ketosteroid estimations after the use of kaolin are the same as those obtained from untreated aliquots of urine.

Summary. A method is described for the adsorption of gonadotrophic hormones from urine onto kaolin and their subsequent elution with ammonium hydroxide. The relatively small volume of the eluate reduces the amount of alcohol needed to precipitate the hormone from solution, the final extracts are less toxic and can be prepared in a shorter time. The hormone assays are comparable to those obtained with the usual alcohol precipitation method.

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17145. Streptomycin-Producing Capacity of Different Strains of *Streptomyces griseus.**†

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Since the first announcement[1] that certain strains of *Streptomyces griseus* produce the antibiotic streptomycin, pertinent questions have been raised in regard to the ability of this organism to produce the particular antibiotic: 1. Is this a characteristic property of all strains of the species *S. griseus?* 2. How widely distributed is the capacity for production of streptomycin among actinomycetes? 3. Did the original strain of *S. griseus,* first isolated in this country in 1915 by Waksman and Curtis,[2] also possess the capacity for producing streptomycin? (The last question is of considerable theoretical and historical interest, since it has a bearing upon the identity of the streptomycin-producing strains isolated in 1943 with the original 1915 isolate, as well as upon the fact that had the subject of antibiotics been recognized at that time, it would not have taken that long to recognize the actinomycetes as highly important producers of antimicrobial agents.)

The first two questions were soon answered. It was established[3] that only certain few strains of *S. griseus* are capable of producing streptomycin, whereas most of the other strains that can easily be isolated from natural substrates are either unable to produce any antibiotics at all or may give rise to antibiotics other than streptomycin, such as grisein,[4] and that certain actinomycetes, other than *S. griseus,* also possess the capacity of forming streptomycin.[5]

In an attempt to answer the third question, the original 1915 culture, which has been kept alive by frequent transfers since its isolation in the collection of the New Jersey laboratories under the ATCC number 3326, was tested for its antibiotic-producing capacity. The same culture was deposited in 1920 in the Baarn collection in Holland; it recently was obtained from that collection and given the number 3326a. Repeated tests for their antibiotic-producing capacity revealed the fact that the culture kept in New Jersey was totally unable to produce either streptomycin or any other antibiotic substance, whereas the corresponding Baarn culture was capable of forming an antibiotic

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1 Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

2 Waksman, S. A., and Curtis, R. E., *Soil Sci.,* 1916, **1**, 99.

3 Carvajal, F., *Mycologia,* 1946, **38**, 596; Waksman, S. A., Reilly, H. C., and Johnstone, D., *J. Bact.,* 1946, **52**, 393.

4 Waksman, S. A., Reilly, H. C., and Harris, D. A., *J. Bact.,* 1948, **56**, 259.

5 Johnstone, D. B., and Waksman, S. A., *J. Bact.,* 1948, **55**, 317.

agent, which was different, however, from streptomycin.[4] This difference may have been due to the fact that in the New Jersey collection the culture was kept on synthetic media and in the Baarn collection organic media are used. The difference pointed definitely to changes in the antibiotic-producing capacity during the 30-year period that the culture has been kept in the two collections.

Recently, Kelner[6] also examined the original *S. griseus* culture No. 3326. He confirmed the observation that the New Jersey strain is largely inactive antibiotically. When x-rayed conidia of this organism were plated out on various media and tested against bacteria, however, several active mutants were obtained, one of which apparently produced streptomycin. Comparison of the antibiotic spectrum of this mutant with that of known streptomycin-producing strains of *S. griseus* showed these spectra to be identical.

These observations appeared to be so important that an effort was made to confirm them and to demonstrate definitely that the antibiotic produced by Kelner's mutant No. 1 was actually streptomycin. This mutant, designated as 3326b, was streaked out on plates containing suitable agar media and, after 24- and 48-hour incubation periods, various test bacteria were cross-streaked toward it. An antibiotic spectrum characteristic of streptomycin-producing strains of *S. griseus* was obtained, as shown in Table I. There was only limited growth of the streptomycin-resistant strains of *E. coli,* and there was good growth of streptomycin-dependent strains. This fully confirmed the streptomycin-producing capacity of Kelner's mutant. The presence of a narrow zone of inhibition of the streptomycin-dependent culture points to the formation of another antibiotic other than streptomycin, a fact well established for the various streptomycin-producing cultures.

TABLE I.
Antibiotic Spectrum of Mutant 1 of 1915 *S. griseus* Culture (3326b), as Determined by Agar Cross-streak Method.
Zone of inhibition in millimeters.

Test organism	Nutrient agar	Nutrient glucose agar
Escherichia coli	24	19
" " sr*	3	7
" " sd*	6-25†	7-22†
Bacillus mycoides	24	21
Staphylococcus aureus	22	15
Bacillus subtilis	28	23

*sr—streptomycin-resistant; sd—streptomycin-dependent.
†First figure—inhibition zone; second figure—growth zone.

To confirm definitely the fact that the antibiotic produced by the mutant of the 1915 culture was streptomycin, it was necessary to produce this antibiotic in suitable culture media, isolate it, concentrate it and test it. This was carried out by the usual procedures developed for streptomycin.[1] The results presented in Table II prove definitely that the antibiotic produced by the mutant of the 1915 culture (3326b) is identical with that of the 1943 isolate of the streptomycin-producing culture (3463). When varying amounts of the metabolite solutions of the two cultures were added to agar media and the latter streaked with various test bacteria, similar results were obtained, except for some minor quantitative differences. After 5 days' incubation a cup assay of 111 μg of streptomycin for the 3326b culture gave 150 *E. coli* dilution units, 250 *B. mycoides* units, 150 *S. aureus* units and 1,500 *B. subtilis* units; the corresponding results for 110 cup assay units for 3463 were 150, 500, 250 and 1,000, which were very close to the foregoing if one recognizes the limitations of this method of testing. The isolated and concentrated streptomycin preparations obtained from the two cultures also gave nearly identical results.

When streptomycin-dependent strains of *E. coli* were streaked on media containing the two preparations, comparable growth was obtained from both preparations and was very similar to growth with similar amounts of pure streptomycin added to other plates. This was true of both the culture filtrates and the concentrates obtained from them. The dependent strains of *E. coli* were affected alike by both preparations, whereas the resistant strains were not affected at all by either.

Other supporting evidence bearing upon the

[6] Kelner, A., *J. Bact.*, 1949, **57**, 73.

TABLE II.
Identity of Antibiotic Produced by Culture 3326b with That of Streptomycin Produced by Known Streptomycin-producing Culture 3463.

Incubation, days	Streptomycin, μg/ml	Dilution units per ml			
		E. coli	*B. mycoides*	*S. aureus*	*B. subtilis*
		Culture filtrate of 1915 mutant (3326b)			
3	59	75	150	75	500
4	108	100	250	250	750
5	111	150	250	150	1,500
		Crude isolated preparation of 3326b			
	867*	1,500	3,000	1,500	>10,000
		Culture filtrate of streptomycin-producing culture (3463)			
3	41	30	100	75	300
4	64	75	150	75	750
5	110	150	500	250	1,000
		Crude isolated preparation of 3463			
	770*	1,000	3,000	500	10,000

* Culture filtrate adsorbed on Norite, dried, eluted with acid alcohol, treated with ether, to yield aqueous concentrate.

problem under consideration may be reported here. It had been suggested previously[7] that the sensitivity of streptomycin-producing strains of *S. griseus* to a specific actinophage could be used for establishing the capacity of such strains to form streptomycin. The original 1915 culture of *S. griseus* (3326) was not sensitive to this particular phage; this was taken to indicate that there was a definite correlation between phage sensitivity of *S. griseus* strains and ability to form streptomycin, a fact confirmed by a lack of sensitivity to phage by other, freshly isolated, non-streptomycin-producing strains of *S. griseus*. When Kelner's mutant was tested for sensitivity to this phage, it was found to be highly sensitive. This is illustrated in Table III. The streptomycin-producing, including the two original isolates (3463, 3464), a spore isolate of one of these (3464a), and a freshly isolated culture (3481), were all phage-sensitive as measured by the rate of cell multiplication. Both forms of the original 1915 isolate (3326, 3326a), a grisein-producing strain of *S. griseus* (3478), and another non-streptomycin-producing culture (3482) were all insensitive to this phage, as shown by lack of multiplication or by actual adsorption by the mycelium of the organism. On the other hand, the Kelner mutant of the 1915 isolate (3326b) was phage-sensitive.

The identical behavior of the mutant of the 1915 culture with that of the streptomycin-producing strains of *S. griseus* is thus established unequivocally. Other evidence of similar cultural characteristics, sporulation, and biochemical properties further confirmed the identity of the two cultures.

These results thus suggest the probability that the 1915 isolate of *S. griseus* (3326) possessed originally the capacity of producing streptomycin, and that this capacity was lost during the more than 30 years that this culture was kept in the culture collection, growing on artificial, mostly synthetic, media. The ability of streptomycin-producing *S. griseus* cultures to mutate readily, even under ordinary conditions of culture, has been well established.[8] At least two mutants are now recognized, one of which does not sporulate and does not produce streptomycin, and one of which produces a red vegetative mycelium and forms an antibiotic substance distinct in nature from streptomycin.[4]

Summary. Evidence is presented that the original culture of *S. griseus* which was isolated from different soils, in 1915-1916, in the laboratories of the New Jersey Agricultural Experiment Station must have possessed the capacity for producing streptomycin. This capacity was lost upon continuous growth of

[7] Waksman, S. A., Reilly, H. C., and Harris, D. A., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 617.

[8] Schatz, A., and Waksman, S. A., *Proc. Nat. Acad. Sci.*, 1945, **31**, 129.

TABLE III.
Effect of Actinophage of Streptomycin-producing *S. griseus* on Various Strains of This Organism.

Culture No.	Nature of strain	Spot test* for phage		Submerged growth effect† Phage per ml × 10⁷, after days of incubation			
		Nutrient agar	Dextrose-asparagine agar	0	3	5	7
3326	N. J. culture of 1915 isolate	—	—	6	3	4	4
3326a	Holland culture of 1915 isolate	—	—	6	<0.4	<0.04	0.004
3326b	Kelner's mutant of 1915 isolate	+	+	6	100	105	186
3463	Streptomycin-producing soil culture of 1943	+	+	6	30	31	119
3464	Streptomycin-producing chicken throat culture of 1943 isolate	+	+	6	210	320	570
3464a	Spore isolate of last	+	+	6	34	260	204
3478	Grisein-producing culture	—	—	6	2	2	3
3481	More recent independent isolate of a streptomycin-producing culture	+	+	6	257	420	400
3482	Non-streptomycin-producing culture	—	—	6	0.2	0.01	0.03

* Phage placed on 24-hour vegetative streaks growing on nutrient and dextrose-asparagine agars, incubated an additional 24 hours, and examined for lysis.
† Phage added to freshly inoculated flasks of medium, grown under submerged conditions.

the culture upon artificial media for more than 30 years. When irradiated, this culture yielded a mutant that was as potent a producer of streptomycin as the streptomycin-yielding strains of *S. griseus* isolated in 1943.

This assumption was fully confirmed by the characteristic antibiotic spectrum of the two cultures, their behavior to streptomycin-dependent and streptomycin-resistant mutants of *E. coli* and other bacteria, and sensitivity of the two cultures to the same actinophage.

These results further point to the accuracy of the identification of the streptomycin-producing cultures of *S. griseus* with that of the original 1915 isolate, based on literature description alone, in spite of the fact that the type culture available in two collections showed distinct changes from the original description.

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17146. Effect of Tetraethylammonium Bromide on Adrenal Medulla.

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Acheson and Moe[1] have shown that tetraethylammonium ion will block autonomic ganglia. Since the adrenal medulla is generally accepted as ganglionic in nature, the transmission of impulses across the ganglion and subsequent release of epinephrine should be blocked by the injection of tetraethylammonium bromide. Some evidence for this has been shown by the action of tetraethylammonium on morphine hyperglycemia in dogs,[2] which closely resembles the effect on such hyperglycemia produced by surgical removal of the adrenal medulla.[3] In both cases mor-

[1] Acheson, G. H., and Moe, G. K., *J. Pharm. and Exp. Therap.*, 1946, **87**, 220.

[2] Morrison, J. L., *Fed. Proc.*, 1947, **6**, 359.

[3] Bodo, R. C., Co Tui, F. W., and Benaglia, A. E., *J. Pharm. and Exp. Therap.*, 1931, **61**, 48.