

# On the effect of Carbon Tetrachloride upon etherized *Drosophila virilis*

[Diptera-Drosophilidae]

(with 3 Tables)

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## CONTENTS

I. Introduction. — II. Method and material. — III. Recovery time of flies subjected to carbon tetrachloride. — IV. Effect of ether upon the flies. — V. Carbon tetrachloride symptoms upon etherized flies. — VI. Conclusion. — VII. Bibliography.

## I. INTRODUCTION

During the early experiments that were carried to study the effect of higher percentages of carbon dioxide and carbon tetrachloride upon the wing-beat frequency of *Drosophila virilis* the author used various methods of quieting the flies before transferring them to the fumigation chamber; among these he tried the method of subjecting the flies to ether in order to make them convenient to handle. It was noticed that those flies which were subjected to the effect of ether gave remarkably contradicting results as far as the recovery time of ether is concerned. This fact alone made it necessary to investigate the effect of the fumigant at hand upon the etherized and over-etherized flies.

It is well known that ether is used in many fields as an anesthetic. Many workers apply the etherization method in their experiments on living specimens for its practical advantages.

Various authors pointed out the marked effects the anesthetics have upon the carbon dioxide output and the oxygen intake of organisms. In order to study the action of these chemicals upon organisms, Bodine (1923) gave

particular attention to the initial and recovery stages of anesthesia. It has been shown, for example, that ether, in the initial stages of its action, produces in the case of plants and some animals an initial lowering of the rate of carbon dioxide output followed by a rapid increase, and this in turn is succeeded by a fall.

Bodine concluded that marked changes in the rate of carbon dioxide output in grasshoppers are produced by ether and the order in which various parts of an animal recover from ether is — first, the respiratory movements, then movements usually controlled or inhibited by the brain and finally, the brain itself.

After consulting the current literature the author could not find any work which was done on the effect of a fumigant such as carbon tetrachloride upon insects that have been subjected previously to the effect of ether.

## II. METHOD AND MATERIAL

The method which has been used in this study was simply to subject flies selected at random from a rearing stock to the effect of varying concentrations of the fumigant. In order to get variations in the concentration of the fumigant, air is allowed to bubble through columns of liquid carbon tetrachloride and then by the aid of calibrated flowmeters the saturated air delivered to the mixing bottle could be measured in liters per minute. By increasing or decreasing the amount of air saturated with the fumigant in comparison to the total amount of pure air allowed to go into the fumigation chamber one can get considerable variations in the total concentration of carbon tetrachloride given to the insects.

Knowing the vapor pressure of the substance used at a given temperature, its concentration in grams per litre of air could be easily calculated using the equation :

$$\text{Total concentration in grams per litre} = \frac{\text{vapor pressure}}{760} \times \frac{273}{273 + t} \times \frac{1}{22.4} \times \text{Mol. wt.}$$

The gases were allowed to mix in a spacious jar before being introduced to the fumigation chamber which is a wide glass tubing 3 cms. in diameter and 20 cms. in length with an inlet and an outlet for the gases.

## III. RECOVERY TIME OF FLIES SUBJECTED TO CARBON TETRACHLORIDE

Several tests were conducted to study the correlation between the dosage of fumigant used and the recovery time for the insects. The standard experimental procedure was briefly as follows :

Groups of 30 flies were confined in the fumigation chamber. These flies taken from the stock could be from newly emerged to several days old.

After exposing the insects to the known concentrations of the fumigant for a fixed period of time and recording their stupefaction period, they were removed to a clean aerated place where they could be put under continuous careful observation to record their recovery time. The recovery time of 50 per cent of the group was always taken as representative of the group under these test conditions.

The results of these experiments show that the higher the concentration of the fumigant, the longer the recovery time for the insect until one reaches the lethal dosage. Also, the more the insect is exposed to the effect of the toxic action of carbon tetrachloride, the longer will be its recovery period (Table I).

TABLE I  
*Recovery time of flies  
subjected to the effect of carbon tetrachloride*

CONCENTRATION OF CARBON TETRACHLORIDE IN GRAMS PER LITRE	STUPEFACTION PERIOD IN MINUTES	PERIOD OF EXPOSURE IN MINUTES	AVERAGE RECOVERY TIME FOR 50 % IN MINUTES
0.07	11	12	10
0.07	11	20	14
0.12	9	10	12
0.12	9	15	16
0.23	7	8	18
0.23	7	10	22
0.48	4	5	25
0.48	4	7	30

Taking as an example for the effect of carbon tetrachloride a test where the flies were subjected to a concentration of carbon tetrachloride of 0.23 gram per litre, the average recovery time here reached a period of about 20 minutes.

#### IV. EFFECT OF ETHER UPON THE FLIES

It has been found that normal flies etherized only until stupefaction were, with the exception of a few of them, completely relaxed. It removed at that time from the ether, their wings would stay in the normal resting position and their legs would be bent in the manner assumed by flies at rest. Such flies subjected to a slight dosage of ether would recover within an average of 20 minutes after their removal from the anesthetic and then they seemingly behave normal afterwards. Yet flies left for a longer time in the etherizer behaved quite different. Their wings would be folded backwards and upwards and their legs were stretched out and held stiffly away from the body. Usually flies thus over-etherized did not recover from the narcosis and died in that position.

In contrast with this, the flies stupefied by carbon tetrachloride fumes show quite a different way of wing position. The majority were stupefied with their wings held upwards or downwards and not in any way resembling the resting form. The legs were also almost always stretched out completely with apparent vigorous telescopic movements of the abdominal segments.

TABLE II  
*Recovery time of etherized flies*

CONCENTRATION OF ETHER IN GRAMS PER LITRE	STUPEFACTION PERIOD IN MINUTES AND SECONDS	PERIOD OF EXPOSURE IN MINUTES	AVERAGE RECOVERY TIME FOR 50 % IN MINUTES
0.21	1.10	2	12
0.21	1.15	4	20
0.56	0.50	2	18
0.56	0.55	4	29
0.84	0.40	2	25
0.84	0.45	4	38

The standard concentration of ether used for these tests was 0.21 gram per litre and the time of exposure was four minutes. Here, also, it was quite true that by increasing the dosage of ether either by increasing its concentration or by extending the exposure period, prolongation of the recovery time takes place until the lethal dosage or overanesthesia is reached and no recovery but death of the flies follows (Table II).

#### V. CARBON TETRACHLORIDE SYMPTOMS UPON ETHERIZED FLIES

In order to examine the effect of toxic carbon tetrachloride upon the slightly etherized flies, i.e., those subjected to ether concentration of 0.21

TABLE III  
*Recovery time of slightly etherized flies  
(ether concentration of 0.21 gram per litre and 4 minutes)  
after carbon tetrachloride fumigation*

CONCENTRATION OF CARBON TETRACHLORIDE IN GRAMS PER LITRE	PERIOD OF EXPOSURE IN MINUTES	AVERAGE RECOVERY TIME FOR 50 % IN MINUTES
0.07	3	5
0.07	6	3
0.12	3	7
0.12	6	4
0.23	3	8
0.23	6	6
0.48	3	12
0.48	6	18

gram per litre for four minutes, several tests were run subjecting groups of these etherized flies to a known dosage of carbon tetrachloride. These insects show peculiarly unique activity while recovering from the effect of both anesthetics. They start after a comparatively short time of stupefaction, gliding around, dragging their seemingly paralysed legs, by the vibrations of their wings; the first parts recorded showing any sign of recovery were the wings, while in the case of carbon-tetrachloride stupefaction alone, the insects show their first signs of recovery through the activity and movements of their legs, and the flight activity of the wings is the last to be demonstrated.

Several tests made clearly show that slightly etherized flies, when subjected to a dosage of carbon tetrachloride enough to cause stupefaction, would recover in almost less than half the time required for the flies to recover from the effect of carbon tetrachloride alone or even ether alone (Table III).

By reversing the procedure of the above experiments and subjecting the flies first to carbon tetrachloride until stupefaction and then, before they gained recovery, to the anesthesia of ether, the recovery time in these cases was greatly increased. Likewise, when a mixture of the two anesthetics is used upon the flies, it has been found that these flies require a longer period for recovery. So it was only in the case of introducing carbon tetrachloride following ether, that the flies did recover much more sooner than in the case of using either one of the anesthetics alone.

## VI. CONCLUSIONS

1. Stupefaction of *Drosophila virilis* by ether is different from that by carbon tetrachloride, at least as far as the wing positions of the flies are concerned in both cases.

2. Recovery time for the insect concerned increases with the increase of the dosage of either ether or carbon tetrachloride given to the insect.

3. Adult etherized flies recover from a sublethal dosage of carbon tetrachloride in a shorter time than those flies which are subjected only either to a dosage of ether or to a dosage of carbon tetrachloride.

4. If the flies are subjected to a mixture of both fumes, the recovery time here is almost double.

5. The above is also true in cases of flies subjected first to the fumes of carbon tetrachloride and second to the fumes of ether.

6. It is safe to assume that the rate of diffusion of ether from the insect body is helped by the fumes of the fumigant bringing the insect sooner to the normal condition.

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