THE ACTION OF ULTRAVIOLET RADIATIONS ON THE ANTAGONIC SYSTEM STAPHYLOCOCCUS 31A - BACILLUS MEGATERIUM 207

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Previous observations have suggested the possibility that the lysis of Bacillus megaterium cells in mixed suspensions of staphylococcal cells and cells of B.megaterium, can be the consequence of a heterospecific high frequency transfer of genetic material Staphylococcus 31A→B.megaterium 207 (Antohi et al. 1966, Moraru et al. 1966). However this assumption makes it necessary to demonstrate the fact that the lysis is not provoked by a lytic factor which is analogous to a bacteriocin. The more so because Barrow (1963) emphasized upon the antagonic activity of some Staphylococcus strains against some strains of Bacillus as being based on the synthesis of a staphylocin. Moraru et al. (1966) have pointed out that the ability of Staphylococcus 31A to provoke the lysis of B.megaterium 207 is suppressed by nitrogen mustard while the inhibitors of protein synthesis bear no effect on it. These results support the hypothesis of genetic material transfer and invalidate the possibility that the bacilli lysis be the attribute of a synthesis of a bacteriocin-like lytic factor by Staphylococcus.

Since ultraviolet (UV) treatment of donor cells in the E.coli K_{12} system inhibits the transfer of genetic material (Gross 1965) and since these radiations act as an inductor on the bacteriocin synthesis (Jacob et al. 1951, 1952, Jacob, 1954, Ivanovics & Alföldi 1955) in the present work the UV action on the lytic ability of Staphylococcus has been studied.

The present study started from the following reason: if the bacilli lysis is a consequence of the transfer of genetic material, UV should necessarily inhibit the lytic ability of staphylococci and likewise if the lysis were produced by a bacteriocin (staphylocin) then UV should eventually increase the lytic ability.

MATERIALS AND METHODS

Strains of Staphylococcus 31A (received from P.H. L.S. London), B.megaterium 207 and B.megaterium 216 (kindly provided by Ivanovics, Szeged, Hungary) were used. The strains were grown in nutrient agar + glucose 0.5% for 20 hours at 37° and then they were suspended separately in nutrient broth + glucose 1% pH 7.4 in a concentration of 10^9 cells/ml. UV irradiations were carried out by means of a low tension Astralux Original Vienna 500 watts lamp at a 25 cm distance. During irradiation time, the 6 mm thick suspensions were stirred with the aid of a magnetic stirring at an approximate frequency of 1000 r.p.m. Though the suspensions in nutrient broth required a prolonged UV treatment, we chose broth instead of inorganic buffered solutions since the lysis of bacilli could take place rapidly only in complete medium (Antohi et al. 1965).

Volumes of 20 ml of staphylococcal suspensions were irradiated separately for 30 seconds, 1 minute, 2 minutes, 3 minutes and 4 minutes respectively. Volumes of 5 ml were collected out of each of the suspensions which were then mixed each with a 5 ml B.megaterium 207 suspension.

5 ml suspension of unirradiated staphylococci were mixed with 5 ml suspension of B.megaterium 207 cells in order to obtain a control mixture.

All mixtures were then incubated at 37° for 8 hours in a thermostat bath. Every 60 minutes samples of 1 ml were collected out of each of the mixtures, in order to determine the moment of bacilli lysis and their biological inactivation. With the purpose of marking the presence or the absence of bacilli, a 0.9 ml volume of each 1 ml sample was centrifuged for 10 minutes at 4000 r.p.m. smears being then taken from the sediment which were stained by Gram technique. The disappearance of bacilli in the smears was a test which pointed out the moment of lysis.

With a view to quantifying the biological inactivation of bacilli, a 0.1 ml volume of each of the 1 ml samples was diluted 10^{-2}, 10^{-3}, 10^{-4} and the dilutions were dispersively inoculated in an Alföldi solide medium (Antohi et al. 1965) distributed in Petri dishes. The dishes were incubated at 37° for 20 hours the number of Bacillus colonies being then scored. The Alföldi solide medium was a selective medium which allowed only for the growth of B.megaterium 207 from the Staphylococcus - Bacillus mixtures.

In order to bring into relief the asymmetrical action of UV on the Staphylococcus 31A - B.megaterium 207 system, suspensions of B.megaterium were irradiated in the manner

already described for the staphylococci. They were then mixed with unirradiated suspensions of staphylococci, the mixtures were incubated for 8 hours at 37^{o} and every 60 minutes, samples for smears were collected with a view to observing the moment of lysis.

To check up the conditions of irradiation in our work we have use of the induction of megacin synthesis by B.megaterium 216 megacinogenic (Ivanovics, 1962). A fresh culture of B.megaterium 216 was suspended in nutrient broth + glucose 1% in a concentration of 10^{9} cells/ml. A 50 ml suspension was irradiated with UV (in conditions described above) for 3 minutes. The suspension was then introduced into a 500 ml Erlenmeyer flask and incubated for 4 hours at 37^{o} with a continuous gently shaking. Having been centrifuged at 5000 r.p.m. for 30 minutes the free-cell supernatant was tested for megacin production through dropwise technique applied on megacin sensitive B.megaterium 207 which was spread in Alföldi solide medium distributed in Petri dishes.

RESULTS AND DISCUSSION

Under the above mentioned experimental conditions the UV irradiations of B.megaterium 216 megacinogenic has had as effect the induction of a synthesis of megacin which was active even in a dilution 1/2000 on megacin sensitive B.megaterium 207.

The UV treatment of staphylococci before the mixtures were made had as effect a marked delay in the bacilli lysis and in their biological inactivation.

A morphological analysis of smears has proved the fact that the moment of bacilli lysis occured after 3 hours of

incubation with the control mixture and with mixtures in which staphylococcal cells were irradiated for 30 seconds and 1 minute respectively. The UV treatment of staphylococcal suspensions for 2 and 3 minutes (before mixing) had as effect a delay in the moment of lysis of bacillary cells. Moreover, if in the case of the control mixtures the bacilli lysis was rapid and almost complete, in the case of staphylococci irradiation the decrease in the number of bacilli began only later (after 4 or 6 hours respectively) while a significant number of bacilli persisted in the smears after 8 hours of incubation.

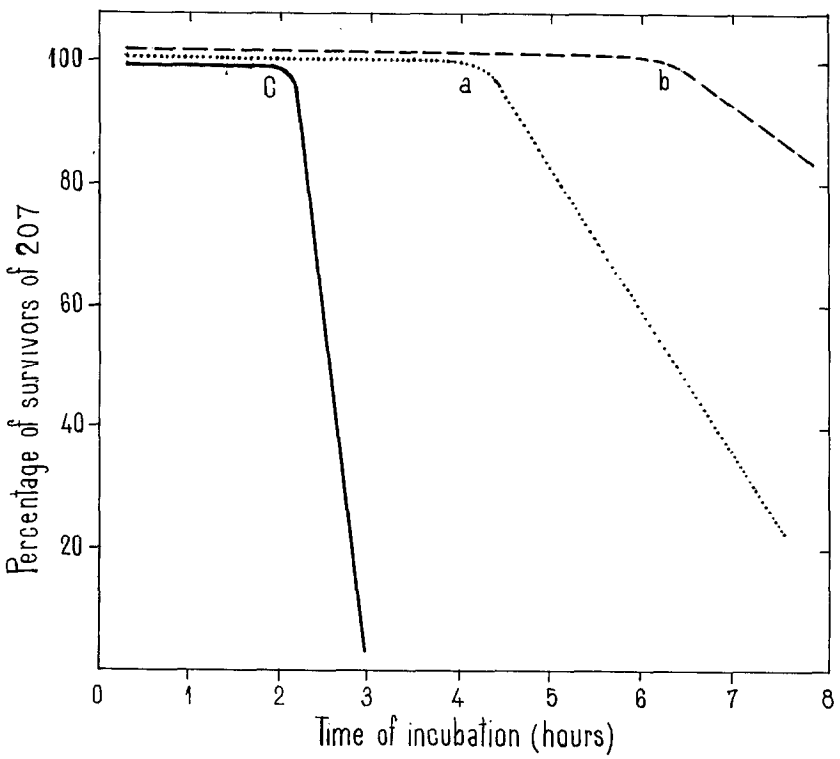

Fig.1.- Survival of Bacillus megaterium 207 mixed with irradiated and unirradiated staphylococcal cells.
C = curve of control mixture (unirradiated staphylococci and unirradiated bacilli).
a and b = survival curves for mixtures in which Staphylococcus 31A cells were irradiated for 2 minutes and 3 minutes respectively.

The inoculation of samples taken successively at an hour's interval in an Alföldi selective medium has shown the following: i) the dilution of mixtures allowed for the growth of isolated colonies of B.megaterium 207 as well as for their counting; ii) as regards the control mixture, the number of colonies was equal both for the samples taken after 1 hour and for those taken after 2 hours; for the samples taken after 3,4, ... 8 hours no colony appeared in the 10^{-3} and 10^{-4} dilutions (see fig.1, C); iii) as for mixtures carried out with staphylococci irradiated for a 2 and 3 minutes, the samples taken during the first 4 hours and first 6 hours respectively produced the same number of colonies as "0" time; after these time-intervals the number of colonies decreased progressively, faster for the first mixture and slower for the second. Nevertheless even after an 8 hours'interval a number of colonies persisted, which stood for a percentage of survivors plotted in fig.1 (curves a and b); iv) samples taken from the mixture consisting of 4 minutes irradiated staphylococci and unirradiated bacilli gave the same number of colonies in all cases collected at a time-interval of 8 hours, so that the graphic representation would be a straight horizontal line.

These results clearly point to the fact that UV irradiation of staphylococci (in doses which are inductive of bacteriocinogenesis) had as result a decrease in the lytic ability. Although the lysis of bacilli is strongly inhibited by a UV treatment of staphylococci, fusion bridges between heterologous cells come into being, the differentiation of these organelles analogous to syngamy being uninhibited by UV.

The effect of UV on the Staphylococcus 31A - B.megaterium

207 system is asymmetrical since the irradiation of bacillary cells does not inhibit the lysis of bacilli by the unirradiated staphylococci.

The inhibition of bacilli lysis in Staphylococcus 31A X B.megaterium 207 mixtures through UV irradiation of staphylococcal cells, the asymmetrical effect of UV in a Staphylococcus 31A - B.megaterium 207 system, the inhibition of lysis under conditions of starvation for the Staphylococcus (Antohi et al. 1965), as well as the inhibitive effect of alkylating agents and the ineffectiveness of protein synthesis inhibitors (Moraru et al. 1966), strongly support the following assumptions:

1) The lysis of bacilli in a Staphylococcus 31A - B.megaterium 207 mixtures cannot be ascribed to a bacteriocin.

2) A genetic material is transfered through the fusion bridges, from the staphylococci to the bacilli.

3) An associated biosynthesis of the genetic material in staphylococcal cells is necessary for the transfer.

4) The lysis of bacilli is the consequence of a lethal heterozygosis. Although this heterozygosis ends up in a bacilli lysis, no lytic factor is released in the medium.

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