XXVII. THE AEROBIC AND ANAEROBIC METABOLISM OF THE COMMON COCKROACH (*PERIPLANETA ORIENTALIS*). II

By WILLIAM KERSHAW SLATER (*Beit Memorial Research Fellow*).

*From the Department of Physiology and Biochemistry,
University College, London.*

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It has recently been shown [Davis and Slater, 1926] that when the common cockroach (*Periplaneta orientalis*) is placed in an atmosphere of nitrogen or some other inert gas, it goes into debt for oxygen, in a manner similar to that which has been observed in the case of striated muscle. This phenomenon was thought to be worthy of further investigation, in the hope that the superficial similarity might prove to be the expression of a common fundamental metabolism.

The metabolic process involved in the case of vertebrate muscle has been shown by Hill, Meyerhof and their co-workers to consist of two separate reactions, viz. (1) an initial breakdown of glycogen to lactic acid, and (2) the transformation of a portion of the lactic acid back again into glycogen, at the expense of the combustion of the remainder. The first reaction is anaerobic, and is the source of energy in the organism, the second is aerobic, and serves simultaneously as a means of removing the lactic acid, and of conserving the energy available in the glycogen. The efficiency of the total process depends largely upon the ratio of the lactic acid returned to glycogen to the lactic acid burnt. During oxygen lack the tissue obtains its energy in the normal manner, the lactic acid being allowed to accumulate. When oxygen again becomes available, this lactic acid is removed by synthesis and oxidation, the organism using an excess of oxygen, equal to that which it would have used during the period of anaerobiosis. In the case of vertebrate muscle, the ratio of lactic acid burnt to that resynthesised to glycogen is given by Furasawa and Hartree [1926] as 1:4.4, from which it can be calculated that a debt of 1 cc. of oxygen corresponds approximately to 6 mg. of lactic acid.

Working upon the assumption that a similar process is involved in the metabolism of the cockroach, it is possible, knowing the oxygen debt, to calculate (1) the lactic acid which should be found in the tissue, and (2) the volume of carbon dioxide which would be evolved if the lactic acid were completely neutralised by carbonate. This latter figure will be identical with the carbon dioxide which is retained when the lactic acid is subsequently removed.

An investigation is at present being carried out with a view to estimating the lactic acid occurring in conjunction with a given oxygen debt after a period of anaerobiosis. The present communication deals with a more detailed study of the respiratory exchanges following anaerobiosis in order to show, firstly, whether carbon dioxide is retained, and secondly, if this proves to be the case, whether the volume of the retained gas bears any relationship to the calculated amount of lactic acid present.

It may appear at first sight that it would be simpler and more direct to measure the carbon dioxide evolved during anaerobiosis, but practically it is easier to compare over a given recovery period the excess oxygen used and the carbon dioxide retained.

METHOD.

The measurements were made by means of the micro-respiration apparatus previously described [Slater, 1926]. This enabled the oxygen uptake and the carbon dioxide output to be determined simultaneously with an accuracy greater than 1 mm.³.

Since the apparatus was originally described, some alteration has been made in the method adopted for ascertaining changes in the volume of the gases. The respiration vessel, in which the carbon dioxide is measured electrically by means of the Shakespear katharometer, is now connected to a small open manometer, instead of the original Haldane blood gas apparatus. At the bottom of the U of the manometer is a small rubber bag, which can be compressed by means of a plate and thumb screw. As the volume of the gases in the respiration chamber alters, the bag is compressed or relaxed, so as to bring the liquid on each side of the U back to the same level. By calibrating the arm of the U on the closed side, the changes in volume may be read at atmospheric pressure. To avoid errors which may be introduced during the experiment, by changes in barometric height or in the temperature of the thermostat, a second bulb as similar to the respiration vessel as possible and connected to a similar manometer, is placed in the thermostat alongside the actual apparatus. The changes in the volume of the air in this vessel are applied as a correction to the actual readings obtained for changes in the volume of the respired gas mixture. The new manometric arrangement has the advantage that it is small, and can be sealed directly on to the tube from the respiration vessel, thus avoiding all rubber joints, and permitting, if necessary, that the whole apparatus be shaken. This method of measuring volume changes is based upon that described by Warburg [1923].

The cockroaches were caught in the building, and were used immediately (except in those cases where they were kept for a few days on a glucose diet in order to raise their respiratory quotient) to ensure that they were in no way damaged by being kept in captivity. The respiratory quotient of the freshly caught insects was rarely above 0.85, probably due to the fact that

in the building where they were caught, there was abundant food of fat and protein, but hardly any carbohydrate.

The insect selected for the experiment was weighed, and dropped into the lower part of the respiration vessel. Care was taken in attaching this to the upper portion that the antennae were not trapped in the ground glass joint. The apparatus was then placed in a thermostat kept at 25°, and left until the cockroach had settled into a quiet state. When the necessary conditions had been attained an oxygen-carbon dioxide mixture was led through the apparatus until the reading of the katharometer showed that all the air had been expelled. The taps were then closed and readings taken of the carbon dioxide percentage and the total volume. When the readings became steady they were continued for an hour in order to get a value for the resting metabolism under the conditions of the experiment. A stream of nitrogen was next passed through the apparatus until the cockroach ceased to struggle. The vessel was then closed for from 30 mins. to 1 hour. As will be seen later, for purposes of comparison it is not necessary that the anaerobiosis should be absolute. Such a condition would be difficult to obtain with the type of apparatus which was in use. At the end of the anaerobic period the nitrogen was rapidly replaced by the oxygen-carbon dioxide mixture, and readings commenced as soon as possible and continued until the respiratory quotient again reached its normal value.

RESULTS.

In Table I are given the chief details of 7 experiments.

Table I.

Experiment	1	2	3	4	5	6	7
Weight of cockroach (g.)	0.13	0.07	0.19	0.22	0.18	0.12	0.08
Condition of cockroach	Glucose-fed	Freshly caught	Freshly caught	Freshly caught	Freshly caught	Glucose-fed	Starving for 1 month
% carbon dioxide at the beginning of the experiment	1.70	1.76	1.44	1.47	0.83	0.60	0.35
Normal respiration:							
Oxygen mm. ³ per g. per hour	314	375	292	258	398	366	225
Carbon dioxide mm. ³ per g. per hour	315	365	242	241	277	342	200
Post anaerobic respiration (period of excess oxygen intake):							
Oxygen mm. ³ per g. per hour	370	433	350	359	458	416	288
Carbon dioxide mm. ³ per g. per hour	262	384	249	260	305	346	209
Excess oxygen used mm. ³ per g. per hour	56	58	58	101	60	50	63
Carbon dioxide retained mm. ³ per g. per hour	108	39	51	82	32	46	54

In the table the rise in oxygen intake after anaerobiosis is clearly marked in each experiment, but this value is not in proportion to the time during which the cockroach was in nitrogen. This is due to the difficulty experienced

in obtaining the complete removal of oxygen by the method used. The nitrogen always contained traces of oxygen, and the shape of the respiration vessel rendered the complete removal of the gases previously present a matter of considerable difficulty. As however the results are used only for the comparison of the oxygen debt replacement and the carbon dioxide retention over the same period of recovery, the efficiency of anaerobiosis is immaterial, provided always that it does not vary outside those limits which may be expected to produce profound tissue derangement.

It was shown [Davis and Slater, 1926] that the normal oxygen uptake at 25° was about 500 mm.³ per g. per hour. In the present experiments the values obtained are all below this average figure. No positive explanation of this difference can be advanced, but it is tentatively suggested that it is due to the fact that the present experiments were carried out at a definite carbon dioxide pressure. Enough data are not available in support of this view to render it more than a working hypothesis, although the results of the experiments in Table I agree with it as far as they have been carried. With the exception of Exp. 7, where the cockroach was distinctly abnormal, owing to being without food for over a month, the oxygen intake appears to be reduced with higher percentages of carbon dioxide. (It is to be noted that in Exp. 7, the weight of the insect is only 0.08 g., *i.e.* it is not fully matured and hence according to previous determinations its oxygen uptake should be approximately 600 to 700 mm.³ per g. per hour.)

To obtain the carbon dioxide retention during the post anaerobic phase, it is necessary to calculate the carbon dioxide which should have been evolved during this period. This value is easily obtained if we assume that the normal metabolism is going on with the recovery process superadded; for under these conditions the carbon dioxide evolved will be equal to the normal plus that from the oxidation of the lactic acid. The combustion of the lactic acid has a unit respiratory quotient, and hence the carbon dioxide evolved is equal to the excess oxygen taken in. Thus we have

$$\text{CO}_2 \text{ output after anaerobiosis} = \text{Normal CO}_2 \text{ output} + \text{O}_2 \text{ debt.}$$

The carbon dioxide retained, as given in the last row of figures in Table I is obtained by subtracting the output found from the output calculated in this way. The retention of carbon dioxide is clearly shown in each experiment. In Table II are shown the respiratory quotients for the same experiments, again illustrating the carbon dioxide retention.

Table II.

Experiment	1	2	3	4	5	6	7
Normal R.Q.	1.00	0.98	0.83	0.93	0.70	0.93	0.89
Post anaerobic R.Q.:							
Calculated	1.00	0.98	0.86	0.95	0.73	0.94	0.91
Found 1st half hour	0.60	0.87	0.72	0.65	—	—	—
2nd "	0.83	0.91	0.68	0.63	0.62	0.82	0.53
3rd "	1.03	0.98	0.73	0.74	0.71	0.83	0.95
4th "	0.96	0.97	0.83	0.83	0.81	—	0.78

It will be seen that in the majority of cases, the respiratory quotient has returned to normal at the end of 2 hours. The figures given for the calculated respiratory quotient for the post anaerobic period allow for the excess oxygen used liberating its own volume of carbon dioxide.

There remains now only the consideration of the relation between the oxygen debt and the carbon dioxide retained. If the figure given above—1 cc. of oxygen equivalent to 6 mg. of lactic acid—is used to calculate the lactic acid present in the tissue, the results shown in row 3 of Table III are obtained.

Table III.

Experiment	1	2	3	4	5	6	7
Excess oxygen mm. ³	56	58	58	101	60	50	63
Lactic acid corresponding to excess oxygen, mg.	0.336	0.348	0.348	0.606	0.360	0.300	0.378
Carbon dioxide retention calc. from lactic acid, mm. ³	84	87	87	151	90	75	94.5
Carbon dioxide retention found, mm. ³	108	39	51	82	32	46	54
Ratio CO ₂ found/CO ₂ calc.	1.2	0.46	0.59	0.54	0.36	0.60	0.57

From the lactic acid, the volume of carbon dioxide which should be retained during the recovery process can be calculated, and is given in row 4. The accuracy of these figures depends on two unknown factors, viz. (1) the extent to which carbonates are involved in the neutralisation of the lactic acid, and (2) the efficiency of the oxidative recovery process. For purposes of the calculation it has been assumed that the whole of the neutralisation is by carbonates, and that the efficiency of the recovery process is the same as that of striated muscle. It is improbable that the first assumption is correct, and there is no precedent for the second. Fortunately these unknown factors will affect all the results proportionately, and if we determine the ratio between the carbon dioxide found and that calculated, it should, on the assumption that the carbon dioxide retention is due to the removal of the lactic acid, be approximately constant. This ratio is given in row 5, and, with the exception of Exp. 1, the values lie between 0.36 and 0.60, with a mean of 0.52, and a percentage mean deviation of 14.6. For the type of experiment under consideration, the agreement is sufficiently good to lend support to the view that the oxygen debt and the carbon dioxide retention are related through the removal of accumulated lactic acid. In the case of Exp. 1 it can only be suggested that some unknown error has been introduced, but as it was the only experiment which fell outside the series, it was considered reasonable to neglect it for the time being.

SUMMARY.

(1) After a period of anaerobiosis the cockroach not only uses an excess of oxygen, but also retains a considerable volume of carbon dioxide.

(2) The oxygen debt and the carbon dioxide retention are shown to be related through the removal of lactic acid.

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