THE MECHANISM OF THE 'WELSCH PHENOMENON'

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(With 2 Figures in the Text)

Welsch (1949, 1950) described experiments in which he mixed broth cultures of a normal Streptomycin-sensitive *Staphylococcus aureus* with a streptomycin-resistant strain, and after a period of incubation made successive subcultures into streptomycin-free broth. From time to time samples were withdrawn from these subcultures and plated on streptomycin-agar. At the commencement of the experiment the streptomycin-resistant forms were in excess of the sensitive forms, but after a series of subcultures the number of colonies of the resistant strain had fallen to a very low figure and the sensitive strain almost completely dominated the picture. The results of this experiment is often referred to as 'The Welsch phenomenon' and Welsch considered that the end-point obtained gave the proportions of sensitive and resistant cells in the populations of a normal culture, and that in such cases there was a biological equilibrium between the two forms. He found that the rate of disappearance of the resistant form from such an artificial mixture was governed by the size of the inoculum used in the subcultures; when it was small, few subcultures were necessary to produce the end result.

Welsch, however, was unable to explain the underlying mechanism. He considered several possible explanations but with negative results. In 1952 he wrote: '...pure cultures of totally resistant organisms grow just as quickly and just as well as normal cultures'. Welsch was unable to show an 'antagonistic action of a normal against a resistant culture' and had to admit that 'no evidence of a nutritive deficiency could be found in the resistant organism and no direct transformation of resistant cells into sensitive ones could be induced by cultivating them in the presence of either autolysates or nucleic acids extracted from normal cultures'.

Linz & Lecocq (1951) confirmed Welsch's findings in their experiments with several other bacterial species and considered that the phenomenon had a general application. Moreover, these two authors (1953) obtained similar results in *in vivo* tests with tubercle bacilli in guinea-pigs. They, like Welsch, were unable to explain the mechanism but they appeared to be convinced that the phenomenon was not associated with any difference in the multiplication rates of sensitive and resistant strains.

The experiments to be described were undertaken to explain the mechanism underlying the Welsch phenomenon.

MATERIALS AND METHODS

The following cultures were used:

Escherichia coli B and two mutants of this strain isolated by the author (Banič, 1956) by a modified replica plating technique. One of these was resistant to 5 μg. streptomycin and the other to 1000 μg. streptomycin in agar.

Staph. aureus Oxford and a variant of this strain with an induced (*in vitro*) resistance to 800 units of penicillin.

Six resistant strains of *Staph. aureus* as isolated from patients; three of which were resistant to penicillin only, one to penicillin and streptomycin and two to penicillin, streptomycin, aureomycin, terramycin and chloromycetin.

Broth cultures of the antibiotic sensitive and resistant strains were prepared from fresh single colony isolations from plate cultures. After 24 hr. incubation 0·1 ml. each of the broth cultures of the sensitive and resistant strains were added to a single tube of broth which was then incubated for 24 hr., after which 0·1 ml. of the well-mixed broth culture was transferred to a fresh tube of broth. The procedure was repeated every 24 hr. and at chosen intervals samples were taken from the cultures, diluted $1:10^5$ and each of two plates, one of agar containing the appropriate antibiotic and the other plain nutrient agar, were inoculated with 0·05 ml. of the diluted culture. After 24 or 48 hr. incubation the colonies on each plate were counted and the ratio of sensitive to resistant cells determined.

RESULTS

Expt. 1

With mixtures of *Esch. coli* B and the mutant, resistant to 5 μg. of streptomycin, the sensitive strain overgrew the resistant after seven subcultures.

Expt. 2

With mixtures of *Esch. coli* B and the mutant, resistant to 1000 μg of streptomycin, the resistant mutant disappeared from the culture after five subcultures.

Expt. 3

With a mixture of *Staph. aureus* Oxford and the strain with an induced resistance to 800 units of penicillin the adapted strain disappeared after only two subcultures.

Expt. 4

With respective mixtures of the *Staph. aureus* Oxford and the three naturally penicillin-resistant staphylococci, isolated from patients, it was found that the normal Oxford sensitive strain became dominant with the disappearance of the resistant strains after seven subcultures.

Expt. 5

Experiments with the Oxford strain of *Staph. aureus* and the two strains of staphylococci resistant to penicillin, streptomycin, aureomycin, terramycin and chloromycetin showed that the resistant strain could not be detected after fourteen subcultures.

Expt. 6

The two staphylococcal strains resistant to penicillin only and the strain resistant to both penicillin and streptomycin were next examined in mixtures. Both of the strains resistant to penicillin only outgrew the strain resistant to penicillin and streptomycin in seven subcultures. The same two dominant strains were then mixed with strains resistant to the whole five antibiotics under test, but in this experiment the results were unexpected: the strains resistant to the five antibiotics appeared to have a selective advantage; one strain dominated after seven and the other after fourteen subcultures.

Expt. 7

In order to compare the size of colonies of sensitive and resistant strains under the same conditions 24 hr. broth cultures of *Esch. coli* B and the mutants resistant, respectively, to 5 and 1000 μg. streptomycin were diluted $1:10^5$ and 0·05 ml. of each diluted suspension was inoculated onto separate plates of plain nutrient agar. The plates were examined after 14, 24 and 36 hr. incubation. After 14 hr. the colonies of the sensitive strain of *Esch. coli* B had a mean diameter of just over 0·5 mm., the colonies of the strain resistant to 5 μg. streptomycin were decidedly smaller but well defined. With the strain resistant to 1000 μg., however, the colonies at this stage were too small to be defined. After 24 and 36 hr. incubation the differences in size were obvious to the naked eye. Fig. 1A shows the colonies of *Esch. coli* B (sensitive strain) after 36 hr. incubation while Fig. 1B at the same magnification shows the colonies of the mutant resistant to 1000 μg. streptomycin after the same period of incubation.

The staphylococcal cultures showed a similar picture. The normal strain of *Staph. aureus* Oxford after 14 hr. incubation yielded colonies of about 0·5 mm. diameter, while those of the strain resistant to 800 units of penicillin only became visible after 24 hr. incubation and Fig. 2A, B illustrates the difference in colony size of the two strains after 36 hr. incubation. All the other antibiotic resistant strains of staphylococci formed somewhat smaller colonies than the sensitive Oxford strain.

Expt. 8

24 hr. broth cultures of the normal *Esch. coli* B and the mutant resistant to 1000 μg. streptomycin on the one hand, and the Oxford strain of *Staph. aureus* and the strain resistant to 800 units penicillin on the other hand, were diluted $1:10^5$. Each diluted suspension was inoculated onto three agar plates in measured amounts of 0·05 ml. to each plate. The plates were incubated for 36 hr. and the colonies counted.

The results with the coliform strains were according to expectations; the resistant strain yielded lower counts than the sensitive strain indicating that the former grew at a slower rate than the sensitive strain. This was confirmed by the naked-eye appearance of the two broth cultures of the same age; the culture of the resistant strain was less turbid than that of the sensitive strain.

In the case of the staphylococcal cultures, however, the results were contrary to expectation. When the plates inoculated with the resistant strain were examined, it was found that although the size of the colonies was smaller than those on the plates inoculated with the sensitive Oxford strain, the total colony counts were higher. The experiment was twice repeated with similar results. Nevertheless,

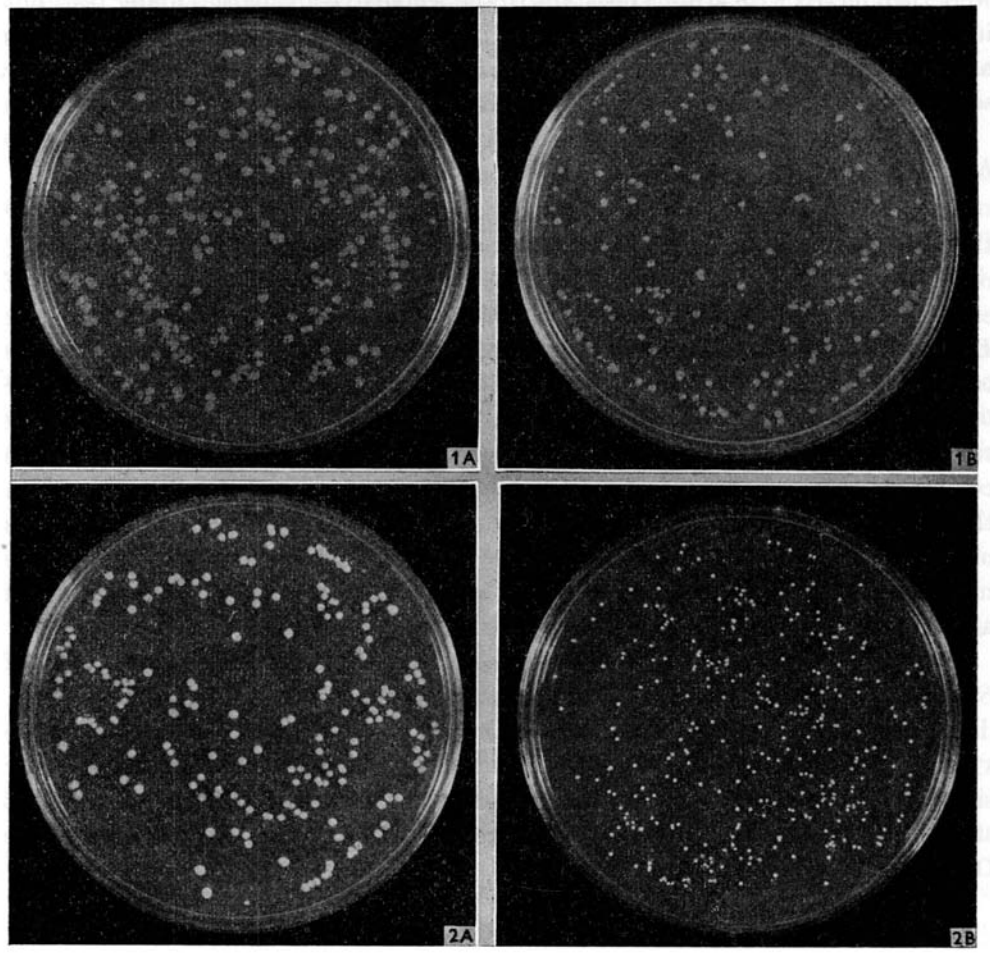

Fig. 1. A, shows the size of colonies of the sensitive strain of *Esch. coli* B; B, shows the size of colonies of *Esch. coli* B strain resistant to 1000 μg. streptomycin.

Fig. 2. A, shows the size of colonies of the sensitive strain of *Staph. aureus* Oxford; B, shows the size of colonies of the *Staph. aureus* Oxford strain resistant to 800 units of penicillin.

the naked-eye turbidity of the broth culture of the resistant strain was significantly less than that of the Oxford strain. This was confirmed by putting up fresh broth cultures of the two strains in parallel in which the size of the inocula was approximately equal. After 24 hr. incubation the respective turbidities were measured in a Beckman electric spectrophotometer at a wave length of 425 μm. in cells 1 cm. wide. The reading with the sensitive Oxford strain was 1·22, whereas with the

resistant strain it was 0·85, results which support the naked-eye readings and the thesis that the resistant strain grows more slowly than the sensitive strain.

The agreement between the two methods of estimating growth rates of different strains of *Esch. coli* and the discrepancy in the case of *Staph. aureus* cannot be properly explained but some suggestions are discussed below.

DISCUSSION

The experiments described above provide evidence to show that the Welsch phenomenon depends on the more rapid growth of the normal antibiotic-sensitive bacterial strain as compared with a resistant mutant. In an artificial culture mixture of a sensitive and resistant strain, the number of successive subcultures required before the dominating sensitive strain emerges in almost pure culture, depends on the size of the inoculum used as the transfer in the successive subcultures. The smaller the inoculum the fewer the number of daily subcultures required before the more rapidly growing sensitive strain completely dominates the slower growing resistant strain.

The demonstration of the differences in the size of colonies of sensitive and resistant strains of the same age offered good evidence of the slower growth of the resistant mutant, and the turbidity measurements on broth cultures confirmed the correctness of this conclusion, but further efforts to obtain confirmation by colony-counting methods were not uniform in their results.

One of the most important assumptions on which the value of colony counts for assessing the number of viable cells in a bacterial suspension depends is that each colony is derived from a single living bacterial cell. While it is generally true that in suspensions of *Esch. coli* the organisms are present as separate bacterial cells it is by no means the case in fluid cultures of *Staph. aureus*, where they tend to grow in clusters of varying numbers of cells. It is obvious, therefore, that colony counts of a particular fluid culture of *Staph. aureus* may well give an inaccurate measure of the number of viable cells present. Whether there was, in fact, any significant difference in the average number of bacterial cells in each cluster of the sensitive Oxford strain of *Staph. aureus* as compared with the resistant strain is not known, but if on shaking the respective broth cultures there was a tendency for the clusters of the Oxford strain to break up less readily than those of the resistant strain, the number of viable units would be smaller and consequently the colony counts would be lower. This would be a possible explanation and, although it cannot be substantiated, the suggestion receives support from the results of the turbidity examination of the suspensions, which indicated that in cultures of the same age the suspension of the resistant strain contained fewer bacterial cells than that of the sensitive strain. That is to say the resistant strain multiplied more slowly.

The selective advantage of sensitive over resistant strains offers the hope that strains, whose sensitivity has been altered by chemotherapy, will sooner or later regain their sensitivity once the particular chemotherapeutic agent is withdrawn. It seems reasonable to regard a strain resistant to penicillin only as having a

selective advantage over a strain resistant to both penicillin and streptomycin and the experiments appeared to show that this was the case. Comparative tests with more complex forms of antibiotic resistance were more difficult to interpret, and it would seem that this point requires investigating on a wider basis before a reasonable explanation can be offered.

SUMMARY

The disappearance of the resistant mutant from an artificial mixture of normal antibiotic-sensitive organisms and a resistant mutant after varying numbers of subcultures is known as the Welsch phenomenon. Welsch's observations have been confirmed and experiments devised to show that the resistant strain disappears because it grows more slowly than the normal sensitive strain which eventually, during the successive subcultures, overgrows it.

REFERENCES

BANIČ, S. (1956). *Schweiz. Z. Path.* **19**, 792.
LINZ, R. & LECOCQ, E. (1951). *C.R. Soc. Biol., Paris*, **145**, 143, 149.
LINZ, R. & LECOCQ, E. (1953). *Ann. Inst. Pasteur*, **85**, 451.
WELSCH, M. (1949). *C.R. Soc. Biol., Paris*, **143**, 1282.
WELSCH, M. (1950). *Bull. Acad. Med. Belg.* **15**, 454.
WELSCH, M. (1952). *Bull. World Hlth Org.* **6**, 173.

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