bacteriostasis were used; excessive drug concentrations¶ did not augment its production, (b) the amount formed varied directly with the total number of bacterial divisions, (c) PAB reduced the production of chromogen in direct, linear proportion to the PAB/Drug ratio used; addition of infusion broth to this medium prevented its formation (and also bacteriostasis).

The production of this diazotizable substance is intimately associated with bacteriostasis; it is also formed with bacteriostatic concentrations of sulfapyridine and sulfathiazole but its detection is difficult with the less potent sulfanilamide which requires far greater concentrations¶ for bacteriostasis.[5]

Isolation of the new substance is in progress. It is stable to boiling, dialyzable, and extractable with butyl alcohol.

Summary and Conclusions. During growth of *Es. coli* in synthetic medium with bacteriostatic concentrations of sulfonamide, a diazotizable substance is produced. This is not p-aminobenzoic acid; in fact, its production is prevented by those amounts of PAB necessary to block the action of sulfonamides. The substance is not produced in the absence of sulfonamide nor when bacteriostasis does not occur.

During bacteriostasis by sulfonamides in this medium there is a change in the metabolism of *Es. coli* so that a metabolite with a diazotizable grouping similar to the therapeutically active group of the drug is produced. Experiments are in progress to determine whether the production of this substance is specifically elicited by the drug or whether it is a normal metabolite which accumulates when a sulfonamide interferes with the growth of the organisms.

¶ Colorimetric differences are not accurately detectable when high concentrations of drug are tested, since the errors may exceed the increment sought.

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New Crystalline Forms of Tomato Bushy Stunt Virus.

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From the first report on the crystallization of tobacco mosaic virus[1] until recently, only 2 additional crystalline forms of purified viruses have been reported, the non-birefringent dodecahedra[2,3] of tomato bushy stunt virus (TBS) and the faintly birefringent plates of tobacco necrosis virus.[4] These were obtained by means of crystallization with electrolytes such as ammonium sulfate. A new crystallization technic for large proteins employing hydrophilic colloidal materials such as heparin, starch, etc., has resulted in the crystallization of a hitherto uncrystallized hemocyanin, the usual crystalline form of the anisotropic viruses such as tobacco mosaic virus, and new crystalline forms of TBS[5] and of a tobacco necrosis virus.[6]

In continuing studies on the crystallization of TBS by means of heparin and other substances, two previously unobserved crystalline forms of this virus were obtained. One form was obtained using heparin under conditions somewhat different from those reported previously. To a solution of TBS in

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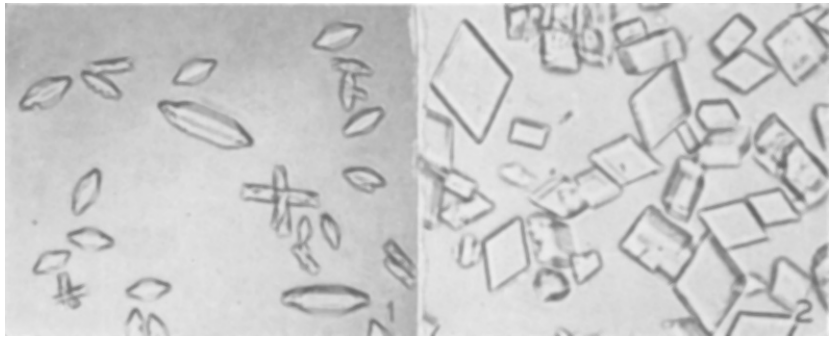

FIG. 1. FIG. 2.

FIG. 1.
Tomato bushy stunt virus crystallized by means of sodium heparinate. × 239.
FIG. 2.
Tomato bushy stunt virus crystallized by means of sodium sulfonate of polyanethole (Liquoide Roche). × 534.

water at a concentration of 4.0 mg per cc the solid sodium heparinate† was slowly added at room temperature and dissolved with rubbing until an opalescence due to protein precipitation was observed. This appeared at a concentration of about 5% with respect to heparin. The mixture was cooled in an ice-bath until the amorphous precipitate completely redissolved, and was then stored at 4° overnight. Well defined crystals, some of which were 0.2 mm in length, appeared in that time. Warming this mixture to room temperature and rechilling resulted in a new larger crop of smaller crystals of the same shape. The new non-birefringent crystals, shown in Fig. 1, appear to be eight-sided prisms whose ends are tapered by joint triangular planes. After being washed several times with 3 M ammonium sulfate, the crystals were dissolved in water and analyzed for carbohydrate and phosphorus as described previously.[5] There was no evidence of combination of virus with heparin from the carbohydrate to phosphorus ratio. The sedimentation constant of the dissolved crystals was normal for TBS,[7] $S_{20} = 129 \times 10^{-13}$. This was estimated at 3 mg per cc in 0.1 M borate buffer at pH 7.1 by Dr. M. A. Lauffer of this laboratory. The redissolved crystals showed no significant change of virus activity as estimated on *Nicotiana glutinosa* L., and on cowpea.

The search for other effective crystallizing agents resulted in the testing of Liquoide Roche, the sodium sulfonate of polymerized anethole. Under conditions comparable to those just described, but at a Liquoide concentration of approximately 4%, another non-birefringent crystalline form appeared. The rhombohedral crystals are shown in Fig. 2.

The property of possessing numerous crystalline forms under varying conditions is common to many of the simpler proteins, such as edestin, insulin, ribonuclease, etc. This property of tomato bushy stunt virus once again emphasizes its relationship to substances which are expected to behave according to chemical and physical laws. It can be expected that an examination of these various well shaped forms by X-ray technic should yield considerable information concerning certain intrinsic properties of this virus molecule.

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[7] Lauffer, M. A., *J. Physic. Chem.*, 1940, **49**, 1137.