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THE REACTIONS OF BACTERIA TO CHEMOTHERAPEUTIC AGENTS*

BY

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Some of my predecessors in this lectureship have been able to choose subjects for it in which the late E. H. Kettle was himself interested. I have not been able to do this, and my subject is in fact one which was only just beginning to develop at the time of his death in 1936. If he had survived to see the remarkable changes which have taken place in medicine during the past 15 years, it would have been interesting to hear his comments on them. He had one of the most penetrating intellects of his time, and could grasp a subject in all its bearings when others saw only the surface of it. Those of us who were privileged to be his colleagues owe a good deal in our attitude to our subject, and even some of our opinions and beliefs, to what we learned from him in ordinary daily conversation. It was largely in this simple way that he exerted his influence and trained his colleagues, and for them it was a most agreeable process, because his comments on men and things were often highly entertaining as well as the product of a profound and original mind.

I have asked myself what his attitude would have been to advances in therapeutics which now make it possible to cure most bacterial infections. While I am sure that he would have welcomed them as everyone else has, I think it might have been with a tinge of regret that the conquest of this type of disease has come in just this way, without any addition to our knowledge of the process of infection and of the factors determining its onset and course. Some fundamental discovery in the field of immunity, could it have had the same practical results, would have been intellectually more satisfying.

Bacteria react in four ways to contact with chemotherapeutic drugs. These reactions may be described shortly as suppression, habituation, dependence, and stimulation. The first of these is best described by the general term "suppression," because it occurs in two forms: the effect may be directly lethal or it may involve simply the inhibition of further growth. This reaction is the aim of treatment, and will normally follow if the latter has been properly directed. The second, habituation or acquired resistance, is a path of escape from the suppressive influence of the drug which has been discovered by some species, often with inconvenient and even disastrous results.

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Acquired Resistance to Sulphonamides

It is interesting to speculate on where we should stand now if the sulphonamides had remained our only resource in treating bacterial infections. There is no doubt that almost all species can become resistant to these drugs and that resistance to one drug involves resistance to all others. Its mechanism is known to be the formation by the organism of a sulphonamide inhibitor, shown in some cases to be *p*-aminobenzoic acid itself (MacLeod, 1940; Mirick, 1942; Landy *et al.*, 1943; Landy and Gerstung, 1944). On the other hand, this change occurs much more readily in some species than in others.

The first organism in which resistance was observed was the gonococcus: indeed, from the very inception of sulphanilamide treatment for gonorrhoea in 1936 a few cases failed to respond, and it is interesting that some resistant strains were found among 50 cultures isolated before this treatment had been introduced (Schmith and Reymann, 1940). These originally resistant strains naturally became increasingly prevalent, and the proportion of treatment failures steadily increased, especially during the war, reaching such figures as 50% (Campbell, 1944), 77% (Abrahamson, 1945), and 85.8% (Dunlop, 1949). There is ample proof that clinically resistant disease is due to strains demonstrably drug-resistant *in vitro* (Petro, 1943). Mahoney and Van Slyke (1945) went so far as to inoculate male volunteers with sensitive and resistant cultures in order to prove that their behaviour in the body would be the same.

It is surprising and very fortunate that the closely related meningococcus has behaved so differently. Sulphonamides are used in the treatment of cerebrospinal fever with the same success as at the time of their introduction 14 years ago: in fact, this is the only acute bacterial infection for which they still are used in preference to penicillin. If resistant strains of meningococcus were to appear they would surely have done so during mass sulphadiazine prophylaxis in the American armed Forces, which had such disastrous results in other directions; but Schoenbach and Phair (1948), who examined many strains isolated during this period, found none

unduly resistant, and no clear evidence even of a tendency to diminishing sensitivity.

The two most important sulphonamide-sensitive species—the pneumococcus and the haemolytic streptococcus—have behaved in a way intermediate between these extremes. Resistant strains of each have appeared, but they have not been common: how common they might be to-day but for the advent of penicillin no one can say. Resistance was observed in the pneumococcus comparatively early, and it seems that of these two organisms it has much the greater tendency to undergo this change. That pneumococci can be habituated to sulphapyridine without loss of virulence was first shown by MacLean, Rogers, and Fleming (1939); that such habituation may occur in patients treated for pneumonia and other conditions was shown by several groups of workers in the United States (e.g., Lowell *et al.*, 1940) during the early part of the war. It was also shown by Julianelle and Siegel (1945), in a very careful study, that mass sulphadiazine prophylaxis can produce a high prevalence of strains of pneumococci of progressively increasing resistance. It is quite possible that, but for the antibiotics, our main resource in the treatment of pneumonia would again be serum.

Haemolytic streptococci, the earliest object of sulphonamide attack, were long in betraying their capacity to develop resistance to it. This has been observed in only a few of the Griffith types of *Streptococcus pyogenes*, notably XII, XVII, and XIX, and their occurrence suggests dissemination from a few original foci rather than widespread habituation in treated individuals. They were first identified as causing cross-infection in wards where wounds and burns were under treatment (Francis, 1942). Much their widest sphere of activity was in the American armed Forces, in which hundreds of thousands of men were given 1 g. of sulphadiazine daily, mainly with the object of preventing haemolytic streptococcus infections. This treatment at first succeeded, but later highly resistant strains appeared and became widely prevalent (Epidemiology Unit 22, 1945; Damrosch, 1946), the last state of the units concerned being thus considerably worse than the first. Of the present distribution and frequency of such strains we have no information at all.

Acquired Resistance to Penicillin

There is one strong point of resemblance between sulphonamide-resistance and penicillin-resistance: the major difficulty occasioned by each is in infection by a single species—the gonococcus and *Staphylococcus pyogenes* respectively. Moreover, in each case selection and not adaptation is the mechanism by which resistant strains have become prevalent. The arguments supporting this view in connexion with staphylococci have been well expressed by Barber (Barber, 1947; Barber and Rozwadowska-Dowzenko, 1948; Barber et al., 1949), who is also responsible for much of our knowledge of the high prevalence of resistant strains in hospitals. Owing to cross-infection and the frequency of the carrier state in nurses, a single resistant strain of a particular phage type, often 52A, may achieve overwhelming predominance and cause serious trouble. On the other hand, in the population as a whole resistant strains are not yet so common: the proportion causing infections in out-patients in two London hospitals have recently been found to be 12.5% (Forbes, 1949) and 6.6% (Griffiths et al., 1949).

It is often asked whether infections due to these resistant staphylococci are amenable to treatment with penicillin given in very large doses. The answer may be found in the work of Gilson and Parker (1948), who showed that the amount of penicillinase formed by these organisms varies nearly a thousandfold. It is on the formation of this enzyme that their resistance depends: hence, presumably, clinical resistance must vary correspondingly, and strains forming the larger amounts must be uninfluenced by any concentration of the drug attainable in the body.

Resistance to penicillin in other species presents no such problem as this. It is of two kinds, one being a purely artificial condition produced in the laboratory, with no apparent counterpart in clinical infection. Thus Miller and Bohnhoff (1945, 1947a) have raised the resistance of both gonococci and meningococci to prodigious levels, but no such organism has ever been recovered from a treated patient. Haemolytic streptococci of Groups B and C can be made somewhat more resistant *in vitro* (Gezon, 1948), as can pneumococci both *in vitro* and in mice (Sesler and Schmidt, 1942; Eriksen, 1945), but there is no suggestion that such strains are being encountered in patients. Haemolytic streptococci of Group A are incapable of developing resistance, and there is fortunately no evidence that *Treponema pallidum* can do so.

These encouraging facts have led some authorities to contest the common belief that the widespread use of penicillin, often in inadequate doses, must eventually produce strains of bacteria other than staphylococci which are resistant to the drug. It is true that no such change has yet been shown to be in progress: indeed, the studies of Finland and his colleagues (Jackson *et al.*, 1950), who compared the sensitivity of 88 strains isolated before and 100 well after the introduction of penicillin, afford satisfying proof that this is not happening among pneumococci. It nevertheless remains a fact that some organisms do become more resistant in the body during

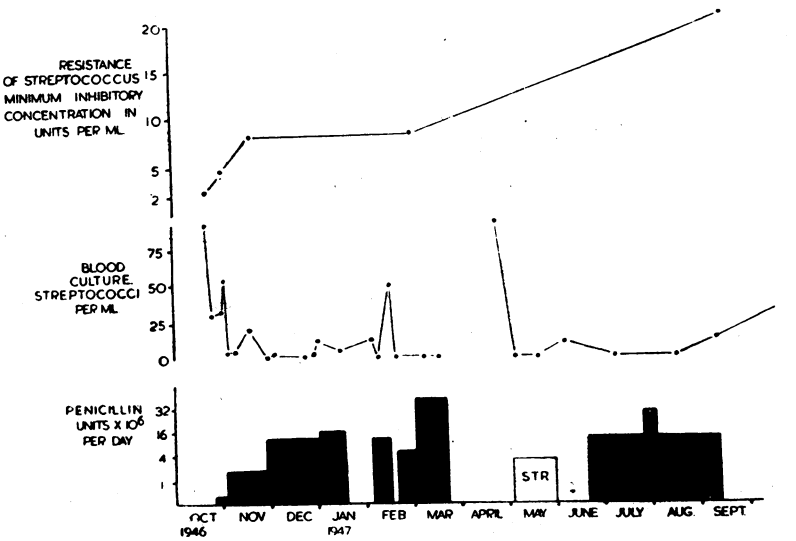

FIG. 1.—Course of events in an ultimately fatal case of subacute bacterial endocarditis due to a streptococcus which underwent a tenfold increase in resistance to penicillin during prolonged treatment with this and other agents. (STR = streptomycin; *p*-aminohippuric acid was also used at some stages in treatment.)

unsuccessful treatment. An example of this was a case of endocarditis lenta described by Dowling *et al.* (1946), in which the streptococcus responsible became progressively more resistant during treatment. We have had a similar experience in this hospital, with the difference that the streptococcus responsible for the endocarditis was originally a resistant one, being inhibited only by 2 units per ml. During prolonged treatment, which included the administration of several thousand pounds worth of penicillin, for one period at the rate of 40 million units daily, as well as other drugs, the degree of resistance of the streptococcus slowly increased: eventually it was inhibited only by 20 units per ml. (Fig. 1).

I have also observed several cases of actinomycosis in which the organism isolated after a period of unsuccessful penicillin treatment was of diminished sensitivity, the difference being about tenfold. In none of these patients is it conceivable that substitution of another organism had occurred, and not habituation of the original one.

Acquired Resistance to Streptomycin

We are faced here with a quite different and indeed unexampled situation, as all species of bacteria can become highly resistant to this drug, often with great rapidity, not only *in vitro* but *in vivo*. Several authors (e.g., Finland *et al.*, 1946; Bondi *et al.*, 1946) have reported the recovery of resistant organisms after only one day's treatment. We have observed this in a *Bacterium aerogenes* urinary-tract infection, the course of which I followed by making quantitative cultures of the urine at frequent intervals (Fig. 2). Only two hours after the first dose of 0.5 g. (repeated four-hourly) the viable count in the urine had fallen from 135,000,000 to only 30,000 per ml.—showing, incidentally, that the action of high concentrations of streptomycin is rapidly bactericidal in the body as well as in the test-tube (Garrod, 1948). The count, repeated at two-hourly intervals during that day, persisted at about the same low level, but after 24 hours had risen again to 5,000,000, and these organisms proved to be 1,000 times more resistant to the drug than those recovered originally. There is evidence (Alexander and Leidy, 1947a) that this remarkable change is due to the existence of a minute proportion of resistant cells in the original population, and it might therefore be possible to predict the result of treatment by cultivating a very large inoculum in drug-containing medium.

I have attempted this in connexion with urinary-tract infections by making pour-plate cultures of the high-speed centrifuged deposit of 10 ml. of urine in agar containing 1,000 μg. of streptomycin per ml. This inoculum is about 1,000 times larger than is usually employed in cultivating urine, and might therefore reveal the presence of a small number of resistant bacteria. These were not detected in any of the following cases in cultures made in the usual way. In 14 cases the heavily inoculated streptomycin plates were sterile, and 12 of these were cured with streptomycin. Treatment was also successful in four patients whose cultures yielded very few colonies (1, 2, 4, and 19). It failed completely in the only two whose streptomycin plates contained numerous colonies, some of which proved also in subculture to be streptomycin-dependent. So far as they go, these findings suggest that the method can be made to yield useful information.

In order to prevent the development of resistance so far as possible, treatment should be vigorous, and for infections other than tuberculosis it need only be so brief that toxic effects have not to be feared. It should also be well timed, particularly in relation to operations, since if it fails there will be no second chance.

Resistance to streptomycin is permanent: hence it needs no great prophetic powers to visualize a time when most of the sensitive bacteria in the civilized world will have acquired it. There are already signs of this: Wilhelm *et al.* (1949) found 85% of strains of *Bact. aerogenes* from urinary-tract infections to be resistant, and in our own experience in this hospital it is becoming common to find resistant strains of this and other coliform bacilli in the urine of patients who give no history of having had the drug. This must mean that they are being disseminated from treated patients. Whether on this account streptomycin should be reserved for treating tuberculosis, as has been seriously suggested, in order to preserve its usefulness for this most important purpose as long as possible, is a matter of opinion. It is of course well known that tubercle bacilli, like other bacteria, can become resistant, although at a slower rate corresponding to their more leisurely growth: this, indeed, is well recognized as the main obstacle to the successful treatment of tuberculosis. Fortunately it appears that the simultaneous administration of *p*-aminosalicylic acid greatly reduces the frequency with which strains of tubercle bacilli become resistant.

Acquired Resistance to Aureomycin and Chloramphenicol

It was originally claimed as one of the advantages of these newer antibiotics that resistance to them does not develop. It is too early to decide how untrue this belief was, but there are signs that the usual process of disillusionment has begun. Certainly, acquired resistance to these drugs is slow to develop, moderate in degree, and not necessarily permanent. Lankford and Lacy (1949), Long *et al.* (1950), and others have found it impossible to habituate staphylococci to "aureomycin" to any serious extent *in vitro*. On the other hand, there are two reports of acquired resistance *in vivo* during treatment, one such case being described by Long *et al.* (1949) and a series by Foley *et al.* (1950). It seems that in some of Foley's cases substitution rather than habituation

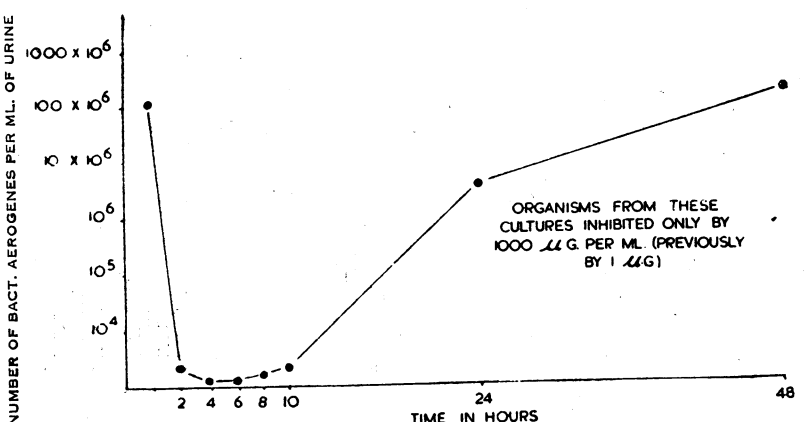

FIG. 2.—Effect of streptomycin (0.5 g. four-hourly, starting at 0 hours) on bacterial content of urine in a case in which treatment failed.

may have occurred, but the mere fact that any staphylococcus can possess 64 times the normal resistance is discouraging.

Increases in resistance to aureomycin or chloramphenicol ranging up to about fiftyfold in various species of Gram-negative bacilli have been reported, either produced deliberately or occurring in treated patients. We have seen in this hospital a fifteenfold increase in resistance to chloramphenicol in *Bact. aerogenes* during four days' treatment for urinary-tract infection. How often this kind of thing will occur remains to be seen: so far it seems to be exceptional. Terramycin appears to behave similarly, and it is interesting that organisms made resistant to it also become resistant to both aureomycin and chloramphenicol (Herrell *et al.*, 1950).

Dependence

The strange phenomenon of nutritional dependence on a substance normally lethal is another aspect of the peculiar behaviour of streptomycin. It was first demonstrated in meningococci by Miller and Bohnhoff (1947b), who recognized two types of resistant colonies, one of which proved to consist of organisms that failed to grow at all in the absence of the drug. They behaved in the same way *in vivo*: mice inoculated with them died if treated with streptomycin, but otherwise survived. Soon after this Paine and Finland (1948) showed that dependent mutants could be obtained from various other species. Lenert and Hobby (1949) have also shown that *Mycobacterium tuberculosis* can become dependent.

These cultures are not a mere laboratory freak: they can often be found among resistant organisms in infected urine, and Miller and Bohnhoff (1949) recovered them not only from the pharynx and faeces of treated animals but from the throats of nurses administering the drug. It is theoretically possible that a patient infected with such organisms could be made worse by streptomycin treatment, and improve on its cessation. The patient described by Spendlove *et al.* (1948), from whose sputum dependent tubercle bacilli were isolated 96 days after treatment was begun, is said to have become worse during further treatment and to have remained *in statu quo* since it was stopped.

Further studies of such patients would be of much interest, but in any case it seems highly improbable that converting the organism to dependence and then starving it by withdrawal of the drug will be found a successful method of treatment. Bacterial populations in which such changes are occurring are highly heterogeneous, including organisms possessing varying degrees of simple resistance and some which are still sensitive as well as dependent cells. The observed effects of treatment are a compound of the separate effects on these three types of organism, and their separate assessment seems unlikely to be feasible.

Streptomycin is not the only antibiotic on which organisms can become dependent. Gocke and Finland (1950) have obtained a resistant variant of *Bact. friedländeri* which is strictly dependent for growth on a critical concentration of chloramphenicol. C. P. Miller (personal communication, 1949) has even obtained variants of meningococci which are dependent on penicillin, although only conditionally: they require penicillin for growth in a deficient medium, but grow without it in a richer one.

Stimulation

I propose to deal more fully with the fourth type of bacterial reaction, not because it is more important—although I believe it to be much commoner and more significant than is generally believed—but because it is much less familiar. This is the opposite of the desired effect: the stimulation of bacteria instead of their suppression. There is good evidence that this effect is produced by concentrations lower than those required to inhibit growth: these are naturally very low for sensitive bacteria, but they may be among the highest attainable in the body if the effect is exerted on naturally resistant organisms.

If this is so, it is only an illustration of the Arndt-Schulz law, which, stated shortly, is "Kleine Dosen reizen, grosse Dosen lähmen": in other words, poisons are stimulants in small doses. Schulz (1888) formulated this law as it applies to bacteria over 60 years ago, and since then many workers (Hüne, 1909; Fred, 1911; Hofmann, 1922; Branham, 1929) have shown that low concentrations of a great variety of germicides accelerate bacterial growth. These substances include salts of mercury and other heavy metals, phenols, halogens, arsenical compounds, dyes, and ether. It is an alarming thought that many common disinfectants, if mere traces of them are present, may apparently have precisely the opposite effect to that desired.

Does the Arndt-Schulz law apply to antibacterial chemotherapeutic agents? There is a good deal of evidence that it does. Such as there is with reference to the sulphonamides is unconvincing: Lamanna (1942) used the cup-plate technique, and describes a zone of heavier growth between the zone of inhibition and that of normal growth which he attributes to stimulation. In interpreting this and many other observations made by this method it must be remembered that colonies at the boundary of the inhibition zone can obtain an extra supply of nutriment from this unoccupied area, which may sometimes be the whole explanation of their greater size. There has been little suggestion from the clinical side that sulphonamides can behave in this way, but in one of the U.S. Naval training centres in which sulphadiazine prophylaxis led to the prevalence of resistant haemolytic streptococci, scarlet fever and other infections became more common in treated than untreated men. The authors of the report on this epidemic (Epidemiology Unit 22, 1945) discuss the possibility that the drug was acting as a growth stimulant to these resistant streptococci.

If penicillin can stimulate bacterial growth it is perhaps a more serious matter, and there is a good deal of evidence that it can. From the clinical point of view this evidence rests largely on the occurrence of what have been called "superinfections" during penicillin treatment. It is well known that during local treatment of the mouth and throat the normal flora is largely replaced by resistant coliform bacilli (Long, 1947). The same thing can happen elsewhere with more serious results: Weinstein (1947) and Appelbaum and Leff (1948) report cases in which resistant Gram-negative organisms appeared as the cause of pneumonia during penicillin treatment for an originally sensitive infection. The full account given by Stanley (1947) of 10 cases of *Pseudomonas pyocyanea* infection is interesting: he does not seem to suspect that penicillin had anything to do with producing or aggravating these infections, but in fact seven of his patients, six of whom died, were treated with penicillin either shortly before or during the progress of the infection.

How is this substitution of resistant for sensitive organisms to be explained? Some authors apparently

take the view that the elimination of existing bacteria leaves a kind of vacuum which is bound to be filled by others. This view seems justified only if we assume that the normal balance is maintained by antibiosis. It is certainly not the explanation of all cases, because during penicillin treatment resistant bacteria may appear where no bacteria at all were before. This seems particularly apt to happen in the urinary tract during the excretion of penicillin given for disease elsewhere. Three of our patients under treatment for bacterial endocarditis have developed urinary infections due to *Ps. pyocyanea* for no apparent reason other than the presence of the drug in the urine. We have also seen a rapidly fatal pyaemia due to this organism develop during the treatment of a case of actinomycosis, the portal of entry being the vein into which the drug was being given by continuous drip (Christie and Garrod, 1944).

If penicillin does stimulate or accelerate bacterial growth it should be possible to demonstrate this *in vitro*. This has in fact been done by several different methods. Pratt and Dufrenoy (1947) and Van Marwyck (1949) used the agar diffusion technique, obtaining a zone of enhanced growth external to that of inhibition: that caution is required in interpreting this appearance has already been pointed out, but the results of Eriksen (1945), who obtained a zone of enhanced growth with no inhibition zone internal to it, suggest that such appearances may be due to true stimulation. Curran and Evans (1947) found that the germination of spores and the growth of staphylococci and streptococci in a skim-milk medium were accelerated by low concentrations of both penicillin and streptomycin. Miller *et al.* (1945) cultivated *Staphylococcus aureus* in broth containing different concentrations of penicillin, estimating the amount of growth turbidimetrically: sub-inhibitory concentrations enhanced it, and it is interesting that maximum enhancement was produced by an amount of penicillin only about one-fifth of that required to inhibit growth completely. The difference between the stimulating and inhibiting concentrations of ordinary germicides is far wider than this. There are also two recorded examples of experimental *in vivo* stimulation: Randall *et al.* (1947) found that subcurative doses of penicillin increased the mortality from *Salmonella typhi* infection in mice, and Foley and Winter (1949) showed that penicillin increases the mortality of chick embryos inoculated with *Candida albicans*.

I have made some observations recently on the acceleration of bacterial growth by penicillin, using the method of Aoki *et al.* (1949), which is simply a comparison of the size of colonies on penicillin-containing and normal media. There is no doubt that such acceleration occurs: it is caused by appropriate concentrations in both sensitive and resistant species, such as 0.004 unit per ml. for a staphylococcus (Fig. 3) and 4 units or more for *Ps. pyocyanea* (Fig. 4). But the differences in colony size are variable, and some of the factors responsible for this remain to be worked out. One is certainly the age of the culture used for inoculating the plates: it seems that penicillin particularly stimulates the growth of relatively dormant cells from older cultures. Another is the medium: the poorer this is, the greater the effect. The greatest contrast in colony size I have seen was in *Ps. pyocyanea* growing on a medium consisting simply of urine solidified with agar. A third appears to be temperature, the contrast being more marked in cultures maintained a few degrees below 37° C. It is interesting that stimulation should be enhanced by three factors in themselves unfavourable to growth: two of these seem to afford a clue to the mechanism whereby previously dormant bacteria may become active in the body.

I have hitherto deliberately avoided any mention of the tubercle bacillus in this connexion. Like *Ps. pyocyanea* it is highly resistant to penicillin, and forms penicillinase: does it react to the drug in the same way? It is some years since Ungar and Muggleton (1946) claimed to have shown that penicillin stimulates the growth of *Myco. tuberculosis* in a liquid medium: Iland and Baines (1949) could not confirm this result, but they used higher concentrations of penicillin, and their

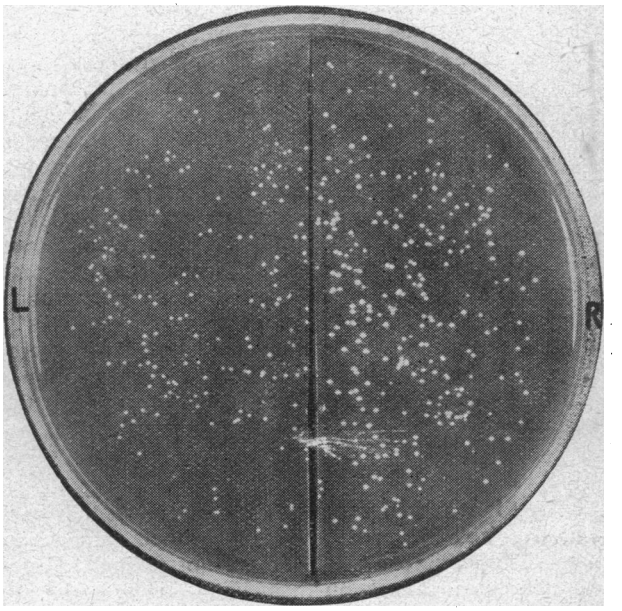

FIG 3.—Divided agar plate culture of *Staph. aureus* (Oxford "H" strain, of normal penicillin sensitivity). Medium in the right half contains 0.004 unit of penicillin per ml. Incubated at 35° C. for 17 hours.

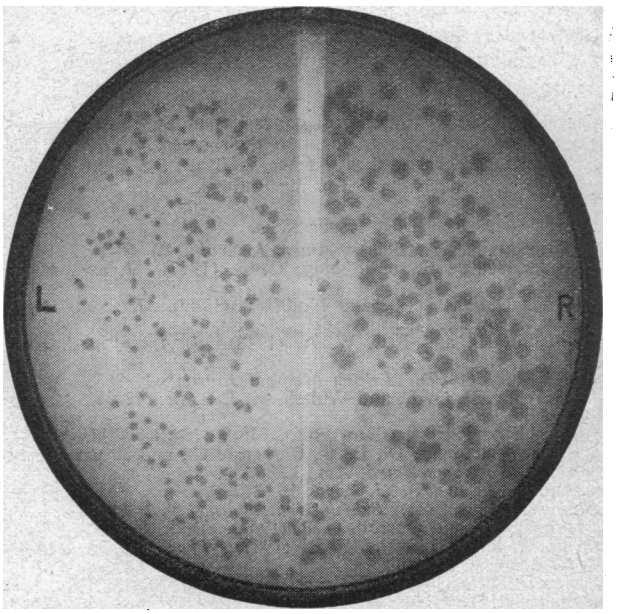

FIG. 4.—Divided agar plate culture of *Ps. pyocyanea* inoculated from a broth culture 1 week old, and incubated at 37° C. for 17 hours. Medium in the right half contains 4 units of penicillin per ml. Not only are the colonies larger on this side, but pigment formation was much more pronounced.

criticism of Ungar and Muggleton's work ignores some of the controls used by these workers. Rivière *et al.* (1947) also reported that penicillin greatly accelerates death from tuberculosis in guinea-pigs, but penicillin is so exceptionally toxic to this animal that uninoculated but treated controls have shown an unduly high mortality in the hands of other workers, and Rivière's conclusion is not accepted. My colleague, J. W. S. Blacklock, and I have been much interested in this problem, and he has recently carried out similar experiments in mice, but without being able to show that penicillin aggravated the disease.

Evidence from the laboratory is therefore inconclusive and I hesitate, as a mere laboratory worker, to import clinical evidence into this argument. Nevertheless one cannot but be impressed when a patient under treatment with penicillin for a staphylococcal lung abscess is found to have tubercle bacilli in his sputum, none having been present before, and dies of acute tuberculosis one month after the starting of treatment. I have certainly formed the impression from the examination of specimens and from post-mortem findings in a number of cases that during treatment with penicillin for some other condition a previously unrecognized or relatively quiescent tuberculous focus may become active: either bacilli appear where none were found before, or their numbers increase enormously. This is only an impression, and it may be false: I can therefore go no further than to suggest that if penicillin is used in a patient who incidentally has tuberculosis the possible effects on that disease are worth watching.

Conclusion

I hope I have said enough to convince you that bacteria are displaying some versatility in their response to chemotherapeutic drugs. They are not taking the present widespread attack on them lying down: some are defending themselves very effectively, and some are even turning our weapons to their own advantage. So far the supply of new antibiotics has more than matched the capacity of bacteria to resist them, but if this supply should cease—and presumably the number yet to be discovered is limited—the time may come when a few of the more enterprising species will flourish more or less unhindered.

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The Chemical–Biological Co-ordination Centre of the National Research Council of the United States has announced the publication of the third volume in its series of reviews concerned with chemical-structure–biological-activity relationships. *Histamine Antagonists* (122 pp., $1.50), by Drs. Frederick Leonard and Charles P. Huttrer, discusses the pharmacological properties, methods of *in vitro* and *in vivo* evaluation, methods of synthesis, structure-activity relationships, mechanism of action, and clinical application of histamine antagonists. More than 1,000 compounds which have been tested for potential antihistaminic activity are listed in 48 classified tables including structures, activities, and literature references. Emphasis is placed on the analysis of structure-activity correlations within the different classes of compounds and on generalizations which can be deduced. The review contains 224 references.