The estimates of activities were made by comparison of the 50 per cent. inhibition concentrations for treated and untreated preparations assayed simultaneously. Under these conditions, 50 per cent. inhibition was achieved by approximately 0.003 ppm of gramicidin or modified gramicidin, a concentration considerably lower than the solubility of these substances. The modified gramicidin gave a steeper dosage response curve than did gramicidin.

In earlier tests with an 18-hour incubation period, the formaldehyde-treated gramicidin was 200 to 400 per cent. more active than gramicidin itself. Subsequently, it was found that this increase was apparently a reflection of the increased solubility of the derivative, since the 50 per cent. inhibition point of the unmodified gramicidin under these conditions was approximately 1 ppm and thus above the solubility range (see below).

The solubilities were estimated as follows: Suspensions of excess gramicidin and its derivative in "medium II" were shaken for two days and permitted to stand for an additional five days. The solutions were filtered and compared in antibacterial activity with solutions of known concentration. By this technique, gramicidin and its derivative were found to be soluble to the extent of approximately 0.4 and 1.4 ppm, respectively.

This increase in water solubility may, in itself, be an advantageous modification, inasmuch as the slow rate of diffusion of gramicidin has appeared to limit its usefulness. For example, Hotchkiss reports that experimental intraperitoneal pneumococci infections could be satisfactorily controlled by gramicidin injected intraperitoneally but not through other routes of administration.[1]

Products of decreased hemolytic activity were obtained from several lots of pure gramicidin and from various crude preparations. The latter were (1) the material that could be extracted from tyrothricin[7] with 50 per cent. acetone-ether, (2) once-crystallized gramicidin prepared from this preparation, and (3) a product from which tyrocidine and other basic substances had been removed by precipitation with phosphotungstic acid. Gramicidin contaminated with tyrocidine, as obtained by the acetone-ether extraction, was not reduced in hemolytic activity to the same extent by formaldehyde as were the other preparations. This reduction could be achieved by a repetition of the formaldehyde treatment.

Gramicidin is distinguished among proteins and polypeptides by containing approximately 40 per cent. tryptophane, but no polar groups known to react reversibly with formaldehyde, such as basic or amide groups.[3] Ross and Stanley[8] and Kassanis and Kleczkowski[9] have shown that the chromogenic property of tryptophane in the Folin reaction is decreased during formaldehyde treatment. Baudouy[10] suggested that proteins containing tryptophane and histidine form complexes with formaldehyde from which the latter can not be recovered. The mode of reaction of formaldehyde with the indole residues of gramicidin and proteins will be described in another place.

The observation that the hemolytic and toxic activity of gramicidin can be lowered without decrease of its antibacterial properties demonstrates that these functions are not necessarily dependent upon the same molecular configuration. In this respect, the formaldehyde reaction with gramicidin is analogous to that with bacterial toxins, in which case the toxicity but not the antigenic activity is reduced.

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THE FREE TRYPTOPHANE CONTENT OF HUMAN URINE*

DURING studies on the metabolism of tryptophane by the rat, samples of human urine were used in standardizing the microbiological method which was being used for the estimation of tryptophane. It was immediately evident that tryptophane figures much lower than the average figure of 281 mg per day reported by Albanese and Frankston[1] were obtained. The results of a more extensive study on the tryptophane content of human urine are reported in this paper.

EXPERIMENTAL

Twenty-four-hour collections of urine were made from nine subjects who consumed average diets. The total urine volume was noted, toluene added as a preservative, and the samples stored in a refrigerator until the following day.

[7] We are indebted to Wallerstein Laboratories for a generous supply of tyrothricin and gramicidin.

[8] A. F. Ross and W. M. Stanley, *Jour. Gen. Physiol.*, 22: 165, 1938.

[9] B. Kassanis and A. Kleczkowski, *Biochem. Jour.*, 38: 20, 1944.

[10] Baudouy, *Compt. rend.*, 214: 692, 1942.

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[1] A. A. Albanese and J. E. Frankston, *Jour. Biol. Chem.*, 157: 59, 1945.

The tryptophane basal medium used for the microbiological assay was the same as that used in the nicotinic acid assay,[2] except that tryptophane was omitted and nicotinic acid was added at a level of 4 μg per tube. *L. arabinosus* was used as the test organism and the standard curve was obtained for a range of 0–25 μg of l-tryptophane per tube. This standard is prepared from a stock solution containing 100 μg/cc of l-tryptophane. No detectable loss in tryptophane activity has been observed when stock solutions have been stored for a period of 6 months at refrigerator temperature (toluene added as a preservative). This has been found by comparing the stability of the stored stock solutions with freshly prepared solutions. In all other respects the assay procedure was identical to that described by Schweigert *et al.*[3]

The urine was diluted with nine volumes of water and the diluted urine was added at levels of 0.5, 1.0, 2.0 and 3.0 cc per tube with duplicate tubes at the 1.0 and 2.0 cc levels. Excellent checks were obtained at all levels tested. Suitable portions of eight collections were extracted with an equal volume of ethyl ether in order to remove any indole present, since indole has appreciable tryptophane activity for *L. arabinosus*.[4,5,6] The results obtained before and after ether extraction are given in Table 1 as the number of mg of l-tryptophane excreted per day.

Due to the fact that the urea in urine may have inhibited the organism, a solution containing 2 μg of l-tryptophane and 6 mg of urea per cc was prepared. Since a 1–10 dilution of the urine samples was used the urea content of the tryptophane-urea solution would be equivalent to 60 mg per cc of undiluted urine. This amount of urea is in excess of the average amount (25 mg/cc) reported by Hawk and Bergeim[7] to be present. The growth response produced by the solution of urea and tryptophane was compared over a 10-fold range with an l-tryptophane solution of the same concentration. No inhibition could be detected at any of the points on the entire curve.

Six recoveries of tryptophane were carried out to further check the reliability of the method used. Two μg of l-tryptophane were added per tube in the presence of 4 different levels of the diluted sample of urine. The following recoveries were obtained: 101, 95, 100, 96, 101 and 100 per cent. of the added tryptophane.

The amount of l-tryptophane excreted per day by 9 individuals ranged from 12.6 to 30.5 mg per day. The values obtained for some of the same subjects for different collection periods also are within this range (Table 1). The amount of tryptophane activity due to the indole content of urine was found to be negligible. Albanese and Frankston[1] also reported that little of the activity was due to the indole present in the urine samples. The results on assays performed a week later on samples of urine stored as described earlier were comparable to those obtained when the tryptophane determinations were carried out immediately after collection. This indicates that samples of urine may be stored for short periods if necessary prior to tryptophane analysis.

TABLE 1

AMOUNT OF FREE TRYPTOPHANE EXCRETED IN THE URINE BY NORMAL MALE SUBJECTS

Subject and collection no.	mg/day of l-tryptophane excreted: Direct analysis	mg/day of l-tryptophane excreted: After ether extraction	Subject and collection no.	mg/day of l-tryptophane excreted: Direct analysis	mg/day of l-tryptophane excreted: After ether extraction
A–1	16.0	15.2	C–2	24.0	...
A–2	12.6	13.3	D–1	20.8	19.9
A–3	16.1	...	E–1	25.8	24.3
B–1	21.0	20.7	F–1	13.1	12.9
B–2	30.3	28.9	G–1	13.1	12.1
B–3	18.3	...	H–1	30.2	...
C–1	30.5	...	I–1	15.5	...

DISCUSSION

The values obtained (Table 1) are approximately one tenth of those reported by Albanese and Frankston.[1] They also reported that the amount of tryptophane excreted was roughly proportional to the body weight of the individual. Our data show no correlation between body weight and tryptophane excretion.

The reasons for these differences are not readily apparent, although one of the major factors may be that different methods were used for the estimation of tryptophane. *L. arabinosus* does not respond to d-tryptophane and it is possible that an appreciable amount of this isomer is excreted in the urine. If this is true, the chemical method would give high values, since both the d- and l-isomers are measured by this method. Other indole containing compounds than those tested by Albanese and Frankston may be excreted in the urine and measured as tryptophane by their method. Any tryptophane or indole derivatives present in combination with other molecules that are excreted in the urine probably would possess little

[2] W. A. Krehl, F. M. Strong and C. A. Elvehjem, *Ind. and Eng. Chem.*, Anal. Ed., 11: 346, 1943.

[3] B. S. Schweigert, J. M. McIntire, C. A. Elvehjem and F. M. Strong, *Jour. Biol. Chem.*, 155: 183, 1944.

[4] R. D. Greene and A. Black, *Proc. Soc. Exp. Biol. and Med.*, 54: 332, 1943.

[5] Unpublished data.

[6] E. E. Snell, *Arch. of Biochem.*, 2: 389, 1943.

[7] P. B. Hawk and O. Bergeim, "Practical Physiological Chemistry," 11th edition, p. 600. The Blakiston Company. 1944.

or no activity for *L. arabinosus* and may be measured by the chemical method. The microbiological method is undoubtedly more specific for l-tryptophane.

SUMMARY

The amount of l-tryptophane excreted per day by 9 different normal male subjects was determined by a microbiological method with *L. arabinosus* as the test organism. From 12–30 mg per day were excreted as contrasted to an average value of 281 mg per day reported by Albanese and Frankston. A negligible amount of indole in the urine could be demonstrated. Satisfactory values and recoveries of l-tryptophane were obtained at different levels of the test sample.

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THE ELECTRON MICROGRAPHY OF CRYSTALLINE PLANT VIRUSES[1]

IT has recently been shown that metal shadow-casting[2] greatly facilitates the electron microscopic study of elementary particles of viruses[3] and of macromolecular substances.[4] Using these techniques we have been examining preparations of purified plant viruses known to form well-defined crystals and we find it possible to record the orderly way in which their particles deposit from solution. Evidently with such a direct method of observation one can approach many fundamental problems of crystal formation that have heretofore been inaccessible to investigation.

Electron micrographs have been made with an RCA Type EMB instrument of ultracentrifugally purified viruses of tomato bushy stunt,[5] tobacco necrosis[6] and southern bean mosaic[7] diseases. Their preparations behave similarly but not in all ways alike, and each has shown ordered arrangements of its particles as seen under the electron microscope. Fig. 1 is a typical photograph of southern bean mosaic virus. The specimen was made by allowing an aqueous suspension of the purified virus to evaporate on the usual collodion-covered screen, which was then shadowed at a five-to-one angle with 8A of gold. The particles seen in this figure, unlike those in such a non-crystalizable substance as limulus hemocyanin[4] are for the most part closely packed in an ordered array. In some places the array is only one layer deep and can be thought of as a two-dimensional crystal, at other points these ordered layers are stacked one above another to produce the three-dimensional regularity that is true crystallinity. Other photographs show the same regularity continuing and becoming more exact as the number of layers of particles increases; well-defined crystal faces and edges have developed on the thicker preparations.

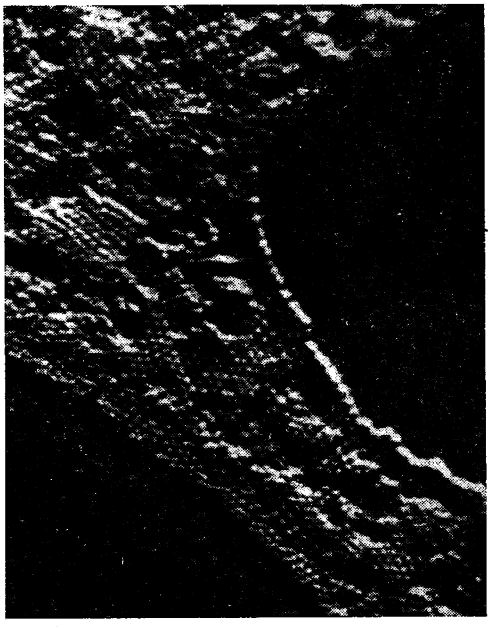

FIG. 1. A shadowed electron micrograph of elementary particles of southern bean mosaic virus showing their ordered arrangement. Magnification is about 58,000 times.

The bushy stunt and tobacco necrosis viruses display similar but not identical regular arrangements of their particles. The bushy stunt virus particles, for instance, have a greater tendency to cover the entire substrate before piling up into three-dimensional aggregates and their two-dimensional patterns have a higher symmetry than that shown by the bean mosaic virus. This accords well with the fact that bean mosaic virus crystals are orthorhombic[7] when developed to ordinary microscopic size, while the bushy stunt virus can form crystals with cubic symmetry.[5] It would appear that in these instances at least we can directly see how the symmetry of a crystal is related to, and determined by, the "shape" of its constituent elementary particles.

[1] From the Department of Physics, from the Virus Laboratory, Department of Epidemiology, School of Public Health, University of Michigan, and from the Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J. Aided in part by a grant from the National Foundation for Infantile Paralysis, Inc.

[2] R. C. Williams and R. W. G. Wyckoff, *Jour. Applied Physics*, 15: 712, 1944.

[3] *Idem, Proc. Soc. Exp. Biol. and Med.*, 58: 265, 1945; SCIENCE, 101, 594, 1945.

[4] R. C. Williams and R. W. G. Wyckoff, *Nature*, in press.

[5] F. C. Bawden and N. W. Pirie, *Brit. Jour. Exp. Path.*, 19: 251, 1938.

[6] W. C. Price and R. W. G. Wyckoff, *Phytopathology*, 29: 83, 1939.

[7] W. C. Price, SCIENCE, 101: 515, 1945.