

EFFECTS OF CARBON DIOXIDE ON GLUCOSE INCORPORATION IN FLATWORMS

by James S. McDaniel, Austin J. MacInnis, and Clark P. Read

ABSTRACT

The rates of glucose-¹⁴C incorporation into polysaccharide under CO₂ and CO₂-limited atmospheres were determined in flatworms of three Orders of Turbellaria, three of Cestoda, and one of Trematoda. Carbon dioxide inhibited glucose incorporation in *Neochildia fusca* (Acoela), a free-living species, and in the ectosymbiotes *Bdelloura candida* (Tricladida) and *Stylochus zebra* (Polycladida). The presence of carbon dioxide increased glucose incorporation in the parasitic tapeworms *Lacistorhynchus tenuis* (Trypanorhyncha), *Calliobothrium verticillatum* and *Orygmatobothrium dohrnii* (Tetraphyllidea), and *Tetraphyllus erostris* (Cyclophyllidea) and the trematode *Cryptocotyle lingua* (Digenea). The results suggest that the rates of glycogen synthesis in some flatworms vary with the level of available carbon dioxide in the environment.

INTRODUCTION

It has been reported that carbon dioxide has marked effects on carbohydrate metabolism in parasitic flatworms (Fairbairn et al., 1961; Kilejian, 1963; Read, 1967), and carbon dioxide fixation has been reported in both free-living and parasitic flatworms (Prescott and Campbell, 1965; Flickinger, 1959; Hammen and Lum, 1962). Kilejian (1963) reported that carbon dioxide was without significant effect on glucose uptake or net glycogenesis in the acanthocephalan *Moniliformis dubius*, although lack of carbon dioxide sharply depresses these functions in some tapeworms (Fairbairn et al., 1961; Read, 1967), and slightly depresses them in others (Von Brand and Stites, 1970). It thus seemed desirable to ascertain the effects of carbon dioxide on carbohydrate metabolism in a representative group of flatworms. Since some flatworms do not synthesize measurable glycogen in short-term experiments,

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the incorporation of uniformly labeled glucose- ^{14}C into worm glycogen was examined.

MATERIALS AND METHODS

Free-living and symbiotic flatworms were collected in the vicinity of Woods Hole, Massachusetts. The acel turbellarian *Neochildia fusca* was collected from mud samples. *Bdelloura candida*, a triclad symbiote of the horseshoe crab *Limulus polyphemus*, was collected from the gill books of naturally infected hosts. *Stylochus zebra*, a polyclad ectosymbiote of the hermit crab (*Pagurus pollicaris*), was collected from crab-inhabited *Busycon* shells. The trypanorhynch tapeworm *Lacistorhynchus tenuis* and the tetraphyllidean tapeworm *Calliobothrium verticillatum* were collected from naturally infected dogfish (*Mustelus canis*). The tetraphyllidean *Orygmatobothrium dohrnii* was collected from the sand shark (*Carcharias taurus*). The cyclophyllidean tapeworm *Tetrabothrius erostrus* and the digenean trematode *Cryptocotyle lingua* were collected from the herring gull (*Larus argentatus*).

The flatworms were incubated in the following solutions to which sufficient sodium bicarbonate was added to maintain a pH of 7.4 when the animals were gassed with carbon dioxide: *Neochildia*, *Bdelloura*, and *Stylochus* in artificial seawater (Cavanaugh, 1964) containing 6 mM tris-maleate buffer (pH 7.4); *Lacistorhynchus*, *Calliobothrium*, and *Orygmatobothrium* in elasmobranch saline containing 25 mM tris-maleate buffer at pH 7.4 (Read et al., 1960); and *Tetrabothrius* and *Cryptocotyle* in Ringer's solution containing 25 mM tris-maleate buffer at pH 7.4. For carbon dioxide-limited incubations, bicarbonate was omitted and 30% KOH was added to a center well in each flask. All incubation media consisted of 20 ml containing 40 micromoles of glucose uniformly labeled with 1 microcurie of ^{14}C . Incubations were carried out at 20°C, except for those involving the avian parasites, *Tetrabothrius* and *Cryptocotyle*, which were performed at 38°C.

At the end of incubations, worms were quickly rinsed three times in 150 ml of the appropriate salt solution, blotted on hard filter paper, placed in a measured volume of 80% ethanol, and extracted for at least 24 hours. The ethanol-extracted worms were dried at 95°C for 12 hours, weighed, and dissolved in warm 1% NaOH. Aliquots were used for estimation of protein by the method of Lowry et al. (1951). To the remaining material KOH was added, to a concentration of 20%. After warming to 75°C, ethanol was added, to a final concentration of 50%. The precipitated alkali-stable polysaccharide was redissolved in water and an aliquot removed for carbohydrate estimation. The remaining polysaccharide was reprecipitated in 50% ethanol five times and aliquots removed for counting of radioactivity and for carbohydrate estimation. Carbohydrate was determined by the method of Dubois et al. (1956).

RESULTS AND DISCUSSION

The incorporation of glucose carbon in the species of flatworms studied is presented in table 1, along with data on the total ethanol-precipitable carbohydrate found in these forms. Carbon dioxide had effects on glucose incorporation in all cases. In all three turbellarians, more glucose was incorporated in the absence than in the presence of carbon dioxide, while the four cestodes and one trematode showed higher rates of glucose incorporation in the presence of carbon dioxide. Among these species, carbon dioxide fixation is known to occur in *Bdelloura* and *Stylochus* (Hammen and Lum, 1962). McDaniel and Dixon (1967) showed carbon dioxide to have an inhibitory effect on glucose incorporation in redia of *C. lingua*, whereas the opposite effect occurs in the adult.

The present data suggest that the significance of carbon dioxide fixation in the metabolism of these forms is not identical. The disparity may be related to significant differences in the terminal electron transport mechanisms of the parasites. The cestodes and the trematode examined in these experiments live in the digestive tract of vertebrates, probably under very low oxygen and high carbon dioxide tensions. The turbellarians, on the other hand, may live under relatively high oxygen tensions with modest carbon dioxide tensions. The free-living acoel, *N. fusca*, is found on the surface of mud bottoms. When the oxygen tension begins to drop, however, as in a collected bucket of mud, they quickly move away from the mud surface. If the bucket of mud is gently aerated, they remain on the mud surface.

TABLE I
EFFECT OF CARBON DIOXIDE ON GLUCOSE-¹⁴C
INCORPORATION IN FLATWORMS

SPECIES	ALKALI-STABLE POLYSACCHARIDE ^a	INCORPORATION ^b		
		No CO ₂	Plus CO ₂	Percentage of change
<i>Neochildia fusca</i>	791.1 (3) ^c	4.4	1.8	- 59
<i>Bdelloura candida</i>	234.0 (2)	8.5	6.7	- 21
<i>Stylochus zebra</i>	270.2 (4)	6.2	4.5	- 27
<i>Lacistorhynchus tenuis</i>	930.2 (1)	173.7	219.7	+ 27
<i>Calliobothrium verticillatum</i>	1069.6 (4)	245.2	323.4	+ 32
<i>Orygmatobothrium dohrnii</i>	514.3 (3)	17.8	43.5	+144
<i>Tetrabothrius erostrus</i>	5173.4 (3)	42.7	50.8	+ 19
<i>Cryptocotyle lingua</i>	762.6 (1)	21.6	36.4	+ 69

a. Expressed as nMoles glucose/mg. protein

b. Radioactivity as nMoles glucose/ μ Mole glycogen/2 hrs.

c. Number of replicates in parentheses.

The findings in the present experiments suggest the difficulty of interpreting a general phenomenon, such as carbon dioxide fixation, with regard to its significance in the overall metabolism or ecology of a specific organism, or organisms. In addition they reveal possible regulatory phenomena worthy of future, more detailed investigation.

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