permits the direct determination of the volume sprayed per unit time.

This gun has been used successfully in the enumeration of purified influenza virus, strain PR8. Data concerning the number of virus particles per egg ID_{50} unit correlated well with the findings of Donald and Isaacs (J. Gen. Microbiol., **10**, 457, 1954).

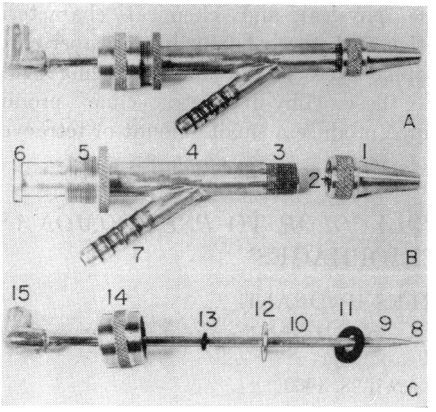

Figure 1. Spray gun used in electron microscopy. *A.* Assembled spray gun. *B.* Outer envelope. *C.* Insert tube. The component parts have the following specifications: *1.* Nozzle head 3/8 inch threads, 0.030 inch aperture. *2.* "O" ring, 3/8 inch outside diameter (o.d.) and 1/4 inch inside diameter (i.d.). *3.* 3/8 inch threads, tapered end. *4.* Brass tubing 5/16 inch o.d.; 0.035 inch wall. *5.* 9/16 inch threads, movable; C*14* threads over *B5*. *6.* Flanged end, 1/2 inch diameter; 3/32 inch long. *7.* Side outlet for hose connection 1/4 inch diameter and 1 1/2 inch long, 30° angle. *8.* Modified 27 gauge needle, 0.006 inch aperture. *9.* 1/8 inch solid round brass with hole drilled to receive needle; turned and soldered into tubing. *10.* 1/8 inch brass tubing, 0.014 inch wall. *11.* Rubber gasket 1/2 inch diameter, 1/8 inch hole, 1/32 inch thick. *12.* Brass washer 1/2 inch diameter, 1/8 inch hole, 1/32 inch thick. *13.* "O" ring 1/4 inch o.d., 1/8 inch i.d. *14.* Packing nut, 9/16 inch threads, with 45° chamfer. *15.* "L" fitting with taper ream.

BIOSYNTHESIS OF TETRACYCLINE BY 5-HYDROXYTETRACYCLINE-PRODUCING CULTURES OF *STREPTOMYCES RIMOSUS*

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Coproduction by selected strains of *Streptomyces aureofaciens* of tetracycline and 7-chlortetracycline has been reported from a number of laboratories (Backus, et al., Ann. N. Y. Acad. Sci., **60**, 86, 1954; Gourevitch et al., Antibiotics & Chemotherapy **5**, 448, 1955; Roland and Sensi, Farmaco (Pavia) (Sci. Ed.), **10**, 37, 1955). But no reports are available of the coproduction by cultures of *Streptomyces rimosus* of tetracycline as well as the chemically related 5-hydroxytetracycline. During a study of antibiotic production by a number of 5-hydroxytetracycline-producing cultures including *S. rimosus* (NRRL 2234), we found that traces of an antibiotic with mobility equivalent to tetracycline in several paper chromatographic systems were present in the fermented media. Among the chromatographic systems which distinguished this antibiotic from 5-hydroxytetracycline but not from authentic tetracycline were: (a) stationary phase, pH 3.0, 0.3 M phosphate solution; moving phase, $CHCl_3$:CH_3NO_2:pyridine (10:30:3); (b) stationary phase, water; moving phase, water saturated methyl-*iso*-butyl ketone: formic acid (10:1); and (c) stationary phase, pH 4.6 McIlvaine's buffer; moving phase, ethyl acetate. The position on the paper chromatograms of the tetracycline-like antibiotic was determined by observation of the chromatograms under ultraviolet light, and by bioautography using *Staphylococcus aureus* as test organism. There was approximately 100 times as much 5-hydroxytetracycline as tetracycline-like material in all fermentation samples examined. Limited variation in the composition of the culture medium thus far has had no marked effect on this ratio.

The new antibiotic was separated from the fermented medium by solvent extraction procedures followed by chromatography on Celite

(Diatomaceous earth filter-aid manufactured by Johns-Manville) columns (in a manner similar to that described by Doerschuk et al., J. Am. Chem. Soc., **81**, 3069, 1959) using pH 4.4 phosphate buffer as stationary phase and a mixture of chloroform and *n*-butanol (4:1) as moving phase. A small amount of material was crystallized and found to have the same infrared spectrum as a sample of pure tetracycline. The rotation, $[\alpha]_D$ −241°, agreed well with the value found with pure tetracycline, and the spectral shifts found after treatment of solutions of the new material with 0.2 N HCl and 0.2 N NaOH were identical with those found when pure tetracycline was treated under these conditions. These physical and chemical characteristics together with the behavior in the paper chromatographic systems provide convincing evidence that these 5-hydroxytetracycline producing cultures produce a small amount of tetracycline.

SUSCEPTIBILITY OF *PSEUDOMONAS POLYCOLOR* TO *PSEUDOMONAS AERUGINOSA* BACTERIOPHAGES[1]

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Although *Pseudomonas aeruginosa* and *Pseudomonas polycolor* are listed as separate species in *Bergey's Manual of Determinative Bacteriology* (Breed, Murray, and Smith, 7th ed., 1957, The Williams & Wilkins Company, Baltimore), no constant characteristics other than source (i.e., *P. polycolor* from plants, *P. aeruginosa* from animals, sewage, and soil) have been found to distinguish between them.

Elrod and Braun (J. Bacteriol., **44**, 633–645, 1942) were able to produce typical disease in tobacco plants using *P. aeruginosa*. They also found *P. polycolor* to be pathogenic for mice. Both species produced pyocyanin, grew well at 37 C, and were indistinguishable serologically. Haynes (J. Gen. Microbiol., **5**, 939–950, 1951) reported that the two species were identical in their ability to grow at 42 C, to convert potassium gluconate to potassium ketogluconate, and to produce slime in a potassium gluconate medium.

TABLE 1

Activity of Pseudomonas aeruginosa bacteriophages on Pseudomonas polycolor

P. polycolor Culture	Bacteriophages*																		
	2	3	7	9	11	16	21	27	31	44	68	73	91	95	109	111	113	116	249
PP2 (Calif.)	0	2+†	4+	0	0	0	4+	0	0	4+	4+	0	0	0	1+	2+	0	3+	0
PP2 (England)	0	3+	4+	0	0	0	3+	0	0	4+	4+	0	0	0	1+	2+	0	4+	1+
NCTC 9433	0	1+	4+	0	0	0	3+	0	0	4+	4+	0	0	0	1+	2+	0	2+	1+

* Used at routine test dose (highest dilution which causes confluent lysis of propagating culture).

† 4+ = Confluent lysis, 3+ = nearly confluent lysis, 2+ = many discrete plaques, 1+ = few discrete plaques, 0 = no lysis.

In the course of investigations of bacteriophage typing of *P. aeruginosa*, we used 20 bacteriophages, 19 of which were isolated in our laboratory from sewage and one (249) isolated from a lysogenic culture by R. E. Brame, Brooke Army Hospital, Fort Sam Houston, Texas. Three cultures of *P. polycolor* were also tested. Two of these were subcultures of the same original strain (PP2, isolated from tobacco in the Philippines in 1925) but had been maintained in separate stock culture collections in two different laboratories. One of the PP2 cultures was received

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