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H.E. Goto  
*Imperial College, London*

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## THE EFFECT OF CARBON DIOXIDE ANAESTHESIA ON COLLEMBOLA

H. E. Goto

Department of Zoology, Imperial College, London, S.W.7, England

In a contribution to the *Michigan Entomologist* by Snider, Shaddy and Butcher (1969), a method of carbon dioxide anaesthesia for some soil arthropods was described as an alternative to etherization which frequently gives rise to unpredictable mortality. They stated (page 359) that "CO<sub>2</sub> has very little long-range effect on most species."

Anaesthetization by carbon dioxide has been used on various species of Collembola, particularly of the genus *Folsomia* Willem, 1902, by myself and my associates for about fifteen years. Until recently no ill effects on the cultures, as a result of this treatment, has been observed or suspected.

As part of an investigation into the cause of sudden and fairly frequent sporadic increases in the incidence in our cultures of numerous abnormal specimens, such as those described, for example, in Goto and Ögel (1961), the effect of regular daily anaesthetization with carbon dioxide was examined. Although more extensive experimentation is needed, a brief comment is given here as a warning of possible side-effects that might cause serious misinterpretation of quantitative data derived from specimens that have been subjected to carbon dioxide anaesthesia in culture.

Over a period of four months the numbers of eggs of *Folsomia candida* Willem, 1902 (Isotomidae) laid in two containers were recorded. The specimens in one of these was anaesthetized daily with carbon dioxide, the others were used as controls. The containers used were essentially as described previously (Goto, 1961) but were larger and provided with plastic screw tops. The two culture jars were freshly made up at the same time in identical ways with equal quantities of plaster/carbon mixture in each. The same amount of water was added to each container so that the plaster was almost but not quite completely saturated (complete saturation, as recommended by me in 1961, is to be avoided as it encourages the growth of fungal hyphae on the surface of the plaster and on the food). An equal number of drops of distilled water was added weekly to each container from a small pipette to replace that lost by evaporation during the daily inspection period when the jars were open. Equal quantities of stabilized wheat germ (Bemax) were placed in the containers for food. These were replaced at weekly intervals and any remaining observable traces of the old food were removed. Stabilized wheat germ is now used as an alternative to the yeast previously recommended. On a few occasions tyroglyphid mites, all belonging to the species *Tyrophagus putrescentiae* (Schränk, 1781), appeared in the cultures. The mites were killed immediately by contact with a hot needle and the bodies removed. In this way no mites were in the containers for more than about 24 hours. They did not occur more frequently in one or other of the vivaria. There is some evidence that these mites may have an effect on the Collembola in culture. The jars were maintained together at a temperature of between 19° and 23° C. and were subjected to normal day-length illumination.

Both jars were opened simultaneously each day. Carbon dioxide was passed into the treated container by means of a small-bore glass tube until all specimens were anaesthetized (the point of anaesthetization was judged by the cessation of movement—excluding occasional minor limb twitches). The tube was then removed and the specimens allowed to recover before both jars were closed. The containers were examined daily for eggs, care being taken to keep the two jars open for equal lengths of time. The eggs were removed as soon as they were found, so no estimate of viability of the treated eggs in comparison to the controls could be made. There were no observable structural or colour changes in the two groups of eggs. No deaths occurred during the four-month period of this pilot experiment.

The monthly egg totals are shown in Table 1.

Table 1. A comparison of the number of eggs produced by *Folsomia candida* Willem under controlled conditions and when subjected to daily carbon dioxide anaesthesia.

	November 15-30	December	January	February 1-19	Totals
Controls	662	1747	2206	943	5558
Treated	696	1678	1534	637	4545
Difference	+34	-69	-672	-306	-1013
% drop	(5.1)	3.9	30.5	32.4	18.2

The experiment was discontinued after the 19th of February owing to the temporary impossibility of carrying out daily counts. It is anticipated that experimentation will be renewed on a larger scale and broadened to investigate not only egg viability but also the subsequent history of the individuals reared from both the treated and the control eggs.

In this experiment the eggs were preserved in alcohol but in a parallel experiment they were allowed to hatch and the populations to build up in both containers. Fortnightly samples were taken at random from both jars and examined for morphologically abnormal specimens. The incidence of abnormality was very low in both containers. It was, in fact, slightly higher in the untreated batch, although statistically insignificantly so.

No interpretation of the above figures will be attempted at this stage but they indicate that carbon dioxide anaesthesia should be undertaken with caution until further details are available.

I am grateful to Miss Zara Springthorpe for much of the tedious counting and for maintaining the cultures.

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