

the Shwartzman reaction in rabbits. However, the purified fraction appears to be more potent than the filtrate in producing the reaction, since intravenous injection of a 1:10 dilution of the stock solution of the purified fraction produced the reaction at practically all sites of endermal injection of both filtrate and of purified fraction while intravenous injection of the undiluted filtrate elicited the reaction only in those sites that had received the highest concentration of filtrate or of purified fraction.

It is of interest to note that, so far as the purified fraction is concerned, the greater the amount of tumor-affecting agent employed in the preparatory injections the more pronounced is the Shwartzman reaction. It may also be pointed out that, in this experiment, a positive Shwartzman reaction was obtained with an amount of purified fraction (0.0005 mg.) that is close to the minimal amount (0.0003 mg.) effective in eliciting hemorrhage in mouse-tumors.

### 8747 P

#### Chemical Composition of *Bacterium tumefaciens*.

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*Bacterium tumefaciens* is the etiological agent of plant tumors, known as crown-gall disease.<sup>1</sup> The importance of investigating the chemical composition of this organism and the pathological effects of its various constituents is apparent. Although the etiology of plant tumors probably does not parallel that of animal cancer, the fact that a chemical stimulant of cellular proliferation apparently is synthesized by this microorganism is reminiscent of certain results obtained with chemically well-defined carcinogenic agents. Doubtless, a deeper knowledge of the mechanism underlying the development of plant cancer would also be of value for a better understanding of malignant growth in general.

A virulent strain of *B. tumefaciens* (strain 6NIS<sup>6</sup>) was cultivated on sterile bean-broth in the dark at room temperature for 14 days. The washed bacteria were suspended in a mixture of equal amounts

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<sup>1</sup> For discussion and literature compare M. Levine, *Am. J. Cancer*, 1931, **15**, 1410.

of alcohol-ether which extracted the fat and phosphatide. The defatted organisms then were extracted with chloroform, in order to remove the wax fraction which, however, in this bacterium is very small and was not further examined. The separation and purification of the lipids were carried out according to the method of Anderson.<sup>2</sup> The distribution of lipids in 3 batches of bacteria of the same strain grown at different times is given in Table I. It will be seen that the results are remarkably uniform. The dry weight of the bacteria examined, in each case, was between 2 and 3 gm.

TABLE I.  
Lipids of *Bact. tumefaciens* (in % of total dry bacteria).

Fraction	Experiments		
	I	II	III
Fat	5.7	5.8	5.6
Phosphatide	1.6	2.0	1.8
Wax	0.8	—*	—*
Bacterial residue	91.9	92.2	92.6

\*Not isolated.

The *acetone-soluble fat* is a yellow viscous oil. Its constants, determined according to the micro-method described by one of us<sup>3</sup> were: iodine No. 95.0, saponification No. 201.2, acid No. 53.2, ester No. 148.0. On saponification (carried out in N<sub>2</sub>) the following components were obtained from 174 mg. of fat: 15.6 mg. of unsaponifiable matter (9% of the fat) and 142.9 mg. of fatty acids (82.1% of the fat). The unsaponifiable fraction showed no selective absorption in the ultraviolet region in alcohol-chloroform (2:1) solution; there was only an end-absorption around 2350 Å. About 10% of the unsaponifiable fraction consisted of a sterol mixture which melted unsharply around 80°, was precipitated by digitonin and gave a positive Liebermann-Burchard reaction and a negative Rosenheim reaction. This sterol may have been carried over from the medium. The fatty acids (142.9 mg.) were separated into the solid and liquid acids according to the Twitchell procedure. The solid acids weighed 30.0 mg. and had the iodine value 43.3; the liquid acids weighed 106.4 mg. and had the iodine value of 51.6. The fat apparently contains unsaturated fatty acids of high molecular weight, as is also the case with the fat of *C. diphtheriae*.<sup>4</sup>

The *phosphatide* was purified by repeated precipitation from its ethereal solution by means of acetone. It formed in the cold a

<sup>2</sup> Anderson, R. J., *J. Biol. Chem.*, 1927, **74**, 525.

<sup>3</sup> Chargaff, E., *Z. physiol. Chem.*, 1931, **199**, 221.

<sup>4</sup> Chargaff, E., *Z. physiol. Chem.*, 1933, **218**, 223.

somewhat sticky yellow powder which on slow heating started to soften at 67° and melted completely at 125°. It contained N 2.2%, P 3.4%.

From the defatted bacteria a *polysaccharide* was obtained which amounted to about 3.5% of the material. This substance contained 65.3% of reducing sugars (calculated as glucose).

A detailed report on the chemical composition of the fractions mentioned and on their biological behavior will be presented at a later date.

The various substances isolated are being tested, at present, as to their effect on the Paris daisy, castor bean, geranium, Bryophyllum and two species of tobacco. Preliminary tests made on the Paris daisy, geranium and Ricinus indicate that the phosphatide acts as a growth-stimulant, whereas the fat is much less active.

## 8748 C

### Some Physical and Chemical Properties of Desiccated Stomach.

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Since the introduction of the use of desiccated hog stomach in the treatment of pernicious anemia by Sturgis and Isaacs,<sup>1</sup> Sharp,<sup>2</sup> Conner,<sup>3</sup> and Wilkinson,<sup>4</sup> efforts have been made to fractionate the material and determine the nature of the active principle. In the following experiments whole hog stomach was finely ground, dried at a temperature below 30°C., and the fat extracted with petroleum benzine. With this method 100 gm. of fresh stomach yield 14-15 gm. of dry powder. This material is stable, retaining its anti-anemic potency for months. It is non-hygroscopic but on the addition of water swells and forms a milky suspension.

This material was used as a starting point for further manipulations. In each case the substance was given to one or more suitable patients with pernicious anemia having the following qualifications: a macrocytic anemia with a red blood cell count of less than 2.5 millions per cubic millimeter; no free hydrochloric acid in the

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<sup>1</sup> Sturgis, C. C., and Isaacs, R., *J. A. M. A.*, 1929, **93**, 747.

<sup>2</sup> Sharp, E. A., *J. A. M. A.*, 1929, **93**, 749.

<sup>3</sup> Conner, H. M., *Proc. Staff Meet. Mayo Clinic*, 1929, **4**, 319.

<sup>4</sup> Wilkinson, J. F., *Brit. Med. J.*, 1930, **1**, 236.