sarcoma 180 tumor has a direct effect on the adrenal or the thymus glands or both. Studies are now in progress which will determine this point.

It must be emphasized that results obtained with transplanted tumors (particularly such atypical growths as sarcoma 180) should be interpreted with reserve. Only when similar observations are made in an inbred strain of animals bearing a spontaneous form of cancer should any general conclusions be made regarding the adrenal changes and their relation to the natural disease.

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Use of Streptomycin-dependent Strains of Bacteria for Demonstrating the Ability of Microorganisms to Produce Streptomycin[1]

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Attention was previously directed (*6*) to the fact that streptomycin-producing strains of *Streptomyces griseus* can be identified by their sensitivity to a specific actinophage. Since this actinophage does not act upon other actinomycetes, however, the method could not be used to demonstrate streptomycin production by organisms other than *S. griseus*. For this purpose another method is suggested. It is based on the ability of certain bacteria, grown in media containing large amounts of streptomycin, to give rise to a mutant which is able to grow only in the presence of streptomycin in the medium.

The production by various bacteria of two types of mutants, one resistant to streptomycin and able to grow in ordinary media and another unable to grow in media free from streptomycin, was first demonstrated by Miller and Bohnhoff (*2, 3*) and later confirmed by Kushnick, *et al.* (*1*) and Paine and Finland (*4*). The latter spoke of the first type of variant as "resistant" and of the second as "dependent," to distinguish the two from the original, or "sensitive," culture. These strains may be designated as *rs, ds,* and *ss,* respectively.

In a comprehensive study on the distribution of resistant and dependent cells of *Escherichia coli* in a broth culture (incubated for 28 hrs at 28° C) of this organism, it was found that one resistant and one dependent cell were present among 1,500,000,000 normal sensitive cells. This was determined by plating the normal culture upon an agar medium containing 15 μg/ml of streptomycin. The *E. coli ds* grown on nutrient agar or in broth containing streptomycin produced the typical gram-negative rods; these were somewhat thinner and slightly longer than the cells of *E. coli ss,* the sensitive mother culture, from which the *E. coli ds* was isolated. When transferred to media free from streptomycin, the *E. coli ds* cells failed to divide, although they increased enormously in length. Streptomycin appears to act for this culture as a growth factor essential for cell division rather than as a substrate or a nutrient, since no destruction of streptomycin takes place.

The growth of *E. coli ds* in ordinary streptomycin-containing broth could be measured by making turbidimetric readings, using a Cenco Sheard-Sanford Photelometer. When the influence of streptomycin concentration in the medium upon the growth of this organism was measured, a definite correlation was obtained, up to a certain point, between the concentration of the antibiotic and the growth, as measured by turbidity, of the culture. This is brought out in Table 1. Frequently the control or streptomycin-free broth, especially when heavily inoculated, showed a certain increase in turbidity; this is primarily, at least at first, a result of the increase in the size of the cells in the inoculum rather than of the actual multiplication of the cells.

TABLE 1

RELATION BETWEEN CONCENTRATION OF STREPTOMYCIN AND GROWTH OF STREPTOMYCIN-DEPENDENT STRAIN OF *E. coli*

Concentration of streptomycin in broth (μg/ml)	Incubation (hrs)			
	10	24	51	73
	Turbidimetric readings*			
0	0	0	0	0
1	0	2	9	9
5	1	6	12	12
10	2	10	14	14
20	4	13	17	19
30	5	14	20	23
40	6	15	22	26
50	6	15	21	25
100	7	16	21	24

* One ml of a suspension of an 18-hr-old streptomycin-dependent culture (*E. coli ds*) containing 37×10^5 viable cells was used as the inoculum.

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A comparative study was first made of the effect of isolated and purified antibiotics upon the growth of *E. coli ds* (Table 2). A highly purified preparation of streptomycin and a crude preparation of a streptomycin-like material were compared with two forms of purified streptothricin. The latter antibiotic was used, first, because it is similar in antibacterial and in certain other properties to streptomycin, and second, because certain organisms may produce a mixture of streptomycin and streptothricin (*5*). The results obtained show that, whereas the two forms of streptomycin supported good growth of *E. coli ds*, the streptothricin preparations did not permit any growth of this culture.

TABLE 2

EFFECT OF DIFFERENT ANTIBIOTICS UPON THE GROWTH OF A STREPTOMYCIN-DEPENDENT STRAIN OF *E. coli**

Incubation of culture (hrs)	Antibiotic added				
	No streptomycin	Pure streptomycin (10 μg/ml)	Crude streptomycin-like material (10 μg/ml)	Streptothricin (34 μg/ml)	Streptothricin (63 μg/ml)
240	0	27	30	0	0
384	0	27	30	0	0

* Growth of organism in nutrient broth is expressed as turbidimetric readings.

A comparative study was next made of crude culture filtrates of several antibiotic-producing actinomycetes (Table 3). Only the streptomycin-producing filtrate (3463) permitted the growth of *E. coli ds*; the supplementary addition of streptomycin to these cultures resulted in a more rapid initiation of the growth of the organism. The culture filtrates of the other three organisms, which did not produce streptomycin, did not permit the growth of *E. coli ds*; these filtrates included 3495, a mutant obtained from the streptomycin-producing *S. griseus*, but no longer producing any streptomycin; 3527, a grisein-producing culture; and 3530, a streptothricin-producing culture. The addition of streptomycin to these culture filtrates favored the growth of *E. coli ds*, thus indicating that the antibiotics found in the culture filtrates of these organisms did not interfere with the growth-promoting effect of streptomycin; at most, there was a delay, as in the case of 3527, due, no doubt, to the initial inhibiting effect of the grisein produced by this culture upon the growth of the *E. coli ds*.

Results similar to those reported in this paper have been obtained recently by R. J. Canderlinde and D. Yegian, of the Ray Brook State Tuberculosis Hospital, using an agar-streak method for the growth of streptomycin-dependent strains of different bacteria.

TABLE 3

EFFECT OF CRUDE CULTURE FILTRATES OF DIFFERENT ACTINOMYCETES ON THE GROWTH OF A STREPTOMYCIN-DEPENDENT STRAIN OF *E. coli*

Incubation of culture (hrs)	Pure streptomycin		Filtrate 3463†		Filtrate 3495‡		Filtrate 3527§		Filtrate 3530‖	
	Cells alone	Cells + SM*	Filtrate alone	Filtrate + SM	Filtrate alone	Filtrate + SM	Filtrate alone	Filtrate + SM	Filtrate alone	Filtrate + SM
24	0	0	0	0	0	0	0	0		
48	0	12	0	13	0	10	0	0		
74	0	16	1	19	0	14	0	0		
96	0	19	12	21	0	19	0	0		
120	0	20	17	21	0	21	0	1		
264	0	15	19	20	0	21	0	20	0	29
336	0	20	19	23	0	29	0	21	0	34

* Cells = suspension of streptomycin-dependent bacterial culture 8 hrs old and containing 480 visible cells/ml. Where the culture filtrates were used, the filtrate alone indicates that the antibiotic was present only in the form of the culture filtrate; filtrate + SM indicates that purified streptomycin (10 μg/ml) was also added.

† Streptomycin-producing *S. griseus* culture; final diluted broth contained 6.4 μg/ml of streptomycin.

‡ Mutant obtained from streptomycin-producing *S. griseus* culture and not producing any streptomycin; diluted broth contained 1,000 *S. aureus* units/ml.

§ Grisein-producing culture gave 388 grisein units/ml in diluted broth.

‖ Streptothricin-producing cultures gave a dilution of 12–47 units of streptothricin/ml of broth.

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Precipitin Reactions in Experimental Histoplasmosis and Blastomycosis

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Skin tests and complement-fixation reactions have been used as diagnostic aids in suspected cases of histoplasmosis and blastomycosis. The antigens employed consisted mainly of either the filtrates of broth cultures of the mycelial phase or suspensions of the yeast-like forms of the causative organisms. Recent studies by various workers have shown that the former, which is the saprophytic form grown at room temperature, was nonspecific for infections caused by the homologous fungus. The latter, which is the parasitic form when grown at 37° C, appeared to be more specific as an antigen in the complement-fixation reaction than in the skin tests.

Investigations on the precipitin reaction in histoplasmosis (*3*) and blastomycosis (*1, 2*) have been limited