

NARCOSIS AND ASPHYXIATION IN SOME SPECIES AND MUTANTS OF *DROSOPHILA*

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(With Eight Text-figures)

I. INTRODUCTION

This paper is concerned with (1) the relationship between narcosis and asphyxiation, (2) the physiological analysis of the insect organism, (3) the influence on recovery from narcosis or asphyxiation of age, pH of food, etc., and (4) the differences in narcosis in *Drosophila* species, subspecies and mutants.

(1) Different physiological schools seek to explain the effects of narcosis in various ways, such as asphyxiation, solution of the narcotic in lipoids, its absorption or permeation, etc. In this paper some similarities and differences between asphyxiation and narcosis are described, while experiments dealing with their interaction are discussed.

(2) Narcotization is an excellent method for the physiological analysis of organisms, as was first emphasized by Claude Bernard (1875). The trachealization of the central nervous system in insects is especially favourable for study, since the circulatory system does not interfere as it does in vertebrates. Volatile narcotics can pass almost directly from the atmosphere to the nervous tissue, as can oxygen after asphyxiation (Kalmus, 1935).

In this paper I attempt to explain some of the results obtained on the assumption that macrophases of different lipid and water content exist in the fly.

(3) The influence of age on the resistance of an individual to oxygen lack is well known for mammals (e.g. Reiss & Haurowitz, 1929). Here I show that young insect imagines remain active with a lower oxygen supply than adult individuals, and that the recovery curves in etherizing experiments differ for newly hatched and older *Drosophila* flies. In addition to the age of the individual fly, the age of the culture bottle must be taken into consideration as a factor influencing narcosis. An attempt has been made to imitate the influence on narcosis of changes in the food medium by varying the pH of special media.

(4) In a laboratory where many stocks of *Drosophila* are kept and studied, this material provides an opportunity of trying to establish differences in narcotizability between species, subspecies and mutant races. Such differences have been established (Sekla, 1937), and are here investigated.

II. CRITERIA FOR THE DEGREE OF ASPHYXIATION OR NARCOTIZATION. RECOVERY TIME

Narcosis and suffocation, although quite different in their action on vertebrates, show striking similarities in insects. Reduction of oxygen below a certain level or increase of concentration of a narcotic (ether, chloroform, carbon dioxide) above a certain level,

both result in an immobilization which can be maintained for some time, and reversed by bringing back the concentration to that of normal air. However, there are certain differences in behaviour at the beginning of both processes. A gradual decrease of oxygen does not cause initial excitation corresponding to the dyspnoea of a mammal, but leads to a decreased mobility, and gradually to complete immobilization (Csik, 1939). On the other hand, etherization or treatment with chloroform or carbon dioxide provokes a very marked initial excitation. Near the limiting concentration this excitation stage may last for a considerable time before sudden immobilization occurs, and several phases of unco-ordinated and violent fluttering may alternate with immobilization. Similar but less well-marked behaviour may occasionally be observed during recovery from heavier narcosis; it is absent during recovery from oxygen want.

Asphyxiation supposedly causes immobilization by greatly reducing the production of nervous impulses and muscular energy. It may be caused by removal of oxygen from the air by means of a vacuum or an inert gas, or by poisoning the respiratory ferment by carbon monoxide or hydrocyanic acid gas. The action of the former on insects has been investigated by Haldane (1927), and that of the latter on *Drosophila* by Bliss (1935) and Broadbent & Bliss (1936). Their results will be considered later.

During narcosis the narcotic (ether, chloroform, carbon dioxide, etc.) accumulates on the loci where nervous excitation is produced, i.e. on structures within, or on the surface of, the nervous cells, and it is assumed that its specific action is the prevention of the permeation of those ions, on which the primary process of excitation depends.

The blocking of the cell surfaces becomes effective when the accumulation reaches a concentration specific for different narcotics; this concentration, the 'local limiting concentration', cannot be determined directly. It is, however, characterized by the external limiting concentration in the gas. The local limiting concentration is below a concentration to be called the 'local saturation concentration'.

Three theoretical criteria are available for the study of narcosis.

(1) The *stupefaction time* can be measured, i.e. the time from the beginning of narcotization to the moment when co-ordinated locomotion ceases. As it is difficult to time the cessation of movement of many flies included in a glass vessel, stupefaction time was measured only occasionally.

(2) The *minimum concentration* of the narcotic *in the air* necessary to immobilize the flies when permanently applied can be determined. Only approximate values can be obtained, mainly for the following two reasons: (a) The production of gas mixtures containing volatile anaesthetics of defined concentration is difficult. (b) There is no sharp change in the behaviour of a fly in the neighbourhood of this concentration, and apparently no stable equilibrium is ever reached between the concentrations of the phases of body and air. Similarly, in a *Drosophila* species the maximum concentration of oxygen causing immobilization varies within a wide range, between 1 and 4% (Csik, 1939).

(3) The most exact and easily determinable criterion is the *recovery time*, i.e. the period from the end of narcotization to the moment when a defined co-ordinated reaction-movement towards the light, either spontaneous or after stimulation—is regained by the fly.

In a narcosis experiment two steps follow one another:

(1) During the 'influx period' the flies are kept in the narcotizing vapour or gas, the narcotic flows in and reaches certain concentrations at different places in the body. The

first part of this phase is the 'stupefaction period' lasting from the start of the experiment till the moment of complete immobilization, when the local limiting concentration is reached. The second part is the super-narcotization period.

(2) During the 'efflux period' the narcotic flows out of the body, now kept in air or specially prepared gas mixtures, till its concentration reaches zero. Generally only the first part of this phase is observed, i.e. the period till the defined locomotor reaction is regained, and it is called 'recovery time'. The 'remainder' of the efflux period is important when flies are repeatedly used in experiments (p. 251), and it must be remembered that it is not identical with the time necessary for recuperation.

III. METHODS

Two different techniques were adopted according to the nature of immobilization:

(A) *Gases and vacuum.* Selected batches of six to thirty flies were enclosed in glass tubes between cotton-wool stoppers and subjected for a few seconds to several hours to a stream of single gases, gas mixtures, or evacuated by means of a pump. The pressure of the gas as well as the speed of the pump were kept fairly constant. The following figures are taken from a carbon dioxide experiment (Table 1).

Table 1. *Recovery time from 25 sec. exposure to concentrated carbon dioxide. Three subsequent treatments of six flies (Drosophila melanogaster or +). 4 October 1941. T = 21° C.*

Individual	1st narcosis sec.	2nd narcosis sec.	3rd narcosis sec.	Total sec.
1st ♀	104	93	95	292
2nd ♀	93	100	99	292
3rd ♀	86	101	93	280
1st ♂	81	91	110	282
2nd ♂	108	90	88	286
3rd ♂	106	88	104	298
Total	578	563	589	1730

Statistical treatment of these figures shows that the differences, both between individual flies and different narcoses, are well below the expected values, and it is clear that the method is quite reliable.

(B) *Ether and chloroform vapours.* Equal batches of flies were transferred from a vial to a broad-necked jar of 335 c.c. capacity, and the jar closed and shaken. Ether or chloroform had previously been pipetted into the jar by means of a pipette graduated in hundredths of c.c., and the jar closed and shaken. After a measured time the flies were removed from the jar to a piece of paper, selected in groups, and the individual recovery times were noted with a stop-watch.

The goodness of the experimental method, as shown by repetition, is demonstrated on p. 241 (Table 2).

Statistical treatment of these figures shows that the differences between narcoses are not significant. But there is some indication of real differences in recovery of different individuals.

In experiments using very low or very high dosages of narcotic it is impossible to determine mean recovery times, as in the first instance some flies are not immobilized at all, whereas in the latter some flies die. However, the recovery of half the individuals

Table 2. *Recovery time of six Drosophila simulans from 1½ min. exposure to air containing 12.75 vol. % ether. T = 21.5° C. The second treatment took place 30 min. after the first. The third treatment 1½ hr. later.*

Individual	1st narcosis min.	2nd narcosis min.	3rd narcosis min.	Mean min.
1st ♀	9.0	10.1	9.8	9.6
2nd ♀	10.1	7.2	8.3	8.5
3rd ♀	10.9	8.8	10.9	10.2
1st ♂	11.0	10.3	11.8	11.0
2nd ♂	8.2	9.2	9.2	8.9
3rd ♂	7.6	7.9	10.2	8.6
Mean	9.2	8.9	10.0	9.5

can still be timed exactly under these extreme conditions. Therefore the *median recovery time* was used in most experiments. At medium concentrations there is very little difference between the mean and the median.

IV. RECOVERY FROM ASPHYXIATION

Recovery times after removal of oxygen for a measured time did not differ greatly, either for a vacuum or an indifferent gas (nitrogen, hydrogen). As the technique is easier, the results of exposure to hydrogen are the most regular. The results of an experiment of this type performed on *D. melanogaster* or + are shown in Fig. 1.

By plotting the logarithms of the asphyxiation time (a) against median recovery time (r) a straight line is obtained of the formula

$$r = 184.5 \log a - 147.6. \quad (1)$$

According to this formula recovery would be instantaneous after exposure to hydrogen for 6.3 sec. The formula indicates that after a short period of asphyxiation most of the oxygen stored in the flies' tissues has been consumed, and thus a stationary stage is reached where no more oxygen can be removed; the recovery from this state remains fairly constant for a considerable time.

V. RECOVERY FROM CARBON DIOXIDE NARCOSIS

By plotting the logarithms of the influx time (a) of carbon dioxide against median recovery time (r) a straight line similar to that for hydrogen is obtained; its formula is

$$r = 62.5 \log a - 25.0. \quad (2)$$

According to this equation recovery would be instantaneous after exposure to carbon dioxide for 2.5 sec.

Fig. 1 shows that the carbon dioxide recovery curve is less steep than the hydrogen curve. Both cross at an exposure period of about 10 sec. where there is little difference in recovery time. After exposure for less than 10 sec. flies treated with hydrogen recover quicker, after exposure for more than 10 sec. carbon dioxide-treated flies start moving first.

The exponential relation between recovery time and time of exposure to hydrogen can be explained by assuming that an asphyxial metabolite (perhaps lactic acid) is formed at a steady rate during exposure to hydrogen, and this immobilizes the fly when it reaches

a certain threshold. The substance is destroyed at a rate proportional to its concentration when the fly is replaced in air; similarly carbon dioxide diffuses in at an almost constant rate and diffuses out at a rate proportional to its concentration.

VI. EFFECT OF NITROGEN AND HIGH AIR PRESSURE

The rate of recovery after exposure of 100% nitrogen at normal pressure was much the same as after treatment with hydrogen. In pressure experiments performed on human beings, including myself (Case & Haldane, 1941), I observed that at 10 atm. air pressure

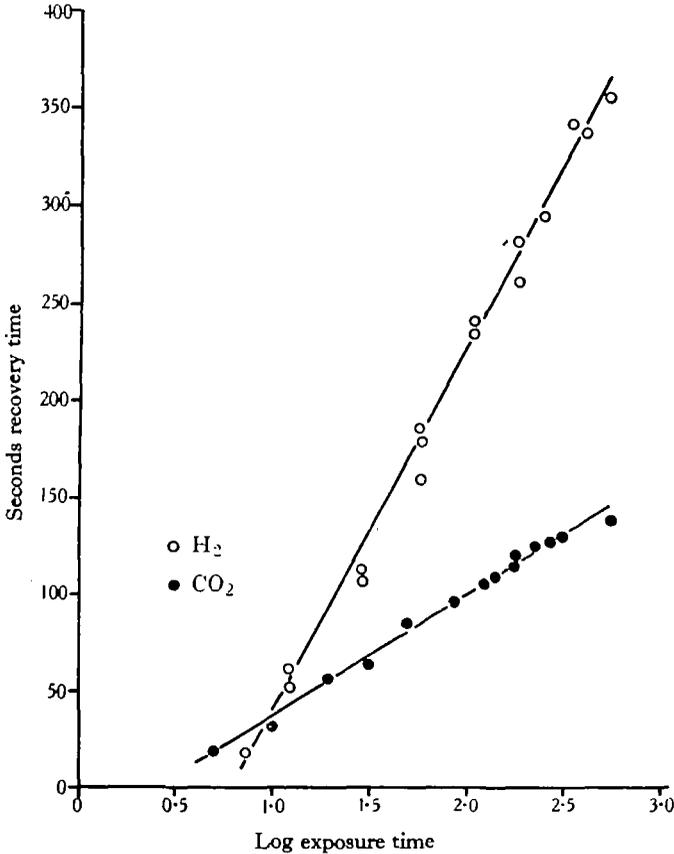


Fig. 1. Median recovery time plotted against log of median exposure time. *D. melanogaster* ♀♀. Hydrogen and carbon dioxide.

Drosophila flies became very lethargic and did not fly even when stimulated. This effect corresponds to the narcotizing effect on human beings under the same conditions, where it is generally ascribed to the action of the 8 atm. nitrogen (Behnke, Thomson & Motley, 1935).

VII. RECOVERY FROM ETHER NARCOSIS

Recovery curves after ether narcosis vary considerably according to the material used. The only general statement which can be made is that any increase in ether dosage, either by increasing influx time or concentration, increases recovery time. Two methods have

been selected to demonstrate the results obtained: (a) isonarcotic curves, (b) curves plotting dosage against recovery time.

(a) *Isonarcotic curves*

The same recovery time can be obtained experimentally with the influx of a high narcosis concentration for a short period as with a lower concentration effective for a longer time. Curves connecting points of equal recovery time may be called isonarcotic curves. A number of them is shown in Fig. 2. They are of the same type as the curves for survival from exposure to poisonous gases given by Flury & Zernik (1931).

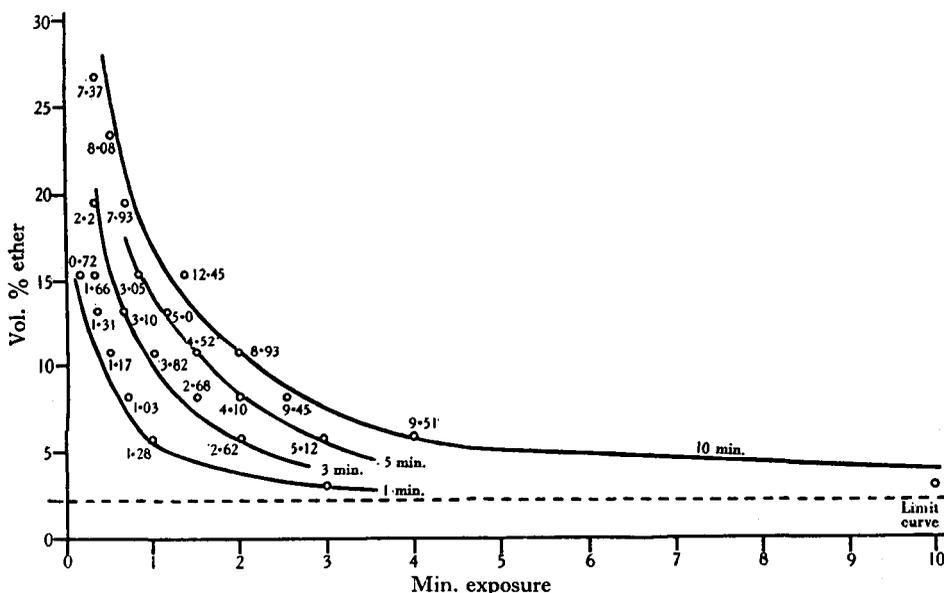


Fig. 2. Isonarcotic curves for equal duration of ether narcosis. *D. melanogaster* or + ♀♀.

(b) *Dosage-recovery curves*

Fig. 3 shows the median recovery times of batches of *D. pseudoobscura* plotted against ether dosages. The dosage is calculated as the product of the difference between actual and limiting (2.4 vol. %) ether concentration and time. Recovery time in this species increases in a wide range in direct proportion to the ether dosage. The formula is

$$r = 0.73a(c - 2.4) - 2.91. \quad (3)$$

This formula again resembles Flury's and Zernik's formula (p. 100) for the action of poisonous gases. Similar results were obtained with *D. virilis*, but rarely with *D. subobscura*. Thus it appears that there is a fundamental difference between the shape of recovery curves from asphyxiation or carbon dioxide intoxication and etherization. The former show a logarithmic relation between dosage and recovery time, the latter a linear relation. However, in other species (e.g. *melanogaster*, *simulans*, and sometimes *subobscura*) or under different conditions, curves of quite a different type are obtained after etherization, some being logarithmic, others showing a point of inflexion.

This behaviour can be explained on two assumptions:

(1) Narcosis takes place so long as the ether concentration on the primary loci of stimulation, i.e. an aqueous phase, is above a certain level.

(2) Ether can pass into and out of this aqueous phase, not only into the outside air but into a lipid phase inside the animal. During narcotization ether passes from the air mainly through the aqueous phase, into the lipid phase, where the presence of ether will prolong recovery only after the partial pressure of ether in it is equivalent to that needed for narcosis in the aqueous phase.

The lipid phase can be large or small in quantity relative to the aqueous phase, and the exchange of ether between it and the air can take place to a different degree through

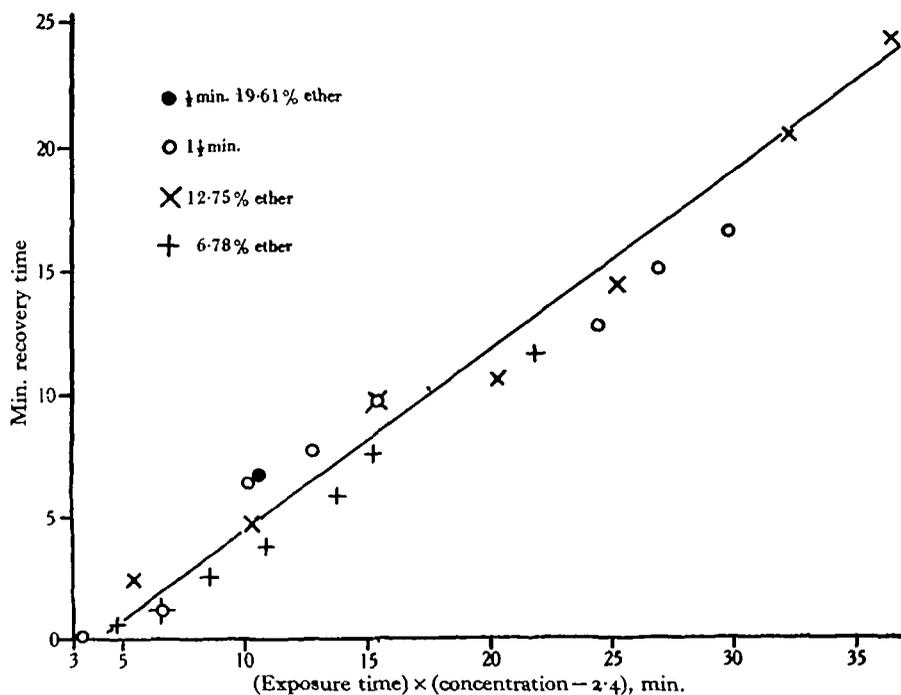


Fig. 3. Product of (median exposure time) and (ether concentration minus 2.4) plotted against median recovery time. *D. pseudoobscura* ♀♀.

the aqueous phase or directly. By varying one or both of these factors most of the types of the curves observed can be explained.

Figs. 4 and 5 show some examples of simpler curves obtained by plotting recovery time against vapour concentration at constant influx periods or against influx time at constant ether concentration.

Both figures show that the recovery curves of *D. melanogaster* or + males after small ether dosages (short time or low concentration) are sigmoid; large ether dosages give recovery times the curve of which corresponds only to the upper part of the sigmoid, as the point of inflexion is very near the X-axis, or even below it. This latter type of curve resembles somewhat the logarithmic curves obtained with hydrogen or carbon dioxide. However, there is one difference; if the dosage is increased still further the curve rises again steeply and becomes almost vertical, i.e. after very heavy ether dosages

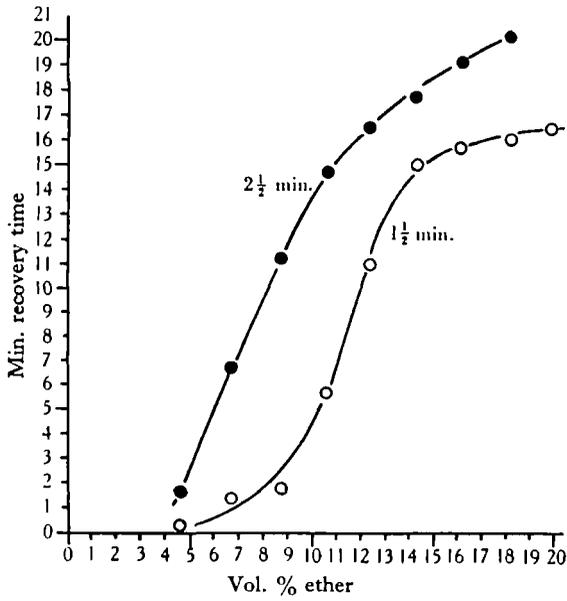


Fig. 4. Median recovery time plotted against ether concentration. *D. melanogaster or + ♀♀*.

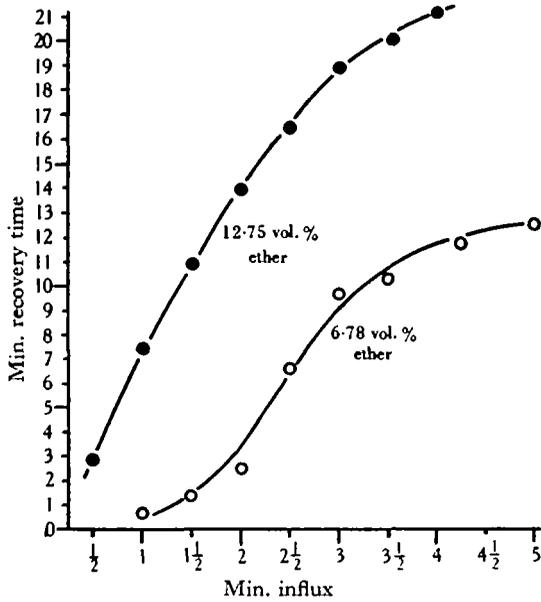


Fig. 5. Median recovery time plotted against median etherization time. *D. melanogaster or + ♂♂*.

flies do not recover at all (Fig. 6a). This is probably due to an irreversible process which is quite independent of narcosis. Now it is easy to imagine that this ether poisoning sometimes starts at dosages where the horizontal branch of the recovery curve is not yet reached. The result (Fig. 6b) is a curve which immediately gets steeper instead of decreasing its slope. As the upper ends of such curves do not depict narcosis, we need not discuss them further.

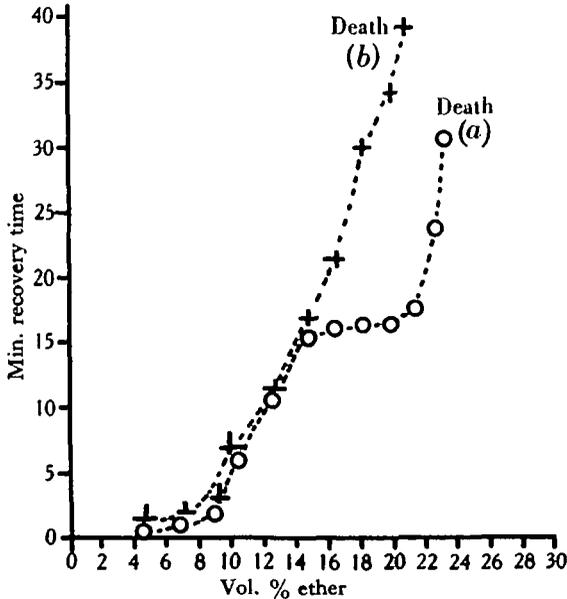


Fig. 6. Two different batches of *D. melanogaster* or + ♂♂ showing different resistance to heavy ether concentrations; 1½ min. etherization, median recovery times.

VIII. DIFFERENCES IN RECOVERY TIME DUE TO SEX AND AGE

The wide range of variation usually observed in the recovery times of individuals from one culture as well as the differences of recovery curves, can be markedly reduced by the control of several factors.

The size of the individuals of a species does not seem to have much influence on recovery time. More important are the sex and age of the flies. After narcosis with low and medium ether dosages, females generally recover more quickly than males from the same bottle. After application of high concentrations this behaviour is reversed. In *D. melanogaster* and *simulans*, where morphological sexual differences are most apparent, the difference in recovery also seems to be most marked. In the other species observed (*subobscura*, *funnebris*, *pseudoobscura*, *miranda*, *virilis*, *montium*) it is smaller, but still significant. The difference of recovery between the sexes at low and medium concentrations may be a little greater than stated by this method, as females run much more slowly than males, and therefore may be considered as being sluggish for a longer period. A slight difference, possibly significant, in the mean recovery times between virgin and mated females in *D. melanogaster* was observed, the virgins recovering more slowly.

The influence of age on the recovery time is rather complex. There is no doubt that fundamental changes in ether resistance occur during the first hours after emergence

in *D. virilis*, during the first few days). After the application of low and medium concentrations the very young flies recover much earlier than the older ones, and frequently they do not become narcotized at all. At high concentrations the opposite behaviour may be observed, the young flies remaining narcotized several times longer than the older control animals. One possible explanation of this behaviour is that parts of the tracheal system are still filled with liquid for some hours after emergence, so that the ether penetrates more slowly into the central nervous system; but once in it would also take longer to get out again.

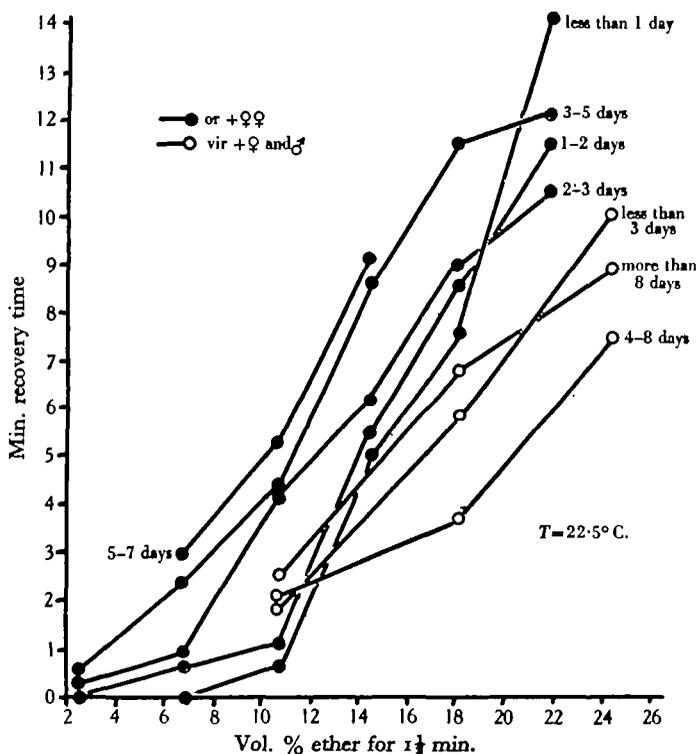


Fig. 7. Recovery times of *D. melanogaster* or + and *D. virilis* + of different ages.

After these changes during the first few hours of life, which show considerable individual variation (see also p. 240), a slight lengthening of the period of recovery can be observed for several days (Fig. 7), where the lengthening of recovery time at high dosages of flies less than 1 day old (3 days in *D. virilis*) can still be noticed.

IX. THE pH OF THE CULTURE MEDIUM AS A CONTROLLING FACTOR

It was found that older flies (10 or more days old) sometimes recover more quickly at medium ether concentrations than do medium-aged flies, and that different results were obtained when flies from bottles of different ages or from bottles and vials were compared. Also the first comparative measurements of species and mutants often gave contradictory results. This was explained by the fact that the 'age' of the culture influences recovery time independently of the age of the individual flies. Later it appeared that, apart from variations in the amount of food and moisture available, the actual reaction of the medium on which the flies are kept is the main factor responsible for the ageing of a culture.

Bridges & Darby (1933) showed that the pH of unbuffered *Drosophila* media decreases from about 5.8, where it lasts 1-2 days after preparation, to 3.6-3.8 during the subsequent 1-3 days, where it remains for about 3 days. Finally, it rises to 3.8-4.8. It was thought that these changes in pH might be responsible for the changes of recovery time correlated with the age of the culture. In experiments to test this the effect on recovery of slightly more extreme values of pH was investigated. Flies from the same culture were put simultaneously on a medium consisting of 10% molasses soaked in cellulose cotton (a material similar to that used by Spencer (1937)). The molasses were mixed with hydrochloric acid and calcium chloride powder or sodium hydroxide solution till a definite

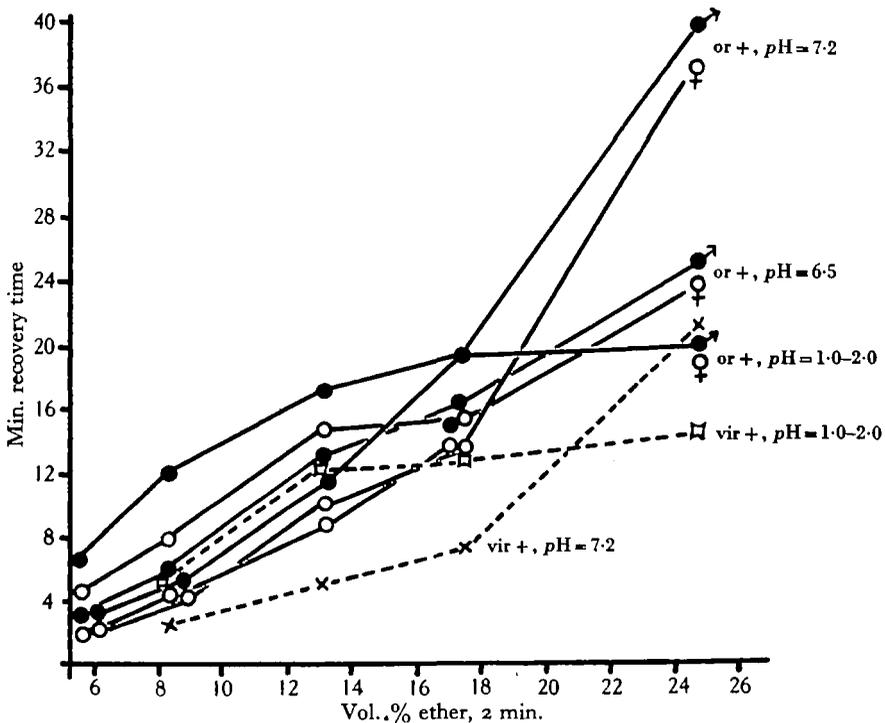


Fig. 8. Median recovery times of *D. melanogaster* and *D. virilis* kept on media with different pH .

pH was reached. This was measured by means of Clarke's colour indicators before and after the flies were put into it. The pH of the media changes very little during the presence of the flies (1-4 days).

Fig. 9 shows that the recovery time of flies of *D. melanogaster* and *virilis* is greatly influenced by the pH of their food.

After slight and medium etherization the flies kept on an acid medium recover much later than after being on a neutral or alkaline medium. After heavy dosages this behaviour is reversed. As the limiting concentration observed did not change very much, this behaviour will be regarded tentatively as a consequence of differences in the speed of ether permeation according to the pH . It is probable that the pH of the *Drosophila* tissues follows that of the food to some extent. According to this conception an acid state increases the influx of ether as well as the efflux, but as the latter lasts longer under the conditions of our experiment the recovery time is increased compared with the control

low and medium concentrations. At high concentrations, however, a similar state of ether saturation, even if only preliminary and local, seems to be reached in the flies from both the acid and alkaline cultures. According to this hypothesis efflux is slower under neutral or alkaline conditions, and hence the acid flies are expected to recover more quickly after exposure to high ether concentration. The following experiments serve to illustrate this.

(a) *The influence of exposure to carbon dioxide before and after ether narcosis*

The most convenient means of making the nervous tissue of the flies acid for short periods is by the application of carbon dioxide. As already described, carbon dioxide itself narcotizes the flies if applied in high concentrations. But a combined narcotic effect can be excluded, as in the following experiment.

$T=23^{\circ}\text{C}$. 2-3 days old *D. simulans* + flies of both sexes were divided into three batches; one of these was marked by cutting the wing tips of the flies. One batch of the uncut flies was treated for 1 min. with 100% carbon dioxide and recovered in less than 1 min. The second batch of flies was subjected to 100% carbon dioxide for 1 min., and during recovery the flies were mixed with the third batch and etherized. The average recovery times from narcosis effected by 10.86% ether lasting for 1½ min. were: for seven females narcotized while recovering from 1 min. exposure to carbon dioxide 5.9 ± 0.8 min.; for eight males treated in the same way 8.8 ± 0.7 min.; for six females narcotized simultaneously as control, 4.1 ± 0.8 min.; for seven control males, 4.8 ± 0.8 min. The differences are significant in both sexes.

Similar results were obtained with *D. melanogaster* and *virilis*.

The shortening of recovery time from ether narcosis by application of carbon dioxide (well established for man) was described for *Drosophila* by Kalmus (1935). It should be noted that the effect of carbon dioxide on recovery time can also be explained by the action of carbon dioxide in increasing respiration; nevertheless, 100% carbon dioxide should cause apnoea rather than hyperpnoea.

It is possible to explain the quicker recovery from ether narcosis of newly-hatched flies by a more anoxybiotic stage of metabolism at this age (see also the influence of carbon monoxide on p. 251).

Lack of oxygen (vacuum, hydrogen, nitrogen, carbon monoxide) and other factors may also shorten the recovery time, the explanation being that the flies become acid during recovery, especially at interfaces in the tracheal-nervous system. The results of two experiments on the shortening of recovery time by means of carbon dioxide are given below:

$T=18^{\circ}\text{C}$. *D. subobscura pl pp op*, 1 day old, narcotized in 10.86% vol. ether for 1½ min. Twelve flies first brought into 100% carbon dioxide for 1 min. recovered after 7.6 ± 0.77 min. Eleven flies brought directly into the atmosphere (control) recovered after 12.2 ± 1.31 min., the difference of 4.6 min. being significant.

Recovery can also be hastened by putting the etherized flies into a tube with expired air which contains between 3 and 4% carbon dioxide, and it can be shown that neither the draught nor the moisture and increase in temperature resulting from the application of expired air are the cause of this shortening. Application of vaporous hydrochloric, acetic or formic acid, gave results which were not significant. Experiments on the

shortening effect of hydrogen, nitrogen, carbon monoxide, coal gas and acetylene have been described by Kalmus (1935).

(b) *Shortening of the recovery time after hydrogen asphyxiation by application of carbon dioxide*

Drosophila flies immobilized by administration of hydrogen recover earlier when subjected to carbon dioxide. This effect resembles that of carbon dioxide in artificial respiration in man. It seems that immobilization through oxygen lack is only in small part due to the accumulation of carbon dioxide, other waste products (perhaps lactic acid) having a greater effect. The following results were obtained in an experiment designed to show this:

$T = 22^\circ \text{C}$. *D. subobscura* pl pp op flies immobilized in a stream of hydrogen for 2 min : nine flies brought for 1 min. into 60% carbon dioxide (approx.) recovered in air after 2.7 ± 1.03 min. Nine control flies brought immediately into air recovered after 4.7 ± 1.14 min. The difference of 2.0 min. is significant. Similar results were recorded when the asphyxiation was caused by treatment in a vacuum.

(c) *Effects of carbon dioxide on recovery from hydrocyanic acid immobilization*

The stupefaction and recovery of *Drosophila* after treatment with hydrocyanic acid has been thoroughly investigated by Bliss & Broadbent (1935, 1936). Recovery time is a linear function of the sum of the logarithm of concentration and influx time. Table 3 shows that carbon dioxide treatment of *Drosophila* before exposure to hydrocyanic acid increases the recovery time, whereas carbon dioxide treatment of the hydrocyanic acid-immobilized flies shortens recovery.

Table 3. *Recovery times of Drosophila melanogaster after treatment with hydrocyanic acid (15 sec. in a killing bottle) and carbon dioxide (90 sec.). $T = 21^\circ \text{C}$.*

Treatment	CO ₂ before HCN	HCN alone	CO ₂ after HCN	CO ₂ alone
Recovery of 5 out of 10 ♀♀	12 min. 16 sec.	9 min. 59 sec.	4 min. 37 sec.	2 min. 14 sec.

The first three recovery times were measured from the moment the flies were removed from hydrocyanic acid, the last one from the time they were removed from carbon dioxide.

(d) *Shortening of recovery from ether narcosis by application of carbon monoxide and coal gas before narcosis*

By subjecting *Drosophila* flies to carbon monoxide or coal gas a shortening of recovery time from a subsequent ether narcosis can be obtained. It seems that the carbon monoxide, by blocking some of the respiratory catalysts, reduces for some time the ability of the organism to make use of atmospheric oxygen and turns it over to a partly anoxidative metabolism. Haldane (1927) proved that a wax moth (*Galleria mellonella*) needs more oxygen for the maintenance of its motor activity in the presence of carbon monoxide than it needs in air. In *Drosophila* a slight sluggishness of movement can be observed for several hours after the flies have been exposed for 1 min. to coal gas. This is most striking at low temperatures where the chill coma temperature (Mellanby, 1939) appears considerably raised. It may be that the presence of the carbon monoxide renders the

flies more acid and therefore shortens recovery time. It is therefore plausible to assume that flies treated with carbon monoxide take on some of the qualities of newly hatched flies for several hours. The result of a typical experiment was as follows:

$T = 22.5^{\circ}\text{C.}$: twelve 1-2 days old *D. subobscura* treated with carbon monoxide for 50 sec. and recovered after 3-5 min., were narcotized after 6 min. for $1\frac{1}{2}$ min. in an atmosphere of 10.86 vol. % ether. They recovered after 4.25 ± 1.10 min., untreated control flies after 5.59 ± 1.12 min., the difference being significant. Similar results were obtained with *D. melanogaster oregon F₅*, *D. simulans* and *D. subobscura*, but not with *D. virilis*.

(e) *The effect of previous narcotization on recovery time*

In certain circumstances, if the narcosis is not too heavy and the flies are allowed to recuperate completely on their usual food, no significant differences in the individual recovery times can be observed, as already shown on p. 241. Pearl and Parker (1922) showed that no sensible alteration of the duration of life follows the ether narcosis, as practised by *Drosophila* workers, even when repeated four times. However, when heavy dosages of narcotic are applied recovery times are altered in the intervals before recuperation is complete. Two antagonistic effects can be observed which sometimes counter-balance each other.

The first is a *summation* effect; it occurs when the second narcosis starts before all the narcotic from the first has been completely eliminated from the animal's body, i.e. during the efflux remainder period. This is an effect easy to produce, and needs no further explanation. A second etherization towards the end of recovery time results in a marked excitation state followed by prolonged narcosis. Secondly, a *shortening* of recovery time occurs during the recuperation, presumably after all ether has gone, a result which is more difficult to demonstrate. However, as it is cumulative, the decrease in recovery time becomes significant after several repetitions of narcotization. The following figures denote recovery times of the same seven *D. melanogaster* flies etherized four times in 10.86 vol. % ether for $1\frac{1}{2}$ min.; the intervals between the narcotizations were about 20 min. in each case. The four recovery times were: 9.12 ± 1.50 min., 5.93 ± 1.51 min., 5.01 ± 1.69 min., and 4.60 ± 1.23 min. The recovery time of the eight control flies etherized for the first time and simultaneously with the seven flies in the fourth narcotization was 8.18 ± 1.63 min. The differences between the first and last recovery time (4.62 min.) and between the last recovery time of the seven flies and the recovery of the eight control flies (3.58 min.) are significant ($p < 0.01$).

X. DIFFERENCES IN RECOVERY TIME OF SPECIES, RACES AND
MUTANTS OF *DROSOPHILA*

There are definite differences in recovery from etherization in *Drosophila*, but although this fact is generally recognized, no one has so far attempted to analyse these differences. Most batches of flies taken from two bottles will differ significantly in recovery time, when carefully examined throughout the concentrations (see Fig. 5), owing to differences in age, pH of culture, proportion of sexes, etc. Neither are differences in the recovery of flies emerging from one culture always due to genetical factors. Thus the F_1 generation of w females and $+$ males in *D. melanogaster* is composed of phenotypically white-eyed males and red-eyed females. As females recover earlier than males from low or medium ether dosages, the difference in recovery time between the red and white-eyed flies cannot

be ascribed to gene mutation. Similar fallacies are to be expected in all sex-linked mutants. The offspring from a culture may emerge at different times according to the phenotype. In such a case differences in recovery time due to age might be mistakenly ascribed to the effects of gene differences. A serious difficulty is the different behaviour of mutants, races, and species during and after recovery. The definition of recovery as used in this paper (see p. 240) can be applied to all flies, though it does not mean the same in all cases. Immobilization occurs in different ways: *melanogaster*, *simulans*, *pseudobscura*, and the other small species usually become narcotized gradually, whereas *subobscura*, *virilis* and *funebis* are often immobilized at one stroke. If a mixed batch of *melanogaster* and *virilis* is etherized and then brought back into atmospheric air the *melanogaster* flies are sluggish but not fully narcotized, whereas all *virilis* flies remain motionless. But when the latter are touched with a needle, they start to walk normally, whereas *melanogaster* flies are far from the defined state of recovery for several minutes.

The classification of the degrees of narcosis by most *Drosophila* workers is rather superficial. The desirable degree is defined by immobilization and normal wing position. Flies whose wings are distorted, e.g. dorsally extended in a position similar to that of a butterfly, are usually regarded as dead. This is not always true, even after heavy ether dosages, and does not apply at all to immobilization by carbon dioxide, hydrogen, coal gas, etc., where this position is mostly reversible, as it is after stupefaction with hydrocyanic acid gas (Bliss, 1935). The reversion to the normal wing position occurs suddenly, usually before recovery, but later after heavy dosages.

Differences in the general liveliness of different species, races and mutants also add to the difficulties of comparison. The larger species are much less active and never in constant movement, as is usual with *D. melanogaster*. Wingless mutants obviously cannot flutter as winged individuals often do during recovery.

It is, nevertheless, possible to compare the recovery times of the different *Drosophila* species under specified conditions. If one tries to arrange the species of *Drosophila* according to the time they need for recovery one obtains different sequences dependent on the narcotic, and a different one again for asphyxiation.

Recovery from asphyxiation, achieved by a vacuum or by hydrogen, is significantly quicker in *D. virilis* + than in *D. subobscura*, and quicker in this species than in *melanogaster* or *simulans w* (23° C. 3-5-day-old flies kept together in one bottle for 24 hr. Hydrogen flowing through for 3 min.).

Differences in recovery between *melanogaster or* + and *dp eb* were not constant. There is a striking difference in recovery time after the asphyxiation of *D. virilis sinensis* and *americana*. 3-day-old *sinensis* flies (wings clipped) recovered from 3 min. exposure to hydrogen after 3.79 ± 0.62 min., *americana* flies which had been kept in the same bottle after 5.50 ± 0.81 min.

Great differences of resistance to hydrocyanic acid poisoning in *Drosophila* species can be detected by measuring the recovery time. Thus in a single experiment ten *melanogaster or* + flies, ten *virilis* + and 10 *subobscura* + flies were kept together for 24 hr. and then shaken in a hydrocyanic acid killing bottle for 15 sec. The *virilis* flies were not immobilized at all, whereas the median recovery time of the *melanogaster* flies was 4.9 min., and that of the *subobscura* flies more than 20 min.

Recovery from carbon dioxide narcosis takes about the same time in the different species of *Drosophila*.

Consistent differences were obtained by simultaneous application of carbon dioxide to *melanogaster* or + and *b vg* or *subobscura* + *subobscura pl pp op*. The multiple mutants of both species recovered significantly slower than the normal flies after 1–15 min. of carbon dioxide narcotization. *D. virilis americana* recovers slightly more slowly than *sinensis*. A stock of *D. melanogaster*, which has been described by L'Héretier & Teissier (1937) as particularly susceptible to carbon dioxide, showed behaviour similar to that of other stocks and had apparently lost this peculiar property.

Recovery from ether narcosis may be the same within wide ranges of dosage in distinct species (*D. melanogaster* and *D. simulans*), races (*D. pseudoobscura* A and B) and mutants (*melanogaster* + and eye-colour mutants, *subobscura* + and *int.*). But even in these species after a very high dosage of ether (such as is sometimes applied by geneticists), significant differences can be observed, probably due to differences in the fat content of the flies. Between other species (*melanogaster*, *subobscura*, *virilis*), mutants (especially multiple mutants, e.g. *subobscura* + *subobscura pl pp op*) and races (*virilis* +, *virilis americana*) significantly different recovery-time curves throughout concentrations could be registered even when conditions were made as alike as possible. Table 4 summarizes some numerical data of recovery times.

Table 4. *Drosophila*-recovery time of nine to fourteen flies of each species kept together for 48 hr. on 10% molasses (pH 6.8). $T = 23^{\circ} \text{C}$.

$1\frac{1}{2}$ min. influx vol. % ether	<i>virilis</i> + (<i>sinensis</i>) min.	<i>pseudo-</i> <i>obscura</i> B min.	<i>melanogaster</i> or + min.	<i>simulans</i> w min.	<i>subobscura</i> + min.	<i>virilis</i> <i>americana</i> + min.
6.78	0.30 ± 0.43	1.96 ± 1.10	3.83 ± 1.31	3.88 ± 1.09	5.84 ± 1.18	2.74 ± 0.41
10.86	2.48 ± 1.15	4.93 ± 0.87	6.33 ± 1.19	6.00 ± 1.16	5.84 ± 0.97	5.13 ± 1.90
14.57	7.54 ± 2.01	8.74 ± 1.91	9.62 ± 1.43	9.73 ± 1.35	11.36 ± 1.55	10.10 ± 1.93
23.32	14.05 ± 2.35	15.28 ± 2.70	15.60 ± 2.75	16.72 ± 2.42	18.72 ± 2.84	15.15 ± 2.07

These and similar figures indicate the following order of recovery in the lower range of etherization described: *virilis* + (*sinensis*) recover most quickly, then comes a group consisting of *melanogaster*, *simulans*, *montium* and *pseudoobscura*. Still slower in recovery are *subobscura*, *miranda* and *immigrans*. *Virilis americana* is the slowest to recover, in spite of the fact that *virilis virilis* is the quickest of all. After stupefaction all *sinensis* flies show normal wing position, all *americana* 'butterfly' position (p. 252). Spencer (1940) observed an extreme difference in the stupefaction time of the two races.

Experiments to elucidate the influence of single mutant genes on recovery time have not been very satisfactory. Some preliminary results of experiments are as follows:

(1) Some mutant genes or combinations do not influence the recovery from medium ether dosages of 3–5-day-old flies, e.g. *w*, F_5 , *v*, *sc* in *melanogaster*, *int. op.*, *th int* in *subobscura*.

(2) Other genes lengthen recovery *e*, *b* in *melanogaster*, *pl pp op*, *ho* in *subobscura*.

(3) A shortening of recovery time occurs in flies homogeneous for *dp* or *vg* in *melanogaster* and *v* in *subobscura*.

XI. SUMMARY

1. Narcosis and asphyxia in insects can be investigated by measuring the recovery time. This and other terms are defined, and suitable criteria of recovery are given.

2. Simple techniques for the etherizing and gassing of *Drosophila* batches are described and the validity of the quantitative results obtained is shown.

3. The recovery time is increased by the time of influx and by the concentration of the narcotic. The shapes of the curves obtained in experimental series are logarithmic (carbon dioxide asphyxiation), straight line (ether in some species), concave, convex or sigmoid (ether). It is suggested that the different forms of ether recovery curves are different parts of essentially similar curves, which one might explain by the joint action of two macrophases, one aqueous and one lipid.

4. Physiological factors determining recovery times are: (a) sex: females recover earlier from ether narcosis than males; (b) age: young flies recover earlier than older ones; (c) lack of food and moisture, which increases the recovery time; and (d) chemical reaction: flies kept on acid food remain longer narcotized than flies bred on an alkaline medium.

5. Carbon dioxide lengthens recovery from ether narcosis and hydrocyanic acid immobilization when applied before the influx time and shortens it when applied during recovery time. If administered during recovery, it also shortens the recovery from asphyxiation.

6. Carbon monoxide and coal gas administered before narcotization can shorten the recovery time from ether narcosis.

7. Under specified conditions corresponding to those used during narcosis by *Drosophila*-workers some differences in recovery time after etherization due to genetical differences could be established. Significant differences also exist between some *Drosophila* species, races and mutants in their resistance to carbon dioxide, asphyxiation and hydrocyanic acid gas.

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