Metabolism of C^{14}-labeled Urea[1]

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The work of Bloch (*1*) using N^{15}-labeled urea indicated that a small portion of the nitrogen of ingested urea was transformed to ammonia and protein N, while the majority of the labeled urea was excreted as such. To determine the fate of the carbon in the urea molecule, C^{14}-labeled urea was synthesized (*3*), and the gross metabolism was studied in a series of mice using the method described by Roth, Leifer, Hogness, and Langham (*4*).

Five-tenths mg of C^{14}-labeled urea equivalent to 60,000 cps/mg was injected intraperitoneally into each of 9 mice (CF-1 strain). At various time intervals the radioactivity in samples of urine was determined by direct plating (*2*). Exhaled CO_2 was absorbed in 20% NaOH. The carbonate formed was converted to $BaCO_3$ and the radioactivity determined. At various time intervals animals were sacrificed, and the radioactivity was determined by burning and measuring activity in the form of $BaCO_3$.

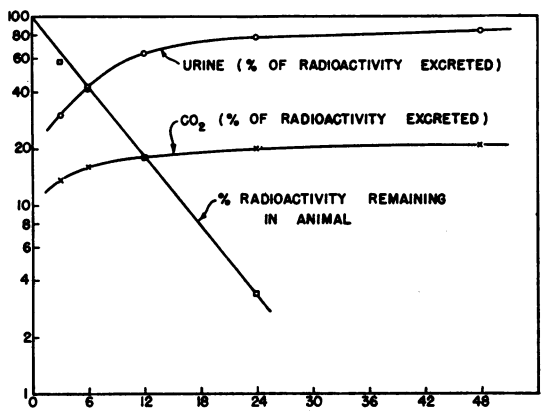

FIG. 1

The per cent of radioactivity excreted in the urine and in the exhaled CO_2 as a function of time is shown in Fig. 1. At 48 hrs one can see from this figure that the sum of activities in urine and CO_2 add up to 105%. This can be explained by an error involved in the measurement of the initial injection volume. Subtracting the excreted amounts from the initial amount injected, the per cent of radioactivity remaining in the animals at various times after injection was calculated. The results are presented graphically in Fig. 1. From the graph the biological half-life of urea was found to be 5 hrs.

The urine was analyzed using the method of filter paper chromatography with the following developing solvents: (a) butanol-water, (b) butanol-3% NH_4OH, (c) butanol-ethanol-water, (d) hexanol-water, (e) hexanol-ethanol-water, (f) phenol-water, (g) phenol-NH_4OH (3%), (h) s-collidine-water. Radioautographs of the paper strips were then made on No Screen or Blue Brand X-ray film in order to identify radioactive metabolites.

Fig. 2 shows a typical radioautograph obtained for 4 solvent systems and established the fact that the radioactive carbon in the urine is contained only in the urea.

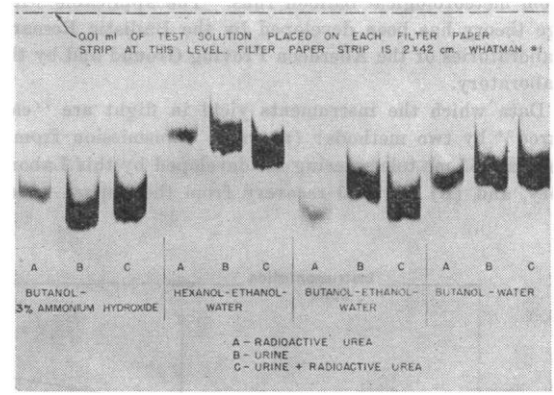

FIG. 2

The results indicate that 20.8% of the injected C^{14} in urea appears in the exhaled CO_2. The very rapid initial excretion of radioactive CO_2 indicates that a large per cent of urea is degraded in the first 3 hrs. Whether this is due to elevation of the urea level or to the mode of injection is unexplained.

The bulk of the urine was collected under toluene, but, to ascertain that the CO_2 collected was not due to bacterial decomposition of the small fraction of urine on the side of the cage, the caudal halves of 2 mice injected with C^{14} urea were isolated in airtight sheaths and the exhaled CO_2 collected. The CO_2 collected during the first 20 min after injection was found to contain radioactive carbon in the same amounts as in the previous experiment.

Tissue analyses indicate that the injected C^{14} urea is uniformly distributed on a weight basis within 25% in the liver, spleen, heart, muscle, brain, and blood, as might be expected, since urea is freely diffusible. The kidneys, however, contained two to three times the amount of radioactivity of the other tissues tested, presumably because of the concentrating action of the tubules.

The mechanism of the formation of CO_2 has not been established. Whether this goes by reversal of the Krebs urea cycle or through another mechanism, such as direct hydrolysis, remains to be established.[2]

References

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2. HOGNESS, J. R., ROTH, L. J., LEIFER E., and LANGHAM, W. H. *J. Amer. chem. Soc.*, 1948, **70**, 3840.
3. MURRAY, ARTHUR, III, and RONZIO, ANTHONY R. (To be published.)
4. ROTH, L. J., LEIFER, E., HOGNESS, J. R., and LANGHAM, W. H. *J. biol. Chem.*, 1948, **176**, 249.

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[2] Since this paper was submitted for publication, similar results using C^{14}-labeled urea have been obtained at the Southern Research Institute, Birmingham, Alabama (personal communication, Dr. Howard E. Skipper).