thopaed. **27**, 213 (1963).

2. Hurley, L. S., Everson, G. J., Wooten, E., and Asling, C. W., J. Nutr. **74**, 274 (1961).

3. Hurley, L. S., Wooten, E., and Everson, G. J., J. Nutr. **74**, 282 (1961).

4. Hurley, L. S. and Asling, C. W., Anat. Record **145**, 25 (1963).

5. Hurley, L. S., Wooten, E., Everson, G. J., and Asling, C. W., J. Nutr. **71**, 15 (1960).

6. Erway, L., Hurley, L. S., and Fraser, A., Science **152**, 1766 (1966).

7. Hurley, L. S., Shrader, R., and Gowan, J., *in* Fifth European Symposium on Calcified Tissues, Bordeaux, 1967, S.E.D.E.J., Paris, in press.

8. Hurley, L. S., Federation Proc. **27**, 193 (1968).

9. Hurley, L. S., J. Nutr. **91**, 27 (1967).

10. Hurley, L. S. and Swenerton, H., Proc. Soc. Exptl. Biol. Med. **123**, 692 (1966).

11. Swenerton, H. and Hurley, L. S., J. Nutr. **95**, 8 (1968).

12. Aubert, J. P. and Milhaud, G., Biochim. Biophys. Acta **39**, 122 (1960).

13. Milhaud, G., Remagen, W., Comes de Matos, A., and Aubert, J. P., Rev. Franc. Etudes Clin. Biol. **5**, 254 (1960).

14. Bronner, F., Trans. N. Y. Acad. Sci. **29**, 502 (1967).

15. Assoc. Official Ag. Chemists. "Official Methods of Analysis" (W. Horwitz, ed.), 8th ed., p. 378, Washington, D. C. (1955).

16. Radin, N. and Granza, A. L., Clin. Chem. **10**, 704 (1964).

17. Moukhtar, M. S., Cherian, A. G., Milhaud, G., and Aubert, J. P., *in* "Proc. First European Bone and Tooth Symposium," p. 103. Macmillan (Pergamon) London, (1964).

Received Sept. 3, 1968. P.S.E.B.M., 1969, Vol. 130.

Characteristics of the Aminoaciduria Resulting from Cycloleucine Administration in Pair-Fed Rats* (33673)

R. A. GOYER, J. O. REYNOLDS, JR., AND R. C. ELSTON

Department of Pathology and Genetics Curriculum, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27515

Cycloleucine (1-aminocyclopentane carboxylic acid) is known to inhibit transport of amino acids into cells. Initially, interest in the mode of action of this compound was prompted by its carsinostatic effect in human carcinomas (1, 2) and on certain experimental ascites tumors (3). The effect on ascites tumor cells is associated with a decreased rate of incorporation of amino acids into protein, which in turn is the result of competition between cycloleucine and naturally occurring amino acids for transport into cells (4). The competition for transport in this system is greatest for glycine, a monoamino-monocarboxylic acid, least for lysine, a dibasic amino acid and intermediate for valine and leucine (4). Cycloleucine does not appear to interfere with other steps in protein synthesis (5).

Employment of cycloleucine for the treatment of multiple myeloma in humans results in greatly increased urinary excretion of the dibasic amino acids, lysine, ornithine, arginine and the sulfur-containing amino acids, cystine (6). Plasma levels of these amino acids are not elevated suggesting that the aminoaciduria is the result of impairment of renal tubular transport. The specificity of the competitive action of cycloleucine on the active transport of amino acids in the renal tubule appears to differ, therefore, from that occurring in ascites tumor cells, but does resemble the transport defect characteristic of cystinuria, an inborn error of metabolism in humans.

The present study was undertaken to define the effect of cycloleucine on the transport of amino acids in the renal tubule of the rat. Plasma levels and renal clearances of fifteen amino acids were measured after three daily injections of cycloleucine in doses

* Supported in part by Research Grants No. HE-03140 and AM-12061 and Contract No. PH-43-68-74 from the United States Public Health Service.

TABLE I. Effect of Cycloleucine on Urinary Excretion of Dibasic Amino Acids and Cystine[a] in Pair-fed Rats.

	Animal groups			
	Expt. I Regular diet[b] 6 pairs of rats		Expt. II Regular diet[b] + 2% cystine 4 pairs of rats	
Amino acid	Control	Experimental	Control	Experimental
Cystine	None	None	None	0.40 ± 0.47
Lysine	1.21 ± 1.42	39.1 ± 44.97	1.53 ± 0.84	23.4 ± 11.4
Ornithine	0.84 ± 0.61	3.64 ± 4.13	0.74 ± 0.48	2.64 ± 0.32
Arginine	0.84 ± 0.77	9.54 ± 11.97	0.36 ± 0.17	4.38 ± 2.98

[a] Values are mean and SD of 24-hr urinary amino acid excretion in μmoles.
[b] Experimental and control rats were pair-fed.

comparable to that employed in the treatment of multiple myeloma in patients.

Materials and Methods. Two experiments were performed. In the first experiment six pairs of 200 g Sprague-Dawley male rats were housed in metabolism cages and pair-fed. A member of each pair received a daily intraperitoneal injection of cycloleucine, 300 mg/kg of body weight for 3 days. Cycloleucine was obtained from K & K Laboratories, Inc., Plainview, New York. Diet consisted of pulverized laboratory chow *ad libitum* for the experimental rats. Food intake was weighed each day and the control member of each pair was fed an identical amount. Urine samples collected during the third 24-hr period were preserved with thymol crystals and refrigerated at 4°.

In the second experiment the diet was supplemented with 2% cystine. Four pairs of rats were pair-fed and cycloleucine was administered as in the first experiment. Plasma as well as urine samples were obtained to calculate renal clearances of amino acids on three of these pairs. At the end of the third 24-hr period the animals were anesthetized with an intraperitoneal injection of pentobarbital, 5 mg/100 mg of body weight, and heparinized blood samples were collected by intracardiac aspiration. Plasma was separated from the blood and deproteinized for amino acid analysis with 5% sulfosalycilic acid.

Amino acids were measured by ion-exchange column chromatography employing a Technicon[1] automatic amino acid analyzer and a 120 × 0.6 cm column containing Chromobead[1] type B resin. Amino acids were eluted with 0.2 *M* sodium citrate buffer with a pH gradient from 2.88 to 5.0. Elution time was 22 hr.

Results. A large increase in urinary excretion of dibasic amino acids followed the administration of cycloleucine to rats fed a regular diet. In every pair, the level of dibasic amino acid excretion was higher for the experimental animal than for the control animal. Thus, for experiment I, in which there were six pairs of rats, the result was significant, using a simple nonparametric test, at the 2% level $[(\frac{1}{2})^6 < 0.02]$. The observed means and standard deviations are given in Table I. Cystine is not normally detectable in rat urine and was not present in this experiment. In order to verify that the observed increase in urinary excretion of dibasic amino acids did, indeed, result from competition of cycloleucine for the renal tubular transport site for the dibasic amino acids and cystine the experiment was modified by adding 2% cystine to the diet. The results of Expt. II (Table I) show that cystinuria as well as dibasic aminoaciduria occurs following cycloleucine administration. Here again, the level of excretion was higher for the experimental animal than its control in every pair; the results are thus significant at the 6.25% level.

In the second experiment, plasma as well

[1] Technicon Corporation, Chauncey, New York.

TABLE II. Plasma Levels of Amino Acids in Rats Given Cycloleucine for 3 Days and in Pair-Fed Controls.*

Amino acid	Control animals	Experimental animals
Threonine	24.8 (20.6 –27.6)	32.6 (24.4 –42.6)
Serine	30.8 (27.1 –33.8)	32.1 (25.6 –36.1)
Proline	8.88 (6.88–12.2)	19.7 (15.0 –22.8)
Glycine	30.1 (18.6 –38.4)	21.8 (17.9 –23.4)
Alanine	34.6 (33.2 –36.9)	28.1 (24.0 –36.0)
Valine	15.5 (13.5 –17.0)	23.5 (23.2 –24.0)
Methionine	3.9 (3.12– 4.51)	13.9 (11.0 –16.3)
Isoleucine	6.16 (4.99– 7.29)	9.61 (8.48–11.1)
Leucine	10.93 (8.68–13.0)	18.7 (17.1 –20.4)
Tyrosine	5.77 (4.26– 6.84)	5.44 (4.7 – 6.18)
ϕ-Alanine	6.46 (5.51– 7.20)	8.17 (7.72– 8.66)
Histidine	8.36 (6.77–11.3)	10.05 (9.08–11.5)
Ornithine	9.2 (8.71– 9.50)	8.23 (7.44– 9.0)
Lysine	32.0 (16.7 –41.4)	36.3 (34.2 –38.2)
Arginine	8.75 (5.82–10.6)	19.6 (15.1 –22.2)

* Values are mean and range of three animals in μmoles/100 ml.

as urinary amino acids were measured (Table II). Increases [of fivefold or more (Fig. 1)] in renal clearances of the dibasic amino acids following cycloleucine administration in the presence of unchanged plasma levels (except possibly for arginine, Table II) suggests impairment of renal tubular reabsorption for this group of amino acids. Cystine was not detectable in plasma from cystine-supplemented rats receiving cycloleucine although cystinuria was present. Lesser increases in renal clearances of serine, glycine, alanine, tyrosine, phenylalanine, and histidine accompanied by normal plasma levels is also consistent with reduced renal tubular reabsorption for these amino acids. Small increases in clearances of the branched-chain amino acids, valine, isoleucine, leucine, and proline may have resulted from "overflow" since plasma levels are elevated (Table II). Data for threonine is equivocal and plasma methionine is elevated whereas renal clearance is decreased.

Discussion. The aminoaciduria which follows cycloleucine administration in pair-fed rats is of two types. For a larger number of amino acids, it is entirely the result of impaired renal tubular reabsorption. For others, there is some degree of overflow from elevated plasma levels. Evaluation of the renal tubular component of "mixed" aminoaciduria requires accurate measure of glomerular filtration rate. This parameter was not measured in this experiment.

The most marked abnormality consisted of a massive dibasic aminoaciduria and a cystinuria similar to that observed by Brown (6) in humans receiving cycloleucine for the treatment of multiple myeloma. Plasma levels of lysine and ornithine were changed whereas plasma arginine tended to be elevated in his patients and in the animals in this experiment. This pattern of aminoaciduria is also characteristic of the genetic defect, cystinuria, which is believed to result from a defect in the transport mechanism common to these amino acids (7). However, unlike cystinuria, decreased renal tubular reabsorption of several monoamino-monocarboxylic amino acids also occurs following cycloleucine administration. Although there is evidence to suggest that some of these amino acids, particularly glycine (8) may compete for the transport site for the dibasic amino acids, it is believed that this group of amino acids shares an independent transport system (9). Reasons for the elevation of the branched-chain amino acids and the imino acid proline is not apparent from this experiment but it is of interest that cycloleucine does compete with members of this group of amino acids (valine and leucine) for uptake into ascites tumor cells (4). The unchanged or elevated plasma levels of amino acids observed in this experiment is consistent with the observations of Clark (10), that levels of body amino acid pools are not altered with cycloleucine administration if adequate dietary intake is maintained.

Studies on the manner in which cycloleucine impairs amino acid transport indicate that this compound competes with natural amino acids for transport sites or carriers (4). The results of the present experiment suggest that cycloleucine competes more

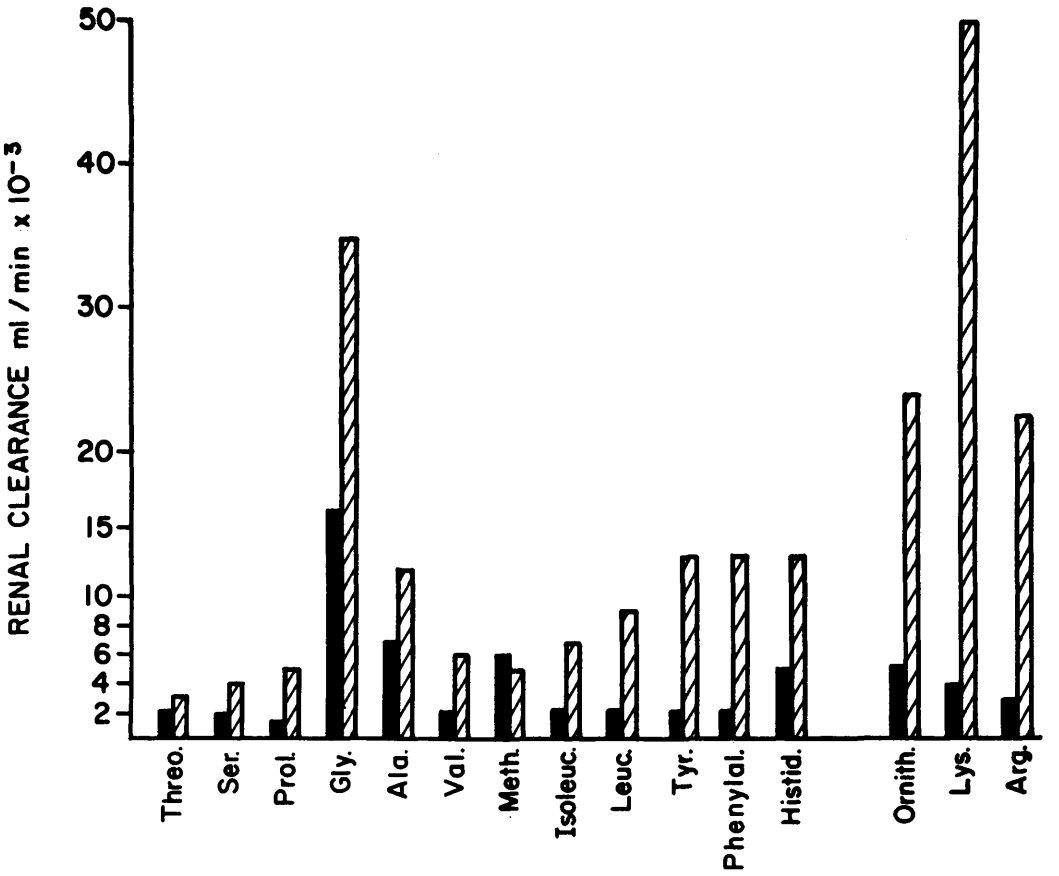

FIG. 1. Twenty-four-hr renal clearances of amino acids from control rats and rats following three daily intraperitoneal injections of cycloleucine.

effectively for transport in the renal tubule with the dibasic amino acids than with other groups of amino acids.

Summary. Cycloleucine administration to rats produces a generalized aminoaciduria which is largely the result of failure of renal tubular reabsorption. The greatest competition for renal tubular transport appears to be with the site for the dibasic amino acids.

1. Benefiel, W. W., Helsper, J. T., and Sharp, G. S., Cancer Chemotherapy Rept. **9**, 21 (1960).

2. Ross, R. B., Noll, C. I., Ross, W. C. J., Nadkarni, M. V., Morrison, B. H., Jr., and Bond, H. W., J. Med. Pharm. Chem. **3**, 1 (1961).

3. Goldin, A., Venditti, J. M., Kline, I., and Mantel, N., Cancer Res. **21**, 27 (1961).

4. Sterling, W. R. and Henderson, J. F., Biochem. Pharmacol. **12**, 303 (1963).

5. Liang, M., Irvin, J. L., and Wilson, J. E., J. Elisha Mitchell Sci. Soc. **81**, 25 (1963).

6. Brown, R. R., Science **157**, 432 (1967).

7. Knox, W. E., *in* "The Metabolic Basis of Inherited Disease" (J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, eds.), 2nd ed., pp. 1262–1282. McGraw-Hill, New York (1966).

8. Webber, W. A., Brown, J. L., and Pitts, R. F., Am. J. Physiol. **200**, 380 (1961).

9. Milne, M. D., Brit. Med. J. **1**, 327 (1964).

10. Clark, A. J., Matsutani, O., and Swenseid, M. E., Proc. Soc. Exptl. Biol. Med. **124**, 1093 (1967).

Received Sept. 23, 1968. P.S.E.B.M., 1969, Vol. 130.