artery D via the left subclavian artery A, inflow tube B, flowmeter C and outflow tube F. The left common carotid artery E is then also cannulated. At this point, the aortic end of the brachiocephalic trunk H is cannulated and connected to the inflow tube B. The aorta is then ligated at J, just beyond the origin of the left subclavian artery, then cannulated at K and connected to the outflow tube F. When the aorta is ligated at J just before being cannulated, an excessive rise of blood pressure in the aorta and its branches above the ligature at J is prevented by allowing blood to escape via tube L into a buffer reservoir M, the pressure of which is adjusted by a sphygmomanometer bulb N. The amount of blood allowed in the reservoir M is such as to maintain the blood pressure in the aorta and its branches above the ligature at J only slightly above the level of the blood pressure before the ligature of the aorta, the level being read from a mercury manometer S. When the aorta has been cannulated at K, the screw clamp O compressing the outflow tube F is gradually released and blood is slowly pumped back into the dog from the buffer reservoir M. This is done slowly and gradually to prevent any sudden or marked drop in blood pressure in the aorta and its branches above ligature J. When the procedure is completed, the blood flows from the aorta, via the brachiocephalic trunk H and the left subclavian artery A into the inflow tube B and the flowmeter C. From the flowmeter C the blood flows into the common carotids D and E and the descending aorta at K, via the outflow tube F. It can be seen that the blood flowing through the flowmeter is the output of the left ventricle except the coronary blood flow and the amount of blood drained by the few small arteries originating from the aorta between R and J. A tubing, T, which allows shunting of the blood past the rotameter, is clamped when the cardiac output is being measured and is only used to record a zero flow deflection without interfering with the circulation. The buffer reservoir M has also been found useful in damping the pulsation of the flow. The arterial blood pressure is recorded photographically by a Gregg manometer, P. This new technic of continuously recording the output of the left ventricle in the anesthetized dog with cerebral circulation intact has been used successfully in several studies.

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Susceptibilities of Pleuropneumonia-like Organisms to Some Selective Bacteriostatic Agents. (17969)

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(Introduced by Stuart Mudd)

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Although pleuropneumonia-like organisms (PPLO) are occasionally isolated in pure culture from various pathological processes, more often these organisms are present along with bacteria of various kinds. Frequently it is very difficult and sometimes it is impossible to isolate the PPLO in pure culture because they require an enriched medium and an incubation period of a few days. The small size of the colonies of the PPLO compared to that of ordinary bacteria together with the inability to fish off colonies of the PPLO with an inoculating needle contributes to the difficulty of isolating them in pure culture. It would be highly advantageous if some substance could be added to the culture medium which would inhibit bacteria of various kinds but allow the PPLO to grow. The determinations of the susceptibilities of PPLO from human sources to some of the

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TABLE I.
Action of Selective Bacteriostatic Agents on PPLO and Bacteria in Solid Media.

		Basic fuchsin				Crystal violet		Potassium tellurite		
Strains	Control	1:25,000†	1:50,000	1:100,000	1:200,000	1:100,000	1:250,000	1:10,000	1:25,000	1:50,000
07	127*	0	0	30	97	110	114	100	107	103
09	132	0	0	14	117	132	140	133	133	135
36	3	0	0	<1	3	1	2	0	0	2
37	6	<1	3	5	5	2	5	3	5	6
43	5	0	2	4	4	1	5	5	5	5
48	3	0	1	3	3	2	3	1	3	4
60	11	0	1	13	13	0	12	5	10	12
Campo "L"	66	0	13	60	67	63	59	62	58	61

* Numbers indicate the mean colony count in 6 low power microscopic fields.
† Numbers indicate final dilution of selective agent in the media.

TABLE II.
Action of Selective Bacteriostatic Agents on PPLO and Bacteria in Liquid Media.

		Basic fuchsin			Crystal violet	Potassium tellurite	
Strains	Control	1:25,000†	1:50,000	1:100,000	1:100,000	1:10,000	1:50,000
07	>300*	>300	>300	>300	>300	>300	>300
09	>300	106	>300	>300	>300	>300	>300
37	>200	0	>200	>200	>200	>200	>200
48	>200	>200	>200	>200	>200	5	>200
60	>200	>200	>200	>200	>200	0	>200
Campo "L"	>200	2	94	>200	>200	>200	>200

* and † explained in footnotes to Table I.

various chemicals frequently employed in culture media for their selective bacteriostatic action are herein reported because they offer help in overcoming some of the above-mentioned difficulties.

Penicillin is the only antibiotic which has been shown repeatedly(1-4) to be ineffective in inhibiting growth of PPLO. Dienes(2) has pointed out two possible sources of errors in isolating the PPLO resulting from penicillin being present in the culture medium. When bacteria are inhibited by the presence of penicillin in a solid culture medium, the bacterial colonies may be very small and the organisms may swell into round bodies which are indistinguishable from those in colonies of PPLO. The other source of error is that in the presence of penicillin bacteria of several species have been observed to dissociate into the L-type of growth. These instances have been summarized in a previous paper(3). Since penicillin has these disadvantages, substances other than the antibiotics were investigated; namely, basic fuchsin, brilliant green, crystal violet, Nile Blue A, potassium tellurite, sodium azide, and thionin.

Since the bacteriostatic effect of agents may vary in liquid and solid media, both types of media were investigated. For the liquid medium Bacto-heart infusion broth, pH 7.8 was employed. The solid medium was essentially Bacto-heart infusion agar in which the tryptose was replaced by Bacto-peptone. Work in progress and which will be reported later indicates that a medium containing an infusion from beef heart muscle and Bacto-peptone is best for growing the PPLO. To

1. Salaman, M. H., *et al., J. Path. and Bact.,* 1946, v58, 31.

2. Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 165.

3. Leberman, P. R., Smith, P. F., and Morton, H. E., *J. Urol.,* 1950, v64, 167.

4. Hatch, M. H., A symposium on current progress in the study of venereal disease, U. S. Government Printing Office, 1949, 183.

both the liquid and solid media was added ascitic fluid to 25 per cent of the final volume. The various chemicals were added to give the final concentrations as indicated in Tables I and II. For each strain of PPLO a block of agar containing numerous colonies was ground with a glass rod in a test tube containing 5 ml of 2% solution of Bacto-proteose peptone No. 3. After allowing the particles of agar to settle, 0.05 ml of the suspension was transferred to the surface of each plate and was then spread over the surface of the medium with a bent glass rod so as to inoculate the entire surface of the medium uniformly. The plates were incubated aerobically at 37°C for 3 days. The average number of colonies per 6 low power microscopic fields was determined. In the case of liquid media 0.1 ml of the suspension was added to 10 ml of the test medium and this was incubated aerobically for 3 days at 37°C. Since no appreciable amount of turbidity develops when PPLO grow in liquid media, a standard 4 mm platinum loopful of the incubated test cultures was streaked over the surface of a plate of the above-mentioned solid medium without inhibitors. These subculture plates were incubated and examined as described above.

Eight strains of PPLO from humans were tested. Strains 07, 09, and Campo "L" had been propagated on artificial media longer than the remaining 5 strains. Strains 07 and 09 were isolated from the urethra and were received from Johns Hopkins Hospital through the courtesy of I. G. Schaub. Campo "L" was received from the Massachusetts General Hospital by the courtesy of L. Dienes. The remaining 5 strains were isolated by ourselves from patients in the Urology Clinic of the Hospital of the University of Pennsylvania. Strains 36, 37, and 43 were isolated from prostatic secretions, strain 48 from urethral discharge of a male, and strain 60 from a cervical exudate. Eight representative strains of gram-negative and gram-positive organisms were also tested on the same media containing maximum concentrations of the selective agents which did not inhibit PPLO. The other concentrations were unimportant for determining the selectivity of the bacteriostatic agents.

Nile Blue A proved to be too toxic for PPLO to be considered as a selective agent favoring the isolation of these organisms. In the solid medium concentrations of Nile Blue A of 1:100,000 to 1:1,000,000 completely inhibited 7 of the 8 strains of PPLO and the eighth strain, Campo "L", grew slightly in the lesser concentration of the dye. Essentially the same results were obtained with the same concentrations of the dye in liquid media. It has occasionally been reported that colonies of PPLO have been detected on media employed for isolating the gonococcus from clinical material. From these results it is obvious that those media employed for cultivating the gonococcus which contain Nile Blue A are unsuitable for detecting PPLO.

Thionin was another dye which proved to be too toxic for PPLO to be employed as a selective agent. Concentrations of 1:10,000 and 1:25,000 were completely inhibitory in both the solid and liquid media. A concentration of 1:200,000 was toxic for some of the strains.

Brilliant Green inhibited completely the growth of PPLO on the solid medium in concentrations of 1:25,000 to 1:200,000. In the liquid medium in concentrations of 1:100,000 and 1:200,000, the PPLO and the gram-negative bacteria were not inhibited but the gram-positive organisms were inhibited completely with the exception of *C. xerose* which was uninhibited.

The greatest concentration of sodium azide in the solid medium which did not inhibit growth of the PPLO was 1:25,000 and at this concentration 3 of the 4 gram-positive organisms were uninhibited.

Of the substances tested basic fuchsin, crystal violet, and potassium tellurite gave the best results in selectively inhibiting either the gram-negative or gram-positive organisms while at the same time not inhibiting the PPLO. In the solid medium basic fuchsin, 1:200,000, and crystal violet, 1:250,000, did not inhibit the 4 gram-negative bacteria, *E. coli, P. vulgaris, A. aerogenes,* and *Ps. aeruginosa,* but did inhibit the 4 gram-positive organisms, *S. aureus, S. albus, St. fecalis* and

C. xerose. The 1:50,000 concentration of potassium tellurite inhibited the 4 gram-negative bacteria but did not inhibit the 4 gram-positive organisms. Usually greater concentrations of the substances were required to produce the same effect in the liquid medium; basic fuchsin, 1:100,000, and crystal violet, 1:100,000 inhibited the 4 gram-positive organisms but permitted growth of the 4 gram-negative bacteria and the PPLO. Potassium tellurite, 1:50,000, inhibited *E. coli* and *A. aerogenes* but not *P. vulgaris* and *Ps. aeruginosa* among the gram-negative bacteria and in addition inhibited the gram-positive organisms except for *C. xerose.*

Discussion. To our knowledge the only other work on the susceptibility of PPLO to bacteriostatic agents is that of Edward(5). Whereas he observed that neither of his 2 strains isolated from mice was inhibited by 1:5,000 sodium azide, we observed that 5 of our 8 test strains were inhibited. The 3 of our strains not inhibited by 1:5,000 sodium azide were strains which had been on artificial media for some time. Our strains from humans, even the recently isolated strains, appear to be slightly more resistant to potassium tellurite and crystal violet than were the two mouse strains tested by Edward. Like Edward, we found that PPLO were inhibited by brilliant green in 1:100,000 concentration. In addition, Edward investigated thallium acetate but it appears to offer no advantages over potassium tellurite which is commonly used to inhibit gram-negative organisms.

Summary. Strikingly, the PPLO do not behave either as gram-positive or gram-negative microorganisms in that they are not inhibited on solid medium by a 1:250,000 dilution of crystal violet which normally inhibits gram-positive microorganisms nor by a 1:50,000 dilution of potassium tellurite which normally inhibits gram-negative organisms. By being able to inhibit both gram-positive and gram-negative microorganisms the isolation of PPLO from mixed cultures can be greatly facilitated. Generally, strains which have been cultivated on artificial media for some time are more resistant to the bacteriostatic substances than are the recently isolated strains.

5. Edward, D. G., ff., *J. Gen. Microbiol.,* 1947, v1, 238.

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Production, Purification, and Some Properties of *Clostridium histolyticum* Collagenase.* (17970)

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Jennison(1) reported that *Clostridium histolyticum* produced a proteinase that was able to digest "native" collagen. In a previous communication from this laboratory(2) the activity of *Cl. histolyticum* filtrates in the degradation of collagen was confirmed, as well as the lack of activity of other proteinases on unmodified collagen.

In this communication a reproducible procedure will be described for the isolation and partial purification of a proteinase(s) from *Cl. histolyticum* which is very active in the solubilization of native collagen. A method will be described wherein filtrates with little or no lethal toxin but adequate "collagenase" activity may be produced in large batches. The final product purified by the method to be described is 400 times as active per unit nitrogen as the original filtrate. The separation of a proteolytic enzyme which has no collagenase activity and which is activated

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1. Jennison, M. W., *J. Bact.,* 1945, v50, 349.

2. Neuman, R. E., and Tytell, A. A., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 409.