for bacterial DNA[1] there results the value $\sigma_{GG} = 5 \times 10^{-3}$, well below the upper limit set by the density gradient experiments. Accordingly, such statistical heterogeneity is not ruled out.

STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. X. CHARACTERIZATION AND CLASSIFICATION OF SPECIES WITHIN THE STREPTOMYCES GRISEUS GROUP

BY SELMAN A. WAKSMAN

INSTITUTE OF MICROBIOLOGY, RUTGERS, THE STATE UNIVERSITY

Communicated May 13, 1959

In 1948, a detailed description of the streptomycin-producing organism was published[13] under the name *Streptomyces griseus* (Krainsky) Waksman and Henrici. In the eleven years that have since passed, there has accumulated a very extensive literature dealing with this organism and a number of related forms. Numerous varieties have been described, and a certain degree of confusion has arisen. It was thereby established that we are dealing here not with a single species, but with a group of closely related forms. Because of the great economic importance of this group, its re-evaluation and reinterpretation is justified at this time.

Nomenclature of the Streptomycin-Producing Organism.—In 1916, Waksman and Curtis[11] isolated from different soils cultures of an organism which they believed to be similar to a culture previously isolated and described as *Actinomyces griseus* by Krainsky.[5] Waksman[10] soon recognized, however, that the two organisms were slightly different. Since no type culture of Krainsky's organism has ever been available for comparison, all the descriptions subsequently published in the various editions of Bergey's *Manual of Determinative Bacteriology* were based upon that of Waksman and Curtis,[11] later modified by Waksman,[10] and by Waksman *et al.*[13] When Waksman and Henrici[12] proposed, in 1943, to create a new genus *Streptomyces*, this organism was named *Streptomyces griseus*. The strain to which this name was applied carries our laboratory number 3463. It was the first streptomycin-producing culture and has been designated as the type culture (by original designation) of the new species *Streptomyces griseus* Waksman and Henrici.

The original proposal to create a new generic name *Streptomyces* was made by Waksman and Henrici in a manuscript submitted to the *Journal of Bacteriology* in April, 1943, and to Dr. R. S. Breed in May, 1943, for publication in the sixth edition of Bergey's *Manual*. This name first appeared in the *Journal of Bacteriology* in October, 1943; unfortunately, the sixth edition of Bergey's *Manual*, which contained a detailed description of *Streptomyces griseus* was not published until 1948.

Krassilnikov[6] also recognized a difference between the culture isolated by Krainsky and that of Waksman and Curtis. The organisms exhibiting spiral formation were regarded as characteristic of Krainsky's culture; the nonspiral forming strains characteristic of the streptomycin-producing *S. griseus* were regarded as a subspecies of *A. globisporus*, and designated as *A. globisporus streptomycini*, later changed to *A. streptomycini*.[7] Krainsky's culture, however, has never been seen by

Krassilnikov, and because Krainsky did not describe the morphology of the aerial mycelium it will remain unknown forever whether his culture formed spirals or not. Furthermore, the color of the aerial mycelium and the pigment of Krainsky's *A. griseus*, as described by him and as can be seen in the color photograph of his publication, resembles the streptomycin-producing culture more than the *A. griseus* of Krassilnikov. Since the Waksman and Henrici characterization of the streptomycin-producing organism, based upon the description by Waksman and Curtis, is now universally accepted, there is hardly any justification for changing the name.

Harada *et al.*[3] concluded as well that Krassilnikov was wrong in placing the streptomycin-producing organism with *A. globisporus* for the simple reason that the former produces oval to cylindrical spores, whereas the latter produces round spores, as its name indicates.

Baldacci and Comaschi[1] suggested that Krainsky's original culture of *A. griseus* appears to have been a strain of *S. viridis* Lombardo Pellegrino. They state:

"The comparison between Krainsky's and Waksman's descriptions gives evidence —as even Waksman has partially pointed out—to the difference of proteolytic activity and, according to our opinion, the very important difference of the color of the sporulating colonies which are greenish in Krainsky's description. If we accept Waksman's correction of the species and compare his descriptions with our strains, we find a perfect identity. Since it is impossible to compare the original strain of Krainsky with the others, the acceptance of the correction proposed by Waksman offers the advantage of maintaining the name 'griseus' for an actinomycete so largely spread out and studied in laboratories, so that we agree with it according to this meaning."

The name *Actinomyces griseus* was applied by Waksman and Curtis to an organism supposedly the same as the *Actinomyces griseus* Krainsky. Later it was concluded that the two organisms belonged to distinct species. This made the binary combination *Actinomyces griseus* Waksman and Curtis a later homonym and hence not legitimate. No name was proposed to replace it.

The conclusion may, therefore, be reached that the proper designation of the streptomycin-producing organism is *Streptomyces griseus* Waksman and Henrici. The use of *griseus* as the specific epithet in the new species *Streptomyces griseus* is legitimate and correct. In support of this conclusion, the note appended to Rule 26 in the Bacteriological Code may be quoted: "Where a new epithet is required, an author may, if he wishes, adopt an epithet previously given to the taxon in an illegitimate combination, if there is no obstacle to its employment in the new position or sense; the resultant combination is treated as a new name."

Major characteristics of the S. griseus Group.—The following are the major characteristics of the *S. griseus* group:

1. Morphology: Sporophores straight, forming tufts. Spores oval to cylindrical; surface of spores smooth. 2. Aerial mycelium: Color yellowish-gray to water-green to grass green. 3. Soluble melanin pigment on protein media: None. 4. Proteolytic activities: Strong.

Species and Varieties Belonging to the S. griseus Group.—

1. *Streptomyces griseus* Waksman and Henrici. Type culture 3463. Synonyms: *Actinomyces globisporus* Krassilnikov, 1941. *Actinomyces globisporus*

subsp. *streptomycini* (Waksman) Krassilnikov, 1949. *Actinomyces streptomycini* Krassilnikov, 1957.

(*a*) Produces streptomycin; some strains also produce cycloheximide.
(*b*) Resistant to streptomycin and to streptomycin-producing cultures.
(*c*) Produces green or yellow soluble pigments on calcium malate and succinate media.
(*d*) Sensitive to a specific phage.

2. *Streptomyces griseinus* n. sp. Type culture 3478.

(*a*) Produces grisein.
(*b*) Sensitive, by cross-streak, to *S. griseus*.
(*c*) No soluble pigment on calcium malate or succinate media.
(*d*) Resistant to the common *S. griseus* actinophage.

3. *Streptomyces purpureus* (Burkholder, 1955) emend. Synonyms: *S. vinaceus* Ciba 1951; *S. puniceus* Patelski, 1950; *S. floridae* Bartz *et al.*, 1951; *S. californicus sensu* Routien and Hofmann, 1951; *S. griseus* var. *purpureus* Burkholder *et al.*, 1955.

(*a*) Produces viomycin.
(*b*) Aerial mycelium tends to be white.
(*c*) Substrate mycelium purple.
(*d*) Resistant to viomycin and streptomycin.

4. *Streptomyces coelicolor* (Müller, 1908) emend. Kutzner and Waksman.[8] Type culture available at CBS (Holland). Synonym: *S. griseus* (Krainsky) Waksman and Curtis; not *S. violaceoruber*.

(*a*) Produces an antifungal antibiotic.
(*b*) Sensitive to streptomycin.
(*c*) Some strains produce a blue pigment on potato plug and certain agar media.
(*d*) Sensitive to a specific phage.

5. *Streptomyces chrysomallus* Lindenbein, 1952. Type culture 3657.

(*a*) Produces actinomycin C; some strains also produce cycloheximide.
(*b*) Substrate mycelium on various media orange to red to purple.
(*c*) Soluble pigment on various media faint yellow to golden yellow. Var. *Streptomyces chrysomallus* var. *fumigatus* Frommer, 1959.

6. There is a sixth type culture (No. 3533) belonging to the *S. griseus* group and that is frequently found in nature. This culture produces streptocin, an antibiotic active against trichomonads, fungi, and gram-positive bacteria. This culture has not been studied sufficiently to raise it to species status.

It is interesting to note here that, in a recent publication, Koreniako and Nikitina[4] divided the *S. griseus* group into four subgroups on the basis of antibiotic sensitivity, cross resistance, and phage sensitivity. These subgroups correspond to 1, 2, 4, and 6 of the species presented here.

Descriptions of Streptomyces griseus and Streptomyces griseinus.—

Streptomyces griseus Waksman and Henrici, 1943. Type culture 3463. (Waksman and Henrici, Bergey's *Manual*, 6th ed. (1948), 948; Waksman, S. A., H. C. Reilly, and D. Harris, *J. Bacteriol.*, **56**, 259 (1948).

Morphology: Sporophores straight, produced in tufts. Spores spherical to oval, 0.8 by 0.8–1.7 μ. Surface smooth.

Sucrose nitrate agar: Growth poor, thin, spreading, colorless, becoming olive-buff. Aerial mycelium thick, powdery, water-green. Pigment insoluble.

Nutrient agar: Growth abundant, almost transparent, cream colored. Aerial mycelium powdery, white to light gray. No soluble pigment.

Glucose agar: Growth elevated in center, radiate, cream colored to orange, erose margin.

Starch media: Growth thin, spreading, transparent. Hydrolysis strong.

Potato: Growth wrinkled, yellowish to brownish. Aerial mycelium white, powdery.

Gelatin: Greenish yellow or cream colored surface growth with brownish tinge. Rapid liquefaction.

Milk: Cream colored ring; coagulation with rapid peptonization; becoming alkaline.

Cellulose: Scant to fair growth.

Nitrate: Poor utilization. Reduction to nitrite.

Production of H_2S: Negative.

Phage sensitivity: Sensitive to a specific phage.

Antagonistic properties: Strongly antagonistic. Produces an antibiotic streptomycin, active against a large number of bacteria and actinomycetes, but not against fungi and viruses, and cycloheximide active upon fungi. Resistant to streptomycin-producing organisms and to streptomycin.

Habitat: Soils, river muds, throat of chicken.

Streptomyces griseinus (Reynolds and Waksman), n. sp. (Reynolds, D. M., and S. A. Waksman, *J. Bacteriol.*, **55**, 739–751 (1948); Okami, Y., *J. Antibiotics* (Japan) **3**, 95–97 (1950).)

Morphology: Straight sporophores produced in clusters or tufts, without spirals. Spores rod shaped, 1.0–1.8 by 0.8–1.0 μ.

Sucrose nitrate agar: Substrate growth wrinkled, reverse cream colored to brownish. Aerial mycelium white to cream colored with light greenish tinge. Lesser tendency to grass-green coloration, more of a cream color. No soluble pigment.

Potato: Growth wrinkled, yellowish white. Aerial mycelium grayish white with olive tinge. Slightly brownish pigmentation of plug.

Gelatin: Growth cream colored with brownish tinge. Aerial mycelium absent, or scant, white. No soluble brown pigment. Rapid liquefaction.

Milk: Growth cream colored. Coagulation and peptonization.

Starch media: Colorless to cream colored growth. Aerial mycelium grayish olive. Rapid hydrolysis.

Tyrosine agar: No pigment produced.

Nitrate: Good utilization. Reduction to nitrite.

Phage sensitivity: Not sensitive to the phage specific against streptomycin-producing strains.

Antagonistic properties: Produces the antibiotic, grisein.

Remarks: Okami[9] reported that grisein-producing strains of *S. griseus*, here designated as a new species *S. griseinus*, grow more steadily in synthetic media containing glucose, glycerol, and sucrose than do streptomycin-producers. According to Benedict and Lindenfelser,[2] streptomycin strains of *S. griseus* form green and yellow pigments on synthetic calcium malate and calcium succinate media, respectively, whereas the grisein strains form no pigment at all on these media or only a light pink pigment.

[1] Baldacci, E., and G. F. Comaschi, *Mycopathol. Mycol. Appl.*, **7**, 278 (1956).

[2] Benedict, R. G., and L. A. Lindenfelser, *Antibiotics & Chemotherapy*, **1**, 512–517 (1951).

[3] Harada, Y., S. Itagaki, S. Kubo, and S. Tanaka, *Bull. Agr. Chem. Soc. Japan*, Abstr. **22**, 12 (1958); **23**, 1, 8–9 (1959).

[4] Koreniako, A. I., and N. I. Nikitina, *Mikrobiologiya*, **28**, 14 (1959).

[5] Krainsky, A., *Zentr. Bakteriol. Parasitenk.*, II, **41**, 649 (1914).

[6] Krassilnikov, N. A., "Guide to the Identification of Bacteria and Actinomycetes," *Acad. Sci. USSR*, Moskau, 1–830 (1949). (Trans. in part by J. B. Routien, Chas. Pfizer & Co., 1957, p. 119).

[7] Krassilnikov, N. A., *Ann. inst. Pasteur*, **92**, 597 (1957).
[8] Kutzner, H. J., and S. A. Waksman, *J. Bacteriol.* (in press).
[9] Okami, Y., *J. Antibiotics*, **3**, 95–97 (1950).
[10] Waksman, S. A., *Soil Sci.*, **8**, 71 (1919).
[11] Waksman, S. A., and R. E. Curtis, *Soil Sci.*, **1**, 99 (1916).
[12] Waksman, S. A., and A. T. Henrici, *J. Bacteriol.*, **46**, 337 (1943).
[13] Waksman, S. A., H. C. Reilly, and D. A. Harris, *J. Bacteriol.*, **56**, 259 (1948).

*COMPENSATIONS IN ELECTRON EXCITATION EFFECTS IN p-p AND p-n SCATTERING**

BY M. DEWIT, C. R. FISCHER, AND W. ZICKENDRAHT

YALE UNIVERSITY

Communicated by Gregory Breit, May 6, 1959

Introduction.—The existence of appreciable probabilities of excitation of atomic electrons in *p-p* and *p-n* scattering caused by Coulomb Excitation and by the acceleration effect has been pointed out by Breit.[1] If the nucleon motion is treated by means of classical mechanics, these excitation effects are of little practical interest for the interpretation of *p-p* and *p-n* scattering in terms of nuclear forces, the changes in the energy and direction of the nucleons being small. The proton disappearing from the elastically scattered beam reappears with almost the same energy and at almost the same angle as an inelastically scattered one. This compensation is replaced[1] in quantum mechanics by the combination of the incoherent scattering with a modification in the coherent scattering caused by changes in phase shifts δK_L of the coherent wave. For any channel with sharp total angular momentum there appears a cross product term between the wave caused by δK_L and the usual elastically scattered wave as in Equation (2) of Reference 1. Estimates showed[1] that for the channels examined in special cases there was no compensation of incoherent by coherent effects. The possibility of the presence of nonnegligible modification in low energy *p-p* and *p-n* scattering due to quantum mechanical corrections to the excitation effects was therefore suggested.[1] On the other hand, it was pointed out[1] that the shortness of the proton and neutron wave lengths in the high energy experiments must produce agreement with classical treatment and therefore the classical mechanics type of compensation of elastic by inelastic processes.

In the present note an account is given of calculations for monopole, dipole, and quadrupole Coulomb Excitation. These were carried out according to the same general plan as that used by Breit.[1] For each of the three multipoles the effects of the different channels were combined and finally the incoherent contributions were added to the effects of the cross product terms on the coherent scattering. Employing the asymptotic expression $kr\text{-}\eta \ln 2kr - (L\pi/2) + \arg \Gamma(L + 1 + i\eta)$ for the phase of the regular Coulomb function and 1 for its amplitude the result was found to be zero. This indicates that the compensation of elastic and inelastic scattering effects present in the classical mechanics treatment carries over to some extent into the quantum mechanical problem. This circumstance was explained by Breit[2] as the result of a formal relationship between classical and