draw attention to the possibility of preventing virus disease in the future by means rather different from orthodox immunological methods.

Recent Progress in Prophylaxis of Other Virus Infections

I will conclude with some very brief items of news concerning other viruses not already mentioned.

Mumps.—Mumps virus has now been grown in fertile eggs and has been shown to resemble influenza A and B, fowl-plague, and Newcastle disease of fowls in its ability to agglutinate red blood cells of certain species. These five viruses seem to form a natural group. Like influenza, mumps can be modified by egg-passages, and there are hopes that an attenuated virus suitable for immunization of man may be developed (Enders *et al.*, 1946). Gamma-globulin prepared from mumps convalescent serum has been stated to be of value in preventing orchitis when given early in the disease (Gellis *et al.*, 1945).

Measles.—Measles virus has also been cultivated in eggs, but its presence can be recognized only by transfer back to rhesus monkeys or human volunteers. Egg-passage attenuates it for children, but one cannot yet advocate its practical use for immunization. Its attenuation is not quite enough to satisfy us; on the other hand, the immunity produced is far from complete (Stokes *et al.*, 1943). Prevention or attenuation of measles by immune serum is an old story. It may be noted, however, that for this purpose concentrated gamma-globulin is better than placental globulin (Greenberg *et al.*, 1945).

Rubella.—This has not yet been transmitted to laboratory animals, and the gaining of knowledge about it lags accordingly. Literature is piling up (Editorial, 1947) concerning the occurrence of congenital abnormalities such as cataract in babies born of mothers who suffered from rubella in the early months of pregnancy. Common sense will warn us to shield such mothers at all costs from unnecessary risk of exposure to the disease.

Rabies.—It has been claimed that animals may be experimentally protected against rabies more effectively by concentrated immune rabbit-serum than by traditional vaccines (Habel, 1945). Many people wonder whether, if Pasteurian vaccines do prevent rabies, the effect is not due to something like the interference phenomenon I discussed earlier rather than to immunization in the usual sense. I say advisedly "if" they are effective, for I note with surprise how little notice is officially taken of the late L. T. Webster's (1942) challenge in his book on rabies. He maintained that there was very slender scientific evidence that such vaccines prevented rabies at all when given after infection.

Smallpox.—Experience in the recent war confirms our belief that, because of varying susceptibility and immunizability, vaccination cannot be depended upon to protect an individual, but (and may no one quote the first half of this sentence away from its context) vaccination is still a reliable means of preventing and controlling smallpox outbreaks, since it will protect most subjects. It has been known for some years that vaccinia grown in tissue cultures or in fertile eggs can be obtained free from all bacteria, and that such a vaccine will immunize man (Buddingh, 1943); but this method of vaccine manufacture has not yet been taken up and seriously tested in comparison with calf or sheep lymph.

Encephalitis.—Viruses cause several forms of mosquito-borne encephalitis in America and the Far East. There is, also, in Russia a tick-borne encephalitis related to the louping-ill of sheep which occurs on the Scottish borders. Against all of these infections formalized vaccines seem to confer a considerable degree of protection. The cause of sporadic encephalitis in this country is unknown.

The Common Cold.—Work on the aetiological agent at the common cold research unit at Salisbury continues. We have not yet succeeded, as two groups of American workers claim to have done, in growing a virus in eggs. Nor, I need hardly say, have we yet produced a vaccine.

More virus vaccines in the future are likely to be made in fertile eggs. At present yellow-fever vaccine, so grown, is of proved value; so is influenza vaccine within the limits I have discussed. Nearly all the development in this field is going on overseas. I believe that far-sighted manufac-

turers of biological products in this country would do well to gain experience in this field and contribute their share to its development.

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STREPTOMYCIN RESISTANCE IN PULMONARY TUBERCULOSIS

BY

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Strains of *Mycobacterium tuberculosis* isolated from patients before treatment are remarkably uniform in their sensitivity to streptomycin (Middlebrook and Yegian, 1946; Youmans and Feldman, 1946; Medical Research Council, 1948c). However, during the course of treatment strains often develop a variable degree of resistance to the antibiotic (Youmans *et al.*, 1946; Report to Council on Pharmacy and Chemistry, 1947; Canada, 1947; Hinshaw and Feldman, 1947; Fisher, 1948; Sadusk and Swift, 1948; Medical Research Council, 1948b). A controlled series of cases of pulmonary tuberculosis has been treated with streptomycin under the auspices of the Medical Research Council, and the result of these trials is reported elsewhere (Medical Research Council, 1948a). Among the group of patients receiving streptomycin, 13 were treated at the Brompton Hospital, and from these were obtained sufficiently detailed data on the development of streptomycin resistance to justify a further report.

Method of Investigation

Type of Case.—Patients were aged from 15 to 30 years and suffered from acute progressive bilateral pulmonary tuberculosis of recent origin. Cavitation was present in all. Before treatment 11 had numerous acid-fast bacilli in direct smears of the sputum; 2 had acid-fast bacilli present only in sputum concentrated or in small numbers in direct smears. There were 10 women and 3 men.

Dosage of Streptomycin.—Every six hours 0.5 g. was given intramuscularly. In the earlier part of the trial treatment was continued for six months, but this was later

*In receipt of a grant from the Medical Research Council.

modified to four months. As a result, of the 13 cases 8 had four months' treatment, 3 had between four and five months' treatment, and 2 had between five and six months' treatment.

The *observation period* was six months, except for one patient from whom a resistant strain of *M. tuberculosis* was isolated for the first time during the seventh month.

Clinical Data.—Clinical examinations were made on each patient at monthly intervals and included an x-ray examination of the chest. Patients were weighed weekly, except some who were very ill, and the erythrocyte sedimentation rate (Westergren) was measured fortnightly.

Assessment of Clinical Progress.—Each patient's progress was assessed by scoring based on the clinical data and on the temperature. As a measure of x-ray progress the independent verdicts of the Medical Research Council panel were taken. Marks were awarded as follows:

	Great Improvement	Improvement	No Change	Worse	Much Worse
Cough		+1	0	-1	
Quantity of sputum		+1	0	-1	
General condition	+2	+1	0	-1	-2
X-ray film	+4	+2	0	-2	-4

Temperature: This was based on the average of evening temperatures each month.

Fall from over 100° F. (37.8° C.) to under 99° F. (37.2° C.) +2

Over 1° F. (0.55° C.) fall +1

1-2° F. (1.1° C.) rise -1

More than 2° F. rise -2

E.S.R.: (In all relevant cases the initial E.S.R. was 40 mm./hour or over):

Reduced to under 50% +1

Increased by over 100% -1

Weight changes in a two-months period:

Gain of 10 lb. (4.5 kg.) or over +3

" " 7 to 9 lb. (3.17 to 4.08 kg.) +2

" " 3 to 6 lb. (1.36 to 2.72 kg.) +1

Loss of 3 to 6 lb. -1

" " 7 to 9 lb. -2

" " 10 lb. or over -3

No doubt many will differ about the importance allotted to the various clinical observations, but the method does form a basis of comparison between cases, and it provides a clinical scale for correlation with the development of streptomycin resistance in the strains of *M. tuberculosis* isolated.

Degree of Cavitation.—The estimate of the degree of cavitation shown on the initial x-ray films was made by Dr. Peter Kerley for the Medical Research Council.

Sputum Examinations.—These were made at short intervals, often twice a week and never less than once a month. During the period at which streptomycin-resistant strains first occurred many cultures failed to grow *M. tuberculosis*, so that the intervals between positive cultures were longer than had been hoped for.

Technique of Testing for Sensitivity to Streptomycin.—The sputum was treated with 4% sodium hydroxide, centrifuged and neutralized, and the deposit inoculated on to Löwenstein-Jensen slopes, which were incubated for nine weeks. The strains of *M. tuberculosis* isolated were subcultured into a synthetic medium containing Tween 80 (Honeywell and Stein, St. James's Square, London) and albumen (Dubos and Davies, 1946), and an inoculum from this was put into bottles of the same medium containing streptomycin. The minimum level of streptomycin inhibiting growth after 10 days' incubation was taken and compared with the level necessary to inhibit a standard strain (H37Rv), which was treated in the same manner. When, for instance, the concentration of streptomycin inhibiting the organism under test was 32 times that inhibiting the

standard strain the streptomycin resistance of the test organism was said to be 32 times that of H37Rv. This comparison is taken as the basis of the measurement of the degree of resistance. The technique is fully described elsewhere (Medical Research Council, 1948c).

Degree of Resistance.—In order to obtain comparative figures in analysing our results we have taken the degree of resistance as the average (actually the geometric mean) of all the resistance tests carried out on each case after a plateau level, as described below, had been obtained.

Date of Development of Resistance.—By "date of development of streptomycin resistance" we mean the first day of streptomycin treatment on which a culture was obtained that proved to be four or more times less sensitive to streptomycin than H37Rv. To compare the different cases we have thought it best to take the mean between the last day on which a sensitive culture was obtained and the first day yielding a resistant culture. We have called this the "mean day of resistance development."

Results

Strains Isolated before Streptomycin Treatment.—From most cases two positive cultures were obtained before streptomycin treatment. These strains were either two times less sensitive than H37Rv, two times more sensitive, or equal in sensitivity, except one strain, which was four times less sensitive.

Development of Streptomycin Resistance.—The times taken for resistance to develop and the degree of resistance attained are recorded in Table I, and examples are illustrated in Fig. 1. Certain comments can be made on these

TABLE I.—*Degree of Streptomycin Resistance and the Time at which it Developed*

Case No.	Last Day when Sensitive Culture was Isolated	First Day when Resistant Culture was Isolated	"Mean Day of Resistance Development"	Degree of Streptomycin Resistance	Clinical Progress Before Development of Streptomycin Resistance	Clinical Progress After Development of Streptomycin Resistance
87	22	42	32	251.2	-1	-2
100	36	42	39	309.2	+3	+3
86	37	47	42	97.9	+6	+6
92	40	46	43	10,000 or more	+5	+5
97	35	51	43	10,000 or more	+4	+4
94	33	61	47	85.1	+7	+4
99	36	60	48	10,000 or more	-2	-7
83	47	49	48	77.6	+6	-1
79	56	76	66	31.6	+7	+9
95	56	77	66.5	40.7	+7	+7
82	65	74	69.5	3.4	+6	+0
89	28	189	108.5	64.0	+11	+6

The "mean day of resistance development" is the mean day between the last sensitive and the first resistant culture. The "degree of streptomycin resistance" is the average of all the tests done after resistance had risen to a plateau level: the figures are in multiples of the streptomycin resistance of the control strain (H37Rv), and were obtained by taking the antilogarithms of the average of the logarithms of the original figures. The method of assessing clinical progress is described in the text.

results. In all cases there was a period following the beginning of treatment during which the strains of *M. tuberculosis* remained sensitive to streptomycin. In 12 out of 13 treated cases there was later an indication of increased resistance to streptomycin. In 11 out of these 12 the strains were 32 or more times less sensitive. In the twelfth case (Case 82) the strains remained approximately four times less sensitive over a period of more than six months after beginning treatment. Subsequently 1 ml. of an eight-day growth in Dubos-Davis medium of a strain isolated from this case before treatment was started was inoculated into a 25-ml. quantity of the same medium containing 1,000 µg. of streptomycin. From this flask a strain was obtained after six weeks' incubation which, when tested by the technique used in this investigation, was 2,000 times less

FIG. 1.—Examples of development of streptomycin resistance in cases under treatment. Note that Cases 82 and 95 are included as unusual examples. Cases 83 and 86 are more typical.

sensitive than H37Rv. This work was performed by Miss M. E. Davies at the Postgraduate Medical School of London. It is thus clear that this strain is potentially capable of producing highly resistant variants. The thirteenth case, in which resistance was not observed to develop, yielded a sensitive culture on the 59th day and subsequent cultures were negative. A further case (No. 89) yielded a sensitive culture on the 28th day. Repeated cultures were then sterile till the 189th day, when two grew strains 64 times less sensitive than H37Rv. Since then all subsequent cultures have failed to yield *M. tuberculosis*.

In most cases the level of resistance rose fairly rapidly to a maximum value and then remained at about the same level. After resistance first appeared isolated sensitive strains were obtained in three cases within 15 days, but not after that time. One of these (Case 97) was of special interest. The first resistant culture was obtained on the 51st day of treatment, and was over 4,000 times less sensitive than H37Rv. The sputum was then negative for a fortnight, at the end of which it became blood-stained. This specimen of sputum was noticeably more purulent than previous specimens and contained numerous acid-fast bacilli. On two occasions cultures yielded strains which were sensitive. After this the sputum became negative by smear and culture, and has remained so for a further 14 months.

Streptomycin resistance was observed to develop in cultures from three patients whose sputa subsequently became negative (Cases 79, 89, and 97).

Numbers of Tubercle Bacilli in the Sputum and Development of Resistance.—Fig. 2 shows that, if bacilli persisted in the sputum, in most cases the number diminished under treatment to a minimum occurring at or after the time that resistance developed. After this the number again increased.

Additional Notes on the Development of Resistance.—In one case (No. 92) sensitive tubercle bacilli were cultured from urine after more than three months' treatment and after the strains from the sputum had been consistently highly resistant for six weeks. In Case 99, dying eight days after finishing treatment and whose sputum cultures had been at least 4,000 times less sensitive than H37Rv, 18 cultures were taken post mortem from different parts of the lungs and all showed the same high degree of resistance.

In two cases resistance tests were done on individual colonies from cultures which routine tests had shown to be resistant. In the first case (No. 92) the culture was the first one shown to be resistant, and was obtained seven days after the last one known to be sensitive. By the routine test the culture was 128 times less sensitive than H37Rv. The growth was a heavy one, and from it nine colonies were selected and tested individually for sensitivity. Four were of the same sensitivity as H37Rv, two were four times less sensitive, two were 64 times less sensitive, and one was 128 times less sensitive. In the second case (No. 94) the culture was the second one shown to be resistant. It was obtained 33 days after the first resistant culture and 61 days after the last sensitive one. Growth was moderate, and the culture was 64 times less sensitive than H37Rv by

FIG. 2.—Degree of positivity of sputum in relation to development of streptomycin resistance. Ordinates indicate degree of positivity, 0=negative to smear and culture; 1=culture only positive; 2=positive on concentration; 3=moderately positive on direct smear; 4=strongly positive on direct smear. The arrows indicate when the first resistant culture was obtained. The monthly positivity figures are based on three examinations in most cases. Other results are indicated only where they are important.

the routine test. Fifteen colonies were selected for individual testing. One was 16 times, 5 were 32 times, 8 were 64 times, and one was 128 times less sensitive than H37Rv. It is of interest that in this case, where the culture was obtained at least a month after resistance had developed, a

higher proportion of the colonies were resistant than in the first case, where the culture was the first one shown to be resistant.

Analysis of Results

An attempt was made to correlate the mean day of development of resistance, the degree of resistance attained, and the clinical progress both before and after the development of resistance. Using the system of scoring previously described, Spearman's rank correlation coefficient was calculated and a test of significance applied, making the appropriate adjustment for the small number of cases. Student's $t = \rho \sqrt{\frac{n-2}{1-\rho^2}}$ (Kendall, 1943). The results are indicated in Table II. This coefficient was used rather than the usual

TABLE II.—Correlation of Streptomycin Resistance of Tubercle Bacilli with Certain Factors

Correlation between	Cases, including Case 89		Cases, excluding Case 89	
	Correlation Coefficient ρ	Probability of Occurrence by Chance	Correlation Coefficient ρ	Probability of Occurrence by Chance
Progress before resistance development and mean day of resistance development	0.6713	0.02-0.01	0.5727	0.1-0.05
Progress after resistance development and mean day of resistance development	0.4056	0.2-0.1	0.2727	0.4-0.3
Progress before resistance development and degree of resistance	-0.7133	0.01-0.001	-0.7091	0.02-0.01
Progress after resistance development and degree of resistance	-0.3497	0.3-0.2	-0.2182	0.6-0.5
Mean day of resistance development and degree of resistance	-0.6748	0.02-0.01	-0.6318	0.05-0.02
Progress before resistance development and progress after	0.6014	0.05-0.02	0.5182	0.2-0.1
Average temperature before treatment and mean day of resistance development	-0.2815	0.4-0.3	-0.1932	0.6-0.5
Average temperature before treatment and degree of resistance	0.5000	0.1-0.05	0.4796	0.2-0.1
Degree of cavitation and mean day of resistance development	-0.3776	0.3-0.2	-0.2273	0.5-0.4

Note.—Correlation coefficients in bold type are considered statistically significant, since the probability of their occurrence by chance is less than the conventional 1 in 20.

correlation coefficient, since it seemed possible that the degrees of resistance developed were not arranged in a normal distribution. One of the determinations of the mean day of development of resistance was obtained only within very wide limits (Case 89). Two sets of figures are given, one excluding and the other including this result. There was an apparent relation between the mean day of resistance development and the clinical progress before development of resistance if the values include results from this case. If, however, these results are not included the correlation coefficient is only suggestively high. Cases which did poorly developed resistance early (Fig. 3).

There was a definite relation between the clinical progress before resistance developed and the degree of resistance that eventually occurred (Fig. 4). Those cases doing well in the early stages of treatment developed a low degree of resistance, while those doing poorly produced more highly resistant organisms. This correlation was significant. There was also a significant correlation between the mean day of resistance development and the degree of resistance eventually achieved. The higher the degree of resistance the more rapidly did it appear (Fig. 5).

There was no significant correlation between the mean day of resistance development or the degree of resistance that developed and the clinical progress after that development; nor was there any demonstrable relation between the time at which resistance developed and the general con-

dition on admission as assessed either on general clinical grounds or as the average evening temperature before treatment was begun. A "dot-diagram" suggested a possible relation between the degree of resistance and the average evening temperature; those with higher average temperatures tended to develop higher degrees of resistance, and vice versa. This relation was not, however, statistically significant. There was no significant relation between the

FIG. 3.—Relation of mean day of resistance development to clinical progress before resistance had occurred. The "boxes" indicate the main groups into which the cases fell.

mean day of resistance development and the extent of cavitation before treatment, though there was some suggestion that those with lesser degrees of cavitation developed resistance later, and vice versa. No relation could be shown between the degree of resistance and the extent of the cavitation. Our failure to demonstrate these relations is not, however, conclusive, owing to the small number of cases.

Clinical Effect of Resistance Development.—The clinical progress of our individual patients during their streptomycin course, before and after the development of resistance, is shown in Fig. 6. It will be seen that in only two cases did any great change in the clinical course coincide with the first demonstration of streptomycin resistance (Cases 83 and 86). In Case 82, in which there was an apparent change, resistance did not rise above four times that of H37Rv during the relevant period. Two cases which went downhill from the start (Nos. 87 and 99) perhaps showed some slowing in the deterioration before resistance developed and thereafter deteriorated more rapidly, but one cannot say that this acceleration might not have occurred if no streptomycin had been given.

Factors other than Streptomycin Resistance which may affect the Response to a Course of Streptomycin.—As we have not in most of our cases been able to demonstrate

a close relation between the development of resistance and the subsequent clinical course, we have considered what other factors might be involved. Two obvious factors are persistent cavitation and the patient's own defences. This latter factor is very difficult to assess, but we have attempted a rough estimate by comparing the patient's progress before streptomycin treatment with the extent of his disease as

FIG. 4.—Relation of degree of streptomycin resistance to clinical progress before resistance occurred. The "boxes" indicate the groups into which the cases fell.

FIG. 5.—Relation of degree of streptomycin resistance to mean day of resistance development. The "boxes" indicate the groups into which the cases fell.

shown radiologically. We have tried to assess for each patient the relative importance to the outcome of the streptomycin course of residual cavitation, the patient's own defences, and, where it occurred, streptomycin resistance. Such an estimate can, of course, be only very rough, but it may serve to suggest the different factors involved.

In three of the eight cases doing well the patient's own defences were thought to have contributed significantly. In four, however, residual cavities made the ultimate outlook doubtful. In the seven cases whose organisms developed streptomycin resistance this did not seem to have affected the issue.

Of the five cases doing relatively badly the development of streptomycin resistance was of obvious importance in two and residual cavitation of importance in one of these and in three others. In two cases streptomycin treatment seemed to have resulted in a slight degree of improvement, in two we thought it had perhaps retarded a downhill course, and in one we were unable to attribute to it any significant benefit.

Discussion

Theoretically there seem to be two possible mechanisms by which strains of tubercle bacilli resistant to streptomycin might appear in the sputum. On the one hand the entire surviving bacterial population might become gradually adapted to growth in the presence of the drug. On the other hand the patient's population of tubercle bacilli before treatment with streptomycin might contain organisms with varying degrees of resistance, the more highly resistant being few in number. Under treatment these would have an advantage over the sensitive strains and might gradually come to replace them.

The first hypothesis presupposes that all strains of tubercle bacilli are initially of equal sensitivity to streptomycin but are capable of gradually adapting themselves to growth in its presence. This adaptation would be most likely to occur if the organisms were exposed to concentrations of streptomycin small at first but gradually increasing. Such conditions would exist if there were parts of the lung, such as cavities, in which streptomycin did not act. Radiating from these areas there would be gradients of antibiotic activity, and strains of tubercle bacilli initially sensitive might little by little grow outwards from the protected areas as they became adapted to higher and higher concentrations of streptomycin. In its pure form this hypothesis is improbable. Not only has such a mechanism been excluded in the development of resistance to penicillin (Luria, 1947) but Pyle (1947) and Davies (personal communication—1948) have shown that strains of tubercle bacilli isolated from the sputum before treatment are not of uniform streptomycin sensitivity; if sufficiently large inocula are used strains highly resistant to streptomycin can be obtained.

If the other hypothesis is correct and streptomycin-resistant organisms come to dominate the patient's population of tubercle bacilli by a process of selection of a few strains innately resistant, we need not presuppose gradients of antibiotic activity. Nevertheless if there were areas relatively protected from the effects of streptomycin, in these the general population of tubercle bacilli would continue to increase and there would therefore be more chance of resistant strains emerging. In addition it might be that gradients of antibiotic activity favoured the growth of moderately resistant strains, and that in these strains selection of highly resistant forms was more likely to occur. Whether such gradients exist is of practical significance; for only if they do is it worth while searching for streptomycin derivatives with a greater diffusibility or a greater activity at low pH.

There is no direct evidence that there are gradients of streptomycin activity in certain areas of the tuberculous lung, but observations have suggested that streptomycin resistance occurs more readily under the physical conditions associated with human pulmonary tuberculosis. Streptomycin-resistant organisms are rarely isolated from treated cases of tuberculous meningitis. Of 15 cultures obtained from the Medical Research Council series after the second month of treatment only three showed a rise in streptomycin resistance (Medical Research Council, 1948b). The technique of estimating the degree of resistance was identical with that described in this paper, and in a number of cases the strains were retested by one of us. In guinea-pig tuberculosis treated with streptomycin the development of drug

If bacteriostatic levels of streptomycin are attained within cavities the continuing isolation of sensitive bacilli from the sputum in the early stages of a course of streptomycin might be due to the presence of dormant viable organisms which are coughed up owing to physical factors such as the disintegration of caseous masses. Although streptomycin seems to have a bactericidal effect on a rapidly growing culture at approximately the same level as it is bacteriostatic, it does not appear to be bactericidal to bacilli suspended in a non-nutrient medium (Middlebrook and Yegian, 1946; Garrod, 1948).

There is another factor of possible importance in the development of streptomycin-resistant strains and the assessment of their clinical significance. It may be that streptomycin-sensitive tubercle bacilli have, in the absence of streptomycin treatment, an advantage over the resistant strains as a result either of innate biological superiority or of weight of numbers.

Karlson, Feldman, and Hinshaw (1947) found that guinea-pigs infected with strains of streptomycin-resistant tubercle bacilli survived longer than those infected with similar doses of sensitive organisms. This appears to be the only published evidence suggesting the lesser virulence of streptomycin-resistant strains.

The correlations obtained from the data in this paper are consistent with either of the mechanisms postulated to explain the development of resistant strains or with the suggestion that both mechanisms might be involved. Thus, if the development of resistance depends on a selective increase of a small number of resistant strains, within a milieu where streptomycin was active the larger, streptomycin-sensitive section of the patient's population of tubercle bacilli would be attacked both by the patient's own defences and by

the streptomycin. The resistant organisms, on the other hand, would be affected by the patient's defences alone. This differential effect might account both for the late development of resistance in cases doing well initially and, if highly resistant strains were rarer than moderately resistant, for the high degree of resistance found in organisms from patients with poor initial response. Alternatively, it may be that the clinical condition is affected, even in the early stages of treatment, by a proportion of resistant organisms as yet insufficient to show in our test.

If, on the other hand, the development of streptomycin-resistant strains is facilitated by gradients of antibiotic activity within the lungs, it may be that the lesions present in those of our patients who showed a poor initial response favour the rapid production of resistant organisms; our results suggest a possible correlation between the initial degree of cavitation and the time at which resistant strains appeared. The same physical conditions might also favour the evolution of highly resistant organisms, though here we have not been able to demonstrate a correlation.

From this discussion it is plain that at present we can only make tentative suggestions about the mechanism by which streptomycin-resistant strains of tubercle bacilli appear in the sputum of cases of pulmonary tuberculosis under treatment with streptomycin. The problem is perhaps best summarized in the form of questions which still await a definite answer:

FIG. 6.—Clinical progress before and after development of streptomycin resistance. The figure covers a four-months period from the beginning of streptomycin treatment. With Case 89, in which resistance was shown to have developed only on the 189th day, and with Case 90, in which the sputum became negative before resistance occurred, clinical progress in the first two months is compared with that in the second two months.

resistance has so far been reported in only two of the many experiments carried out (Feldman *et al.*, 1947, 1948). The contrasting findings might be due to the existence in human pulmonary tuberculosis of foci of bacilli in caseous areas or cavities less readily penetrated by streptomycin, though in tuberculous meningitis there are often regions isolated by fibrous tissue from the streptomycin circulating in the cerebrospinal fluid (Smith *et al.*, 1948). Another, and perhaps more probable, explanation is that the local aggregates of bacilli tend to be larger in human pulmonary tuberculosis, thus increasing the chance of a resistant strain being present.

There is also some evidence that streptomycin does in fact penetrate cavities. Steenken and his colleagues (1947) found a concentration of streptomycin averaging 7.7 $\mu\text{g./ml.}$ in fluid from Monaldi drainage, the average blood concentration being 13.3 $\mu\text{g./ml.}$ In our experience the strain H37Rv is inhibited by about 1 $\mu\text{g./ml.}$ when growing in Dubos-Davis medium adjusted to pH 6, so that it seems likely that the streptomycin concentration in cavities is adequate for bacteriostasis in spite of the acidity of tuberculous pus. It must be remembered that Steenken's figures relate only to a type of cavity suitable for treatment by Monaldi drainage. Schwabacher (1948), however, claims that sputum inhibits the action of streptomycin; in addition, it may be that tubercle bacilli are unaffected by streptomycin when growing within cells (Barski, 1948).

1. Are there gradients of streptomycin concentration or antibiotic activity within the lung? If so, do these favour the development of resistant strains?

2. Is there in the tuberculous patient a competition for survival between sensitive and insensitive organisms? In the absence of streptomycin treatment have the sensitive strains any advantage?

3. Do the sensitive bacilli isolated from the sputum in the early stages of a course of streptomycin represent a population actively increasing? If growth has ceased, do the viable bacilli in the sputum represent dormant organisms coughed up owing to physical factors such as the disintegration of caseous masses? What proportion of bacilli seen in a sputum smear are actually viable at different phases of treatment?

4. What is the proportion of resistant tubercle bacilli in the sputum when resistance shows in our tests? What is the proportion of bacilli resistant at the end of treatment, and does this correlate with clinical progress?

5. What is the distribution curve of resistance in the pre-treatment population of tubercle bacilli? Does this vary from case to case, and how does it change during the course of treatment?

The clinical significance of the development of resistance is still uncertain. In only two of our cases was there a clear change for the worse coinciding with the isolation of resistant organisms, whereas resistant strains were obtained from three cases in which cultures subsequently became negative. Other cases continued to improve in spite of the isolation of streptomycin-resistant organisms. In the main M.R.C. series, of which our cases formed a part, a considerable proportion of the treated cases continued to improve after the second month, the mean date of emergence of resistance being the 53rd day. This continued improvement might be due to the streptomycin continuing to act on a residuum of sensitive organisms or to the patient's defences taking over when the streptomycin ceased to be effective.

Summary

Increased resistance to streptomycin was observed in strains of tubercle bacilli isolated from 12 out of 13 cases of severe bilateral pulmonary tuberculosis treated with 2 g. of streptomycin a day for four months or more.

The rise in resistance varied from 4 to over 4,000 times that of the control strain H37Rv. Resistant strains were isolated from the 42nd day of treatment onwards. In the individual case resistance usually rose fairly rapidly to a plateau and then remained at about the same level.

When bacilli persisted in the sputum the numbers at first fell but after resistance had developed tended to rise again.

Streptomycin-resistant strains were isolated from three cases which subsequently became sputum-negative.

The time at which resistance developed and the degree of resistance attained were related to clinical progress before, but not after, resistant strains had been found. In cases which did poorly at first resistant strains were usually isolated early and attained a higher degree of resistance.

The higher the degree of resistance the more rapidly did it appear.

Only two cases showed any great change in clinical course coinciding with the time when resistant strains were first found.

The bearing of these results on the problem of the mechanism of development of streptomycin resistance and its clinical significance is discussed.

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STREPTOMYCIN IN GONORRHOEA

WITH ITS EFFECTS UPON DARK-FIELD POSITIVE LESIONS OF SYPHILIS

BY

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In the field of venereology streptomycin has great potentialities which have yet to be adequately explored. It apparently has some action against syphilis, but venereologists hope that this will prove slight, for streptomycin will then assume many of the present indications for sulphonamides in the treatment of venereal diseases in which syphilis is suspect but not proved (Willcox, 1948). Moore (1947) considers that the drug is unlikely to be clinically useful in syphilis, and points to the work of Dunham and Rake (1946), who found that the antisiphilic effect of penicillin G was 3,000 times greater than that of streptomycin, though Herrell and Nichols (1945) treated four cases of early syphilis with doses of 1.2–10 g. of streptomycin and obtained a temporary absence of treponemata from the dark-field. Herrell (1947) later believed these results to be inconclusive and considered that so far there was no indication that the drug should be used in the treatment of this disease. Fiske and Gruhzt (1946) gave rabbits 1,000–3,000 subtilis units of streptomycin in three divided doses daily for 13–20 days and found no evidence whatever of any antispirechaetal action.

These observations are of considerable interest, for all reports indicate that streptomycin is particularly effective against gonorrhoea. This disease is at present being well controlled by penicillin and there is no clinical evidence of the development of penicillin resistance by the gonococcus on any substantial scale: many of the suspected cases so far reported have proved on investigation to be due not to the gonococcus itself but to a simultaneously acquired non-specific urethritis (Parkhurst *et al.*, 1947). The possibility that penicillin treatment of gonorrhoea might modify or mask an incubating syphilitic infection has been prominent in the minds of clinicians for some years, and indeed has been responsible for prolonging the period of