

# THE EFFECT OF CARBON MONOXIDE ON THE RESPIRATION OF ARTIFICIALLY BIVOLTINIZED SILKWORM EGGS\*

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IN earlier publications of this series (Wolsky, <sup>1, 2, 3</sup>) the theory has been advanced (based on concepts of Rumström and other concerning sea-urchin eggs, *e.g.*, <sup>4, 5</sup>) that the increase in the intensity of respiration that occurs in silkworm eggs at fertilization is due to a change in the submicroscopic structure of the cytoplasm of these eggs (see also Wolsky<sup>6</sup>). This is supposed to remove some barriers between respiratory enzymes and their substrates and to make the enzymes (the amount of which does not change) more "saturated", *i.e.*, heavier engaged in their catalytic activity than before fertilization. This would explain the fact that before fertilization the respiration cannot be blocked with mixtures of oxygen and carbon monoxide (one of the specific poisons of the iron containing respiratory enzyme known as Warburg's "Atmungs-ferment" or cytochrome-oxydase) whereas it becomes susceptible to this poison at fertilization when the rate of respiration is almost doubled. The explanation which the theory offers is the following (see also Fig. 1):

(1) Before fertilization the cytochrome oxidase is in surplus and only a fraction of it is actively engaged in the catalysis of respiration. Therefore the carbon monoxide (which competes with oxygen for the enzyme so that they "partition" it among themselves according to Warburg's formula) combines with surplus only leaving enough of the enzyme free to combine with oxygen and to keep up the respiration at the level which is normal in this stage.

(2) After fertilization a far greater portion of the cytochrome-oxydase is "on duty", *i.e.*, becomes engaged in the catalysis of respiration and the reserve is greatly reduced. Therefore a similar treatment with oxygen-carbon monoxide mixtures will affect not only the reserve of the enzyme but also that part which is needed for the upkeep of the respiration. Consequently the rate of respiration will be reduced.

It is clear that this explanation supposes a close correlation between the rate of respiration and its susceptibility to carbon monoxide:

the higher the rate of respiration the stronger should be the inhibiting effect of a certain carbon monoxide-oxygen mixture. This in turn indicates that an argument could be furnished in favour of the theory if the rate of respiration could be raised artificially in silkworm eggs and the effect of a certain carbon monoxide-oxygen mixture upon this increased respiration would prove to be

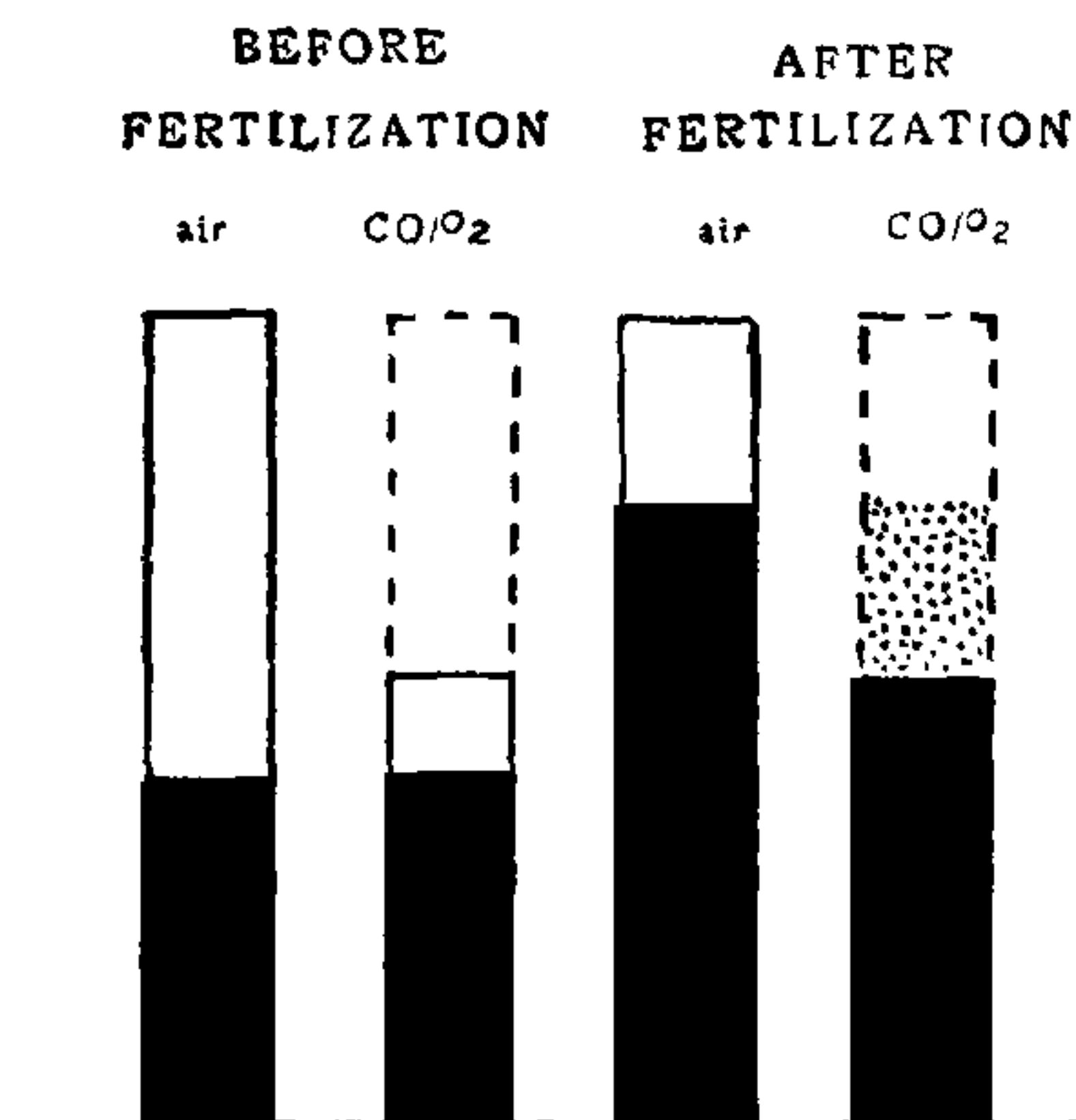


FIG. 1. Graphic representation of the theory put forward to explain the different effect of carbon monoxide on the respiration of silkworm eggs before and after fertilization. The columns represent the total amount of the cytochrome oxidase. The dark zone of the columns represents the portion which is actively engaged in the catalysis of respiration, the light zone represents the reserve. The part of the columns drawn with full lines (and jet black in the dark zone) denotes the portion of the cytochrome oxidase available for respiration, *i.e.*, not poisoned by carbon monoxide; the part drawn with broken line (and dotted in the dark zone) represents the portion poisoned by carbon monoxide. (The total amount of cytochrome oxidase given in the graph is fictitious; probably it is greater in comparison to the part engaged in respiration, *i.e.*, the light zone of the columns should be higher in comparison to the dark one, stronger than upon the normal rate. The possibility of carrying out this experiment was given when it was found that the treatment, by which eggs of univoltin races† are artificially bivoltinized, enhances the respiration of these eggs (see Wolsky.) The treatment consists in bathing the eggs in 50 per cent. hydrochloric acid for 10

\* Contributions to the knowledge of the respiratory mechanism of silkworm eggs. IV.

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† Races which have one generation a year.



minutes within 24 hours after they have been laid and afterwards washing them in running water for at least 30 minutes. Eggs of the univoltin race "Moretaina" treated in this way have been—after checking their oxygen uptake in air<sup>†</sup>—put in mixtures on carbon monoxide and oxygen and their oxygen uptake measured in these mixtures. At the same time eggs of the same raft which were not treated with hydrochloric acid were also used for similar experiments to compare the effect of the same carbon monoxide-oxygen mixtures on bivoltinized and not-bivoltinized eggs. Carbon monoxide and oxygen were mixed in the ratio of 9:1 and 8:2 respectively. The measurements were made with the method described before (Wolsky<sup>1</sup>). Typical results of the experiments are given in Table I.

TABLE I

*Oxygen uptake of not bivoltinized (control) and bivoltinized silkworm eggs in cu. mm. per hour per 500 eggs, in air and in carbon monoxide-oxygen mixtures, at 26° C. The column n gives the rate of oxygen consumption in the carbon monoxide-oxygen mixtures expressed in percentage of the consumption in air*

Number of Experiment	Not bivoltinized			Bivoltinized			CO/O <sub>2</sub> ratio
	Air	CO/O <sub>2</sub>	n	Air	CO/O <sub>2</sub>	n	
20/7 A	20.0	15.4	77	29.0	19.0	66	9:1
20/7 B	15.0	13.0	87	22.4	16.6	73	
10/8 A	22.4	16.0	71	36.0	24.4	62	
10/8 B	20.4	14.0	72	34.2	21.4	63	8:2
21/7 A	20.0	21.0	100	27.0	22.2	82	
21/7 B	20.0	20.0	100	26.0	20.4	75	
9/8 A	24.0	24.2	100	32.2	30.2	94	

These results show that the artificial bivoltinization with hydrochloric acid has increased the respiration in all cases quite considerably which confirms the earlier results. Indeed the increase is in some cases even greater than reported before. Together with this increase the susceptibility of the respiration to carbon monoxide also increases. Using a mixture of 90% CO & 10% O<sub>2</sub> the so-called residual respiration (n) expressed in percentage of the normal is about 75 per cent. for not-bivoltinized eggs but only about 66 per cent. for bivoltinized eggs. Using a mixture of 80% CO & 20% O<sub>2</sub> the difference becomes still more striking as

this mixture does not at all affect the oxygen uptake of not-bivoltinized eggs (n=100) but depresses the uptake of bivoltinized eggs (n=75 to 94). This is very nearly the same difference which we have between fertilized and unfertilized eggs. These figures definitely prove that an experimentally increased respiration becomes automatically more susceptible to CO than normal respiration and this speaks greatly in favour of the theory put forward to account for the difference in carbon monoxide-susceptibility before and after fertilization.

There is however one more point which needs some explanation. According to the abovementioned theory the limiting factor of the oxygen-consumption in carbon monoxide mixtures should be the amount of cytochrome-oxydase which is not poisoned by CO. As this must be the same both in bivoltinized and not-bivoltinized eggs (the treatment with hydrochloric acid could not have changed the amount of cytochrome oxydase in bivoltinized eggs), we should expect the rate of oxygen consumption to be lowered to exactly the same level in both kinds of eggs. But in fact this is never the case. The bivoltinized eggs although relatively stronger affected by carbon monoxide than the not-bivoltinized ones (as expressed in the lower values of n), nevertheless consume more oxygen in the CO-poisoned condition than the not-bivoltinized controls in the same condition (as the comparison of the absolute figures show). For example in Expt. 20/7B the oxygen uptake (in cu. mm per hour for 500 eggs) of not-bivoltinized eggs is lowered by carbon monoxide from 15.0 to 13.0 (n=87). In the case of the bivoltinized eggs the oxygen consumption should have been depressed by the same treatment to the same level, i.e., from 22.4 to 13.0. But instead it is lowered only to 16.6 which figure is even higher than the oxygen uptake of not bivoltinized eggs in air, although it still means a relatively stronger depression of oxygen uptake than observed in not-bivoltinized eggs (n=73).

The deviation of the data from the theoretical expectation can have two possible explanations:

(1) In the bivoltinized eggs a certain degree of cytolysis may have set in. This is quite possible in view of the drastic treatment. According to earlier results (Wolsky<sup>2</sup>), cytolysis in silkworm eggs is connected with an increase of oxygen uptake, but

<sup>†</sup> Earlier experiments have shown that the oxygen uptake in a nitrogen-oxygen mixture of 9:1 is the same as in air (cf. Wolsky<sup>1</sup>).



this increase is not susceptible to carbon monoxide (autoxydation of necrotic substances?). Thus it is possible that a cytolytic effect of the bivoltinizing treatment is responsible for the increased amount of carbon monoxide resistance respiration.

(2) It is possible that the same factor which is supposed to be responsible for the increase of the respiration catalysed by cytochrome-oxidase (i.e., change in the sub-microscopic structure of the egg-cytoplasm) may have also increased some respiratory processes which are catalysed not by cytochrome-oxidase but by some carbon monoxide-resistant respiratory enzymes, e.g., the yellow enzyme of Warburg. This explanation is in fact an additional argument in favour of our theory as it supposes that the changes in respiration are due to non-specific causes which affect more than one single enzyme system. Such an unspecific factor would be in the first line a change in the sub-microscopic structure of the egg-cytoplasm.

#### SUMMARY

Artificially enhanced respiration of freshly laid fertilized silkworm eggs (treated with hydrochloric acid for bivoltinization) is more susceptible to carbon monoxide than the normal respiration. The significance of

this phenomenon and the details of the results are discussed.

1. Wolsky, A., "Beiträge zur Kenntnis des. Atmungsmechanismus der Seidenspinnereier. I. Vergleich der Atmung verschiedener Entwicklungsstadien, mit besonderer Berücksichtigung der Kohlenmonoxydwirkung". *Rivista di Biologia*, 1941, **31**, 209. 2. —, "Beiträge... etc. II. Über den Sauerstoffverbrauch geschädigter unbefruchteter Eier," *Magyar Biol. Kut. Munk.* (Publications of the Hungarian Biological Research Institute), 1939, **11**, 375. 3. —, "Beiträge... etc. III. Die Wirkung der Behandlung mit Selzsäure auf die Atmung." *Magyar Biol. Kut. Munk.* (Publications of the Hungarian Biological Research Institute), 1942, **14**, 445. 4. Runnstrom, J., "Atmungsmechanismus und Entwicklungserregung bei dem Seeigelkeim," *Protoplasma* (Berlin), 1930, **10**, 106. 5. Brock, N., Druckrey, H., and Herken, H., "Der Stoffwechsel des geschädigten Gewebes" III. (Zugleich Beitrag zur Frage der Entwicklungserregung am Seeigeler.) *Nauyn-Schmiedeberg's Archiv*, 1938, **188**, 451. 6. Wolsky, A., "The physiology of development in Insects," *Proc. Nat. Inst. Sci. India* 1949, **15**, 67. 7. —, "The effect of carbon monoxide on the oxygen consumption of *Drosophila melanogaster* pupæ." *Journ. Exper. Biology*, 1938, **15**, 225.

## SUGARCANE (*SACCHARUM OFFICINARUM*) TOP SILAGE AS FEED FOR CATTLE

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THE problem of adequate supply of nutritious fodder for livestock in India during the dry periods of the year is very baffling. After the monsoon, the cattle have to depend, for greater part of the year, for roughage supply, principally on coarse and bulky fodders like straws which are nutritionally deficient. One of the solutions of the problem seems to be in devising methods for the conservation of surplus green fodder in times of abundance, especially during monsoon months. Hay making and ensilage, if widely undertaken, may solve the problem to a great extent by providing alternative fodder of satisfactory nutritive value.

In previous articles (Kehar and Sahai, 1949; Paul and Rangaswamy, 1947) observations on the nutritive value of sugarcane tops have been reported. As already stated

the estimated yield of sugarcane tops in India roughly works out to be 16 million tons annually. At present most of it goes to waste. As this huge production of cane tops is available only during the cane crushing season extending over three to four months, it is not possible to utilise the entire produce as such for feeding cattle. The additional quantity of cane tops after meeting the requirements of the livestock in those parts will be available for conservation by a suitable method. Hence measures must be adopted to conserve the surplus material, to be utilised during periods of scarcity. The observations reported in this article relate to the conservation of sugarcane tops by ensilage and the nutritive value of the ensiled product.

Green sugarcane tops chaffed into 5" to 6" bits were filled, in pits of 8' × 5' × 4'