

COMBINED DRUG TREATMENT OF TUBERCULOSIS. IV. BACTERIOLOGIC STUDIES ON THE SPUTUM AND RESECTED PULMONARY LESIONS OF TUBERCULOUS PATIENTS

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The altered response to antituberculous agents of both the tubercle bacilli cultured from the sputum and from excised pulmonary lesions during and after therapy with these drugs has aroused the interest of the bacteriologist, the pathologist and the clinician. Therefore, many studies of this problem based upon clinical material subjected to wide varieties of drug-treatment regimens have been reported. These investigations have indicated that a significant proportion of clinically inactive lesions resected from drug-treated, sputum-negative patients still contain viable tubercle bacilli (1-7). For this reason surgical excision of roentgenographically demonstrable residual lesions is widely practiced.

The purpose of this paper is to present a comparison of the preoperative and postoperative sputum results, including drug-susceptibility testing, with similar bacteriologic studies carried out on the specimens of pulmonary tissue removed at surgery from 220 tuberculous patients. These bacteriologic findings have also been correlated with the drug-treatment regimens used at National Jewish Hospital before and after October 1, 1954, including the results of bacteriologic examination of resected pulmonary lesions from 37 patients who had been originally treated with the high dosage combined-drug regimen described in the third paper of this series (8).

MATERIAL AND METHODS

A. Examination of sputum. Digestion of the sputum (10 ml. or less) was performed by addition of "activated pangestin"¹ (Difco), 1.0 ml., and sodium bicarbonate 10

¹ Pangestin (Difco) is activated in the following manner: Dissolve-suspend one part of "pangestin" in 25 parts of distilled water; centrifuge at high speed in the angle

per cent, 1.0 ml. The specimen was diluted with distilled water to 20 ml., and incubated in a water bath at 40° C. for 30 minutes. Following this, "activated pangestin," 1.0 ml., was again added and the specimen reincubated in the same manner after dilution with distilled water to about 45 ml.; the specimen was centrifuged at 2,500 rpm for 30 minutes. The supernatant fluid was discarded, and a smear of the concentrate was made and stained by the Ziehl-Neelsen technique. Two hundred oil immersion fields were examined for acid-fast rods.

Specimens were divided into three groups according to the number of acid-fast rods (AFB) seen per oil immersion field: I. Containing < 1 AFB per oil immersion field. II. Containing 1-10 AFB per oil immersion field. III. Containing > 10 AFB per oil immersion field.

During examination of the stained films from the concentrate, further digestion and decontamination with weak alkali was performed, as described elsewhere (9).

Sediments from specimens of Group I were inoculated undiluted and at 10⁻¹ dilution; Group II, 10⁻¹ and 10⁻²; Group III, 10⁻² and 10⁻³.

In each case, after dilution, approximately 0.1 ml. was inoculated directly onto each of four quadrants of a Felsen plate which contained the 7H-10 solid agar medium plus 1.0 µg. of malachite green per ml. (9). The quadrants were as follows: Quadrant 1, no drug (control); Quadrant 2, 2.0 µg. streptomycin per ml.; Quadrant 3, 0.2 µg. isoniazid per ml.; and Quadrant 4, 2.0 µg. para-aminosalicylic acid per ml. This provided direct drug-susceptibility testing, as well as routine culture, on every sputum specimen.² The definition of drug-susceptibility employed in this report is: A strain or population of tubercle bacilli is susceptible to the action of streptomycin or isoniazid if less than 1 per cent of those

centrifuge in the cold, pour off the clear, amber-colored supernate. Mix this solution with an equal volume of a 12 per cent aqueous solution of magnesium sulfate.

² Prior to 1955, this last quadrant was used to test 10 µg. isoniazid per ml. During part of 1955, this quadrant was used to test pyrazinamide, 50 µg. per ml. Since that time, para-aminosalicylic acid has been used in this quadrant. Therefore, the results of susceptibility to para-aminosalicylic acid (PAS) test are not reported here.

members yielding colonies on the control are able to proliferate on culture media containing either 0.2 μ g. of isoniazid per ml. or 2.0 μ g. of streptomycin per ml. Similarly, any tubercle bacilli which multiply on media containing these concentrations of drugs, are drug-resistant, whether they derive directly from a wild population or are members of a population which has been selected by exposure to either of the drugs.

B. Examination of tissue. Immediately following surgical resection, each specimen was subjected to pathological examination in the fresh state.³ The preoperative roentgenograms were correlated with the findings of the resected specimen. Different sets of sterile instruments were used for obtaining bacteriologic specimens from each of the lesions so that the possibility of cross-contamination was minimized. Caseous lesions which measured less than 3 mm. in diameter were not studied individually, but were sometimes included with a predominantly fibrous lesion for culture. Impression smears were made from the necrotic material of the lesion. Abundant necrotic tissue which included a portion of the wall of the lesion was excised for culturing. When small lesions were encountered, the major portion was so used. The relationship of the lesion to the bronchi was then determined. The remaining pulmonary tissue was processed for histologic study.

Each bacteriologic specimen was macerated with sterile dissecting scissors and sterile forceps, and placed in a test tube with 5 ml. of filter-sterilized 0.2 per cent bovine serum albumin in distilled water and further macerated in a Teflon-glass grinder (11). The Felsen plates used for this study were prepared in the same manner as those used for culture of the sputum, and contained the same concentrations of drugs. Each of the four quadrants of a Felsen plate, containing the 7H-10 agar medium with or without drugs, was inoculated with 0.1 ml. of this suspension. Similarly, a 10⁻¹ dilution was made and inoculated onto the quadrants of a second Felsen plate.

Both sputum and tissue cultures were incubated for 21 days at 36° C. under an atmosphere of 2 to 5 per cent CO₂ in air. Some authors have emphasized the importance of keeping cultures of surgical specimens over very long periods of time in order to be certain that colonies of tubercle bacilli (especially isoniazid-resistant organisms) would not appear at a later date (12). However, evidence has been presented elsewhere that the culture medium and techniques employed in the studies described here give a reliable index of the viable bacterial count within three weeks (9). Cultures of several of the surgical specimens described here which were smear-positive and culture-negative at 21 days were further incubated for long periods of time (up to 11 months). None of these yielded colonies.

Determinations of catalase activity as a routine pro-

cedure on cultures of tubercle bacilli recovered from resected specimens was begun on May 1, 1956. These determinations were done as previously described (13).

DEFINITIONS

A. Histopathologic terms. The lesions were classified as open cavities, "filled" cavities, nodules and lung parenchyma (predominantly scar tissue).

Necrotic lesions which apparently at one time had been open and possessed a communicating bronchus, but which did not exhibit an empty space, were classified as "filled" cavities. Localized, gross areas of tuberculous tissue, regardless of their physical state, with no evident bronchocavitary communication were classified as nodules. Fibrous or scar tissue, occasionally with minute sclerotic foci, was classified as lung parenchyma.

It is important to emphasize that classification of a cavity as "filled" does not necessarily imply that the cavity was functionally closed.

B. Bacteriologic conversion of the sputum. For the purpose of this communication, sputum conversion to the bacteriologically negative state is defined to have occurred when both negative smears and negative cultures have been obtained on at least three consecutive monthly sputa with no subsequent positives. The date of conversion is defined as the date of the first of such a series of negative smears and cultures.

CLINICAL MATERIAL

Bacteriologic study, including drug-susceptibility testing, was performed on 298 individual lesions in 229 consecutive surgically resected specimens from 220 patients between October 1, 1954, and October 1, 1957.

Beginning on October 1, 1954, the application of high dosage streptomycin (20 mg. per Kg. per day) in combination with high dosage isoniazid (16 mg. or more per Kg. per day) together with pyridoxine, 50 to 100 mg. daily, was used in the treatment of all patients excreting typical tubercle bacilli susceptible to these drugs. Para-aminosalicylic acid was added when indicated to elevate the isoniazid levels. This approach to chemotherapy of pulmonary tuberculosis has been described in detail in the third paper of this series (8). Prior to that time, conventional dosages of streptomycin (1 Gm. every three days) combined with intermediate to high doses of isoniazid (8 mg. or more per Kg. per day) were used.

Original treatment cases are defined as those who received three weeks or less of chemotherapy with streptomycin, isoniazid or para-aminosalicylic acid, either singly or in combination, prior to admission. There was a total of 73 patients considered as original treatment cases: 24 admitted prior to October 1, 1954, and 49 admitted after October 1, 1954. Populations of tubercle bacilli isolated from the sputa of these 73 original treatment patients were both isoniazid- and streptomycin-susceptible at the time of admission. Six of the nine original treatment patients whose chemotherapy dated from before October of 1954 were excreting isoniazid-resistant tubercle bacilli at surgery. The remaining three

³ Between 10-1-54 and 6-15-56, surgical specimens were submitted to deep freezing prior to bacteriologic study, according to the method previously described by Hobby and co-workers (10).

TABLE I

Summary of 200 patients according to status of chemotherapy on admission and bacteriologic status of sputum prior to resection

Preoperative bacteriologic status	Original treatment		Retreatment	Total
	Before 10-1-54	After 10-1-54		
Positive	9*	2†	96	107
Converted	15‡	47§	51	113
Total	24	49	147	220

* Six of these were excreting isoniazid-resistant organisms at surgery. The remaining three were sputum-negative but not converted as defined.

† These two patients were negative but not converted at surgery.

‡ One lesion of one patient was culture-positive.

§ All resected specimens from these 47 patients yielded negative cultures.

|| Lesions from four patients were culture-positive.

plus the two positives treated after October of 1954 were bacteriologically negative but not converted, as defined.

Retreatment cases are defined as those patients who had received antituberculous chemotherapy for more than three weeks prior to admission. One hundred and forty-seven patients comprised this group. Ninety-six patients were excreting drug-resistant populations of tubercle bacilli on admission. And, 33 patients were either bacteriologically negative or yielded insufficient numbers of colonies of tubercle bacilli to permit satisfactory drug-susceptibility testing (Tables I and II).

RESULTS

At the time of surgical resection 107 patients were not "sputum converted": Eleven of them belonged to the group of 73 original treatment patients (15 per cent) and 96 belonged to the group of 147 retreatment patients (65 per cent). The remaining 113 were sputum-negative and

TABLE II

Bacteriological status of 147 retreatment cases at the time of admission and at resection

Bacteriologic status on admission	Bacteriologic status at time of surgery		Per cent converted	Total patients
	Positive	Converted		
Positive and drug resistant	96	9	9	105
Positive and drug susceptible	0	9	100	9
Insufficient growth for testing, or sputum negative*	0	33	100	33
Total	96	51		147

* Sputum-negative or too few colonies grew for reliable drug-susceptibility testing.

"converted." Sixty-two patients in this group were first treated with antimicrobial agents at the National Jewish Hospital (85 per cent of the total number of original treatment patients) and 51 belonged to the "retreatment" group (35 per cent of the total number in this group).

Among the 51 "retreatment" patients who were converted before the time of surgical resection, 33 either had been sputum-negative since admission to hospital or were excreting very few organisms in the sputum. None were excreting populations of tubercle bacilli which were susceptible to streptomycin and isoniazid at the time when they were transferred to higher dosage combined-drug treatment (streptomycin and isoniazid). And the remaining nine patients represented 8.5 per cent of 105 retreatment patients who were excreting drug-resistant populations of tubercle bacilli on admission to hospital (Table II).

It is also shown in Table I that 15 (60 per cent) of 24 original treatment patients admitted prior to October 1, 1954, were sputum-converted before surgical resection, while 47 (96 per cent) of 49 original treatment patients admitted after that date, and treated with high dosage of both streptomycin and isoniazid, were sputum-converted before surgical resection.

Of the 298 lesions studied, 144 (48 per cent) were positive on culture; three-fourths of these were cavitory lesions. Of the remaining 154 culture-negative lesions, one-half were positive on smear. The number of solid lesions in this culture-negative group was twice as great as the number of cavitory lesions (Table III).

In 206 out of the total of 220 patients, there was complete agreement in the bacteriologic results of the study of the preoperative sputum specimens and of the resected specimens. Anomalous

TABLE III

Two hundred ninety-eight lesions from 220 patients according to type and bacteriology of lesion

Type of lesion	Bacteriology			Total
	Z.-N. 0* cult. 0	Z.-N. + cult. 0	Z.-N. + or 0 cult. +	
Cavitory†	35	27	116	178
Solid	44	48	28	120
Total	79	75	144	298

* Z.-N. = Ziehl-Neelsen stain of impression smear.

† Both "open" and "filled" cavities.

TABLE IV

Bacteriologic findings of resected lesions according to pre-operative sputum status (220 patients)

Sputum status before surgery	Z.-N. 0 cult. 0	Z.-N. + cult. 0	Z.-N. + or 0 cult. +	Total
Positive	5*	4*	98	107
Converted	54	54	5*	113
Total	59	58	103	220

* Anomalous results (see text, Discussion).

results were obtained in 14 (7 per cent) of the cases (Table IV).

The nine patients classified as sputum-positive prior to surgery, but whose tissue failed to yield tubercle bacilli on culture, have been analyzed (Table IV). Two patients, consistently sputum-positive, yielded a solid lesion and an open cavity; both patients remained sputum-positive after surgery. It is quite clear the lesions responsible for the positive sputa were not resected. Two patients, consistently sputum-negative for five months, had one positive sputum immediately prior to surgery. Smear positive solid lesions were encountered in each case. Such observations are consistent with those of Canetti (14), who reports that solid caseous lesions may contain numerous visible bacilli while few and sometimes none of them may be viable. Five patients had three consecutive negative sputa prior to surgery, but had not been negative for the full period of three months. These might have been defined as "converted" had surgery been delayed.

All patients except the first two remained sputum-negative following surgery. Thus, in reality, only two of the nine patients definitely yielded anomalously culture-negative tissue, a 2 per cent disagreement between sputum and resected tissue bacteriology among 107 sputum-positive patients.

The five patients who were negative prior to resection, but whose pathologic specimens were positive on culture (Table IV), were originally treated with conventional dosage regimens of streptomycin and isoniazid at National Jewish Hospital prior to October, 1954, or at other institutions (Table I). Three of these specimens contained open cavities and a fourth specimen contained a "filled" cavity. Streptomycin-susceptible, isoniazid-resistant tubercle bacilli were cultured from

TABLE V

Bacteriologic findings—139 resected lesions from 113 sputum converted patients

Lesions	Z.-N. 0 cult. 0	Z.-N. + cult. 0	Z.-N. + or 0 cult. +	Total patients
Open cavity	31	12	3	46
"Filled" cavity	0	9	1	10
Nodule	26	40	0	66
Lung parenchyma*	13	3	1	17
Total	70	64	5	139

* Fibrous tissue occasionally containing minute sclerotic foci.

each specimen. The fifth patient had never been sputum-positive at this hospital, but aspiration of a large pleural effusion yielded streptomycin-susceptible, isoniazid-susceptible tubercle bacilli on culture. A sub-pleural nodule in the resected lung yielded organisms with drug-susceptibility characteristics identical to those cultured from the fluid. All five patients remained sputum-negative after surgery.

The 139 lesions from the 113 converted cases are analyzed in Table V. All but three of the 46 open cavities were negative on culture and three-fourths of them were negative on smear as well. On the other hand, nine of the 10 "filled" cavities were positive on smear and negative on culture; one was positive on culture. No "filled" cavities were negative on smear and culture. Although there were no culture-positive nodular lesions in this group, about two-thirds of them were positive on smear.

In marked contrast to these results are the 159 lesions studied from 107 sputum-positive patients (Table VI). One hundred and two of 104 open cavities were positive on culture. Ten of 18 "filled" cavities were positive on culture, and six

TABLE VI

Bacteriologic findings—159 resected lesions from 107 sputum-positive patients

Lesion	Z.-N. 0 cult. 0	Z.-N. + cult. 0	Z.-N. + or 0 cult. +	Total
Open cavity	2	0	102	104
"Filled" cavity	2	6	10	18
Nodule	4	3	21	28
Lung parenchyma*	3	0	6	9
Total	11	9	139	159

* Fibrous tissue occasionally containing minute sclerotic foci.

of the remaining eight were positive on smear. Seventy-five per cent of 28 nodular lesions were positive on culture, and three of the remaining seven were positive on smear.

Analysis of the drug-susceptibility patterns of the populations of tubercle bacilli recovered from sputum of unconverted patients and from their resected specimens is presented in Table VII. Identical patterns of drug susceptibility of populations of tubercle bacilli recovered from the sputa and resected lesions were found in 94 patients. There were two patients whose surgical specimens yielded anomalous results. Apparently one of these patients was excreting tubercle bacilli from two foci. Prior to resection 22 consecutive highly positive sputum specimens had been obtained from this patient. The bacterial populations in 18 of these specimens were resistant to isoniazid and resistant to 2 μ g. streptomycin per ml. The remaining four specimens contained organisms resistant to isoniazid, but completely susceptible to streptomycin. The resected specimen contained a "filled" cavity with isoniazid-resistant, streptomycin-susceptible organisms. After resection the sputum remained positive and contained only isoniazid-resistant and streptomycin-resistant tubercle bacilli. The preoperative sputum of the second patient contained tubercle bacilli resistant both to streptomycin and to isoniazid. Streptomycin-susceptible, isoniazid-resistant tubercle bacilli were cultured from a resected open cavity. Apparently this lesion was the source of the highly positive preoperative sputum since the patient became sputum-negative immediately after surgery. No explanation of this discrepancy is evident.

Catalase activity was determined on tubercle bacilli recovered from positive cultures from the surgical specimens and from the preoperative sputum of 54 patients (Table VIII). The re-

TABLE VII

Comparative results of drug-susceptibility testing tubercle bacilli from preoperative sputum and resected lesions (96 patients)

Source	SM-S* INH-S†	SM-R INH-S	SM-S INH-R	SM-R INH-R	Total patients
Sputum	0	2	16	78	96
Lesions	0	2	18	76	96

* SM-S (or R) = streptomycin-susceptible (-resistant).

† INH-S (or R) = isoniazid-susceptible (-resistant).

TABLE VIII

Comparative results of catalase testing tubercle bacilli from preoperative sputum and resected lesions (54 patients)

Source	Catalase activity			Total patients
	High* +++++	Moderate† +++ to +++	Reduced or absent 0 to +	
Sputum	4	9	41	54
Lesions	4	7	43	54

* Photochromogenic "yellow" bacilli.

† Typical isoniazid-susceptible tubercle bacilli.

sults indicate a high degree of correlation. Photochromogenic or "yellow" bacilli were recovered from the preoperative sputa and resected specimens of four patients. Of the remaining 50 patients who had been excreting typical tubercle bacilli, there were two whose organisms from the surgical specimen showed somewhat less catalase activity than did the organisms recovered from the preoperative sputum.

DISCUSSION

Numerous studies have been done to correlate the bacteriology of resected tuberculous tissue with the various chemotherapeutic regimens, the duration of chemotherapy, the incidence and duration of sputum conversion prior to resection, and the effect of chemotherapy upon the type and the size of the lesion. Results of these studies have indicated that the longer the duration of chemotherapy and of sputum conversion prior to resection, the lower the incidence of viable organisms recovered from lesions resected from sputum converted patients. These studies have also indicated that there was a higher incidence of viable organisms recovered from open cavitory lesions of sputum-negative patients.

The results of our study generally confirm these findings with the exception that the recovery of viable organisms in the lesions of sputum converted patients was very low in our series. The most striking difference between our results and those of other investigators in this field (1-7, 10, 14) is our failure to recover viable organisms from the open cavitory lesions resected from sputum-negative patients treated with a high dosage combined-drug regimen of streptomycin and isoniazid. It has been suggested (15, 16) that for permanent arrest of tuberculous infection a re-

sidual open cavitory lesion may be a more desirable end result of chemotherapy than a "filled" cavity. This is supported by the material presented in Table V. Not one of the 10 filled cavities removed from sputum-negative patients was negative on both smear and culture. On the other hand, 65 per cent of the 46 open cavities resected from this group of patients were negative on both smear and culture and only three of these lesions yielded positive cultures, all from patients originally treated with conventional as opposed to high dosage, combined-drug regimens.

The question may be raised as to whether the bacteriologic methods employed in this study are as effective in isolating small numbers of tubercle bacilli as those employed by other investigators. However, the percentage of negative cultures on surgical specimens, obtained from patients whose preoperative sputum specimens were definitely positive, was very low in this study, about 2 per cent—indeed, as low as any recorded in the literature. Furthermore, broader evaluation of the bacteriologic methods employed in this study has revealed them to be at least as efficient in isolating tubercle bacilli from the sputum as any of the more conventional methods (9).

Middlebrook and Cohn (9) have reported that 26 per cent of patients at National Jewish Hospital with bacteriologically proven tuberculosis never had positive concentrate smears of the sputum or gastric contents. They have also reported that less than 3 per cent of those sputum specimens with positive smears yielded negative cultures (including contaminated cultures) in our laboratories. These experiences speak for a very high degree of effectiveness of the methods employed in this study for cultivating small numbers of tubercle bacilli.

The incidence of positive cultures of resected pulmonary tissue from sputum-negative patients accumulated in the literature as well as in our series is presented in Table IX. It is evident that our series presented the lowest incidence (5 per cent) of positive cultures from sputum-negative patients.

There was a high degree of correlation (98 per cent) between the drug susceptibilities of tubercle bacilli recovered from the culture of the resected specimen, on the one hand, and of the tubercle bacilli recovered from the preoperative sputum,

TABLE IX
Reported incidence of positive cultures of resected pulmonary tissue from sputum-negative patients

Ref. no.	Investigators	No. of patients	Total positive
			%
(1)	Hurford and Valentine (1957)*	50	38
(2)	Wayne and Salkin (1956)	201	15
(3)	Bell (1956)	147	40
(5)	Stewart <i>et al.</i> (1956)†	47‡	23
(7)	Adler <i>et al.</i> (1955)	103§	18
(8)	National Jewish Hospital	113	5

* Only closed lesions reported.

† Only open cavities reported.

‡ Only lesions yielding drug-susceptible organisms included.

§ Probably not all patients sputum-negative at the time of surgery.

on the other. This may be contrasted with the results of a similar study by Kennedy, Vandeviere, Melvin and Willis (17), who reported only 67 per cent agreement.

There was also a high degree of correlation between the results of catalase tests on cultures of tubercle bacilli from sputum and from resected specimens (18). The finding of a high percentage of culture-positive sputa and lung lesions yielding only isoniazid-resistant catalase-diminished or catalase-negative mutants of tubercle bacilli is not surprising, since nearly all patients in this study had been treated at this institution with at least 8 mg. isoniazid per Kg. per day for several months prior to surgical resection (19). This was done regardless of the results of isoniazid-susceptibility tests, for reasons presented elsewhere (20).

One-quarter of the original treatment patients whose chemotherapy was begun prior to October 1, 1954, were chemotherapeutic failures excreting isoniazid-resistant organisms before the time of resection. Although they were originally treated with isoniazid, 8 or more mg. per Kg. per day, streptomycin was not given on the highly effective dosage schedule of 1 to 2 Gm. daily for 90 days or more later employed routinely at this institution, as described elsewhere (8). In contrast to the bacteriologic results obtained in these patients were those obtained in the 49 patients who received their original chemotherapy at National Jewish Hospital after October 1, 1954, with high doses of daily streptomycin and isoniazid (Table I).

The two patients in this last group who were sputum-positive at the time of resection required

emergency thoracotomy early during their original course of chemotherapy. The remaining 47 patients were all converted on chemotherapy alone, and without bed rest, prior to resection. All these patients, while they were still positive, continued to excrete only streptomycin-, isoniazid-susceptible tubercle bacilli, though in progressively diminishing numbers, until the time of sputum conversion. No positive tissue cultures were obtained from the resected tissues. The postoperative follow-up period on these patients ranges from eight months to more than two and one-half years, without evidence of roentgenographic or bacteriologic relapse in any of these individuals.

Thirty-seven of these 49 patients had moderately advanced or far advanced bilateral disease with cavity size ranging from 1.5 cm. to greater than 7 cm. They belong to a special study [part of Group I described in the third paper of this series (8)]. The duration of chemotherapy prior to resection in these patients was less than 12 months, an average of 209 days, ranging from 159 to 353 days, but longer than four months. Our results in this group of cases are in disagreement with those of other investigators who suggest that 12 months of combined chemotherapy are probably necessary to assure negative cultures on surgical specimens from such cases (5).

The discrepancy between our results and the results of other investigators may, indeed, be due to important differences in the dosage of drugs employed (3 to 5 mg. of isoniazid per Kg. per day, and streptomycin, 40 to 60 mg. per Kg. [2 to 3 Gm.] per week in most other studies, in contrast with isoniazid, 16 mg. per Kg. per day, and streptomycin, 140 mg. per Kg. per week, for these patients). The earliest bacteriologic and pathologic evidence of the superiority of the use of 7 over 2 or 3 Gm. streptomycin per week in combined drug therapy was reported by D'Esopo and co-workers (6). The lowest incidence of positive cultures of resected tissue from sputum converted patients in their studies was obtained when streptomycin was used in dosage of 1 Gm. daily with para-aminosalicylic acid.

In view of the low incidence of positive cultures from the resected specimens of patients rendered sputum-negative by intensive combined drug treatment, doubt may be raised as to what purpose is

served by surgical resection of residual pulmonary lesions of such patients (21). Nevertheless, even if it is to be assumed that the culture techniques employed in this study were perfectly effective in detecting any viable tubercle bacilli, it must be emphasized that only a small sample of each lesion was actually submitted to culture. Therefore, it would seem unwise to take a dogmatic position on this issue in the current state of our knowledge.

SUMMARY

1. Bacteriologic studies on the preoperative sputum and on individual pulmonary lesions of 220 patients subjected to resection for pulmonary tuberculosis have been analyzed. Two hundred six (93 per cent) of these were in agreement in that the sputum and the surgical specimens were either both negative on culture or both positive on culture.

2. Discrepancy between the results of drug-susceptibility studies performed on the preoperative sputa and on the resected tissue specimens was found in two of 96 patients.

3. There was a high degree of correlation (95 per cent) between the catalase activity of organisms recovered from preoperative sputa and the catalase activity of tubercle bacilli recovered on culture of resected specimens.

4. There was a low incidence (10 per cent) of sputum conversion on chemotherapy among the retreatment cases excreting tubercle bacilli resistant to streptomycin or isoniazid, or to both agents.

5. The high incidence of bacteriologically negative results on surgically resected specimens from sputum-negative patients originally on high dosage combined-drug treatment with isoniazid and streptomycin corroborates other evidence for the effectiveness of this approach to the chemotherapy of pulmonary tuberculosis.

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