The Effects of Cold Acclimation upon the Oxygen Consumption of Two Species of Free-Living Nematodes[1]

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Abstract: Two species of free-living nematodes, *Panagrellus redivivus* and *Turbatrix aceti*, were cultured axenically at control (20 C) and cold (10 C) temperatures. Oxygen consumption of worms from each population was measured manometrically on days 2 through 8 after exposure to these temperatures. In both species, the slope of the oxygen consumption curve for the controls was greater than that of the worms exposed to the cold on day 2. The slope of the curve of the cold-exposed worms gradually increased until day 7. At this time, the slope of the oxygen consumption curve from the cold-exposed worms exceeded or equaled that of the controls. This is taken as an indication of the onset of the cold-acclimated state in both species of worms by day 7.

For many years, a number of parameters have been used to denote the onset of the acclimation of a poikilothermal organism to various environmental conditions, particularly to temperature (1, 9, 10). Among the criteria utilized have been such rate functions as heart beat (20), ciliary gill movements (19) and metabolic rate; e.g., rate of an enzymatic reaction (7, 10, 11).

In most species of poikilothermic animals, exposure to a cold but nonlethal temperature will result in an initial decrease in metabolic rate according to Van't Hoff's principle (1). However, after a certain period of time during which the organism has been exposed to cold, it often responds by acclimating to the new temperature. This is frequently manifested as an increased metabolic rate (10).

In the present study, two species of free-living nematodes were acclimated to cold. Metabolic rates of acclimated and control worms were compared, and rates of acclimation were determined in both species.

MATERIALS AND METHODS

Culture methods: Axenic cultures of *Panagrellus redivivus* and *Turbatrix aceti* were maintained continuously in 50-ml cylindrical separatory funnels lined with glass wool and stoppered with cotton balls wrapped in cheese cloth (6). At weekly intervals, the worms were subcultured aseptically by draining the worms and the old medium from each funnel and adding them to another funnel containing fresh medium.

The worms were exposed to a temperature of 20 C until use in this experiment. They were subjected to a photoperiod of 12 hr of light and 12 hr of dark throughout the study.

The basic medium consisted of an autoclaved aqueous solution of 4% soya peptone and 3% yeast extract (2) to which heated liver extract (17) was added to make up 10% of the volume of the medium (2). This medium was used for *P. redivivus*. For *T. aceti*, glacial acetic acid was added to make up 3% of the basic medium (14).

The cultures were checked periodically for signs of bacterial or fungal contamination by using agar plates of brain-heart infusion or thioglycollate medium incubated at room temperature for 1 week. Contamination in a culture was readily apparent, however, because it caused a subsequent discoloration of the medium.

Cold acclimation: Each population of worms harvested from the funnels received approximately 5 ml of fresh medium. The medium of each species was divided evenly between two sterile 125-ml Erlenmeyer flasks, which then were covered with aluminum foil. One flask was returned to the control (20 C) temperature, whereas the second was introduced to the cold (10 C).

On days 2-8 following the initiation of the treatment, two 0.5-ml aliquots of each worm-medium mixture were removed, each of which was pipetted into the main compartment of a Warburg reaction vessel for manometric studies. From time to time, aliquots were extracted and counted to verify accuracy of the

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procedure. There were no significant changes in number of worms over the time period studied when compared on a statistical basis.

Each reaction flask also contained 1.5 ml of 1 M phosphate buffer (pH 8) in the main compartment, 1 ml of 10% glucose in the sidearm, and a piece of fluted filter paper soaked with 0.2 ml of 20% KOH in the center well (22). After equilibration of the reaction vessel in the Warburg apparatus for 15 min, the substrate was tipped into the main compartment, and oxygen consumption was measured for 105 min.

Originally, the rate of oxygen uptake was measured at 37 C. However, *T. aceti* failed to consume detectable quantities of oxygen at this temperature. Therefore, all subsequent oxygen consumption measurements were made at 30 C for both species.

The experiment subsequently was repeated using worms obtained from a second subculture.

RESULTS

The control of *P. redivivus* consumed considerably greater amounts of oxygen than did the worms exposed to the cold (Fig. 1-A). The difference between oxygen consumption of the two groups decreased gradually until very little difference appeared by day 5. On day 6, although the difference was very slight between the two groups, the cold-exposed worms consumed more oxygen per unit time than did the controls. This trend was accentuated by day 7. No further difference was detected by day 8.

A lag in oxygen consumption following the addition of exogenous glucose was evident in some of the curves (Fig. 1-A). In the control worms, this lag either was not apparent (days 2-4) or persisted for only 5-10 min after glucose supplementation (days 5-7). On the other hand, relatively long lag periods of 45 min (day 2), approximately 30 min (day 3-4) and 10 min (day 5) were revealed in the curves obtained from the cold-exposed worms. No lag period appeared after 6 or 7 days of cold exposure.

The results obtained from *T. aceti* are not as clear-cut as are the data from *P. redivivus*. At days 2 and 3 (Fig. 1-B) little difference was observed between the oxygen consumption of the control and that of the cold-exposed worms. In both cold-acclimated and control groups, oxygen uptake, although greater after the addition of exogenous glucose, was very low. The control worms consumed slightly more oxygen per unit time by day 4 and much more by days 5 and 6; oxygen consumption by control worms decreased again by day 7. Enzyme accumulation apparently peaks after day 4, hence the notable increase in oxygen consumption on day 5.

Whereas little oxygen was consumed by cold-exposed *T. aceti* (Fig. 1-B) on days 2-4, uptake increased slightly on day 5 and considerably more on days 6 and 7. By day 7, the rate of oxygen consumption by worms exposed to the cold was appreciably greater than that of the controls. Again, no further difference was detected on day 8.

Unlike the linear curves obtained for *P. redivivus* (Fig. 1-A) which are typical of oxygen consumption curves, those obtained by plotting oxygen uptake of *T. aceti* versus time often showed a distinct plateau in the first few days after the initiation of the experiment (control: day 2; cold: days 2-5). Subsequently, however, the more conventional, linear pattern gradually replaced the horizontal nature of the lines, the slopes becoming greater and greater with time (with the exception of that of the day 7 control, where the slope decreased with respect to that of day 6).

DISCUSSION

Typically, the amount of oxygen consumption increased in the control worms of both species shortly after the initiation of the experiment (*P. redivivus*: days 2-4; *T. aceti*: days 2-5). Gradually, the slopes obtained from the worms subjected to the cold increased with time until they clearly exceeded those of the control worms by day 7. This is the classic picture of cold acclimation as presented by many authors, in which some rate function (usually rate of oxygen uptake) increases at a given temperature in a cold-acclimated animal with respect to that of a control. Precht (9) described five patterns of temperature acclimation, ranging from supra-optimal compensation (type 1) to inverse compensation (type 5). The most common pattern is one in which the normal rate is greater in cold- than in warm-acclimated animals (9). This pattern was observed in this experiment for both *P. redivivus* and *T. aceti*. Most frequently, partial compensation (type 3) is observed (9, 10), but often the rate function approaches perfect compensation (type 2) (10). Bullock (1) pointed out that this type of compensation to temperature by various rate functions has the

over-all effect of conferring relative homeostasis to poikilotherms that can acclimate to changing temperatures over a nonlethal temperature range.

In the oxygen consumption-time plots for both *P. redivivus* and *T. aceti*, a change occurred in the relative positions of the curves from the control and the cold-exposed worms. In both species, the curve obtained from the cold-exposed worms increased with time until day 7, when the rate of oxygen consumption was appreciably greater than that of the controls. Since no further difference was noted between the two curves on day 8, we assumed

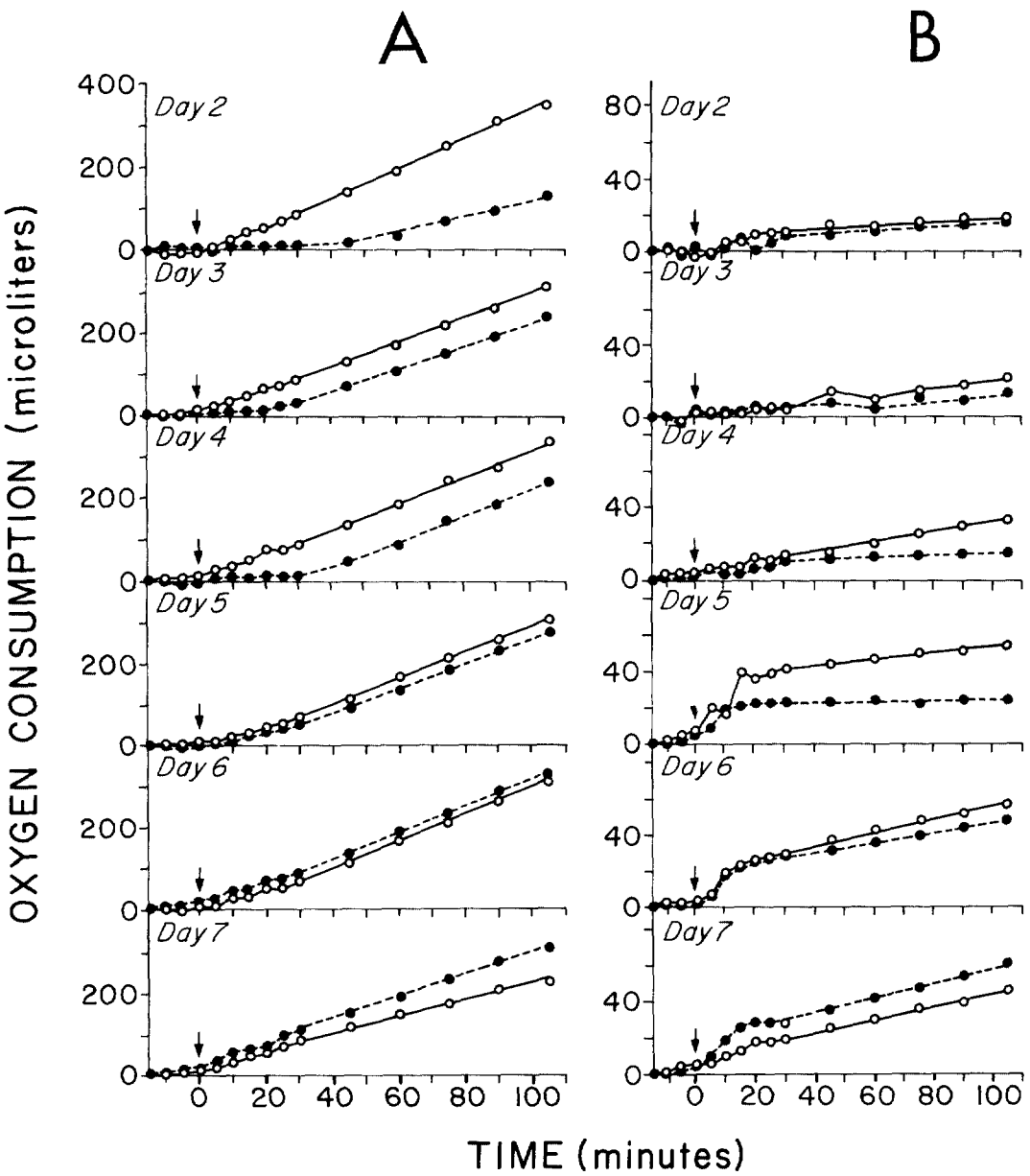

FIG. 1. **A.** Rate of oxygen consumption by *Panagrellus redivivus* from day 2 through day 7 following exposure to experimental conditions. Control: o————o. Cold-exposed: •————•. Arrows indicate addition of glucose. **B.** Rate of oxygen consumption by *Turbatrix aceti* from day 2 through day 7 following exposure to experimental conditions. Control: o————o. Cold-exposed: •————•. Arrows indicate addition of glucose.

that both species had acclimated to 10 C in 7 days.

The length of time required for the onset of the cold-acclimated state is a species-specific character, which may be subject to seasonal variation and/or physiological state of the organism (3, 8). The blowfly, *Calliphora erythrocephala*, acclimates to 10, 20 or 30 C in 8 days (21); the crayfish, *Orconectes virilis*, to 5 C in 1-3 weeks (8); *Ascaris suum* to 25 or 30 C in 11 days (23); and the American cockroach, *Periplaneta americana*, within 3 weeks (3); all of these data are based on measurements of oxygen uptake. In view of these data, a 7-day period for acclimation of *P. redivivus* and *T. aceti* to 10 C is not surprising. It is reasonable to assume, however, that a different time period might have been found had the worms been maintained at a temperature other than the one used (20 C) prior to exposure to the cold.

The lag period observed in some of the curves could be explained by a requirement for enzyme synthesis induced by adding the exogenous glucose. In the worms subjected to the cold for only 2 days, the rate of metabolic processes had been decreased as a direct consequence of the cold; therefore, the rate of protein synthesis would also be reduced considerably. However, as the metabolic rate accelerates with the onset of cold acclimation, the rate of enzyme synthesis also increases. It is possible that no lag period is evident at day 7 due to the presence of sufficient enzymes in the system at this time to accommodate the exogenous glucose.

A very different picture arises in the case of *T. aceti*. Here, no oxygen consumption was observed when measured at 37 C and only a small amount of oxygen was taken up on day 2 at 30 C. According to these data, the assumption made by Ells (4) and Read (*personal communication*) that *T. aceti* is unable to metabolize glucose is substantiated under the conditions which these authors utilized. This lower capacity (or absence of it) to metabolize glucose might also explain the low consumption of oxygen by *T. aceti* in comparison to *P. redivivus*. It is probable that the enzymes capable of breaking down glucose are in low concentration in *T. aceti* and, therefore, the organism cannot respond readily to glucose administration. However, as the medium is depleted of nutrients with time, the rate of oxygen consumption rises appreciably in both control and cold worms. Concomitantly, the plateau originally observed disappears and is replaced by a linear curve that indicates gradually increasing oxygen consumption after day 2 and 5 in the control and cold-exposed worms, respectively. All of these factors, the low level of oxygen consumption forming a plateau which is replaced by a linear curve of increasing slope, could indicate a change from one predominant metabolic pathway (A), in which glucose is not used directly, to another (B), in which glucose is freely metabolized. Thus, the low plateaus of oxygen uptake may represent the presence of a small amount of enzyme(s) capable of metabolizing glucose but rather insignificant with respect to the enzyme systems currently being utilized. This initially minor pathway would gain importance as the substrate(s) necessary for pathway A disappears from the medium.

The apparent failure of *T. aceti* to metabolize exogenous glucose in relatively fresh medium is of interest in itself and is paradigmatic of the atypical nature of the metabolism of many species of free-living nematodes. For example, Ells and Read (5) found evidence for an incomplete tricarboxylic acid (TCA) cycle in *T. aceti*. The free-living nematode *Caenorhabditis briggsae* does not seem to utilize carbohydrates completely, thereby suggesting either the total absence or a limited functioning of the TCA cycle (18). These two species also appear to have an electron transport system that is different from that of most animals (12, 18). Rothstein and his co-workers have demonstrated the enzymes isocitrate lyase and malate synthetase in *C. briggsae* (15) and in *P. redivivus, T. aceti* and *Rhabditis anomala* (see 18), thus suggesting the presence of the glyoxylate cycle in these nematodes. The synthesis of certain "essential" amino acids (valine, lysine, threonine, tyrosine, leucine, isoleucine and histidine) has also been shown to occur in *C. briggsae* (16).

It is possible that the failure of *T. aceti* to utilize exogenous glucose reflects a dependence upon an alternate carbon source such as acetate or other short-chain fatty acids. In this respect, it has been suggested that short-chain fatty acids rather than carbohydrates are indeed the major energy-yielding substrates in this species (12). Moreover, incubation of *T. aceti* with ^{14}C-acetate in whole medium results in the formation of radioactive glycerol (13). When these worms are incubated with labeled acetate in water, however, the predominant product is glucose rather than glycerol. This dichotomy in

metabolic products formed resulting from a difference in the composition of the medium, may substantiate the observation made in the present paper that glucose utilization increases as the medium is depleted of nutrients presumably necessary for the maintenance of type A metabolism.

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