The effect of the dye neutral red on a strain of *Staphylococcus aureus* in the presence of sunlight

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SYNOPSIS A strain of *Staphylococcus aureus* was isolated, the growth of which in the presence of neutral red was inhibited by sunlight. This phenomenon was not shown by any of a number of other strains of the same or different genera.

The present study was undertaken after the accidental finding of a peculiar effect of sunlight on a particular strain of *Staphylococcus aureus* on MacConkey agar plate.

In May 1973, a technician after inoculating an ear swab on a blood agar (Oxoid) plate and a MacConkey agar (Oxoid) plate, left both on the bench in the presence of sunlight for about 20 minutes before incubation. When the plates were examined the following morning, an unusual appearance was noted on the MacConkey agar plate only. The patient's name and the date of inoculation has been written in black marking ink on the underside of the petri dish; the plates had been left inverted on the bench and this writing had cast a shadow on the culture medium and inoculum beneath. Where the shadow had been cast a sharply delineated, luxuriant growth of *Staphylococcus aureus* occurred on incubation, but elsewhere on the plate the growth was scanty (figure).

Materials and Methods

Apart from the property described here, the strain (laboratory number 3715/73) was a typical coagulase-positive *Staphylococcus aureus* of phage type NT/29/71. It was sensitive to erythromycin, tetracycline, cloxacillin, and neomycin, but resistant to penicillin by the disc diffusion method. Also tested for inhibition by sunlight in the presence of neutral red were other 28 strains of *Staphylococcus aureus*, nine strains of *Staphylococcus albus*, four strains of *Proteus*, and four strains of *Pseudomonas* species isolated in the diagnostic laboratory.

The ability of the strains to grow on the media

Fig The appearance of the original MacConkey agar plate inoculated with the particular strain of *Staphylococcus aureus* under test. This plate was exposed to sunlight for 20 minutes and incubated overnight at 37°C.

listed in table I was tested by pouring the media into presterilized petri dishes 90 × 15 mm in diameter (Sterilin Limited), and flooding them with different strains under test, the size of the inoculum being 10⁵ organisms/ml. The plastic plates were labelled with a Pentel felt tip pen containing blue, black, or red ink. The plates were inverted and exposed to sunlight for 10 to 60 minutes as in the original observation so that felt-pen labels cast a shadow on the medium beneath. They were then incubated at 37°C overnight.

In an attempt to reproduce the phenomenon artificially, Spekker filters were placed over the plates during exposure to sunlight or ultraviolet

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Medium No.	Composition of Medium
1	Nutrient agar (Oxoid, code CM 3, Lab-Lemco powder, yeast extract, peptone, sodium chloride, agar no. 3)
2	Blood agar base (Oxoid, code CM 55, Lab-Lemco, peptone, sodium chloride, agar no. 3)
3	Salt agar (blood agar base as above + 5% sodium chloride)
4	Cystine, lactose electrolyte-deficient medium (CLED—Oxoid, code CM 301, peptone, Lab-Lemco, tryptone, lactose, L-cystine, bromothymol blue, agar no. 1)
5	Blood agar (Oxoid, code PM 1055B—ready poured)
6	Lysed blood agar (Oxoid—DST agar, code PM 1261LB, ready poured)
7	Chocolate agar (blood agar base + sterile heated defibrinated horse blood)
8	Blood agar base + 0.5% bile salt
9	Blood agar base + 1% lactose
10	Blood agar base + 0.5% bile salt + 1% lactose
11	Blood agar base + 0.007% neutral red
12	Blood agar base + 0.5% bile salt + 0.007% neutral red
13	Blood agar base + 1% lactose + 0.007% neutral red
14	MacConkey agar (Oxoid CM 76, peptone, lactose, bile salt, neutral red, agar no. 3)

Table I Composition of media tested for inhibition of growth by sunlight

light (253.7 nm, energy emitted 37 microwatt/sq cm). Control plates were set up without having Spekker filters placed over them in both experiments.

Results

The figure shows the appearance of a MacConkey agar plate inoculated with the original strain of *Staphylococcus aureus* exposed to sunlight for 20 minutes and incubated overnight at 37°C. There was dense growth on areas shadowed by the felt-pen writing on the plate.

This effect was not found when this organism was inoculated on media 1-10 (table I). Table II shows that when the components of MacConkey medium were separated, only combinations containing neutral red showed the phenomenon. A shadow cast over the sunlit MacConkey medium for up to an hour before inoculation with this particular staphylococcus failed to reproduce the phenomenon.

Plates shaded from sunlight by filters also showed the phenomenon which was not affected by the colour of the filters or of the ink marker.

When the plates were exposed directly to ultraviolet light with the lids off the usual bactericidal effect of ultraviolet light was observed. The plates were sterile on incubation after exposure for

30 to 40 minutes. When plates were exposed to ultraviolet irradiation exactly in the same way as described above for sunlight no effect could be shown.

Table III shows the influence of time on the appearance of the phenomenon.

Time of Exposure of Plate to Sunlight (minutes)	Growth on Exposed Area of MacConkey Plate	Phenomenon Demonstrated
10-20	Good	No
20-30	Moderate	Yes
30-40	Scanty	Yes
40-50	Very scanty	Yes
50-60	None	Yes

Table III Effect of time of exposure to sunlight on occurrence of growth of *Staphylococcus aureus*

Discussion

An inhibitory effect of the dye neutral red in the presence of sunlight on a particular strain of *Staphylococcus aureus* was observed. It has been reported in the past that neutral red may exert an inhibitory effect on the growth of *Escherichia coli* in samples of water and that the action is most probably on the bacterial cell wall (Childs and Allen, 1953). Also it has been shown that the pre-exposure of nutrient agar plates to sunlight inhibits the growth of a number of strains of *Staphylococcus aureus* (Waterworth, 1969). Had the effects been due to the protection of bacteria from sunlight by the writing on the plastic petri dish or superimposed filters, the same effect would have been produced on any transparent medium capable of supporting the growth of this or any other strains of staphylococci. But the effect was seen only on media containing neutral red and astonishingly so far only this

Medium	Phenomenon Demonstrated
BAB ¹ + 0.5% bile salt	No
BAB + 1% lactose	No
BAB + 0.007% neutral red	Yes
BAB + 0.5% bile salt + 1% lactose	No
BAB + 0.5% bile salt + 0.007% neutral red	Yes
BAB + 1% lactose + 0.007% neutral red	Yes

Table II Effect of constituents of MacConkey agar to demonstrate the phenomenon with *Staphylococcus aureus*

¹BAB = blood agar base (Oxoid, as described above)

particular strain has shown this phenomenon. Evidently dye and sunlight worked together inhibiting this unusual strain.

Plenty of sunshine in the months of June and to some extent in August enabled us to carry out these tests. Unfortunately, the effects of different dyes or of glass petri dishes could not be tested due to cloudy weather. Also the actual difference in the intensity of sunlight and ultraviolet light used in these tests is not known. Further attempts to reproduce the phenomenon by exposure to artificial light sources of different intensities are in progress.

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