tate. This result suggests that in isotope feeding experiments, analysis of the blood glucose and lactate may yield information comparable to that obtained by the degradation of liver glycogen. Additional experiments to put this question to a more critical test are in progress.

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17222. Activation of Antifungal Extracts of Actinomycetes by Ultrafiltration Through Gradocol Membranes.

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In the course of the purification of extracts from broth cultures of two soil actinomycetes showing antifungal activity, it was observed that solutions which were passed through gradocol membranes often possessed greater activity than the original solutions. Striking results were obtained repeatedly and under varying conditions. Since there may be a wide application in studies of antibiotics, it seems well to report the original observations. The extent and nature of the phenomenon are under investigation.

Materials and methods. Microorganisms. Two distinct actinomycetes, Nos. 47205[1] and 48240, isolated from soil and found to be antagonistic to fungi pathogenic for man, were grown in liquid media dispensed in 250-ml amounts in 1000-ml Erlenmeyer flasks. Strain No. 47205 was grown in glycerol-yeast extract broth containing 0.15% agar for from 9 to 12 days at approximately 28°C, and No. 48240 in glucose-tryptone broth for from 12 to 17 days at the same temperature. The composition of the media varied from the original formulas[1] only in that distilled water replaced the tap water. Four pathogenic fungi, *Candida albicans* No. 4567, *Cryptococcus neoformans* No. 45215, *Trichophyton mentagrophytes (gypseum)* No. 45141, and *Trichophyton rubrum (purpureum)* No. 4507 were employed as test microorganisms.

Antifungal tests. The extracts were assayed by the agar-diffusion or cup test and by agar-streak or agar-dilution tests.[2] Glucose-tryptone agar[1] was used throughout. The time of incubation varied according to the rate of growth of the particular test microorganism and the type of test. Thus in the diffusion tests in which *C. albicans* was employed, incubation for 8 to 10 hours was adequate, while with *C. neoformans*, 22 to 24 hours were necessary. The agar-streak tests with all 4 test microorganisms were incubated 72 hours. All tests were incubated at approximately 28°C.

Ultrafiltration. Gradocol membranes were prepared from nitrocellulose in the form of Parlodion according to the method described by Bauer and Hughes.[3] The average pore diameter of the several membranes varied from 52 to 64 mμ.* The solutions were passed through the membranes under positive pressure. After filtration, the membranes were washed by passing through some of the pure solvent. They were then removed from the filter and extracted with a small volume of 95% ethyl alcohol. Whenever the pH of a solution was determined before ultrafiltration, it was in the acid range.

Experimental. Different types of solutions showing antifungal activity were subjected to

[1] Schatz, A., and Hazen, E. L., *Mycologia*, 1948, **50**, 461.

[2] Reilly, H. C., Schatz, A., and Waksman, S. A., *J. Bact.*, 1945, **49**, 585.

[3] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol,*. 1934, **18**, 143.

* In one experiment with a coarse membrane, 378 mμ, ultrafiltration did not result in activation of the solution.

TABLE I.
Activation of Antifungal Activity of Butyl Alcohol Extractives of Actinomycete Culture Filtrate by Ultrafiltration.

Actinomycete No. 48240 Test material	Vol., ml	Dilution titer*	
		C. albicans	*C. neoformans*
Original aqueous solution of butyl alcohol extractives†	10 Approx.	10	640
Ultrafiltrate	7	160	>320
Aqueous washings	10	80	320
" ext. of membrane	5	20	80
Alcohol " " "	10	80	160
Original alcohol solution of butyl alcohol extractives†	4.5 Approx.	40	640
Ultrafiltrate	3.5	640	640
Alcohol washings	5.5	160	160
" extract of membrane	4.5	160	80

* Highest 2-fold serial dilution to give a zone of inhibition of at least 10 mm in diffusion or cup test. Figures represent reciprocals of the dilutions.
† Aliquot of butyl alcohol extract of 1/5/49.

TABLE II.
Activation of Antifungal Activity of Ethyl Acetate Extractives of Actinomycete Whole Culture by Gradocol Membranes.

Actinomycete No. 48240 Test material	Vol., ml	Dilution titer*	
		C. albicans	*C. neoformans*
Original alcohol solution	12	<5	1,280
Ultrafiltrate	12	160	20,480
Alcohol washings	12	20	1,280
" extract of membrane	6	160	320
Original sol. shaken with membrane scraps	25	10	10,240
Alcohol extr. of membrane scraps	25	<5	1,280

* Highest 2-fold serial dilution to give a zone of inhibition of at least 10 mm in diffusion or cup test. Figures represent reciprocals of the dilutions.

ultrafiltration. For the initial concentration of active material, whole culture, culture filtrate, or pellicle was extracted with organic solvents; or culture filtrate was treated with charcoal and the charcoal eluted with organic solvents. In each case the organic solvents were removed by distillation *in vacuo* and the residue was dissolved in either water or 95% ethyl alcohol for ultrafiltration.

The effect of ultrafiltration on both a water solution and an ethyl alcohol solution of the butyl alcohol extractives from a culture filtrate of No. 48240 is shown in Table I. Not only was the activity of the ultrafiltrate much greater than that of the solution placed on the filter, but the alcohol extract of the membrane showed marked activity, especially against *C. albicans*.

In another experiment an alcohol solution of the ethyl acetate extractives of whole culture was divided into two portions: one was passed through a gradocol membrane and the other shaken with small scraps of membrane. The results are presented in Table II. Both solutions were activated; but the activity in the one filtered through the membrane was greater, particularly against *C. albicans*. In the original solution no activity against *C. albicans* was demonstrated in a 1:5 dilution; after ultrafiltration the solution inhibited growth in a dilution of 1:160. When filtrates of the broth cultures of the actinomycetes were treated with charcoal (Darco G 60) to remove the active agent and the charcoal was eluted with water or organic solvents, no activity against *C. albicans* could be demonstrated by diffusion tests. Ultrafiltration, however, resulted in activation of these eluates against *C. albicans* to a dilution of 1:640. The protocol of such an experiment is given in Table III.

Enhancement of activity was not peculiar

TABLE III.
Activation of Antifungal Activity of Charcoal Eluates of Actinomycete Culture Filtrate by Ultrafiltration.

Actinomycete No. 48240 Test material	Vol., ml	Dilution titer*	
		C. albicans	*C. neoformans*
Original alcohol solution of charcoal eluates	10	0	80
Ultrafiltrate	9	640	640
Alcohol washings	10	40	40
" extr. of membrane	5	320	320

* Highest 2-fold serial dilution to give a zone of inhibition of at least 10 mm in diffusion or cup test. Figures represent reciprocals of the dilutions.

TABLE IV.
Activation of Antifungal Activity of Extractives from Actinomycete Culture Pellicle by Ultrafiltration.

Actinomycete No. 47205 Test material	Vol., ml	Dilution titer*	
		C. albicans	*C. neoformans*
Original alcohol solution	10	<5	>320
Ultrafiltrate	Approx. 7.5	320	640
Alcohol washings	10	40	160
" extr. of membrane	5	160	160

* Highest 2-fold serial dilution to give a zone of inhibition of at least 10 mm in diffusion or cup test. Figures represent reciprocals of the dilutions.

to a single actinomycete nor to one culture medium. Similar results obtained with No. 47205 are shown in Table IV. In this case extractives obtained from the pellicle with ethyl acetate were chromatographed on alumina and an alcoholic eluate subjected to ultrafiltration. The original solution was without activity against *C. albicans* but the ultrafiltrate inhibited growth in a dilution of 1:320. By the agar-streak test enhancement of antifungal activity of the ethyl acetate extractives of No. 48240 culture filtrate was also demonstrated against *C. albicans*, *C. neoformans*, *T. gypseum*, and *T. purpureum*. (Table V).

Water or alcohol ultrafiltrates of gradocol membranes are inactive indicating that the phenomenon is not due to a principle in the filter itself.

Discussion. It is not uncommon for the activity of antibiotic substances to be reduced or lost unaccountably during the course of purification. A few such observations that seem to be more or less related to our own are noted briefly. Woodruff and Foster[4] traced the loss of antibacterial activity in bacillin to the presence of an inhibitor of peptide nature that occurs naturally in many complex organic substances. Cavallito and others[5] found that sulfhydryl compounds had an inactivating effect upon some antibiotics. In an examination of the separation of penicillins by the method of counter-current distribution, Craig and his colleagues[6] observed that the use of ethyl acetate led to a certain degree of inactivation. They suggested that this might be due to a particular lot of ethyl acetate. Another observation that may be related was made by Junowicz-Kocholaty and others:[7] namely, that in the purification of sulfactin, activity apparently disappeared in butyl alcohol extracts, but reappeared subsequently in chloroform extracts. On the other

[4] Woodruff, H. B., and Foster, J. W., *J. Bact.*, 1946, **51**, 371.

[5] Cavallito, C. J., Bailey, J. H., Haskell, T. H., McCormick, J. R., and Warner, W. F., *J. Bact.*, 1945, **50**, 61.

[6] Craig, L. C., Hogeboom, G. H., Carpenter, F. H., and duVigneaud, V., *J. Biol. Chem.*, 1947, **168**, 665.

[7] Junowicz-Kocholaty, R., Kocholaty, W., and Kelner, A., *J. Biol. Chem.*, 1947, **168**, 765.

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.

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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.