THE EFFECT OF CARBON MONOXIDE ON THE OXYGEN CONSUMPTION OF DROSOPHILA MELANOGASTER PUPAE

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(With Three Text-figures)

The aim of these investigations is a study of the effect of carbon monoxide on developmental processes in *Drosophila*. Such a study involves, of course, a determination of the carbon-monoxide action in a quantitative manner, and for this purpose it seemed to be most suitable to undertake a study of the oxygen uptake in different pupal stages, first under normal conditions and then in various mixtures of oxygen and carbon monoxide.

The wild type stock of *D. melanogaster* used in these experiments was kindly supplied by the Swedish Institute of Animal Breeding, Wiad, and it was inbred for ten generations (brother-sister matings) before starting the experiments, in order to obtain a uniform population. Thereafter cultures were made every second day from eggs collected within 2 hr. periods. (Care was taken to avoid overcrowding.) By this arrangement it was possible to obtain pupae in the required stages at almost any time. The stocks were reared at room temperature on fermented banana (with baker's yeast) by a method similar to that described by Henshaw & Henshaw (1933). The oxygen consumption was studied chiefly in four different pupal stages, which hereafter will be called stages I–IV. These stages may be characterized as follows: I = early stage, imaginal body not yet formed, main trunks of the larval tracheal system still visible (about 5–10 hr. old, when reared at constant 25°C temperature (cf. Strasburger, 1935)); II = external organization of the imaginal body just completed, but no signs of coloration (about 25–30 hr.); III = the coloration of the eyes begins, the eyes are about chrome yellow (about 50–60 hr.); IV = late stage, eyes red, bristles begin to darken (about 75–85 hr.). (The adult flies hatch in about 90–100 hr. at constant 25°C temperature.) These stages were selected in such a way that they are about at equal time intervals within the pupal period, and at the same time they represent characteristic stages as regards oxygen uptake. When pupae of these stages were selected from the culture bottles, they were washed, sterilized, etc., according to the prescriptions of Poulson (1935) and Dobzhansky & Poulson (1935). At the end of each experiment the pupae were dried in an oven,
and the dry weight of all pupae used in one vessel was measured together, to 0.1 mg. on an analytical balance.

Oxygen consumption was measured in manometers of the Warburg type, having very small gas spaces (1.47–1.64 cm.\(^3\)). The vessels were of conical shape and were provided with a small well for KOH and a small side arm with glass stopper for the purpose of blowing through gas mixtures. In the great majority of the experiments five pupae were used in each vessel, in some of the earlier experiments ten pupae. They were put into the vessel on a piece of wet filter paper, \(0.5 \text{ cm.}^2\) in size. For the absorption of the CO\(_2\) produced, 0.1 cm.\(^3\) 5% KOH was used in each vessel. Experiments were carried out in a water-bath at 25°C, regulated with an accuracy of \(\pm 0.05^\circ\) C. The manometers were not shaken because the pupae were immediately exposed to the gases in the vessel (not submerged in fluid), and also because preliminary experiments showed that shaking was superfluous. Readings were taken at 1/1 hr. intervals. Results have been recalculated in mm.\(^3\) per hr. per mg. dry weight.

In each experiment the normal oxygen consumption of the pupae was first measured, then the vessel was filled with one of the gas mixtures in order to measure the residual oxygen consumption, or "Atmungsrest", in that particular gas. It was found that filling the vessels with carbon monoxide does not alter the vessel constants. Three different mixtures of oxygen and carbon monoxide were used: (1) 5% O\(_2\) + 95% CO (CO/O\(_2\) = 19), (2) 10% O\(_2\) + 90% CO (CO/O\(_2\) = 9), (3) 15% O\(_2\) + 85% CO (CO/O\(_2\) = 5.67). In the following pages these mixtures will be called according to their CO/O\(_2\) ratio, gases 19, 9, and 5.67 respectively. The mixtures were made of commercial O\(_2\) and of CO which had been generated by dropping concentrated formic acid into concentrated sulphuric acid, heated over a water-bath. The developing gas was washed by bubbling it through 25% KOH. The gases were collected and mixed over concentrated NaCl solution in gasometers of 1000 cm.\(^3\) content, calibrated to 10 cm.\(^3\). No gas analysis of the mixtures was made.

RESULTS

Concerning the oxygen consumption under normal circumstances (called hereafter N.c. from "normal consumption"), the findings of earlier investigators have been confirmed. It is well known that the rate of oxygen uptake of holometabolous insects during the pupal period can be expressed by a U-shaped curve, i.e. it is high at the beginning, then falls off rather rapidly and finally rises again, sometimes to the initial level or even higher. (For a detailed discussion see Needham (1929), Wigglesworth (1934).) This had been shown also to be valid for Drosophila pupae (Bodine & Orr, 1925; Clare, 1925; Poulson, 1935; Dobzhansky & Poulson, 1935). My figures for N.c. per hr. per 1 mg. dry weight are summarized in Table I (first row for every stage). As can be seen, the N.c. in stage I is about twice as much as in stages II and III, which differ but little from each other, while in stage IV the N.c. not only reaches the original level, but is even somewhat higher. Thus a U-shaped respiratory curve results (Fig. 1, curve a). At the same time the average
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Table I. Summary of experiments on the effect of carbon monoxide upon oxygen consumption of Drosophila melanogaster pupae at 25°C.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Dry weight in mg. Average with S.D. and percentage S.D. (in brackets)</th>
<th>Gas mixture (CO/O₂)</th>
<th>No. of experiments</th>
<th>Oxygen consumption in mm.³ per hr. per 1 mg. dry weight. Average with S.D. and percentage S.D. (in brackets)</th>
<th>Residual oxygen consumption (R.C.) in percentage of normal uptake of the same pupae. Average with S.D. and percentage S.D. (in brackets)</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.44 ± 0.11 (23.7)</td>
<td>Air</td>
<td>26</td>
<td>4.66 ± 11 (23.8)</td>
<td>55.6 ± 6.2 (11.2)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5.67</td>
<td>9</td>
<td>10</td>
<td>2.53 ± 0.68 (27.0)</td>
<td>39.5 ± 7.0 (17.8)</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>10</td>
<td>5</td>
<td>2.12 ± 0.83 (39.0)</td>
<td>21.1 ± 2.6 (12.3)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.85 ± 0.05 (5.9)</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>II</td>
<td>0.44 ± 0.10 (22.5)</td>
<td>Air</td>
<td>16</td>
<td>2.12 ± 0.54 (23.0)</td>
<td>56.5 ± 9.9 (17.5)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>5.67</td>
<td>9</td>
<td>3</td>
<td>2.28 ± 0.33 (34.0)</td>
<td>42.0 ± 1.9 (4.5)</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>4</td>
<td>3</td>
<td>0.93 ± 0.54 (25.0)</td>
<td>21.3 ± 3.6 (16.9)</td>
<td>15</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.47 ± 0.05 (10.6)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>III</td>
<td>0.41 ± 0.08 (20.6)</td>
<td>Air</td>
<td>10</td>
<td>2.12 ± 0.47 (20.5)</td>
<td>66.6 ± 2.8 (4.1)</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>5.67</td>
<td>3</td>
<td>3</td>
<td>1.60 ± 0.06 (3.9)</td>
<td>50.0 ± 7.3 (14.9)</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>4</td>
<td>3</td>
<td>1.15 ± 0.10 (8.7)</td>
<td>30.5 ± 2.9 (9.5)</td>
<td>82</td>
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<tr>
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<td></td>
<td>0.72 ± 0.05 (7.0)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IV</td>
<td>0.36 ± 0.07 (20.5)</td>
<td>Air</td>
<td>25</td>
<td>5.05 ± 0.84 (16.5)</td>
<td>67.1 ± 6.5 (9.9)</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>5.67</td>
<td>11</td>
<td>3</td>
<td>3.10 ± 0.86 (37.5)</td>
<td>57.0 ± 3.2 (5.6)</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>2.38 ± 0.62 (26.0)</td>
<td>20.2 ± 3.0 (10.3)</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>10</td>
<td>3</td>
<td>1.48 ± 0.63 (42.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Curves showing oxygen consumption during metamorphosis. a, in air; b, in gas 5.67; c, in gas 9; and d in gas 19. Abscissa: pupal age in hours. Ordinate: oxygen consumption in mm.³ per hr. per 1 mg. dry weight.

dry weight of pupae decreases somewhat with age, from 0.44 to 0.36 mg., a fact also observed by previous authors (Bodine & Orr, 1925; Poulson, 1935). It will also be noticed that there is a considerable variation among the individual data both for dry weight and N.C., the percentage standard deviation ranging between 16.5 and 23.8. But it must be pointed out that this fact is not a measure of experi-
mental error, because the differences between the individual readings within an experiment were usually smaller, about 10% in general. Thus the variation is an expression of the scatter of individual data, probably due to variations in physiological conditions of the pupae as well as small fluctuations in age (about ± 5 hr.). The findings of Clare (1925) illustrate how different the N.C. of the pupae can be, when they are reared under different environmental conditions, and Poulson (1935) and Dobzhansky & Poulson (1935) have demonstrated small differences between the males and females of Drosophila.

The present data agree fairly well with those given by earlier investigators when the latter are recalculated on the basis of dry weights. The calculation can only be approximate, because the fresh weights are very variable. The ratio of dry to fresh weight is roughly 1:2:2.

If we now compare the figures for N.C. with those for oxygen consumption in various oxygen-carbon monoxide mixtures (called hereafter R.C. from "residual consumption", which is the "Atmungsrest" of Warburg), we find about the same range of variation. Only in four cases is the standard deviation greater than 30% and only one of these exceeds 50%. Thus in general the variations seem to express again the physiological scatter of individual data, rather than experimental errors. That this must be so becomes evident if we consider R.C. in percentage of the previously measured N.C. of the same pupae. We see that the averages of the so calculated individual data show less standard deviation than the absolute figures themselves, which means that in the individual cases the plus variants of the average N.C. correspond in general to plus variants of the average R.C., and similarly the minus variants of both go to a certain extent parallel with each other. Such a calculation of the percentage R.C. for each experiment, instead of its calculation from the average N.C. of all experiments, carried out within a given developmental stage, eliminates a considerable source of error. But it must be borne in mind that, as a consequence of this procedure, the absolute figures for N.C. and R.C. respectively will not give exactly the percentage values presented in the table, simply because the figures for N.C. are based on another range of individual data than the various figures for R.C. The same concerns the graphic representation of the data as plotted against time (Fig. 1).

From the data we see that the oxygen consumption of Drosophila pupae is strongly inhibited by carbon monoxide. In gas 5-67 the consumption is from 55.6 to 67.1% of the N.C. of the same pupae, in gas 9 from 39.5 to 57.0%, and in gas 19 only from 21.1 to 30.5%. In some experiments the complete reversibility of the carbon-monoxide effect was shown. After the treatment the original N.C. values were practically reached again, when the pupae were replaced in normal atmosphere.

To see if the low partial pressure (tension) of oxygen in the gas mixtures causes decrease in the oxygen consumption of the pupae, a series of experiments was undertaken with corresponding mixtures of oxygen in nitrogen instead of carbon monoxide. A report on these experiments will be published elsewhere; here it suffices to state that if 10 or 15% oxygen is present in the nitrogenous mixtures, there is no measurable decrease in the oxygen uptake. In a gas mixture of 5%
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oxygen and 95% nitrogen a slight decrease can be detected, but it is less than 10%, the average being 7.2% (standard deviation ± 1.2, or 16.7%). The bearing of this on the data will be discussed below.

In some experiments the light reversibility of the carbon-monoxide effect was studied (cf. Warburg, 1926). A 60 W. electric bulb was used as the source of light at a distance of 10–15 cm. It was found that illumination diminished the effect of carbon monoxide to a certain degree, but no complete reversal could be obtained. Fig. 2 illustrates the results. According to the concentration of carbon monoxide in the gas mixtures, 20–50% of the respiratory inhibition could be reversed under

![Graph showing light reversibility of carbon-monoxide effect.](image)

the experimental conditions. This incompleteness of the light reversibility must be due simply to the opacity—and in later stages also to the pigmentation—of the pupae, which prevents the light-penetration into deeper regions of the respiring tissues. Under such circumstances no detailed quantitative or spectroscopic study on light reversibility has been attempted. It is interesting in this connexion that Bodine & Boell (1934a) found "no significant effects" of light on the carbon-monoxide reaction of developing grasshopper eggs (*Melanoplus differentialis*). From this and other facts they conclude (Bodine & Boell, 1934b) that the respiratory mechanism as postulated by Warburg may be not valid for their material at all. However, knowing the technical difficulties connected with such illumination
experiments, it seems to the present writer that Bodine and Boell's negative result is probably due to opacity of the grasshopper eggs or to other external factors, rather than to fundamental differences of the respiratory mechanism.

**DISCUSSION**

The degree of inhibition of the oxygen uptake by carbon monoxide seems to be quite considerable in *Drosophila* pupae, when compared with other material. Thus, for example, in fertilized sea-urchin eggs the r.c. in gas 5·67 is about 90%, in gas 9 about 75% and in gas 19 still about 50% (Runnström, 1930, 1932; Örström, 1932, 1935), i.e. some 25–30% higher than the corresponding values for *Drosophila* pupae. Örström (1935), studying the effect of carbon monoxide on the oxygen consumption of yeast with five different substrates, found that only with acetaldehyde is the inhibition stronger than in *Drosophila* pupae; with sodium acetate the inhibition is about equal. Bodine & Boell (1934a, b), using the eggs of the grasshopper *Melanoplus differentialis*, found that in actively developing eggs the oxygen uptake can be inhibited by carbon monoxide to a somewhat less extent than in *Drosophila* pupae (percentage r.c. in gas 9, 53·4; in gas 19, 33·5), whereas in resting (diapause) eggs the oxygen consumption cannot be inhibited at all. This clearly demonstrates the principal difference between diapause and metamorphosis, because the latter is, though externally a "resting" stage, in fact an actively developing phase in insect ontogeny.

Further analysis of the data reveals two main facts, which should be discussed here. The first concerns the relation between carbon-monoxide concentration and intensity of respiratory inhibition. In this respect the data agree well with the formula of Warburg (1927), according to which \[
\frac{n}{1-n} \cdot \frac{CO}{O_2} = K,
\]
where \(n\) is the "Atmungsrest" or residual respiration (called r.c. in this paper) in percentage of normal respiration. The formula means that varying the CO/O\(_2\) ratio, the \(n/1-n\) ratio should vary in a proportional manner, so that the product of the two ratios should yield a constant \(K\). The \(K\) was calculated for all cases (Table I, last column), and it is clear that its values agree fairly well within a given stage. There is a small but apparently systematic deviation in so far as (with the exception of stage IV) \(K\) increases somewhat as the CO/O\(_2\) ratio decreases. This, however, can be accounted for if we take into consideration the fact that in gas mixtures of high CO/O\(_2\) ratio the respiratory inhibition is not entirely due to the effect of carbon monoxide, but partly also to the reduced oxygen tension. As mentioned, if the partial pressure of oxygen becomes as low as in gas 19, this causes on the average 7·2% decrease of the normal oxygen consumption. Correcting for this we get in stage I an r.c. of 28·3%, in stage II 28·5%, in stage III 37·3% and in stage IV 36·4%. The corresponding \(K\) values would be 7·4, 7·6, 11·6 and 10·8. As we see, these corrected \(K\)'s not only compensate the excess of \(K\), calculated on the basis of lower CO/O\(_2\) ratios, but are even somewhat higher. However, the correction is not quite exact in this way, because the effect is *not* a simple summation of the effects of carbon-
monoxide concentration and reduced oxygen pressure. Also it should be taken into account that the R.C. figures for gas 9 may also need some slight correction, which, however, lies within the limits of the probable error and cannot be well detected in corresponding oxygen-nitrogen mixtures. (To obtain a proportional correction, the difference between N.C. and oxygen consumption in a gas mixture of 10% oxygen and 90% nitrogen should be about 3% only.) Thus we are justified in taking the highest observed $K$ in each group as valid, and may say this is practically constant within a given stage, i.e. not varying appreciably with varying the CO/O$_2$ ratio from 19 to 5.67. This means that Warburg's formula is valid for *Drosophila* pupae. The bearing of this is that Warburg's formula can be valid only when the concentration of the oxidizing enzyme system, combining with carbon monoxide and oxygen respectively, is the limiting factor in the activity of the respiratory mechanism. In other words, $K$ is only constant when the oxidizing system is "saturated" with substrate. The new formula for the kinetics of respiration, derived recently by Runnström & Klein (private communication), here gives practically the same result as the Warburg formula, because the oxidizing system is saturated.

The other main point emerging from the data presented here concerns the changes of the R.C. values (and $K$ based on them) during metamorphosis. For example, in gas 5.67 the difference of the percentage R.C. between stages I and II is only 0.9 and between stages III and IV only 0.5, whereas it is from 10.1 to 11.5 between these two respective groups. The differences clearly come out in the value of $K$ and can be presented graphically. If we plot percentage R.C. against CO concentration for the four different stages, the corresponding points for stages I and II are almost identical, but considerably lower than the corresponding points for stages III and IV, which again are very near to each other. Thus two very definite lines can be drawn, one representing the percentage R.C. at various CO concentrations in stages I and II, and another representing the same in stages III and IV (Fig. 3). The two lines are separated from each other by a quite definite distance.

It seems of some importance that this change in R.C. occurs between stages II and III, because here lies the "turning point" of the U-shaped respiratory curve, i.e. the point at which oxygen uptake begins to rise again. The importance of this fact becomes obvious if we consider that for the shape of the respiratory curve in insect pupae during metamorphosis no satisfactory explanation has been given so far. The suggested explanation (cf. Needham, 1929) that the abrupt fall in oxygen consumption coincides with the process of histolysis, whereas the rise in the consumption is due to the development of the imaginal tissues (histogenesis), does not hold well, at least for *Drosophila* pupae. Dobzhansky & Poulson (1935) pointed out recently, in a fine analysis of the relation between oxygen consumption and developmental stages, that "the processes of histolysis are consummated even before the consumption of oxygen by the pupa reaches its minimum, and that the adult organs become anatomically complete before half of the pupal development has elapsed, thus before the oxygen consumption takes a decided upswing". The
other suggestion put forward by Wigglesworth (1934), that the U-shape might be
due to temporary anaerobic conditions in the tissues during the reorganization
of the tracheal system, is likewise untenable, for this would also involve, to a certain
extent, morphological parallelisms which do not seem to exist in Drosophila pupae.
My histological sections show that in stage III the tracheal system of the imago
is quite developed, although the oxygen consumption in this stage is at almost
exactly the same level as in stage II, when the breakdown of the larval organization,
including the tracheal system, is completed.

The change in the intensity of the CO effect at the “turning point” of the
respiratory curve indicates some connexion between this change and the shape of
the curve. Of course it remains obscure how this connexion can be causally explained

![Fig. 3. Graphs showing residual oxygen consumption in percentage of normal consumption plotted
against CO/O2 ratios in different stages. ▲ stage I, △ stage II, ● stage III, ○ stage IV. Abscissa:
ratios of CO/O2. Ordinate: residual oxygen consumption in percentage of normal consumption.
The two dotted lines indicate the average distance between points of stages I—II and III—IV
respectively.](image)

and to discuss the theoretical possibilities would be out of place here. (Cf., however,
Szörényi & Tschepinoga (1936), who have recently shown that in trained muscles
the oxygen consumption increases and at the same time the respiration becomes
more resistant to HCN!)

There are, however, some more concrete points which should be considered
here. As pointed out above, in Drosophila pupae the oxidizing enzyme system is
saturated during the whole period of metamorphosis. Such being the case, all
explanations for the changes occurring in the respiration of the pupae, i.e. changes
of the N.C. (the shape of the respiratory curve) and of the R.C. (change of K at the
“turning point” of the respiratory curve), must be concerned with the oxidizing
enzyme system itself. (By this system we understand both the iron-containing
pheohemine compound, called “Atmungsferment” by Warburg, and the so-called
“carriers” which are the cytochromes of Keilin, cf. Keilin, 1929.)

This means that the decrease of the intensity of oxygen consumption during
the first half of metamorphosis and the subsequent increase during the second half
must be due to changes in the amount or activity of the Warburg-Keilin system. Either a part of it is first destroyed (possibly during histolysis) and subsequently rebuilt during the second half of metamorphosis, or else respiratory poisons, which partly inactivate the enzyme system, accumulate gradually at the beginning and later are gradually removed.

As to the question of what changes in the Warburg-Keilin system would give the change of $K$ observed at the "turning point" of the respiratory curve (without changing the degree of saturation), there are two possibilities, which might apply to the pheohemine or to the cytochromes, linked with it: (1) there may be changes in the physico-chemical properties of the medium in which the Warburg-Keilin system is reacting, affecting the velocity constants of the reactions or the solubility of the gases; (2) there may be a qualitative change in the Warburg-Keilin system, which alters the velocity constants of its reactions, so that it reacts more readily with $O_2$ and less readily with CO. At the moment we have no means of deciding which of these two possible mechanisms is at work in the pupae. They may play the role equally well, or may produce the effect in combination with each other.

**SUMMARY**

1. The oxygen consumption of *Drosophila melanogaster* pupae during metamorphosis can be expressed by a U-shaped curve, as stated by earlier authors; that is, the consumption is high at the beginning, then falls off rapidly, and from the middle of the pupal period onwards it rises again till about the original level.

2. The oxygen consumption is strongly inhibited by carbon monoxide in all pupal stages. The inhibition is proportional to the percentage of CO and $O_2$ in the gas mixtures and can be expressed by the formula of Warburg (1927) $\frac{n}{1-n} \cdot \frac{CO}{O_2} = K$. The validity of this formula means that in *Drosophila* pupae the oxidizing enzyme system (Warburg-Keilin system) is saturated during the whole period of metamorphosis.

3. The CO effect decreases markedly at the time when the oxygen uptake begins to rise again (the "turning point" of the respiratory curve). Accordingly the value of $K$ increases at this stage. The bearing of this is discussed.

4. The CO effect on oxygen consumption is sensitive to light and can be counteracted to a certain extent by illumination (the limiting factor being probably the opacity of tissues).

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