THE RESPIRATION OF *TYROGLYPHUS FARINAE*

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(With Two Text-figures)

This investigation into the reactions of *Tyroglyphus farinae* to varying concentrations of carbon dioxide and oxygen and to the presence of small quantities of hydrocyanic acid, was carried out in order to obtain some insight into the respiratory activities of the mite, very little being known of its physiological reactions. As a necessary preliminary, its reactions to humidity were determined. *T. farinae* belongs to the family Tyroglyphidae and respires by direct diffusion through the body surface, since no tracheae are present. These mites infest stored food material, being found on grain, flour and meal. They are associated with the micro-organisms of decay and it might be expected that the gaseous conditions under which they live differ considerably from those of ordinary air. It was decided, therefore, to isolate cultures and to investigate the gaseous changes produced by the organisms in the culture and the effect of such changes on the mites present. The respiratory quotient in normal air was also determined, together with the quotient obtained in the presence of an *M*/1000 solution of potassium cyanide. The reactions of the mite to varying concentrations of carbon dioxide, oxygen and water vapour in the atmosphere were also determined.

A. HUMIDITIES

Small quantities of culture were placed in wide specimen tubes, the mouths of which were covered with fine silk material to enclose the animals. These tubes were placed in desiccators containing sulphuric acid of known specific gravity, and the desiccators kept in an incubator at 20° C. Five tubes were used in each case. At intervals, the tubes were removed and examined for live mites under a low-power binocular microscope. Mites cultured in flour and on whole grain were used.

Table 1

Time after	R.H. %						
	20	45·5	58·3	65·5	74	80	90
1 day	D	A	A	A	A	A	A
2 days	.	D	A	A	A	A	A
3 days	.	.	A	A	A	A	A
4 days	.	.	D	A	A	A	A
5 days	.	.	.	A	A	A+	A+
6 days	.	.	.	A	A	A+	A+
7 days	.	.	.	A	A	A+	A+
8 days	.	.	.	A	A	A+	A+
9 days	.	.	.	A	A+	A+	A+

Abbreviations: A = alive, D = dead, A+ = alive and breeding.

The lower limiting factor of humidity was found to lie between R.H. 58·3% and R.H. 65·5% at 20° C. This result appears to be in accordance with the work of A. M. Hora (1933) on *Glycyphagus domesticus* de Geer, where a lower limit of R.H. 70% at 25° C. was obtained. It is difficult to obtain results at humidities above 90% owing to condensation which tends to drown the mites when tubes are removed for inspection.

B. GASEOUS CHANGES PRODUCED BY ENCLOSING THE CULTURES

A strong culture of mites in flour was placed in a conical flask (in this case about an ounce of culture was used) which was joined to another by a wide-bore tube. Into the second flask, sulphuric acid of sp. gr. 1·2 could be run from a tap funnel; a delivery tube led from the first flask into a beaker of glycerine. The two flasks stood in a water-bath at 25° C. A certain amount of carbon dioxide would be lost by solution in the acid, and, owing to the delivery tube being in glycerine, there would be a humidity gradient from the flask containing acid to the surface of the glycerine in the tube. Even so, the humidity in the flask remained sufficiently high to prevent death by desiccation, as would have happened if glycerine had been used in the place of the sulphuric acid. At intervals, 20 c.c. of air were displaced, collected over glycerine to prevent further loss of carbon dioxide, so that the actual concentration above the culture might be known. This air was collected in a small eudiometer tube, transferred to the clamp of a levelling apparatus, so that all volumes could be read with the levels inside and outside the tube equal. The tube having been levelled in a tall jar of glycerine, the volume was read. To absorb carbon dioxide, large drops of 20% potassium hydroxide solution were injected up the tube by means of a bent pipette of 5 c.c. capacity. Three consistent readings were obtained before a 5% solution of pyrogallic acid in 5% potassium hydroxide was added to absorb the oxygen. The temperature was noted at each reading, and any

necessary correction having been made, the percentages of carbon dioxide and oxygen present were calculated and plotted on a graph of gas concentration against time. The respiratory quotient for the culture as a whole was calculated, and is seen to drop as the quantity of carbon dioxide increases. The graph reproduced shows the type of changes which took place in the atmosphere over the flour in this series of experiments.

Table 2

Time days	CO_2 %	O_2 %	R.Q.
8	3·553	17·26	0·95
20	6·77	13·56	0·969
27	7·263	11·9	0·798
32	8·872	6·403	0·607
36	9·754	6·340	0·66
44	8·437	6·21	0·57
50	9·001	5·00	0·56

The carbon dioxide concentration rose and became steady between 9·75 and 10·5%, the oxygen content having dropped to 6·3–5·00%. On opening the cultures, the mites were found to be anaesthetized. If the cultures were given access to air, the mites recovered in a few hours.

Newstead and Morris (1920) exposed *T. farinae* to pure carbon dioxide and found that the mite was anaesthetized but not killed. Few times of exposure, however, are given. The anaesthetic effect in isolated cultures might either be due to the increased carbon dioxide concentration or the low oxygen concentration. On comparison with the results of (E) it became evident that the anaesthetic effect was probably due to accumulation of carbon dioxide since the mites are tolerant of low concentrations of oxygen.

The respiratory quotient for the enclosed cultures was found to be in the neighbourhood of 0·9 at first, which is in agreement with the R.Q. for mites in the absence of culture medium, as determined in the series of experiments (C). In point of fact, a R.Q. of this order is only what one would expect for animals living in a medium very rich in carbohydrate which must form the principal respiratory base.

The R.Q. of isolated cultures begins to drop as the carbon dioxide concentration above the culture rises; this may be due to increased loss of carbon dioxide in the sulphuric acid used to regulate the humidity and to displace the air samples for analysis, or it may be due to the mites beginning to oxidize a fat as anaesthesia approaches.

C. RESPIRATORY QUOTIENT IN ORDINARY AIR

Thirty to forty mites were enclosed in a hypodermic syringe, the open end of the needle being immersed in glycerine. After intervals of 6, 12 and 24 hr. a small bubble of air was injected into a Krogh micro-analysis apparatus as modified by Campbell & Taylor (1935). This air bubble was analysed for carbon dioxide and oxygen and the respiratory quotient calculated.

Table 3. *Respiratory quotient over air at laboratory temperatures of* 10–17° C.

0·8830	0·8900	1·2900	0·8700
1·1700	0·9212	1·1600	1·1670
0·7264	0·9363	0·7457	0·7800
0·7300	0·9500	0·8000	0·9500
0·9520	0·8900	0·9170	0·9450
1·1300	0·8300	0·8900	

Total 21·5236

Variance = 0·02272 S.E. S.D./$\sqrt{n}$ = 0·03144
S.D. $\sqrt{v}$ = 0·1508 Mean = 0·9358

The mean R.Q. 0·9358 with a standard error of 0·03144 would indicate a normal process of aerobic respiration of a carbohydrate base. This R.Q. is in close agreement with that obtained in the early stages of experiment (B). The mites do not show any alteration in R.Q. due to lack of food in intervals up to 24 hr., since all the R.Q.'s obtained showed the same sort of variation whether after 6, 12 or 24 hr.

D. THE RESPIRATORY QUOTIENT IN THE PRESENCE OF POTASSIUM CYANIDE

The same apparatus was used for investigating the effect of hydrocyanic acid, a drop of *M*/1000 potassium cyanide solution being suspended on the plunger.

Table 4

No. of mites	R.Q. after 12 hr.	R.Q. after 24 hr.
40	1·69	0·86
40	1·62	0·88
35	1·48	0·85
35	1·28	0·93
40	1·72	0·93
40	1·8	0·85
35	1·2	1·16
30	1·2	1·17
30	1·077	1·07
30	1·1	Mean 0·966
30	1·1	Variance 0·0154
30	1·04	S.D. 0·1241
35	1·12	S.E. 0·0415
30	1·14	
30	1·14	
30	1·14	
30	1·1	
40	1·37	
40	1·6	
	Mean 1·323	

The mean quotient obtained at the end of 12 hr. was 1·323. The actual quotient obtained in any experiment depends apparently on the number of mites used. No results below unity were obtained. From this it seems evident that the absorption of oxygen is largely cyanide-sensitive, and that the animals respire by means of a cytochrome and cytochrome oxidase which is sensitive to cyanide (Keilin, 1929), as is the normal case in aerobically respiring organisms.

The return after 24 hr. to a R.Q. which shows close agreement with that obtained in ordinary air, indicates that, with this degree of inhibition of the oxidation of reduced cytochrome, the animals survive long enough for the production of fresh cytochrome oxidase, or else destroy the absorbed cyanide by some process of tissue metabolism.

E. REACTIONS OF *T. FARINAE* TO LOW CONCENTRATION OF OXYGEN

Tubes were set up as for the humidity experiments and stood over alkaline pyrogallol solution in glass-stoppered jars. The stoppers were well greased and a seal of heavy engine oil was run into the grooves between the stoppers and the top edges of the jars. The bottles were opened one at a time and the tubes examined for live mites. Cultures on whole grain and in flour were used. In the case of the flour, $\frac{1}{4}$ in. of flour in a $2\frac{1}{2} \times \frac{3}{4}$ in. specimen tube was used.

The tubes over pyrogallol were assumed to be in an atmosphere of low oxygen concentration and saturated with aqueous vapour. After 24 hr. the mites were alive and feeding; after 36 hr. a number were dead; after 48 hr. there were still a few alive. After 72 hr. all were dead in the case of cultures in flour.

Table 5

Time hr.	State of culture in flour	State of culture on grain
12	Alive and feeding	Alive but still
24	Alive and feeding	Alive but still
36	Most alive, a few dead	Alive but still
48	Majority still, a few sluggish	Majority dead
60	Majority alive but still	Dead
72	All dead	

The fact that *T. farinae* can in some cases, at any rate, survive 48 hr. in an atmosphere of low oxygen concentration, would at first sight seem to indicate that it is capable of anaerobic respiration for a limited length of time. This is in accordance with the results obtained in (D). It also seems to confirm the view that inactivity of mites in isolated cultures is due to the accumulation of carbon dioxide rather than the diminution in oxygen. However, a comparison of the results obtained when using mites cultured in flour or on whole grain and with the results of (F) shows conclusively that the activity of mites in tubes of flour over pyrogallol is due to the slowness with which an equilibrium is set up, and not to the ability of the mites to respire anaerobically to such an extent as to remain active.

The time lag of the atmosphere in flour in coming to an equilibrium with the outside atmosphere is seen when the results of (E) and (F) are compared; mites remaining active in flour stood over pyrogallol for at least 36 hr.; whereas the effect of atmospheric nitrogen on exposed mites is to produce an almost instantaneous anaesthesia. For this reason, it is important to use either mites picked out on a needle or else a heavily infected grain when determining the R.Q. or the relation to humidity.

F. REACTIONS TO VARYING CONCENTRATIONS OF CARBON DIOXIDE AND OXYGEN IN AIR

Mites were placed in an observation tube and air of known composition as regards to carbon dioxide and oxygen run through it. The air was mixed in the apparatus shown in Fig. 1. This apparatus was used as follows: The gas chamber was filled

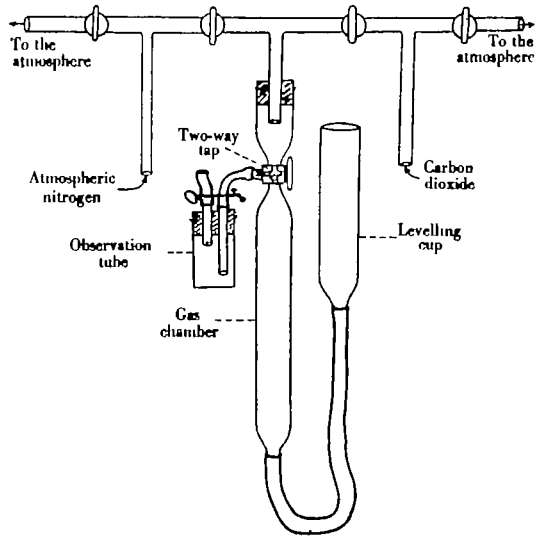

Fig. 1

with glycerine and by means of the levelling cup, known quantities of air, carbon dioxide and atmospheric nitrogen were drawn in. The gases drawn in were washed by being aspirated through water. The atmosphere thus mixed could then be displaced by means of the levelling cup into the tube containing the mites. Since the volume of air passed through the observation tube was many times its own volume, it was assumed that the

final atmosphere would be the same as that mixed in the apparatus. The observation tube was then sealed with a spring clip and put into a water-bath at 20° C. At intervals the mites were observed under the microscope. To avoid error in mixing the atmosphere, care was taken to pass plenty of carbon dioxide and nitrogen through the two-way tap before starting the actual mixing. Carbon dioxide was generated by the action of sulphuric acid on sodium carbonate and passed through two wash bottles of distilled water before passing into the apparatus. Atmospheric nitrogen was prepared by using a Winchester quart with 100 c.c.

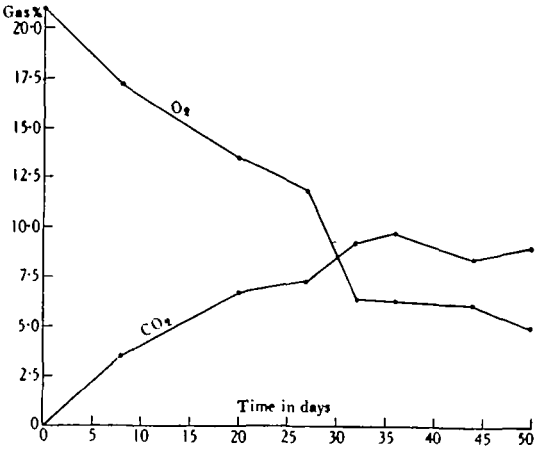

Fig. 2. Type of gaseous change in the atmosphere over enclosed cultures

of alkaline pyrogallol in it; this was shaken up repeatedly and the bottle allowed to stand for 24 hr. The gas was displaced into the apparatus by running distilled water into the Winchester from a large tap funnel. Care was taken to end all measurements of volume with the levels of glycerine in the gas chamber and the levelling cup equal. If the experiments were to last for more than 24 hr. a fragment of a grain was placed in the observation tube to supply food, and to prevent alterations due to respiration accumulating, the atmosphere was renewed every 24 hr.

These experiments were repeated upwards of 20 times with consistent results.

From the results of this series of experiments it is seen that the value of carbon dioxide as a factor limiting activity is in the neighbourhood of 30%. Concentrations above this produce an almost instantaneous anaesthesia. For oxygen this value lies between 1·2 and 0·8%.

It is immediately obvious that these figures are at variance with those obtained in series (B), where the culture stabilized with an external atmosphere of 10% carbon dioxide and 5% oxygen. I am of the opinion that the micro-atmosphere inside the flour had, when these external conditions had been reached, an entirely different constitution. The concentration of carbon dioxide must have been much higher than 10% or the concentration of oxygen much lower than 5% to produce anaesthesia and eventually death. The fact that flour which has stood over pyrogallol—an active absorbent of oxygen—contained active mites after 36 hr., whereas pure nitrogen has an instantaneous effect, indicates the slowness with which the micro-atmosphere is affected by external conditions; so that it is not really surprising that the atmosphere above an isolated culture should be very slow to show the increase in carbon dioxide and the diminution in oxygen which must be going on inside it.

The results obtained in the series (F) indicate that these mites have a very high resistance to carbon dioxide accumulation and to lack of oxygen. This would be an obvious adaptation to the conditions under which they live. The

Table 6

Dosage		Conditions within an hour
Carbon dioxide	Oxygen	
100	0	M−, almost instantaneously
37·5	12·5	N− −, more often, complete immobility
30	14	N− −, more often, complete immobility
25	15	N−, majority still
20	16	N−, differing very little from normal
15	17	N
0	2·4	N, possibly a little sluggish
0	1·6	N−
0	1·2	N− −, sometimes N−
0	0·8	M, sometimes N− −
0	0·4	M, immediately

Table 7

Gas concentration		Time and condition after dosage				
CO_2	O_2	12 hr.	1 day	2 days	3 days	4 days
100	0	M	M	M	M	M*
27·5	14·5	N− −	M	M	M	M
20	15	N−	M	M	M	M
20	16	N	N	N	N	N
0	2·0	N	N	N	N	N
0	1·6	N−	N−	N−	N−	N−
0	1·2	N−	N−	N−	N−	N−
0	0·8	N− −	N− −	M	M	M
0	0·4	M	M	M	M	M
0	0	M	M	M	M	M*
10	5	N	N	N	N	N

* Dead after 72 hr.

Abbreviations used in Tables 6 and 7: N denotes normal movement of mites; N−, sluggish walking movement; N− −, feeble twitching of limbs; M indicates immobility.

accumulation of carbon dioxide and lack of oxygen inside large masses of grain and flour must be very important factors in the physiological processes of the organisms living in them. The low concentration of the oxygen at which mites remain active would indicate the presence of an oxygen carrier in the fluid of the vacuolated packing tissue of the body. This activity at low oxygen concentration is met with in some insect larvae where it would seem to be correlated with the presence of haemoglobin. No such pigmented carrier is visible in *T. farinae* (Harnisch, 1937).

CONCLUSIONS

1. *Tyroglyphus farinae* is not viable at R.H. of less than 65·5% at 20° C.

2. The normal R.Q. is in the neighbourhood of 0·9, indicating respiration of a carbohydrate base.

3. The oxygen absorption is in part cyanide-sensitive, which suggests the presence of a 'cytochrome'-'cytochrome oxidase' mechanism.

4. Anaesthesia can be produced by concentrations of carbon dioxide above 30% of the atmosphere and by low oxygen concentrations below 1·2%.

5. The external atmosphere over flour is no guide to the internal conditions within the flour, even when both are enclosed.

6. If exposed to pure carbon dioxide for 72 hr. the mites are killed.

7. The mites will survive 48 but not 72 hr. exposure to atmospheric nitrogen.

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