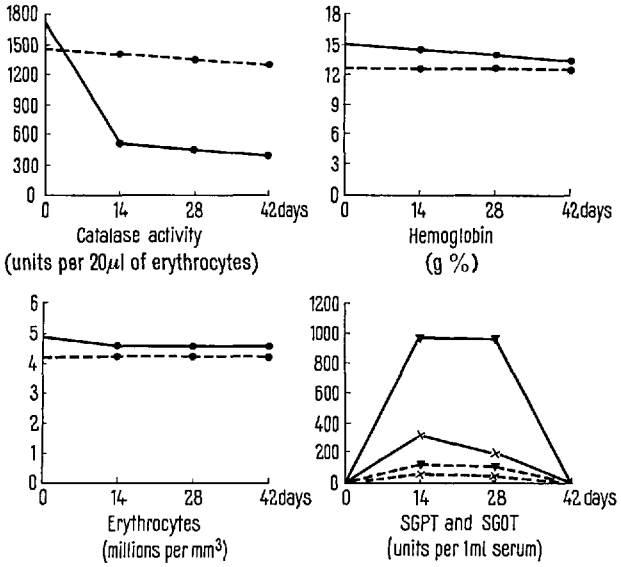

Fig. 1. Erythrocytes catalase activity as compared with other changes in dog blood in the course of chronic liver poisoning. Solid line = intoxicated dogs. Broken line = control dogs. ▽-▽-▽ SGTP activity. ×-×-× SGOT activity.

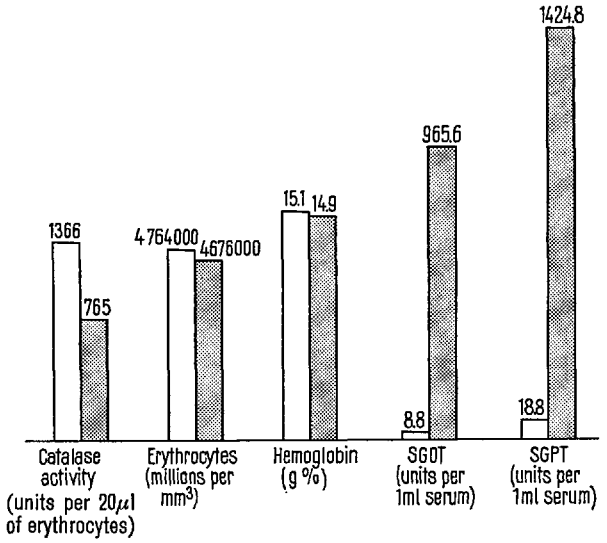

Fig. 2. Erythrocytes catalase activity as compared with other changes in dog's blood in the course of acute liver poisoning. White columns = values before liver injury, black solumns = values after poisoning.

DIOGUARDI[8] recently revealed that the activity of a great number of enzymes in erythrocytes may vary in larger limits in people with liver diseases than in healthy subjects. Moreover, in this author's opinion, adenosine triphosphate (ATP) concentration in erythrocytes decreases in liver diseases.

The results presented indicate a relationship between liver function and erythrocyte catalase activity. Further investigations, however, are needed in order to elucidate the role of the liver in the biosynthesis of this enzyme[9].

Résumé. Nous avons trouvé que l'activité de la catalase des erythrocytes chez les cirrhotiques et chez les chiens intoxiqués par CCl_4 est considérablement diminuée, tandis que le numéro globulaire et le taux d'hémoglobine ne représentent que des changements très peu signicatifs.

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II. Clinical Department of Internal Diseases and Department of Physiological Chemistry of the Medical Academy in Bialystok (Poland), March 1, 1963.

[8] N. DIOGUARDI, Europ. Symp. med. Enzymol. (Milano 1960), Proc. (Karger, Basel-New York 1961), p. 234.

[9] *Acknowledgments.* The author is indebted to L. JAROSZEWICZ, who kindly performed transaminase determination, and to L. REJNIAK (Department of Pathological Anatomy) for his help in the evaluation of autopsy data.

Distribution of Antibiotic-Producing Actinomycetes in Danish Soils

This paper reports results obtained in a study of the antibiotic activity displayed by 660 strains of the genus *Streptomyces* isolated from Danish soils.

Experimental Methods. Soil samples: Soil samples were collected at depths not exceeding 10 cm in 19 localities of uncultivated soil and other natural habitats in Denmark (Table I).

Media: A composite synthetic medium was used for the isolation – dextrose 5 g, soluble starch 1 g, glycerol 4 g, casein 1 g, asparagin 1 g, yeast-extract (Difco) 0.5 g, dibasic potassium phosphate 2 g, sodium chloride 1 g, sodium nitrate 1 g, magnesium sulphate ($7H_2O$) 1 g, calcium carbonate 0.5 g, agar (Difco) 16 g, tap water 1000 ml – adjusted to pH 7.0 after autoclaving (121°C/20 min).

For the maintenance of the cultures a potato-dextrose agar medium with 0.5% peptone was used (pH 7.0 after sterilization). A heart infusion-peptone-yeast-extract agar, pH 7.2 (after BELCOVE and SANTOW[1]), was used for the agar tests against bacteria, and for the anti-fungal analysis a dextrose-peptone-yeast-extract agar (pH 6.8).

Test organisms: In the antibacterial analysis, the main test organism was *Staphylococcus aureus* (strain 209 p) and in the antifungal tests *Saccharomyces ellipsoideus*. Other test organisms were *Bacillus subtilis, Mycobacterium phlei, Escherichia coli* and *Aspergillus niger.*

Isolation of strains of Streptomyces: Vegetation, withered leaves and stones were removed from the collecting place. The soil samples were taken aseptically. The pH of the soil was measured electrometrically. The water content of the samples was determined by drying 10 g of the soil at 98°C for 24 h. The soil was plated out as soon as possible after sampling, most often on the same day. The standard dilutions for plating-out of the soil samples were 1:250000, 1:500000 and 1:1000000. To the first flask of dilution, one drop of Tween 80 was added and so many

[1] A. S. BELCOVE and S. SANTOW, Antibiotics and Chemotherapy 6, 585 (1956).

Table I. Numerical distribution and activity of 660 strains of streptomyces isolated from Danish soils

Source of soil	Number of isolated strains	Number of active strains	Number of strains active against			
			B	F	b	f
Decaying seaweeds, pH 6.3–7.7	284	97	76	47	50	21
Deciduous forest soils						
Limestone rock, pH 7.5–8.2	133	54	24	41	13	30
Mould, pH 4.5–5.1	22	11	6	9	2	5
Humus, pH 3.8–5.5	47	11	6	8	3	5
Clay, pH 5.1–5.6	33	12	5	10	2	7
Coniferous forest soils						
Humus, pH 4.0–4.1	14	5	1	4	1	4
Sand, pH 4.3	20	12	7	12	0	5
Old grassland soil, pH 6.5–6.9	48	25	11	22	3	14
Heath soil, pH 4.2–4.7	34	18	11	16	2	7
Highmoor, pH 3.4–3.6	1	0	0	0	0	0
River shore soil, pH 7.1	10	9	5	6	3	4
Anthill, in coniferous forest, pH 4.7	14	11	6	9	2	5
Total	660	265	158	184	81	107
Percentage	100	40.1	23.9	27.9	12.3	16.2

B = total activity against bacteria F = total activity against fungi.
b = activity only against bacteria. f = activity only against fungi.

Table II. Results of antibiotic tests with six test organisms

Test organism	*S. aureus*	*E. coli*	*B. subt.*	*M. phlei*	*S. ellips.*	*A. niger*
Total number of strains in the test	503	503	503	413	503	445
Number of active strains	107	37	63	45	154	110
Percentage	21.3	7.4	12.5	10.9	30.6	24.7

glass beads (3.5 mm) that the bottom of the flask was covered. After shaking the flask for 10 min, the above-mentioned dilutions were produced. From each dilution 6–8 plates were made. Each soil sample was thus evaluated on 18–24 plates. The plates were incubated at 23°C for 14 days.

Isolations were made daily after the first six days of incubation. All colonies of *Streptomyces* were transferred to pure culture studies on potato-agar slants. The pure cultures were evaluated on a morphological basis, using the systematic criteria of BALDACCI[2] and WAKSMAN[3]. Only the strains differing *inter se* for each soil were included in the antibiotic tests.

Antibiotic analysis: For detecting antimicrobial activity, the method of LANDERKIN[4] was used. At first, the streptomycete was transferred—with the least possible spore mass—to one or two spots of an agar plate; after this, the plate was incubated for 72 h at 23°C. Then a suspension—made of 5 ml test agar which was melted and cooled to 52°C and 1 ml of a young culture of the bacteria (24 h) or of the yeast—was poured on to the surface of the plate so carefully that, when setting, the suspension would reach exactly the edge of the *Streptomyces* colony. The suspension of *Aspergillus niger* was made as a suspension of spores in sterile water with Tween 80. The antibacterial test plates were incubated at 37°C, and the antifungal ones at 23°C. The inhibition zones were measured after 12, 24, 48 and 72 h, respectively.

Results. The results obtained are presented in Table I and II. Of the total of 660 strains of *Streptomyces*, 265 (40%) showed an inhibitory effect. Antibacterial activity was demonstrated for 158 strains (24%) and antifungal capacity for 184 strains (28%). A purely antibacterial effect was found in 81 strains (12%), and a purely antifungal ability in 107 strains (16%).

The distribution of antibiotic activity had no particular ecological dependence, but the isolates from decaying seaweeds, limestone rock soil and old grassland soil displayed activities in between 34% and 53% of the strains isolated. Only 7% were active against Gram-negative bacteria, and the antibiotic activity against the other four test organisms listed above was smaller than against the two main test organisms. From the bottom layer of a pond, of two lakes, as well as from samples taken from the sea bed, no *Streptomyces* were isolated.

All active strains were handed over to the drug industry for closer examination and identification of the antibiotics produced. It is interesting to note that both antibiotics described and several antibiotic substances not previously known have been isolated by this screening method.

Zusammenfassung. 660 *Streptomyces*-Stämme wurden von natürlichen dänischen Standorten isoliert und auf ihre antibiotische Aktivität untersucht. Ablagerungen von Tang, waldbedecktem Kreidefelsboden sowie altem Grasland gaben die grössten Werte.

T. V. NISSEN

The Royal Technical University of Denmark, Copenhagen (Denmark), April 24, 1963.

[2] E. BALDACCI, C. SPALLA, and A. GREIN, Arch. Mikrobiol. *20*, 347 (1954).
[3] S. A. WAKSMAN, Bact. Rev. *21*, 1 (1957).
[4] G. B. LANDERKIN, Canad. J. Pub. Health *38*, 90 (1947).