scanner–photorecorder. Control of the processing of the film has indicated that a high degree of stability exists between the film records. A transfer characteristic from such a negative to the photometric target is shown in Fig. 23. The latitude of the film (SO-337) is so great that the entire video transfer characteristic may be recorded on the linear portion of the film.

As a preliminary check on the use of the camera as a photometer, the scene luminance was measured for parts of the lunar surface surrounding the pad upon which the photometric target was mounted. By fitting the measured scene luminance to the photometric function derived from the telescopic measurements of Fedorets (*5*), an estimate of 7.7 percent for the normal albedo was derived for the parts of the surface that appeared to be undisturbed by the pad (Fig. 9); the estimated albedo for the disturbed areas was about 2 percent lower. In general, the terrain exhibits the gross luminance values expected from the telescopically determined average of the photometric function of the maria. As further data are obtained in the lunar afternoon, the local normal albedo can be determined more precisely.

The light scattered from the spacecraft is a particular problem in evaluating the luminance of the lunar surface. Areas in the immediate vicinity of the spacecraft (within 2 m) are partly illuminated by light scattered from the spacecraft, especially at low Sun angles. The contribution of light from the spacecraft fills in the shadows to such a degree that an additional photometric target mounted on the B omniantenna was clearly discernible when the chart was averted from Sun.

Several color surveys were made with the three filters, beginning the 3rd day after touchdown; their main purpose was to ascertain whether or not there are color differences in the vicinity of the spacecraft; the data so obtained permit estimates to be made of the spectral reflectances. Again, to check and maintain the calibration of the camera-filter combination, the photometric target was observed with each filter before and after the survey (Fig. 15). Preliminary examination of the prominent mottled rock lying just southwest of the spacecraft (Fig. 21) indicates that any color differences that may be present in the surface of the rock are very small. Much more careful processing of the video data is necessary before subtle color differences can be measured.

Lunar Surface Electrical Properties

Five days after the touchdown of Surveyor I, the radar signal-strength data were still under detailed analysis to determine the average radar cross section in the vicinity of the landing site. The radar frequencies used by the spacecraft were 9300, 12,900, and 13,300 Mc/sec. If it proves possible to deduce the effective reflectivity, effort will be made to calculate some of the electrical characteristics of the lunar surface. No conclusions, however, are yet warranted.

References and Notes

1. R. W. Shorthill and J. M. Saari, "Isotherms in the equatorial region of the totally eclipsed Moon," Rept. DI-82-0530, Boeing Geo-Astrophysics Laboratory, Seattle, Wash., April 1966.
2. A. E. Wechsler and P. E. Glaser, in *The Lunar Surface Layer*, J. W. Salisbury and P. E. Glaser, Eds. (Academic Press, New York and London, 1964), pp. 389–410.
3. L. D. Jaffe, in *ibid.*, pp. 355–80.
4. H. Knudsen, Hughes Aircraft Co., suggested working from the outer-canister temperature on compartments A and B to establish lunar-surface temperatures; M. Gram, Jet Propulsion Lab., carried out the preliminary thermal analysis.
5. V. A. Fedorets, "Photographic photometry of the lunar surface," in *Reports of the Astronomical Observatory of the Charkow State University* (1952), vol. 2.

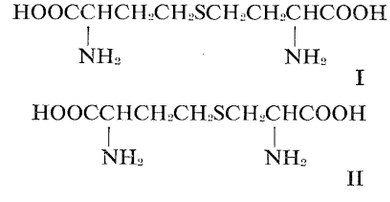
15 June 1966

Homolanthionine Excretion in Homocystinuria

Abstract. *Patients with homocystinuria excrete in their urine small amounts of an amino acid indistinguishable from authentic* L-*homolanthionine. This compound could be formed from homocysteine and homoserine by a condensation analogous to that normally leading to cystathionine. The only other known occurrence of homolanthionine in nature is in a methionine-requiring mutant strain of* Escherichia coli.

Mudd and coworkers (*1*) have shown that the basic metabolic defect in patients with homocystinuria is a lack of activity of the enzyme cystathionine synthase in the liver. This enzyme, which is normally present both in liver and in brain (*2*), condenses homocysteine and serine to form cystathionine. Homocystinurics show, at autopsy, an absence or virtual absence of cystathionine from the brain (*3*), and presumably cystathionine synthase activity is greatly reduced in their brain cells as well as in their livers.

Since in homocystinurics the major known degradative pathway of methionine metabolism is blocked at the step of cystathionine synthesis, these patients develop abnormally high blood and tissue concentrations of methionine and homocysteine, and both amino acids overflow into the urine. However, the total amounts of these sulfur amino acids and their known metabolites excreted in the urines of homocystinurics account for only a fraction of that portion of the daily dietary intake of methionine which is not utilized in protein synthesis (*4*). We have, therefore, searched for other ninhydrin-reactive, sulfur-containing metabolites in the urines of homocystinuric children in an effort to explain this discrepancy. We have identified one such metabolite as 5-amino-4-imidazolecarboxamide-5′-*S*-homocysteinylriboside (*5*). We now report tentative identification of another urinary metabolite as homolanthionine (I), a higher homolog of cystathionine (II).



I

II

A urine specimen containing 100 mg of creatinine was obtained from a 4-year-old mentally defective homocystinuric girl (D.S.). It was concentrated to a small volume by lyophilization, and the concentrate was applied to the surface of a column (45 by 2.16 cm) of Dowex 50 × 8, buffered with pyridine acetate at *p*H 3.50 (0.1*M* in pyridine). The same buffer was then pumped through the resin column at a flow rate of 50 ml per hour and at 50°C, and 10-ml fractions of effluent were collected. Appropriate fractions were pooled, and the volatile buffer was removed under reduced pressure on a rotary evaporator. On subsequent paper chromatography, the zone between 670 and 730 ml effluent volume contained a ninhydrin-positive compound which reacted positively to the chloroplatinate spray reagent (*6*). The unknown compound failed to react to sodium nitroprusside after prior exposure either to sodium cyanide or to methanolic NaOH. These reactions sug-

Table 1. Homolanthionine concentration (micromoles per gram of creatinine) in the urines of seven homocystinuric children from three unrelated families.

Patient	Age (yr)	Homolanthionine (μmole/g)
D. S.	4	140
Cr. S.	8/12	135
T. C.	10	104
E. C.	9	93
R. B.	17	67
S. B.	13	78
J. B.	11	81

gested that the compound was a thioether. The unknown compound was separated from other substances present in the effluent zone by chromatography on paper in two different solvents, and approximately 1 mg of chromatographically pure unknown was obtained.

The purified urinary compound has been tentatively identified as homolanthionine on the basis of the following criteria. Its mobility (R_F) was identical to that of authentic L-homolanthionine when chromatographed on paper in each of five different solvents. The R_F values were as follows: 0.18 in a mixture of pyridine, acetone, ammonium hydroxide, and water (45:30:5:20); 0.14 in a mixture of isopropanol, formic acid, and water (75:12.5:12.5); 0.14 in a mixture of *n*-butanol, acetic acid, and water (12:3:5); 0.11 in a mixture of 2-methyl-3-butyn-2-ol, formic acid, and water (75:5:20); 0.02 in a mixture of isopropanol, ammonium hydroxide, and water (8:1:1).

When the compound isolated from urine and authentic homolanthionine was chromatographed on the short column of the Technicon amino acid analyzer, in the buffer gradient system for protein-hydrolyzate analysis (*7*), both were eluted as single peaks 5 minutes after norleucine. When chromatographed on a long column (127 cm) on the analyzer, with the buffer system recommended by Efron (*8*), the urinary compound and authentic homolanthionine both were eluted as single peaks 14.5 minutes before norleucine. When sulfur was removed from the urinary compound and authentic homolanthionine with Raney nickel (*9*), in each case only one amino acid that did not contain sulfur was produced. This was α-aminobutyric acid as shown by paper chromatography and its characteristic elution time on the amino acid analyzer.

Removal of sulfur with Raney nickel should yield α-aminobutyric acid alone, not only from homolanthionine but also from two other closely related thioethers, β,β-dimethyllanthionine and β-methylcystathionine. These two compounds were not available for comparison by paper and ion-exchange chromatography with the substance isolated from homocystinuric urine. It is most unlikely that the urinary compound could be β,β-dimethyllanthionine, since this thioether is not a derivative of homocysteine. The possibility does exist that the urinary compound might be β-methylcystathionine, the thioether which would result from a condensation of homocysteine and threonine.

The optical rotatory dispersion curves of the urinary compound and of authentic L-homolanthionine were very similar when measured in aqueous solution in a Jasco Model ORD/UV-5 instrument at wavelengths between 208 and 240 mμ. Due to the very small amounts of authentic L-homolanthionine and of the purified urinary compound available, aqueous solutions were too dilute to yield measurable values at wavelengths greater than 240 mμ. For authentic L-homolanthionine $[\alpha]_{210}^{26\text{-}30}$ was 2020, and it was 1800 for the compound isolated from urine. These observations are consistent with the identification of the latter as L-homolanthionine. Final proof that the urinary compound is L-homolanthionine must await a synthesis of β-methylcystathionine and a demonstration that this substance has different chromatographic or optical rotatory properties from the compound we have isolated from homocystinuric urine.

A search was then made for homolanthionine in the urines of seven homocystinuric children by means of chromatography on the amino acid analyzer. It was necessary to use the short-column system (*7*) when the original urines were chromatographed, since in the long-column system (*8*) homolanthionine emerges at the same point as leucine, which is normally present in small amounts in urine. The patients belonged to three unrelated families, and the clinical details have been described (*10*). Each of the seven homocystinurics excreted presumed homolanthionine in quantities varying from about 0.07 to 0.14 mmole per gram of creatinine (Table 1).

The only other occurrence of homolanthionine in a living system was reported by Huang (*9*), who found that this amino acid was accumulated by cells of a methionine-requiring mutant of *Escherichia coli*. He isolated L-homolanthionine from the fermentation broth of submerged cultures of this mutant, and considered it a by-product formed by the cells from L-homocysteine and L-homoserine, as a result of an enzymatic block preventing the normal synthesis of methionine from homocysteine.

Homolanthionine presumably is formed in human homocystinuria by a condensation of homocysteine and homoserine, analogous to the condensation of homocysteine and serine to form cystathionine occurring in normal individuals. Possibly a second condensing enzyme is present in man, with homolanthionine formation taking place only when tissue concentrations of homocysteine become abnormally high. An attractive alternative is that homocystinurics do not suffer from an absence of cystathionine synthase, but rather have an altered form of the enzyme which has greater activity for presence of homoserine than for serine, its normal substrate.

If homolanthionine results from the condensation of homocysteine and homoserine, the source of the latter requires explanation. Homoserine is formed as an intermediate in the biosynthesis of threonine from aspartic acid in bacteria and plants, but not in mammalians. There is evidence, however, for the formation of α-keto-γ-hydroxybutyric acid from pyruvate and formaldehyde in mammalian liver, and transamination of this α-keto acid would result in the production of homoserine (*11*).

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References and Notes

1. S. H. Mudd, J. D. Finkelstein, F. Irreverre, L. Laster, *Science* **143**, 1443 (1964); J. D. Finkelstein, S. H. Mudd, F. Irreverre, L. Laster, *ibid.* **146**, 785 (1964); S. H. Mudd, J. D. Finkelstein, F. Irreverre, L. Laster, *Biochem. Biophys. Res. Commun.* **19**, 665 (1965).
2. S. H. Mudd, J. D. Finkelstein, F. Irreverre, L. Laster, *J. Biol. Chem.* **240**, 4382 (1965).
3. T. Gerritsen and H. A. Waisman, *Science* **145**, 588 (1964); D. P. Brenton, D. C. Cusworth, G. E. Gaull, *Pediatrics* **35**, 50 (1965).
4. L. Laster, S. H. Mudd, J. D. Finkelstein, F. Irreverre, *J. Clin. Invest.* **44**, 1708 (1965).
5. T. L. Perry, S. Hansen, H. P. Bär, L. MacDougall, *Science* **152**, 776 (1966).

6. I. Smith, *Chromatographic and Electrophoretic Techniques* (William Heinemann, London, 1960), vol. 1, p. 98.
7. Technicon Chromatography Corporation, Chauncey, N.Y., technical bulletin (1965).
8. M. Efron, personal communication (1965).
9. H. T. Huang, *Biochemistry* **2**, 296 (1963).
10. T. L. Perry, H. G. Dunn, S. Hansen, L. MacDougall, P. D. Warrington, *Pediatrics* **37**, 502 (1966); H. G. Dunn, T. L. Perry, C. L. Dolman, *Neurology* **16**, 407 (1966).
11. A. Meister, *Biochemistry of the Amino Acids* (Academic Press, New York, 1965), vol. 2, p. 678.
12. Supported by grants from the Medical Research Council of Canada. We thank Dr. H. T. Huang, International Minerals & Chemical Corporation, Wasco, California, for a sample of authentic L-homolanthionine; Dr. M. Darrach and R. Craybill for the optical rotatory dispersion determinations; P. D. Warrington for technical assistance; and Drs. J. G. Foulks and J. P. Kutney for helpful discussions.

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Lysosomal Nature of Juxtaglomerular Granules

Abstract. *Demonstration by a combined histochemical and electron-microscopic technique of the ultrastructure of and acid phosphatase within the juxtaglomerular granules in kidneys of normal man and rat and in those from members of both species with renal hypertension indicate the identity of juxtaglomerular granules as lysosomes.*

Hypertension produced by decreased or altered renal blood flow is associated with an increase in pressor substance in the kidneys and blood (*1*). Correlation of renal pressor activity with the number of granules in renal juxtaglomerular cells (JGC) (*2*), identification of renin by immunofluorescence (*3*), and the location of pressor activity in juxtaglomerular granules (JGG) (*4*) suggest that renin is associated with JGG. Although the function of the JGC is influenced by blood volume, by electrolyte concentrations, and perhaps by cells of the macula densa of the distal nephron and unmyelinated nerve fibers within this cellular complex, little is known concerning the synthesis or release of renin.

Study of the ultrastructure of JGC of ischemic renal tissue obtained by needle biopsy from six patients with renal hypertension, and from a similar number of rats after unilateral renal artery constriction (*5*), revealed abundant electron-opaque JGG enclosed within solitary, smooth limiting membranes, as well as within saccules of the Golgi apparatus. Paracrystalline forms of JGG and lipofuscin droplets were prominent in JGC of man, but not in those of the rat. In both species, the endoplasmic reticulum, principally of the smooth variety, was frequently dilated. Its matrix was often comprised of flocculent, slightly electron-opaque material. The ultrastructural appearance of JGG, the relationship of these granules to Golgi structures, the presence of lipofuscin within the cells, and the proteolytic nature of renin prompted inquiry into the lysosomal nature of the granules.

Renal biopsies were performed on two patients: (i) a 50-year-old man with renal hypertension and (ii) a 45-year-old normotensive man. In addition, ischemic kidneys from five rats with hypertension (160 mm-Hg) induced by unilateral renal artery constriction and a similar number of normotensive rats were examined. A combined histochemical and electron-microscopic method for identification of acid phosphatase activity was used (*6*), except that tissue was first fixed in cold 8-percent glutaraldehyde in sodium cacodylate, *p*H 7.4, for 3 hours at 4°C.

The reaction product for acid phosphatase appeared as electron-opaque deposits within many limiting membranes as well as within the matrix of mature JGG, within occasional Golgi lacunae, and around lipofuscin droplets in JGC of all tissues examined; the deposit was most conspicuous in JGC of kidneys from the man and rats with hypertension (Figs. 1 and 2). No precipitate was noted in control sections incubated in buffer and lead nitrate in the absence of glycerophosphate substrate.

These observations appear unrelated to those of Finegan (*7*), who, using light-microscopic techniques, observed alkaline phosphatase in some cells of the juxtaglomerular apparatus lying between JGC and the macula densa. Of the many species that were examined, only the rat had demonstrable alkaline phosphatase in its kidney, and the functional significance of the enzyme was uncertain.

Although caution is theoretically warranted in regarding as lysozymes all particles which contain acid phosphatase, the presence of the enzyme within granules or vacuoles that are limited by a solitary, smooth membrane conforms with the biochemical and morphological definition of these subcellular particles. Accumulating evidence indicates the lysosomal nature of secretory vacuoles in a variety of tissues (*8*). The demonstration of acid phosphatase within Golgi lacunae of JGC indicates a source for the acid phosphatase (and perhaps other lysosomal enzymes) in JGG. This interpretation is consistent with the general hypothesis which identifies the

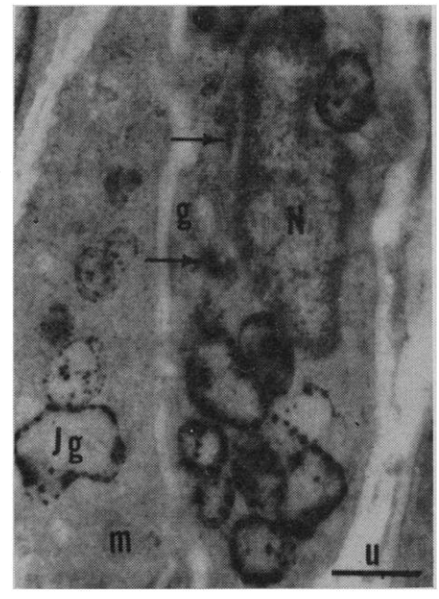

Fig. 1. Reaction product of acid phosphatase in limiting membrane and matrix of juxtaglomerular granules (*Jg*) of portions of two juxtaglomerular cells of ischemic kidney from hypertensive rat. Reaction is also evident in portions of Golgi (*g*), indicated by arrows, but not in the nucleus (*N*) or mitochondria (*m*).

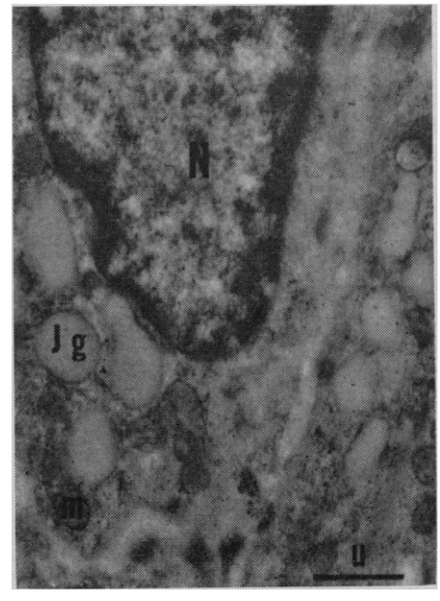

Fig. 2. Portions of juxtaglomerular cells from a control section of the kidney depicted in Fig. 1, incubated in buffer and lead nitrate without substrate. No reaction product is evident within juxtaglomerular granules (*Jg*). The nucleus (*N*) appears larger than in Fig. 1 due to plane of section.