Utilization of ^{14}C-labeled *E. coli* by the Rat Cecum and After Force-feeding[1] (36353)

M. G. YANG, W. G. BERGEN,[2] A. E. SCULTHORPE, AND O. MICKELSEN

Department of Food Science and Human Nutrition and Department of Animal Husbandry, Michigan State University, East Lansing, Michigan 48823

In the cecum of rats, almost 5% of the dietary energy was converted to volatile fatty acids (VFA) (1). These VFA disappeared from the cecum indicating that they were being absorbed by the animal. Support for this came from the finding that a large portion of the VFA containing radioactive carbon atoms appeared as respiratory CO_2 when the VFA were placed directly in the cecum. Undoubtedly, the major portion of the cecal VFA came from fermentation activities of the cecal bacteria since secretion of one of the VFA, acetic, into the cecum or anywhere into the gastrointestinal tract is insignificant.[3] The quantity of VFA in the cecum was, however, increased by the addition of cellulose to the diets of rats (2). This latter finding suggests that there might have been an increase in the number or activity or both of the microorganisms in the cecum. Whether the microflora in the cecum are an important source of nutrients for the host animal is not known. However, when the animal practices coprophagy, the microflora in the feces contribute nutrients since digestion of the flora takes place in the upper sections of the gastrointestinal tract. The primary aim of this investigation was to determine whether a common bacterial cell such as *E. coli* can be utilized by the host when they are placed in the ceca of rats. Secondarily, it was our aim to determine the extent of digestion of *E. coli* when force-fed to rats.

Methods. Male Sprague-Dawley rats were fed a nutritionally adequate diet made up primarily of corn-soy mixture used routinely in our laboratory.[4] The diet and water were available at all times until the introduction of *E. coli* into the cecum which was performed at 9:00 AM. The rats weighed on an average 486 g (Table I) and were about 3.5 months old. Prior to the experiment, they were maintained in a room with temperature controlled near 27° and light from 8:00 AM to 8:00 PM and darkness from 8:00 PM to 8:00 AM.

Escherichia coli, strain K12 was cultured in a mineral salt medium containing glucose-U-^{14}C (3, 4). The radioactive cells resulting from this culture were washed 4 times and the pellet of cells obtained from the last centrifugation was suspended in saline. The radioactivity of this final suspension of cells was 731,752 dpm per 0.1 g. Structural integrity of the cells was examined under oil immersion using 1000× magnification. A suitable aliquot of the ^{14}C-labeled cells in suspension was disrupted by repeated (6 times) freezing and thawing and homogenization in a Potter Elvehjem homogenizer. The cell homogenate was treated with 10% (final concentration) trichloroacetic acid (TCA). The TCA soluble and precipitable portions were

[1] Michigan Agricultural Experiment Station Journal No. 5332.

[2] Department of Animal Husbandry.

[3] S. C. H. Chen and M. G. Yang, unpublished data.

[4] Percentage composition of the grain diet was: ground corn, 60.7; soybean meal (50% protein), 28.0; alfalfa meal (17% protein), 2.0; fish meal (65% protein), 2.5; dried whey (67% lactose), 2.5; limestone (38% Ca), 1.6; dicalcium phosphate (18.5%P, 22 to 25% Ca), 1.75; iodized salt, 0.5. Supplementary minerals and vitamins and amino acids were added to provide per kg of diet: (in mg) Mn, 121; Fe, 95; Cu, 7; Zn, 4; I_2, 4; Co, 2; choline chloride, 400; Ca pantothenate, 6; riboflavin, 3; niacin, 33; menadione, 2; DL-methionine, 500; (in μg) vitamin B_{12}, 7; (in IU) retinyl palmitate, 8010; vitamin D_2, 750; alpha-tocopherol 5.

TABLE I. Body Weights and Radioactivities of *E. coli* Injected into the Ceca of Rats together with the Radioactivity in their Expired CO_2, Cecal and Large Intestinal Contents and Carcasses.

Rat no.	Body wt (g)	Activity of *E. coli* placed in cecum (dpm)	Total activity in expired CO_2 in 4 hr	Activity remaining in cecum + large intestine	Activity in carcass
			(% of dose placed in cecum)		
1	497	3,775,840	3.9	75.1	29.3
2	489	3,820,477	5.8	73.0	24.1
3	466	4,127,813	5.3	81.1	28.7
4	488	3,714,373	4.9	85.5	23.3
5	494	3,780,231	4.2	87.7	25.5
6	480	3,780,963	5.4	27.9	52.1
Average	486	3,833,283	4.9	71.7	30.5
SD ±	11	± 14,855	±0.7	±22.2	±10.8

separated by centrifugation and radioactivity determined.

In the first experiment a known quantity of the radioactive *E. coli* suspension was injected directly into the exposed ceca of rats. The ceca were exposed by means of a 2 cm midventral line incision of the ether-anesthetized animal. After introducing the *E. coli* into the cecum the incision was closed with autoclips[5] and the rat placed in a respiratory chamber previously described (5). The hyamine hydroxide trap arrangement for the respiratory CO_2 was similar to the one used in other trials (1); however, the traps in the present studies were changed every 20 min for 4 hr. At the end of the collection period, the rat was killed by overetherization and the cecal and large intestinal digesta removed quantitatively. This and the carcass were kept frozen until all samples for the experiments were collected for radioactivity determinations. The same procedures were repeated until six rats were completed.

In the second experiment another six comparable rats (Table II) maintained in the same manner as in the first experiment were each gavaged at 3:00 PM with a known quantity of the radioactive *E. coli* suspension (0.4550 to 0.5430 g). The feces and urine of the rats were collected separately for 24 hr. During this time the rats had access to the grain diet and water. At the end of the collection period, the rats were killed by overetherization and the entire gastrointestinal contents removed and together with the carcasses, urine and feces saved for radioactive analyses.

Radioactivities of all samples were determined by means of a channel ratio technique employing a liquid scintillation detector counter.[6] The solvent for the samples was dioxane containing 10% naphthalene, 1% PPO,[7] 0.025% POPOP and 4% Cab-O-Sil to hold the samples in a fine suspension. All carcasses were first autoclaved for approximately 5 hr and homogenized in 250 ml of distilled water with a Waring Blendor. A known quantity of this homogenate (50 g) was further disintegrated in 250 ml of distilled water with a Polytron[8] homogenizer. Similar treatment was employed for all samples except that autoclaving was not used since feces, urine and the various gastrointestinal contents were easily disintegrated with the Polytron. The final Polytron prepared samples are suitable for direct pipetting into the scintillation vials for counting.

Representative samples of the homogenized carcasses were also fractionated to de-

[5] Autoclips purchased from Clay-Adams, Inc., NY.

[6] Unilux I, Nuclear Chicago, Chicago, IL.

[7] PPO or 2,5-diphenyloxazole and POPOP or *p*-bis[2-(5-phenyloxazolyl)]-benzene purchased from Nuclear Chicago, Chicago, IL, Cab-O-Sil, a gelling agent purchased from Packard Instrument Company, Inc., Downers Grove, IL.

[8] Polytron model PT10-35 with a PT20ST was purchased from Brinkmann Instruments, Inc., Westbury, NY.

TABLE II. Body Weights and Radioactivities of *E. coli* Given to Rats by Gavage. Radioactivities of Gastrointestinal Contents, Feces, Urine and Carcass Are Also Given.

Rat no.	Body wt	Activity of *E. coli* force-fed (dpm)	Activity in feces	Activity in gastro-intestinal contents (% of that force-fed)	Activity in urine	Activity in carcass
7	462	3,329,472	4.3	6.8	5.3	39.7
8	473	3,563,632	5.5	3.2	4.7	50.5
9	492	3,972,413	10.1	6.1	5.6	31.6
10	489	3,578,267	7.1	2.3	4.8	47.8
11	522	3,863,651	6.4	2.1	3.4	46.4
12	454	3,746,570	9.6	4.7	4.6	45.6
Average	482	3,675,834	7.3	4.2	4.7	43.5
SD	±25	± 23,489	±2.2	±2.0	±0.8	±6.9

termine distribution of radioactivities in the end product. For this purpose carcass samples were exhaustively extracted for total lipids using chloroform–methanol (2:1). After removing lipids, the lipid-free residues were treated with either hot or cold trichloroacetic acid (TCA). The final concentration of TCA was 5%. The hot and cold TCA soluble and insoluble fractions were then obtained by centrifugation. These and the lipid fractions were then counted in a similar manner as that used for counting radioactivities in the homogenized carcass sample.

Results and Discussion. Microscopic examination of the labeled *E. coli* revealed that the cells were structurally intact in that no broken cells or cell wall material were noted. Furthermore since there were questions raised as to the viability of the cell preparation as used in the experiments another culture of the *E. coli* was made. The method of culturing and all aspects of procedures in obtaining the final cell preparation were exactly duplicated. A total count of cells using a Petroff–Hauser counting chamber under phase microscope and a viable count using standard plate count agar with pour plating technique were made (Standard Methods Agar was obtained from BBL, Cockeysville, MD). Data obtained from the triplicate serial count revealed that 100% of the cells were viable. When the *E. coli* were disrupted and homogenized, 92.7% of the total radioactivity was recovered in the TCA precipitable fraction and only 7.3% of the radioactivity was in the soluble fraction. In addition the radioactivity in the final washing of the radioactive cells was only 1/40,000 of the radioactivity in the cells. This strongly indicates that radioactivity of the cell preparation used in the experiments was an integral part of the cells and not soluble in the saline used in washing and that the preparation contained only traces of radioactive contaminants. Thus, the appearance of radioactivity in the respiratory CO_2 or in the carcass in excess of 7.3% of the dose when labeled *E. coli* were placed into the cecum, must be due mainly to a cecal and perhaps large intestinal breakdown of the *E. coli* macromolecules with a subsequent absorption of breakdown end products. In the first experiment, wherein radioactive *E. coli* were placed directly into the cecum of rats, nearly 5% of the radioactive dose was recovered in the respired CO_2 in 4 hr of collection (Fig. 1 and Table I). Furthermore, an average of 30.5% of that placed in the cecum was deposited in the carcass (Table I). The chemical forms in which the ^{14}C was absorbed from the cecum was not determined. Hoogenraad *et al.* (4) placed radioactive *E. coli* directly into the rumen of sheep and found that the *E. coli* cells were rapidly degraded and fermented by the microbiota in the rumen to VFA. Since the cecum in many respects is similar to the rumen, most likely a portion of the radioactivity found in the rat carcass 4 hr after the *E. coli* administration

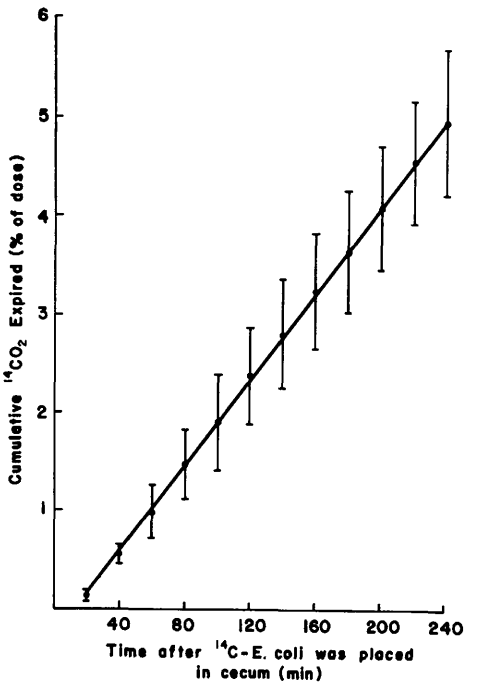

FIG. 1. Cumulative expiratory $^{14}CO_2$ after placing radioactive *E. coli* in ceca of six rats. Bars represent ± SD.

into the cecum came from absorbed VFA. The extent of contribution in the form of amino acids, vitamins or VFA produced by the degradation of the *E. coli* must be determined in future work.

Four hours after the introduction of the radioactive *E. coli* into the cecum, 71.1% still remained in this segment and the large intestine (Table I). The percentages of the dose remaining in the cecum and large intestine were variable among the six rats and ranged from 27.9 to 87.7. Undoubtedly, the variation partly resulted from the surgical trauma associated with exposing the cecum for the injection of *E. coli*.

In the second experiment, 24 hr after the rats were force-fed the radioactive *E. coli*, the carcasses of these rats contained 43.5% of the radioactive dose (Table II); 4.2% of the radioactive dose remained in the gastrointestinal contents, while 7.3% and 4.7% of the radioactive dose were recovered in the feces and urine, respectively (Table II). Since respiratory CO_2 was not collected in this study, total recovery of ^{14}C activity was not made; however, the unaccounted portion of the label was most likely lost in the respiratory CO_2 during the 24 hr period. Assuming the radioactivities not recovered in the feces or gastrointestinal tract were digested and absorbed, the digestion coefficient for this *E. coli* preparation appeared to be close to 88.5% [100% — (4.2% + 7.3%)]. The net utilization value for this bacterium was 83.8% (digestion coefficient — urinary excretion) (Table II). Not surprisingly this second experiment showed that *E. coli* can be digested and absorbed by animals when used as a dietary source. Since microbial cells contain generally 60% crude protein (6), the primary end products of intestinal breakdown are likely to be amino acids, whereas in the cecum a portion of the *E. coli* constituents could be absorbed as VFA if we assumed that cecal activities are similar to those reported for the rumen (4).

Fractionation of the carcass samples indicates that about 44% of the ^{14}C was in the form of lipids whether the rats were force-fed the radioactive *E. coli* or when the *E. coli* were placed in the cecum (Table III). No statistical differences were found in the data obtained from the use of the hot or cold TCA as precipitant of the carcass samples. For this reason, the data were combined and presented as TCA soluble and TCA insoluble materials (Table III). On the average, 29 to 36% of the carcass radioactivity was in the TCA soluble materials and 20 to 27% in the TCA insoluble fractions. Thus, these data strongly indicate that the rat incorporated the products absorbed from the degradation of *E. coli* into lipids, protein and other carbon-containing materials.

Summary. Radioactive *E. coli* produced by means of adding glucose-U-^{14}C into the culture medium was placed into the cecum of six adult male rats. The rats were then placed in metabolic chambers for the collection of respiratory CO_2. At the end of 4 hr, almost 5% of the dose appeared as CO_2 indicating that the *E. coli* were digested in and absorbed from the cecum. Further evidence that this occurred came from the finding that 30.5% of the dose appeared in the carcass of

TABLE III. Percentages of the Total Radioactivity in the Lipid and TCA Soluble and Insoluble Fractions Obtained from Carcasses of Rats Force-fed Radioactive *E. coli* and When the *E. coli* Were Placed in Their Ceca.

E. coli placed in cecum				*E. coli* force-fed			
Rat no.	Lipids	TCA soluble[a]	TCA insoluble[a]	Rat no.	Lipids	TCA soluble[a]	TCA insoluble[a]
1	27	36	37	7	36	36	28
2	59	28	13	8	49	22	29
3	54	33	13	9	47	37	16
4	48	43	9	10	34	28	38
5	48	48	4	11	56	23	21
6	31	35	34	12	42	31	27
Average	44	36	20	Average	44	29	27
SD	±13	±7	±14	SD	±8	±6	±7

[a] Hot and cold TCA were used. However, data were combined since there were no statistical differences between the two methods.

the rats killed at the end of the metabolic CO_2 collection period. About 70% of the dose remained in the cecum and large intestine at this time which is the portion not absorbed. Another six comparable rats were gavaged with the radioactive *E. coli*. Carcass and fecal analyses and measurements of activities in the gastrointestinal contents indicate that 24 hr after gavage, the rat retained 43.5% of the gavaged dose. They did not digest 7.3% (fecal activity) plus 4.2% (remaining in the gastrointestinal tract) for a total of 11.5%. Thus, the digestion coefficient for *E. coli* is 88.5% and net utilization is 83.8% since 4.7% of the gavaged dose appeared in the urine in 24 hr. Fractionation of carcass samples revealed that the rat utilized the products obtained from the digestion or degradation of the radioactive *E. coli* force-fed or placed in the cecum for the formation of lipids, protein and other carbon-containing materials.

1. Yang, M. G., Manoharan, K., and Mickelsen, O., J. Nutr. **100**, 545 (1970).

2. Yang, M. G., Manoharan, K., and Young, A. K., J. Nutr. **97**, 260 (1969).

3. Roberts, R. B., Cowie, D. B., Abelson, P. H., Bolten, E. T., and Britton, R. J., "Studies of Biosynthesis in *Escherichia coli*," p. 5. Carnegie Institute of Washington, Washington, D.C. (1955).

4. Hoogenraad, N. J., Hird, F. J. R., White, R. G., and Long, R. A., Brit. J. Nutr. **24**, 129 (1970).

5. Biochemistry Division Staff, University of Illinois, "Experimental Biochemistry." Stipes Publishing Company, Champaign, IL (1959).

6. Hungate, R. E., "The Rumen and Its Microbes." Academic Press, New York (1966).

Received Sept. 27, 1971. P.S.E.B.M., 1972, Vol. 139.