with T3 have shown various steps in the development of new bacteriophage particles in the protoplasm of infected and lysed cells. After 15 minutes incubation fields like those of Fig. 2, 3 and 4 are common. The lysed cell of Fig. 2 resembles that of Fig. 1 in showing its protoplasm extruded from the broken membrane. Much of this protoplasm has, however, already been converted into new particles of T3. The conversion is still more complete in Fig. 3 where the cluster of coccoid T3 apparently is developing from one or more central particles. This cluster recalls a bacterial microcolony resulting from several divisions of a single cell. A still later stage wherein the newly formed particles have commenced to disperse is shown in Fig. 4. After a few more minutes incubation the formation of bacteriophage has progressed so far at the expense of the lysed protoplasm that it is hard to find either the protoplasmic masses or the clumps of bacteriophage evident in these earlier photographs. Many electron micrographs like the foregoing have been obtained; they exhibit T3 particles in small groups and chains that suggest they have arisen by the same kind of self-reproduction which is often seen in bacterial growths and which appears to underlie the multiplication of the T2 coli bacteriophage.

Summary. Cultures of young, rapidly growing susceptible colon bacilli obtained after hourly transplants to fresh broth appear quickly lysed by the addition of T3 bacteriophage. Electron micrographs show that the bacterial membranes rupture very soon after adsorption of bacteriophage and that colonies of new phage particles rapidly appear in the protoplasmic masses. This protoplasm from young cells is the same assemblage of spherical macromolecules seen after lysis with T2 phage. As was the case with this other phage the observed phenomena are those to be expected of particles behaving as if they were minute microorganisms multiplying by division at the expense of the bacterial protoplasm.

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17113. Effect of Chemical Irritation of the Peritoneum on Transmural Migration of Intestinal Organisms.

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For reasons already given elsewhere[1] it is believed that the peritonitis which develops during peritoneal irrigation for the treatment of acute renal failure is due to the transmural migration of bacteria from the intestine. While there is no evidence that this can occur in the absence of severe damage to the intestinal wall, it might be possible as a result of the combined effect of the uremic state and the irritating properties of the dialyzing fluid. In the absence of clinical evidence for or against such a hypothesis, animal experiments were performed to see if transmural migration of intestinal bacteria can be induced (1) in normal animals subjected to chemical irritation of the peritoneum and (2) in normal and uremic animals exposed to the intraperitoneal injection of the fluids used for peritoneal irrigation in acute renal failure. The results of the first part of these studies are herewith reported.

Method. Healthy rabbits and dogs received sterile physiologic saline solution or the fluid used for peritoneal dialysis of uremic patients, in a volume sufficient to grossly distend the peritoneal cavity twice daily up to 2 weeks. Peritoneal fluid was aspirated as completely as possible just before each infusion. The as-

[1] Frank, H., Seligman, A., Fine, J., *Annals Surg.*, 1948, **128**, 3.

TABLE I.

Effect of Intraperitoneal Injection of Sterile Monolate Solution and of Sterile Gum Tragacanth Suspension in Rabbits and Dogs.

Day		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Gross pathology
		Monolate.																				
R_1	Injection (cc)	1	1	1	2	2		3	2	2	2	—	—	—	—	—						Fibrinous peritonitis.
	Culture	—	—	—	—	+		+	+	+	+	+	+	+	—	—	S					
R_2	Injection (cc)	2	2	2	3	3	3		3	3	3											Fibrinopurulent peritonitis.
	Culture	—	—	—	—	+	+		+	+	+	S										
R_3	Injection (cc)	1	2	2	2	2	2		3	3	3	3	3	3		3	3	3				,, ,,
	Culture	—	—	—	—	+	+		+	+	+	+	+	+		+	—	+	S			
*R_4	Injection (cc)	2	2	2	2	2	2		2	2	2	2	2	2		2	2	3	4	4	—	Multiple adhesions. No active inflammation.
	Culture	—	—	—	—	—	—		—	+	+	+	+	+		+	+	+	+	+	+	
*R_5	Injection (cc)	2	3	3	3	4	4		4	4	4	4	—	—		—	—	—	—	—	—	,, ,, ,, ,, ,,
	Culture	—	—	—	—	—	+		+	+	+	+	+	+		+	—	—	—	—	—	
*R_6	Injection (cc)	2	3	3	3	3	3		3	3	3	3	—	—		—	—	—	—	—	—	,, ,, ,, ,, ,,
	Culture	—	—	—	+	+	+		+	+	+	+	+	+		+	—	—	—	—	—	
*R_7	Injection (cc)	2	3	3	3	3	3		3	3	3	3	—	—		—	—	—	—	—	—	,, ,, ,, ,, ,,
	Culture	—	—	—	+	+	+		+	+	+	+	+	+		+	+	—	—	—	—	
D_1	Injection (cc)	4	5	5	5	5	8		8	—	5	5	5	5		—						Fibrinopurulent peritonitis.
	Culture	—	—	—	—	+	+		+	+	+	+	+	+		+	S					
D_2	Injection (cc)	5	5	5	5	5	5		—													,, ,,
	Culture	—	—	—	+	+	+		+	S												
D_3	Injection (cc)	5	5	5	5	5	5		5	—												,, ,,
	Culture	—	—	—	+	—	—		+	+	S											
		Tragacanth.																				
R_1	Injection (cc)	10	10	—	—	—																,, ,,
	Culture	—	—	+	+	+	S															
R_2	Injection (cc)	10	—	—	—																	,, ,,
	Culture	—	—	+	+	S																
R_3	Injection (cc)	10	—	—																		Fibrinopurulent hemorrhagic peritonitis.
	Culture	—	—	+	S																	
D_1	Injection (cc)	40	40	40	40	40	40		40	—	—	—	—	—		—	—					Subacute peritonitis.
	Culture	—	—	—	—	—	—		—	—	—	—	—	—		—	—	S				
D_2	Injection (cc)	40	40	40	40	40	40		—	—												Fibrinopurulent peritonitis.
	Culture	—	—	—	+	+	+		+	+	S											
D_3	Injection (cc)	40	40	40	40	—	—		—	—	—	—	—	—		—						Fibrinous peritonitis.
	Culture	—	—	—	—	+	+		+	+	—	—	—	—		—	S					
D_4	Injection (cc)	40	40	40	40	40	40		40	—	—	—	—	—		—	—					,, ,,
	Culture	—	—	—	—	—	—		—	—	—	—	—	—		—	—	S				

The volume of solution injected each day is indicated by the number. Blank spaces prior to S indicate that no injection or culture made on that day. R = Rabbit. D = Dog. — = Sterile culture. + = Presence of *E. coli in* peritoneal fluid. S = Sacrifice of animal.

* R_4—Cultures positive >6 days, followed by 8 negative cultures and sacrifice of rabbit on 36th day. R_5, R_6, R_7—Cultures negative >9 days and sacrifice of rabbits on 32nd day.

pirated fluid was always found devoid of bacteria by aerobic and anaerobic culture. A moderate leukocytic response was regularly present after the first few infusions. Postmortem examination of some of the animals after 2 weeks showed no grossly recognizable changes in the peritoneum.

A severe inflammation of the peritoneum was then produced in a second group of animals by the intraperitoneal injection of sterile 5% monolate (monoethanolamine oleate), which is strongly irritating to endothelial cells. Since a single dose of 4 cc of monolate killed rabbits within a few hours, a graded dose of from 1-4 cc was injected daily into each of a series of rabbits. Each of a series of dogs received 5 cc of monolate daily.

Another series of rabbits and dogs received a daily dose of 5 cc and 40 cc of sterile 2½% gum tragacanth suspension[2,3] respectively, for a number of days. Samples of peritoneal fluid were taken daily, with sterile precautions, from all animals. The animals were sacrificed on the day following the last injection or at some later date. When the tap was dry, 5 or 10 cc of sterile saline were injected and, after a few minutes, a portion of this fluid was withdrawn. All specimens were smeared and cultured immediately in tryptone broth, on agar and on endoplates. The same small loop was used for all inoculations, so that positive plates would permit a roughly quantitative measure of the degree of contamination. Broth and plates were observed for as long as 10 days.

Contamination of peritoneal fluid from the irritant itself or from the skin at the site of puncture was avoided by sterile technic and by the finding of no growth in cultures made from the irritant and from the skin. Daily blood cultures were also done on several animals throughout the experiment to exclude bacteremia as a source of peritoneal contamination. Identification of bacteria was made by standard bacteriologic technics.

Results Table I. Cultures of all animals injected with monolate or gum tragacanth if positive contained *Escherichia coli* and never any other organism. The number of colonies on direct plates was always less than 50 colonies per plate. *E. coli* was found regularly in the peritoneum of all rabbits and dogs beginning on the 4th to 9th day of injection. Once the microorganism appeared, it continued to be present until the injections were stopped. In four rabbits the culture remained positive for from 3 to 7 days after the last injection, and sterile for from 9 to 14 days thereafter. These rabbits showed extensive intraperitoneal adhesions without active inflammation, while those sacrificed shortly after the last injection showed diffuse fibrinopurulent and adhesive peritonitis. That the fibrinopurulent peritonitis was produced by the monolate rather than by *E. coli*, is indicated by finding in several rabbits and dogs sacrificed after only 2 or 3 days of monolate injections that cultures taken each day and at postmortem were sterile despite the presence of a marked fibrinopurulent peritonitis.

Gum-tragacanth Experiment (Table I): Three rabbits injected intraperitoneally with one dose of gum-tragacanth showed a fibrinopurulent peritonitis within 24 hours and *E. coli* was recovered at the same time. In dogs, however, the results were somewhat different. Four dogs, injected once, revealed sterile peritoneal cultures each day up to the time when they were killed on the 2nd, 4th, 5th, and 7th days respectively. At autopsy they all showed mild degrees of fibrinopurulent or fibrinous peritonitis. The results were the same in 2 more dogs which received daily doses of tragacanth for 4 days and for 7 days respectively. In another which received a daily dose of 40 cc of tragacanth for 6 days, *E. coli* first appeared on the 4th day and continued to be present in the peritoneal exudate for 4 more days. At postmortem he showed severe fibrinopurulent peritonitis. In still another dog similarly treated for 4 days *E. coli* was first found on the 5th day and continued to be present for 4 more days, after which sterile cultures were obtained during 5 subsequent days. Autopsy revealed a resolving fibrinous peritonitis. That the positive cultures were in no instance due to bacteremia, contamination from the skin at the

[2] Steinberg, B., Goldbatt, H., *Int. Med.*, 1927, **39**, 449.

[3] Steinberg, B., *Am. J. Clin. Path.*, 1936, **6**, 253.

site of injection or of the irritant itself was shown by uniformly negative cultures from these sources.

Discussion. In spite of the large number of investigations which have been made on the permeability of the intestinal wall for bacteria, conclusive evidence of transmural migration of intestinal bacteria has not been produced. A normal intact serosa is generally regarded as impermeable, but this may not be the case where there is inflammation of the peritoneum due to a foreign body or a chemical irritant. For example, the presence of bile or a sponge in the peritoneal cavity may result in peritonitis or abscess formation, with organisms of intestinal origin present in the absence of a visible break in the continuity of the intestinal serosa. However, convincing proof that in such instances no break in continuity, however small, was responsible is difficult to produce.

Experimental data are contradictory. When a foreign substance, such as melted agar or sodium nucleinate[4] or broth[5] is placed in a serous cavity, intravenously injected cocci are found occasionally in the cavity containing the foreign substance but not in the other cavities. Thus an irritant in the peritoneal cavity may attract and retain intravenously injected cocci, but such organisms obviously must have entered the cavity via the circulation. Peritonitis following the feeding of virulent cocci usually does not occur.[6-12] In several instances where this did happen transmural migration was not proved, because bacteria may have entered the circulation via the inflamed intestinal wall.[13-17]

The recovery of the organism responsible for primary peritonitis from the intestinal wall[18,19,16] or in the stool[20,21] does not establish the case for transmural migration, because all of the involved areas may have been invaded from the blood stream. The evidence that the intestinal serosa when intact can be traversed by bacteria has therefore been equivocal.

In our experiments the sterility of the injected substance and of the skin, ascertained by daily cultures, exclude an external source for the bacteria found in the peritoneal cavity of our animals. Invasion via the blood stream is unlikely. To exclude this remote possibility daily blood cultures from two rabbits and one dog were taken during the entire time of the experiment and yielded no growth. In view of these observations the regular finding of a typical intestinal microorganism in pure culture in the peritoneum inflamed by chemical irritation is strongly in favor of its origin from the gut by transmural migration, in spite of the absence of any signs at postmortem examination of mucosal inflammation. Why only *E. coli* and none of the other intestinal bacteria was recovered is difficult to say. Pure *E. coli* peritonitis is rare.[22,23,13] The fact that *E. coli* was the usual contaminant or infecting organisms in patients subjec-

4 Pawlowski, P., *Zentralblatt f. Chir.*, 1887, No. 48; *Virchows Arch.*, vol. 117, 1889, quoted by Künzel, H., *Münchn. Med. Woch.*, 1904, **51**, 1920-1921.

5 Peiser, A., *Beitr. z. Klin. Chir.*, 1907, **55**, 484.

6 Austerlitz, F., Landsteiner K., Sitzungberichte der kaiserlichen Akademie der Wissenschaften in Wien, 1889.

7 Markus, H., *Z. f. Heilkunde*, 1899, **29**, 427.

8 Neisser, M., *Z. f. Hyg.*, 1906, **54**, 363.

9 Opitz, E., *Z. f. Hyg.*, 1904, **48**, 67.

10 Selter, H., *Z. f. Hyg.*, 1896, **22**, 12.

11 Bail, M., *Arch. f. Klin. Chir.*, 1900, **62**, 369.

12 Hornemann, E., *Z. f. Hyg.*, 1911, **69**, 39.

13 Lenander, K., Nyström, G., *Z. f. Klin. Med.*, 1906, **63**, 263.

14 Geirsvald, S., quoted by Lenander, K. G., Nyström, G.[13]

15 Felsen, J., Osofsky, A. G., Pharyngogenic Hematogenous Streptococcic Peritonitis, *Arch. Surg.*, 1935, **31**, 437.

16 Weil, S. A., Saphir O., *Am. J. Dis. Child.*, 1932, **43**, 611.

17 Montgomery, A. H., *Surg. Gyn. Obst.*, 1925, **21**, 798.

18 Escherich, T., *Jahrb. f. Kinderheilkunde*, 1889, **49**, 167.

19 Flexner, S., *The Johns Hopkins Hospital Bull.*, 1895, **6**, 64.

20 Obadalek, W., *Deutsche Z. f. Chir.*, 1929, **220**, 307.

21 Obadalek, W., *Deutsche Z. f. Chir.*, 1931, **233**, 587.

22 Welsh, quoted by Flexner.[19]

23 Fishbein, M., *Am. J. Med. Sciences*, 1912, **144**, 502.

ted to peritoneal irrigation for uremia suggests that the mechanism of invasion in these patients was similar to that in the foregoing experiments.

Summary. Chemical peritonitis induced by repeated injections of monolate or gum-tragacanth in rabbits and dogs results in the appearance of *E. coli* in pure culture, in small to moderate numbers, in the inflamed peritoneum. Once this contamination is established, it continues for some time after the injections are discontinued. Eventually all cultures are sterile.

By exclusion of all other possible sources of contamination of the inflamed peritoneum, it appears that the *E. coli* found in the peritoneal exudate is derived from the intestinal lumen by transmural migration.

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17114. Transmural Migration of Intestinal Bacteria During Peritoneal Irrigation in Uremic Dogs.*

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Peritonitis due to *Escherichia coli* in pure culture occurs readily during peritoneal irrigation for the treatment of acute renal failure.[1] Clinical data suggested that the peritonitis was due to invasion of the peritoneum by the transmural migration of *E. coli* from the intestinal lumen.[1]

In a previous experimental study[2] it was demonstrated that transmural migration of *E. coli* can be induced by the intraperitoneal injection of strong irritants, but that bacterial peritonitis does not ensue. It was then considered desirable to determine whether the irrigating fluid used in treating acute renal failure can also provoke a bacterial invasion of the peritoneal cavity from the gut. The data to follow demonstrate that bacterial contamination is produced following intraperitoneal administration of this irrigating fluid in uremic dogs but not in normal dogs and that prophylactic oral antibiotic therapy is effective in preventing it.

Method. Normal dogs, and dogs made uremic by removal of both kidneys 24-72 hours previously, were treated as follows: Fifteen hundred to 3000 cc of sterile irrigating fluid‡ was injected into the peritoneal cavity by needle puncture under rigid sterile precautions. The fluid, as it escaped from the needle, just before and just after the puncture, was inoculated into dextrose broth. At the same time, swabs of the site of puncture of the skin, which had been prepared by shav-

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† With the technical assistance of Sunya Gordon and Dorothy Kaufman.

1 Frank, H. A., Seligman, A. M., and Fine, J., *Ann. Surg.*, 1948, **128**, 561.

2 Schweinburg, F. B., and Heimberg, F., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 146.

‡ The 2 solutions used for infusion were those which had been used in patients for peritoneal irrigation.[1] Each was used in about half of the experiments. As there was no difference in their effect, the results are not considered separately.

	Solution A (g/l)	*Solution B* (g/l)
NaCl	7.25	7.4
KCl	0.19	0.05
$CaCl_2$	0.095	0.05
$MgCl_2 \cdot 6H_2O$	0.2025	0.22
$NaHCO_3$	1.5	0.77
Dextrose	9.5	5.0
Gelatin§	4.75	10.0
pH after sterilization	7.9	7.2-7.6

§ We are indebted to the Atlantic Gelatin Company for the gelatin used in these experiments.