of the tissues and of the blood in equilibrium

with them. Further, a more acid blood will be required to maintain the increase in respiratory movements necessary to remove the additional carbon dioxide. It is true that the tendency exists to consider the change in reaction necessary for this to be trivial [Douglas & Havard, 1932], but that it would occur to some extent would be generally admitted. If now on a complete change-over from fat to carbohydrate this alteration in hydrogen-ion concentration, when equilibrium is established, causes a reduction of 7 p.c. in the intensity of the catalytic oxidative activity, the isodynamic equivalence would automatically result for these two substances.

If this be the explanation of the isodynamic law the following two demonstrable consequences follow:

(1) Any artificially produced increase in acidity of the tissues (and blood) should cause a fall in oxygen consumption, no matter what substances are being burnt.

(2) It should be possible to find an association between the respiratory quotient and blood reaction, indicating that an adequate fall in *p*H occurs with rising respiratory quotient.

The first of these points is here dealt with; the second is treated in a subsequent communication.

Indications of the complexity of the control of metabolism have been shown, as already indicated, in the study of the influence of temperature on oxygen consumption. This has been shown, in the anæsthetized animal, to be different in the three temperature ranges 22–29°; 29–34°; 34–40° [O'Connor, 1936*a*]. As reasons exist for believing that the second and third of these phases—though more especially the third—are in action at normal body temperature in the unanæsthetized animal, it was of importance to examine the influence of *p*H on at least these two. Observations in all three phases have in fact been made.

Methods

The examination of the influence of *p*H was done with rabbits under urethane anæsthesia (2 g. per kg.). Urethane alters the course of the metabolic phases, detected by temperature analysis, from their normal relations, but this has at least one advantageous result. The third of these phases normally extends in the rabbit from about 41 to 38° [O'Connor, 1936*b*]. Under urethane it extends down to about 34° before giving place to the second phase. The greater range makes the investigation easier. As it is the most important it has been fully examined.

The rabbits were artificially ventilated and immersed in a bath of saline at a suitable temperature. The artificial respiration was maintained by a Starling "Ideal" pump driving air into the inlet tube of a tracheal cannula. The outlet tube of the tracheal cannula, the dead space of which was made as small as possible, led through a mixing bottle and a Bohr meter to the outlet port of the pump. A continuous sample of the mixed expired air was drawn off throughout the period of observation, and was subsequently analysed. From this analysis, an analysis of the inspired air and the volume of the expired air, the oxygen consumption was determined in the usual way and expressed as c.c. in 5 min. for a rabbit of 2 kg. The determinations (over 20 min. periods) are correct to a coefficient of variation of 3 p.c. [O'Connor, 1936*a*].

The *p*H of the blood was altered in several ways: (*a*) by changing the depth of respiration, (*b*) altering the CO_2 content of the inspired air to air containing CO_2 (up to 8 p.c.) in a Douglas bag connected to the inlet port of the pump, (*c*) by intravenous injections of HCl up to 50 c.c. $N/10$ in 0·6 p.c. saline, (*d*) by intravenous injections of $NaHCO_3$ up to 250 c.c., 5 p.c. Care was taken to vary the sequence of the blood reactions as far as possible.

The *p*H of the blood was determined from the Henderson-Hasselbalch equation in the form

$$p\mathrm{H} = p\mathrm{K}' + \log \frac{[\mathrm{HCO_3'}]}{[\mathrm{CO_2}]} - 0{\cdot}5\sqrt{\mu}.$$

For this equation there was required the partial pressure of CO_2 in the alveolar air and the total CO_2 concentration of the plasma.

The partial pressure of CO_2 was obtained by collecting automatically a small portion (about 0·3 c.c.) of the last of each expiration. This was done in the following way. A mercury filled sampling tube was attached by fine tubing to a side tube on the outgoing branch of the tracheal cannula near its beginning. A few drops of the mercury contained in the sampling tube were allowed to escape at the end of each expiration by an electro-magnet controlled from a cam on the pump wheel. The contents of the sampling tube of alveolar air were subsequently analysed.

The CO_2 content of the plasma was obtained by collecting a sample (about 3 c.c.) of carotid blood at the end of each period of observation. This sample was collected without contact with air under paraffin oil in a centrifuge tube lined with paraffin wax and containing a particle of heparin. The blood was immediately sealed off with molten paraffin wax, cooled on ice and centrifuged. The CO_2 content of 1 c.c. of the plasma was determined by an application of the diffusion method of

Conway & Byrne [1933]. This method, due to Conway & Isaacson (unpublished), consists essentially in allowing the CO_2 to diffuse over from acidified plasma into standard barium hydroxide and titrating. It gives results corresponding to the Van Slyke constant volume gasometric method.

From application of these analyses to the formula the pH was determined. The value of pK′ was taken as 6·33 at 38°, and increased by 0·005 for each degree below this temperature. The dissolved CO_2 at the prevailing alveolar air CO_2 tension and body temperature was determined from the Bunsen solubility coefficient in water as given by a graph drawn from the data of Bohr [1899], altered by a factor so as to correspond with the value of 0·510 for serum at 38° [Van Slyke *et al.* 1928]. The value of $0{\cdot}5\sqrt{\mu}$ was calculated from the average NaCl content of rabbit's plasma and the bicarbonate concentration as given by analysis. It varied between 0·17 and 0·22.

Comparisons with results obtained with the glass electrode give very good agreement at 38°, provided that 15 min. were allowed to establish equilibrium when carbon dioxide was inhaled.

Results

(1) *Influence of* p*H on oxygen consumption at* 36°. For reasons already given the temperature zone to which most attention was directed was that between 35 and 38°. The experiments on this zone are in three groups. In the first group, half-hour intervals were allowed for equilibrium after each change of conditions, and the actual periods during which the oxygen was determined were 20 min. With such times but few observations could be made on each animal. Eighty were obtained on twenty-one rabbits. It then appeared desirable to do a larger number on each animal, lest in seeking to avoid errors arising from uncompleted equilibrium, confusion be produced through the different levels in oxygen consumption in different animals. A second series was done in which the intervals were reduced to 15 min. and the periods to 8. Forty-four observations were obtained on six rabbits. With one of these curare was used. In a third series, in which we had the assistance of Dr O. Fitzgerald, the pancreas with the duodenum or a loop of jejunum was removed 2 hours previous to the first of the observations which were made at intervals similar to the second series. There were seventy-eight observations on seventeen animals.

The results obtained in the second group of observations are given in Fig. 1, in which the data from individual rabbits are indicated by dis-

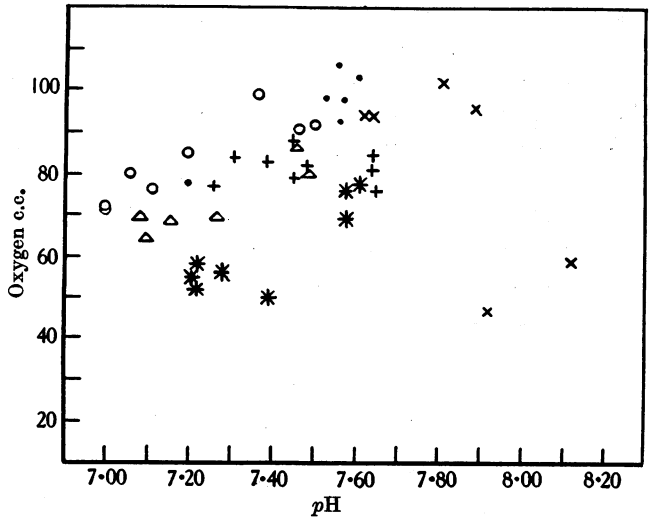

Fig. 1. The influence of acidity on oxygen consumption at about 36°; second group of observations. The separate experiments are indicated by different marks. In the experiment marked by asterisks curare was used.

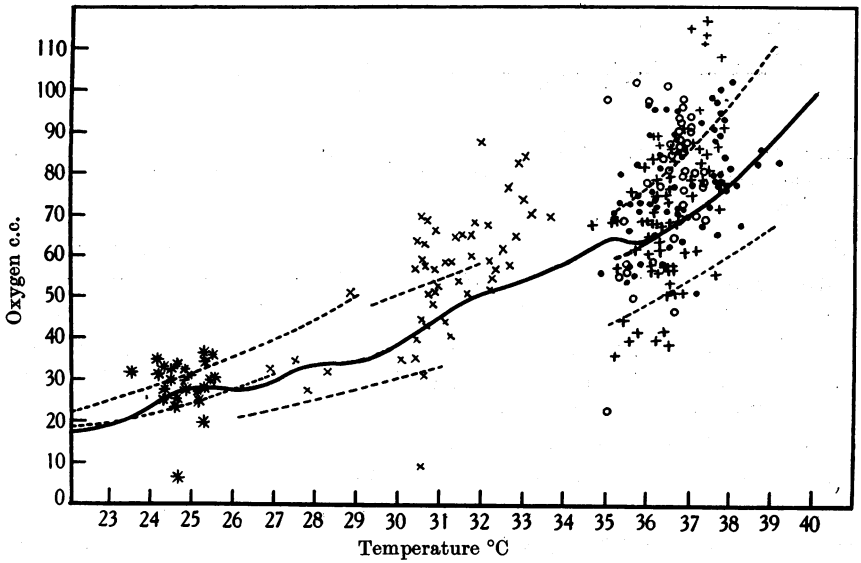

Fig. 2. The relation of the observations on oxygen consumption to rectal temperature (see text). All data were obtained from quiescent rabbits, i.e. shivering was prevented, if necessary, by curare.

tinctive marks. From this group it appears that the oxygen consumption rises with rising *p*H to 7·6, at which point it becomes approximately steady or reaches a maximum. When we come to consider the quantitative relationship between *p*H and oxygen consumption, the first difficulty that arises is that when the metabolism falls in a bath at constant temperature, the body temperature also tends to fall. This tends to exaggerate the decline in oxygen consumption. The extent to which change in temperature may influence oxygen consumption may be judged from Fig. 2. In this the heavy line [Conway *et al.* 1937] in-

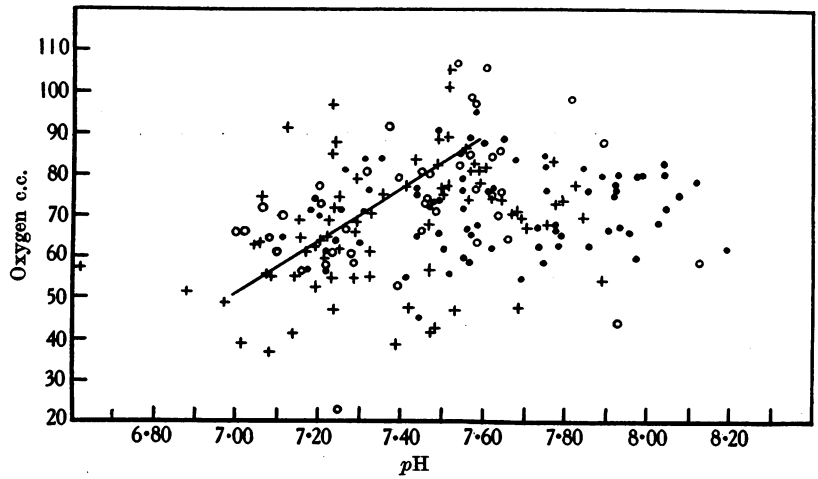

Fig. 3. The influence of acidity on oxygen consumption at 36°. The dots, circles and crosses indicate the three groups of observations alluded to in the text. The line is the regression line.

dicates the average results of over 600 determinations, and the pairs of dotted lines [O'Connor, 1936*a*], the limits within which the most reliable values lie when no intentional alteration in *p*H was produced. In order to correct for any possible influence of temperature, the data have been adjusted to a temperature of 36° in accordance with the thick line in Fig. 2 with the slight extrapolation back to 35°.

The results of all the observations in the region of 35–38°, corrected according to this scheme, are given in Fig. 3. Inspection of this graph gives the impression that the fall in oxygen consumption which begins with falling *p*H at 7·6 is at first slight but becomes more pronounced at about 7·3. This aspect of the result will be considered subsequently, but the immediate interest is to establish the existence of the fall and to measure its average amount as accurately as possible. This was done

by calculating the statistical correlation between oxygen and *p*H up to 7·6.

The statistical results may be summarized as follows: Number of observations, 132. Mean *p*H, 7·32. Standard deviation of *p*H, 0·19. Mean oxygen consumption, 68·5 c.c. Standard deviation of the oxygen consumption, 15. Correlation coefficient, $+0{\cdot}41 \pm 0{\cdot}08$. Regression of oxygen consumption on *p*H, 3·3 c.c. per 0·10 *p*H. From this it appears that a fall of *p*H from 7·4 to 7·3 would produce a fall in oxygen consumption of about 4·5 p.c.

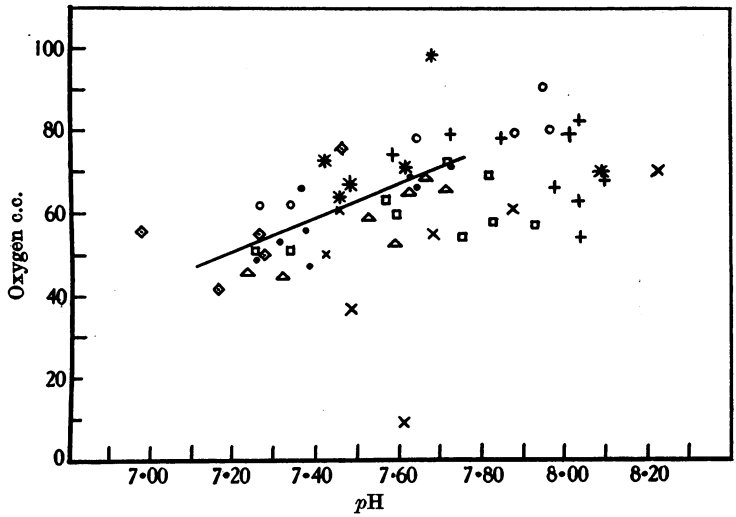

Fig. 4. The influence of acidity on oxygen consumption at 31°. The separate experiments are indicated by different marks. The line is the regression line.

(2) *Influence of* p*H on oxygen consumption at* 31°. Since at rectal temperatures below 34° anæsthetized rabbits almost always shiver, it is necessary, in order to obtain comparable figures, to use curare in addition to the urethane. Fifty-seven observations were obtained from eight rabbits for periods of about 8 min., and with intervals of 15 min. The results, corrected to a temperature of 31° according to Fig. 2, are given in Fig. 4 in which the separate experiments are again distinguishable. The effect of *p*H is very similar to that shown at 36°. The rise in oxygen consumption with rising *p*H appears, it is true, to continue to *p*H 7·7 rather than 7·6, but obviously no stress can be laid on this. The statistical analysis of the influence of *p*H up to 7·74 gives the following: Number of observations (excluding the one very low value from a moribund rabbit),

39. Mean and standard deviation of *p*H, 7·46 ± 0·18. Mean and standard deviation of oxygen consumption, 61 ± 11·5 c.c. Correlation coefficient, +0·61 ± 0·10. Regression of oxygen, 3·9 c.c. per 0·1 *p*H. A change from *p*H 7·3 to 7·2 will cause a fall of about 6·5 p.c.

(3) *Influence of* p*H on oxygen consumption at* 25°. Different from the two previous cases are the results obtained at 25°. There are twenty-six observations on five rabbits obtained during periods of 9–17 min. and

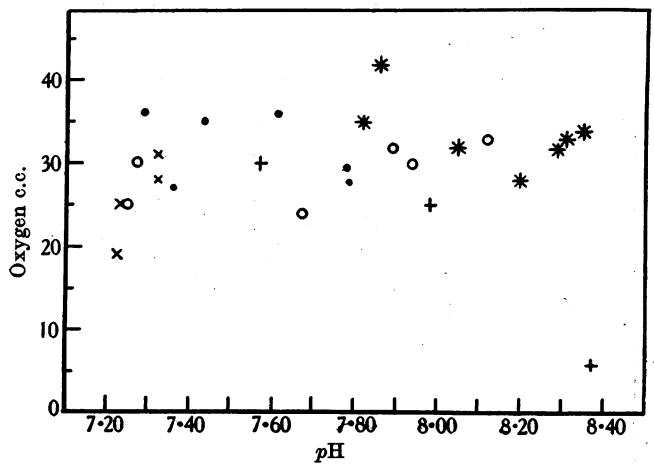

Fig. 5. The relation of oxygen consumption to acidity at about 25°. The separate experiments are indicated by different marks. No correction for temperature has been attempted.

with intervals of 15–50 min. Curare was used. As can be seen from Fig. 5, in which the separate experiments are distinguished, *p*H between 7·2 and 8·3 has no effect on oxygen consumption. Attempts to drive the *p*H outside these limits caused death of the animal.

Discussion

If the activity of organs be associated with their oxygen consumption and if, further, the rate of oxidation be in the main governed by the active concentration of the oxidative catalysts, it follows that the oxidative catalysts control the fundamental activity of organs, i.e. the energy expended in them. From this it follows—omitting the unlikely possibility that the energy consumption controls the concentration of the catalysts—that the isodynamic equivalence must result from an influence on the oxidative catalytic system. If the suggestion put forward in the communication, that this control is exercised through the in-

fluence of the blood (and tissue) *p*H be correct, the first condition is that the blood *p*H must be below 7·6, as, above this, *p*H has no marked effect on oxygen consumption. In fact in the rabbit 7·6 is just above the upper limit of usual arterial *p*H which, according to Hawkins [1924], is 7·58. In man the highest normal value seems to be 7·52 [Earle & Cullen, 1929]. If now we assume that when fat and protein were being metabolized, 15 p.c. of the oxygen was used for protein, and that, this remaining unchanged, the fat was completely replaced by carbohydrate, the oxygen consumption should fall 6 p.c. for complete constancy of energy production. Assuming an initial *p*H of 7·4 and using a range of four standard deviations as the limits of the regression coefficient calculated as prevailing at 36°, it appears that the oxygen consumption will fall between 3 and 5·8 p.c. for a fall of 0·1 *p*H. For isodynamic equivalence a fall of between 0·11–0·20 *p*H would therefore be necessary. Such a fall is well within the range of the rabbit's normal [Hawkins, 1924], and is not impossible in man.

It is improbable that the regression coefficient, which is of course an average over the whole range of *p*H to which it applies, gives the true relation of oxygen and *p*H. A better approximation to this will be given by a graph drawn through the means of the oxygen consumption in Fig. 3 in arrays of 0·10 *p*H. If this is done it will appear that a change as small as 0·08 at a *p*H of 7·30 would be sufficient to produce the isodynamic law. *p*H differences of this amount have been met with in man in the course of a single day during which there is no reason to believe that the entire range of the respiratory quotient has been covered [Cullen & Earle, 1929; Shock & Hastings, 1934]. There is consequently no improbability involved in the theory that the fall in oxygen consumption on a carbohydrate diet may be produced by a fall in *p*H. Evidence pointing to the existence of a fall of this type will be dealt with in a following communication. That the effect of acidity on oxidation may be produced through an influence on catalysts, gains in plausibility from the fact that *p*H has a similar influence on catalysts known to be involved in oxidations, such as indophenol oxidase [Gräff, 1922], and succinicodehydrase [Cook & Alcock, 1931].

There is a corollary argument arising out of the facts presented above which suggests a further test of the theory. At a temperature of 25° *p*H has no influence on oxygen consumption. If now the oxygen consumption of cold-blooded animals be controlled at this temperature by the same catalytic system—and inspection of the observations of Benedict [1932] on the CO_2 production of reptiles indicate that this is so—

it would follow that, at this temperature, cold-blooded animals would not exhibit the isodynamic law. Data are, however, not available for this test.

SUMMARY

The intensity of oxidation in the body must depend in the first instance on the concentration and activity of the oxidative catalysts. As the law of isodynamic equivalence, applied to carbohydrate and fat, implies a diminution of the amount of oxidation when carbohydrate is being oxidized, it is suggested that this decrease is due to an influence on the oxidizing catalysts of a more acid reaction of the blood and tissues resulting from the increased production of carbon dioxide during carbohydrate oxidation.

The influence of artificial alteration of the *p*H of the arterial blood on the oxygen consumption of the anæsthetized rabbit was examined at three temperatures. At 36° and at 31° a fall of *p*H below about 7·6 causes a fall in oxygen consumption of approximately 5 p.c. for a fall in *p*H of 0·1.

For isodynamic replacement of fat by carbohydrate a fall of *p*H of at least 0·08 or more probably 0·11–0·20 would be necessary. Changes in *p*H of this order may occur normally.

At 25° variation in *p*H between 7·2 and 8·3 has no influence on oxygen consumption.

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