TABLE V.
Activation of Antifungal Activity by Ultrafiltration Demonstrated by Agar-streak (Agar-dilution) Tests.

Dilution of material	Growth of test fungi							
	C. albicans		*C. neoformans*		*T. gypseum*		*T. purpureum*	
	Original*	Ultra-filtered	Original*	Ultra-filtered	Original*	Ultra-filtered	Original*	Ultra-filtered
1:40	4+		—		—		—	
1:80	4+	—	—	—	±	—	±	—
1:160	4+	—	—	—	2+	—	2+	—
1:320	4+	—	—	—	3+	—	3+	—
1:640	4+	—	—	—	3+	—	3+	—
1:1,280	4+	2+	±	—	3+	—	3+	±
1:2,560		3+		2+		—		±
Controls	4+		4+		4+		4+	

* The ethyl alcohol solution of ethyl acetate extractives from No. 48240 culture filtrate.
— = No visible growth.
± = Least detectable growth.
+ = Scanty, but definite growth.
2+ = Moderate growth.
3+ = Good growth.
4+ = Maximum growth.

hand, Melin and Wikén[8] reported that aqueous extracts of pure forest litter that were somewhat active against staphylococci became more active when autoclaved or passed through a Seitz filter pad.

Whatever the explanation of the present results, it is evident that the ultrafiltration technic may be applied profitably in the study of antibiotics.

Summary. The antifungal activity of some partially purified extracts of two actinomycetes was sharply increased by filtration through gradocol membranes.

We wish to thank Mr. James Quigley for carrying out all the procedures in ultrafiltration.

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8 Melin, E., and Wikén, T., *Nature*, 1946, **158**, 200.

17223. Relation of a Specific Strain of Salmonella to Ulcerative Cecitis of Rats.

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Although *Salmonella enteritidis* is found very constantly in the lesions of ulcerative cecitis of rats[1] some doubt remains as to whether this organism is the sole cause of the disease. The possibility that some other agent, perhaps a virus, may be concerned is suggested by certain findings; the arguments pro and con can be summarized briefly as follows:

(1) *Arguments in favor of Salmonella being the specific etiological agent in cecitis of rats.* a. *Salmonella enteritidis* has been found by all workers to be invariably associated with the lesions.[2] b. Salmonella may be obtained from the enlarged mesenteric lymph nodes associated with cecitis.[2] c. Cecitis may be prevented by rations containing either sulfaguanidine or sulfasuxidine.[3] d. The le-

1 Buchbinder, L., Hall, L., Wilens, S. L., and Slanetz, C. A., *Am. J. Hyg.*, 1935, **22**, 119.

2 Bloomfield, A. L., and Lew, W., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 179.

sions can be cured by ingestion of streptomycin which inhibits (*in vitro*) in high dilution the strains of salmonella recovered from the cecum.[4] e. Attempts to isolate a virus have so far been fruitless.[5]

(2) *Arguments which suggest that some other agent may play a part.* a. The anatomy of the lesions—early involvement of lymph nodes and later localized granulomatous lesions are not known to be caused by members of the salmonella group. b. The large cysts containing clear fluid so often found as part of the cecitis picture do not yield salmonella or other organisms by ordinary culture technic.[2] c. Salmonella isolated from the lesions and fed in large dosage to healthy rats produce a violent diffuse enteritis, rather than the usual picture of cecitis.[2] d. Some of the animals which recovered from such an acute enteritis were later found to have typical lesions of chronic cecitis but with no greater frequency than uninoculated controls.[2] e. Cecitis in the offspring can be prevented by treatment of the mothers with sulfonamides during pregnancy and lactation. It is not clear why such a procedure should protect against salmonella infection.[6] f. Rats fed a diet deficient in Vitamin B develop cecitis less often than controls given a normal diet.[7] This result suggests the possibility of a virus infection and is just the opposite of what one would expect with salmonella.

Experiments. The following studies were therefore made in the attempt to define more precisely the role of salmonella in chronic ulcerative cecitis of rats. The first group of experiments was designed to determine relation of the development of lesions to the presence of salmonella and to the titer of specific antibodies.

Seven groups of 6 young rats were used. Each group was housed in a separate cage and was fed the stock diet of the laboratory as desired. At the age of 2 months and thereafter at intervals of 2 weeks one group was sacrified. Under ether anaesthesia and with aseptic technic the abdomen was opened and the cecal region was carefully examined for suspicious lesions. The cecum was then excised, opened, and searched for ulceration. Material for culture was taken from the cecum, the swab being thoroughly rubbed over the mucosa. Except in the case of the first 2 groups the animals were then exsanguinated by cutting the abdominal vessels from which blood was collected for serological tests.

Stool cultures were made on MacConkey (Difco) and salmonella-shigella (Difco) agar plates, from which colonies were sub-cultured in Russell double sugar tubes and in liquid carbohydrate media. Various strains were finally tested with salmonella diagnostic sera (Lederle). Sera to be tested for agglutinins were stored in the refrigerator. Serial dilutions were set up against a live suspension of 24 hour growth of the salmonella isolated from cecal lesions. The tubes were held for 2 hours in the water bath at 37°C placed over-night in the refrigerator and then read at room temperature.

Results. The results are summarized in Table I. Aside from the normal bowel flora the only organisms isolated were members of the salmonella group. These were obtained from 9 animals among a total of forty-two. In each case the characteristics were identical and conformed to *Salmonella enteritidis*, Group D of the Kauffmann-White Schema. Furthermore 6 strains which were identified in the salmonella typing laboratory of the State Department of Public Health all had the same antigenic formula: – (I), IX, XII . . . : g . . . ; —

A study of Table I shows several points of interest. There was no visible cecitis in the 2 to 3 months old groups. From 3½ months on, with the exception of the 4 months group, cecitis was evident in approximately one-half the animals. Organisms of the salmonella group were recovered in only one instance in which gross lesions were not evident, (Rat I of Group IV), and then only

[3] Bloomfield, A. L., and Lew, W., *ibid.*, 1941, **48**, 363; 1942, **51**, 28.

[4] Bloomfield, A. L., and Lew, W., *ibid.*, 1948, **69**, 11.

[5] Schultz, E. W., personal communication.

[6] Bloomfield, A. L., and Lew, W., *Am. J. Med. Sci.*, 1943, **205**, 383.

[7] Bloomfield, A. L., and Lew, W., *J. Nutrition*, 1943, **25**, 427.

TABLE I.
Relation of Lesions of Cecitis to Presence of Salmonella, and Titer of Agglutinins in Rats of Various Ages.

Rat. No.	Lesions	Culture Salmonella	Agg. titer
	Group I—2 mo.		
6 rats	None	All negative	Not done
	Group II—2½ mo.		
6 rats	None	All negative	Not done
	Group III—3 mo.		
6 rats	None	All negative	4, 0, 0, 0, 4, 8
	Group IV—3½ mo.		
Rat No. 1	No cecitis	10	32
2	Walls of cecum seem a little thick, no ulcer	50	8
3	No ulcer seen but wall a little thick and there is a mesenteric cyst 0.5 cm	10	32
4	No cecitis	0	0
5	Frank cecitis with ulcer 1 cm in diam.	Innumerable	32
6	No cecitis	0	0
	Group V—4 mo.		
6 rats	None	All negative	8, 4, 4, 4, 4, 0
	Group VI—4½ mo.		
Rat No. 1	No cecitis	Negative	8
2	,, ,,	,,	8
3	Marked thickening of cecum with ulcer 0.5 cm, large cysts and adhesions	Many	16
4	No cecitis	Negative	32
5	,, ,,	,,	64
6	Typical ulcer 0.5 cm	Many	8
	Group VII—5 mo.		
Rat No. 1	No ulcer, thickening of cecal wall and cyst 0.5 cm	A few	32
2	Extreme lesions, huge ulcer	Innumerable	32
3	No cecitis seen	Negative	8
4	,, ,, ,,	,,	16
5	Marked lesion, ulcer 1 cm	Innumerable	16
6	No cecitis seen	Negative	0

a few colonies were obtained. The number of salmonella usually paralleled the extent of the lesions; when the lesion was slight a few up to 50 organisms per plate were obtained, when the lesion was advanced there were always "many" to "innumerable" colonies. The point of greatest importance however, is that as rats grow and develop cecitis salmonella are already present in the earliest lesions; they do not appear only after cecitis is advanced.

The agglutinins are of special interest. Among the 8 animals with visible cecitis 2 had a titer of 8, two had a titer of 16, and 4 had a titer of 32. Among 21 animals without visible lesions and with negative cultures for salmonella, however, 14 showed titers varying from 4 to 64. Furthermore, one-half the animals in the 3 month old group showed agglutinins at a time when no cecitis was yet to be seen and no cultures were positive.

It is clear then that there is an early and frequent, although slight, immunological reaction to salmonella enteritidis in these rats at a time before lesions of cecitis are visible, and before it is possible to isolate salmonella by our technic. The question was pursued further by examining the sera from even younger

TABLE II.
Agglutinin Titer Against *Salmonella enteritidis* of Young Healthy Rats.

Age 2 months		Age 1 month		Age 12 days	
Rat No.	Titer	Rat No.	Titer	Rat No.	Titer
2A-F	8, 4, 16, 4, 8, 16	1A-F	8, 8, 32, 8, 8, 4	1-6	0, 4, 0, 4, 8, 8
				Mother	8

rats. All the animals were healthy and none showed cecitis. Six were 2 months old, 6 were one month old, and 6 were 12 days old. The eyes were not yet open in the last group and they had not yet been weaned. Serum from the mother of this last group was also tested. The results are shown in Table II. It is seen that even the younger rats may already show specific agglutinins against salmonella at a time long before cecitis can be demonstrated or salmonella isolated. The presence of such agglutinins are therefore of little diagnostic significance and it will be of interest to look for similar antibodies in a rat colony entirely free from cecitis.

Further evidence for the causal role of salmonella was then sought in the following experiments. Sixty rats about 2 months old were divided into 3 groups of 10 test animals and three groups of litter-mate controls. On 5 consecutive days one solid loop of growth from a 24 hour agar slant of salmonella enteritidis isolated from rats with cecitis was shaken up in the water bottles of the test animals. Culture of the water thereupon showed heavy growth. None of the animals however, developed any clinical indisposition with these doses and they all gained weight normally. After one, 2, and 3 months when the rats were 3, 4, and 5 months old one set of test animals with its controls were sacrificed under ether anaesthesia and the degree of cecitis, if any, was noted. The results are shown in Table III. It is seen that one month after ingestion of salmonella there was very little cecitis in either test animals or controls. After 2 months definite cecitis was present in some animals, a little more in the test group than in the controls; but after 3 months cecitis was rampant in the culture-fed animals with not a single definite lesion in the controls.

Discussion. The above experiments were designed to define more exactly than has heretofore been done the significance of *Salmonella enteritidis* in cecitis of rats. The invariable relationship of salmonella to the lesions was confirmed; no culture from a visible lesion, even very early, failed to yield the specific organism. On the other hand salmonella were never recovered from the cecum unless a lesion was present except in one instance (animal No. 1 of the 3½ months group) and then only a few colonies were isolated. Specific agglutinins were uniformly present in the sera from animals with cecitis, but since such agglutinins were frequently present without either cecitis or a positive culture for salmonella, the significance of these antibodies is uncertain. On the whole higher titers were obtained from animals with frank lesions than from healthy rats.

These observations demonstrate conclusively that no cecitis occurs in this colony without the presence of the specific salmonella. However, this finding does not entirely exclude the association of some other unidentified agent in producing the infection. The inoculation experiments are of interest in this connection. Rats fed salmonella did in the end show much more cecitis than untreated controls but the lesions developed only after what appeared to be a long latent period of from one to 3 months. Where are the specific bacteria during this long interval? There is some suggestion that the earliest lesion of the cecitis syndrome is an adenitis of the regional nodes, (Table III) and that the actual cecal lesion only develops after a considerable interval. If this is true the long latent period may be explained, but such a course of events is unknown with salmonella infections. We plan to explore this possibility in further studies. Meanwhile it seems clearly established that ingestion of the specific salmonella promoted the development of cecitis which in turn never occurred in the absence of the

TABLE III.
Incidence of Cecitis in Rats Fed Salmonella Enteritidis, and in Untreated Controls.

Degree of cecitis in rats aged 3 mo.		Degree of cecitis in rats aged 4 mo.		Degree of cecitis in rats aged 5 mo.	
Test animals	Controls	Test animals	Controls	Test animals	Controls
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	?	0
?	0	0	0	++	0
?	0	?	0	+++	0
?	0	?	0	+++	0
?	0	++	0	+++	0
+	0	++	?	++++	0
+	?	++	++++	++++	?

0 = No ulcer, no glands, no cysts.
? = No ulcer, slight or doubtful enlargement of glands, no cysts.
+ = Ulcer to 0.5 cm, enlarged glands and/or cysts.
++ = Ulcer 0.5 to 1.0 cm, enlarged glands and/or cysts.
+++ = Ulcer 1.0 to 2.0 cm, enlarged glands, cysts, adhesions, and matting.
++++ = Huge ulcers, enlarged glands, cysts, adhesions, and matting.

organisms. Of special interest is the fact that in spite of the extensive infestation of the colony healthy carriers are of greatest rarity.

Summary. 1. A specific strain of salmonella has been invariably obtained on culture from the ulcers of rat cecitis. 2. Salmonella were not obtained from the cecum in the absence of lesions with one exception. 3. Agglutinins for the specific strain of salmonella are found in most of the rats in this colony although the titer is on the average higher in animals with visible cecitis. 4. Feeding the specific salmonella in appropriate doses was followed after a long latent period by development of cecitis in many of the test animals. 5. The association of some other synergistic agent is not fully excluded but seems at present unlikely.

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17224. Aminopterin and Response of Frog Oviducts to Estradiol. II. Histological Studies and Mitotic Counts.*

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In a previous paper,[1] the gross changes in the immature female frog oviduct following parenteral administration of estradiol benzoate† alone and in conjunction with folic acid‡ and with a folic acid antagonist, aminopterin,‡ were described. Aminopterin (4-amino pteroylglutamic acid) inhibited the growth and differentiation response of oviducts of newly metamorphosed female frogs, *Rana clamitans*, to estradiol. The aminopter-

* This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

1 Goldsmith, E. D., Schreiber, S. S., and Nigrelli, R. F., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 299.

† The estradiol benzoate was generously supplied by Dr. F. F. Yonkman and Dr. F. L. Mohr of Ciba Pharmaceutical Products.

‡ The 4-amino pteroylglutamic acid was generously supplied by the late Dr. Y. SubbaRow, and the folic acid by Dr. T. H. Jukes and the late Dr. A. L. Franklin of Lederle Laboratories.