acid, and the second by the fact that any polyvalent base would yield fictitiously high rather than fictitiously low calculated osmotic activities. Correction for non-solvent volume would not account for more than 6-7 m.E./K. However, it may be that the fourth assumption does not hold for anacid juices, particularly of pyloric origin. The high total nitrogen content of such secretion samples suggests that nitrogenous constituents of relatively low molecular weight may account for an appreciable part of the osmotic deficit.

Summary. 1. In general, pilocarpine-stimulated greater curvature gastric pouch juice has a lower osmotic activity, and high total nitrogen content than does histamine-stimulated juice. 2. Fasting juices show highly variable relations between total nitrogen content and osmotic activity. 3. Pyloric pouch fasting juice has in our experience very much higher total nitrogen content than does greater curvature pouch juice. The osmotic activity of the former is nevertheless hypertonic with respect to blood. 4. There appears to be a partial correlation between total nitrogen content and osmotic activity of gastric juice. 5. Calculated and observed osmotic activities are in reasonable agreement for six greater curvature pouch samples in which free acid was present. 6. Calculated values were 12 to 24 m.E./K. lower than the observed osmotic activity values for three pyloric pouch juices without free acid. It is suggested that part of the deficit results from neglecting in the calculation the contribution of the nitrogenous constituents.

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An Analysis of Tobacco Mosaic Virus for Biotin, Riboflavin and Pantothenic Acid.

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Investigation into the structure of filterable viruses has revealed the presence of some amino acids,[1, 2] certain enzymes such as catalase

[1] Ross, A. F., *J. Biol. Chem.*, 1941, **138**, 741.

[2] Knight, C. A., and Stanley, W. H., *J. Biol. Chem.*, 1941, **141**, 39.

and phosphatase,[3, 4] as well as other important biologically active compounds, notably biotin and riboflavin.[5, 6] The presence of these latter growth-promoting substances has been demonstrated only in a highly organized virus, namely, the elementary bodies of vaccinia.[5, 6] It, therefore, became of interest to determine whether less complex viruses, such as tobacco-mosaic virus which could be obtained in a highly purified form might contain such substances. Investigation of such a virus for the presence of highly active chemical groupings might well furnish valuable information concerning the metabolic relationship between the virus molecule and host-tissues. Accordingly, highly purified tobacco-mosaic virus was examined for the presence of biotin, riboflavin, and pantothenic acid. Microbiological methods of assay for these vitamins are extremely sensitive and determinations can be made with a reliable degree of precision.

Methods. A sample of tobacco-mosaic virus suspended in 0.1 M total phosphate buffer pH 7.0 was obtained through the kindness of Professor E. J. Cohn of the Department of Physical Chemistry, who received it from Dr. Lauffer of the Rockefeller Institute for Animal Research at Princeton. A five-fold dilution of this material was then assayed for riboflavin by the method of Snell and Strong[7] and for pantothenic acid by the method of Pennington, Snell and Williams.[8] Aliquots of a five-fold dilution of unhydrolyzed virus and virus hydrolyzed with 5% HCl for 1 hour at 15 lb pressure, neutralized and brought to pH 4.5 were also tested for biotin by the procedure of Snell, Eakin and Williams.[9]

Results. No evidence of the presence of riboflavin or pantothenic acid in unhydrolyzed solutions of tobacco-mosaic virus was obtained. Biotin, however, was present in appreciable amounts and further work was then undertaken to investigate its relationship to the virus molecule.

The virus solution was filtered through a graded collodion-filter (540 mu) to remove certain bacteria found to be present, and then

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6 Hoagland, C. L., Ward, S. M., Smadel, J., and Rivers, T. M., *J. Exp. Med.*, 1941, **74**, 133.

7 Snell, E. E., and Strong, F. M., *Ind. Eng. Chem. Anal. Ed.*, 1941, **11**, 346.

8 Pennington, D., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1940, **135**, 213.

9 Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.

thrown down in the ultracentrifuge (800 rps for 15 minutes). The supernatant fluid was removed, the virus washed once with distilled water, and again deposited by centrifugation. During the ultracentrifugation, the presence of a single sedimentation-peak gave definite indication of the purity of the virus preparation. A micro-Kjeldahl determination of the filtered virus material, before ultracentrifugation, gave a value of 1.37 mg of nitrogen per ml.

Several fractions were obtained after ultracentrifugation, namely, the original supernatant fluid, the supernatant fluid after the first washing, and the resuspended, centrifuged, washed virus. Assay of each component for biotin revealed, as may be seen in Table I, that the major portion of this substance remained in the *original* supernatant fluid and accordingly was not intimately associated with the virus.

A small amount of biotin was present in the washed, resuspended virus. This, we believe, may have been due to slight residual retention of the original supernatant fluid by the virus, probably by absorption. No increase in biotin was noted upon pressure-hydrolysis of the virus. An increase would presumably have occurred if the biotin were an integral part of the virus molecule.

Although it has been demonstrated that biotin and riboflavin are both present in high concentrations in the purified elementary bodies of vaccinia, we have been unable to obtain evidence for their presence in significant amounts in the purified tobacco-mosaic virus. We believe that the large amount of biotin found in the original supernatant fluid of the preparation we examined probably was

TABLE I.
Biotin Assay of Centrifuged Tobacco Mosaic Virus.

	Micrograms $\times 10^{-6}$ biotin per 1.37 mg of nitrogen
(1) Virus sol'n, filtered, centrifuged, but *not* hydrolyzed	162
(2) Virus sol'n, filtered, centrifuged, and hydrolyzed	143
(3) Original supernatant fluid (obtained by centrifuging the filtered virus)	835
(4) Supernatant from the first H_2O washing	93
(5) Sum total of (2), (3) and (4)	1071
(6) Virus sol'n, filtered and hydrolyzed, but *not* centrifuged	1044

Notes: (a) The large amount of biotin found in (3) indicates that the major portion was present in the original fluid in which the virus was dissolved and was not intimately associated with the virus.

(b) The close check between (1) and (2) indicates that no increase in biotin resulted on hydrolysis of the virus. This is further confirmation that biotin is not intimately associated with the tobacco-mosaic virus molecule.

(c) The sum total of (2), (3) and (4) checks very well with (6) indicating that the experimental error of the method was less than 3%.

derived either from the original tobacco-plant material or from certain bacteria which were isolated from the solution of virus before filtration through the collodion filter. Accordingly, we suggest that great caution must be observed in interpreting the significance of analyses of materials derived from living tissues when subjected to delicate procedures of assay.

Conclusions. The presence of riboflavin, pantothenic acid, and biotin could not be demonstrated in significant amounts in moderately concentrated solutions of purified tobacco-mosaic virus.

We are deeply indebted to Dr. Oncley and Mr. Gordon, of the Department of Physical Chemistry, for performing the ultracentrifugation, to Dr. Robinson, of the Massachusetts State Antitoxin and Vaccine Laboratory, for the micro-Kjeldahl determination, and to Dr. Enders, of the Department of Bacteriology, for helpful suggestions in this investigation.

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Sulfanilylguanidine in Control of Salmonella Infection and Carrier State in Mice.

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The presence of an enzootic Salmonella infection in our laboratory colonies of mice has prompted an investigation into the etiology, control, and nature of this disease. Data gathered from animal autopsies and bacteriological studies indicate that in 2 strains the infection follows different courses. The 2 strains of mice, Akh and Rfi, were developed by Furth of Cornell University Medical School for his studies of leukemia. Seventy to 80% of the Akh animals die of lymphoid leukemia if they live to be over 8 months of age. The Rfi mice carry myeloid leukemia and 1% die of the disease. Bacteriological studies of stool cultures revealed a significantly higher incidence of infection in Rfi mice than in Akh animals. Autopsies performed on 26 Akh mice in a 2-months' period revealed gross evidence of Salmonella infection in only 2, whereas gross evidence of this disease was seen in 17 animals in a series of 35 Rfi mice autopsied during the same period. The typical lesions seen at autopsy were numerous, irregularly distributed, white areas of focal necrosis, variable in