

BIOCHEMICAL REFLECTIONS ON A NON-CHEMICAL CONTROL
METHOD. THE EFFECT OF CONTROLLED ATMOSPHERE ON
THE BIOCHEMICAL PROCESSES IN STORED PRODUCTS INSECTS.

by

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The toxicity of carbon dioxide is species dependent and various animals can adapt to the gas either by selection or by other mechanisms. A very short and complete summary of data given in Table 1 supports this statement.

About 10 years ago we became interested in the subject of the effect of biochemical effects of CO_2 on stored products insects due to the use of the gas for the storage of grain (Jay and Pearman, 1971).

A competitive inhibitor of succinic acid oxidase, a major component of the Krebs cycle (Kasbekar 1966), was believed at one time to be connected with the knockdown effects of carbon dioxide on the corn earthworm (Edwards 1968). Later work of the same author, however, discounted this belief (Edwards and Batten, 1973) so that further investigation was called for.

Since CO_2 apparently prevented the insect from using oxygen (Navarro 1975), we undertook research in that direction. 0-24-h-old pupae of *Ephestia cautella* (Wlk.) were used since this moth is a major storage pest and the pupae are more resistant to CO_2 than the adults or the egg.

Our first investigation was on the pyruvate and lactate levels. As is shown in Table 2 (Friedlander and Navarro 1979), in hypoxia both lactate and pyruvate increase so that their ratio does not change significantly from normal. Hypercarbia, however, reduces their ratio to about 25% of the control value, thus indicating a drastic change in the redox state; this points to a lesion of the electron transport chain, possibly due to a change in the permeability of the mitochondrial membrane (Wyatt 1963; Jacey, Messier and Schaefer 1972). Indeed, a determination of the adenosine nucleotides (Table 3) revealed a drastic decrease of ATP and the energy charge (Atkinson 1968), a parameter we shall return to.

Since no increase in citrate, malate (Table 4) and glutamate, but rather decreases of these metabolites were found, other lesions were considered. Our attention was called to the NADPH-producing pathways, especially since CO_2 is either a substrate or a product in these (Fig. 1). Thus we found that the reversible decarboxylation of 6-phosphogluconic acid was 62% inhibited in an atmosphere containing more than 10% CO_2 . The "malic" enzyme was completely inhibited at concentrations around 40% CO_2 , thus stopping the anaplerotic production of NADPH, i.e. of reducing power. (Friedlander, Navarro, and Silhacek 1983).

In Stryer's (1981) words, "the basic strategy of metabolism is to

form ATP, reducing power and building blocks for biosynthesis." Since we have shown CO_2 to cause a fall of ATP and NADPH levels, we can now explain a number of phenomena that resulted from exposure of the pupae to the gas.

The Energy Charge has been defined by Atkinson (1968) as in Fig. 2. This parameter is shown to affect the velocity (V in the figure) of ATP-requiring and ATP-producing processes as shown in Fig. 3. This leads to the understanding of the following:

1. Exposure of 0-24-h-old pupae to 90% CO_2 for the period of 24 h is not lethal but delays eclosion 3-4 days. Development is connected to protein synthesis which depends on the availability of ATP, i.e. a high energy charge. Lowering the energy charge should slow down protein synthesis and development, which has been shown by us to be the case (Friedlander and Donahaye, unpublished).
2. Glutathione, a tripeptide involved in a variety of biosynthetic and detoxification reactions, especially in the detoxification of methyl bromide and similar fumigants (Starratt and Bond 1981). It remains more or less at a constant level for the first 72 h of the pupal life. However, if the pupae were exposed to 90% CO_2 for 24 h, the level fell to 35% and continued to fall for 48 h after the organism's return to normal atmosphere. At that point the glutathione returned to its control value (Fig. 4). (This incidentally coincided with the resumption of development). This can be rationalized if we assume that the biosynthesis of glutathione, which maintains the steady state of the tripeptide, is inhibited. Now there are a number of steps in the biosynthesis and/or the recycling of glutathione where ATP is required: (i) The formation of the peptide bonds requires ATP; (ii) Glutamic acid, which is required for the synthesis, is split off from polypeptides, or from 5-alkyl-glutathione (in the course of the formation of mercapturic acids), in the form of the cyclic pyroglutamic acid which requires ATP for the opening of the pyrrolidine ring in order to re-form glutamic acid (Meister and Tate 1976); (iii) The lowering of the energy charge will cause some of the glutamic acid present to be oxidised to ketoglutarate by allosteric regulation (Stryer 1981), thus further reducing available substrate for the biosynthesis.

Taking these steps, jointly or separately, into account one would expect a reduction of glutathione in the system, which we have found to occur indeed.

3. The reacylation of choline in the course of neural transmission requires ATP (Fig. 5). Indeed, Navon and Agrest (1968) found reduced levels of ATP in the central nervous system of rats with chronic hypercapnia. This might explain some of the neurotoxic effects of CO_2 .
4. Reduction of produced NADPH will also affect the mixed-function-oxidase mediated detoxification of insecticide (Fig. 6).

The data presented here indicate multiple sites of action for CO₂, thus making the build-up of tolerance more difficult. In addition they can be helpful in further investigation of biochemical mechanisms in the insect, especially in estimating the value of CO₂ as a possible synergist for fumigants (Ali Niazee and Lindgren 1969).

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Table 1. Effects of CO₂ on various species

Species	CO ₂ concentration	Effect	Ref.
<u>Mammals:</u>			
Man	10%	Immediate loss of consciousness. Death after 5 minutes	(1)
Mouse	64%	Death after 5 minutes.	(2)
Mouse	50%	Survives less than four hours.	(2)
Rabbit, domestic	50%	Dead in 23 minutes	(2)
wild	50%	Dead in 71 minutes	
<u>Insects:</u>			
E. cautella (pupae)	21%	At high Rh, can survive more than 6 days exposure.	(3)
Rhagius inquisitor L.	16%	Survives for more than 4 months without apparent ill effects.	(4)

- References: (1) Merck Index, ninth edition.
 (2) Hayward and Lisson (1978).
 (3) Navarro and Calderon (1973).
 (4) Pasche and Zachariassen (1973).

Table 2. Pyruvate, Lactate and Pyruvate/Lactate ratios in pupal tissues exposed to modified atmospheres

N ₂	O ₂	CO ₂	Pyr.	Lac	Pyr/Lact
80	20	-	0.270	1.080	0.25
90	10	-	0.257	1.687	0.15
99	1	-	0.621	2.705	0.23
-	20	80	0.219	5.045	0.04
-	10	90	0.239	4.305	0.06

Table 3. Adenine nucleotide levels ($\mu\text{mole/g}$ insect \pm S.E.) in *E. cautella* pupae exposed to various gas compositions

Gas composition (%)			ATP	ADP	AMP	Energy charge
N ₂	CO ₂	O ₂				
80	-	20	0.942 (± 0.016)	0.271 (± 0.019)	0.078 (± 0.009)	0.84 (± 0.005)
90	-	10	1.092 (± 0.038)	0.297 (± 0.022)	0.063 (± 0.006)	0.85 (± 0.008)
99	-	1	0.642 (± 0.029)	0.329 (± 0.017)	0.107 (± 0.005)	0.75 (± 0.007)
-	80	20	0.458 (± 0.019)	0.391 (± 0.005)	0.159 (± 0.006)	0.65 (± 0.014)
-	90	10	0.407 (± 0.013)	0.343 (± 0.032)	0.131 (± 0.007)	0.66 (± 0.015)

N.B. Values given are the means of four determinations.

S.E. = Standard Error

(Friedlander and Navarro 1979)

Table 4. Citrate and malate levels ($\mu\text{mole/g}$ insect \pm S.E.) in the tissue of *E. cautella* pupae exposed to various gas compositions

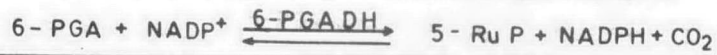
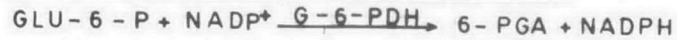
Gas composition (%)			Citrate	Malate
N ₂	O ₂	CO ₂		
Treated pupae				
80	20	-	3.148 (±0.065)	1.055 (±0.045)
90	10	-	2.974 (±0.140)	1.372 (±0.074)
99	1	-	0.871 (±0.091)	1.755 (±0.115)
-	20	80	0.660 (±0.051)	0.275 (±0.082)
-	10	90	0.464 (±0.029)	0.508 (±0.048)
Untreated pupae				
			3.601 (±0.173)	1.468 (±0.070)

N.B. Values given are the means of four determinations.

S.E. = Standard Error
(Friedlander and Navarro 1974)

PRODUCTION OF REDUCING POWER (NADPH)

1. PENTOSE PHOSPHATE SHUNT (OXIDATIVE PART)



2. "MALIC" ENZYME

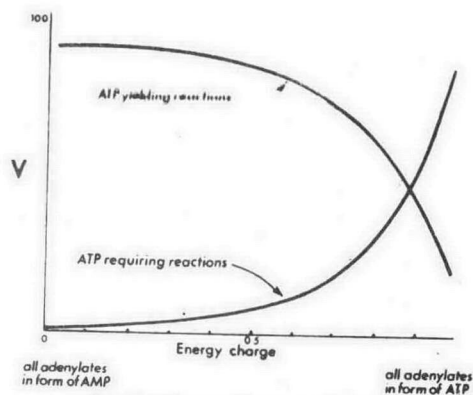


Fig. 1

$$E = \frac{[\text{ATP}] + \frac{1}{2}[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$$

E=ENERGY CHARGE

Fig. 2



The effect of energy charge upon ATP-yielding and ATP-requiring enzyme reactions. Energy charge, defined as the concentration ratio $(ATP + \frac{1}{2} ADP) / (AMP + ADP + ATP)$, is a measure of the degree to which AMP is "charged" with high-energy phosphate bonds. This ratio is usually maintained between about 0.7 and 0.9. In this range, small changes in energy charge cause large changes in reaction velocities.

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Fig. 3

RECUPERATION OF GLUTATHIONE LEVELS AFTER 24h EXPOSURE TO 90% CO₂

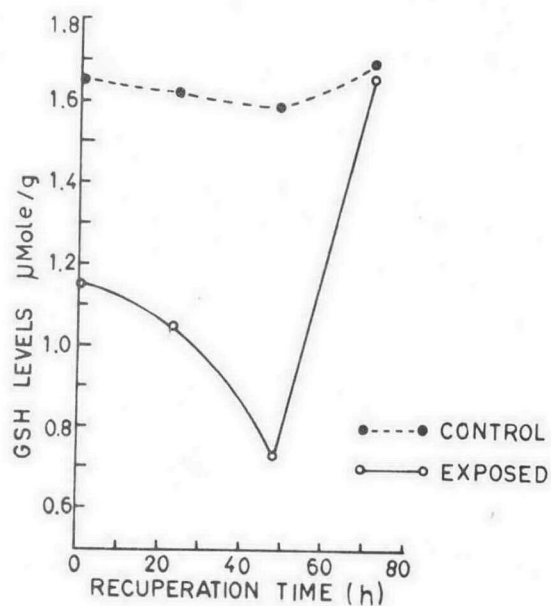


Fig. 4

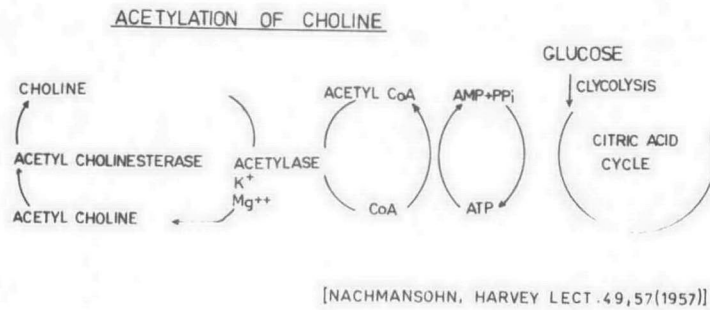
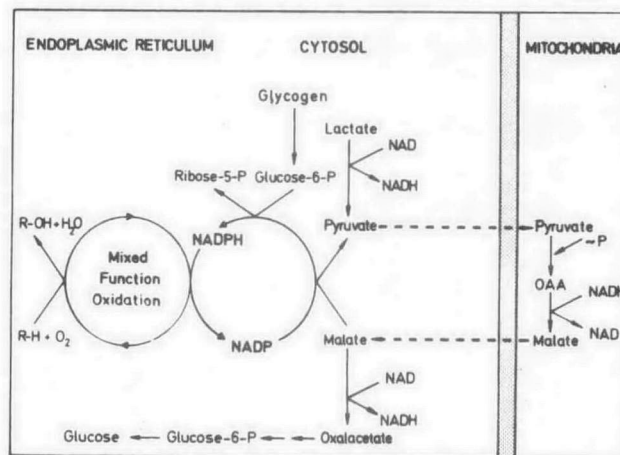


Fig. 5

MIXED FUNCTION OXIDATION AND INTERMEDIARY METABOLISM Thurman et al. 1974



Scheme depicting the site for extramitochondrial NADPH generation and its interaction with the pathway of gluconeogenesis and mixed function oxidation. R-H: substrates for mixed function oxidation; R-OH product; ~P: energy-rich phosphate representing ATP required for carboxylation of pyruvate.

Fig. 6

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