# *In vitro* Metabolism of DL-Alanine-2-C-14 and Glycine-2-C-14 by *Trichinella spiralis* Larvae.* (22288)

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(Introduced by Donald D. Van Slyke.)
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Recent work from this laboratory(1) has shown *in vivo* incorporation of carbon-14 by immature and encysted *Trichinella spiralis* larvae from mice fed $C^{14}$-labeled amino acids. When glycine-2-C-14 and dl-alanine-2-C-14-labeled diets were fed to mice with Trichinella infections of 14 and 56 days' duration, the larvae incorporated $C^{14}$ indicating that they were exchanging metabolites with the host. Encysted larvae in 180-day infected mice also incorporated significant levels of $C^{14}$ from the tissues of mice fed glycine-1-C-14 and dl-alanine-1-C-14 diets, showing an active metabolism by well encapsulated muscle larvae.

In the present study, Trichinella larvae were isolated from host tissues by pepsin digest and cultured in media containing dl-alanine-2-C-14 or glycine-2C-14. This report concerns *in vitro* incorporation of $C^{14}$ by muscle larvae and metabolism of $C^{14}$ into larval protein from $C^{14}$-labeled amino acids.

*Materials and methods.* Trichinella larvae were obtained from stock mice with infections of 4 months' duration. The muscle larvae were freed from their cysts by pepsin digest, separated from undigested materials and washed to remove soluble digestion products, as previously described(1). The Krebs-Ringer bicarbonate solution was prepared using the procedure outlined by Cohen(2). Normal mouse serum and Krebs-Ringer solution were used to prepare 4 types of media as follows: a) a 50-50 mixture of mouse serum and Krebs-Ringer solution containing glycine-2-C-14; b) a 50-50 mouse serum and Krebs-Ringer solution containing dl-alanine-2-C-14; c) Krebs-Ringer solution with glycine-2-C-14; and d) Krebs-Ringer solution with dl-alanine-2-C-14. A 2 ml sample from each medium was saved to determine $C^{14}$ content prior to incubation with Trichinella larvae. Beckman pH meter determinations of the media gave pH values of 7.68 for the serum Krebs-Ringer media and pH 7.61 for the Krebs-Ringer media. The values for total $C^{14}$ activity and free amino acid concentration in the media are shown in Tables I and II. Aseptic technic was used to prepare 4 series of 50 ml culture flasks containing 10 ml of the $C^{14}$-labeled media. Approximately 100,000 larvae were introduced into each culture flask by pipette suspension in 0.5 ml of 0.85% saline. Penicillin G Potassium (Lederle) 1,000 units and 1,000 $\mu$g of Dihydrostreptomycin Sulfate (Lilly) in 0.2 ml volume were added to each flask to control bacterial growth. Thus, each flask contained a total volume of 10.7 ml of the $C^{14}$-labeled media. The serum-Krebs-Ringer culture flasks were incubated at 37.5°C for periods of 3, 6, 12, 24, and 48 hours. The Krebs-Ringer culture flasks were incubated at the same time for periods of 6, 24, and 48 hours. During incubation the flasks were shaken at 50 cycles per minute. At the end of each incubation period, the larvae were separated from the culture media by centrifugation at 1,000 rpm. The larvae were washed 6 times in 50 ml distilled water and the supernatant drawn off by aspiration. Live Trichinella larvae settle out rapidly, while the dead larvae, if present, float and are lost in the supernatant. Microscopic examination of the washed larvae just prior to lyophilization revealed only motile larvae. The following procedure was devised to obtain total soluble and insoluble larval protein for $C^{14}$ analysis. Lyophilized larvae (7 mg) samples were weighed in specially modified 12 ml centrifuge tubes. The tubes were fitted with removable base tips. The larval material in the base tip was suspended in 0.15 ml of 10% sodium tungstate

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TABLE I. Influence of Incubation Time upon Glycine-2-C-14 Metabolism by Trichinella Larvae.

| Composition of glycine-2-C-14 media | Serum and Krebs-Ringer solution | | | | | Krebs-Ringer solution | | |
|---|---|---|---|---|---|---|---|---|
| Total activity/flask (cpm) | $3.808 \times 10^6$ | | | | | $3.1576 \times 10^6$ | | |
| Total wt of free glycine/flask ($\mu$g) | (86.7† + 95‡) = 181.7 | | | | | 71.9† | | |
| Hr of incubation | 3 | 6 | 12 | 24 | 48 | 6 | 24 | 48 |
| Total wt in mg lyophilized larvae | 40.14 | 34.11 | 37.22 | 31.24 | 26.09 | 35.01 | 36.22 | 27.80 |
| Total wt in mg larval carbon | 15.99 | 13.92 | 14.42 | 12.80 | 10.71 | 15.44 | 14.21 | 11.16 |
| cpm/mg carbon lyophilized larvae | 1,938 | 3,284 | 8,145 | 27,895 | 47,846 | 1,481 | 30,697 | 58,158 |
| Total activity (cpm) lyophilized larvae* | 30,998 | 45,713 | 117,451 | 357,056 | 512,430 | 22,867 | 436,204 | 649,043 |

* These values represent total $C^{14}$ content in larvae recovered from each culture flask.
† Added glycine is calculated from No. of counts per flask and specific activity of glycine-2-C-14 (19.965 $\mu$c/mg).
‡ Estimated from content of free glycine of normal mouse plasma(5).

TABLE II. Effect of Incubation Time upon Dl-Alanine-2-C-14 Metabolism by Trichinella Larvae.

| Composition of dl-alanine-2-C-14 media | Serum and Krebs-Ringer solution | | | | | Krebs-Ringer solution | | |
|---|---|---|---|---|---|---|---|---|
| Total activity/flask (cpm) | $1.143 \times 10^6$ | | | | | $0.776 \times 10^6$ | | |
| Total wt of free alanine/flask ($\mu$g) | (127† + 285‡) = 412 | | | | | 87† | | |
| Hr of incubation | 3 | 6 | 12 | 24 | 48 | 6 | 24 | 48 |
| Total wt in mg lyophilized larvae | 36.26 | 36.07 | 36.78 | 29.74 | 40.46 | 33.17 | 27.89 | 27.41 |
| Total wt in mg larval carbon | 15.85 | 15.38 | 15.85 | 12.77 | 16.92 | 14.79 | 12.26 | 11.42 |
| cpm/mg carbon lyophilized larvae | 190 | 525 | 734 | 901 | 1,602 | 214 | 2,055 | 2,300 |
| Total activity (cpm) lyophilized larvae* | 3,011 | 8,075 | 11,633 | 11,505 | 27,106 | 3,165 | 25,194 | 26,266 |

* These values represent total $C^{14}$ content in larvae recovered from each culture flask.
† Added dl-alanine is calculated from No. of counts per flask and specific activity of dl-alanine-2-C-14 (4.08 $\mu$c/mg).
‡ Estimated from content of free l-alanine of normal mouse plasma(5).

and precipitated with 1 ml of ⅔ *N* $H_2SO_4$. The precipitated larval protein was centrifuged into the tip of the tube at 2,200 rpm. The material in the tip was resuspended in 0.15 ml sodium tungstate, precipitated again with acid and centrifuged. The process was repeated once more and the samples dried in a high vacuum oven at 30°C. The centrifuge tips containing the larval protein† were removed and placed in Van Slyke carbon combustion tubes for analysis. All lyophilized larvae and larval protein samples were analyzed for $C^{14}$ using the methods of Van Slyke *et al.*(3,4).

*Results.* The first experiment was designed to test *in vitro* the ability of *T. spiralis* larvae to metabolize glycine-2-C-14 when cultured in a serum-Krebs-Ringer medium and a chemically defined Krebs-Ringer medium. The total activity (cpm) of $C^{14}$ per flask and period of incubation for each culture are shown in Table I. It is evident from the data that the larvae actively incorporate glycine-2-C-14 throughout the 48-hour period of incubation in both types of media. The larvae cultured in the Krebs-Ringer medium incorporated more $C^{14}$ than the larvae cultured in the serum-Krebs-Ringer medium. This is of interest in that the total activity (cpm) per flask was higher in the serum-Krebs-Ringer medium than in the Krebs-Ringer medium. This may indicate an acceleration of glycine-2-C-14 metabolism when it is the only amino acid present in the medium and also suggests that the larvae utilized unknown metabolites present in the serum. The amounts of serum free amino acid (glycine) and $C^{14}$-labeled glycine per flask are shown in Table I. When isotope dilution of the $C^{14}$-labeled amino acid by the free amino acids of serum is considered, the actual uptake of glycine by the larvae in the serum media may be greater than indicated by the $C^{14}$ values. The incorporation of $C^{14}$ by larvae in the Krebs-Ringer culture during the first 6 hours was less than half the amount found in the larvae incubated for 6 hours in the serum-Krebs-Ringer medium. Apparently, the larvae in the chemically defined Krebs-Ringer medium required a period of adaptation to the medium and in the following 6- to 24-hour period of incubation incorporated glycine-2-C-14 at a more rapid rate.

In the second *in vitro* experiment, the metabolism of dl-alanine-2-C-14 by Trichinella larvae was studied. The total activity (cpm) of $C^{14}$ per flask and period of incubation for each culture are shown in Table II. The larvae were taken from the same pool of isolated muscle larvae used in the first experiment. The cultures were incubated at the same time as the cultures in the first experiment. The data in Table II indicate that the larvae were able to metabolize dl-alanine-2-C-14 although not as extensively as found with glycine-2-C-14. During the first 6 hours of incubation, the larvae incorporated more $C^{14}$ from the dl-alanine-2-C-14 serum-Krebs-Ringer medium than from the Krebs-Ringer medium. Although the $C^{14}$ activity (cpm) of the serum-Krebs-Ringer medium was higher than the Krebs-Ringer medium, the 6- and 24-hour period of incubation demonstrated a more rapid rate of $C^{14}$ uptake by the larvae in the Krebs-Ringer culture, than in the serum-Krebs-Ringer culture. If, in the 48-hour serum cultures one assumes that the same proportion of the l-alanine, added with the free amino acids of the serum, is incorporated as of the l-alanine in the added C-14-dl-alanine, the total amount of alanine incorporated is estimated to be about 5 times the amount incorporated in the 48-hour Krebs-Ringer cultures.

Table III shows the per cent incorporation of $C^{14}$ into total larval protein by Trichinella larvae cultured 48 hours in the 4 types of media. A higher concentration of $C^{14}$ was found in the total protein obtained from larvae cultured in either glycine-2-C-14 or alanine-2-C-14-labeled Krebs-Ringer media as compared with the $C^{14}$-labeled serum-Krebs-Ringer media. Although the total $C^{14}$ level of the serum-Krebs-Ringer media was higher than the Krebs-Ringer media (Tables I and III), the larvae incorporated less $C^{14}$ into protein when they were incubated in the

† The material precipitated by tungstic acid will be referred to as "protein"; it presumably includes lipids, purines and primidines of nucleic acids.

TABLE III. Influence of Composition of Media upon Protein Metabolism of 48 Hour Incubated Trichinella Larvae.

| Amino acid | Glycine-2-C-14 | | Dl-alanine-2-C-14 | |
|---|---|---|---|---|
| Media | Serum and Krebs-Ringer solution | Krebs-Ringer solution | Serum and Krebs-Ringer solution | Krebs-Ringer solution |
| cpm/mg of lyophilized larvae | 19,634 | 23,362 | 670 | 959 |
| cpm in protein from 1 mg lyophilized larvae | 13,660 | 19,712 | 212 | 545 |
| % $C^{14}$ incorporated into larval protein | 69.6 | 84.4 | 31.7 | 56.8 |

serum media, indicating that they utilized metabolites present in normal mouse serum.

The $C^{14}$ activity from glycine-2-C-14 was incorporated into larval protein material in higher quantity than alanine-2-C-14 regardless of the media used. Glycine, in addition to its incorporation into protein peptides as glycine, may be converted into other amino acids which are eventually incorporated into protein. Furthermore, there is the possibility of incorporation of glycine into purines and pyrimidines of the nucleo-proteins. The higher values of $C^{14}$ activity from alanine-2-C-14 in nonprotein larval material suggests that the larvae are able to convert alanine into nonprotein components, such a glycogen, glucose-1-$PO_4$ and others *via* the pyruvate pathway.

*Summary.* 1. *Trichinella spiralis* larvae incorporated $C^{14}$ when cultured *in vitro* in a serum-Krebs-Ringer medium or Krebs-Ringer medium containing either dl-alanine-2-C-14 or glycine-2-C-14. 2. Carbon-14 analysis of the larvae revealed a progressive uptake of the $C^{14}$-labeled amino acids in both types of media through 48 hours of incubation. 3. The larvae incorporated more $C^{14}$ activity (cpm) from glycine-2-C-14-labeled media than from dl-alanine-2-C-14-labeled media. 4. Of the total $C^{14}$ incorporated by larvae cultured 48 hours in glycine-2-C-14-labeled media about 70 to 84% was precipitable by tungstic acid, and was presumably chiefly in the proteins. Larvae cultured in the dl-alanine-2-C-14-labeled media incorporated about 32-57% of their total $C^{14}$ content into material precipitable by tungstic acid.

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## Effect of Intravenous Administration of Lactate on Blood Pyruvate Level in Man. (22289)

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The role of circulating lactate in body metabolism is not entirely clear. On the one hand, the concept of the Cori cycle implies that most or all circulating lactate becomes glycogen in the liver; on the other hand, there is ample evidence that lactate can serve as a source of energy in some organs(1). Knowledge of whether the blood pyruvate