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CHANGES IN ISONIAZID RESISTANCE OF TUBERCLE BACILLI AFTER CESSATION OF TREATMENT

REPORT BY THE LABORATORY SUBCOMMITTEE OF THE TUBERCULOSIS CHEMOTHERAPY TRIALS COMMITTEE, MEDICAL RESEARCH COUNCIL*

M.R.C. ISONIAZID TRIAL: REPORT No. 6

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The use of isoniazid in the treatment of pulmonary tuberculosis became widespread in the spring of 1952. It was soon shown that when this drug was given alone bacterial resistance developed frequently and rapidly (Medical Research Council, 1952); approximately two-thirds of positive cultures from patients at the end of a three-month course of isoniazid alone were resistant to the drug, many of them to a high degree. Much confirmatory evidence followed from other countries (Dye, 1953; Ferebee and Long, 1953; Lotte and Poussier, 1953; Swedish National Association Against Tuberculosis, 1953).

There were also early reports that strains which developed resistance to isoniazid *in vitro* might revert to sensitivity when contact with isoniazid ceased (Pansy, Stander, and Donovick, 1952; Barnett, Bushby, and Mitchison, 1953a). These reports were followed by others presenting evidence that after treatment with isoniazid had been discontinued resistant cultures from patients might also revert either to a lower level of resistance or to full sensitivity (Nitti, 1952; Ashino, 1953; Nigoghossian, 1953; Petit, 1953a, 1953b). These observations were in clear contrast to the usual persistence of streptomycin-resistant strains in patients following treatment with streptomycin alone (Tucker, 1949; Canada, Allison, D'Esopo, Dunner, Moyer, Shamaskin, Tempel, and Charter, 1950; Ferebee and Appel, 1951; Ashino, 1953).

The question of possible reversion of the resistance of strains from individual patients after treatment with isoniazid alone has been studied in the current Medical Research Council trial. In an earlier report (Medical Research Council, 1953b) data were presented for 35 patients with pulmonary tuberculosis who were treated for three months with isoniazid alone but who received no further isoniazid during the next three months. Results of sensitivity tests were available for each of these patients at the end of isoniazid treatment and also three months later. It was concluded that, "over a three-month period after treatment with isoniazid had been stopped, there was no evidence of any general reversion either to a lower level of isoniazid resistance or to sensitivity, nor evidence of any general increase in the proportion of resistant strains." This conclusion is in conflict with the reports already referred to.

As a result of a follow-up of patients one year after entry to the trial, it has now become possible to extend the period of observation. Two main groups of patients have been studied: those treated with isoniazid alone for three months who received no further isoniazid for nine months, and those treated with isoniazid alone for six months who received no further isoniazid for six months. To give a fuller picture for each group the results of cultures as well as of sensitivity tests have been analysed at the beginning and at the end of the follow-up period.

Of the 264 patients with various forms of pulmonary tuberculosis and varying degrees of illness who were originally treated with isoniazid alone and for whom results were analysed in the earlier report (Medical Research Council, 1953b), 174 are included in the present analysis. Of the 90 patients excluded, 54 received isoniazid, either alone or in combination, at some time during the follow-up period; the remaining 36 patients had no culture

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The trial was co-ordinated by the late Dr. Marc Daniels and by Dr. Wallace Fox, of the Council's Tuberculosis Research Unit.

The results were analysed and the report was prepared by Dr. Fox and Dr. Ian Sutherland, of the Council's Statistical Research Unit, with the collaboration of Dr. D. A. Mitchison.

undertaken either at the beginning or at the end of the follow-up period, or, in a few instances, failed to complete at least a three-month course of isoniazid alone.

Results of Cultures

In Table I are presented the results for the group of 134 patients who were followed for nine months after a three-month course of treatment with isoniazid was stopped. During the follow-up period 79 of these patients received chemotherapy, which did not include isoniazid, the average duration of drug treatment being 3.3 months. Of 69 patients who were culture-negative at three months, 62 were also culture-negative at 12 months. It is of interest to note in passing that none of the strains isolated at 12 months from the other seven patients was resistant. Of 22 patients with sensitive or doubtfully resistant organisms at three months 19 were

TABLE I

RESULTS OF CULTURES AND ISONIAZID-SENSITIVITY TESTS AT THE END OF A THREE-MONTH COURSE OF ISONIAZID ALONE AND NINE MONTHS LATER, THE PATIENTS HAVING RECEIVED NO ISONIAZID BETWEEN THE TWO TESTS

Result of Test at Three Months	Patients with Cultures Examined	Result of Test at 12 Months							
		Culture-negative (No Sensitivity Test Possible)	Patients Culture-positive with Sensitivity Tests						
			Total Results Available	Sensitive	Doubtful	Resistant			
				No Growth on 0·2 μg. per ml.	Growth on 0·2 μg. per ml. (Not on 1)	Growth on 1 μg. per ml. (Not on 5)	Growth on 5 μg. per ml. (Not on 10)	Growth on 10 μg. per ml. (Not on 50)	Growth on 50 μg. per ml.
Culture-negative (no sensitivity test possible) ..	69	62	7	6	1	0	0	0	0
Sensitive: No growth on 0·2 μg. per ml. ..	13	11	2	0	1	1	0	0	0
Doubtful: Growth on 0·2 μg. per ml. (not on 1)	9	8	1	0	1	0	0	0	0
Resistant: Growth on 1 μg. per ml. (not on 5)	13	6	7	1	1	1	2	1	1
Growth on 5 μg. per ml. (not on 10)	3	1	2	0	0	0	1	1	0
Growth on 10 μg. per ml. (not on 50)	7	5	2	0	0	0	1	1	0
Growth on 50 μg. per ml.	20	11	9	0	1	1	1	2	4
Total	134	104	30	7	5	3	5	5	5

TABLE II

RESULTS OF CULTURES AND ISONIAZID-SENSITIVITY TESTS AT THE END OF A SIX-MONTH COURSE OF ISONIAZID ALONE AND SIX MONTHS LATER, THE PATIENTS HAVING RECEIVED NO ISONIAZID BETWEEN THE TWO TESTS

Result of Test at Six Months	Patients with Cultures Examined	Result of Test at 12 Months							
		Culture-negative (No Sensitivity Test Possible)	Patients Culture-positive with Sensitivity Tests						
			Total Results Available	Sensitive	Doubtful	Resistant			
				No Growth on 0·2 μg. per ml.	Growth on 0·2 μg. per ml. (Not on 1)	Growth on 1 μg. per ml. (Not on 5)	Growth on 5 μg. per ml. (Not on 10)	Growth on 10 μg. per ml. (Not on 50)	Growth on 50 μg. per ml.
Culture-negative (no sensitivity test possible) ..	15	12	3	2	0	0	0	0	1
Sensitive: No growth on 0·2 μg. per ml. ..	0	0	0	0	0	0	0	0	0
Doubtful: Growth on 0·2 μg. per ml. (not on 1)	2	0	2	0	2	0	0	0	0
Resistant: Growth on 1 μg. per ml. (not on 5) ..	3	2	1	0	0	1	0	0	0
Growth on 5 μg. per ml. (not on 10)	1	1	0	0	0	0	0	0	0
Growth on 10 μg. per ml. (not on 50)	4	0	4	0	0	2	1	1	0
Growth on 50 μg. per ml.	10	3	7	1	0	2	0	0	4
Total	35	18	17	3	2	5	1	1	5

culture-negative at 12 months. In contrast, of 43 patients with resistant strains at three months, 20 were culture-positive at 12 months.

Table II presents analogous material for the group of 35 patients who were followed for six months after a six-month course of isoniazid alone. During the follow-up period 12 of these patients received chemotherapy, which did not include isoniazid, the average duration of drug treatment being 3.0 months. As in the previous group, the culture-negative patients nearly all remained culture-negative, while patients with resistant organisms at six months were frequently still culture-positive at 12 months.

Bacterial Resistance to Isoniazid in Patients No Longer Receiving Isoniazid

The usual approach of other workers in studying the possibility of reversion of bacterial resistance has been to consider the results of periodic sensitivity tests in patients who yielded organisms resistant to at least 5 μg. of isoniazid per ml. at the end of a course of treatment (Ashino, 1953; Petit, 1953a, 1953b). However, in such patients, the presence of organisms of a lower degree of resistance, together with unavoidable variations in technique at later examinations, might give a false impression of a general reversion of strains towards lower degrees of resistance, or even to sensitivity. For this reason it is advisable, in addition, to investigate possible increases in degree of resistance of strains which are sensitive or mildly resistant at the end of a course of treatment; in these patients chance variations will tend to give an impression of a general increase in levels of resistance. In the present study, therefore, the sensitivity results for every patient from whom a positive culture was isolated at the beginning and at the end of the follow-up period have been included in the tables. The resistance of each culture was tested on Löwenstein-Jensen slopes containing isoniazid in concentrations of 0, 0.2, 1, 5, 10, and 50 μg. per ml. (Medical Research Council, 1953a).

In Table I are presented the results for the 23 patients with positive cultures both at the end of a three-month course of isoniazid alone and nine months later, the patients having received no isoniazid in the interval. (There was no evidence that the results of the sensitivity tests had been affected by the other chemotherapy which was administered to 14 of these patients.) The results of the two sensitivity tests were similar for strains from 16 of the 23 patients (the same or within one concentration of isoniazid (tube) in the sensitivity test); the strains from four patients were less resistant (a decrease of two or more concentrations) and from three were more resistant (an increase of two or more concentrations) at the end of nine months.

Table II gives corresponding results for the 14 patients with positive cultures both at the end of a six-month course of isoniazid alone and six months later, the patients having received no isoniazid in the interval. (Again there was no evidence that the results of the sensitivity tests had been affected by the other chemotherapy which was administered to six of these patients.) The results of the two sensitivity tests were similar for strains from nine of the 14 patients: the strains from five patients were less resistant (a decrease of two or more concentrations of isoniazid (tubes) in the sensitivity test) at the end of the follow-up; no patient yielded a strain which was more resistant.

The results of these sensitivity tests have been combined in Table III, together with those from

TABLE III

RESULTS OF ISONIAZID-SENSITIVITY TESTS AT THE END OF A COURSE OF ISONIAZID ALONE OF AT LEAST THREE MONTHS AND AT LEAST SIX MONTHS LATER, THE PATIENTS HAVING RECEIVED NO ISONIAZID BETWEEN THE TWO TESTS

Result of Test at End of Course of Isoniazid Alone	Total Results Available	Result of Test at End of Follow-up Period					
		Sensitive	Doubtful	Resistant			
		No Growth on 0·2 μg. per ml.	Growth on 0·2 μg. per ml. (Not on 1)	Growth on 1 μg. per ml. (Not on 5)	Growth on 5 μg. per ml. (Not on 10)	Growth on 10 μg. per ml. (Not on 50)	Growth on 50 μg. per ml.
Sensitive: No growth on 0·2 μg. per ml.	2	0	1	**1**	**0**	**0**	**0**
Doubtful: Growth on 0·2 μg. per ml. (not on 1) ..	5	0	3	1	**0**	**0**	**1**
Resistant: Growth on 1 μg. per ml. (not on 5) ..	9	**2**	1	2	2	**1**	**1**
Growth on 5 μg. per ml. (not on 10)	3	**0**	**0**	1	1	**1**	**0**
„ „ 10 „ „ „ („ „ 50)	7	**0**	**0**	**2**	2	3	**0**
„ „ 50 „ „ „	16	**1**	**1**	**3**	**1**	2	**8**
Total	42	3	6	10	6	7	10

Strains which showed a definite change in the level of resistance (whether an increase or a decrease) are indicated in bold type

the remaining five of the 174 patients studied, who received four, four, four, five, and six months of isoniazid alone, and were followed for 11, nine, eight, seven, and seven months respectively. Thus the table presents pairs of sensitivity results at the beginning and at the end of a follow-up period of not less than six months without isoniazid for 42 patients who had received that drug alone for at least three months. The results of the two sensitivity tests were similar for strains from 28 of the 42 patients: the strains from 10 patients were less resistant (a decrease of two or more concentrations of isoniazid (tubes) in the sensitivity test) and from four were more resistant (an increase of two or more concentrations) at the end of the period. Thus the resistance of some strains, notably those growing on 50 μg. of isoniazid per ml., decreased, whereas of a few it increased. It is of interest to summarize the situation by examining the distribution of levels of resistance at the beginning and at the end of the follow-up period. At the beginning of the period, when treatment with isoniazid ceased, 16 of the 42 strains showed resistance at the highest level and two were sensitive. By the end of a period of at least six months without isoniazid, 10 of the 42 strains showed resistance at the highest level and three were sensitive. This presentation of the data might be considered to offer some evidence of reversion.

Discussion

Recent experimental findings suggest that tubercle bacilli which are resistant to isoniazid might be expected to propagate themselves less successfully in human tuberculous lesions than do sensitive bacilli. Resistant cultures have been shown to have abnormal nutritional requirements *in vitro*, probably connected with abnormalities in their porphyrin metabolism (Fisher, 1954a, 1954b; Middlebrook, 1954). In certain media they may fail to grow, may grow slowly or, having once grown, they may die out more rapidly than sensitive strains (Fisher, 1952; Karlson and Ikemi, 1952; Barnett and others, 1953a, 1953b; Middlebrook and Cohn, 1953). Furthermore, although there are differences of opinion about the degree of animal pathogenicity of resistant strains from patients (Bloch, Widelock, and Peizer, 1953; Peizer, Widelock, and Klein, 1953; Steenken and Wolinsky, 1953), many of these strains have been demonstrated to contain a proportion of bacilli of low pathogenicity in guinea-pigs and sometimes in mice (Barnett and others, 1953a, 1953b; Barry, Conalty, and Gaffney, 1953; Middlebrook and Cohn, 1953; Mitchison, 1954). Unfortunately it cannot be considered certain that these findings imply that resistant bacilli are also of low pathogenicity in man.

If resistant bacilli are less pathogenic than sensitive bacilli in man, it would be expected, if no isoniazid were given, that patients with resistant strains would become culture-negative more rapidly than similar patients infected with sensitive strains. At first sight there is evidence against this view in the finding (Table I) that 20 out of 43 patients with resistant strains at the end of three months' treatment with isoniazid remained culture-positive at a year, whereas only three out of 22 patients with sensitive or doubtfully resistant strains were positive at that time. However, 11 (26%) of the 43 patients with resistant strains had extensive cavitation on admission compared with only one (5%) of the 22 patients with sensitive or doubtfully resistant strains. Similarly 19 (44%) of the group of 43 patients had an average evening temperature of 99° F. or more in the pre-treatment week compared with four (18%) of the group of 22 patients. In other words, those patients who developed resistant strains were more seriously ill than those who did not, and the culture results at 12 months may therefore be attributable to this. The possibility remains that resistant bacilli are less virulent than sensitive strains, and that the findings at 12 months in the resistant group of patients might have been even less favourable had the organisms been fully pathogenic.

There is experimental evidence bearing upon the stability of isoniazid resistance. Strains composed *entirely* of highly resistant bacilli can be subcultured for at least six months in the absence of isoniazid or passaged through untreated animals without becoming less resistant (Knox, King, and Woodroffe, 1952; Barnett and others, 1953a, 1953b). However, both abnormal nutritional requirements and low animal pathogenicity were found in highly resistant bacilli (growth on 50 μg. per ml.) as compared with those with a lower degree of resistance (Fisher, 1952; Mitchison, 1953, 1954; Middlebrook, 1954). There is evidence that in patients under treatment with isoniazid (as with streptomycin and with sodium P.A.S., Colwell, Pitner, and Moravec, 1951) the strains within the lesions often consist of a mixture of bacilli with different degrees of resistance (Stewart, 1954). If a relationship between the degree of isoniazid resistance and pathogenicity, similar to that in experimental animals, occurs in man, it should follow that the highly resistant bacilli would be outgrown and successive cultures from a patient would then tend to become less resistant. The occurrence of reversion *in vitro* (Pansy and others, 1952; Barnett and

others, 1953a) can also be explained in terms of differential growth of the sensitive and resistant organisms.

In the present analysis, although there has been no general reversion of resistant strains, either to a lower level of resistance or to sensitivity, some patients with highly resistant strains (growth on 10 or 50 μg. of isoniazid per ml.) yielded less resistant or sensitive strains subsequently. On the other hand, some patients with sensitive or doubtfully resistant strains yielded resistant strains at the later test. As a result, the frequency distribution of strains according to level of resistance remained largely unaltered at the end of a follow-up period, without isoniazid treatment, of at least six months. It may nevertheless be misleading to regard an apparent increase in the level of resistance of a sensitive or doubtfully resistant strain in a subsequent test as the strict opposite of an equivalent decrease in the level of resistance of a highly resistant strain in a subsequent test. A specimen from a treated patient generally consists of a mixture of organisms of different degrees of resistance, and the sensitivity test usually indicates the highest level of resistance present, even if these organisms represent only a small proportion of the total (Colwell and others, 1951; Stewart, 1954). Thus a small increase in the proportion of resistant organisms in a predominantly sensitive mixture may suffice to yield a highly resistant result at a later examination. On the other hand, even a very considerable reduction in the proportion of resistant organisms in a substantially resistant mixture may leave the level of resistance in a subsequent test unaltered.

The findings in the present report, over a period of at least six months after stopping isoniazid, accord with the earlier experience in this trial (Medical Research Council, 1953b) for a follow-up period of three months. Coates, Meade, Steenken, Wolinsky, and Brinkman (1953), referring to a group of 43 patients treated for at least 16 weeks either with isoniazid or with iproniazid, state that "data available on some of these patients up to six months after treatment was discontinued, indicate that resistance to the I.N.H. drugs persists after the termination of treatment." Widelock and Robins (1954) treated a group of patients with isoniazid alone for eight months. They found that the level of resistance of strains isolated during a follow-up period of three months remained the same. On the other hand, Petit (1953a, 1953b) presents evidence that resistant strains often revert towards sensitivity, and Ashino (1953) that they usually do. Both these authors studied only patients whose cultures had moderate or high degrees of resistance (growth on slopes containing at least 5 μg. of isoniazid per ml.) and they did not take into account any tendency for sensitive or doubtfully resistant strains to become resistant. Nevertheless, this selection of strains cannot entirely explain the discrepancy between their findings and those reported here. In view of the abnormal nutritional requirements of highly resistant bacilli, it is possible that differences in the media used, as well as differences in technical methods, may have contributed to the discrepancies among these observations.

The conflicting evidence on reversion indicates the need for reports on further series of patients. Patients with sensitive strains at the end of a course of chemotherapy should be studied as well as those with resistant strains, and the levels of resistance of strains isolated subsequently should be investigated at regular intervals in some detail. Any information from serial studies on the proportions of bacilli of different levels of resistance in each strain isolated would be of great value.

When all the information available at present is considered, it may be concluded that once a patient has developed a strain resistant to isoniazid it is not justifiable to assume that loss of resistance will occur. Furthermore, even if some resistant strains are largely composed of bacilli with low pathogenicity both in man and animals, there is evidence that they usually contain a few fully pathogenic bacilli which would be capable of initiating fresh lesions (Peizer and others, 1953; Steenken and Wolinsky, 1953; Mitchison, 1954). It follows that an important aim in treatment with isoniazid should always be to prevent the emergence of drug-resistant organisms by combining isoniazid with other chemotherapeutic agents in appropriate dosage.

Summary

In a follow-up of patients with pulmonary tuberculosis, who had been treated with isoniazid (isonicotinic acid hydrazide) alone, information was collected on changes in isoniazid resistance of cultures of tubercle bacilli after isoniazid therapy was stopped.

Two main groups of patients were studied; one group had received isoniazid (200 mg. daily) for three months, and was then followed for a period of nine months without isoniazid; the other group had received isoniazid (200 mg. daily) for six months and was followed for a further six months without isoniazid. Results of sensitivity tests were available both at the beginning and at the end of the follow-up period for 23 patients in the first group and for 14

patients in the second group. Corresponding results were also available for five more patients followed for periods varying between seven and 11 months without isoniazid.

When treatment with isoniazid stopped, 16 of the 42 strains from these patients showed resistance at the highest level (growth on 50 μg. of isoniazid per ml.), 24 showed some lower degree of resistance, and two were sensitive. At the end of the follow-up period the strains from some patients were resistant at a higher level than previously, and from others were resistant at a lower level. Of the 42 strains, 10 then showed resistance at the highest level, 29 at lower levels, and three were sensitive. It is concluded that over a period of at least six months there is little evidence of general reversion of resistant strains either to a lower level of resistance or to sensitivity. Since the disappearance of isoniazid resistance after its development cannot be counted upon it is important to prevent its emergence by the use of suitable drug combinations.

Some of the limitations in the interpretation of isoniazid sensitivity tests are discussed.

Dr. D. A. Mitchison's laboratory, Postgraduate Medical School of London, served as reference laboratory for drug-resistance tests. Dr. E. H. Bailey, Southern Group Laboratory, supplied culture medium where desired. The isoniazid used throughout the trial was supplied as " nydrazid " by E. R. Squibb and Sons.

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