Breakdown of Urea in the Rat. (20662)

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At the present time there is an apparent conflict in the evidence regarding the stability of urea in the animal organism, especially in the rat. Bloch(1) found that when urea-N^{15} was fed to rats over a period of 3 days the N^{15} concentration in the excreted ammonia was only 4% and that in the liver protein was less than 2% of that in the urinary urea. This led him to believe that urea once formed in the rat was subject to little breakdown. The more recent experiments of Kornberg and Davies(2), carried out in the cat led to quite similar results. In contrast to these results obtained with urea-N^{15}, Leifer and coworkers (3) found that when urea-C^{14} was administered to mice 21% of the C^{14} appeared in the respiratory air in 48 hours. Skipper and coworkers(4) found that in the same species about 9% appeared in the respiratory air in 10 hours and a total of 80% of the C^{14} was recovered in the urine after 24 hours. These data indicate a great lability of urea in the mammal and are in agreement with the nutritional data of Lardy and Feldott(5) and Rose and coworkers(6) who have shown that the growth of rats, on diets containing nitrogen in the form of essential amino acids only, could be improved by adding urea or ammonium salts to the diet. Since urease is present in the gastric mucosa of most species(7-9), it is tempting to attribute the breakdown of urea which has been observed, to the action of this enzyme. However, it is equally possible that in the intact animal the major part of the decomposition is due to the action of the intestinal microflora.

The object of the present experiments was to determine the site of urea breakdown in the intact rat and to assess its significance. The results show that urea-C^{14} when injected into normal rats is decomposed to a considerable extent into carbon dioxide, up to 40% appearing in the respiratory gases in the first 24 hours. The eviscerated rat, however, breaks down urea at a comparatively slow rate. Isolated tissues from rats were also found to cause comparatively little urea breakdown, although the reverse was the case with washings from the intestine. It is clear from these experiments and from those of Bloch, who used urea-N^{15}, that in the rat, urea breakdown occurs continually at a rather rapid rate due to the activities of urease in the intestinal flora and gastric mucosa. The ammonia is again reconverted into urea. This conversion has been repeatedly demonstrated both *in vivo* (10) and by enzymatic experiments.

TABLE I. Metabolism of Urea-C^{14} in Rats. Experiments were on normal rats except Ip.3 in which the animal was pretreated with succinyl sulfathiazole for 2 days and in Sc.2 in which the animal was eviscerated. The experimental period was 24 hr except in the eviscerated animal in which the period was 9 hr.

Exp.*	Wt of rat, g	Dose of urea,† mg	Fraction of dose excreted in exp. period: Urine, %	Fraction of dose excreted in exp. period: Respired air, %
Ip.1	230	4.5	43.8	40.4
2	250	4.5	67.5	30.5
3	244	4.75	69.5	18.9
Sc.1	240	4.75	75.6	21.2
2	250	2.5	24.9	3.6

* Ip. and Sc. indicate inj. of urea made into the peritoneal cavity or subcut.

† In terms of radioactivity dose varied between 1.4 and 2.8 $\times$ 10^6 counts/min.

Methods. The urea-C^{14} was purchased from the U. S. Atomic Energy Commission to whom our thanks are due. Before use it was diluted with suitable amounts of inert urea. The Long-Evans rats used in the *in vivo* experiments were adults with weights shown in Table I. They were maintained on a diet of Purina laboratory chow prior to the experiment. After the injection of the urea, which was made either subcutaneously or into the peritoneal cavity, the animals were kept in an all glass metabolic cage through which a slow stream

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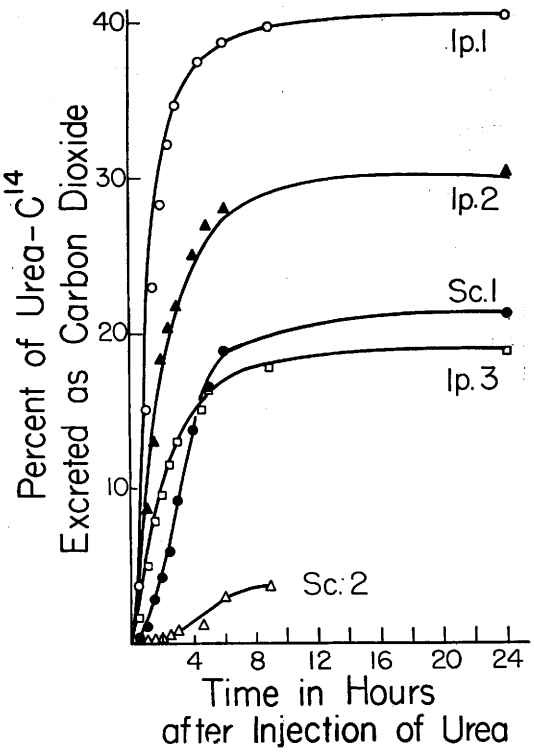

FIG. 1. Cumulative excretion of carbon dioxide-C^{14} following the administration of urea-C^{14} to rats either into the peritoneal cavity (Ip.) or subcutaneously (Sc.). In exp. Ip.3 the animal was treated with succinyl sulfathiazole, and in Sc.2 an eviscerated animal was used.

of carbon dioxide-free air was passed. The expired carbon dioxide was collected in sodium hydroxide using 2 milli-equivalents of the base per minute of collection time. Urine was collected in dilute hydrochloric acid contained in flasks connected to the metabolic apparatus. The rat used in experiment Ip. 3 was pretreated with succinyl sulfathiazole for two days (1 ml 10% solution twice a day) prior to the administration of the urea, and that used in experiment Sc. 2 was eviscerated following an overnight fast. Our thanks are due to Dr. W. O. Reinhardt for performing the operation. Rat tissues and washings from the intestine used in the *in vitro* experiments were incubated in flasks with an inlet tube equipped with a sintered glass tip. The outlet tubes were provided with absorbers for carbon dioxide which was swept over with a slow stream of oxygen. For medium, Krebs-Ringer-phosphate buffer containing 0.4 g glucose per 100 ml was employed at a temperature of 38°. For the determination of radioactivity the carbon dioxide was precipitated as the barium salt which was washed, dried and then sedimented on to aluminum discs. Aliquots of urine were dried on glass discs at room temperature. All radio activities were measured in duplicate on a Geiger-Müller counter, and were corrected for self-absorption.

Results and discussion. The extent to which the C^{14} from urea was excreted in the urine and expired air by rats under several conditions is shown in Table I, and the time course of the appearance of the C^{14} in the carbon dioxide is shown in Fig. 1. From these data it is clear that there is a considerable and quite rapid decomposition of urea to give carbon dioxide in the intact rat, and that the amount decomposed is less when the substance is given subcutaneously. It is also evident that the decomposition was reduced by pretreating the animal with succinyl sulfathiazole, and

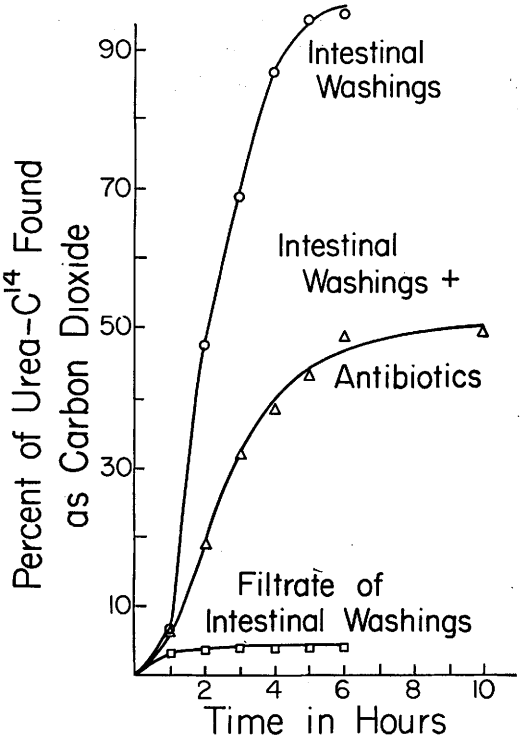

FIG. 2. Cumulative formation of carbon dioxide from urea-C^{14} by washings from the intestine. Antibiotics used/10 ml, penicillin 50000 units, dihydroxystreptomycin 2 mg, sulfasuxidine 4 mg, sulfathiazin 1 mg.

TABLE II. Metabolism of Urea in Isolated Tissues from the Rat.
About 1 g wet wt of tissue was incubated in Krebs-Ringer-Phosphate containing 0.4% glucose and 1 *mg* of urea-C^{14}, 3.2 to 5.6 × 10^5 c.p.m. and the CO_2 formed was aerated into base. Samples were taken at hourly intervals.

Interval, hr	Fraction of urea-C^{14} appearing as CO_2		
	Liver slices, %	Kidney slices, %	Stomach sections, %
0-1	.15	.16	.04
0-2	.23	.19	.09
0-3	.24	.23	.14
0-4	.24	.41	.16
0-5	—	—	.20
0-6	—	.49	.30

especially by evisceration. In the last mentioned condition there was little appreciable decomposition of the urea at all, particularly in the first 3 or 4 hours of the experiment. Subsequently, the rate appeared to increase which may be due to contamination with bacteria.

The production of respiratory carbon dioxide follows monomolecular reaction kinetics from 0.5 to about 5 hours after the injection of the urea when the substance is given intraperitoneally, the half time being of the order of one hour. Gould and coworkers(11) found a half-time for loss of carbon dioxide following the injection of bicarbonate to be about 15 minutes. Hence, the rate governing process must be that for urea decomposition.

The *in vitro* experiments with liver, kidney and stomach tissue show that there is little decomposition of urea by these tissues(Table II). It is evident that these organs could not account for the extensive urea breakdown observed in the *in vivo* experiments. However, when intestinal washings were incubated with urea considerable decomposition occurred in a few hours. This decomposition was not found when the washings were filtered, nor to such a great extent when antibiotics were present. Taken together with the previous results it appears that the greater part of the urea breakdown must be attributed to the activity of the intestinal flora and not to the tissues of the rat itself.

The indications from these experiments are that urea is not a stable substance in the rat but rather that it undergoes a continual decomposition. There is a type of urea cycle in the animal. Urea being freely diffusible appears in the secretions into the intestine and is in part decomposed there by the microorganisms. The resorbed ammonia is immediately reconverted to urea by the liver.

Sprinson and Rittenberg(12) and others (13) have administered amino acids such as glycine-N^{15} to animals in attempts to deduce rates of turnover of body protein from the rate of excretion of the N^{15}, largely in urea. It is obvious that the urea breakdown-resynthesis mechanism which has been shown to exist may modify the N^{15} excretion rate, and that in animals under different conditions, with different intestinal flora, different rates of N^{15} excretion may exist for this reason alone.

Summary. A rapid breakdown of urea-C^{14} is observed in the intact rat but this is entirely absent in the eviscerated animal. Due to the existence of this breakdown which is immediately followed by resynthesis, there is a type of urea cycle in the intact animal. No significant breakdown of urea occurs in either liver, kidney or stomach, but the intestinal washings rapidly decompose the substance.

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Resistance in Leukemic Cells to an Adenine Antagonist, 6-Mercaptopurine. (20663)

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Transformations have been obtained to resistance or dependence in leukemic cells of the mouse by the use of several antimetabolites: folic acid analogs(1,2-4) and the triazolopyrimidine analog of guanine, 8-Azaguanine(5). Such transformations have been shown to be stable, irreversible and heritable, and have been produced readily in several transplantable lines of lymphocytic leukemia (6). Experimental findings favor the assumption that the variant lines produced by the use of antimetabolites arise by mutation and the role of antimetabolite is merely as a selective agent(7).

Preliminary evidence which suggests that purine analogs act as antagonists of nucleic acid synthesis has encouraged investigation of the role of these compounds in the inhibition of cancerous growths. The adenine analog, 6-mercaptopurine, has been shown to act as a purine antagonist in the metabolism of *Lactobacillus casei*(8). It has also been shown to be a unique inhibitor of Sarcoma 180(9) and of certain mammary adenocarcinomas(10). Limited clinical trials of this compound in advanced leukemia of children have been encouraging(11).

Definite, regular and reproducible inhibition of growth of leukemic cells has been obtained in 2 transplantable lines of acute lymphocytic leukemia. No increase in survival time of mice bearing 2 other transplantable acute lymphocytic leukemias could be obtained. In leukemia L 1210(12), particularly, striking increases in survival time were observed and are described here. Following the methods used previously in obtaining transformations to folic acid antagonists(4) and 8-Azaguanine(5), a resistant subline of leukemia L 1210 has been developed using 6-mercaptopurine as the selective agent. Some of the pertinent characteristics of these L 1210 resistant leukemic cells will be considered.

Transplant generations 202 to 251 of the sensitive subline of leukemia L 1210 have been used as control material for the present study. Beginning with the 202nd transfer, a standard dose of leukemic cells (8 x 10^5 cells) in Lockes' solution was inoculated subcutaneously in the right axillary region of 18-20 g DBA/2 strain mice. These mice subsequently received daily injections, beginning at 24 hours, of the adenine analog, 6-mercaptopurine.† The compound was dissolved in 0.1 N NaOH then diluted with sterile saline so that the constant volume injected subcutaneously was 0.025 cc per g of body weight. Daily injections were continued until palpable growths sufficient for transfer were obtained. During the first 3 transfers the total dosage of analog was 262.5 mg/K (37.5 mg/K x 7 days) and subsequently the total dosage was 525 mg/K (75 mg/K x 7). Transfers of leukemic cells were done at 9 to 11 days during the first 4 transfer generations and later at 7 and 8 days as larger subcutane-

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† This compound furnished by Dr. George Hitchings of The Wellcome Research Laboratories, Tuckahoe, N. Y. A-methopterin and 8-Azaguanine, were supplied by Lederle Laboratories Division, American Cyanamid Co.