LITMOCIDIN, A NEW ANTIBIOTIC SUBSTANCE PRODUCED BY PROACTINOMYCES CYANEUS

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In a search for new antibiotic substances a soil actinomycete was isolated by us from a sample of south Russian soil that produces a powerful antibiotic substance of a new type. In this article a description of this organism is given, together with some details concerning the production of the antibiotic substance and its action upon pathogenic bacteria. A subsequent paper by M. G. Brazhnikova treats of the isolation and purification of litmocidin and outlines its chemical nature.

The antagonist. The strain of actinomycete isolated by us is close to *Actinococcus cyaneus*, isolated by Beijerinck from garden soil in Holland in 1913. It forms well-developed mycelium which later disintegrates into single cells. Colonies produce deep-blue water-soluble pigment, which becomes red at an acid reaction, and blue at an alkaline reaction. A similar litmuslike pigment was recorded by Beijerinck.

However, the conditions for the production of a litmuslike pigment by our microbe and by the *Actinococcus* of Beijerinck are very different. The latter readily produced pigment upon a mineral medium containing glucose and ammonium nitrate. Upon this medium our microbe is unable to produce a litmuslike pigment. The conditions for pigment production by our microbe are outlined in table 1. It is clear that either peptone or tryptone (still better both of them) is required for pigment production in our strain.

In view of important differences in pigment production between our microbe and the *Actinococcus* of Beijerinck, and because of the strong antibiotic action of the litmuslike pigment produced by our microbe (nothing like this is reported by Beijerinck), it is proposed to designate the strain as a new variety, var. *antibioticus*. According to modern classification (Krasilnikov, 1945) the generic name *Actinococcus* should be replaced by *Proactinomyces*, and the complete name of the organism should be *Proactinomyces cyaneus-antibioticus*.

It is to be pointed out that the production of a litmuslike pigment occurs in different groups of actinomycetes, and has been particularly well studied in *Actinomyces coelicolor*. As Kriss (1936) has shown, the pigment of the latter species belongs to the anthocyanines. It is also remarkable that the litmuslike pigment of *A. coelicolor* is devoid of antibiotic action (Krasilnikov, 1945). The anthocyanine pigments of plants are also devoid of antibiotic action, according to our experience.

In the light of all this evidence it appears probable that the antibiotic, litmuslike pigment observed by us represents some specific modification of the common litmuslike pigment. As will be shown in the subsequent paper, the antibiotic

substance recorded by us represents some derivative of anthocyanidine and evidently differs from the familiar anthocyanine pigments of plants. Inasmuch as the new antibiotic substance represents a litmus pigment with antibacterial action, it is proposed to designate it as *litmocidin*.

Formation of litmocidin. To obtain litmocidin, *P. cyaneus-antibioticus* is grown upon a nutritive agar of the following composition: peptone 0.5 per cent, tryptone 0.3 per cent, glucose 1 per cent, NaCl 0.5 per cent, $FeSO_4$ 0.001 per cent, agar 2 per cent, tap water. While growing upon this medium *P. cyaneus-antibioticus* strongly inhibits the growth of staphylococci. The antagonistic organism grows very rapidly, and the litmocidin produced by it reaches maximal concentration in the nutritive agar 48 to 72 hours after inoculation at 28 C. The

TABLE 1

Growth and pigment production by Proactinomyces cyaneus-antibioticus upon various media

COMPOSITION OF NUTRITIVE AGAR	GROWTH	PIGMENT PRODUCTION
1. Peptone, glucose, mineral salts.....	Good	Observed (++)
2. Tryptone, glucose, mineral salts....	Good	Observed (++)
3. Peptone, tryptone, glucose, mineral salts.....	Good	Very strong (++++)
4. Meat extract, glucose, mineral salts.....	Good	No pigment
5. Pea extract.....	Good	No pigment
6. Yeast autolysate.....	Good	No pigment
7. Asparagine, glucose, mineral salts...	Good	No pigment
8. Ammonium nitrate, glucose, mineral salts.....	Good	No pigment

TABLE 2

The inhibition of growth of Staphylococcus aureus in broth by the watery extract of a 48-hour-old agar culture of P. cyaneus-antibioticus

DILUTION OF EXTRACT	1:50	1:100	1:250	1:500	1:750	1:1,000	1:1,500
Growth of staphylococci....	−	−	−	−	−	−	+

inhibitory action upon staphylococci of the watery extract of an agar culture is shown in table 2.

Strain variation. In the course of subsequent cultivation the strain of *P. cyaneus-antibioticus* isolated by us produced inactive variants differing from the basic active strain:

Active strain	Inactive variant
1. *Antibiotic activity.* Abundantly produces litmocidin in agar cultures.	1. No litmocidin or only traces of litmocidin are formed.
2. *Growth.* No sporulating aerial mycelium. Growth characteristic of *Proactinomyces.*	2. Colonies are covered by a thin layer of grayish-white aerial mycelium.
3. *Variation.* Nonsporulating strain gives rise to sporulating variants.	3. No reconversion of sporogenous variants into original asporogenous form has so far been recorded.

The situation observed by us is in a sense the reverse of that recorded by Schatz and Waksman (1945) in *Actinomyces griseus*. For the successful production of litmocidin the cultures of *P. cyaneus-antibioticus* are regularly plated upon nutritive agar; active nonsporulating variants are selected and kept in pure culture.

Unit of activity of litmocidin is that quantity of the substance which is just sufficient to inhibit completely the growth of *Staphylococcus aureus* in 1 ml of broth. One ml of a two-day-old agar culture of *P. cyaneus-antibioticus* contains 2,000 units of litmocidin.

TABLE 3

The bacteriostatic action of litmocidin upon various bacteria in nutritive broth

BACTERIA	DILUTION OF LITMOCIDIN COMPLETELY INHIBITING GROWTH
Staphylococcus aureus 1418	1:4,000,000
Staphylococcus aureus M	1:4,000,000
Staphylococcus aureus 75a	1:2,000,000
Staphylococcus aureus 1623	1:2,000,000
Streptococcus hemolyticus 8	1:1,000,000
Streptococcus hemolyticus T	1:2,000,000
Streptococcus hemolyticus D	1:2,000,000
Streptococcus hemolyticus 5	1: 400,000
Streptococcus hemolyticus 1972	1: 500,000
Streptococcus hemolyticus 11	1:2,000,000
Streptococcus hemolyticus 13	1:1,000,000
Streptococcus viridans	1: 500,000
Mycobacterium tuberculosis	Very strong action
Vibrio comma 23	1:1,000,000
Vibrio comma 15	1:1,000,000
Vibrio comma 72	1:1,000,000
Vibrio comma 1606	1:1,000,000
Vibrio comma 771	1: 500,000
Vibrio comma 99	1:2,000,000
Vibrio comma U	1:2,000,000
Shigella dysenteriae (Shiga)	1: 10,000
Shigella dysenteriae (Flexner)	1: 10,000
Eberthella typhosa	1:1,000
Salmonella paratyphi A	1:1,000
Salmonella paratyphi B	1:1,000
Escherichia coli	1:1,000

As will be shown in the subsequent paper, the active principle of litmocidin is now isolated and purified, and it is available as a dry powder. The activity of this powder is 4,000 units per mg.

Action of litmocidin upon bacteria. The experiments here reported were made with a purified preparation of litmocidin. Usually we made a 0.2 per cent solution of litmocidin in water, which preparation possesses an acid reaction and a red coloration. With a little alkali the reaction was made neutral, and the solution acquired a violet color. The bacteriostatic action of litmocidin upon various bacteria is shown in table 3.

It is clear that litmocidin possesses selective action. It strongly inhibits staphylococci, streptococci, tubercle bacilli, and *Vibrio comma*, whereas its action upon dysentery bacilli is moderate, and upon typhoid and colon bacilli there is practically no action at all. For most strains of staphylococci, streptococci, and *Vibrio comma* the action of litmocidin is not only bacteriostatic, but also bactericidal. It is also remarkable that the antibacterial action of litmocidin is in no way inhibited by the addition of 10 per cent to 30 per cent of human or horse serum to the nutritive medium.

Toxicity of litmocidin. Neutral 0.2 per cent to 0.4 per cent solutions of litmocidin in physiological saline were given intraperitoneally to white mice 20 grams in weight. The observations continued for 5 days, and the corresponding data are presented in table 4. This table shows that a single intraperitoneal administration is not very toxic for mice.

TABLE 4

Toxicity of litmocidin for mice

UNITS OF ACTIVITY PER 20-G MOUSE	NUMBER OF MICE	PERCENTAGE OF MORTALITY
1,000	20	0
1,500	20	0
2,000	15	0
2,500	15	0
3,000	15	0
4,000	25	52
5,000	25	48
10,000	20	100

Chemotherapeutic activity. White mice were infected intravenously with a virulent strain of *Staphylococcus aureus* 1418, which is inhibited by litmocidin *in vitro* in a dilution of 1:4,000,000. Watery solutions of litmocidin were injected subcutaneously into infected mice in amounts of 50 to 500 units, 1 to 3 times per day, for 3 consecutive days. These experiments have shown that litmocidin does not possess chemotherapeutic action on staphylococcal septicemia in mice. The author is indebted to Professor A. Pines for making the chemotherapeutic trial of litmocidin.

SUMMARY

A new variety of *Proactinomyces cyaneus* has been isolated from a sample of south Russian soil and is described as *P. cyaneus-antibioticus*. It produces an antibiotic substance of a new type, representing a derivative of anthocyanidine. This antibiotic is designated as litmocidin. It reveals strong bacteriostatic action upon staphylococci, streptococci, *Vibrio comma*, and tubercle bacilli, and this action in not inhibited by blood serum. Litmocidin is devoid of chemotherapeutic action on septicemia in mice caused by a strain of staphylococcus very susceptible to litmocidin *in vitro*.

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