Effects of Carbon Monoxide on Insects

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This investigation resulted from an interest in the possibility that prolonged exposure to sublethal concentrations of carbon monoxide (CO) would kill the hypoxic cells in tumours. The distance between the capillaries and the necrotic regions would thereby be reduced and this should render the tumours more susceptible to subsequent X-irradiation in high pressure oxygen (O_2).

Our experiments have shown that exposure of mice to low levels of CO stops or retards the growth of tumours carried by the animals. We have found, moreover, that at least in some tumours the proportion of necrosed tissue is increased by CO treatment. Since it is known that CO combines with haem-containing pigments besides haemoglobin (Hb), among them the most common terminal oxidases of the respiratory chain, cytochrome oxidase (CHANCE et al. 1970) and P-450 (ESTABROOK et al. 1970), the possibility that CO might exert some direct toxic effect on the tissues became of interest. Such effects have been claimed by some authors. The visual impairment which results from exposure to CO and which persists after the level of carboxyhaemoglobin (COHb) has declined (HALPERIN et al. 1959) is an example of an effect which cannot be explained simply in terms of reduced O_2 availability to the tissues. An animal can be made independent of its Hb by administering pure O_2 at a pressure of 3 atmospheres (ATM). Sufficient O_2 to supply the tissues then becomes dissolved in the blood. HALDANE (1927) demonstrated that rats in 3 ATM of O_2 plus 1 of CO showed only slight clumsiness of movement although this was enough CO to combine with about 98.3% of their Hb. However, a further ATM of CO (3 ATM O_2 + 2 ATM CO) caused convulsions and death. This Haldane attributed to the excess CO acting as a tissue poison.

Thus there is evidence that CO has effects on mammals besides those due to its combination with Hb. Studies on organisms which lack Hb provide a possible means of demonstrating and assessing such effects. Most insects have no Hb and some work has been done on their sensitivity to CO. HALDANE (1895) reported that a cockroach was unaffected by 18 days exposure to a mixture of approximately 80% CO and 20% O_2. Later HALDANE (1927) showed that 80% CO rapidly inhibited the movement of wax moths provided that the O_2 concentration was below 14%. A CO/O_2 ratio of 5.67 reduced the O_2 consumption of Drosophila pupae by approximately 40%

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(WOLSKY 1938). Isolated silkworm hearts were inhibited in the absence of white light by CO/O_2 mixtures with ratios of 24/1 or greater (HARVEY and WILLIAMS 1958). Diapausing silkworm pupae are known to be resistant to CO, but in injured pupae the respiratory rate is elevated and respiration is then inhibited by exposure to CO/O_2 ratios of 9/1 or more (SHAPPIRIO and HARVEY 1965).

Thus effects of CO on insects have been observed but only when high CO/O_2 ratios were used. Such ratios were of necessity achieved by using lower than normal O_2 tensions or administering the gases at pressures above atmospheric. Except for the isolated observation by HALDANE (1895), the foregoing experiments were all short term. In view of these findings and because of our intention to use low levels of CO over long periods, it was decided to investigate levels of CO which would not be expected to affect insects in the short term, but which might be detrimental when administered for several weeks. O_2 tension was maintained at the normal level of 20%.

MATERIALS AND METHODS

Coccinella septempunctata (the seven-spot ladybird) and *Carausis morosus* (the stick insect) were used. The ladybirds were collected, mainly from Hampstead Heath, London, in May and June of 1971 and immature stick insects, approximately 15mm long, were obtained from a dealer. The ladybirds were fed on aphids and the stick insects on sprigs of privet. The animals were kept in glass jars containing the food material which was kept fresh by placing the stems in small pots of water.

In the case of the CO-treated groups the jars were placed in a glass tank fitted with a glass lid which was sealed with grease. Mixed gases were passed into the tank through a single inlet, and the outlet was connected to a water seal. CO (C.P.grade) was obtained from British Oxygen Company and this was mixed with medical grade O_2, and air from a pump. The gas mixture was passed through the sealed tank at 0.6 litres per min. Analysis of samples showed that maintenance of this flow for 210 min was sufficient to achieve the desired concentration in the tank. The tank then remained sealed until it was necessary to transfer the animals to fresh jars and replenish their food supply.

For the first experiment on ladybirds the CO mixture was passed through the tank for only 90 min after cleaning out and feeding. This would only have achieved a CO concentration of 70%. In subsequent experiments the tank was always gassed for 210 min after opening for cleaning. On weekdays when the tank was not opened (and in the case of the second stick insect experiment on Sundays as well) the tank was flushed with the CO mixture for 1-2 hours in order to maintain the desired levels of the gases.

Cleaning out was done on Mondays, Wednesdays and Fridays for the first ladybird experiment, on Tuesdays and Fridays for the second ladybird and first stick insect experiments, and at intervals of 2-4 days for the remaining experiment. As cleaning took approximately 30 min, the tank was flushed with air for 1 hour prior to opening and 3.5 hours was necessary for the complete flushing of the tank, the insects were free from the desired level of CO for about 5 hours each time they were cleaned out.

The control ladybirds were kept outside the tank but were otherwise similarly treated. Control stick insects were kept in a second identical tank which was cleaned out and gassed with air, flowing at approximately 1 litre/min, in a similar fashion to the CO tank.

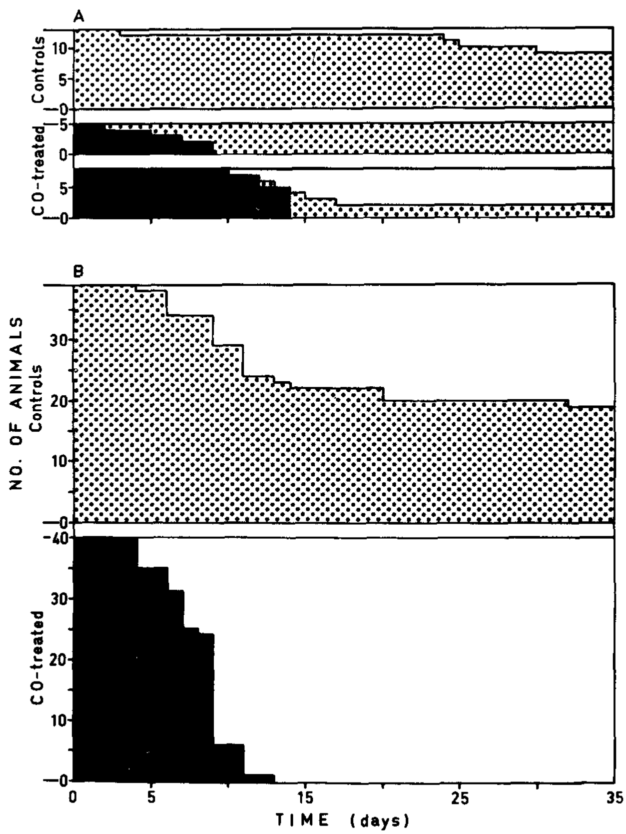

FIG.1. Survival of ladybirds in a mixture of approximately 80% CO and 20% O_2, ▇▇, compared with that of controls in air, ░░. In the first experiment, A, the concentration of CO was 70-80%. Treated ladybirds were exposed for periods of 2 to 14 days. Survivors were then kept under control conditions. In the second experiment, B, 78-80% CO was used and treated insects were left in this until they died.

RESULTS

Fig.1 shows the results of two experiments on ladybirds. The first, A, in which some animals were removed from the CO before death, shows that they all survived when exposure was less than 10 days. 10 days or more in CO hastened death, killing 75% in 18 days. Only 7.7% of the controls died during the same period. In the second experiment, B, all of the treated insects died between the 4th and 14th day, compared with 41% of the controls. It was observed that the ladybirds in CO were less active and consumed less food than controls.

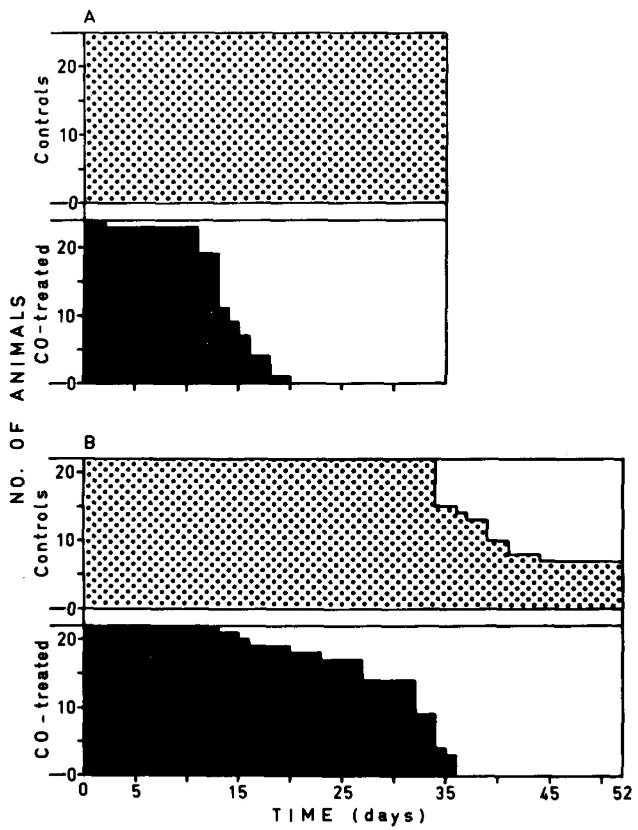

FIG.2. Survival of stick insects in different concentrations of CO, , compared with that of controls in air, . In the first experiment, A, the CO concentration was 78-80%. Stick insects which were initially about 20mm long were used. For the second experiment, B, the gas mixture was 20% CO, 5% O_2 and 75% air. In both cases the treated insects remained in CO until they died.

The results of experiments on immature stick insects are shown in Fig.2. 80% CO caused the death of these animals between the 2nd and 21st day of exposure; none of the corresponding controls died. The animals withstood 20% CO longer than the higher concentration, no deaths occurring before 14 days exposure. However, all the animals were dead by day 37, during which time only 36.4% of the controls had died. Like the ladybirds the CO-treated stick insects consumed less food than the controls.

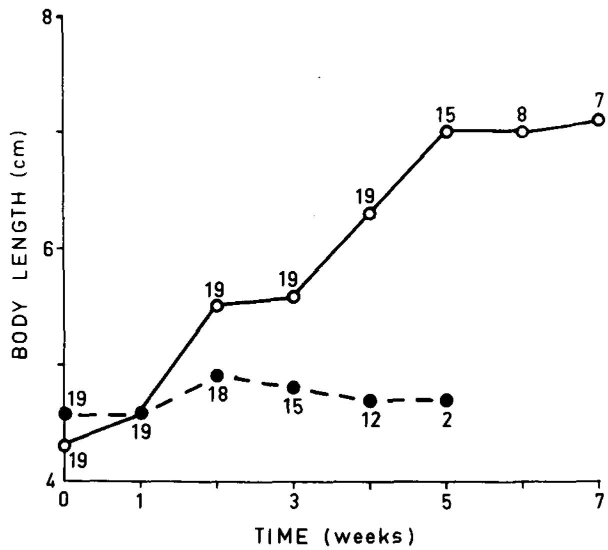

FIG.3. Growth rate of immature stick insects in air, o, or a mixture of 20% CO, 5% O_2 and 75% air, ●. During the course of the second stick insect experiment (see Fig.2B) the length of each insect (i.e. length of head, thorax and abdomen) was measured weekly. Points on the graph repesent mean length of the number of animals indicated.

Measurements of length of the stick insects, see Fig.3, showed that growth was apparently arrested by exposure to 20% CO. Mean length at the beginning of the experiment was 4.6cm for CO-treated animals and 4.3cm for controls, while after 35 days in CO it was 4.7cm for exposed insects and 7.0cm for controls. In accord with this was the fact that regrowth of lost limbs was observed among the control animals but not in those exposed to CO.

DISCUSSION

In vitro studies have shown that 50% inhibition of reactions catalysed by cytochrome P-450 is caused by a CO/O_2 ratio close to 1; reactions catalysed by cytochrome oxidase are somewhat less sensitive to CO (ESTABROOK et al. 1970). Effects on insects, however, have only been demonstrated when CO/O_2 ratios higher than 1 were used.

There is evidence that the cytochrome chain has features which more or less overcome the effects of CO-inhibition of some of its components. Two protective mechanisms, cushioning and branching, have been described (CHANCE et al. 1970). As a result of these the ultimate steady state of the respiratory chain is only slightly affected by CO and the results, using isolated mitochondria, indicate that even when most of the cytochrome oxidase is blocked by the poison the entire chain can still be oxidised at low electron flux rates. The demonstrated resistance of diapausing Cecropia silkworm pupae to CO has been attributed to imbalance in the components of the cytochrome chain, namely to a high relative proportion of cytochrome oxidase (HARVEY and WILLIAMS 1958). Since a similar imbalance exists in developing adults of this species (SHAPPIRIO and HARVEY 1965), perhaps such an argument may be extended to adult insects. Features such as these may account for the apparent resistance of insects to CO.

The results obtained here, however, indicate that insects are more affected by the presence of CO in their environment than has previously been shown. According to earlier workers 80% CO produced no effect provided that the concentration of O_2 was 14% or more (HALDANE 1895, HALDANE 1927). A CO/O_2 ratio of 5/1 did not materially affect the respiratory rate of injured silkworm pupae, although higher ratios did (SHAPPIRIO and HARVEY 1965). The present results show that in an atmosphere of 80% CO and 20% O_2 insects soon become less active and consume less food than controls. Reduced activity was noted during the first day of exposure, and loss of appetite was apparent after 2 days. As little as 20% CO in the environment (i.e. a CO/O_2 ratio of 1) caused an immediate, measureable effect on young stick insects in that their growth was arrested. After prolonged exposure to these levels of CO death occurred. The time of death was apparently related to the concentration of CO, 36 days being necessary for all the animals to be killed in 20% CO, as opposed to only 20 days in 80% CO. It may be noted that stick insects seem to be more resistant than ladybirds. Since susceptibility to CO is known to increase with the respiratory rate (ESTABROOK et al. 1970, SHAPPIRIO and HARVEY 1965), perhaps the greater resistance of stick insects might be a result of their sluggish habit and low metabolic rate. In spite of the immediate effects of CO described, death in our insects was usually delayed; one stick insect lived for 20 days in 80% CO. Further the animals nearly always recovered from sub-lethal exposure.

A possible criticism of these results is that the observed effects might have been due to impurities in the commercial CO used. In this connection it may be noted that SCHNEIDERMAN and WILLIAMS (1954) found that very pure CO caused an effect on the respiration of the Cecropia silkworm which was indistinguishable from that produced by a less pure supply.

It may be concluded that insects can survive high concentrations of CO for several weeks. However, in an environment containing 20% CO the respiratory chain of enzymes although not inactivated seems to be inhibited to such an extent as to soon interfere with the animal's wellbeing. This inhibition eventually results in death. This might in fact be due to starvation as a consequence of lack of energy. It is possible that concentations of CO lower than 20% would also affect insects.

In a mammal CO becomes concentrated in the blood because the affinity of Hb for the gas is higher than that of other substances with which it combines. Workers have, until recently, given little consideration to the question of how much CO is available to the tissues during exposure. The symptoms of poisoning have been attributed to inadequacy of the supply of O_2 to the tissues, and direct effects of CO have been regarded as more or less insignificant. Recently, however, LOUMANÄKI and COBURN (1969), experimenting on rats, demonstrated that only approximately 77% of the CO in the body was actually in the blood. This was with COHb levels of up to approximately 50% saturation. Hypoxia or higher COHb saturations caused larger percentages to go into the extravascular tissues. It is assumed that most of the CO outside the blood is bound to muscle myoglobin, since this has the second highest affinity for the gas.

Because the insects used in the experiments described here lacked myoglobin as well as Hb, the effects must surely have been due to the combination of CO with the respiratory enzymes. They were not due to defficiency in the supply of O_2 to the tissues. Thus availability of CO and O_2 in equal amounts has sufficient effect on the cytochrome chain to prevent growth and depress the activity of an animal, at least in the case of insects. We cannot attempt to assess direct action of CO on the respiratory chain in mammals until it is known how much of the poison combines with the cytochromes in these under various conditions of exposure.

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