Two points are of particular interest. The first is the independent confirmation of Lederberg and Tatum’s demonstration of regular recombination in *E. coli*, K12 (see Lederberg 4). The second is the marked increase in rate of recombination due to the irradiation. The reasons for this increase are unknown, but the fact suggests an interesting adaption, perhaps connected with the abnormal growth pattern of the irradiated cells.


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**STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. VIII. PRODUCTION OF A GRISEIN-LIKE ANTI-BIOTIC BY A STRAIN OF STREPTOMYCIES GRISEUS**

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Since the recent demonstration 1 that certain antibiotic agents produced by actinomycetes possess bacteriostatic and bactericidal properties against *Mycobacterium tuberculosis*, a considerable interest has arisen in a systematic study of similar potentialities among the practically unlimited strains and species of actinomycetes that could be isolated from various natural substrates. 2 3

The present investigations were initiated to determine the presence in the feces of healthy and tuberculous herbivorous animals of actinomycetes which have growth-inhibiting properties against mycobacteria and especially *M. tuberculosis*, and of the production by such organisms of antibiotics which have similar properties.

A culture of an organism belonging to the *Streptomycyes* was isolated from the fresh stool of a healthy heifer, and found to be highly effective. This culture (No. 3510) was tested against four mycobacteria, namely, *M. ranae*, *M. avium*, *M. phlei* and *M. tuberculosis* 607, a fast-growing non-pathogenic strain of the human tubercle bacillus. These tests were made by the agar cross-streak method 4 on three different media: dextrose-asparagine agar, nutrient agar and egg albumin agar. A streptomycin-producing strain of *S. griseus* was included among the organisms for comparison with the more active unknown cultures.
The results presented in table 1 show that the production of active anti-
mycobacterial substances differs with the media used for test purposes.
The various strains of the mycobacteria also differ in sensitivity to the differ-
ent actinomycetes. Whereas culture R60 had, for practical purposes, no
activity against any of the mycobacteria, A47 had considerable activity on
2 of the mycobacteria on certain media, and HF23 had good activity on all
the mycobacteria on nutrient agar, 3510 and the streptomycin-producing
strain of *S. griseus* were active against all the mycobacteria tested. There
was a difference, however, between the last two, 3510 showing only limited
activity on dextrose-asparagine agar. Because of the high level of activity
on various substrates against mycobacteria, 3510 was selected for more
detailed investigation.

**TABLE 1**

<table>
<thead>
<tr>
<th>Streptomyces No.</th>
<th><em>M. ranae</em></th>
<th><em>M. avium</em></th>
<th><em>M. phlei</em></th>
<th><em>M. tuberculosis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DA NU EG</td>
<td>DA NU EG</td>
<td>DA NU EG</td>
<td>DA NU EG 607</td>
</tr>
<tr>
<td>R60</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>A47</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>34 13 0 14 0</td>
</tr>
<tr>
<td>HF23</td>
<td>0 15 0</td>
<td>0 15 0 15 0</td>
<td>6 36 0 0 20 0</td>
<td></td>
</tr>
<tr>
<td>3510</td>
<td>6 20 9</td>
<td>19 9 7 25 12 0</td>
<td>32 11 11 11 11</td>
<td></td>
</tr>
</tbody>
</table>

* DA = dextrose-asparagine agar; NU = nutrient agar; EG = egg albumin agar.

On further study, 3510 was found to belong to the group of *S. griseus*. It
grew well under both static (surface) and agitated (submerged) conditions
and produced an active antibacterial agent. The concentration of the anti-
biotic depended greatly on the composition of the medium. Lysis of the
mycelium appeared after 5 to 7 days in submerged cultures at 28°C, and
after 2 weeks or longer in static cultures.

The antibacterial potency of the culture filtrate and of the isolated crude
preparation may be determined either by the agar-streak dilution method or
by the agar-diffusion or cup method. The rapid development of resist-
ance of certain bacterial cells to the antibiotic precludes the general use of
serial dilution procedures in liquid media. In general, the degree of resist-
ance observed in using the serial dilution assay techniques is as great as that
observed for grisein on some test organisms; however, the capacity of bac-
terial cells to develop resistance to this antibiotic is selective rather than
general, as is the case for grisein. The growth of strains of *E. coli*, for ex-
ample, in dilutions ranging from 1-4 to 1-2048 incubated for 18 hours at
cannot be differentiated from the control tube; whereas strain 3 of
S. aureus under similar conditions will show a clear-cut delineation between
tubes containing growth and those not, at varying dilutions depending upon
the potency of the culture filtrate or powdered preparation used.

The antibiotic spectra of a typical culture filtrate of 3510, using the agar-
streak dilution method, and of a solid preparation obtained from the filtrate
were similar, as shown in table 2. The activity was highly selective in
nature, including both gram-positive and gram-negative bacteria; fungi
were not affected. The culture medium showed occasional activity against
M. tuberculosis 607; this activity was lost, however, on the isolation of the
antibiotic. This points to the possibility that the culture produces a second
antibiotic substance which is not removed from the medium or which is in-
activated in the purification process.

TABLE 2
ANTIBACTERIAL SPECTRA OF THE CULTURE FILTRATES AND OF SOLID PREPARATION OF
3510

<table>
<thead>
<tr>
<th>TEST ORGANISM</th>
<th>CULTURE FILTRATE, UNITS/ML.</th>
<th>CRUDE DRY PREPARATION, UNITS/MG.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>E. coli, streptomycin-resistant strain</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Aerobacter aerogenes</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>Sh. paradysenteriae</td>
<td>300</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Sh. alkalascens</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Salmonella pullorum</td>
<td>300</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>B. mycoides</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B. circulans</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B. cereus</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>30</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Sarcina lutea</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Micrococcus lysodeikicus</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis 607</td>
<td>0-10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

The culture filtrate did not always possess tuberculostatic activity. This
substance appeared to be produced in increasing concentrations in the
medium, after the activity of the major antibiotic had reached a maximum.
This was true especially under submerged conditions of culture and at a
temperature of incubation lower than the usual 28°C, namely, at approxi-
mately 24°C. All attempts, however, to isolate the tuberculostatic factor
from the metabolic solutions failed. By the use of the cup method and
*M. avium* as the test organism with streptomycin as a standard of compar-
ison, 30–50 units/ml. of culture filtrate were commonly obtained. Occa-
sionally as high as 120 units/ml. were observed.

The antibiotic that was isolated from the culture medium had a high
activity against gram-negative enteric bacteria, including streptomycin-
resistant strains of *E. coli*.

The addition of 50 mg. of FeSO₄·7H₂O per liter of nutrient broth yielded
culture filtrates and solid preparations with much greater activity than
those produced by the best meat extract-corn steep liquor media. When
zinc was also added, it was found that there was a critical balance between
this element and iron. When 30–35 mg. per liter FeSO₄·7H₂O, 8–10 mg.
per liter ZnSO₄·7H₂O and 10 g. NaCl per liter were added to peptone-meat
extract media in distilled water, good growth and production of the anti-
biotic were obtained. The addition of 3 g. per liter glucose resulted in
even better growth. When this medium was used in static cultures, 2550
_E. coli*_ units/ml. of culture filtrate were obtained in 4 days at 28° C. The
addition of glucose to unbuffered media inhibits the production of the anti-
biotic, because of the lowering of the pH in the early stages of growth.

When the cell-free culture filtrate was treated with activated charcoal
(7–10 g. per liter) all the antibiotic activity was removed from the filtrate.
Elution of the adsorbate with neutral 95 per cent ethanol yielded 10–15 per
cent of the activity. The eluate was concentrated to dryness in vacuo, or
ethyl ether was added in a separatory funnel and the aqueous layer col-
lected and concentrated to dryness. When the concentrated liquid was
added to acetone, a precipitate was obtained; this was triturated to a pow-
der, washed with ether and desiccated. A yield of 200 to 400 mg. was ob-
tained per liter of medium, depending upon the composition of substrate,
rate of growth and antibiotic-producing capacities of the strain used. The
preparations thus obtained showed an activity of 12,000 to 20,000 *E. coli*_
dilution units per gram. The activity against *Shigella paradysenteriae* and
*Salmonella pullorum* was nearly 10 times as great.

The various biological and chemical properties of the antibiotic point to
its close similarity to grisein, an antibiotic produced by another strain of
*S. griseus.* A comparison of the antibacterial spectra of this antibiotic
with certain others produced by actinomycetes is given in table 3.

The similarity of this antibiotic to grisein may be summarized as follows:

1. Both are produced by strains of *S. griseus*.
2. Both are highly selective in their action against gram-positive and
gram-negative bacteria, and are especially active against enteric
bacteria, antibiotic 3510 having an even narrower antibacterial
spectrum than grisein.
### Table 3
Comparative Antibiotic Spectra of Streptothricin, Streptomycin, Grisein and Antibiotic 3510

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Streptothricin</th>
<th>Streptomycin</th>
<th>Grisein</th>
<th>Antibiotic of 3510</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E. coli*</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E. coli†</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ps. fluorescens</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A. aerogenes</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pr. vulgaris</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. aureus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. mycoides</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. cereus</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. megatherium</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S. lutea</td>
<td>+</td>
<td>+</td>
<td>=</td>
<td>-</td>
</tr>
<tr>
<td>M. lysodeikticus</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Streptomycin-resistant.
† Grisein-resistant.

3. Antibiotic 3510 is accompanied occasionally by tuberculostatic activity, a fact which has never been demonstrated under comparable conditions for the grisein-producing strains of *S. griseus*.

4. Antibiotic 3510 is obtained by the same methods of elution from activated carbon as is grisein. All elution methods that failed with grisein have also failed in the isolation of this substance.

5. Antibiotic 3510 and grisein are similar in their solubility in water and insolubility in organic solvents.

6. The two antibiotics are similar in their heat stability.

7. The activity of neither antibiotic is inhibited by cysteine, glucose and horse serum.

8. The production of both antibiotics is favored by the presence of iron in the medium.

9. The activity of both antibiotics is inhibited by certain concentrations of iron in the test medium.

10. Actinophage active against streptomycin-producing strains of *S. griseus* has no activity either against grisein or the 3510 producing strain.

11. Both antibiotics have a greater effect on streptomycin-resistant *E. coli* cells than on the normal non-resistant laboratory strains of this organism.

12. When cross-streaks are made between actinomycetes producing various antibiotics and the same organisms, certain striking differences are obtained, as shown in table 4.
### TABLE 4

**CROSS-INHIBITION AMONG VARIOUS ANTIBIOTIC-PRODUCING ACTINOMYCETES**

<table>
<thead>
<tr>
<th>CULTURE NO.</th>
<th>ANTIBIOTIC PRODUCED</th>
<th>TEST ORGANISM, INHIBITION IN MILLIMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3465</td>
</tr>
<tr>
<td>3463</td>
<td>Streptomycin</td>
<td>8</td>
</tr>
<tr>
<td>3516</td>
<td>Streptothricin VI</td>
<td>9</td>
</tr>
<tr>
<td>3478</td>
<td>Grisein 3478</td>
<td>5</td>
</tr>
<tr>
<td>3510</td>
<td>Antibiotic 3510</td>
<td>17</td>
</tr>
</tbody>
</table>

*Streaked on agar plate, allowed to grow 48 hours at 28°C., then cross-streaked by test cultures.

13. Streptomycin-resistant *E. coli* cultures as well as grisein-resistant cultures are sensitive to antibiotic 3510.

14. *S. griseus* 3510 is active against the Bodenheimer bacterium, which is resistant to both grisein- and streptomycin-producing strains of *S. griseus*.

Except for certain minor differences, antibiotic 3510 is most similar to grisein. Until this antibiotic has been isolated in a pure form and its chemical composition established, it shall be designated as antibiotic 3510 and may be considered as a grisein-like substance.

When tested against several common bacteria by the use of the cup method, streptomycin was found to give the smallest zones of inhibition against *E. coli*, larger zones against *S. aureus* and the largest zones against *B. subtilis*. Grisein produces the smallest zones with *S. aureus*, larger zones with *B. subtilis* and the largest zones with *E. coli*. Antibiotic 3510 produces smallest zones with *B. subtilis*, larger zones with *E. coli* and the largest zones with *S. aureus*.

Antibiotic 3510 was found to have marked *in vivo* activity. This was established by injecting yolk sacs of 9-day-old chick embryos previously infected by the same route with a suspension of *Salmonella pullorum*. For this purpose crude preparations of the substance assaying by the cup method 45 streptomycin units per milligram were used. Chick embryos with 0.1 ml. of a 10⁸ dilution of a 24-hour heart broth culture representing about 150 cells thus infected die regularly in 18 hours or less.

All the uninfected controls, both untreated and treated, survived, as did the controls injected with sterile physiological saline. All infected controls died in 24 hours. When streptomycin was used, all the infected eggs with µg. died in 24 hours; 1000 µg. allowed 3 out of 5 infected eggs to survive for 24 hours and beyond. The infected eggs treated with 10 units of antibiotic 3510 died in 24 hours; with 100 units, 1 out of 5 survived 24 hours and throughout the experiment; 500 units allowed all the eggs to survive 24 hours, and 4 out of 5 survived from 48 hours through 15 days, at which time the experiment was concluded (table 5).
TABLE 5

COMPARATIVE EFFECT OF STREPTOMYCIN AND ANTIBIOTIC 3510 UPON Salmonella pullorum IN CHICK EMBRYOS

<table>
<thead>
<tr>
<th>NO. OF EMBRYOS TREATED</th>
<th>AMOUNT OF ANTIBIOTIC PER EMBRYO, UNITS</th>
<th>NO. OF EMBRYOS SURVIVING, DAYS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 15</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Uninfected</td>
<td>.. 5 5 5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Infected, untreated</td>
<td>.. 0 0 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Streptomycin</td>
<td>100 0 0 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Streptomycin</td>
<td>500 0 0 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Streptomycin</td>
<td>1000 3 3 3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Antibiotic 3510</td>
<td>10 0 0 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Antibiotic 3510</td>
<td>100 1 1 1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Antibiotic 3510</td>
<td>500 5 4 4</td>
<td></td>
</tr>
</tbody>
</table>

* All embryos tolerated well 1000 streptomycin units or 500 units of antibiotic 3510.

In view of the relatively low toxicity of crude preparations of antibiotic 3510, high solubility in water, relative stability, lack of inhibition by serum, activity in vivo, high level of activity on gram-negative enteric pathogens and activity upon streptomycin- and grisein-resistant bacteria, this antibiotic offers certain possibilities for the control of infections caused by enteric pathogens, especially those resistant to other antibiotics.

Summary.—A grisein-like antibiotic, designated as antibiotic 3510, was found to be produced by a strain of S. griseus isolated from the intestinal contents of a heifer.

Antibiotic 3510 has a very narrow antibacterial spectrum, even narrower than that of grisein. It is active against certain gram-positive and gram-negative bacteria, especially organisms of enteric origin. Bacteria that have been made resistant by serial passage to streptomycin and to grisein are still sensitive to this grisein-like substance.

Antibiotic 3510 shows, in crude preparations, a rather low toxicity for the chick embryo. It is capable of protecting the latter against infections with Salmonella pullorum. In equal concentrations, it appears to be more potent than streptomycin.

The strain of S. griseus which produces the grisein-like antibiotic 3510 forms another antibiotic which inhibits the growth of certain mycobacteria, including M. avium and M. tuberculosis 607.8

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THE GAS TURBINE AND ITS SIGNIFICANCE AS A PRIME MOVER

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Read before the Academy, April 28, 1948

Introduction.—The emergence of the gas turbine as an accepted member of the family of heat engines and prime movers has taken place within the last ten years. Its application to the field of aircraft propulsion, notably in the form of jet propulsion, has been accompanied by much publicity and fanfare. This application, while enormously important, has tended to obscure the wider implications of this development. This paper gives a brief survey of developments which led to the gas turbine and their implications for the future.

In the two centuries and a half during which man has occupied himself with heat engines, there have been only a few events of far-reaching implications, and only three major types of prime movers have reached maturity: the steam engine, the steam turbine and the internal combustion engine. The selection from technological history is somewhat arbitrary, but up to the end of the last century the following events and approximate dates may be singled out as of particular significance: Savary's water raising engine, 1700; Newcomen's atmospheric engine, 1710; Watt's condensing engine, 1770; the caloric engine (Cayley, Stirling, etc.), 1825; Otto's internal combustion engine, 1875; the steam turbine (Parsons and de Laval), 1885; the Diesel engine, 1895. The arrival of the gas turbine is thus an event of the first importance. If it had taken place in a less exciting period of history, it would have been regarded as truly epoch making. Competing as it does with atomic bombs, atomic power, supersonic airplanes and guided missiles, not to mention the political and cultural characteristics of a "time of troubles," its relative significance has been overshadowed by other events.

Survey of Historical Background.—The gas turbine represents the fulfillment of an idea which was born at the beginning of the last century with