

COMPARATIVE STUDIES ON RESPIRATION.

V. THE EFFECT OF ETHER ON THE PRODUCTION OF CARBON DIOXIDE BY ANIMALS.

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The purpose of this investigation is to make a comparison of the CO₂ output of animals with that of plants under the influence of a typical anesthetic such as ether. One obstacle to such a comparative study is found in the fact that the movements of animals are more or less inhibited by anesthetics; such inhibition diminishes the CO₂ output even when it is not due to anesthetics. Hence it is difficult to say how the CO₂ output would be affected by anesthetics if this complication were absent. This difficulty is largely avoided in my studies on frog eggs and *Fundulus* embryos, while in the investigations on tadpoles and on aquatic insects it is partly obviated by the method of experimentation.

No attempt has been made to compare the results with those obtained on animals by other workers, such as those of Tashiro,¹ because the methods were so different as to render a comparison difficult.

Method.

The method used in determining the output of CO₂ is the one described by Haas.² The standard buffer solutions were made with mixtures of phosphates. To 10 cc. of the solution, three drops of 0.01 per cent solution of phenolsulfonephthalein were added; in all the experiments sufficient NaOH was added to bring the pH value to 8.0. As the concentration of NaOH was the same in all cases the

¹ Tashiro, S., A chemical sign of life, Chicago, 1917.

² Haas, A. R. C., *Science*, 1916, xliv, 105.

buffer action was identical in the solutions containing anesthetics and in the controls (the ether has practically no buffer action).

In each case 10 cc. of the solution were placed in a Pyrex glass tube of the same diameter and thickness as the Pyrex glass tubes containing the buffer solutions. The organisms were put in the solutions and the time required to change from pH 8.0 to 7.7 was recorded by means of a stop-watch. After experimentation the solutions were tested by driving out the CO₂ by means of air free from CO₂; invariably the solutions returned to pH 8.0, thus proving that no acids other than the carbonic were responsible for the change in the pH value.

The temperature was kept approximately constant at 20°C. (with variations of less than 1°C.) by means of a water bath.

In presenting the results the rate of respiration is taken as the reciprocal of the time required to change the solution from pH 8.0 to 7.7. For convenience this rate is expressed as per cent of the normal rate, which is always taken as 100 per cent. The normal rate was in most cases practically constant.³

Frog Tadpoles.

The first set of experiments to determine the effects of ether on the carbon dioxide output of frog tadpoles was made with ten concentrations of ether, ranging from 0.007 to 0.55 per cent by volume. The tadpoles chosen were about 20 mm. in length; at this stage, they respire through the internal gills and the skin. A single tadpole was placed in each tube containing 5 cc. of liquid, and the time required to change the solution from pH 8.0 to 7.7 was noted. Fig. 1 (Curve B) shows the effect of ether on respiration, after an exposure of 150 seconds; there was no acceleration of respiration at any concentration: on the contrary there was a decrease from 0.15 per cent ether and upward. It might be thought that this was due to the cessation of the breathing movements, by which the water is carried to the gills, or to the stopping of the muscular movements, especially of the tail. As a matter of fact, however, at these concentrations and

³ For further details see Paper I (Osterhout, W. J. V., *J. Gen. Physiol.*, 1918, i. 171).

with this length of exposure these movements remained practically normal. The measurements of the breathing movements were made by determining the length of the time necessary to count twenty movements. In general the normal mouth movements were about twenty in 11 seconds. On comparing Curves A and B, Fig. 1, it is evident that at these concentrations the decrease in CO₂ output is

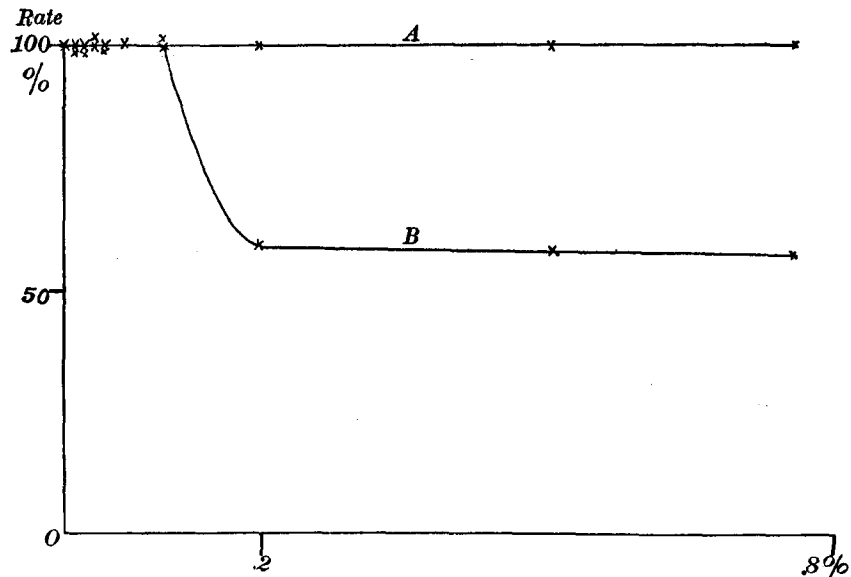


FIG. 1. Curves showing the effect of ether upon the rate of CO₂ production (Curve B) and the rate of mouth movement (Curve A) of frog tadpoles. The normal rate is taken as 100 per cent in each case. The time of exposure to ether was 150 seconds. Average of six experiments. Probable error less than 2.5 per cent of the mean. The figures expressing the per cent of ether should be corrected by multiplying by 0.73.

not due to the decrease in the water supply to the gill system. A quantitative measurement of the body movement was not possible for the reason that the movements of the tail were in many cases too rapid for accurate measurement. However, it may be stated that there was no appreciable decrease in the rate of the body movement at the concentrations in which the retardation of the CO₂ output took place. Thus, in general it may be stated that the de-

crease in CO_2 output takes place in these concentrations before the muscular movements are much affected.

Fig. 2, shows the effect of the 3.65 per cent ether (Curve A) and the effect of the 7.3 per cent ether (Curve B) on the CO_2 output. With

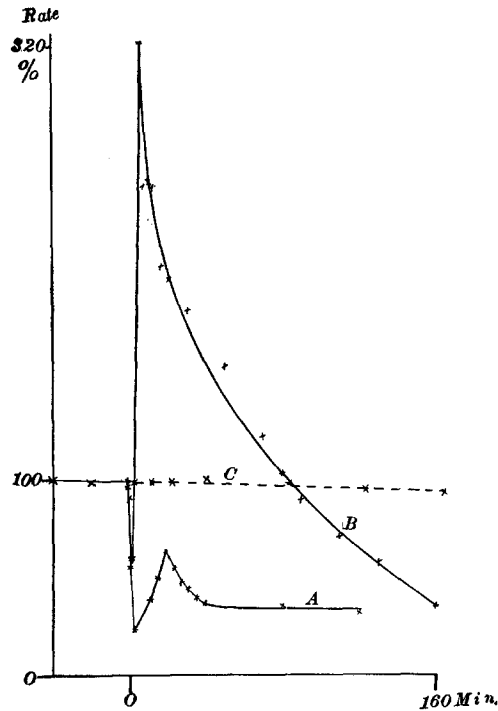


FIG. 2. Curves showing the effect of 3.65 per cent ether (Curve A), and 7.3 per cent ether (Curve B) on the CO_2 production of frog tadpoles, and control in tap water (Curve C, dotted line). The point marked 0 on the abscissa indicates the beginning of exposure to ether for Curves A and B; previous to this the material was in tap water (horizontal part of curves). The normal rate (which is taken as 100 per cent) corresponds to a change from pH 8.0 to pH 7.7 in 50 seconds for Curve A, in 43 seconds for Curve B, and in 58 seconds for Curve C. Each curve represents an average of six experiments. Probable error less than 2.5 per cent of the mean.

3.65 per cent ether a decrease of 81 per cent took place which was followed by an increase; with 7.3 per cent ether a decrease of 50 per cent occurred which was followed by an increase, the maximum of ac-

celeration being about three times the normal; in both cases decrease takes place after the increase. The reason the initial decrease is less with 7.3 per cent ether than it is with 3.65 per cent, is that the acceleration takes place more rapidly in case of the former, thus preventing the further decrease in CO₂ output.

Both 3.65 and 7.3 per cent ether caused peeling of the cuticular layers of the epidermis; this reaches a maximum in 20 minutes in 3.65 per cent ether, and in 5.5 minutes in 7.3 per cent ether. During the first 4 minutes in 3.65 per cent ether no peeling was observed. It might be supposed that this peeling causes an apparent increase in CO₂ production by allowing the CO₂ to escape more freely. However, the extent of the peeling seems to be the same in both the 3.65 and the 7.3 per cent solutions, although the acceleration in the CO₂ output in the former concentration is much greater than the latter. This difference cannot therefore be wholly due to the peeling of the skin.

Both the breathing movements and the body movements cease at these concentrations in less than 10 seconds after immersion of the tadpoles in the solutions. This might be thought to account for the drop in the rate of CO₂ output, but in view of the fact that we find a similar drop in Fig. 1, where the movement remained normal, we are obliged to conclude that the cessation of the muscular movement cannot account entirely for the falling off in the CO₂ output. This conclusion is confirmed by the experiments on frog eggs.

Aquatic Insects.

In order to avoid the disturbances due to the peeling of the epidermis, an aquatic insect was chosen. This is the common whirligig beetle, *Dineutes assimilis* Aube. It possesses a chitinous covering which prevents any peeling. 7.3 per cent ether solutions were used. Fig. 3, Curve A, shows a 94 per cent decrease in CO₂ output, after which an increase takes place followed again by a decrease.

The activity of these insects is much greater than that of tadpoles and a correspondingly greater drop in CO₂ output is to be expected as the result of anesthesia.

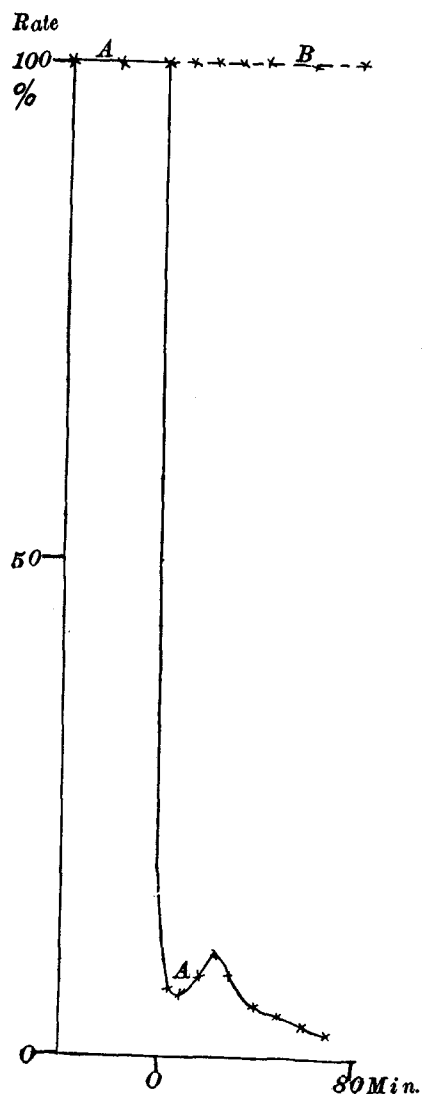


FIG. 3. Curves showing the effect of 7.3 per cent ether (Curve A) on the CO_2 production of an aquatic insect (*Dineutes*), and control in tap water (Curve B, dotted line). The normal rate is taken as 100 per cent. The point marked 0 on the abscissa indicates the beginning of exposure to ether for Curve A; previous to this, the material was in tap water (horizontal part of curves). The normal rate (which is taken as 100 per cent) corresponds to a change from pH 8.0 to pH 7.7 in 38 seconds for Curve A and in 41 seconds for Curve B. Each curve represents an average of six experiments. Probable error less than 2.5 per cent of the mean.

Frog Eggs.

In order to check the above results, frog eggs in the blastopore stage were chosen. The CO_2 output of the jelly (free from any traces of eggs) was first tested. It was found that in the time required for

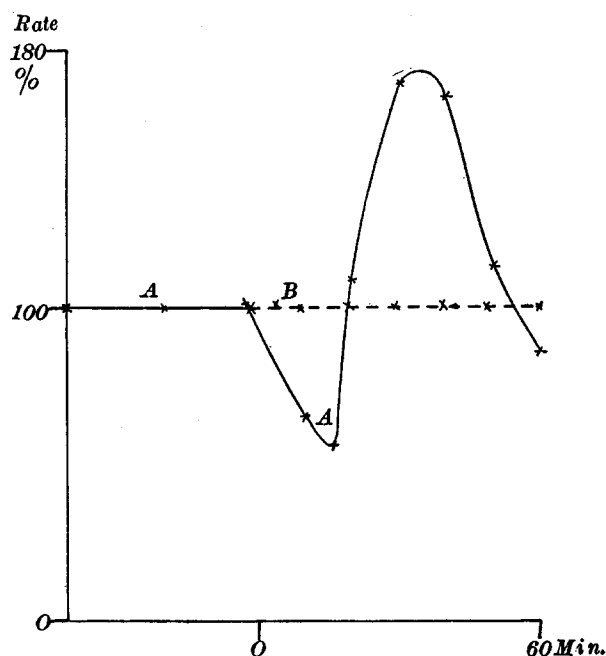


FIG. 4. Curves showing the effect of 7.3 per cent ether (Curve A) on the CO_2 production of frog eggs, and the control in tap water (Curve B, dotted line). The normal rate is taken as 100 per cent. The point marked 0 on the abscissa indicates the beginning of exposure to ether for Curve A; previous to this the material was in tap water (horizontal part of curves). The normal rate (which is taken as 100 per cent) corresponds to a change from pH 8.0 to pH 7.7 in 157 seconds for Curve A and in 142 seconds for Curve B. Each curve represents an average of six experiments. Probable error less than 2.5 per cent of the mean.

the experiments, no appreciable change in the pH value took place, thus proving that the jelly contains no organisms respiring sufficiently to interfere with the accuracy of the experiments. Fig. 4, Curve A, shows a 44 per cent retardation, followed by an increase in the rate of the CO_2 output. There was no disintegration of any portion of the

eggs which might correspond to the peeling of the epidermis in case of the tadpoles, but owing to the presence of cilia, the decrease in CO₂ output may be partly influenced by the cessation of ciliary movement as result of anesthesia.

Fundulus heteroclitus.

In order to understand the true nature of the effect of the production of CO₂ by animals it was necessary to avoid completely the disturbances due to ciliary and muscular movement, and to peeling of the skin. The embryos of the fish, *Fundulus heteroclitus*, 2 days old, were chosen for this purpose; at this stage of embryonic development there are no muscles, no cilia, and no skin, and furthermore the heart beat has not yet commenced. 1 day before the experiment was carried out, the jelly surrounding the membrane was removed, so that the embryos would not adhere to each other during the course of experimentation.

Fig. 5, Curve A, shows the effect of 0.73 per cent ether solution on the CO₂ production of the fish embryos. A gradual decrease takes place to about 46 per cent of the normal, after which the rate remains almost constant during the rest of the experiment. Fig. 5, Curve B, shows the effect of 3.65 per cent ether solution. There is a slight decrease at first, which is followed by an increase of carbon dioxide; this is succeeded by a decrease. Curve C shows the effect of 5.48 per cent ether; a tremendous increase of the carbon dioxide output takes place. If there is any decrease at the start it must be so brief as to escape observation. The rate soon reaches a maximum, after which it falls rapidly.

During the increase in 3.65 per cent and 5.48 per cent ether the embryo becomes gradually more opaque; this process begins to be observable after 20 minutes in 3.65 per cent ether and after 4 minutes in 5.48 per cent ether.

These experiments show that a decrease in the carbon dioxide output may take place as a direct effect of the ether on the protoplasmic substance, when complications due to motion are excluded. This effect may play a part in the phenomena observed in the previous experiments (on tad poles, aquatic insects, and frog eggs) where the cessation of motion is involved.

With tadpoles and *Fundulus* embryos the effect of ether at a concentration of about 0.73 per cent is reversible. The *Fundulus* embryos recover, as shown by their subsequent growth and normal development. The tadpoles also recover, as shown by their subsequent normal respiration. The increase in carbon dioxide production in 3.65

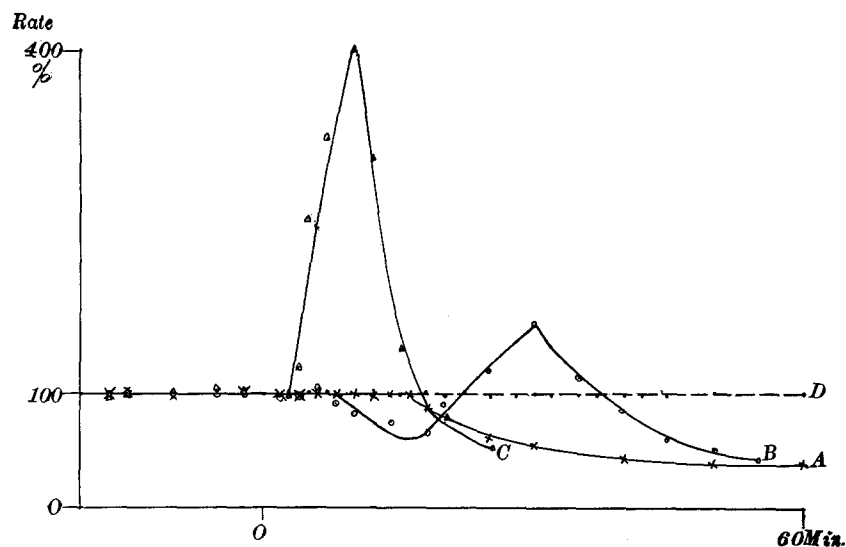


FIG. 5. Curves showing the effect of 0.73 per cent ether (Curve A), 3.65 per cent ether (Curve B), and 5.48 per cent ether (Curve C), on the CO_2 production of *Fundulus* embryos, and control in tap water (Curve D, dotted line). The point marked 0 on the abscissa indicates the beginning of exposure to ether for Curves A, B, and C; previous to this, the material was in tap water (horizontal part of curves). The normal rate (which is taken as 100 per cent) corresponds to a change from pH 8.0 to pH 7.7 in 128 seconds for Curve A, in 119 seconds for Curve B, in 176 seconds for Curve C, and in 111 seconds for Curve D. Each curve represents an average of six experiments. Probable error less than 2 per cent of the mean.

and 7.3 per cent ether is accompanied by irreversible changes leading to death. If *Fundulus* embryos were removed from the ether about 75 seconds after the increase began in 3.65 per cent ether, they failed to recover. In tadpoles the recovery is no longer possible 47 seconds after the increase has begun. In frog eggs similar results were obtained.

It is evident from these experiments that in animals the effect of ether is of two types: (1) the decrease in the output of carbon dioxide, which is reversible; (2) the increase of the output of CO₂ which is irreversible.

It was shown by Loeb and Wasteneys⁴ that 1 per cent ether produces narcosis in the 1 week old embryos of *Fundulus*, and there can be little question that this is also the case in embryos used in my experiments. The question arises whether the decrease in the output of carbon dioxide observed in 0.73 per cent ether is sufficient to produce narcosis. In order to test this, experiments were made to determine to what degree the temperature must be lowered in order to reduce carbon dioxide output of 2 day embryos to the same point as in 0.73 per cent ether (that is, to 57 per cent of the normal). It was found that lowering the temperature from 22 to 13°C. produced the desired result. But it was observed that no narcosis was produced by this drop in temperature, for when 8 day embryos were similarly treated, their movements remained practically normal. We must therefore conclude, as did Loeb and Wasteneys⁵ from an experiment on sea urchin eggs, that the decrease in respiration is wholly inadequate to produce narcosis. It is therefore evident that the action of anesthetics producing narcosis is due to some other cause than the effect upon respiration. This is in complete agreement with the striking experiments of Loeb and Wasteneys⁴ on *Fundulus* embryos, where they found that oxidation must be reduced to one-fourteenth of the normal in order to produce narcosis.

A comparison of the data obtained by the writer on the effect of ether on the carbon dioxide production of tadpoles, aquatic insects, frog eggs, and killifish eggs with those described in the preceding articles of this series, as well as with those obtained by Haas,⁶ shows that there exists a difference in plants and animals as regards the effect of ether. With animals, in weaker concentrations, such as 0.73 per cent solution of ether, a decrease in the CO₂ output takes place, and the effect is reversible, while in stronger concentrations, such as 3.65

⁴ Loeb, J., and Wasteneys, H., *Biochem. Z.*, 1913, lvi, 295.

⁵ Loeb and Wasteneys, *J. Biol. Chem.*, 1913, xiv, 517.

⁶ Haas, *Science*, 1917, xlvi, 462.

per cent and 7.3 per cent ether, an increase in the carbon dioxide production takes place, the effect being irreversible. This is different from the effects usually found in plants.

SUMMARY.

1. The experiments on frog tadpoles show that with 0.15, 0.37, and 0.55 per cent ether solutions there is a decrease in CO₂ output. The effect is reversible. With these concentrations the breathing movements and body movements remained normal during the experiment. In 3.65 and 7.3 per cent ether there is a decrease of respiration followed by an increase which in turn is followed by a decrease. The increase may reach about three times the normal rate. The increase in the CO₂ output is accompanied by the peeling of the skin. The effect is irreversible.

2. Experiments on an aquatic insect, *Dineutes assimilis* Aube, show that in 7.3 per cent ether there is a decrease followed by an increase which in turn is followed by a decrease. There is no apparent disintegration of structures in the organism accompanying the increase. The effect is irreversible.

3. The experiments on frog eggs with 7.3 per cent ether show a result similar to that found in aquatic insects.

4. Experiments on *Fundulus* embryos show that with 0.73 per cent ether there is a reversible decrease in the rate of CO₂ production. In 3.65 per cent ether there is a temporary decrease followed by an increase, after which the rate begins to fall off. In 7.3 per cent ether there is an immediate increase amounting to 307 per cent which is followed by a decrease. The increase in the 3.65 and 7.3 per cent ether is accompanied by irreversible changes leading to death. The decrease found in 0.73 per cent ether is not sufficient to cause narcosis, as is shown by experiments on which the same decrease is produced by lowering the temperature.

5. These experiments show that narcosis is not due to asphyxia. The action of anesthetics is due to some other cause than the effect on respiration.

There is a difference between the animals studied and the plants described in this series of articles, since in animals the increase in the

CO₂ output is accompanied by irreversible changes leading to death, while this is not necessarily the case in plants. The reversible (narcotic) action of ether on the animals studied was accompanied by a decrease in the carbon dioxide output; in plants this is not ordinarily the case.

These facts are of considerable interest, but their interpretation must be left to future investigation.