

the number of functioning renal tubules varies directly with the filtration rate (9). Thus the filtration rate may be reduced by the closing of a number of glomeruli. When a glomerulus is closed the tubule collapses, and transport across its epithelium ceases. Functioning normal nephrons remain open, and fluid transport takes place across the tubular epithelium.

Among the reptiles investigated the blue-tongued lizard showed the widest variations in the filtration rate: from 0.7 ml/hr per kilogram of body weight, during dehydration, to 25 ml/hr per kilogram of body weight during water-loading (Table 1). In the saltwater crocodile a water load similarly increased the filtration rate, whereas in the freshwater crocodile little variation was seen in the filtration rate as a result of dehydration or water-loading. A survey of many sections from kidneys of all the reptiles in different physiological conditions showed a rough correlation between the number of open to closed tubular lumina and the glomerular filtration rate.

In the tubules with open lumina from normal, dehydrated, and salt-loaded lizards, the intercellular spaces were also open, but they exhibited different forms (Fig. 1, a and b; Fig. 2). Sometimes the space was widely expanded from the tight junction of the terminal bar to the base of the cell; in other tubules part of the space was closed. No particular shape could be correlated with the physiological conditions. In the tubules with closed lumina the intercellular spaces were closed. In the ouabain-treated lizard, in which active sodium transport presumably was stopped, most proximal tubules had open lumina, but the intercellular spaces were tightly closed. In summary, in proximal tubules the intercellular spaces were closed when the transport was stopped either by no filtration or by poisoning of the sodium pump with ouabain. They were open whenever fluid transport across the epithelium took place.

Urine osmolality varies relatively little in reptiles. Their kidneys do not function as countercurrent multiplier systems, and reptiles can therefore only excrete a urine that is either hypoosmotic or isosmotic to the blood (10). The osmolality of the urine is regulated by the distal tubules and the cloaca. In our experiments urine was collected directly from the ureters, and it is therefore the distal tubular function that has determined its osmolality. In the blue-

tongued lizard, the urine osmolality varied from 43 percent to 79 percent of the blood osmolality. This means that the resorbed fluid was hyperosmotic to the tubular fluid in the water-loaded lizard, but more closely approximated the osmolality of the tubular fluid in the dehydrated lizard. The osmolality varied over a similar range in *Crocodylus porosus* and *C. johnstoni* and varied in both of them more than in *Crocodylus acutus* (Table 1).

The intercellular spaces of the distal tubular cells appeared in many different shapes (Fig. 1, c and d; Fig. 3). The width of the spaces could not be related to the rate of fluid resorption, but, when transport was stopped completely, two-thirds of the apical part of the spaces were closed. This was the case in tubules with closed lumina from normal and dehydrated animals, and in tubules with open lumina from animals treated with ouabain. The shape of the spaces also seemed conspicuously related to the osmolality of the urine. In the water-loaded lizard when the urine osmolality was 45 percent of the blood osmolality the spaces were tightly closed from the terminal bar to the basal lamina (Fig. 3).

Thus, the intercellular spaces in distal as well as proximal renal tubules of reptiles appear to be open when water and solute are resorbed in about the same concentrations as in the lumen. These spaces however are closed when no transport takes place, as in tubules with closed lumina or in tubules poisoned with ouabain. When the urine is significantly hypoosmotic to the blood (when the resorbed fluid is hyperosmotic to the luminal fluid) the spaces between the distal tubular cells are closed apically, or all the way to the basal lamina. This

finding seems to contradict the predictions by Diamond and Bossert (11) that, when the resorbed fluid is hyperosmotic, the spaces should be wide and short whereas an isosmotic resorbed fluid should require long and narrow intercellular spaces. The findings, however, could be consistent with the predictions that active transport into the basal parts of the intercellular spaces makes the resorbed fluid more hyperosmotic.

BODIL SCHMIDT-NIELSEN
LOWELL E. DAVIS

Department of Biology,
Case Western Reserve University,
Cleveland, Ohio

References and Notes

1. P. F. Curran, *J. Gen. Physiol.* **43**, 1137 (1960); J. M. Diamond, *ibid.* **48**, 1 (1964); P. F. Curran, *Fed. Proc.* **24**, 993 (1965).
2. G. I. Kaye, H. O. Wheeler, R. T. Whitlock, N. Lane, *J. Cell Biol.* **30**, 237 (1966); J. M. Tormey and J. M. Diamond, *J. Gen. Physiol.* **50**, 2031 (1967); J. M. Diamond and J. M. Tormey, *Nature* **210**, 817 (1966).
3. L. E. Davis and B. Schmidt-Nielsen, *J. Morphol.* **121**, 255 (1967).
4. T. Olsen, *Nature* **212**, 95 (1966).
5. M. J. Karnovsky, *Exp. Mol. Pathol.* **2**, 347 (1963).
6. J. A. G. Rhodin, *An Atlas of Ultrastructure* (Saunders Philadelphia, 1963); in *Diseases of the Kidney*, M. B. Straus and L. G. Welt, Eds. (Little, Brown, Boston, 1963).
7. B. Schmidt-Nielsen and E. Skadhauge, *Amer. J. Physiol.* **212**, 973 (1967).
8. Analyses of NH_4^+ , Na^+ , K^+ , and Cl^- were also made on the blood and urine samples.
9. R. P. Forster, *J. Cell. Comp. Physiol.* **20**, 55 (1942); B. Schmidt-Nielsen and R. P. Forster, *ibid.* **44**, 233 (1954); W. H. Dantzler and B. Schmidt-Nielsen, *Amer. J. Physiol.* **210**, 198 (1966).
10. B. Schmidt-Nielsen, in *Handbook of Physiology*, D. B. Dill and E. F. Adolph, Eds. (American Physiological Society, Washington, D.C., 1964), section 4, pp. 215-243.
11. J. M. Diamond and W. H. Bossert, *J. Gen. Physiol.* **50**, 2061 (1967).
12. The experimental work was carried out on board R.V. *Alpha Helix* at the Flinders Island Group off the coast of North Queensland, Australia. Supported by NIH grant AM 09975-02. We thank Dr. P. F. Scholander, leader of the "Billabong" expedition, for making this work possible.

28 November 1967

Synthesis of a Sulfur-Containing Amino Acid under Simulated Prebiotic Conditions

Abstract. *Ultraviolet irradiation of an aqueous solution of ammonium thiocyanate produces the sulfur-containing amino acid methionine. Synthesis of this class of biocompound fills another important gap in development of an overall picture of how prebiological chemistry may have evolved on primitive Earth.*

The primordial appearance of nearly all classes of biomonomers can now be accounted for on the basis of several experiments carried out under hypothetical conditions on primitive Earth (1). However, one very important group that has persistently resisted synthesis thus far is the naturally oc-

curing sulfur-containing amino acids (2). Electron-beam irradiation of a gaseous mixture of ammonia, methane, water, and H_2S has led to isolation and identification of the nonproteinaceous, oxidized amino acids taurine, cysteic acid, and cystamine (3). [The use of H_2S in a "primitive atmosphere" is rea-

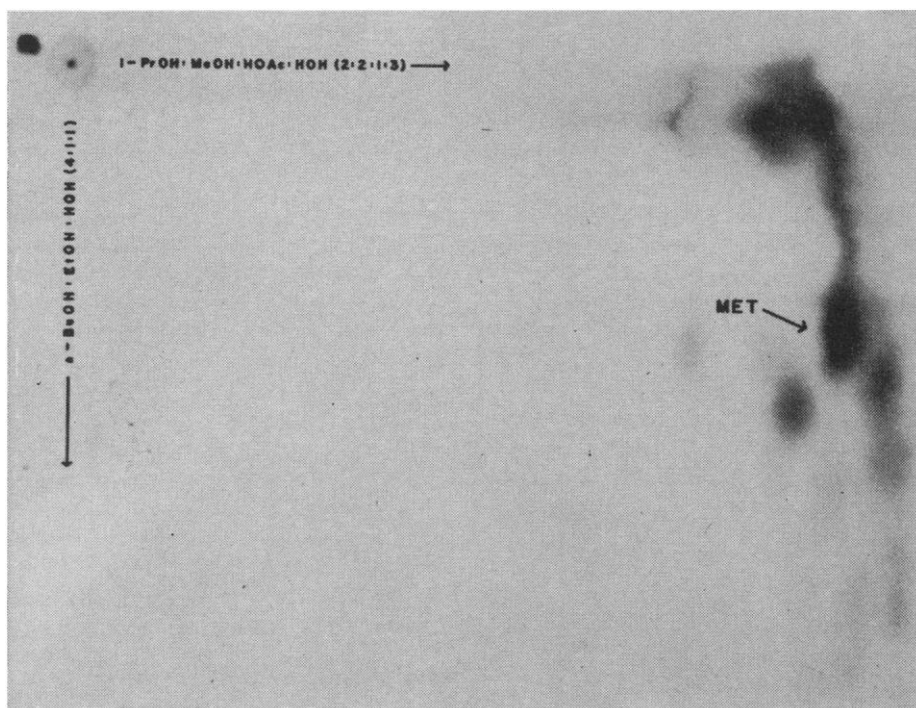


Fig. 1. Autoradiogram of a chromatogram exhibiting methionine synthesized by ultraviolet irradiation of an aqueous solution of ^{14}C -labeled ammonium thiocyanate.

sonable since this compound is found in the juvenile exhalations of volcanoes (4).] When the same gaseous reactant mixture was sparked, ammonium thiocyanate was produced (5). Similarly, the combination of CO_2 , NH_3 , and H_2S in volcanoes reportedly results in production of ammonium thiocyanate (see 6).

Ammonium cyanide, activated by heat or ultraviolet light, yields amino acids and peptides (7). Also, incubation of aqueous solutions of ammonium thiocyanate and formaldehyde reportedly produces amino acids, although none has been identified as a sulfur-containing residue (8).

In a study of the supermolecular morphogenicity of ammonium thiocyanate in the presence of ultraviolet light (9), an examination was made for the possible concurrent synthesis of a sulfur-containing amino acid. In a typical experiment, an aqueous 0.1M solution of NH_4SCN was irradiated for 3 hours with a submerged Pen-Ray Quartz UV lamp (10). The thiocyanate was labeled with ^{14}C , and its radio-purity was confirmed by paper chromatography and autoradiography. The reaction vessel was placed in an ice-water bath during the irradiation. The product was hydrolyzed in 6N HCl for 16 hours in a sealed ampoule under N_2 . A cloudiness found in the irradiated product disappeared upon hydrolysis.

The hydrolyzate was divided into three parts:

1) The first was chromatographed (Whatman No. 1 paper), in parallel with standards of several amino acids, with a 4:1:5 (organic phase) mixture of *n*-butanol, acetic acid, and H_2O as solvent. With autoradiography to locate the labeled compounds and ninhydrin for the standards, one of the products of irradiation exhibited an R_F value very similar to that of methionine in particular (standard R_F , 0.41; experimental R_F , 0.39).

2) The chromatographic procedure was repeated on the second portion of hydrolyzed product, with a 4:3:2:1 mixture of *t*-butanol, methylethylketone, water, and concentrated NH_4OH as solvent. Again there was good coincidence with methionine (standard R_F , 0.74; experimental R_F , 0.75).

3) The third portion of hydrolyzate was mixed with standard methionine and paper-chromatographed in two dimensions: (i) a 4:1:1 mixture of *n*-butanol, ethanol, and H_2O ; and (ii) a 2:2:1:3 mixture of *i*-propanol, methanol, acetic acid, and H_2O . The identity of the unlabeled standard amino acid was confirmed by elemental analysis (theory: C, 40.3; H, 7.4; N, 9.4; S, 21.4; found: C, 40.8; H, 7.2; N, 9.2; S, 20.4). Coincidence of position and shape was detected between a labeled product and the standard methionine

(Fig. 1). This observation substantiated the conclusion that this sulfur-containing amino acid had indeed been produced. The use of labeled reactant eliminated the possibility that any products might in fact be contaminants introduced during synthesis or analysis.

Although the yield was low in this synthesis (less than 1 percent), greater quantities might result from longer irradiation if decomposition did not prove to be a significant factor. The inability of other investigators to observe this amino acid probably reflects its presence in amounts lower than the levels of detectability by the analytical methods employed and the quantity and nature of the reactants used.

Results from related studies (7) suggest two possibilities:

1) Polymers of amino acid precursors may be produced by polymerization of cyanide.

2) The cyanide may act both as reactant for amino acid monomer synthesis and as the condensing agent for the subsequent dehydration coupling of the residues.

The cloudiness observed in our experiments after irradiation may have been due either to true polypeptides or to polyamino acid precursors. In either case, methionine monomer would be expected only after hydrolysis.

GARY STEINMAN

Department of Biochemistry,
Pennsylvania State University,
University Park 16802

ADOLPH E. SMITH

JOSEPH J. SILVER

Physics Department, Sir George
Williams University, Montreal, Canada

References and Notes

1. J. Oro, *Ann N.Y. Acad. Sci.* **108**, 464 (1963) (a review); D. H. Kenyon and G. Steinman, *Biochemical Predestination* (McGraw-Hill, New York, in press).
2. G. Steinman, *Science* **154**, 1344 (1966).
3. A. S. U. Choughuley and R. M. Lemmon, *Nature* **210**, 628 (1966).
4. A. P. Vinogradov, in *The Origin of Life on the Earth*, A. I. Oparin, Ed. (Pergamon, New York, 1959), p. 23.
5. K. Heyns, W. Walter, E. Mayer, *Naturwiss.* **44**, 385 (1957).
6. A. Gautier, *Compt. Rend.* **132**, 932 (1901); **150**, 1564 (1910).
7. J. Oro and S. S. Kamat, *Nature* **190**, 442 (1962); C. V. Lowe, M. W. Rees, R. Markham, *ibid.* **199**, 219 (1963); C. N. Matthews and R. E. Moser, *Proc. Nat. Acad. Sci. U.S.* **56**, 1087 (1966); M. Labadie, R. Jensen, E. Neuzil, *Bull. Soc. Chim. Biol.* **49**, 46 (1967); P. H. Abelson, *Proc. Nat. Acad. Sci. U.S.* **55**, 1365 (1966).
8. A. L. Herrera, *Science* **96**, 14 (1942).
9. A. E. Smith, J. J. Silver, G. Steinman, *Experientia* **24**, 36 (1968); in preparation.
10. Model SCT-1; Ultraviolet Products.
11. Aided by NASA grant NGR-39-009-015 and the National Research Council of Canada.

15 January 1968