A method is described for the production of large numbers of C. briggsae free from culture medium. Various aspects of the respiratory metabolism of these worms have been examined using a variety of techniques.

The respiration of the worms increased rapidly as the amount of oxygen in the gaseous phase of the incubation vessels increased, until a level of 5% O₂ was attained. After this no increase was observed. Cyanide in high concentrations and carbon monoxide inhibited respiration. The effect due to carbon monoxide was reduced in daylight. These substances also inhibited the development and reproduction of the worms, but the effect due to carbon monoxide was reversible.

Preparations of worms subjected to sonic disruption utilised radioactive succinate, converting it to fumaric, malic, lactic and aspartic acids and to alanine.

Spectrophotometric studies of washed homogenates of C. briggsae suggested the presence of pigments similar to a cytochrome b present in parasitic helminths, cytochrome c and traces of cytochrome a. Flavoprotein was detected in the washings.

The implications of these results are discussed.

Previous work on Caenorhabditis briggsae (Nicholas & Jantunen 1964; 1966) has shown that oxygen is an essential requirement and that a worm maintained under anaerobic conditions may be permanently impaired. It was also demonstrated that growth was retarded if the partial pressure of oxygen fell below 30 mm Hg, but very low levels (below about 1.2 mm Hg) were reached before growth was completely inhibited. As far as we are aware, no studies have been published on the terminal respiratory system in C. briggsae. Rothstein (1963) cites unpublished evidence for the presence of a cytochrome of the b type in this nematode, and also makes reference to the effects of high concentrations (10⁻³M) of potassium cyanide (Rothstein & Tomlinson 1962).

Cytochromes of the b type have been demonstrated in Acanthocephala (Bryant & Nicholas, 1966), in Cestoda (Cheah & Bryant, 1966) and in Nematoda (Chance & Parsons, 1963). A detailed study carried out on Moniezia expansa (Cestoda) by Cheah & Bryant (1966) demonstrated that the b-type cytochrome was involved in an electron transport pathway in which fumarate, not oxygen, acted as terminal acceptor in the oxidation of NADH. Studies by Kmetec & Bueding (1961), Kikuchi, Ramirez & Guzman-Barron (1959), and Kikuchi & Ban (1961) strongly suggest the operation of a similar mechanism in Ascaris. Most of these parasites are extremely resistant to cyanide poisoning.
The possibility, therefore, that *C. briggsae* possessed a b-type cytochrome similar to that of parasitic helminths (together with its absolute requirement for oxygen, and its ready availability in axenic culture) made it an ideal organism for study. The present paper deals with experiments which give some indication of the nature of the respiratory mechanisms of this nematode.

**MATERIALS AND METHODS**

[1:4-14C₂] succinate was obtained from the Radio-chemical Centre, Amersham, Bucks, England. Reduced nicotinamide adenine dinucleotide (NADH), adenosine di- and tri-phosphates (ADP, ATP), cytochrome c and antimycin A were obtained from the Sigma Chemical Corp., U.S.A. Analytical grade reagents and glass distilled water were used throughout.

Stock cultures of *Caenorhabditis briggsae* were maintained and handled by the techniques described by Dougherty et al. (1959). The medium used for culture was the basic medium EM 2 (Nicholas, 1963), supplemented with 10% chick embryo extract (CEE) prepared as described by Nicholas et al. (1959).

To produce large numbers of worms free from culture material these basic methods were modified as follows. A small volume of culture (0.3 ml from a Wassermann tube) containing worms in all stages of development was mixed with 100 ml of complete, fresh, sterile culture medium. This was distributed in 5 ml aliquots amongst 20 square-sided screw-capped culture vessels (volume 160 ml, dimensions 4 × 4 × 10 cm) which were laid flat in an incubator at 20° C. This was essential as it was found that, with a large air volume relative to the volume of the culture medium, and with the medium present as a very shallow layer, much higher populations, fewer dead worms, and virtually complete digestion of the chick embryo material were obtained. After 12 or 13 days the cultures were pooled, and the worms collected by gentle centrifugation in a bench centrifuge. At this stage the coarsest particles of CEE in the lower layers were separated from the worms. Less coarse particles were removed by washing twice in 10 ml distilled water, and standing for about two hours in the second wash. The worms were centrifuged, collected and resuspended in 0.5 M sucrose for a few minutes. A further gentle centrifugation resulted in the sedimentation of the CEE, while the worms remained floating. They were again collected and subjected to the same flotation process, after which the suspension medium was diluted to 0.1 M and divided into the appropriate number of aliquots, which were stored at 20° C overnight.

For the manometry experiments, each sample was centrifuged and the volume of suspension adjusted to 3.0 ml with sucrose (0.1 M).

**Effect of different oxygen tensions**

Samples prepared as described in the preceding paragraph were transferred to Warburg flasks containing 0.6 ml KOH (10%) in the centre well and their oxygen utilisation recorded for 90 minutes in air at 20° C, shaking at 80 cycles/