THE TEMPERATURE AND HUMIDITY RELATIONS OF THE COCKROACH

VI. OXYGEN CONSUMPTION

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(With One Text-figure)

I. INTRODUCTION

In recent years a considerable amount of work has been done on the rates and the mechanisms of water loss by evaporation in insects (e.g. Uvarov, 1931; Buxton, 1932; Mellanby, 1935; Ludwig, 1936; Ludwig & Landsman, 1937; Thornthwaite, 1940) and on the behaviour reactions of insects in humidity gradients (e.g. Gunn & Kennedy, 1936; Fraenkel & Gunn, 1940; Pielou, 1940; Wigglesworth, 1941). A certain amount of information has been published about effects of air humidity on metabolic rate (Hennings, 1907; Caldwell, 1925; Uvarov, 1931; and others quoted below), but a clear distinction has not been made between direct effects of dry air and indirect effects due to a desiccated condition of the insects.

Caldwell (1925) used an Osterhout apparatus to measure the carbon dioxide production of mealworms (*Tenebrio molitor* L.) and some other animals when starved and desiccated. Since no special method was used to control the humidity in this apparatus, presumably the air was kept moist by the fluids normally present in it. A few batches of three animals were tested over a desiccation period of 6 days, a very short period for the mealworm (Buxton, 1930), and there were undesiccated controls. The controls did not show a constant metabolic rate; in the first 3 or 4 days the variation was seldom greater than 2 or 3%, but by the sixth day the rate had fallen by 20–30%. This fall was presumably due to the cessation of digestive processes. A similar fall occurred with desiccated animals. Presumably the appropriate way of allowing for this is to compare the change in rate for desiccated animals with that for undesiccated animals starved for the same time. On the third day the average rate for desiccated animals had risen absolutely and was 26% higher than that for undesiccated controls. On the sixth day the desiccated animals were producing slightly less carbon dioxide per gram of original (undesiccated) weight than the controls. Caldwell concluded that there was 'a definite, positive, correlation between desiccation and carbon dioxide production'. The only justifiable conclusion, however, is that after 1–4 days' starvation desiccated animals produced carbon dioxide faster, when tested in moist air, than undesiccated controls.

It was tentatively suggested by Buxton (1930) that mealworms desiccated for long periods can make up some of the lost water by the oxidation of larger amounts of reserve foods; the suggestion was based on the dry weights of small numbers of insects after

* All the experimental work was done by C. A. C.

desiccation at 23° C. Mellanby (1932*a*) found, on the basis of chemical analyses, that no extra metabolism occurred in mealworms desiccated at 30° C. In later work he concluded that there was no greater loss of dry weight in dry air than in moist in *Cimex* (1932*b*), *Tineola* (1934), *Glossina* (1936*a*) and *Tenebrio* (1936*b*). Mellanby (1936 *a*, *b*) further pointed out that the beneficial effect of producing extra metabolic water would be offset by an increase in water loss, for the increased metabolism would require increased opening of the spiracles.

This work on food reserves does not distinguish between the direct effect of dry air on respiration and the indirect effect through desiccation. A temporary increase of respiration at the beginning of desiccation such as Caldwell (1925) found might not be large enough to show in determinations of food reserves utilized over a long period. Consequently the results of Caldwell and Mellanby are not necessarily contradictory.

In our work on the cockroach the rates of oxygen consumption of both undesiccated and desiccated animals were found in moist air and in drier air.

II. METHODS

Only adult male specimens of *Blatta orientalis* L. were used. They were kept at 25° C., the temperature at which respiration was measured. They were deprived of food for 48 hr. before each experiment with a view to measuring standard metabolism. Desiccated animals had been kept, unfed, in dry air for 3 days. Some died during that time, but usually they lived and lost about 25 % of their initial weight (cf. Gunn, 1933).

Oxygen consumption was measured in a Barcroft apparatus with a specially designed bottle (Fig. 1 *e*). A solution of caustic potash of appropriate concentration covered the whole floor of the bottle and served both to absorb carbon dioxide and to control the air humidity. The cockroach could move about in a basket made of perforated zinc but could not touch the potash. The potash solutions were made up to be in equilibrium with atmospheres at 10 and 95 % R.H. according to data given by Buxton & Mellanby (1934).

There is considerable individual variation in rate of oxygen consumption of cockroaches, and in one individual, for example, the rate varies from time to time because of activity, feeding and moulting (Gunn, 1933, 1935 *a*, *b*). It was therefore necessary to design and control the experiments carefully. Two Barcrofts were used together, one for drier air and the other for moist, each with a single animal. After a 20 min. equilibration period and observations usually lasting 60–90 min. the animals were interchanged and tested again. Thus each animal was tested at each humidity, with a short interval between the tests. On the next occasion the Barcroft which had previously been used for moist air was used for drier; this tended to compensate for any calibration error. When a particular animal was tested in more than one pair of experiments the order in which it was subjected to moist air and to dry was alternated in successive pairs of experiments; this was to allow for any trend in rate of respiration.

On the whole the cockroaches were remarkably inactive during the experiments. It is well known that variable activity or any variation in rate of oxygen consumption makes the Barcroft an unsuitable instrument because of delay in absorption of carbon dioxide. In these experiments the manometer differences were plotted against time, and only those which gave a straight line were accepted. Experiments in which activity occurred other than one or two changes of position were rejected.

III. CALIBRATION OF THE APPARATUS

The Barcroft respiration apparatus is usually employed for wet tissues or for animals in a saturated atmosphere. For our experiments, in which the air was sometimes moist and sometimes drier, it is necessary to consider whether the results would be falsified by a humidity effect; when oxygen is absorbed the pressure will fall more in saturated air than in dry because some of the water vapour will liquefy. It is not necessary to repeat the complete calculations leading to the calibration constant, for they have been dealt with by Dixon (1934).

Let V cu.mm. be the volume of each bottle, assumed equal,

v cu.mm. be the volume of oxygen consumed in a given time at pressure $(B-w)$,

B mm. Hg be the barometric pressure,

w mm. Hg be the water vapour pressure in both bottles,

Pe mm. Hg be the pressure in the experimental bottle after the given time,

Pc mm. Hg be the pressure in the control bottle after the same time,

d mm. be the difference of levels along the manometer scale (initially zero) after the same time,

k be the factor required to convert d into a pressure difference in mm. Hg,

a sq.mm. be the cross-sectional area of the manometer tubing.

Since all the experiments were carried out at one temperature, temperature may be omitted from the following equations.

For the control bottle the weight of dry air present remains constant, though the weight of water vapour present in bottle and manometer tubing will increase because of the movement of the manometer fluid.

For the dry air
$$(B-w)V=(Pc-w)(V+\tfrac{1}{2}da),$$
$$Pc=w+\frac{(B-w)V}{V+\tfrac{1}{2}da}. \tag{I}$$

For the experimental bottle the dry air of volume V at pressure $(B-w)$ is reduced by the absorption of air (oxygen) of volume v at pressure $(B-w)$. The weight of air left after the given time therefore originally occupied $(V-v)$ at $(B-w)$; afterwards this expands to fill the bottle, but its space is reduced by the manometer fluid rising:
$$(B-w)(V-v)=(Pe-w)(V-\tfrac{1}{2}da),$$
$$Pe=w+\frac{(V-v)(B-w)}{V-\tfrac{1}{2}da}. \tag{II}$$

The difference of pressure is shown by the manometer
$$kd=Pc-Pe. \tag{III}$$

Substituting from (I) and (II) in (III):
$$\frac{kd}{B-w}=\frac{V}{V+\tfrac{1}{2}da}-\frac{V-v}{V-\tfrac{1}{2}da}.$$

Substituting the following values:
$$k=0{\cdot}06,\quad V=27{,}500 \text{ cu.mm.},\quad a=0{\cdot}7 \text{ sq.mm.},\quad d=200 \text{ mm.},$$

we get
$$v=27{,}430\left(\frac{12}{B-w}+0{\cdot}0054\right).$$

Correcting this to standard pressure

$$v' = \frac{27{,}430}{760}(12 + 0{\cdot}0054(B - w)).$$

With $B = 740$ and w either 0 or 23 mm. Hg

$$\frac{v' \text{ for dry air}}{v' \text{ for moist air}} = 1{\cdot}008.$$

That is to say, a given difference of manometer levels will represent 0·8 % more oxygen absorbed in dry air than in moist. If, therefore, the same amounts of oxygen are used in dry air and in moist the readings will falsely suggest that oxygen is used 0·8 % more rapidly in moist air.

IV. COMPARISON OF TWO TYPES OF BARCROFT APPARATUS

Some doubt has been cast on the suitability of this apparatus for experiments with whole animals. Experiments showed that it gave lower values than a chemical analysis method when used for aquatic insects (Fox, Wingfield & Simmonds, 1937). Earlier experiments were done with Barcrofts which had a small surface of potash for absorption of carbon dioxide (Gunn, 1933, 1935 *a*, *b*), and so a comparison was made between results from that earlier type and the new *basket* type.

The earlier type had the potash in an annulus at the top of the bottle, while the glass floor was covered with a carpet of damp filter paper (Fig. 1 *c*). This carpet was necessary to control the humidity, for in its absence wet faeces deposited during an experiment raised the vapour pressure and spoiled the experiment. The cockroach was prevented from touching the potash by means of a copper wire spiral (Fig. 1 *a*). This apparatus is referred to as the *carpet* type.

The volume of each bottle in the carpet type was 21 c.c., while in two Barcrofts of the basket type it was 27 and 35 c.c. The carpet type could be used only with saturated air, so the basket type had to have weak potash in it, to give a high humidity, in this set of experiments.

Ten experiments were done, in which three normal cockroaches were tested separately, first in one apparatus and then in the other, in conformity with the plan described on p. 125. In the carpet type the average rate of oxygen consumption was 308 ± 9 mm.^{3}/g./hr. (at N.T.P.), and in the basket type 296 ± 3 mm.^{3}/g./hr. (average weight of animals 345 mg.). In the whole series, which included some imperfectly paired experiments, the rates were for the carpet type 331 ± 9 and for the basket type 320 ± 10 mm.^{3}/g./hr. The differences are thus 12 ± 10 and 11 ± 13 mm.^{3}/g./hr., with the higher rate in the carpet type; they are only slightly larger than the calibration error of the apparatus.

It may therefore be stated that the differences between these two types of Barcroft apparatus do not make it improper to compare the results obtained from them. It should be understood that the apparatus used was of the old type, not the newer kind with an external burette as described by Dixon (1934, pp. 6–8). The newer type saves troublesome calibration.

V. DESICCATED ANIMALS

The rates of oxygen consumption of seven cockroaches were measured in basket type Barcrofts in both moist air and dry after loss of about 25 % of their original weight by desiccation. In moist air the average rate was 416 ± 33 and in drier air 358 ± 22 mm.^{3}/g./hr.

Both of these figures are considerably above the values already given for normal animals, the rate for moist air being quite significantly higher. These values were calculated on the basis of the weights of the cockroaches immediately before their respiration was measured; when, however, the rates are recalculated on the basis of the normal weight of the undesiccated animals the results are quite different. In moist air the rate was 323 ± 24 and in dry air 287 ± 11 (Table 1 *a*). That is to say, the rate of oxygen consumption *per animal*, when measured in moist air, does not alter appreciably when the animal is desiccated (cf. Table 1 *b*). The most obvious loss of water from desiccated insects is

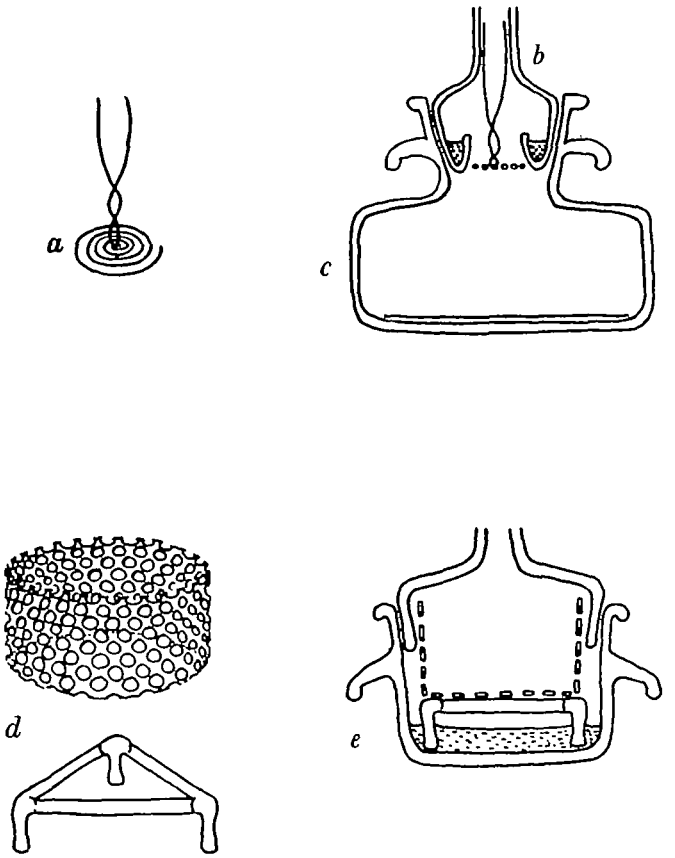

Fig. 1. Details of the two kinds of Barcroft apparatus used. *a*, *b*, *c*, carpet type; *d*, *e*, basket type. *a*, wire coil to prevent the cockroach getting its antennae into the potash; *b*, annular potash container, showing tube for filling and emptying it; *c*, bottle assembled, showing wire coil and potash; *d*, perforated zinc basket and glass stand; *e*, bottle assembled.

loss from the blood (Mellanby, 1939), a tissue which probably does not consume oxygen at any great rate, so there is no impropriety in referring the rate of metabolism to the normal rather than to the desiccated weight. The lower rate obtained for dry air is dealt with in the next section.

There is no evidence, therefore, that desiccation increases the oxygen consumption of a cockroach. A fortiori, there is no evidence that the desiccated condition is relieved by extra production of metabolic water. This agrees with the results of Mellanby, already quoted, for other insects. It is not comparable with Caldwell's (1925) conclusions for the mealworm because his mealworms were hardly desiccated at all.

VI. NORMAL ANIMALS AT TWO HUMIDITIES

The results of properly paired experiments with basket type Barcrofts in which seventeen animals were tested at each humidity are given in Table 1 *b*. It will be seen that rate of oxygen consumption was faster by 36 ± 14 mm.^{3}/g./hr. in moist air than in drier. Making allowance for the humidity error of the calibration (pp. 126–7) this excess is about 12 % of the value for dry air and is $2\frac{1}{2}$ times its standard error. There is a similar difference for desiccated animals (Table 1 *a*) and there seems to be no objection to treating all 24 experiments together. The average excess in moist air is then 36 ± 12 mm.^{3}/g./hr. and, corrected for the calibration effect, the chance probability of this if there is no real difference is less than 1 in 100.

Table 1. *Rates of oxygen consumption of individual cockroaches, each tested both in moist air and in drier air*

Rates in mm.^{3}/g./hr.		Excess rates in moister air	Rates in mm.^{3}/g./hr.		Excess rates in moister air
Moister air	Drier air		Moister air	Drier air	
(*a*) Desiccated animals			(*b*) Normal animals		
425	255	+170	341	168	+173
405	330	+ 75	486	356	+130
288	243	+ 45	313	216	+ 97
304	299	+ 5	284	198	+ 86
272	276	− 4	300	254	+ 46
297	305	− 8	346	302	+ 44
268	302	− 34	362	324	+ 38
Totals 2259	2010	+249	357	326	+ 31
Averages 323	287	+ 36 ± 26·3	357	328	+ 29
			257	246	+ 11
			209	198	+ 11
			369	371	− 2
			373	377	− 4
			268	282	− 14
			242	256	− 14
			343	359	− 16
			246	285	− 39
			Totals 5453	4846	+607
			Averages 321	285	+ 36 ± 13·8
Grand totals: moister air, 7712; drier air, 6856; excess in moister air, +856					
Grand averages: moister air, 322; drier air, 286; excess rate in moister air, +36 ± 12·1					

Examination of Table 1 shows that the distribution about the mean of values of excess oxygen consumption in moist air is markedly asymmetrical. Thus fourteen of the values are below 36 and ten above. Two very high values were derived from experiments in which the rate in moist air was very high or the rate in drier air very low. If these are omitted the average excess is reduced to $23 \pm 9{\cdot}3$ mm.^{3}/g./hr. Successive removal of the next two high values reduces the average excess to $18 \pm 8{\cdot}2$ and $14 \pm 7{\cdot}6$. It is possible that these extreme values are not merely extreme variants in a normal distribution but are due to the intrusion of some large experimental error. Since the average of all the data was 36 ± 12, there is no strong reason for believing the true average to be 36 rather than 24, especially in view of the asymmetry of the distribution. The true average might

be as low as 18. All we can infer is its order of magnitude and not its precise value but in whichever of these ways the data are treated, there is always a larger oxygen consumption in moist air. It is clear from the table, however, that humidity is relatively unimportant amongst the causes of variation in rate of oxygen consumption.

VII. DISCUSSION

The experiments on metabolic rate in two different humidities are of more interest than the others, indicating as they do a higher metabolic rate in moister air both for normal and desiccated animals. There are three kinds of possible reason for the difference found: instrumental reasons, differences of activity and real differences of basal metabolic rate.

The instrumental error due to a humidity effect on the calibration constant has been allowed for. Apart from this a bias would arise if an animal were always tested in moist air before dry air, but that error was carefully avoided. We might regard the error as due to the apparatus if the body temperature of a cockroach were higher in moist air than in dry, for the two sets of experiments would not then have been conducted at the same body temperature and the thermostat would not be adequate. Using the data of Gunn (1933) for the temperature coefficient of oxygen consumption of this species, we find that at about 25° C. a rise of 12% in oxygen consumption is caused by a rise of 1½° C. in temperature.

Now in saturated air, body temperature must be above environmental temperature, for otherwise metabolic heat cannot leave the body; in drier air the body temperature must be somewhat lower. But in these experiments the precise humidities are not known. In moist air there is no reason why the humidity should fall below 95% R.H., and it may be higher because of evaporation from the insect. In drier air evaporation will certainly raise the humidity above 10% R.H., the equilibrium value for the potash; the humidity is unlikely to be uniform throughout the bottle, and no data are available for estimating it. That is to say no precise figure can be given for the difference of body temperature in moist air and drier air in these experiments. Necheles (1924) found differences of 0·4 and 1·6° C. between cockroaches in moist air and in dry at about 25° C., and these differences are of the right order of magnitude to explain the difference in metabolic rate. Koidsumi (1935) found a similar body-temperature difference dependent on humidity in a grasshopper, *Gastrimargus transversus*; he also found a higher metabolic rate, which was of the appropriate size for the body-temperature difference.

A difference of body temperature would alter the calibration constant of the apparatus by altering to some extent the air temperature; but, at the *steady state*, if all the air in the bottle were warmed by 1° C. the error would be only about 0·3% of the rate calculated. On the other hand, if an insect *becomes* active *during* a Barcroft experiment it would warm the air of the bottle and so cause a rise of pressure. A rise of 1° C. would produce, in a bottle of 30 c.c., a pressure increase equivalent to a volume increase of 100 mm.^{3} at constant pressure. This is of the same order as the average oxygen consumption of a blowfly in flight in 2 min. (Davis & Fraenkel, 1940) or of a resting cockroach in 1 hr. It is well known, however, that the Barcroft apparatus gives reliable results only for steady state conditions, owing to lag in absorption of carbon dioxide, and the temperature effect emphasizes this. The heating effect of activity is not a complication in these experiments because experiments with more than the slightest movements were discarded. Apart from the effect of high humidity in raising body temperature and

therefore raising metabolic rate the difference found cannot therefore be traced to instrumental errors.

The second possibility is that the animals were more active in moist air than in drier, as they tend to be in some circumstances (Gunn & Cosway, 1938). The full effect of such a difference of activity could not show in these results, however, because experiments were discarded if the activity amounted to more than a few changes of position; but it is still possible that there was sufficient difference in activity to require more oxygen in moist air.

Since slight differences of body temperature and of activity could explain the higher rate of metabolism in moister air there is no reason to postulate a higher basal metabolism in moister air.

Results such as those given above could have been interpreted in various ways if less care had been taken in designing the experiments. For example, desiccation increases the rate of oxygen consumption per gram, though not per animal, while dry air decreases it. That is to say, if no distinction were made between the effect of dry air during the respiration experiment and the effect of a desiccated condition, then it might have been said either that dry air accelerates metabolism or that it retards it. Divergent conclusions in the literature are likely to be due to a failure to make this distinction.

Ludwig (1937) and Bellucci (1939) found no reliable effect of desiccation on metabolic rate in *Chortophaga viridifasciata* De Geer and larvae of *Popillia japonica* Newman respectively, so long as the rates per animal, not per gram, were used. The analyses of the food reserves of various insects carried out by Mellanby (quoted on p. 125), after prolonged subjection to various humidities, do not show any effect of humidity and desiccation on metabolic rate. They are not comparable with experiments in which rates of oxygen consumption are measured during short subjection to various humidities.

VIII. SUMMARY

1. The carpet type of Barcroft respiration apparatus previously used by Gunn for cockroaches gives results comparable with those now obtained with a new basket type.

2. Desiccated cockroaches use oxygen at the same rate per animal as undesiccated specimens. If, however, the rates are calculated with reference to the weight of the animal at the time of the experiment, since the desiccated animals tested had lost about 25% of their original weight, their rates of oxygen consumption appeared to have gone up.

3. Both normal and desiccated animals used oxygen slightly faster in moist air than in dry. Part of this increase must be attributed to a higher body temperature in moist air at 25° C. than in drier air at 25° C. Part of it may be due to greater activity in moist air than in dry, slight though the activity was in both cases.

4. There is no reason to believe that, at a given body temperature, air humidity influences basal metabolic rate.

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