The Metabolism of Carbon-labeled Urea in the Germfree Rat

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For many years, urea was considered an end product of amino acid metabolism in mammals. With the advent of isotopic techniques (1-3), it was realized that further catabolism of urea occurred, probably due to bacterial action in the gut. Dintzis and Hastings (4) found that by feeding a mixture of various antibiotics to mice, the breakdown of urea to carbon dioxide and ammonia was almost completely abolished. From this, they concluded that enzymatic hydrolysis of urea in mammals is effected only by urease of the bacteria present in the gut. Kornberg et al. (5, 6) also arrived at this conclusion from their experiments in cats. We have subjected this thesis to direct test in the "germfree" animal.

METHODS

In these experiments, 6 healthy, female, white Lobund strain rats were used. They ranged in weight from 250 to 350 gm. Of these, 4 (germfree since birth) were housed in individual wire mesh cages in a standard germfree Reyniers' holding tank (7). The diet was sterilized by steam under pressure. The microbiologic examinations of the germfree animals and tank consisted of weekly sampling of food, feces, cage debris, and anal contents cultured on blood agar plates, Sabouraud's plates, thioglycolate broth, brain-heart infusion broth and plate, and Trypticase soy broth.2 In addition, at time of sacrifice, blood, liver, lung, and spleen, as well as cecal and nasopharyngeal contents, were cultured on the above media. There was no growth in any of the cultures. These animals, then, are termed "germfree", i.e. free from bacteria and fungi by our tests. The other 2 rats were normal controls maintained since birth in a conventional animal room. Their diet was prepared in the same fashion as for the germfree rats.

The C14-urea solution was prepared by dissolving labeled crystalline urea (kindly supplied by Dr. Wright Langham, Los Alamos Scientific Laboratories, Health Division, Los Alamos, New Mexico) in 0.9 per cent sodium chloride solution and aseptically transferring it, with a Swinnny hypodermic adapter,3 to a sterile rubber-stoppered serum bottle. The labeled urea solution was entered into the Reyniers' tank through a germicidal trap. Five rats were given a subcutaneous injection of the sterile C14-urea solution (0.6 ml. of C14-urea, containing 2.5 X 10-5 moles of urea, with a total activity of 1 X 106 c.p.m. as measured at infinite thinness in our Q gas flow counter). The labeled urea, diluted with additional 0.9 per cent sodium chloride solution, was given intragastrically to 1 germ-free rat by a nasopharyngeal tube. The rats were allowed food and water ad libitum up to injection time; no food or water was given thereafter. Immediately after injection, each rat was placed in a metabolic apparatus (8) for collecting expired CO2, urine, and feces. For all the germfree rats, the metabolic apparatus and sodium hydroxide solutions were sterilized by autoclaving inside a Reyniers' tank. The germfree animal was then transferred under sterile conditions from the holding tank to the experimental chamber. These experiments, then, were conducted under germfree conditions. One of the experiments on the control rats was conducted similarly, except, of course, that the animal was not sterile. The other experiment on a control rat was carried out in an ordinary laboratory hood. Radioactivity determinations were made on weighed samples of BaCO3 as described in an earlier paper (9).

RESULTS AND DISCUSSION

The dramatic difference between the amounts of C14O2 expired by the germfree rats and the controls, after the subcutaneous injection of the C14-labeled urea, is shown in Fig. 1. The control animal expired an amount of C14 in 6 hours equivalent to 2 per cent of the injected dose; the germfree rat only 1/25 as much. The specific activities of the CO2 expired by the two types of rats were also strikingly different (Fig. 1, cf. Table I). The pattern of urea metabolism in the germfree rats was similar whether the labeled urea was given subcutaneously or intragastrically (Table II).

The very small fraction of the injected C14 expired by the germfree rat is definite; it is not a methodologic error. Clearly, then, urea does break down at a very slow rate in the rat in the absence of bacteria. But we need not invoke endogenous tissue urease activity to explain this, since we found that urea hydrolyzes spontaneously in vitro to at least this extent at pH 7 at a temperature of 37°.

The studies in vitro were performed by incubating sterile C14 urea solutions which were buffered at pH 7 with phosphate and were covered with mineral oil. After incubation, the mineral...
oil was removed, the pH adjusted to 2 with phosphate buffer, and any volatile material was then washed out with dry N₂ into 2.5 N NaOH. Kinetic analysis revealed that the urea hydrolyzed at a rate of about 0.045 per cent per hour.

Applying this figure to the germfree rat, and assuming that no urea was enzymatically metabolized, as much as 0.18 per cent of the original injected dose might have been expired as C⁴O₂ in 4 hours. But this estimate is clearly too high since (a) the germfree rats excreted 25 per cent of the injected urea in the urine over the 4-hour period, and (b) a previous study by Solomon et al. (10) has shown that when C⁴-labeled bicarbonate is injected into fasted rats after administration of lactic acid by stomach tube, the expired radioactive CO₂ over a 4-hour period accounts on the average, for 50 per cent of the radioactivity injected. In their experiment, less than 2 per cent of the radioactivity was recovered in the urine.

The actual amount of C⁴ expired by the rat given urea over the 4-hour period was 0.02 per cent which although different from a theoretical estimate (0.06 per cent) is of the same order of magnitude, and, actually, is lower and precludes the possibility of enzymatic action.

Our results support unequivocally the thesis that the enzymatic hydrolysis of urea in mammals is effected by urease of their bacteria.

**SUMMARY**

1. C⁴-urea was injected subcutaneously into three germfree and two normal control rats; the labeled urea was given intragastrically to one other germ-free rat.

2. The germfree animals expired only \( \frac{1}{5} \) as much C⁴O₂ as the controls in the first 6 hours after the administration of the urea.

3. The pattern of urea hydrolysis in the germfree rats was the same whether the labeled urea was given subcutaneously or intragastrically.

4. These results support the thesis that the enzymatic hydrolysis of urea in mammals is effected by urease of their bacteria.

**REFERENCES**


The Metabolism of Carbon-labeled Urea in the Germfree Rat
Stanley M. Levenson, Leo V. Crowley, Richard E. Horowitz and Ole J. Malm