

EFFECTS OF CARBON DIOXIDE ANAESTHESIA ON *DROSOPHILA MELANOGASTER**

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Abstract—Carbon dioxide anaesthesia produces significant high mortality in 0- to 3-hr-old flies. Young females are more sensitive to 15 min CO₂ treatment than the males. Reduced fecundity is observed in flies that recovered from CO₂ exposures. However, no effect was observed in *Drosophila* flies that are older than 3 hr. Similarly carbon dioxide does not affect the fertility of the females as percentage of hatching is the same in both the exposed and control flies.

INTRODUCTION

DURING our screening of the various candidate chemosterilants for *Drosophila melanogaster* Meig. it was necessary to isolate and sex the flies before these could be mated. To achieve this emerging flies were collected by a laboratory aspirator from the rearing bottles. The young flies were then anaesthetized with carbon dioxide, sexed, and chemosterilized. The method, however, was found to be unsatisfactory because a high mortality was observed in these CO₂-treated flies. Since carbon dioxide is used routinely in biological laboratories, further work was undertaken to study its effects on the longevity and fecundity of adults in *D. melanogaster*. The results of these investigations form the basis of the present report.

MATERIALS AND METHODS

The *D. melanogaster* flies used in these experiments were obtained from the cultures maintained in the biological laboratories at Université Laval since 1966. These were originally obtained from Sussex, England, through the courtesy of Professor J. H. Sang. The larvae were reared in cornmeal yeast agar medium as described by LEWIS (1960) at $25 \pm 1^\circ\text{C}$. F₁ individuals from the cross male Oregon \times female NB were used in all these tests. Much later, some tests were also conducted with male Oregon \times female Oregon, male NB \times female NB, and male NB \times female Oregon, for comparison. The experimental flies were collected from the rearing bottles by a laboratory aspirator at different times after emergence, sexed, and given a 30 sec treatment with pure CO₂ so as to immobilize them. Then they were submitted to the various experimental treatments described below. For the tests with virgin flies 3 days after emergence, males as well as females were fed upon the Lewis medium. Each group of 1, 2, 3 hr, and 3 day post eclosion virgin

* Contribution No. 86.

flies was then placed in the modified anaesthetic chamber of BROWN and SANG (1965), and connected to a commercial pressurized CO₂ cylinder. The flies were then exposed to the required period at room temperature (22°C), and transferred to growth chambers where they were raised. The control batch received similar treatments with air.

The effects of the CO₂ levels and its effects were measured on the 3-day-old virgin flies as follows. The stream of CO₂ coming from the commercial bottle was mixed in a flask with air supplied by an electrically driven pump. The mixing flask was connected to the anaesthetic chamber. The CO₂ level was adjusted by reducing either the air or CO₂ flow into the mixing flask and was measured by filling a 100 ml cylinder with the gas mixture which was then inverted into a beaker containing 10% potassium hydroxide solution stirred with a magnetic bar. The rise of potassium hydroxide into the cylinder gives the percentage of CO₂ in the gas mixture (PATTON *et al.*, 1968). In some experiments the length of the exposure period was varied. Different groups of 3-day-old virgin flies were exposed to continuous flow of CO₂ for 5, 15, 30, and 45 min respectively.

After treatment, a single pair of flies were placed in a small glass vial (30 mm diameter × 6 mm length) containing a disk (20 mm diameter × 5 mm high) filled with Lewis medium (LEWIS, 1960). The medium, however, contained 2.5 per cent of activated charcoal to facilitate observation and counting of eggs laid, since the charcoal gave a black colour to the medium. The bottom of the disk was dipped in melted paraffin to cover it so as to ensure that feeding and egg deposition would take place on the upper surface only. A grain of active dry yeast was placed on the upper surface of the disk to offer extra food. When the new disk was introduced, the glass vial bottom was placed near a light source before removing the plug. The light attracted the flies and prevented their escape. The disk in the vial was changed daily. The number of eggs laid, the still unhatched eggs after 24 hr incubation at 25°C, and the number of dead flies were carefully counted and daily recorded.

RESULTS

The exposure of 0 to 3 hr post eclosion flies to carbon dioxide for 10 to 15 min brought almost 50 per cent mortality within a week whereas no mortality was observed in the control, unexposed to CO₂, and unanaesthetized flies (Fig. 1). The effect of CO₂ on *Drosophila* could be noted at 24 hr after exposure when almost 20 per cent had a greyish abdomen. After 48 hr more than 20 per cent of the flies had a slightly distended abdomen and 15 per cent had a fully distended abdomen. On dissection this distension was found to be due to accumulation of liquid substances in the intestine. The experiment was repeated much later with 0- to 1-hr-old male Oregon × female NB, male Oregon × female Oregon, male NB × female NB, and male NB × female Oregon (Table 1). These exposed flies showed the same symptoms. However, the pathogenic examination of the dead male and female flies did not reveal any bacterial, fungal, protozoan, or nematode pathogens. The peak of mortality occurred at 72 hr after exposure to carbon dioxide. Ovaries of these carbon dioxide-exposed flies were undeveloped.

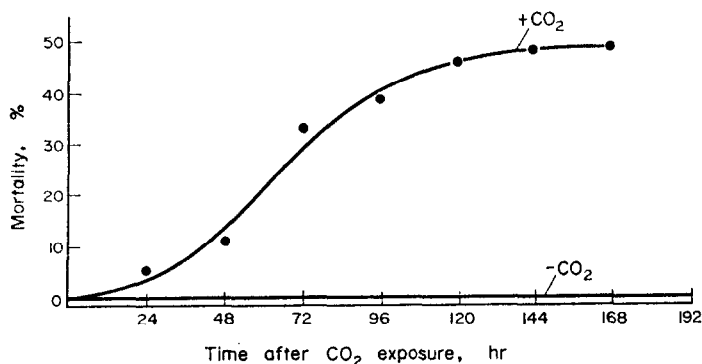


FIG. 1—Mortality observed in *Drosophila* females anaesthetized with carbon dioxide at the age of 0 to 3 hr.

TABLE 1—EFFECTS OF CARBON DIOXIDE ON THE LONGEVITY OF DIFFERENT STRAINS OF *D. melanogaster*

Strains	Age (hr)	Mortality (within 10 days)	
		Males	Females
Male Oregon × female NB	0-1	75	98
Male NB × female NB	0-1	83	100
Male Oregon × female Oregon	0-1	72	94
Male NB × female Oregon	0-1	88	96

Once the peculiar toxic effect of carbon dioxide treatment was established, an attempt was made to find out the exact time after emergence when the flies become resistant to the gas. Percentages of mortality for both sexes, when exposed to carbon dioxide at different ages, are presented in Table 2. The results show that the young females were more sensitive to carbon dioxide than the young males. In both sexes, no toxic effects were observed in 3- to 4-hr-old flies.

From these experiments, the flies (0-3 hr) which recovered from the carbon dioxide treatment (75 pairs of flies) were kept under observation to measure the secondary effects of CO₂-anaesthesia on fecundity and fertility. Observations made over a 10 day period showed a highly significant difference ($t=3.11$) between the fecundity of the control flies (67 eggs/day) as compared to the carbon dioxide-exposed flies (54 eggs/day). However, percentage of hatching was the same in both the exposed and control flies ($t=1.27$).

Since the flies showed an increased resistance with age to the primary effects of carbon dioxide treatment, the secondary effects of carbon dioxide on fecundity of 3 day post eclosion insects were studied (Table 3). It showed that the levels of carbon dioxide did not affect the fecundity of flies. No statistical differences could

TABLE 2—SENSITIVITY OF *D. melanogaster* (MALE OREGON × FEMALE NB) TO CARBON DIOXIDE IN RELATION TO THE AGE AFTER EMERGENCE

Age (hr)	Mortality (calculated on 50 pairs of flies) %	
	Males	Females
0-1	75	98
1-2	16	100
2-3	4	28
3-4	0	0
Control (air)	0	0

TABLE 3—EFFECTS OF CARBON DIOXIDE LEVELS ON THE FECUNDITY OF 3-DAY-OLD *D. melanogaster* (ANAESTHETIZED DURING 10 TO 15 MIN)*

CO ₂ (%)	Eggs/female per day		t
	Mean†	Standard error	
0 (Control-air)	71	14	} 2
25	64	13	
50	65	7	
75	62	7	
100	67	9	

* Male Oregon × female NB.

† Mean number of eggs per day calculated on 20 pairs of flies.

TABLE 4—EFFECTS OF CARBON DIOXIDE EXPOSURE TIME ON THE FECUNDITY OF 3-DAY-OLD *D. melanogaster*

Time (min)	Eggs/female per day		t†
	Mean*	Standard error	
0 (Control-air)	73	7	
5	67	6	1.77
15	59	11	3.29
30	63	12	2.17
45	62	10	2.88

* Mean number of eggs per day calculated on 20 pairs of flies.

† Student's *t*-test made between control (0/min) and each successive exposure time (5 min, 15 min, etc.).

be established among the CO₂ levels administered ($t < 2$). However, the duration of time of exposure, from 15 min upwards, could affect the fecundity of flies ($t > 2$). (Table 4).

DISCUSSION

The results of the present investigations show the toxic effects of carbon dioxide as an anaesthetic agent. The data on the 0 to 3 hr-post eclosion flies indicate that 15 min exposure of carbon dioxide affects the longevity of flies (Tables 1, 2; Fig. 1). This observation is somewhat different from that of L'HÉRITIER (1948, 1958) who showed that *Drosophila*'s sensitivity to CO₂ was due to sigma virus. His sensitive flies were unable to recover even a 30 sec contact with CO₂. Our results differ also from those of McCRADY and SULERUD (1964) since in our experiments all the flies recovered from the 15 min exposure to CO₂ within 10 min. Thus it is difficult to explain these results in the light of CO₂-sensitivity described by L'HÉRITIER (1948, 1958), and of the data of McCRADY and SULERUD (1964) where sigma virus was considered to be the causative factor. Since no infectivity tests could be conducted (though other tests of the dead flies did not reveal the presence of any pathogenic organisms), the presence or absence of sigma virus could not be absolutely ruled out. There is, however, an indirect inference that these effects were not due to virus as our control batches were taken out randomly from the same stocks and these flies laid a normal number of eggs. On the other hand, if it is assumed that our observations are due to sigma virus, it becomes worthwhile to find out how prevalent this virus is amongst the wild populations as well as in the various laboratory cultures.

Toxic effects of carbon dioxide have been described for other insects, such as growth retarding effects in *Blatta germanica* L., as determined by the time required to reach adulthood (BROOKS, 1957). An enhanced mortality due to CO₂ anaesthesia was reported in the Mediterranean fruit fly, *Ceratitidis capitata* (Wied.) (SHERMAN, 1953). HOOPER (1970) demonstrated that CO₂ anaesthesia for 30 min was detrimental to the Mediterranean fruit fly strain tested. In our studies no toxic effect of CO₂ was noted in 3 to 4 hr post eclosion flies.

The highly significant difference observed in fecundity between 0 and 3 hr post eclosion flies (that recovered the CO₂ treatment) and the control are interesting as workers from elsewhere are raising doubts on the desirability of the use of various chilling and anaesthetizing agents. WHITE *et al.* (1970) pointed out the effects of various agents against the codling moth, *Laspeyresia pomonella* (L.), and showed that CO₂ significantly reduced oviposition and percentage hatch, interacted with chilling, and chilling plus staining and negated the beneficial effect of chilling on the percentage hatch. Thus our results on the percentage hatch of eggs differ from those of WHITE *et al.* (1970) as no effect of CO₂ on hatching was observed in our investigations.

Since no attempt at the time was made to study the mode of action of carbon dioxide, it is difficult to fully understand and explain how 3 day post eclosion flies were not affected by different concentrations of CO₂, but that their fecundity was

reduced by different lengths of exposure to CO₂ (Table 4). However, EDWARDS (1968) showed that, in *Heliothis zea* (Boddie), five exposures of 5 to 10 min to 100% CO₂ at 3 day intervals, retarded larval growth by about 27 per cent. The use of 30% CO₂, 20% O₂, and 50% N₂ caused no retardation. Recently LÓPEZ and BALOCK (1970) have showed that 24% of CO₂ in the air in the burial pits produced 85 per cent mortality of the Mexican fruit fly, *Anastrepha ludens* (Loew), while infested mangos handled similarly produced a concentration of 60% CO₂ and 97 per cent mortality. It is, therefore, difficult to state whether the results obtained are CO₂ specific, or due to anoxia felt especially by 0- to 3-hr-old flies. The present investigations do point out that when CO₂ is used as an anaesthesia, it should be administered with great care, at least to young flies (0-3 hr), for it may cause high mortality and reduce the fecundity.

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