knowledge resulted from studies of hull bran, cottonseed proteins, nutritional properties of cottonseed meal, cotton line, and linters. Through the agency of the institute the foundation established research fellowships at the University of Texas, Texas Technological College, and the University of Tennessee, besides sustaining an investigation at the University of North Carolina and a fellowship on cellulose economics at the University of Pittsburgh.

The multiple fellowship on food varieties reached the full technological development of a line of special foods for the feeding of children beyond the strained foods age. The technical glassware fellowship developed methods and apparatus to evaluate "surface hardness" of glass. The heat insulation fellowship assisted in acquiring technical information on fireproof constructional materials and on all-asbestos insulating air ducts. "Kemite" and "Karcite," laboratory constructional materials, were further studied by another fellowship, with special attention to new raw materials, development of additional properties, and novel applications.

The industrial fellowship on meat merchandising, after three and a half years of research, attained the development to commercial status of a new process making an improvement in the palatability of beef through increase in tenderness and juiciness. The natural gas fellowship studied major problems pertaining to conditions of the distribution and utilization of this fuel. The multiple fellowship on organic synthesis entered its twenty-fifth year of continuous, creative activity. On the multiple fellowship on petroleum refining several new devices were evolved for quick and accurate measurements of physical constants of oil hydrocarbons. The pressing machinery fellowship announced a new, safer and purer type of petroleum naphtha and also improvements in filters and stills for purifying the liquids used in the dry-cleaning industry. The multiple fellowship on protective coatings progressed in investigations of the fundamental structure and properties of organic resin films on metals and other surfaces.

Twenty-two years of age, the multiple fellowship on refractories solved the riddle of "mottled" silica brick. The mineral products fellowship designed and supervised the erection of a large plant for manufacturing "Garspar," a new ceramic raw material, and developed two other silica products, "Garbond" and "Gartex." The multiple fellowship on steel applied the principles of carbon wire technology to the manufacture of stainless steel wire with beneficial results and devoted much study to problems of manufacturing seamless tubing. Some new uses for sulfur came from the researches of another fellowship.

Seventeen industrial fellowships began work during the year, and four other fellowships have been accepted and will soon start operation. Eight industrial fellowships concluded their investigations during 1938–39. Among the new fellowships the programs on acid recovery (prevention of stream pollution by waste pickle liquors), air filters, gypsum products, plastics in meter construction, criteria of excellence of pearls, and watch lubrication have already advanced by the acquirement of useful results.

During the calendar year 1938, 17 bulletins, 27 research reports and 43 other papers were published. Twenty-four United States patents and 30 foreign patents on fellowship inventions came to issue. The total publications for the 28 years ended December 31, 1938, have been 20 books, 174 bulletins, 803 research reports and 1,225 miscellaneous articles; 714 United States patents were granted during the same period.

W. A. HAMOR

MELLON INSTITUTE, PITTSBURGH, PA.

SPECIAL ARTICLES

DISINTEGRATION OF TOBACCO MOSAIC VIRUS IN UREA SOLUTIONS

CHANGES are known to occur in the structure of proteins when they are dissolved in concentrated solutions of urea. In the case of egg albumin, the alteration is quite marked,[1] whereas in the case of hemoglobin and of pepsin, the degree of the change is much less, for, although hemoglobin is split into half-molecules,[2] there appears to be no change in the special properties of these proteins. Recently it was reported[3] that solution of tobacco mosaic virus in 6 M urea and 0.1 M phosphate buffer caused a 100-fold increase in the diffusion constant of the material with no change in the virus activity. Since this would indicate a decrease in molecular weight from one of the order of several millions to one of about 100,000, and, since in many previous attempts[4] it had not proved possible to demonstrate the existence of low molecular weight material possessing virus activity, it seemed desirable to reinvestigate the effect of concentrated urea solution on tobacco mosaic virus.

[1] F. G. Hopkins, *Nature*, 126: 328, 383, 1930; M. L. Anson and A. E. Mirsky, *Jour. Gen. Physiol.*, 13: 121, 1929; H. Wu and E. F. Yang, *Chinese Jour. Physiol.*, 5: 301, 1931.

[2] N. F. Burk and D. M. Greenberg, *Jour. Biol. Chem.*, 87: 197, 1930; J. Steinhardt, *Jour. Biol. Chem.*, 123: 543, 1938.

[3] V. L. Frampton and A. M. Saum, SCIENCE, 89: 84, 1939.

[4] W. M. Stanley, in *Handbuch der Virusforschung*, Springer, Wien, 1938.

A solution containing 10 milligrams of tobacco mosaic virus per cubic centimeter in 6 M urea and 0.1 M phosphate buffer at pH 7 was prepared at room temperature and determinations of virus activity, stream double refraction, turbidity and the amount of protein insoluble in 0.1 M phosphate buffer were made after different periods of time. Since it is not possible to measure virus activity in the presence of high concentrations of urea, the solutions used for activity determinations were diluted with 9 parts of distilled water, and portions of these with 9 or more parts of 0.1 M phosphate buffer at pH 7. These solutions were then compared with controls containing the same small amount of urea, 0.1 M phosphate buffer, and appropriate amounts of virus, by means of the half-leaf method using 30 or more *Nicotiana glutinosa* L. leaves in each test. The stream double refraction measurements were made directly on the urea-virus solution by the method previously described.[5] The turbidity measurements were made with a photoelectric colorimeter by comparing a constant depth of the virus-urea solution with a variable depth of a copper sulfate standard. It may be seen from Table I that solution in 6 M urea and 0.1 M phosphate buffer at pH 7 immediately caused a reduction in virus activity and that after 1, 8 and 96 hours only about 25, 1 and 0.1 per cent., respectively, of the original activity remained. It may be seen from Fig. 1 that this reduction in activity was accompanied by decreases in turbidity, in the amount of protein remaining soluble in dilute buffer, and in stream double refraction. Mehl[6] has also noted the decrease in stream double refraction in urea solutions.

TABLE I

Hours in urea	Virus in urea: Gm. protein/cc. in inoculum	Virus in urea: No. of lesions/ half-leaf	Control: No. of lesions/ half-leaf	Control: Gm. protein/cc. in inoculum	Estimate of per cent. reduction in activity
< 0.1	10^{-5}	42.7	38.3	5×10^{-6}	
	10^{-4}	103.9	46.2	10^{-5}	ca 50
1	10^{-4}	60.9	41.2	10^{-5}	ca 75
4	10^{-4}	37.5	10.5	10^{-6}	> 90
8	10^{-4}	12.4	7.8	10^{-6}	ca 99
30	10^{-4}	2.9	0.9	10^{-7}	> 99
96	10^{-4}	0.2	1.9	10^{-7}	> 99.9

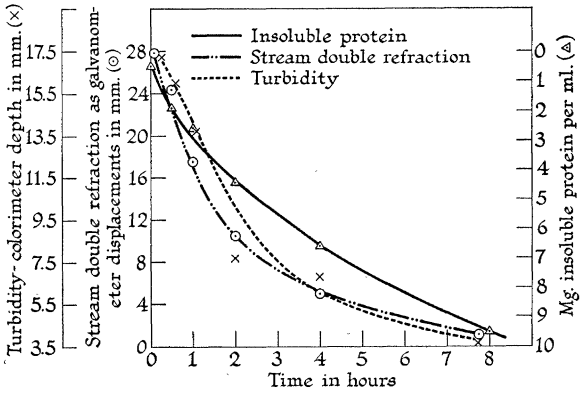


FIG. 1

The fact that the protein insoluble in dilute buffer was found to be free of nucleic acid indicates that the changes described above are due to the degradation of virus nucleoprotein. This disintegration was followed by means of osmotic pressure determinations which indicated an average molecular weight of about 100,000 after 5 days and about 40,000 after 4 weeks. The formation of low molecular weight material was also demonstrated by subjecting virus-urea solutions to high-speed centrifugation. In every one of over 50 experiments the low molecular weight, non-sedimentable material remaining in the supernatant liquids was inactive, and any residual infectivity was always associated with high molecular weight material which sedimented at a rate comparable to that with which ordinary virus sediments. The specific activity of remaining high molecular weight material, whether consisting of traces or of the major portion of the protein, was found to be from 10 to 50 per cent. that of ordinary virus protein. Following extensive disintegration, practically all the protein was found in the supernatant liquid and only traces in the sediment. Dilution of the supernatant liquids containing the low molecular weight protein with distilled water or with 0.1 M phosphate buffer or removal of the urea by dialysis failed to yield solutions possessing virus activity. Therefore, the disintegration does not appear to be readily reversible. The solution of virus in concentrated urea was accompanied by the appearance of a positive nitroprusside reaction, indicative of the formation of free sulfhydryl groups. These were estimated with the porphyrindin dye according to the method described by Greenstein.[7] The titration of a sample prepared by the addition of 2 grams of urea to 2 cubic centimeters of a solution containing 73 milligrams of virus at pH 8 corresponded to 0.70 per cent. cysteine. When 3.2 grams of guanidine hydrochloride were added to 2 cubic centimeters of the virus solution and the hydrogen ion concentration adjusted to pH 7, the titration after 1 hour corresponded to 0.76 per cent. cysteine. These results indicate that all or most of the sulfur[8] in tobacco mosaic virus occurs in the sulfhydryl groups which become free and measurable in concentrated solutions of urea or of guanidine.

The effect of 5 different concentrations each of sodium chloride, potassium phosphate and sodium

[5] M. A. Lauffer and W. M. Stanley, *Jour. Biol. Chem.*, 123: 507, 1938; M. A. Lauffer, *Jour. Phys. Chem.*, 42: 935, 1938.

[6] J. W. Mehl, *Cold Spring Harbor Symposia*, 6: 226, 1938.

[7] J. P. Greenstein, *Jour. Biol. Chem.*, 125: 501, 1938. It is a pleasure to express appreciation to Dr. Greenstein for material and helpful suggestions.

[8] A. F. Ross and W. M. Stanley, *Jour. Am. Chem. Soc.*, 61: 535, 1939.

citrate on the rate of disintegration of virus in 6 M urea at about pH 7.5 was determined by separating the high from the low molecular weight material by high-speed centrifugation after different periods of time. Disintegration proceeded most rapidly in 0.1 M phosphate buffer, slightly less rapidly in 0.05 M phosphate, 0.05 M borate and 0.02 and 0.04 M citrate, and less rapidly in 0.01 and 0.3 M phosphate and 0.004 and 0.12 M citrate. The rate was noticeably slower in 0.024, 0.12, 0.24 and 0.71 M sodium chloride and in 0.24 M citrate, and quite slow in 0.6 M phosphate, in 1.4 M sodium chloride and in the absence of electrolytes. The half-life periods of the native protein in the various urea-electrolyte solutions cover the range from less than 2 hours to more than a week. The effect of 4 different hydrogen ion concentrations on the rate of disintegration in 6 M urea and 0.1 M phosphate buffer was also studied. The rate was greatest at pH 8.2, slower at pH 7.4, much slower at pH 6.4 and almost negligible at pH 5.5. The disintegration of virus protein in 6 M urea and 0.1 M phosphate buffer at pH 7 is similar to the urea denaturation of egg albumin[9] in proceeding more rapidly at 0° than at 25° C., but differs from it by proceeding more rapidly at 40° than at 25° C. The rates of reaction during the degradation of about 90 per cent. of the high molecular weight material at 25° or 40° C. in 6 M urea and dilute buffers at about pH 7 may be described fairly satisfactorily by the equation of a first order reaction. However, the amount of high molecular weight active material remaining after about 50 hours, though quite small, may be as much as 10^6 times that predicted by the first order equation.

It may be concluded that tobacco mosaic virus is rapidly disintegrated in 6 M urea and 0.1 M phosphate buffer at pH 7, with appearance of free sulfhydryl groups, into low molecular weight protein components which contain no nucleic acid, exhibit no double refraction of flow, are insoluble in dilute buffers, and, most important, possess no virus activity. The rate of degradation varies widely with the concentration of urea, the concentration of electrolyte, the type of the electrolyte, the hydrogen ion concentration and the temperature. These results, especially the demonstration of the great effect of small changes in pH or in electrolyte concentration on the rate of disintegration, as well as the earlier work on the degradation of virus in solutions of sodium dodecyl sulfate,[10] may provide information concerning the nature of the forces which hold together the large virus molecule. The conclusion of Frampton and Saum[11] that virus activity is associated with the low molecular weight products obtained from tobacco mosaic virus upon solution in concentrated urea has not been confirmed.

W. M. STANLEY
MAX A. LAUFFER

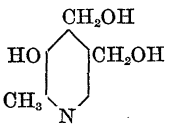
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, N. J.

[9] F. G. Hopkins, *loc. cit.*
[10] M. Sreenivasaya and N. W. Pirie, *Biochem. Jour.*, 32: 1707, 1938.
[11] *Loc. cit.*

SYNTHETIC VITAMIN B_6

THE isolation of vitamin B_6 was reported by Keresztesy and Stevens,[1, 2] of this laboratory, and also by other workers.[3–6]

The structure of vitamin B_6 was studied at this laboratory, and the evidence which led to the structure is described fully in two papers[7, 8] which are about to appear. The structure of vitamin B_6 was shown to be 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine,



I

I. Kuhn and coworkers[9–11] have announced the results of researches which led to the same structure for the vitamin.

In this laboratory, the complete synthesis of the vitamin B_6 has been accomplished, and we wish to describe the results of the comparison of the synthetic vitamin B_6 hydrochloride with the natural vitamin B_6 hydrochloride.[1]

M.p. 206–208° C., mixed melting point with natural vitamin B_6 hydrochloride, 207° C. Positive ferric chloride test.

Anal. Calc. for $C_8H_{12}O_3NCl$: C, 46.72; H, 5.84; N, 6.81. Found: C, 46.55, 46.64; H, 5.57, 5.69; N, 6.83, 6.75. The biological assay also confirms these findings.

A complete report of this work will be published in the near future.

STANTON A. HARRIS
KARL FOLKERS

RESEARCH LABORATORY, MERCK AND COMPANY, INC., RAHWAY, N. J.

[1] Keresztesy and Stevens, *Proc. Exp. Bio. Med.*, 38: 64, 1938.
[2] Keresztesy and Stevens, *Jour. Amer. Chem. Soc.*, 60: 1267, 1938.
[3] Lepkovsky, SCIENCE, 87: 169, 1938; *Jour. Biol. Chem.*, 124: 125, 1938.
[4] Kuhn and Wendt, *Ber.*, 71: 780, 1118, 1938.
[5] Ichiba and Michi, *Sc. Papers Inst. Phys. Chem. Research*, 34: 623, 1014, 1938.
[6] Gyorgy, *Jour. Amer. Chem. Soc.*, 60: 983, 1938.
[7] Stiller, Keresztesy and Stevens, *ibid.*, 61, May, 1939.
[8] Harris, Stiller and Folkers, *ibid.*, 61, May, 1939.
[9] Kuhn and Wendt, *Ber.*, 72: 305, 1939.
[10] Kuhn, Andersag, Westphal and Wendt, *ibid.*, 72: 309, 1939.
[11] Kuhn, Wendt and Westphal, *ibid.*, 72: 310, 1939.