COMPARATIVE STUDIES CONCERNING THE EFFECT OF ALKYLATING AGENTS AND OF INHIBITORS OF PROTEIN SYNTHESIS ON THE ANTAGONIC SYSTEM STAPHYLOCOCCUS 31A - BACILLUS MEGATERIUM 207

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The requirements of an intercellular contact between Staphylococcus cells and cells of Bacillus megaterium for the production of lysis in bacillary cells, as well as the physical evidence of cytoplasmic fusion bridges between staphylococci and bacilli, have suggested the possibility that the phenomenon of lysis of Bacillus cells becomes the consequence of a unidirectional, high frequency heterospecific genetic transfer Staphylococcus 31A ⟶ B.megaterium 207 (Antohi et al. 1965, 1966). However, the presence of an analogous process of syngamy does not in the least refute the possibility that cells of Staphylococcus synthesize a bacteriocin-like factor located, for example, in the fusion bridges and which provokes the lysis of the bacilli.

Since inductory effect of alkylating agents on bacteriocin synthesis is already known (Jacob, 1954) in the present research we have tested the effect of nitrogen mustard in the lysis phenomenon. Because inhibitors of protein synthesis such as streptomycin, chloramphenicol, erythromycin, similary inhibit the synthesis of bacterio-

cins (Hertman and Ben Gurion, 1958), we have studied likewise the effect of the above-mentioned drugs on the lytic ability of staphylococci.

Our studies were prompted by the following reasons: i) If the mechanism of bacilli-lysis is grounded on the synthesis of a bacteriocin by the Staphylococcus, then it would follow that the treatment of staphylococcal cells with alkylating agents should increase the rapidity of the bacilli-lysis in a mixed suspension. The drugs interfering with the synthesis of proteins should decrease or stop in this case the lysis phenomenon altogether. ii) If the lysis is provoked by a transfer of genetic material, in this case the alkylating agents should inhibit the bacilli-lysis while the inhibitors of protein synthesis could eventually have no effect. The latter reason is based on some findings which have hitherto been made known: alkylating agents interfere primarily with DNA synthesis (Lawley and Brookes, 1963); the genetic bacterial transfer is associated to the DNA synthesis (Jacob et al. 1963, Gross, 1965); inhibitors of protein synthesis (chloramphenicol) permit the DNA synthesis once it has begun (Flaks et al. 1959, Luria, 1962).

MATERIAL AND METHODS

Strains of Staphylococcus 31A (provided by P.H.L.S. London) and Bacillus megaterium 207 (provided by Ivanovics, Szeged) were used. The strains were grown in nutrient agar + 0.5% glucose for 20 hours at 37° and suspended separately in nutrient broth pH 7.4 in a concentration of about 3×10^9 cells/ml. The suspensions were divided in 5 ml volumes and the following course was taken: (a) 5 ml suspension of Staphylococcus 31A was mixed with 5 ml suspension of B.mega-

terium 207 (as a control mixture); (b) 5 ml suspension of Staphylococcus 31A were treated with 1000 μg/ml nitrogen mustard for 30 minutes at 37°, and were then centrifuged and the bacterial sediment was resuspended in the same volume of broth, this time without any alkylant. It was finally mixed with 5 ml suspension of B.megaterium 207; (c) 1000 μg/ml of nitrogen mustard was added to 5 ml suspension of B.megaterium 207 and the course described at point (b) was followed, finally mixing it with a 5 ml suspension of untreated staphylococcal cells; (d) three mixtures of 5 ml suspension of Staphylococcus and 5 ml suspension of B.megaterium were carried out. An addition of streptomycin 200 μg/ml in the first, of chloramphenicol 10 μg/ml in the second and 5 μg/ml of erythromycin in the third mixture was then made. Each of the concentration of drugs was inhibitory for the growth of the used strains.

All mixtures were incubated for 8 hours at 37°. Every 60 minutes, samples of 1 ml from each mixture were drawn and were centrifuged for 10 minutes at 4000 r.p.m. Smears were then made of the sediment, and were stained by Gram technique. The presence or the absence of bacilli in the microscopically observed smears were a test for the moment of lysis.

With the purpose of effecting a general control, megacinogenic strain B.megaterium 216 (Ivanovics, 1962) and a streptomycin resistant megacin sensitive mutant of B.megaterium 207 were made use of in their capacity as well-tried antagonic bacteriocin grounded system. For the induction of megacin with the aid of nitrogen mustard, fresh cultures of B.megaterium 216 in nutrient agar were suspended in

nutrient broth in a concentration of 1.5×10^9 cells/ml. 1000 µg/ml nitrogen mustard was added and incubated for 10 minutes at 37° and then centrifuged at 5000 r.p.m. for 10 minutes. The sediment was resuspended again in nutrient broth + 1% glucose. 50 ml of this latter suspension were introduced in a 500 ml Erlenmeyer flask and incubated for 4 hours at 37° with a continuous gently shaking. The suspension was then again centrifuged, the supernatant free of cells was diluted 1/2,1/4,1/8···1/1024, the dilutions being then tested by dropwise technique on cells of B.megaterium 207 spread in nutrient agar. In order to test the effect of streptomycin on the production of megacin, cells of fresh culture of the B.megaterium 216 suspended in nutrient broth + glucose 1%, in a concentration of 1.5×10^9 cells/ml were irradiated with ultraviolets according to Nagy et al. technique (1959). After adding streptomycin 200 µg/ml the suspension was incubated for 4 hours at 37°, it was then centrifuged and the supernatant was tested by the dropwise technique on streptomycin resistant megacin sensitive mutant of B.megaterium 207.

RESULTS AND DISCUSSION

Nitrogen mustard had an inductive effect on the megacin synthesis in the antagonic system B.megaterium 216 - B.megaterium 207, this because the alkylating agent stimulates the production of an active megacin even in an 1/1024 dilution. The streptomycin provoked a 99% decrease in the production of megacin.

Notwithstanding the above effects found in the case of bacteriocin synthesis, nitrogen mustard produced a marked inhibition of the lytic ability of Staphylococcus 31A.

The treatment of cells of Staphylococcus 31A with nitrogen mustard before mixing, had a retarding effect of the lysis of bacillary cells with at least three hours (table 1, b). In the smears carried out at 4,5 and 6 hours of

TABLE 1.

The effect of nitrogen mustard and of drugs on the moment of lysis of the bacilli in the mixtures of cells of Staphylococcus 31A and B.megaterium 207.

0,1,2,3···8 = the time units (extressed in hours) when the samples were collected. + = the presence of bacilli in smears, the same frequency as at 0 time unit being observed. ± = the presence of bacilli in smears, this time in small numbers. 0 = the absence of bacilli in smears.

	Agent	Mixture of cells. Staphylococcus X B.megaterium			0	1	2	3	4	5	6	7	8
(a)	———	31A	X	207	+	+	+	±	o	o	o	o	o
(b)	Nitrogen mustard (only 31A was treated)	31A	X	207	+	+	+	+	+	+	+	±	±
(c)	Nitrogen mustard (only 207 was treated)	31A	X	207	+	+	+	±	o	o	o	o	o
(d)	Streptomycin	31A	X	207	+	+	+	±	o	o	o	o	o
(e)	Chloramphenicol	31A	X	207	+	+	+	±	o	o	o	o	o
(f)	Erythromycin	31A	X	207	+	+	+	±	o	o	o	o	o

incubation the bacillary cells were present with a frequency equal to that exhibited at 0 time. Moreover, the nitrogen mustard had an asymmetric effect because the moment of lysis was not in the least retarded by the treatment of B.megaterium 207 cells (see table 1, c). The moment of lysis for this last mixture was the same as in the case of the control mixture (see table 1, a). Although the treatment of staphylococcal cells retarded the moment of lysis, the Staphylococcus -Bacillus fusion bridges were produced at a high frequency.

Streptomycin, chloramphenicol, erythromycin had no effect on the moment of lysis (see table 1, d, e and f).

These experimental results clearly point to the fact that the lysis of bacillary cells in Staphylococcus 31A X B.megaterium 207 mixture cannot be due to a bacteriocin or to any bacteriocin-like substance.

If the DNA synthesis is primarilly inhibited by alkylating agents, if the DNA synthesis is associated with the transfer of genetic material and finally if the inhibitors of protein synthesis permit the DNA synthesis once it has begun, then the above mentioned results strongly support the hypothesis according to which the lysis of bacilli is the outcome of a high frequency unidirectional heterospecific genetic transfer Staphylococcus 31A⟶ B.megaterium 207. This transfer could form a lethal heterosis conducive to the lysis of bacillary cells which under the circumstances might be the seat of this heterosis.

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