A laboratory study of drug-resistant strains of Mycobacterium tuberculosis occurring in THE CITY OF GLASGOW in 1963.

by

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I N D E X.

A C K N O W L E D G M E N T S.

This study would not have been possible without the co-operation of all the bacteriologists in the area who submitted cultures to the Reference Laboratory, and the clinicians in charge of the cases also made an invaluable contribution by supplying certain information.

The clerical problems inherent in an investigation of this sort were patiently yet quickly disentangled by the Glasgow Central Tuberculosis Department of the Western Regional Hospital Board.

For the clinical information included in the Tables in Chapters VII and VIII I am indebted to the Consultant Chest Physicians in the City, who not only discussed the cases at length with me but also allowed me to quote freely from the case-notes - Drs. J.E. Geddes, G.D. Marshall Clarke, J. Cuthbert, R. Cuthbert, R. Sinclair Kennedy and A. Lees.

I am especially grateful to Dr. L.G. Bruce, Consultant Bacteriologist to the Victoria Group of Hospitals and Consultant to the Reference Laboratory, for permitting me to abstract various figures from the mass of information which has been gathered since the inception of the scheme, and for his constant support, encouragement and advice in the carrying out of this work.

It is a pleasure to record my thanks to Mr. Hugh Grey, Senior Photographer in the Department of Medical Illustration, Victoria Infirmary, who prepared some of the Figures, and to Miss M. Dunn, who typed the manuscript.

Chapter I.

Introduction.

Tuberculosis mortality and morbidity are still high in many parts of the world and the cost to some of the affected countries is immense because of the far-reaching economic and social factors associated with the disease. The World Health Organisation calculated that between 0.5% and 1% of the world's population has infectious pulmonary tuberculosis - figures which represent between 12 and 25 million people (HOIM, 1959). India alone probably harbours 4 to 8 million active cases (Indian Council of Medical Research, 1959).

Against this background the position in the United Kingdom can be objectively assessed. It was suggested by Logan and Benjamin (1957) that 375,000 patients in England and Wales had respiratory tuberculosis and that of this number some 45,000 were infectious. More recent surveys, from the Chief Medical Officer's Annual Report for 1962, showed that over 310,000 tuberculous cases were listed as attending clinics for treatment or supervision. New notifications totalled 20,519, a reduction of 5.7% on the 1961 figure of 21,747. Corresponding statistics for Scotland were 2,842 new cases, 6.4% less than the 1961 total of 3,036.

During /

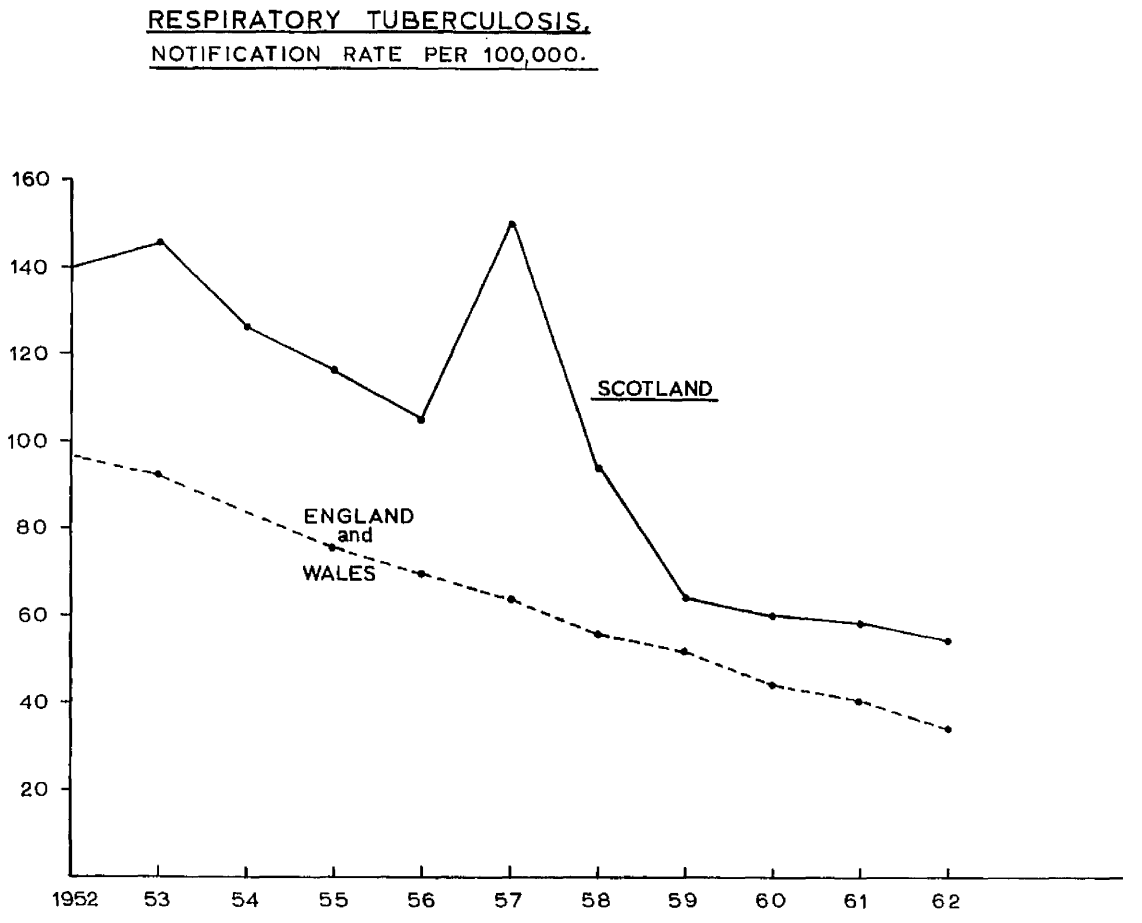


Fig. 1.

Showing the steady downward trend in new notifications of pulmonary tuberculosis. The graph for Scotland is interrupted by a spectacular peak in 1957, when the effect of extensive M.M.R. was felt.

PULMONARY TUBERCULOSIS.
CASE RATE PER 100,000.
1952 - 1962.

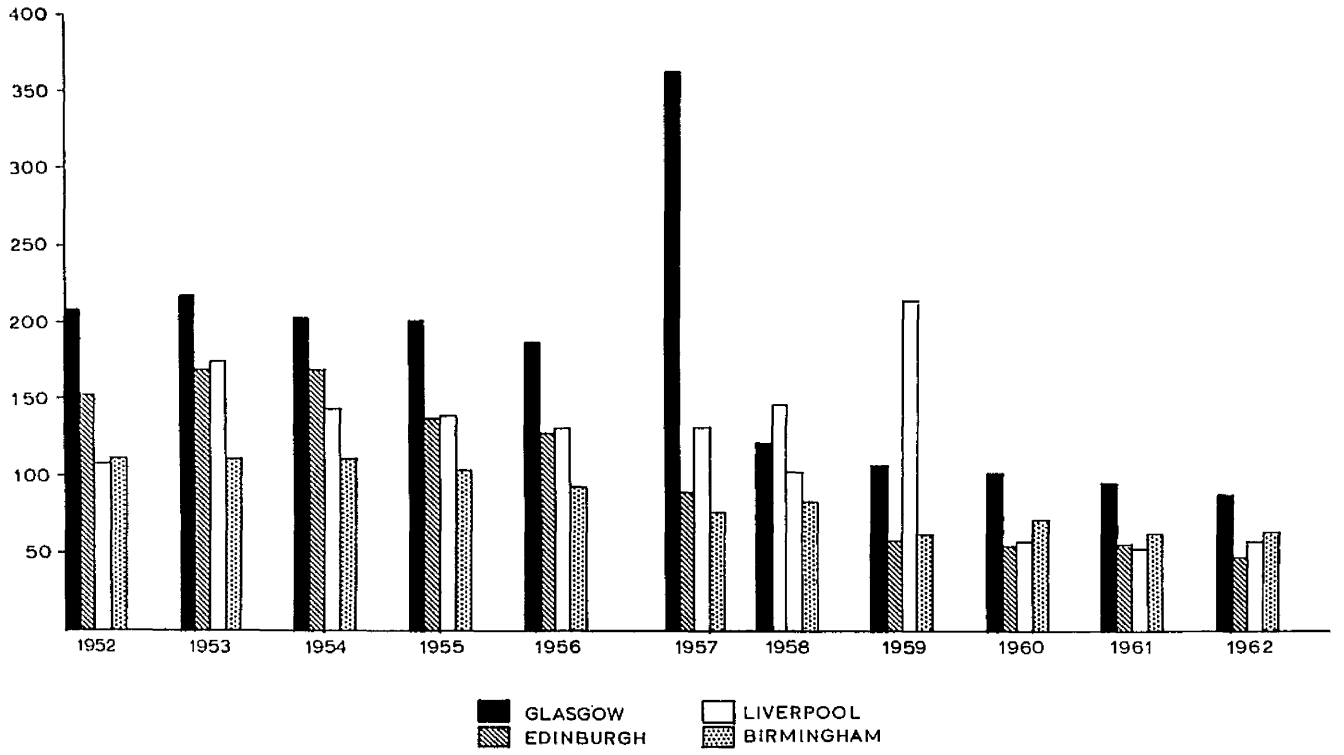


Fig. 2.

PULMONARY TUBERCULOSIS.
DEATH RATES PER 100,000.
1952 - 1962.

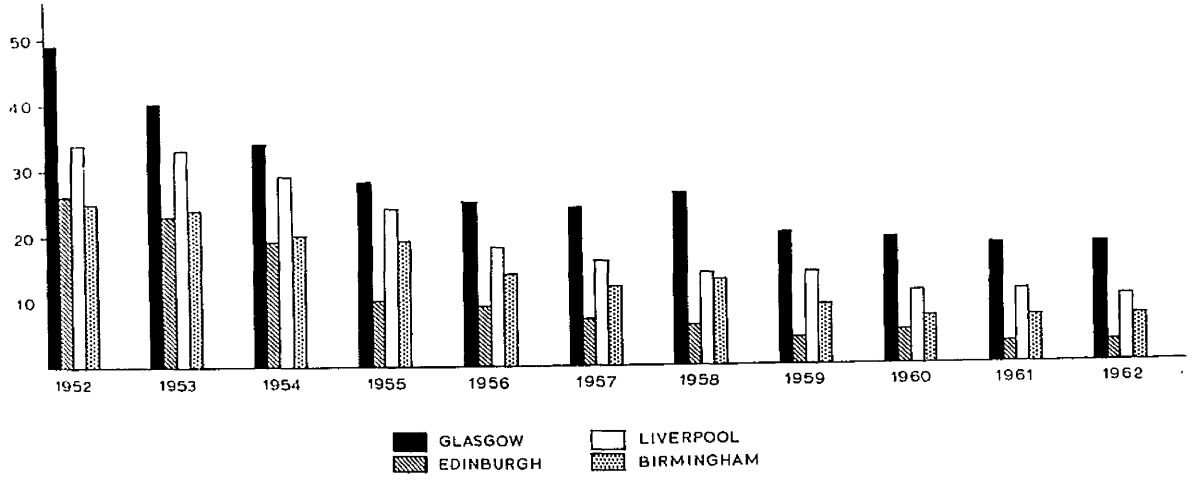


Fig. 3.

/During the 1939-45 war years Scotland's tuberculosis death rate rose, and it was not until 1948 that this trend was reversed; in the next decade there was a fall of 82% in the mortality rate, a figure which compared favourably with that of other countries and allowed Scotland to move from fifth to eighth highest when the death rates for European countries were tabulated. Morbidity figures followed a broadly similar pattern; new notifications increased during the nineteen-forties but levelled off at the end of the decade. The steady fall which ensued was only interrupted by the spectacular "peak" years of 1957-8 when widespread use was made of Mass Miniature Radiography. The downward trend was then resumed (Fig. 1) and today the cumulative effects of improving social conditions, systematic case-finding and the availability of effective chemotherapy and surgery have reduced the whole tuberculosis problem to a size much more manageable than that of 10 years ago.

For many years the City of Glasgow has had the unenviable reputation of being one of the tuberculosis "blackspots" in Great Britain, and in fact the morbidity and mortality rate has been consistently higher in Glasgow than in any other British city (Figs. 2 & 3). New notifications in Glasgow still number more than 1,000 per year, and it is obvious that there is much to be done before the disease can be regarded as finally checked, let alone eradicated. The latest complete figures for tuberculosis in the /

GLASGOW - TUBERCULOSIS

at 31st. Dec. 1962.

Clinic Area and Population	Respiratory tuberculosis.				Non-respiratory tuberculosis
	Sputum+	Sputum-VE	Not tested	Total	
EASTERN Pop. 238,586	563	2163	1160	3886	199
NORTHERN Pop. 205,267	670	2145	449	3264	214
WESTERN AND CENTRAL Pop. 210,266	431	2418	374	3223	210
SOUTH-EASTERN Pop. 218,587	99	1804	185	2088	139
SOUTH-WESTERN Pop. 156,441	162	1406	150	1718	94
TOTAL Pop. 1,029,147	1925	9936	2318	14179	856

TABLE I.

The position in Glasgow at the end of 1962. The figures for 1963 were incomplete at the time of writing.

/ the City apply to 1962 and they are summarised in Table 1 - respiratory and non-respiratory, bacteriologically positive and otherwise, geographical distribution and incidence. Nevertheless, the general trend is downward, but one disquieting feature of an otherwise hopeful current situation is that chemotherapeutic progress may be self-limiting unless careful attention is paid to an aspect of ever-increasing significance - the phenomenon of drug-resistance, primary or acquired.

Drug-resistance and its associated problems are receiving more attention in many centres throughout the world. A recent Leading Article (1964) discussing the relative merits of the various indices of tuberculosis at present in operation (mortality and notification records, mass radiography data, tuberculin test results, etc.) has suggested that most important of all in the future will be the information available from the National Drug Resistance Register. The position in the United Kingdom as a whole has recently been investigated by the British Tuberculosis Association (1963) and this study of a random sample of patients known to have had pulmonary tuberculosis for at least a year showed that 82% of their cultures were resistant to one or more of the standard three drugs and that 41% were resistant to all three. Calculations suggested that in 1960 some 3,500 patients in Great Britain were harbouring organisms resistant to one or more drugs. In the same survey were /

/ were shown the proportions of the population with resistant cultures in different parts of the country, urban, suburban and rural; once again the Glasgow rate of 20.9 per 100,000 was the highest, followed by Barrow-in-Furness with 15.6 and twenty four other area clinics with rates between 2.0 and 11.1 (all per 100,000).

Because of the persistence in Glasgow of these high figures for tuberculosis it was planned to give the problem of drug-resistance special attention and accordingly, towards the end of 1962, it was decided to establish a register of all patients in Glasgow who were excreting drug-resistant strains of Mycobacterium tuberculosis. It was thought that the information necessary for such a register could best be obtained by linking all the bacteriology laboratories of the Western Regional Hospital Board to a central Reference Laboratory with the following principal functions.

1) The subjection of all strains of M. tuberculosis received to a standard investigation.

2) The determination of the overall incidence of strains resistant to streptomycin, para-aminosalicylic acid and isoniazid, or to any combination of these drugs.

3) The periodic review of changes in this incidence, once established.

4) The determination of the sensitivity of these strains to a range of "secondary" drugs - viomycin, cycloserine and ethionamide in the first instance.

An account of the establishment of, the workings of, and the results obtained by this laboratory forms the substance of this thesis.

Chapter II.

Establishment of the Reference Laboratory.

The foundations for this new development were already available at Mearnskirk Hospital, Renfrewshire in the facilities for the diagnosis and assessment of tuberculosis in patients of the Victoria Group of Hospitals - mainly, out-patients at Florence Street Chest Clinic and in-patients at Mearnskirk Hospital.

It was envisaged that regional laboratories would send cultures from the following patients to the Reference Laboratory.

1)	New cases of tuberculosis (of any form)	...	estimated at 1,000.
2)	Known drug-resistant cases	...	estimated at 500.
3)	Suspected drug-resistant cases or those cases showing a changing pattern of drug resistance	...	estimated at 100.
4.)	Cases with persistent positive cultures after an initial six months chemotherapy	...	expected to be few among the originally sensitive strains.

As it was planned to accept cultures from the surrounding counties within the administration of the Western Regional Hospital Board a working estimate of 2,000 - 3,000 cultures per year was made and the laboratory was geared to complete 40-60 tests per week.

It was appreciated at a very early stage that the scheme's full potential as an epidemiological tool could only be realised if the record-keeping were of the same high standard as the technology. Form-filling had to be kept to a minimum for the convenience of the regional laboratories, yet a certain amount of information was /

1423.

WESTERN REGIONAL HOSPITAL BOARD

VICTORIA SECTORAL BACTERIOLOGICAL LABORATORY

MEARNSKIRK HOSPITAL, NEWTON MEARNS, RENFREWSHIRE.

TUBERCLE BACILLUS (DRUG SENSITIVITY) REGISTER.

Surname of Patient ..... SMITH ..... Sex M. ....
(Block letters, please)

Christian names .... JOHN D. ..... Date of Birth ..19./.5./.32.....

Home address ...101 OAKBANK PLACE ..... Nationality ..BRITISH..... ...

....GLASGOW S. 5. ....

Base Clinic ..... FLORENCE ST. ..... Hospital or Clinic No. ..... 18729 ....

Year originally notified as suffering from Tuberculosis ...1960..... .....

Site of lesion from which culture derived ..... PULMONARY .....

Laboratory sending culture ....MEARNSKIRK..... ....

Culture Reference No. 6351972 ..... T.B.R. No. .....1094.....

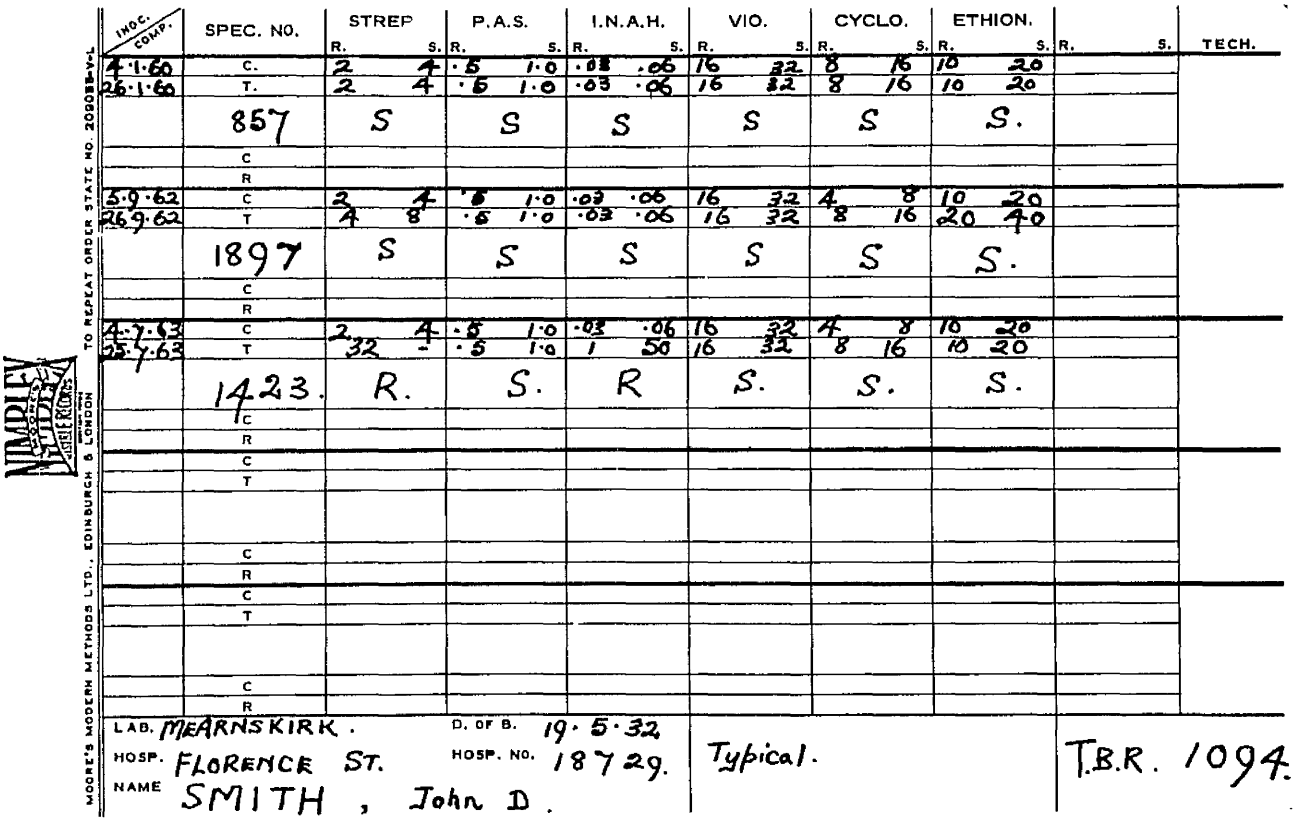
Categories			
A. A new case of tuberculosis	~~YES~~	NO	
B. Organisms considered resistant by local laboratory	YES	~~NO~~	~~NOT KNOWN~~
C. Patient producing positive culture after six months' chemotherapy	YES	~~NO~~	
D. Pattern of resistance appears to be changing	~~YES~~	NO	~~NOT KNOWN~~
E. Relapse case	~~YES~~	NO	

Comments from local laboratory - sensitivity pattern, etc.

RESISTANT TO STREPTOMYCIN and ISONIAZID.

Fig. 4.

A specimen request form.



INOC. COMP.	SPEC. NO.		STREP R.	STREP S.	P.A.S. R.	P.A.S. S.	I.N.A.H. R.	I.N.A.H. S.	VIO. R.	VIO. S.	CYCLO. R.	CYCLO. S.	ETHION. R.	ETHION. S.	R.	S.	TECH.
4·1·60		C.	2	4	·5	1·0	·03	·06	16	32	8	16	10	20			
26·1·60		T.	2	4	·5	1·0	·03	·06	16	32	8	16	10	20			
	857		S		S		S		S		S		S.				
		C															
		R															
5·9·62		C	2	4	·5	1·0	·03	·06	16	32	4	8	10	20			
26·9·62		T	4	8	·5	1·0	·03	·06	16	32	8	16	20	40			
	1897		S		S		S		S		S		S.				
		C															
		R															
4·7·63		C	2	4	·5	1·0	·03	·06	16	32	4	8	10	20			
25·7·63		T	32	-	·5	1·0	1	50	16	32	8	16	10	20			
	1423.		R.		S.		R		S.		S.		S.				
		C															
		R															
		C															
		T															
		C															
		R															
		C															
		T															
		C															
		R															

LAB. MEARNSKIRK. D. OF B. 19·5·32

HOSP. FLORENCE ST. HOSP. NO. 18729. Typical. T.B.R. 1094.

NAME SMITH, John D.

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TO REPEAT ORDER STATE NO. 2008

Fig. 5.

The loose-leaf sheets were ruled on both sides so that the results of 10 tests could be carried, although only 3 are shown here. The number in the margin (e.g.1423) corresponds to that on the right-hand top corner of the request form (Fig. 4.).

/ was necessary for reliable identification of each patient's culture and for the assembly of the desired data. The layout of the form agreed upon is shown in Fig. 4.

After much consideration the following three simple and complementary records were selected:

1) The Register itself - a simple numerical roll allocating a number to each patient who fulfilled the criteria for registration.

2) An alphabetically arranged index, allocating loose-leaf sheets to each patient. This sheet carried the patient's personal data and registration number, and could accommodate the results of ten successive sensitivity tests, making any changes in the patient's drug-sensitivity pattern visible at a glance. Continuation sheets could be added as required. (Fig. 5).

3) A visual punch-card system for rapid analysis of the data - Brisch-Vistem (Carter-Parratt). Cards are labelled with the feature it is desired to record (so that the system is readily expandable) and the information for each patient is entered by selecting the appropriate set of feature cards and drilling a circular hole in the numbered squares - the particular number corresponds to the registration number (Fig. 6). For analysis, the feature under investigation is easily selected, when all the registration numbers of patients fulfilling the particular condition will appear as holes on the appropriate card. Various combinations or sets of conditions can be examined simply by overlaying the selected feature cards and noting which numbers are common to all the cards i.e. which still appear as holes (Fig. 7).

Each new culture reaching the laboratory was first checked against the alphabetical index (frequently, cultures from the same patient appeared from two or more laboratories); genuine new cultures to the /

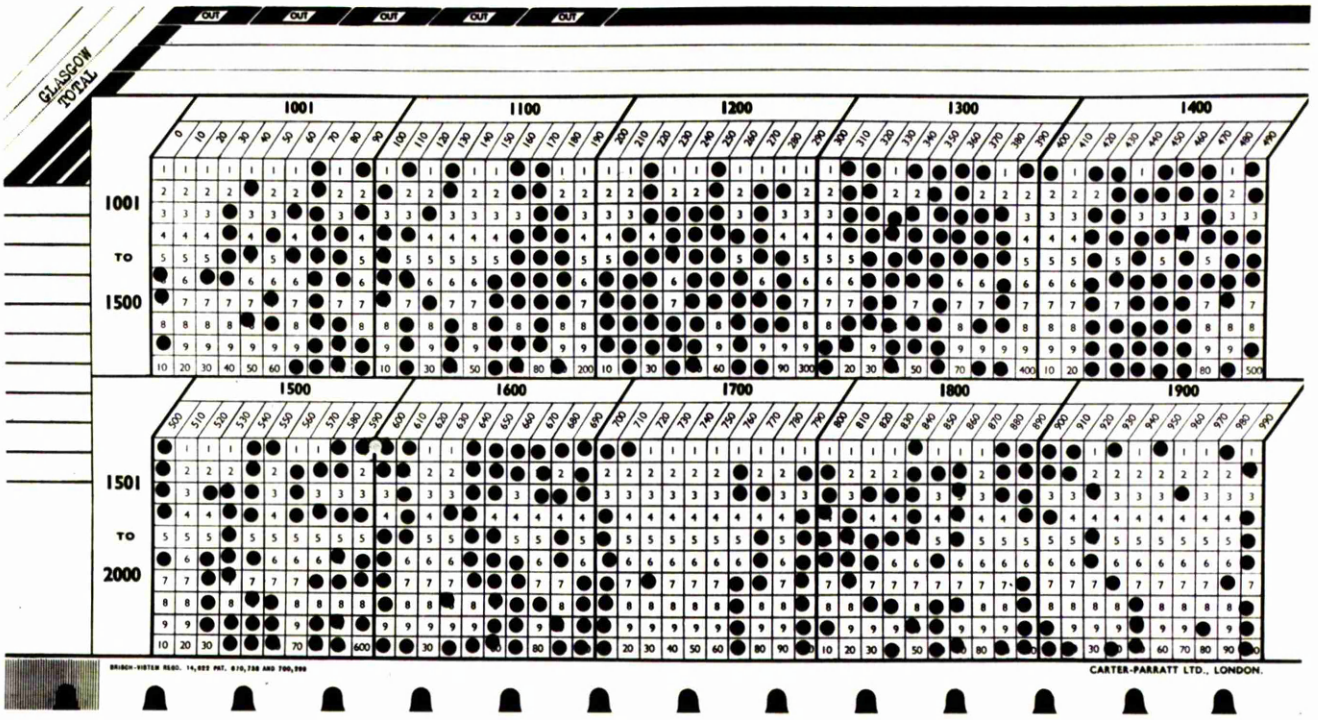

Fig. 6.

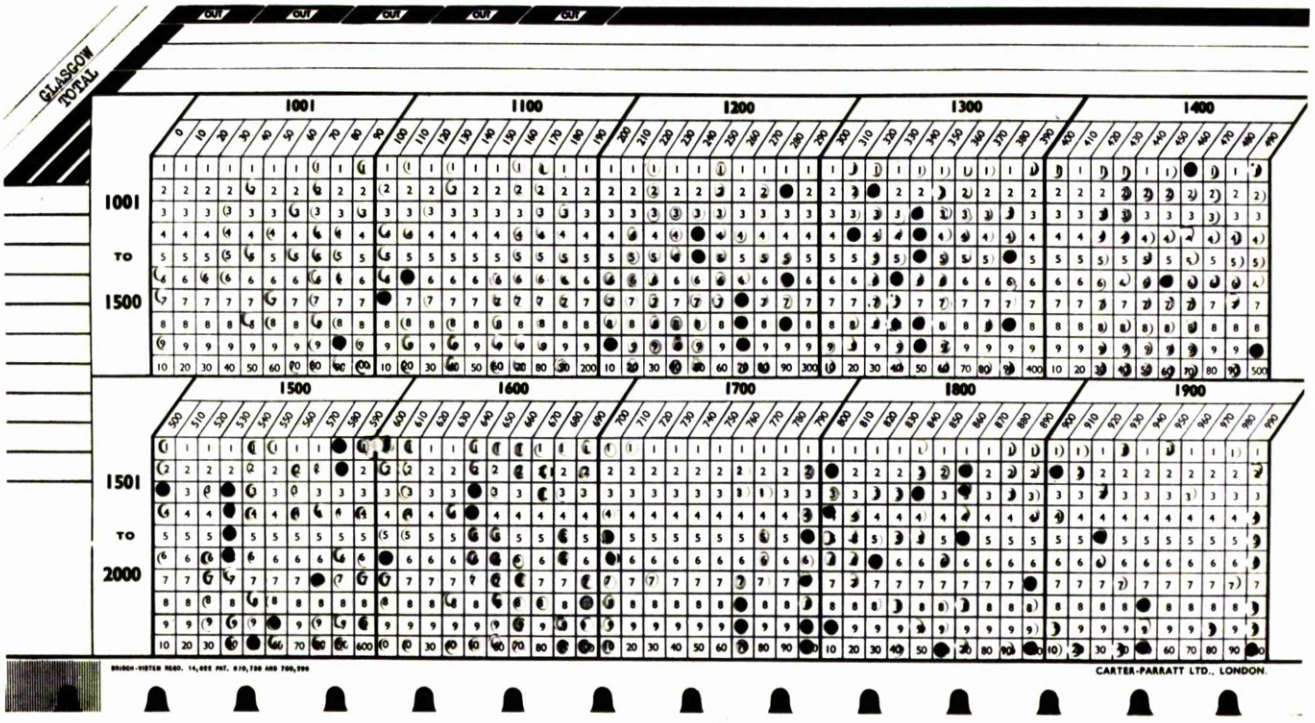

Fig. 7.

Above, the card for "Glasgow" alone; below, the effect produced by overlaying the "Glasgow" card on that for "pulmonary" and that for "resistance to primary drugs".

/ the Reference Laboratory were registered, but cultures reappearing for second or subsequent examinations were simply continued on the corresponding alphabetical sheet with their original registration number. Any changes in personal data or laboratory results were noted.

An annual complete return was planned for the Department of Home and Health for Scotland, together with sectoral abstracts for the supervising Chest Physician of the City of Glasgow, for the City's Public Health Department, and for the Medical Officers of Health of the participating areas. In turn, information was to be sent to the Reference Laboratory about patients who had died, changed their address, etc. In this way it was hoped to maintain the register in an up-to-date condition.

It appears that the only other established project of this kind in the United Kingdom is in Birmingham, whose register has been maintained since 1956 (Thomas 1961). It has been of particular interest to compare Glasgow's figures with those of Birmingham, where the population is similar and the notification rate not much lower. There, the number of patients alive and producing resistant organisms has fallen from 149 at the end of 1957 to 47 in 1961 - a much lower figure than Glasgow's 1963 total of 372.

A Central Register of Drug Resistant Cases has been recommended recently for England and Wales by the Standing Tuberculosis Advisory Committee, and it is understood that the Tuberculosis Reference Laboratory at Cardiff is to collaborate with the Statistics Branch of /

/ of the Ministry of Health in a detailed survey of cases of tuberculosis from whom have been isolated bacilli showing significant resistance to the standard drugs isoniazid, para-aminosalicylic acid and streptomycin.

Chapter III.

Materials and Methods.

Before performing sensitivity tests subcultures of all specimens received were made on Lowenstein-Jensen slopes, using a wide-sweep technique to ensure that a truly representative sample of the original growth was taken.

The subcultures were incubated at 37°C for 10-21 days till actively-growing, pure cultures were obtained. The importance of inoculum size has been stressed by many workers and considerable care was taken to keep this factor constant; nichrome wire (25 s.w.g.) was used to prepare standard loops with an external diameter of 3 mms., and these were checked regularly. It was found by experiment that the amount of culture which would just cover the distal part of such a loop was equivalent to 2 mgms. moist weight. This quantity was transferred to a sterile bijou bottle containing 0.4 ml. sterile distilled water and six small glass beads. Even suspension of the inoculum was ensured by agitation on a Kahn shaker for 5 minutes, and settling was prevented by gentle shaking at intervals during the inoculation of the complete set of tests - one loopful of the fluid suspension was spread evenly over the entire surface of the test medium in each bijou.

Solid media were used throughout, Lowenstein-Jensen medium being prepared according to the method of Mackie and McCartney (1960 a). Streptomycin, para-aminosalicylic acid, isoniazid, viomycin and /

/ and cycloserine were dissolved separately in sterile distilled water and added to bulk Lowenstein-Jensen medium to give the final concentrations shown below. Three millilitre amounts were dispensed in half-ounce screw-capped bottles; the caps were marked with the antibiotic concentration, each antibiotic having a separate colour for ease of identification. The bottles were placed in inspissators in the sloped position, and 30 minutes were allowed for complete heating of the loaded inspissator to 80°C; this temperature was maintained for one hour and then the batch was allowed to cool to room temperature. Antibiotic-containing media were prepared each week and used immediately so that drug deterioration (due to storage) was minimal. Because of its almost complete insolubility in water ethionamide was dissolved in ethylene glycol before being added to the Lowenstein-Jensen medium. Control tubes of medium without antibiotic were incorporated in each test. The final concentrations of the various antibiotics before inspissation were as follows (in micrograms per millilitre).

Streptomycin	1	2	4	8	16	32	
P.A.S.	0.25	0.5	1	2	4	8	
I.N.A.H.	0.03	0.06	0.125	0.25	0.5	1	50
Viomycin	16	32	64	128	256	512	
Cycloserine	4	8	16	32	64	128	
Ethionamide	5	10	20	40	80	160	

Each complete set of tests (Fig. 8) was incubated at 37°C for 21 days before reading. For each week's tests control sets using /

Fig. 8.

A complete set of sensitivity test bottles ready for inoculation.

GLASGOW VICTORIA HOSPITALS

W.S.B. & S. 11668

BACTERIOLOGY REPORT NO. ~~1423~~.....

Patient's Name	Hospital Registration No.	Ward/Dept.
John D. Smith	1 8 7 2 9	Florence St.

Dr./~~Mr.~~	Hospital or Address of Doctor if to G.P.
Physician	

Report on specimen of Positive culture — Date received 31.5.63.

Antibiotic	H37Rv mcgm R.	H37Rv mcgm S.	Test mcgm R.	Test mcgm S.	Resistance Ratio	Interpretation
Streptomycin	2	4	32	-	16	Resistant
P.A.S.	.5	1	.5	1	1	Sensitive
Isoniazid	.03	.06	1	50	32	Resistant
Viomycin	16	32	16	32	1	Sensitive
Cycloserine	4	8	8	16	2	Sensitive
Ethionamide	10	20	10	20	1	Sensitive

Date....25.7.63..... TBR 1094. Signed..... J. D. Baxie .....

Fig. 9.

A specimen report form.

/ using H 37 Rv and a known sensitive strain were included.

Interpretation of results. A strain was regarded as resistant to a particular drug concentration when confluent growth, innumerable colonies, or more than 20 discrete colonies grew at that concentration. Resistance ratios were calculated by dividing the highest concentration to which the test strain was resistant by the highest concentration to which the H 37 Rv was resistant. The criteria used for the classification of strains as "resistant" or "sensitive" were those listed in the Association of Clinical Pathologists' Broadsheet No. 32 (1961). For isoniazid a ratio of 2 or less was regarded as sensitive, 4 or more resistant. For the other antibiotics a ratio of 2 or less was termed sensitive, 8 or more resistant; a ratio of 4 was regarded as "doubtfully resistant" and the test was repeated, thereafter a ratio of 4 or more was classed as resistant.

Stewart et al (1962) have presented evidence that ethionamide sensitivity tests are more accurate if Youmans medium is used in preference to Lowenstein-Jenson, but it was felt that the use of a solid medium was safer in view of the large number of tests to be done on a routine basis; furthermore, clinical correlation of the Lowenstein-Jenson test results was found, on enquiry, to be good.

Two copies of the test result were sent to the laboratory submitting the culture, one for retention by the bacteriologist and the second for transmission to the clinician involved. A specimen report form is shown in Fig. 9.

Chapter IV.

Analysis of drug-resistance figures in respect of streptomycin, para-aminosalicylic acid and isoniazid.

The profound change which the discovery of effective chemotherapeutic agents has brought about in the treatment of tuberculosis is apt to obscure the fact that the oldest of these weapons has been in general use for not much more than a decade and that in this relatively short period little has been definitely established about their fundamental mode of action. It is perhaps only natural that the pressing clinical need for effective remedies has resulted in a great expansion of the search for new antibiotics, but it seems unfortunate that the enquiry into the basic mechanisms of the development of drug resistance should have been pursued only on a substantially smaller scale.

Some of the earliest work in the latter field was undertaken by Umbreit et al. (1951) who suggested that streptomycin's bactericidal action might be due to inhibition of the organism's metabolism in respect of condensation of pyruvate and oxalo-acetate. Other theories attributed the drug's action to inhibition of dephosphorylation of mononucleotides and depolymerisation of nucleic acids, or to inhibition of aerobic and anaerobic deamination of certain nucleic acids. Paine /

/ Paine and Clark (1953) suggested that the salient feature was an interruption of oxidation brought about by preventing the synthesis of certain essential enzymes. The various theories were comprehensively reviewed by Eagle and Saz (1955) but they could only conclude that some of the metabolic effects of streptomycin were not necessarily related to its cytotoxic action, and that no satisfactory explanation had been found.

Difficulty in deciding whether any one biochemical upset was directly attributable to the effect of the antibiotic on cellular metabolism or was simply a secondary manifestation of the dying cell was not confined to streptomycin alone. In investigating the effects of isoniazid Tsukamura and Tsukamura (1964) confirmed their earlier observations of specific inhibition of protein synthesis in mycobacteria exposed to the drug, but they were unable to determine that the effect was a primary one.

A full understanding of the means of development of drug resistance would be of great value in assessing the relative importance of the mutational (genetic) and environmental (adaptative) factors, about which there has been much controversy. McDermott (1962) has come to believe that no one explanation will suffice for all types of drug resistance and he has given a tentative classification of the different forms which may occur:

1) /

1) Genotypic - exemplified by resistance to streptomycin.

2) Enzyme change - the most familiar example is the relationship of staphylococcal penicillinase to penicillin; another possible example is the peroxidase or catalase deficiency of certain isoniazid-resistant strains.

3) Phenotypic (or pseudoresistance) - the unstable form of some isoniazid-resistant strains described in chapter VIII.

Whatever the final explanation of many of the phenomena associated with drug-resistance and encountered both in vivo and in vitro there is no doubt of their great clinical effectiveness and value. Pugsley et al. (1960) have compared the results in active pulmonary disease before and after the introduction of chemotherapy, and the remarkable improvement which they found in the life expectancy of victims of the disease is shown below.

Chemotherapy	Expectation of death in 10 - 20 years if disease		
	Minimal	moderately advanced	far advanced
Before	5%	20%	70%
After	NIL	NIL	11%

In this investigation triple drug therapy was given to 140 patients for an average of 17 months (although most became sputum-negative after 6 months). After 17 months the disease was inactive in 100% of patients with minimal, 97% with moderately advanced, and 81% with far advanced lesions. During the follow-up period of 2 - 5 years the relapse rate was 4%.

Similar /

/ Similar dramatic improvements have been seen in the United Kingdom; the British Tuberculosis Association's enquiry in 1961 showed that the proportion of patients whose disease never became quiescent fell from 4% among those first notified in 1947 to 10.8% among the cases notified in 1954. Encouraged by such trends and the good results of intensive and well co-ordinated treatment in Edinburgh Crofton (1960) has claimed that in ten months judicious chemotherapy will render sputum-negative all patients initially excreting organisms sensitive to two of the three standard drugs. Relapses should be very few indeed. In Birmingham Thomas et al. (1960) were completely successful in treating 530 patients (whose cultures were sensitive to at least two standard drugs) among the 1,000 new cases discovered in 1957-58. Blake (1961) reported 92% success in the initial treatment of 50 patients, but the relapse rate was higher at 11%. An investigation for the Medical Research Council (1962) noted 91% quiescence among 187 patients treated by chemotherapy for a year; in a 3-year follow-up it was found that the relapse rate was 22% among those who took drugs for 12 months only, compared with the much more satisfactory level of 4% among those who continued treatment for 2-3 years. Similar figures were obtained by Dutch workers (Mulder, 1960) who attributed their success to three-monthly changes in the drug regime and enforced bed-rest; the relapses were all of a mild nature and totalled 6.4% of all those originally treated.

Despite these encouraging figures there is no doubt that /

/ that it has proved impossible to attain 100% sputum conversion in some parts of the United Kingdom, and the percentage of successes in the under-developed countries must be very much lower. Even a small proportion of failures is of great significance for many of these will join the ranks of drug-resistant patients (whose numbers are only now being accurately assessed) and there can be no doubt that the development of drug resistance adversely affects the patient's prognosis. Thomas (1961), analysing the fate of tuberculosis patients in Birmingham between 1956-59, found that 5% of those with sensitive bacilli had died by the end of the period, whereas the rates for those with organisms resistant to streptomycin, P.A.S. and isoniazid were 30%, 20% and 28% respectively. For those with strains resistant to two drugs the death rate was 40% and for three drugs, 56%. The same author (1961 b) concluded that the numbers of resistant cases in Birmingham had reached a steady state instead of being gradually reduced as he had thought the previous year; and in 1963 he found it "a little disturbing" to observe a small increase in drug-resistant cases among those first notified in 1959.

The figures for Glasgow* are no less disquieting for it can be seen from Table 2 that a substantial proportion of the drug-resistant cases have been notified in the years of "modern" combined chemotherapy, say, in the last decade. The available records do not allow /

*(Since this work was concerned with all patients notified to Glasgow's Medical Officer of Health it was decided to include in the analysis patients who had been notified as tuberculous but who were excreting anonymous strains of mycobacteria. These numbered 7 out of a total of 372 (less than 2%) and are discussed more fully in chapter VII).

TABLE 2.

Resistance pattern by year of notification.

YEAR	Strep. M	Strep. F	PAS. M	PAS. F	INAH M	INAH F	S + P M	S + P F	S + I M	S + I F	P + I M	P + I F	S+P+I M	S+P+I F	Total M	Total F	Total Cases
pre-1930	-		-		1	0	-		-		1	0	0	1	2	1	3
1930	-		-		-		-		-		-		-		-		0
1931	-		-		-		-		1	0	-		0	1	1	1	2
1932	-		-		-		-		-		-		-		-		0
1933	-		-		-		-		-		-		-		-		0
1934	1	0	-		-		-		-		-		-		1	0	1
1935	-		-		-		-		-		-		-		-		0
1936	-		-		-		-		1	1	-		-		1	1	2
1937	-		-		-		-		-		-		-		-		0
1938	-		-		-		-		-		-		-		-		0
1939	-		-		-		-		-		-		-		-		0
1940	-		-		-		0	1	-		-		1	0	1	1	2
1941	-		-		-		-		1	0	-		-		1	0	1
1942	-		-		-		1	0	-		1	0	1	2	3	2	5
1943	1	0	-		-		-		1	3	-		2	2	4	5	9
1944	1	0	-		-		-		-		-		4	3	5	3	8
1945	2	0	-		0	1	-		-		-		6	3	8	4	12
1946	1	1	-		-		1	0	3	1	1	0	2	2	8	4	12
1947	-		-		-		-		0	1	-		-		0	1	1
1948	0	1	-		-		-		2	3	-		0	4	2	8	10
1949	1	0	1	0	0	1	1	0	2	2	1	1	5	4	11	8	19
1950	3	1	-		1	0	-		2	2	3	1	4	4	13	8	21
1951	2	0	-		2	1	-		2	1	0	1	6	6	12	9	21
1952	3	1	1	0	-		-		3	1	1	1	4	7	12	10	22
1953	1	2	-		1	1	-		3	3	-		9	3	14	9	23
1954	2	0	-		-		0	1	2	1	3	0	4	3	11	5	16
1955	0	1	-		1	0	-		4	3	2	0	10	3	17	7	24
1956	1	0	-		1	0	-		1	2	0	1	5	2	8	5	13
1957	4	2	-		3	1	-		10	2	2	0	12	0	31	5	36
1958	1	0	-		1	1	-		5	0	2	1	3	1	12	3	15
1959	0	1	-		2	1	-		4	0	-		2	1	8	3	11
1960	2	0	1	0	1	0	-		5	1	1	0	6	1	16	2	18
1961	1	0	-		4	1	-		5	0	3	0	1	0	14	1	15
1962	4	1	1	1	7	0	-		3	3	1	0	0	2	16	7	23
1963	7	6	1	0	1	0	1	0	2	0	2	0	5	2	19	8	27
	38	17	5	1	26	8	4	2	62	30	24	6	92	57	251	121	372.
	55		6		34		6		92		30		149		372.		

/ allow separation of primary from acquired resistance for the years up to 1962, but in this work the impression has been gained that small but significant numbers of recently diagnosed cases are still becoming drug-resistant despite apparently adequate chemotherapy. A prospective study now being undertaken will use the present figures as a base-line and thereafter will record the year in which truly acquired resistance developed and the form it took. It is for this reason that few definite inferences about the future trends of drug-resistance can be drawn from Table 2. Nevertheless, certain basic facts emerge. The distribution of cases by the quinquennium in which they were first notified is illustrated in Fig. 10, and the shape of this curve is important in assessing the significance of trends which emerge from other quinquennial reviews* in this work. Today's total of drug-resistant cases is made up of 8 (2.1%) pre-war notifications, 79 (22%) during the war and immediate post-war years and the remainder (75% approximately) from 1950 onwards. In the total of drug-resistant cases the ratio of male : female is rather more than 2 : 1, compared with 1.75 : 1 among new notifications in Glasgow in 1962 and it is interesting to see that although their respective graphs approximate to each other at first their courses are very different after 1950-54. Whether this is the result of a genuine difference in response to /

* In all graphs and histograms for comparisons of 5-year periods figures for 1960-64 have been estimated by increasing the actual 1960-63 figures by a factor of 1.25.

follows p. 17.

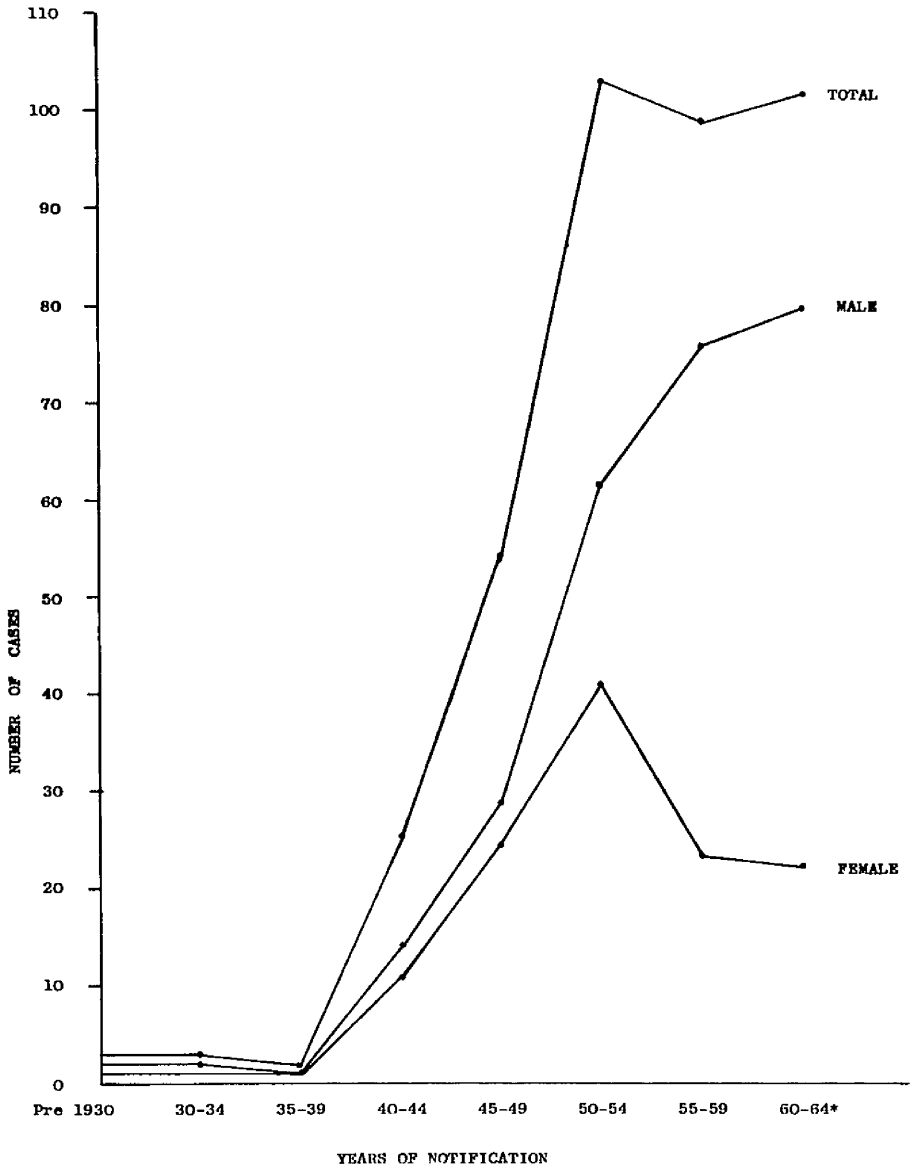

Fig. 10.

face p. 18.

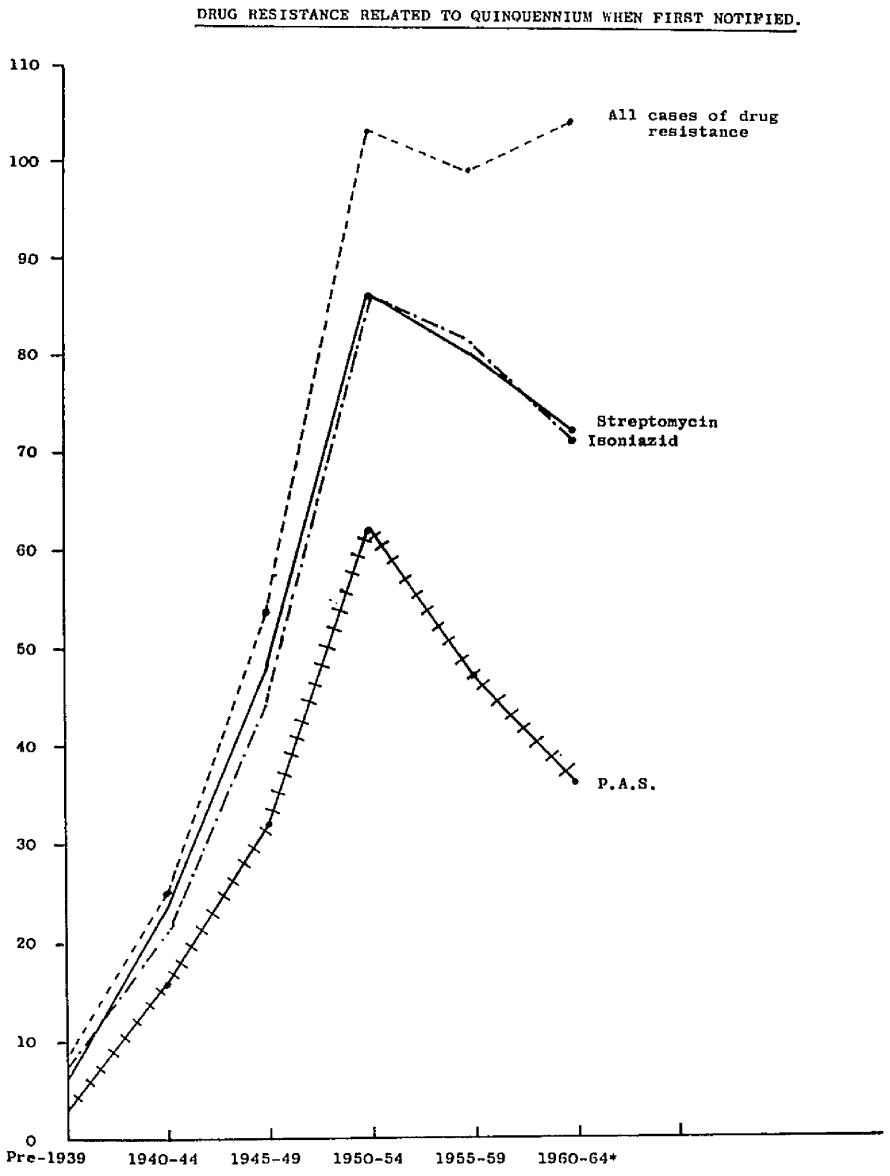

Fig. 11.

/ to chemotherapy will only become evident when drug resistance figures have been systematically followed for some years.

In Fig. 11 the incidence of resistance to each drug is related to the total of resistant cases, again grouped in 5-year periods. The first part of the graph shows no unexpected features; the comparatively low figures for the years up to 1945 represent the survivors from a period when effective chemotherapy was unknown, and the death rate was correspondingly high. Over the next decade the curves for streptomycin and isoniazid follow closely (less so for P.A.S.) the curve for "all cases of drug resistance"; this period saw the gradual introduction of successive antituberculosis agents to which, unfortunately, many cases became resistant in turn. Thereafter, the marked separation of the curves indicates that cases of multiple drug-resistance have become less common among the more recent notifications - a very satisfactory feature which can more readily be appreciated by a detailed study of the changing proportion of cases resistant to one, two or three drugs over this period (Fig. 12). The frequency with which resistance to each drug occurs is illustrated in Fig. 13 and it is interesting to note that the proportion of resistance caused by any one drug is fairly constant no matter in which 5-year period the case was first notified.

Thus, experience in Birmingham and what can at present only be claimed to be knowledgeable conjecture in Glasgow are rather at /

follows p. 18.

PROPORTION OF CASES RESISTANT TO SINGLE AND MULTIPLE DRUGS,

1950 - 1963.

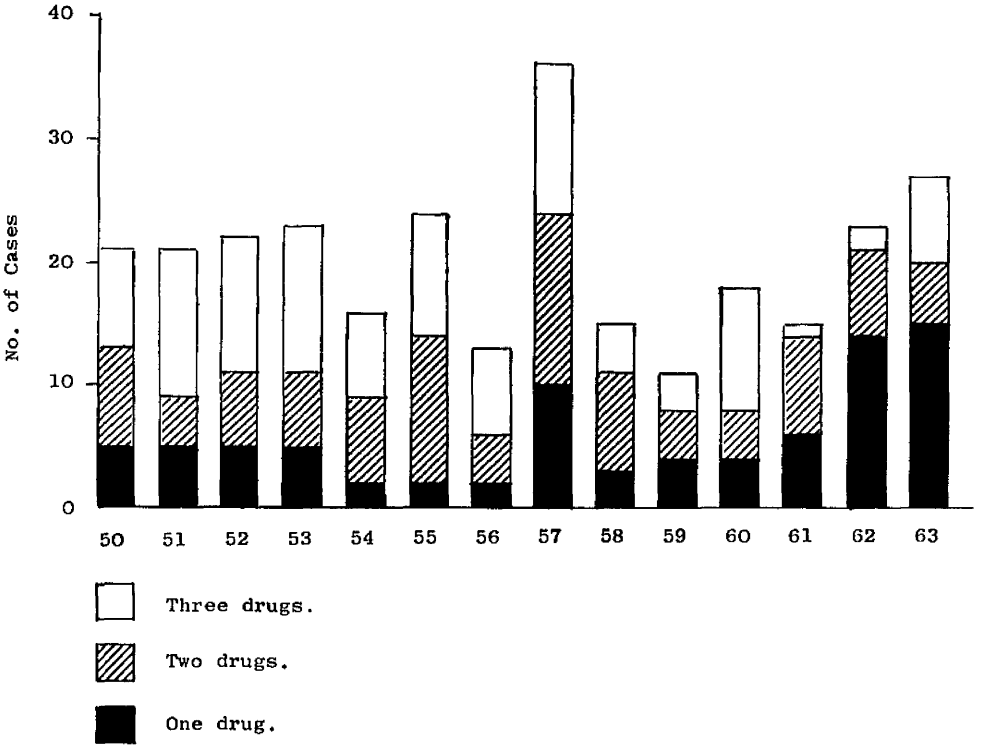

Fig. 12.

face p. 19.

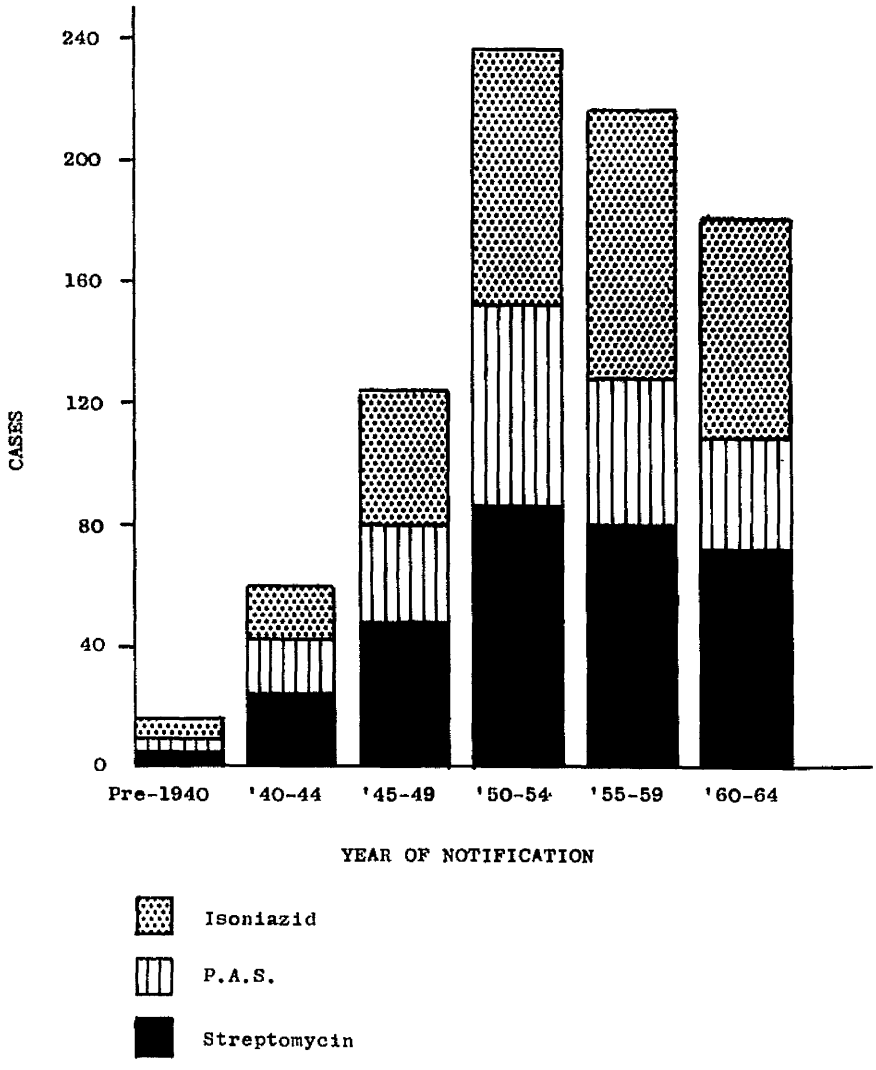

Fig. 13.

/ at variance with the national findings published by the British Tuberculosis Association in 1963. This survey points to the fact that among 430 cultures from "old patients" (those whose sputum was positive at the time of the survey and 12 months previously) only 12 were from patients first notified in 1959-60 and of those only 6 were drug resistant, and claims that the slow decrease in the number of drug resistant cases since the peak years 1950-55 is proof of the success of current anti-tuberculosis measures. The interpretation of the figures obtained is open to criticism on the grounds that it cannot be assumed that all drug-resistance developed in the year of notification (an assumption avoided in the present work); such cases should be classified as examples of primary resistance and should be excluded from any study of acquired resistance. Furthermore, if the year in which drug-resistance was actually acquired (e.g. 1960 for a 1950 notification) is not known it is surely impossible to state whether the current trend is upwards or downwards.

Drug resistant strains (372 in 1963) are found in about 20% of Glasgow patients with positive sputum (1,925 in 1962). Within the City there is some variation from one area to another in the distribution of resistant cases (Table 3). The Eastern and Northern sectors have both the highest resistance rate per 1,000 tuberculosis cases and the highest resistance rate per 100,000 population at risk. The Eastern sector's resistance rate per 1,000 tuberculosis cases is almost double that of the sector with the lowest figure (West and Central) and its resistance rate per 100,000 population is more than /

TABLE 3.

Area	Resistant Cases	Tuberculous Population	Resistance Rate per 1,000 T.B. Cases	Area Population	Resistance rate per 100,000 population.
Eastern	136	3,886	35	238,586	57
Northern	75	3,264	23	205,267	37
West and Central	59	3,223	18	210,266	28
South-Eastern	56	2,088	27	218,587	26
South-Western	46	1,718	27	156,441	29
TOTAL	372	14,179	26	1029,147	36

TABLE 4.

AREA	Sex	S	P	I	S+P	S+I	P+I	S+P+I	Total	TOTAL (M, F) Resistant to 1 drug	2 drugs	3 drugs	ALL CASES.
Eastern	M	21	3	7	1	27	4	36	99	37	44	55	136
	F	5	0	1	1	10	1	19	37	27%	32%	41%	100%
Northern	M	5	0	3	0	12	4	13	37	12	21	23	56
	F	2	0	2	0	4	1	10	19	21%	37%	42%	100%
West and Central	M	3	0	5	1	4	6	14	33	19	17	23	59
	F	7	1	3	0	5	1	9	26	32%	29%	39%	100%
South-Eastern	M	7	0	5	2	17	4	18	53	14	34	27	75
	F	1	0	1	1	8	2	9	22	18%	45%	37%	100%
South-Western	M	2	2	6	0	2	6	11	29	13	12	21	46
	F	2	0	1	0	3	1	10	17	28%	26%	46%	100%
	M	38	5	26	4	62	24	92	251	95	128	149	372
	F	17	1	8	2	30	6	57	121	26%	34%	40%	100%
TOTALS		55	6	34	6	92	30	149	372				

/ than double that of the sector with the lowest incidence (South-Eastern). With one or two exceptions, the pattern of drug resistance tends to be more constant (Tables 4 and 5).

TABLE 5.

Resistance to / Area	Streptomycin	P.A.S.	Isoniazid	Total Cases
Eastern	120 (88%)	65 (48%)	105 (77%)	136
Northern	46 (82%)	28 (50%)	49 (87%)	56
West and Central	43 (73%)	32 (54%)	47 (80%)	59
South-Eastern	63 (84%)	36 (48%)	64 (85%)	75
South-Western	30 (65%)	30 (65%)	40 (87%)	46
All Glasgow	302 (81%)	191 (51%)	305 (82%)	372

Resistance to isoniazid occurs most commonly, except in the Eastern area where streptomycin resistance is more frequent; the latter usually follows isoniazid closely with P.A.S. resistance a good deal less common than either of the others. The unusually low rate for streptomycin resistance and high rate for P.A.S. resistance in the South-Western area are other two features which emerge from this analysis.

With /

/ With the continual clearance of slums and the redistribution of families to new housing schemes and to nearby overspill areas these figures can be relevent for only a short time - especially as open cases of tuberculosis have priority in such movements. But, if the British Tuberculosis Association's estimate of a total of 3,500 cases of drug-resistant tuberculosis in the United Kingdom is correct then Glasgow, with less than 2% of the total population, contains more than 10% of these cases. It is no real solution merely to make geographical changes in the incidence of the disease, and it would be a tragedy if a false sense of security were to develop at national level and result in a premature relaxation of the efforts required to stamp out this disease altogether in Glasgow and certain other localities.

Chapter V.

Analysis of drug resistance figures in respect of viomycin, cycloserine and ethionamide.

The appearance of mycobacterial strains resistant to the standard drugs* has resulted in a search for new and more effective antibiotics, just as the occurrence of penicillin-resistant staphylococci stimulated the production of an immense number of alternative therpeutic agents. As yet the additional range of anti-tuberculosis compounds is not wide, but it was hoped that the principles of therapy derived from experience of the primary drugs would allow fuller use of the newer discoveries by delaying, or even preventing, the development of resistance to them in turn.

A direct comparison of the effectiveness of primary and secondary drugs was made by Saliba and Beatty (1961), and they found that the radiographic and bacteriological progress of 26 patients on pyrazinamide, cycloserine and isoniazid was inferior to that of 15 patients on streptomycin and high doses of isoniazid. Pyridoxine was given to both groups. They concluded that only if the latter scheme was unsuccessful should the secondary drug regime be employed. This is to be remembered when assessing the value of the secondary drugs; it is hardly surprising that few accounts of their use have been /

* Throughout this work the terms "standard" or "primary" drugs indicate streptomycin, P.A.S. and isoniazid; "secondary" drugs indicate viomycin, cycloserine, ethionamide etc.

/ been altogether favourable when they are usually reserved for chronic sputum-positive cases who have developed resistance to one or more of the standard drugs and whose general condition is sometimes poor.

The administration of some secondary drugs has been further complicated by the existence of a certain amount of cross-resistance with the primary drugs. Tsukamura et al. (1962) found that many kanamycin-resistant mutants were less susceptible to streptomycin than non-mutants, and that many viomycin-resistant mutants were less susceptible to kanamycin than non-mutants. As in each case the reverse was not true it was concluded that these drugs should always be given in the sequence, (1) streptomycin (2) kanamycin (3) viomycin. It is fortunate that this order coincides with what has in the past been normal clinical practice, though the use of kanamycin in tuberculosis has only been on a limited scale. It has been interesting to note in the present work that among the 35 strains showing resistance to viomycin only 3 were not also resistant to streptomycin.

Various workers have suggested different combinations of these secondary drugs and no clear cut evidence has emerged as to which is the best scheme. The Royal Netherlands Tuberculosis Association (1963) studied 96 sanatorium patients whose organisms were resistant to one or more of the standard drugs; they were treated by combinations of either 2 of the 3 standard drugs or pyrazinamide, ethionamide, viomycin, cycloserine and kanamycin. Most favoured among the /

/ the latter were regimes which included ethionamide, particularly ethionamide with cycloserine and pyrazinamide. It was stressed once again that any drug to which resistance had been demonstrated should be avoided, and that one new drug should not be added to two others to which resistance already existed. In Scotland, Pines (1962) described the fate of 109 Edinburgh cases whose treatment began between 1953-58. He confirmed Thomas's observation (1961) that multiple drug resistance is associated with a correspondingly worse prognosis, for 31% of the 71 patients in the trial whose organisms were resistant to 2 standard drugs died with a persistently positive sputum compared with 53% of the 38 whose organisms were resistant to all 3 drugs. Nevertheless, he obtained very encouraging results from treatment of the latter by combining pyrazinamide, cycloserine and ethionamide, sometimes adding viomycin and isoniazid. Such multiple therapy, however, could have little application in the treatment of out-patients. Another disadvantage in out-patient therapy is the need to give viomycin by injection, and this drug's popularity has probably been further decreased by rather unfavourable reports such as that of Aronovitch et al. (1961). These workers found that among 38 patients refractory to conventional chemotherapy treatment with viomycin for seven months resulted in bacteriological and radiological improvement only in 3 cases, and a further 7 had to discontinue taking the drug because of adverse side-effects.

Cycloserine came in for similar criticism by Craig et al /

/ Craig et al. (1961) who found that although some of their patients treated with a combination of this drug and isoniazid attained temporary sputum conversion the improvement was never maintained, and 3 of their original 24 cases were unable to tolerate these drugs. Haapanen et al. (1960), however, took a special interest in the patients whose response to therapy was poor and stressed the importance of examining the serum levels attained by the drugs in such cases; they were convinced that adequate doses of pyrazinamide and cycloserine would produce favourable results in a high proportion of patients resistant to the primary drugs.

Many of these reports deplore the frequency with which drug toxicities were met, but Battaglia et al. (1961) were adamant that no toxic manifestations appeared with cycloserine and isoniazid even when the blood concentrations of these drugs were within the accepted therapeutic range and even without the prophylactic use of pyridoxine and anti-convulsants.

Among the earliest experimental work on ethionamide was that of Rist et al. (1959) who showed it (in animals) to have an anti-tuberculosis activity twice that of streptomycin and about a tenth that of isoniazid; its toxicity was about a fifth that of isoniazid. Clinical confirmation of these results followed. Allen et al. (1961), after treating 78 patients suffering from chronic pulmonary tuberculosis with ethionamide and a variety of complementary drugs, /

/ drugs, concluded that it was an useful drug whose value was severely limited by the occurrence of relatively frequent toxic reactions (the cause of the withdrawal of no fewer than 32 cases from this trial alone). The British Tuberculosis Association (1961) agreed that ethionamide, with pyrazinamide or cycloserine, was of considerable value unless intolerance developed (in this series 44 of the original 62 patients withdrew because of side effects). Petty and Mitchell (1962) treated 70 patients whose organisms were resistant to streptomycin and isoniazid with ethionamide and pyrazinamide. For various reasons only 43 continued therapy for 3 to 18 months and more than half of these showed bacteriological, clinical and radiological improvement. Although ethionamide caused gastric disturbance and other side effects in many cases it was found that after temporary withdrawal of the drug it often could be reintroduced gradually and thereafter was tolerated. Clarke and O'Hea (1961) treated "salvage cases" - chronic sputum-positive patients whose organisms were resistant to some or all the standard drugs - with a combination of ethionamide and cycloserine or oxytetracycline and regarded the results as promising, but only for such selected cases. Much more enthusiastic was Slavin's (1962) report of a small series of 11 cases in which ethionamide combined with pyrazinamide or cycloserine showed anti-tuberculosis activity clinically comparable to that of isoniazid and streptomycin. Good early results were obtained by Somner and Brace (1962) who treated 26 patients with /

/ with ethionamide, pyrazinamide and cycloserine and achieved sputum conversion in 22 cases in 7 months. Although they had difficulty in persuading some of their patients that the cure was not worse than the disease they felt that 80% of chronic sputum positive cases could be rendered sputum negative by this form of treatment.

This brief review indicates that not only is there a place, despite their acknowledged limitations, for these drugs in the current antituberculosis armamentarium but also a real need for new and effective drugs, preferably with less toxicity. Already significant resistance has developed to the secondary drugs; 71 cases (almost 20% of the total resistant to the primary drugs) are detailed below. Only one strain was found which showed any form of resistance to the secondary drugs in the absence of resistance to at least one standard drug. This same strain was the only example of primary resistance to any of the secondary drugs, for the other two cases shown as 1963 notifications in Table 6 did not fulfil the criteria set for primary resistance.

It can be seen from Fig. 14 that, in contrast to the situation with the standard drugs, considerably fewer cases of resistance to viomycin, cycloserine and ethionamide occur among the more recent notifications than among those first diagnosed in 1950-54. This finding is probably directly related to the marked reduction in the numbers of cases resistant to two or more of the standard drugs which was noted among those notified from 1955 onwards (Fig. 12), a /

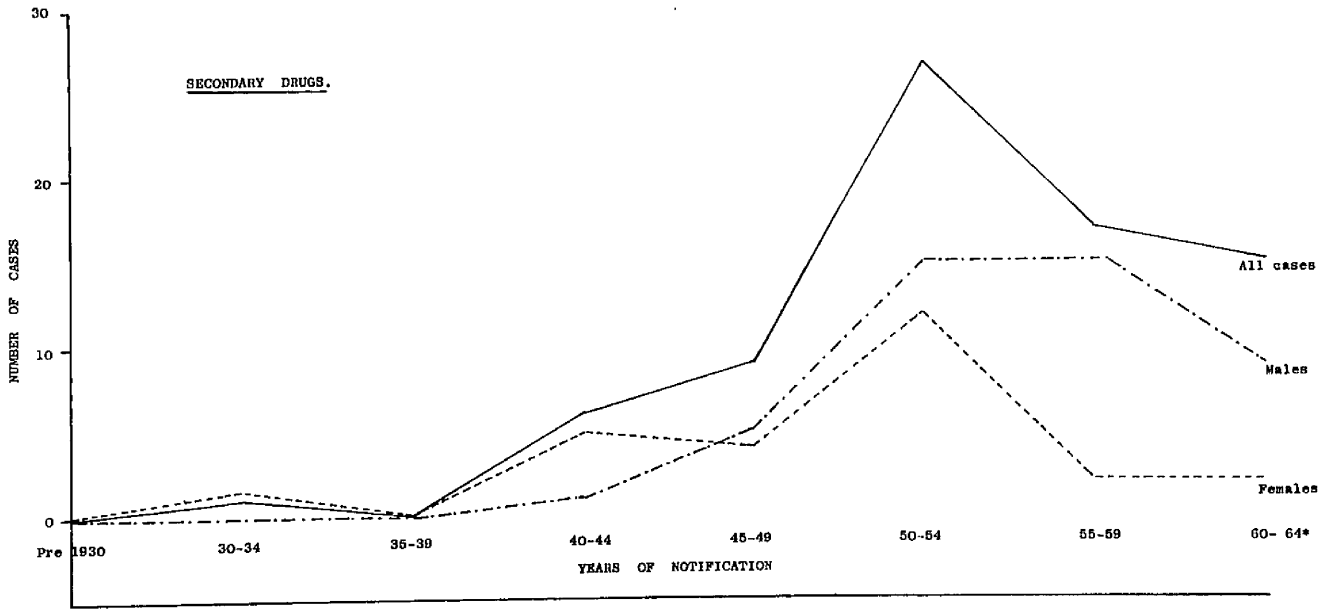

Fig.14.

Resistance to the secondary drugs; numbers of male and female cases related to quinquennium in which first notified.

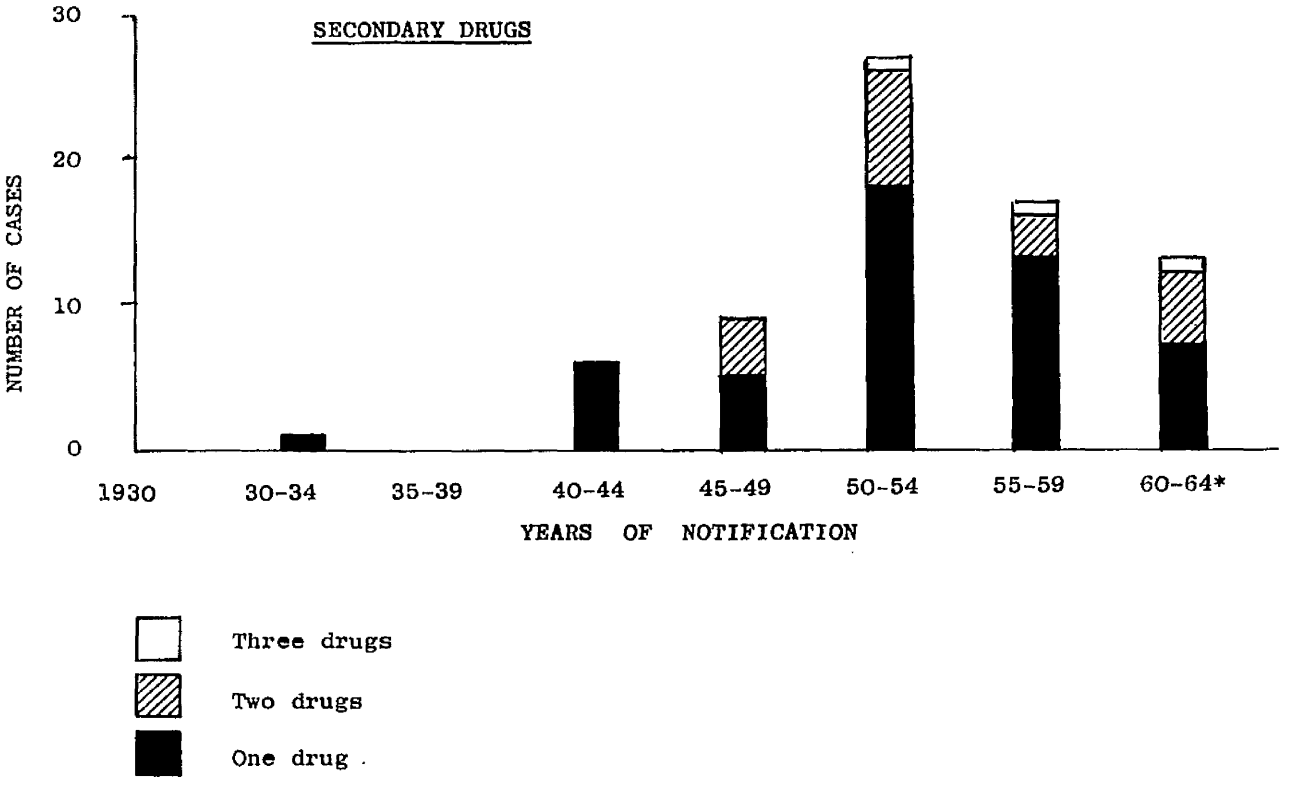
SECONDARY DRUGS
NUMBER OF CASES
30
20
10
0
1930
30-34
35-39
40-44
45-49
50-54
55-59
60-64*
YEARS OF NOTIFICATION
Three drugs
Two drugs
One drug

Fig.15.

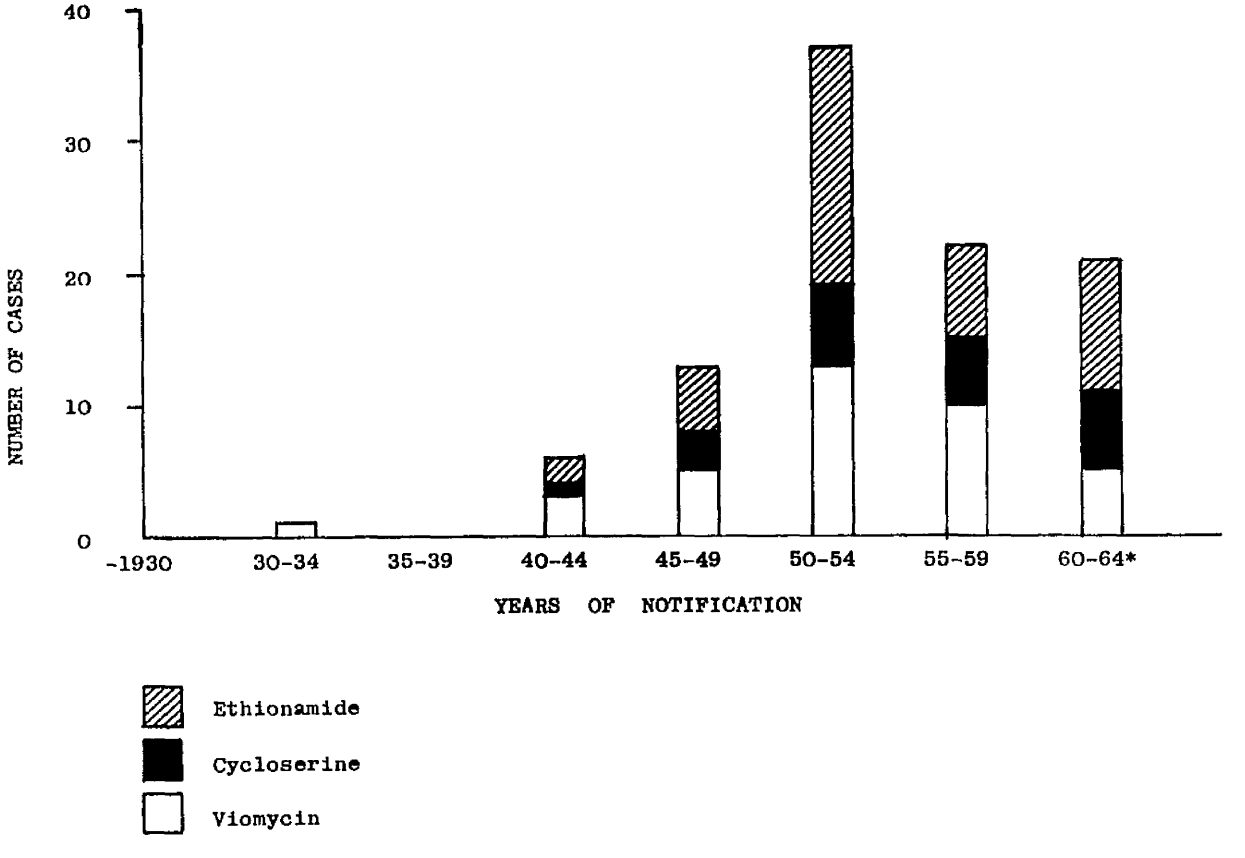
NUMBER OF CASES
40
30
20
10
0
-1930
30-34
35-39
40-44
45-49
50-54
55-59
60-64*
YEARS OF NOTIFICATION
Ethionamide
Cycloserine
Viomycin

Fig. 16.

TABLE 6.

Resistance pattern by year of notification.

V = Viomycin C = Cycloserine E = Ethionamide.

YEAR	V. M	V. F	C. M	C. F	E. M	E. F	V + C M	V + C F	V + E M	V + E F	E + C M	E + C F	V+C+E M	V+C+E F	Total M	Total F	Total Cases.
pre-1930	-		-		-		-		-		-		-		-		0
1930	-		-		-		-		-		-		-		-		0
1931	-		-		0	1	-		-		-		-		0	1	1
1932	-		-		-		-		-		-		-		-		0
1933	-		-		-		-		-		-		-		-		0
1934	-		-		-		-		-		-		-		-		0
1935	-		-		-		-		-		-		-		-		0
1936	-		-		-		-		-		-		-		-		0
1937	-		-		-		-		-		-		-		-		0
1938	-		-		-		-		-		-		-		-		0
1939	-		-		-		-		-		-		-		-		0
1940	1	0	-		-		-		-		-		-		1	0	1
1941	-		-		-		-		-		-		-		0	0	0
1942	-		-		0	1	-		-		-		-		0	1	1
1943	0	1	-		-		-		-		-		-		0	1	1
1944	0	1	0	1	0	1	-		-		-		-		0	3	3
1945	1	1	1	0	1	0	-		0	1	-		-		3	2	5
1946	-		-		-		-		-		-		-		0	0	0
1947	-		-		-		-		-		-		-		0	0	0
1948	-		-		-		-		-		-		-		0	0	0
1949	-		1	0	-		-		1	1	0	1	-		2	2	4
1950	1	1	-		1	1	-		-		-		-		2	2	4
1951	1	1	-		3	0	-		-		0	1	-		4	2	6
1952	0	1	-		-		0	1	1	1	1	0	0	1	2	4	6
1953	1	0	-		3	1	0	1	1	0	-		-		5	2	7
1954	1	0	-		0	2	-		-		1	0	-		2	2	4
1955	3	0	1	0	3	0	-		-		-		0	1	7	1	8
1956	-		-		-		-		-		-		-		0	0	0
1957	1	0	1	0	-		-		-		-		-		2	0	2
1958	1	0	1	0	-		1	0	2	0	-		-		5	0	5
1959	0	1	-		1	0	-		-		-		-		1	1	2
1960	-		1	0	2	0	-		1	0	1	0	-		5	0	5
1961	-		-		2	0	-		-		-		-		2	0	2
1962	-		-		-		-		-		1	0	-		1	0	1
1963	1	0	-		-		0	1	-		-		0	1	1	2	3
	12	7	6	1	16	7	1	3	6	3	4	2	0	3	45	26	71
	19		7		23		4		9		6		3		71		

/ a tendency which would curtail the need for additional chemotherapy. Other factors, however, which would also lower the incidence of resistance among recent notifications are the rarity of primary resistance to these drugs and, it is to be hoped, the genuine reduction in acquired resistance by their careful use.

It is very gratifying to see from Fig. 15 that resistance to all three of the secondary drugs under consideration is very uncommon indeed; only three cases have been recorded, a very different situation from that with the standard drugs.

The frequency with which each drug is involved in resistant cases is shown in Fig. 16. Resistance to ethionamide is most frequently met with (59%), followed by viomycin (49%) and cycloserine (2%).

The distribution in the City of cases showing resistance to the secondary drugs is indicated in Table 7. The numbers are rather small for analysis in quite the same way as the primary drugs in Table 5, and percentage figures are rather misleading. Nevertheless, the absolute numbers of cases follow a pattern not unlike that set by the primary drugs, the largest number being in the Eastern area and the lowest in the South-Western. Attention was drawn to the somewhat ephemeral nature of the corresponding statistics in the previous chapter and, of course, the same reservations are applicable here.

TABLE 7.

AREA	SEX	Viomycin	Cyclo.	Ethion.	C + E	V + E	V + C	V+C+E	TOTAL	Total Viomycin (M+F)	Total Cyclo. (M+F)	Total Eth. (M+F)	ALL CASES
EASTERN	M	6	1	6	0	4	2	0	19	15	6	16	28
	F	3	1	2	1	1	1	0	9	54%	22%	57%	
NORTHERN	M	4	2	2	0	0	0	0	8	8	3	6	15
	F	3	0	3	0	0	0	1	7	53%	20%	40%	
WEST and CENTRAL	M	1	3	2	0	1	1	0	8	6	8	6	12
	F	0	0	0	2	0	0	2	4	50%	67%	50%	
SOUTH-EASTERN	M	1	0	4	1	1	1	0	8	5	3	9	12
	F	0	0	2	1	1	0	0	4	42%	25%	75%	
SOUTH-WESTERN	M	0	0	2	0	0	0	0	2	1	1	3	4
	F	1	0	0	0	0	1	0	2	25%	25%	75%	
	M	12	6	16	1	6	4	0	45	-	-	-	71
	F	7	1	7	4	2	2	3	26				
		19	7	23	5	8	6	3	71	35	21	40	71

CHAPTER VI.

Primary Drug Resistance.

During investigation of the antimicrobial effects of streptomycin it was noted that a small number of naturally drug-resistant cells were present in almost any mycobacterial population. A similar discovery was made when isoniazid came into general use and Hobby and Lenert (1952) demonstrated that isoniazid-resistant clones existed in significant numbers among most, if not all, strains of M. tuberculosis prior to contact with the drug. Provided the number of resistant cells was small the host could eliminate them when the susceptible elements were destroyed by the drug; if, however, the number was large the host might be unable to eliminate them and the destruction of susceptible clones by therapy would simply allow rapid multiplication of the uninhibited mycobacteria until the infecting strain was homogeneously resistant. This artificially induced state was termed acquired resistance, whereas previously untreated patients infected by such strains were said to show primary resistance.

The significance of primary resistance is twofold. Firstly, it presents the clinician with an awkward therapeutic problem, especially if more than one of the standard drugs is involved. Secondly, it is commonly regarded as an index of the control of infection in a community and therefore has value in epidemiological studies. At present the /

/ the latter potentiality cannot be fully realised because, although a vast store of information has accumulated, the different technical methods and different criteria of resistance employed by various workers have prevented international comparisons of the true incidence of primary resistance. Hobby (1962), after an extensive review of European, Asian and American publications in which the incidence of primary resistance to any of the three standard drugs fluctuated wildly, suggested that this difficulty could be remedied if certain specific criteria were adopted for the collection of worthwhile information.

(1) Only patients with newly diagnosed tuberculosis who have received no previous antituberculosis chemotherapy to be classified as "primary resistant".

(2) Drug sensitivity tests to be performed in a single laboratory under carefully defined conditions with precise control of inoculum size, medium, incubation period, drug concentrations, etc.

(3) Differentiation of true M. tuberculosis and other mycobacteria to be carried out.

(4) Resistance standards in laboratory to be related to clinically significant levels.

As these criteria have been fulfilled in the current investigation it is felt that the figures now presented for Glasgow are a true indication of the size of the local problem and should be acceptable as a baseline for study in future years.

The reference laboratory examined cultures from a total of 350 patients who were first notified as suffering from pulmonary tuberculosis during 1963 - rather more than a third of the year's notifications and probably a very large proportion of all the bacteriologically positive cases. It is appreciated that the figures given below may be a little inflated because of a tendency to submit apparently resistant cultures to the reference laboratory rather than sensitive cultures, although the original protocol clearly included ALL cultures (resistant or sensitive) from newly diagnosed patients.

Twenty-three cases were accepted as examples of primary resistance; four of the twenty-seven shown in Table 2 as notified in 1963 were excluded for various reasons. The resistance pattern of these cases is shown in Table 8 related to their sex and age. It should be noted that three male cases are shown as primary resistance to P.A.S. and I.N.A.H., yet Table 2 shows only two male cases of resistance to P.A.S. and I.N.A.H. in 1963; this is because one of the three primary cases developed resistance to streptomycin before the end of 1963 and is therefore included in the "resistant to all three drugs" column.

The overall incidence of primary resistance was 6.6%; 3.7% resistant to one drug, 1.7% to two drugs and 1.2% to three drugs. This compares not too unfavourably with the 4.5% found in the Medical Research Council's* investigation in 1956-7, and the 3.1% recorded by the Public Health Laboratory Service in the 1960 survey; it is appreciably lower than the figure of 11.1% obtained by the British Tuberculosis Association in 1960-1, but this level has been accepted only with reservation by /

* Fox et al. (1957).

TABLE 8.

Decade born	Sex	S.	P.	I.	S + P	S + I	P + I	S+P+I	Total	TOTAL PERSONS
1890-	M	1	-	-	-	-	1	-	2	2
-99	F	0	-	-	-	-	0	-	0	
1900-	M	2	-	1	-	1	2	1	7	7
-09	F	0	-	0	-	0	0	0	0	
1910-	M	1	-	-	1	-	-	1	3	4
-19	F	1	-	-	0	-	-	0	1	
1920-	M	1	1	-	-	-	-	-	2	3
-29	F	1	0	-	-	-	-	-	1	
1930-	M	0	-	-	-	-	-	1	1	3
-39	F	2	-	-	-	-	-	0	2	
1940-	M	0	-	-	-	1	-	0	1	3
-49	F	1	-	-	-	0	-	1	2	
1950-	M	0	-	-	-	-	-	-	0	1
-59	F	1	-	-	-	-	-	-	1	
	M	5	1	1	1	2	3	3	16	23
	F	6	0	0	0	0	0	1	7	
All ages		11	1	1	1	2	3	4	23	

TABLE 9.

	1 drug.	2 drugs.	3 drugs.	TOTAL.
Primary resistance	13	6	4	23
% distribution.	56%	26%	18%	100%
Acquired resistance	82	122	145	349
% distribution	23%	35%	42%	100%
All forms of resistance	95	128	149	372
% distribution	26%	34%	40%	100%

/ by some authorities because the numbers involved were relatively small and it was specifically stated that the series did not include all newly diagnosed and previously untreated patients. In reviewing these figures some reservations must be made before accepting a percentage increase as a sinister omen, for, if the absolute numbers of new cases of pulmonary tuberculosis continue to fall each year and the absolute numbers of cases of primary resistance remain static or fall more slowly the incidence of primary resistance, expressed only as a percentage, will inevitably show an annual increase. A more accurate idea of any trend which may be developing would be obtained by expressing this figure as a rate per unit of population at risk. The marked differences in the drug patterns of cases of primary and acquired resistance are clearly shown in Table 9. The distribution by age and sex of these cases of primary resistance shows little variation from that found among the patients with acquired resistance, but when the figures for the individual drugs are examined certain important discrepancies appear (Tables 10 and 11).

It is evident that primary resistance involving streptomycin is much more frequent than that involving isoniazid, whereas, in the acquired form, isoniazid resistance is slightly more frequently seen. In both forms of resistance P.A.S. involvement is least common. Again, these findings parallel those of the British Tuberculosis Association in 1960-1.

Even /

	S.	P.	I.	S + P.	S + I.	P + I.	S+ P+ I.	TOTAL.
Primary Resistance to	11	1	1	1	2	3	4	23
% distribution	47.8	4.4	4.4	4.4	8.8	12.9	17.3	100%
Acquired resistance to	44	5	33	5	90	27	145	349
% distribution	12.2	1.4	9.2	1.4	26.5	7.4	41.9	100%
All forms	55	6	34	6	92	30	149	372

TABLE 11.

	S.	P.	I.	ALL CASES.
Primary resistance involving drug	20	9	10	23
% of total	87%	39%	44%	
Acquired resistance involving drug	283	181	292	349
% of total	82%	52%	84%	

Even more striking (Table 10) is the high incidence of primary resistance to streptomycin alone, which accounts for almost half of all the cases of primary resistance and amounts to almost four times the frequency of acquired resistance to streptomycin alone. That this is not just a chance finding (attributable to the small number of cases analysed) can be established by brief reference to Tables 12 and 13 which illustrate the same trend throughout the whole of Scotland.

TABLE 12.
(All Scotland).

	S.	P.	I.	S + P.	S + I.	P + I.	S+ P+ I.	TOTAL
Primary resistance to	25	3	7	2	4	6	9	56
% distribution	44.2	5.4	12.6	3.6	7.2	10.8	16.2	100%
Acquired resistance to	63	9	70	12	143	44	236	577
% distribution	11.7	1.6	13.3	2.5	21.9	8.1	40.9	100%
All forms	88	12	77	14	147	50	245	633

TABLE 13.
(ALL SCOTLAND).

Cont'd. /

TABLE 13.
(All Scotland).

	S.	P.	I.	All cases.
Primary resistance involving drug	40	20	26	56
% of total	72%	36%	46%	
Acquired resistance involving drug	454	301	493	577
% of total	78%	52%	85%	

Comparison of the infecting organism's sensitivity pattern to numerous antibiotics has been found useful in following outbreaks of staphylococcal sepsis, especially in the absence of 'phage typing facilities, and it is unfortunate that the above findings indicate that the same simple technique cannot be directly applied to the epidemiology of tuberculosis. Nevertheless, Fox et al (1957), who could only demonstrate a link with a drug-resistant patient or chemotherapy in about half their cases of primary resistance, felt that most, if not all, infections with drug-resistant organisms were due to contact with patients who were excreting such strains. If their theory is correct an explanation must be sought for the differences between the drug patterns of primary and acquired resistance.

It seems most unlikely that the development of any form of drug-resistance should so enhance the virulence of any mycobacterial strain as to make it predominant. More probable is the theory that to-day's pattern of primary resistance reflects the spread of resistant organisms in the community some years ago. Thus, the present high level /

/level of primary resistance to streptomycin alone would be attributed to the spread of streptomycin-resistant strains in the early days of chemotherapy when this drug was the only effective anti-tuberculosis agent available; it is assumed that infections by such strains lay dormant for a variable number of years before manifesting themselves openly. This is not inconsistent with the natural history of the disease; in compiling this Register it was noted that many of the older patients being notified for the first time had fully sensitive strains, and it may well be that a considerable proportion of these were infected in the days before chemotherapy was practised, the infection remaining latent for many years. If this is the true explanation it follows that the pattern of primary resistance in future years will be determined by the forms of drug resistance most prevalent today; in other words, an increase in the incidence of primary resistance to multiple drugs can be anticipated. This possibility should be looked on with some apprehension and should act as a further stimulus to contain the disease effectively and quickly.

CHAPTER VII.

The Anonymous Mycobacteria.

In any investigation of drug-resistant tuberculosis the role of the anonymous mycobacteria should be considered as these organisms are often resistant to antimicrobial drugs. Whether patients producing such organisms should be included in a tuberculosis register is rather a perplexing question, for as yet no absolute agreement has been reached on the nomenclature or clinical significance of these organisms. An accepted definition, quoted by Foreman (1962) is that they are acid-fast bacilli distinct from tubercle bacilli, isolated repeatedly from human morbid material, after due precautions against contamination.

Kalaberder (1961) suggests that the whole question of the status of unclassified mycobacteria would be no more than a laboratory curiosity were it not for the tendency to place them all in a well-defined group independent of other mycobacteria, assuming the lesions in man to be non-tuberculous and therefore of no epidemiological importance. Over-optimism about anti-tuberculosis progress has resulted in the suggestion of the term "mycobacterioses" to cover those diseases whose lesions are substantially identical with those of tuberculosis but whose causative organisms give a poor response to therapy. He stresses the great plasticity and adaptability of mycobacteria, and deduces from the many experiments which he cites that all mycobacteria are capable of /

/ of producing tuberculosis, or a similar disease, in susceptible animals; and that differences observed between strains are simply differences in pathogenic response in a given host, varying between species or between individuals in a species.

Conflicting views are held by Runyon (1959, 1961) who believes that the unclassified mycobacteria represent a number of distinct and essentially stable species, whatever their origin. Tubercle bacilli mutations do not include changes to such strains as are now designated "unclassified". He points out that photochromogens have not appeared as variants during countless laboratory experiments on M. tuberculosis strains, and that only rarely have photochromogens been known to co-exist with or follow infection with true tubercle bacilli. He maintains that these organisms are not tubercle bacilli and that diseases resulting from them should not be designated "tuberculosis". This is probably the most important practical consideration at the present state of our knowledge, for if Kalsbeeder's hypothesis is correct the patient suffering from "anonymous" infection should be treated in a tuberculosis sanatorium as a difficult chemotherapeutic problem, whereas Runyon's theory demands that the patient should be diagnosed and treated as a specific disease entity other than tuberculosis, and should be treated separately from Koch infections. His argument is supported by a number of instances in which cases of "anonymous" infection have been nursed in a tuberculosis ward and have then contracted frank pulmonary tuberculosis. Slender experimental evidence for this view is provided by the work of /

/ of Klugh and Pratt (1962) who showed that immunisation of guinea-pigs with photochromogen strains afforded some protection against tuberculosis, but not as much as B.C.G.; they argued that patients with photochromogen infection should not be placed in contact with patients suffering from virulent tuberculosis.

The answers to these various problems are unlikely to be forthcoming until many more cases have been thoroughly investigated and described in different geographical areas. Although still comparatively rare it has been suggested that they may become commoner as true tuberculosis declines, (Foreman, 1962) and Runyon (1959) has indicated that white men past middle-age are especially prone to such infections - the very group about whom concern with regard to tuberculous infection is being expressed in this country. Because of its obvious clinical importance M. tuberculosis has been singled out for the fullest investigation, and only in the last 10 years has interest in other mycobacterial illnesses arisen. Like many another medical problem it is difficult to decide whether the increase in "anonymous" infections is real or apparent - in other words, whether an absolute increase in numbers has occurred or whether a greater awareness of and consequent improvements in diagnostic methods for these infections have uncovered examples which previously were missed. One wonders whether the adoption of not only the screening procedures for cultures (to be described later) but also alterations in the classical techniques for the decontamination of specimens would result in more frequent isolation of these /

/ these mycobacteria; Hedgecock and Faucher (1961) found a fourfold increase in the numbers of unclassified mycobacteria recovered when 0.2% malachite green replaced 2% NaOH during concentration. Schiff and Farshis (1950) suggested that anonymous mycobacteria have been encountered more frequently since the introduction of streptomycin, but Clark and Brisebois (1962), failed to produce unclassified strains by serial transfer of M. tuberculosis cultures on media containing streptomycin. Kenat et al. (1961) support Christianson and Dewlett's (1960) view that antituberculosis therapy is unlikely to cause this type of infection in an individual.

Marks and Trollope (1960) investigated the incidence of anonymous mycobacteria in England and Wales; of 200,000 sputum specimens cultured 16% were positive for M. tuberculosis, and on further investigation, 2% of these strains proved to be anonymous. The most recent survey for England and Wales, reported by the Public Health Laboratory Service (1962) revealed an incidence of 1.4% of clinically significant anonymous strains and 1.1% of non-significant strains. These low figures must be related to the finding that patients producing anonymous mycobacteria seem to be much less infectious than those with M. tuberculosis, and reports of transmission within families are rare. Single instances of husband-to-wife infection have been recorded by Beck et al. (1963) and by Hosty (1959). Crow et al. (1957), in a study of 69 patients with pulmonary lesions associated with atypical /

/atypical acid-fast bacilli, found that positive tuberculin tests and X-rays of contacts were much less frequent than in contacts of true M. tuberculosis infections. Chapman et al. (1962) felt that evidence was not conclusive in regard to person-to-person transmission and that the skin reactor rate was lower than would have been expected if anonymous infection were spread in the same manner as tuberculosis.

Other workers have considered the results of skin reactions in the apparently healthy, in typical tuberculosis and in anonymous infections. Griffith et al. (1963) concluded from a recent important survey of schoolchildren in Cardiff that a good deal of low grade sensitivity to tuberculin in children who had not had B.C.G. appeared to be due to sensitisation by bacilli of the Battey-avian group. Evidence that tuberculin sensitivity could be derived from sources other than human or bovine tuberculous infections was also obtained during an investigation conducted by the British Tuberculosis Association (1963) in six very different localities in England and Wales. Generally, there was less sensitivity to human than to avian tuberculin: the overall figures were 18.6% and 23.9% respectively (of 6,343 unvaccinated children examined). Results obtained by Mellman and Barness (1962) in Philadelphia showed that cross-sensitisation to the antigens employed may be an important cause of apparent tuberculin sensitivity. The practical implications of such findings are obvious for investigations of tuberculous infections in a community, or when considering chemoprophylaxis for a child who has no other manifestations of a tuberculous infection. Kendig (1961) /

/ (1961) investigated 105 children diagnosed as asymptomatic primary tuberculosis, all with a positive Mantoux test. M. tuberculosis was recovered from 8 patients, and 5 photochromogens and 4 scotochromogens were cultured from other cases. He concluded that although such anonymous infections were widespread in Virginia resultant disease was probably rare, and he accepted the possibility of obtaining a positive tuberculin reaction in these cases. In later work (1962) he stated that although tuberculosis was the major cause of tuberculin sensitivity in Virginia, infection with one of the other mycobacteria might also account for a positive skin test with a diameter of less than 10 mms; and in a series of 2,000 children examined by the same author (1963) he estimated that a false positive Mantoux test was obtained in as many as 25% of the reactors. In Houston, Texas, Hsu (1962) found that infections with anonymous mycobacteria (especially Battey strains) were even more prevalent than with M. tuberculosis, and further studies (1962) convinced her that most of the low-grade sensitivity (discrete papular reaction to the Heaf test) did not represent true tuberculous infection.

In Queensland, Australia, Singer and Rodda (1961) obtained weak reactions to Old Tuberculin in 2% of the children living in the southern part of the state and in 80% of those in the north; the discrepancy between these figures and the known incidence of tuberculosis prompted the re-examination of the whole role of acid-fast bacilli in the area and it was discovered that mycobacteria other than M. tuberculosis occurred commonly in Queensland. These local findings /

/findings were broadly confirmed by Abrahams and Silverstone (1961) who stressed that a single test with low doses of tuberculin was inadequate for the detection of tuberculous infection and was unsatisfactory as a screening test before radiography surveys. In their investigations reactions to PPD-F (M. fortuitum) and PPD-Y (yellow bacillus) were regarded in vaccinated children as cross-reactions to B.C.G.

The origin of these subclinical infections remains unknown. Direct human transmission has been shown to be rare, and an alternative theory is that food, milk and water contaminated by infected soil may be the source of Battey organisms; Seamon et al. (1963) argue that, since the Battey bacillus and M. avium appear to be related, and since the latter can survive high temperatures and survive for long periods in soil, any animal which harbours M. avium (or allied species) may be a hazard to public health. Evidence that human contact with M. avium is more widespread than was previously believed is provided by the prevalence of skin sensitivity to avian derivatives. These reports prompted a search of the current veterinary literature for descriptions of similar infections in animals but only two brief reports of possible relevance were found.

Lesions histologically resembling tuberculous granulomata were described in the skin of cats by Brown et al. (1962) and Wilkinson (1964). Although acid-fast bacilli morphologically resembling M. tuberculosis were seen by these authors in no case were attempts to isolate the organism by culture or guinea-pig inoculation successful. Few technical details are given, and in particular it is not stated whether a range of/

/of incubation temperatures was employed. Wilkinson's account is of particular interest in that two cats, successive pets in the same household, developed identical lesions, suggesting a common source of infection possibly within the house itself. Whether these represent anonymous infections or are more akin to lepromatous lesions cannot be decided on the available evidence.

There can now be no doubt that diseases akin to tuberculosis are caused by organisms other than M. tuberculosis but it has been a fascinating study to see how the first sporadic reports containing tentative theories of cause-and-effect relationship between organism and disease have gradually given way to reviews more numerous and more confident to supply overwhelming clinical evidence establishing the theory. Corper (1918) noted that of 88 strains of M. tuberculosis tested two did not produce progressive disease in guinea-pigs. Twenty years later Branch (1938) described a pathogenic acid-fast bacillus which was definitely not of the human, bovine or avian varieties. Pinner (1935) regarded the majority of 15 strains of "atypical chromogen mycobacteria" as urinary saprophytes but could not completely deny the pathogenicity of a few. Feldman et al. (1943) recorded 20 positive results from 90 cultures from a miner observed over 9 years: in such a chronic case they postulated "penetration" by saprophytes but as no other similar case had been described they felt the time not ripe for a decision on pathogenicity. Buhler and Pollak (1953) isolated a /

/ a "yellow bacillus" from autopsy material from a 21 year old male whose illness resembled miliary tuberculosis, and also from resected lung tissue from a man of 47 years who had had an haemoptysis; surgery resulted in improvement in his condition. In both cases the authors held that the evidence was conclusive for regarding the organism as the sole pathogenic agent. Young (1955) cultured a "yellow bacillus" from the sputum of a 31 year old female who had had an haemoptysis, and an identical organism (alone) was recovered, after right upper lobectomy, from the surgical specimen - again interpreted as satisfactory proof of pathogenicity.

More recent reports of pulmonary disease are those of Nassau and Hamilton (1957) in England, Kanat et al. (1961) in Wales, Engback and Magnusson (1961) in Scandinavia, and Crow et al. (1957), Lewis et al. (1960) and Christianson and Dewlett (1960) in the United States. Similar lesions have been described in Puerto Rican children by Dialkin et al. (1961). Extra-pulmonary disease is now well-documented; cervical adenitis resulting from anonymous mycobacterial infection has been reported by Prissick and Masson (1956) in Canada, by Chapman and Guy (1959) and by Davis and Comstock (1961) in the U.S.A. and by Marsden and Hyde (1962) in England. Generalised non-tuberculous mycobacterioses have been described by McCusker and Green (1962), cutaneous manifestations by Brock et al. (1960) and by Knox et al. (1961) and a fatal case of disseminated osteomyelitis by Yakovac et al. (1961); Weed (1956) reported successful surgery for a case of recurring migratory chronic osteomyelitis associated with what he termed saprophytic acid fast bacilli /

/bacilli but which probably were clinically significant chromogenic strains. The only case of meningitis of this nature yet described seems to be that by Chen and Pathmanathan (1961); the illness proved fatal to the 9-month old infant.

Laboratory confirmation of pathogenicity remains a problem. In many of these studies experimental inoculation of guinea-pigs did not result in progressive disease, although the organism often could be recovered from limited lesions at the injection site and from regional lymph glands. Brown and Clark (1962) investigated the effects of 78 scotochromogen strains by inoculation of the cisterna magna of guinea pigs and produced various degrees of chronic inflammatory change in the meninges. These changes were similar to those caused by tubercle bacilli of low virulence but were regarded as sufficiently distinct from those of true, virulent tubercle bacilli as to be of some value in differentiation. Reikes and Washington (1962) confirmed the relative avirulence of three photochromogen, one scotochromogen and two Battey strains for guinea-pigs, but were able to demonstrate small, non-caseous, epithelioid, granulomas in the tissues of Syrian golden hamsters and Swiss-Webster white mice. Engbaek (1961) produced lesions in the joints or tendon sheaths of rabbits injected intravenously with photochromogen and Battey strains; lesions also developed in mice, and these animals seemed to be the most susceptible to scotochromogen infection.

Thus, the choice of experimental animal is important in determining the "virulence" of these anonymous strains, and there would /

/would appear to be ample evidence for abandoning the tenet that a strain avirulent for the guinea pig alone must also be avirulent for man.

Much effort has gone into devising other methods of classifying these mycobacteria. If satisfactory chemical tests were available they could be employed by laboratories which do not have facilities for animal work, but the large number of methods which have been suggested is in itself evidence that none is entirely satisfactory. Furthermore, many of these tests are aimed only at separating M. tuberculosis from other mycobacteria, or the human variety from the bovine and avian, and do not offer a guide to the further classification of unusual strains. With these limitations in mind there may yet be a place for one or more of these procedures in a preliminary "screening" of cultures. Probably one of the best is the test for niacin production (Konno, 1958; and later modifications). Binding of basic dyes (Nile blue, methylene blue, neutral red, methyl violet) has been employed by Desbordes et al. (1955) and Dubos and Middlebrook (1948). The detection of enzyme activity has also been used as a guide - aryisulphatase (Whitehead et al, 1953), urease (Singer and Cysner, 1952), acylamidase (Bonicke, 1960), penicillinase (Bonicke, 1957), catalase (Knox et al., 1956) and peroxidase (Stief et al., 1958). A relatively simple test is that more recently described by Tsukamura (1962) in which sodium salicylate is incorporated in egg medium at a critical concentration of 500 mcg/ml. to separate strains of M. tuberculosis from other myco-bacteria.

Quite/

/ Quite a different line of study was that adopted by Beck (1961). He noted that antisera prepared against atypical photochromogenic mycobacteria, M. tuberculosis (H 37 Rv), M. phlei and M. smegmatis gave marked cross-reactions with their respective purified protein derivatives - a finding which is not at all surprising in view of the results of skin-sensitivity tests previously described. Beck went on to use a more refined technique (absorption analysis) to distinguish the photochromogenic strains from the others, and it may well be that further technical improvements in the preparation of antisera may make yet more specific classification possible. Serological changes in patients with anonymous infections have been little studied; the electrophoretic patterns of sera from patients with sarcoidosis, anonymous infections and tuberculosis were compared by McCuiston and Hudgins (1960) who found a resemblance between the patterns in the first two conditions but a dissimilarity between those of tuberculosis and anonymous infections.

Opportunities of studying the histological appearances of human lesions produced by the anonymous mycobacteria have been rare but a few surgical and autopsy specimens have been described. Little further information seems likely to be derived from these sources, however, if the experience of Corpe and Stergus (1963) is borne out elsewhere; in a well-devised scheme the opinions of 27 experienced histopathologists were sought on duplicate sets of slides, and less than 10% of the diagnoses correctly selected the anonymous lesions, with a /

/ a similar percentage of success in picking out the truly tuberculous.

From the foregoing review it is obvious that much further work still needs to be done before the inter-relationships of these organisms is understood. An essential part of that work is the detection in routine laboratories of anonymous mycobacteria, so that a full clinical assessment of the patient producing them can be made and the strain referred for fuller investigation. The scheme suggested by Marks and Trollope (1960 b) demands little additional equipment or technical skill of any laboratory already undertaking tuberculosis culture procedures. Their method can be summarised as follows:

1) In the routine examination of Ziehl-Neelsen smears made from mycobacterial cultures note the colour, texture and consistency of the colonies as the film is made; and the depth of staining, bacillary length, degree of dispersion and "cording".

2) Subculture on Lowenstein-Jensen medium in continuous light at 37°C.

3) Subculture on L-J. medium containing 10/ug./ml. thiosemicarbazone.

4.) Subculture on L-J. medium at 25°C.

5) Expose the original culture to light at 37°C for two days.

When the results of these tests are collated it is found that anonymous mycobacteria show two or more of the following features:

(a) Distinctive morphology

(b) Chromogenicity

(c) Growth at 25°C.

(d) Resistant to thiosemicarbazone.

An/

/ An important contribution by Marks and Richards (1962) is a tentative scheme of classification which will include all the pathogenic strains likely to be isolated in the British Isles, based mainly on the methods outlined above but also taking cognisance of temperature requirements, rates of growth, catalase activity, growth in a semi-solid medium and drug sensitivity patterns. The author, while recognising the importance of this idea, believes that the suggested classification includes too many subjective variables to be accurate and introduces involved terminology unlikely to be adopted by clinicians. Although criticised both by Marks and Richards (1962) and by Collins (1962) the terminology originally used by Runyon (1959) has still been favoured by the author, who feels that it should not be abandoned until the anonymous strains can confidently be arranged as named species.

Some progress along these lines has already been made. The photochromogens can be classed as Runyon Group I, and they have also been designated M. kansasii. Many scotochromogens (Group II) are thought to be non-pathogenic, but an important sub-group recognised as a cause of cervical adenitis in children has been named M. scrofulaceum. Non-photochromogens (Group III) include such species as M. avium and M. intracellularis as well as the Battey bacillus. Rapid-growing strains (Group IV) comprise M. smegmatis, M. phlei, M. rhodochrous, M. fortuitum and a number of heterogeneous strains the majority of which are non-pathogenic.

Despite /

/ Despite the suggestion that atypical mycobacteria did not occur in Scotland (Mitchison, 1962) the increasing number of descriptions of these organisms from many parts of the world indicated that a search for them might not in fact be unrewarding; furthermore, it was known that most of these mycobacteria showed varying degrees of drug resistance, and it was imperative that they should be recognised among the other drug-resistant strains which were to be submitted to the Reference Laboratory. Accordingly, every culture suitable for sensitivity testing was also subjected to Marks and Trollope's screening procedure already outlined. Where indicated, certain further investigations were performed - additional temperature range, catalase activity, carbohydrate fermentation, niacin production, animal pathogenicity - and full clinical details were obtained to permit a complete review of the patient's illness.

During 1963, nine hundred positive sputum cultures from Glasgow patients were screened and 6 were found to be atypical strains. In addition, one atypical strain was found among the 95 non-pulmonary cultures examined.. This represents an overall incidence of 0.7%. Five of the six pulmonary isolates were regarded as of pathological significance (0.5% of all pulmonary cultures). The remaining pulmonary and the non-pulmonary strains were thought to be casual isolates. Although excluded by definition from further description in this survey it is interesting to note that the incidence of significant sputum cultures of atypical mycobacteria in the remainder of the West of /

/ of Scotland was 0.46% (3 out of 643).

The results obtained with each of the strains obtained from Glasgow patients are shown in Tables 14 and 15. Certain features are worthy of further comment. Microscopic examination of smears of these cultures was only of limited value, for even in retrospect the morphology of none of the strains was sufficiently abnormal to have allowed a privisional diagnosis of "atypical strain". All the photochromogen strains had identical drug-sensitivity patterns, being sensitive to the secondary drugs; in vivo, they tended to produce cavitational rather than fibro-caseous lesions, with a correspondingly high frequency of haemoptysis. The non-photochromogen strains were resistant to thiosemicarbazone, but the use of this drug in the screening test has certain qualifications. Eight further cultures showed resistance to thiosemicarbazone on repeated testing but revealed no other atypical features and produced progressive and fatal disease when injected into guinea pigs; enquiry into the histories of the patients who had produced these organisms showed that in the past all had had or could have had this drug as part of their chemotherapy. In these circumstances it was concluded that thiosemicarbazone resistance was an acquired feature.

Animal experiments.

Three guinea-pigs were inoculated with each strain (2 mgms. moist weight intreperitoneally). Two of the three photochromogens failed to / produce progressive disease in any of the animals which were sacrificed /

TABLE. 14.

Reg.No. Sex Age	Morphology	Pigment in artificial light.	Incubation temps. + = growth - = no growth				Thiosemi-carbazone	DRUGS R=Resistant S=Sensitive						CATALASE	NIACIN.	Classification.	CLINICAL ASSESSMENT.
			25	37	45	52		Streptomycin	P.A.S.	I.N.A.H.	Viomycin	Cyclo.	Ethion.				
1054 F. 56 yrs.	Long, slender, prominent beading. Easily emulsified.	Bright yellow	±	+	-	-	S	R	R	R	S	S	S	++	-	PHOTOCHROMOGEN GROUP I.	Haemoptysis, lung cavitati Identical organism isolat on several occasions. Undoubtedly pathogenic.
1400 F. 48 yrs.	Medium length Easily emulsified.	Bright yellow	+	+	-	-	S	R	R	R	S	S	S	++	-		Cavitational lesions and haemoptysis. Undoubtedly pathogenic.
1461 M. 51 yrs.	Long, slender, prominent beading. Easily emulsified.	Bright orange-yellow.	+	+	-	-	S	R	R	R	S	S	S	++	-		Cavitational lesions, improved by secondary dr Undoubtedly pathogenic.
455 M. 25 yrs.	Short, stubby. Moderate beading. Difficult to emulsify.	None.	+	+	-	-	R	R	R	R	R	S	R	-	-	NON - PHOTOCHROMOGEN GROUP III	Long-standing lung disea Now on secondary drugs. Undoubtedly pathogenic.
517 M. 48 yrs.	Short. Beading not prominent. Easily emulsified.	None.	+	+	-	-	R	S	R	R	S	S	S	++	-		Clinically identical wit P.T.B. Improved by secondary drugs. Undoubtedly pathogenic.
1033 F. 54 yrs.	Short, cocco-bacilli. Difficult to emulsify.	None.	+	+	-	-	R	R	R	R	R	R	S	++	-		Chronic bronchitic, but serial X-rays NOT suggestive of tuberculos Probably casual isolate.
520 M. 26 yrs.	Medium length, slightly curved.	None.	+	+	-	-	R	S	R	R	S	R	S	++	-	RAPID GROWER Grp. IV.	Casual isolate from urin Presented with albuminur haematuria, sterile pyur Improved without therapy.

TABLE 15

+ = fermentation.
- = no fermentation
W = doubtful reaction.

Sugar	Resistant M. Tuberculosis.	Sensitive M. tuberculosis.	Photochromogen 1054	Photochromogen 1400	Photochromogen 1461	Non-photochromogen 455	Non-photochromogen 517	Non-photochromogen 1033	Rapid grower 520
Adonitol	-	-	-	-	+	-	-	-	-
Arabinose	W	W	-	-	-	+	+	+	+
Fructose	+	+	+	-	+	+	+	+	+
Galactose	-	+	-	-	-	+	+	W	-
Glucose	+	+	W	-	-	+	+	-	W
Glycerol	+	+	+	+	+	W	+	+	+
Inositol	-	-	-	-	-	W	W	W	-
Mannose	-	+	-	-	+	+	W	-	-
Raffinose	-	-	-	-	-	+	+	-	W
Rhamnose	-	-	-	-	-	+	W	W	-
Ribose	+	+	+	+	+	+	+	+	+
Sorbitol	-	+	-	-	-	W	-	-	-
Sucrose	-	+	-	-	+	W	+	+	-
Trehalose	W	+	+	W	+	W	+	+	+
Xylose	W	-	W	W	-	+	+	+	-

/sacrificed at 8, 12 and 16 weeks after inoculation, but the organism could be recovered from glands at the injection site. The third strain produced enlargment of omental glands as well as regional glands in one of the three guinea-pigs, but the disease was not disseminated to the abdominal viscera.

None of the other strains produced lesions in guinea-pigs.

Two mice were also inoculated with each strain (2 mgms. moist weight intravenously). The spleens, livers, lungs and kidneys were examined both macroscopically and histologically when the animals were sacrificed at 6 and 12 weeks after injection, but no granulomatous lesions were found.

Carbohydrate fermentation (Table 15).

The fermentative properties of eachanonymous strain and of drug-sensitive and drug-resistant strains of M. tuberculosis were investigated by the method of Sweeney and Jann (1961). No clear-cut pattern emerged. Nevertheless, there was broad agreement with their findings that the photochromogen strains were less active biochemically then either M. tuberculosis or non-photochromogen strains, that few strains fermented adonitol, inositol, rhamnose and sorbitol, and that few strains failed to ferment ~~fe~~ctose and glycerol. Two possible explanations of such variable results arise; the method itself may be technically unsatisfactory, giving inconstant readings, or there may be genuine differences between strains which appear in preliminary investigations to be identical. Support for the second alternative can be found in the work of Laidlaw and Norris (1964, personal communication) who have used /

/used electrophoresis to demonstrate significant differences in the isoenzyme patterns of strains which otherwise closely resembled each other. Thus it may be that species variation among the anonymous mycobacteria is just as great as among, say, salmonellae.

In conclusion, it can be stated that there is now definite evidence of the occurrence of anonymous mycobacteria in Scotland. The incidence at present is lower than in England and Wales, and perhaps the most surprising feature in this connection is that the mining areas of the Scottish lowlands and East coast do not resemble those of Wales where photochromogen infection is a relatively common sequel to pneumoconiosis. It may be that an answer to this question, and to many of the others which still are pertinent to this subject, will be found when greater attention is paid to these organisms in all bacteriology laboratories so that a comprehensive account of their distribution and behaviour can be made at national level.

CHAPTER VIII.

An investigation of strains showing high levels of resistance to isoniazid.

Among the first investigators to note the inverse relationship between the virulence for the guinea pig and the degree of resistance to isoniazid of strains of M. tuberculosis were Middlebrook and Cohn (1953) in the United States and Barry et al. (1953) and Barnett et al. (1953) in Great Britain. Occasional exceptions to this rule, such as those found in the first series of experiments carried out by Feizer, Minkin and Widelock (1954), were later attributed to the use of mixed isoniazid sensitive and isoniazid resistant populations rather than pure isoniazid resistant clones for the infection of animals. (Feizer, Widelock and Klein (1954)). Morse et al. (1954) investigated this point in detail and satisfied themselves that conflicting reports might be explained by such inconsistencies in laboratory practice. In the same year Cohn et al. showed that the catalase-positive isoniazid-resistant mutant was much more pathogenic for the guinea-pig than its catalase-negative counterpart; failure to differentiate these two might also have been a source of confusion in earlier investigations. The carefully controlled work of Van Dijk (1957), however, suggested that some anomalous results could not be completely explained by variations in experimental conditions; among 44 catalase-negative strains resistant to 1/ug./ml. isoniazid he found three which were fully virulent for guinea pigs, and conversely, in /

/ in another series, he noted three catalase positive strains sensitive to isoniazid which showed decreased virulence for guinea pigs. Oscarsson (1961) also described variations from the expected results in experiments with isoniazid resistant strains, in enzyme activity, drug susceptibility pattern and degree of virulence in guinea pigs.

Nevertheless, the idea that isoniazid resistant strains had reduced virulence (or were "attenuated") for guinea pigs gained general acceptance in the 1950s and soon the theory's therapeutic implications for man came under investigation.

Oestreicher et al. (1955) concluded that isoniazid was an unique chemotherapeutic agent - it eliminated the drug - susceptible components and the host was able to starve the drug-resistant mutants. They felt that if multiplication of tubercle bacilli in vivo could not be avoided at the very least they should be made highly resistant to the direct antibacterial effects of isoniazid. Opposing points of view have been expressed by Gernez-Rieux et al. (1955) who did not anticipate a favourable result in infections with attenuated organisms, and by Feizer et al. (1960) who could find no correlation between the course of the disease and the virulence, catalase activity, and isoniazid susceptibility of the infecting bacilli. A more cautious approach was that of Mulder (1957) who felt that a relationship was probable between bacillary virulence and the clinical course of the disease; he noted that the majority of patients who were improved by chemotherapy /

/ chemotherapy originally had fully virulent bacilli in their sputa, whereas those producing attenuated bacilli seemed only to achieve equilibrium with their infecting organism - cavities and positive sputa persisted and there occurred neither significant clinical improvement nor deterioration.

It is necessary to reconsider the significance of all this work in view of the enthusiastic use of isoniazid alone as a prophylactic measure in some highly developed countries, such as the United States, (Ferebee and Mount (1962), Katz et al. (1962)), and as a therapeutic agent in many of the underdeveloped nations, where its cheapness and ease of administration make it suitable for large-scale administration with relatively little medical supervision. It seems that the well-proved principles of combined chemotherapy aimed at preventing the development of drug-resistance are being sacrificed for two highly controversial reasons; first, that medication with isoniazid alone is better than no antituberculosis treatment at all, and second, that the inevitable production of isoniazid resistance in a proportion of these patients' organisms is of less significance than other forms of resistance, and is a price worth paying for the cure of a proportion of patients. The problems of chemotherapy which face clinicians working in countries with limited supplies of drugs have been well marshalled and fully examined by Fox (1964); he himself (1962) concluded from extensive field studies in Madras that persistent infection with isoniazid resistant (even if catalase negative) strains was dangerous for the /

/ the patient, and that once established, the disease in man is not attenuated and self-limiting as in the guinea pig. Canetti (1962) also has sounded a warning about the danger of INAH resistance arising on a large scale in developing countries, and it was interesting, in discussing this problem with physicians in Glasgow, to find that development of isoniazid resistance was the complication of chemotherapy which worried them most of all.

Further doubt has been cast on the applicability of guinea-pig results to man by the work of Mulder-de Jong (1957) who has shown that the virulence of isoniazid-resistant strains for the hamster is greater than that for guinea pigs, and it can be argued that the virulence of the same mycobacterial strain may be totally different for various susceptible mammals.

Finally, it has been demonstrated by various authors (see Chapter VII) that several species of anonymous mycobacteria, while avirulent or of only limited virulence for the guinea pig, are undoubtedly pathogenic for man.

It is concluded that the ultimate measure of virulence of any isoniazid-resistant strain must be the nature and behaviour of the lesion it produces in each particular host; the laboratory worker can recognise and classify the particular strains by the criteria mentioned above but it must be in conjunction with the clinician that the /

/ the question of pathogenicity is decided. Accordingly, this review of infections in Glasgow caused by strains of mycobacteria resistant to 50/ug./ml. isoniazid contains brief clinical summaries in addition to the results of conventional laboratory investigations (Table 16). The 50/ug./ml. concentration was chosen because this level had been regarded as critical in some of the earlier M.R.C. projects in which this laboratory had participated, and attention was focussed on strains resistant to this concentration of isoniazid alone; numerous other strains highly resistant to isoniazid were found which were also resistant to streptomycin and/or P.A.S., but these were excluded to keep variable factors to a minimum. Experimental conditions also were standardised. To ensure a homogeneously resistant population the inoculum (equivalent to 2 mgms. moist weight) was taken from the slope containing 50/ug./ml. isoniazid and suspended by shaking with glass beads in 0.4 ml. sterile distilled water. The injection was made intraperitoneally above the right groin of guinea pigs, weighing between 400 - 600 grammes. The animals were caged separately and were fed a standard antibiotic-free diet. No animal was sacrificed until at least 8 weeks after inoculation. At autopsy, particular attention was paid to the glands at the inoculation site and in the omentum, to the spleen and to the liver. These viscera were examined histologically, and where the infecting organism could be recovered the sensitivity test was repeated (i.e. after animal passage). In only one of the twelve cases where this was done was there an alteration in the resistance pattern. The exception (942) was of /

Follow p. 98.

TABLE 16.

Reg.No. Sex. Age	Years since first notified	Catalase activity.	ANIMAL: Days after Injection. S=sacrificed D=died of disease.	RESULTS: Autopsy findings.	CLINICAL NOTES.
9 M. 76 yrs	7	-	S 90	No gross lesion, but visceral histology positive.	Extensive X-ray changes in both lungs for 7 years. No clinical doubt of pathogenicity.
57 M. 69 yrs	1	-	S 90	No gross lesion, visceral histology negative.	Undoubtedly virulent; patient deteriorating despite other chemotherapy.
133 M 54 yrs	27	-	S 210	No gross lesion, visceral histology negative.	Bronchitic. Recurrent empyema requiring drainage, never sterilised by drugs.
144 M. 57 yrs	12	-	S 84	Enlarged gland at inoc. site, visceral histology negative.	Clinically typical pulmonary tuberculosis. Improved by chemotherapy.
219 F 41 yrs	4	-	S 170	Enlarged gland at inoc.site, visceral histology negative.	Progressive pulmonary disease, patient still on chemotherapy. Undoubtedly virulent.
412 M 54 yrs	4	-	D 41	Extensive tuberculous lesions.	Developed INAH resistance 1961; more extensive disease on readmission 1963. Various chemotherapy regimes without much improvement.
631 M 72 yrs	1	-	S 170	No gross lesion, visceral histology negative.	Lung lesion suspected in 1941. Intermittent positive sputum; lesion static despite all forms of chemotherapy. Clinically, virulent organism.
634 M 27 yrs	10	-	D 44	Enlarged gland at inoc.site. Visceral histology positive.	Typical progressive tuberculous process. Still on chemotherapy.

675 M 62 yrs	6	-	D	56	Discrete glands at inoc.site, omentum. Liver histology positive.	Irregular attender and drug-taker. Readmitted recently with haemoptysis: progressive disease on X-ray
739 F 48 yrs	6	-	S	168	Caseous gland at inoc.site, visceral histology negative.	Chronic case slowly but steadily deteriorating. Undoubtedly virulent.
745 M 61 yrs	2	-	S	154	Caseous gland at inoc.site, visceral histology negative.	Static lesion on X-ray, intermittent positive sputum; clinically accepted as tuberculosis.
942 M 56 yrs	4	-	D	101	Extensive tuberculous lesions.	Obviously progressive disease despite previous thoracoplasty. Now on secondary drugs.
1074 M 46 yrs	1	-	S	84	Caseous gland at inoc.site, visceral histology negative.	Extensive disease when first diagnosed, sputum now converted to negative. Still requires chemotherapy.
1077 F 36 yrs	12	+	D	51	Extensive tuberculous lesions.	Chronic tuberculous process, lung fibrosis and pleural thickening. Still on chemotherapy.
1186 M 64 yrs	2	+ -	S	140	Caseous gland at inoc.site. Spleen histology equivocal	Mild fibrosis of lungs. Intermittent positive sputum. On chemotherapy.
1323 M 46 yrs	2	-	S	98	No gross lesion, visceral histology positive.	Typical tuberculous appearances on X-ray. Still on chemotherapy.
1324 M 76 yrs	8	-	S	126	Caseous gland at inoc.site, visceral histology negative.	Progress characteristic of chronic tuberculosis, extensive involvement of both lungs.
1494 F 35 yrs	14	-	S	84	Caseous gland at inoc.site, visceral histology positive.	Typical tuberculous lesions aggravated by recent pregnancy during which P.A.S. was discontinued.
1650 F 33 yrs	5	-	D	60	Enlarged gland at inoc.site, visceral histology positive.	Characteristic tuberculous changes, developed INAH resistance in 1962. Marked extension of disease at most recent examination.

/ of especial interest as it was one of the three strains which caused extensive lesions in the guinea-pig

	1 /ug./ml.	I.N.A.H.	50 /ug./ml.
Before animal passage	RESISTANT		RESISTANT
After animal passage	RESISTANT		SENSITIVE

A similar increase in isoniazid susceptibility during animal passage was reported by Feizer et al (1960) in 13 out of 46 strains tested. It may be that this phenomenon can be explained by a certain instability of newly-acquired resistence to isoniazid, for Barnett et al. (1953) noted that isoniazid-resistant variants of strain H37 Rv rapidly reverted to susceptibility if they had been in contact with the drug during only one subculture, and they also reported a substantial fall in the resistance levels of 10 out of 16 strains after three subcultures in the absence of isoniazid.

Another strain (1077) which caused extensive lesions in the guinea-pig was catalase positive, a finding which is in agreement with the contention of Cohn et al. (1954) to which reference has already been made.

The third strain (412) to cause extensive disease must be regarded as exceptional rather than unique if the experience of Van Dijk (1957) is remembered. Technical errors can be ruled out, for none of these strains was accepted as truly resistant unless there was either confluent growth or innumerable discrete colonies on the slope containing 50/ug./ml. isoniazid; borderline cases were excluded. The levels at /

/ at which isoniazid would inhibit these strains were determined:-

5 strains were resistant to 50/ug./ml. but sensitive to 62.5/ug./ml.
3 " " " " 62.5 " " " " " 125 " "
11 " " " " 125 " " " " " 250 " "

None was resistant to concentrations greater than 250/ug./ml. All the cultures were niacin positive.

Returning to the animal autopsy findings in Table 16 it is evident that they are similar to those of earlier workers, and that once again certain inconsistencies appear. Nevertheless, the results can be broadly classified in five main groups, corresponding to different degrees of tissue involvement.

Group	Category	Number of animals
1	Neither gross nor microscopic lesions in glands or viscera	3
2	Gross involvement of glands, but viscera clear	6
3	No gross, but microscopic lesions present	2
4	Gross involvement of glands, microscopic visceral lesions	5
5	Gross involvement of glands and viscera, "extensive lesions"	3

The histological appearances found in groups 3, 4 and 5 are shown in Figs. 17 - 27. (No visceral lesions were present in groups 1 and 2).

The /

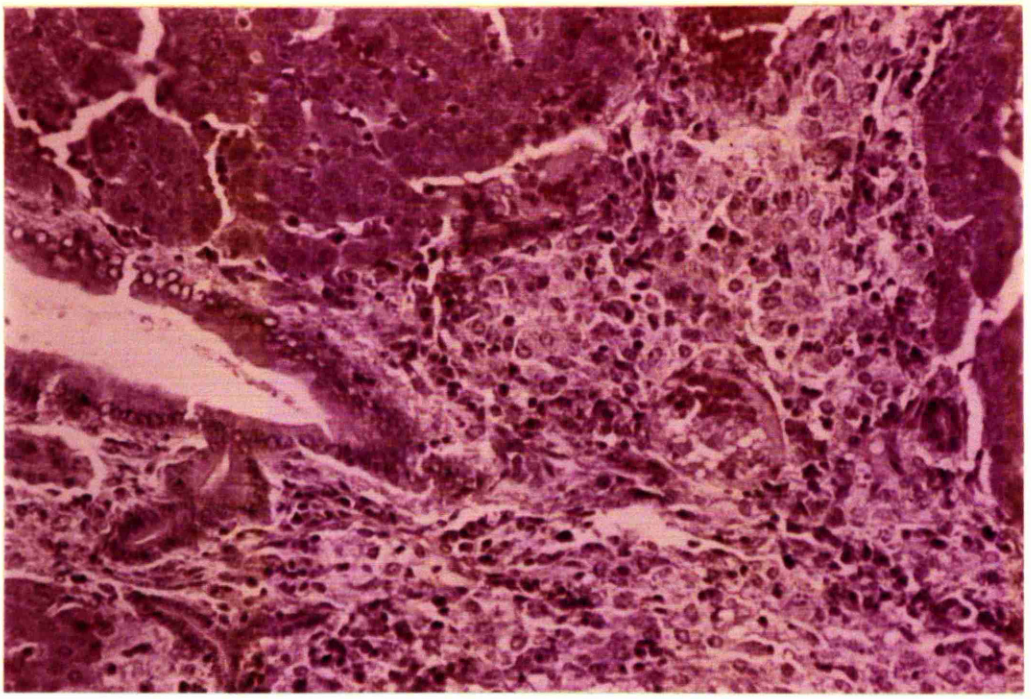

Fig. 17. Liver, H.& E. x 160.

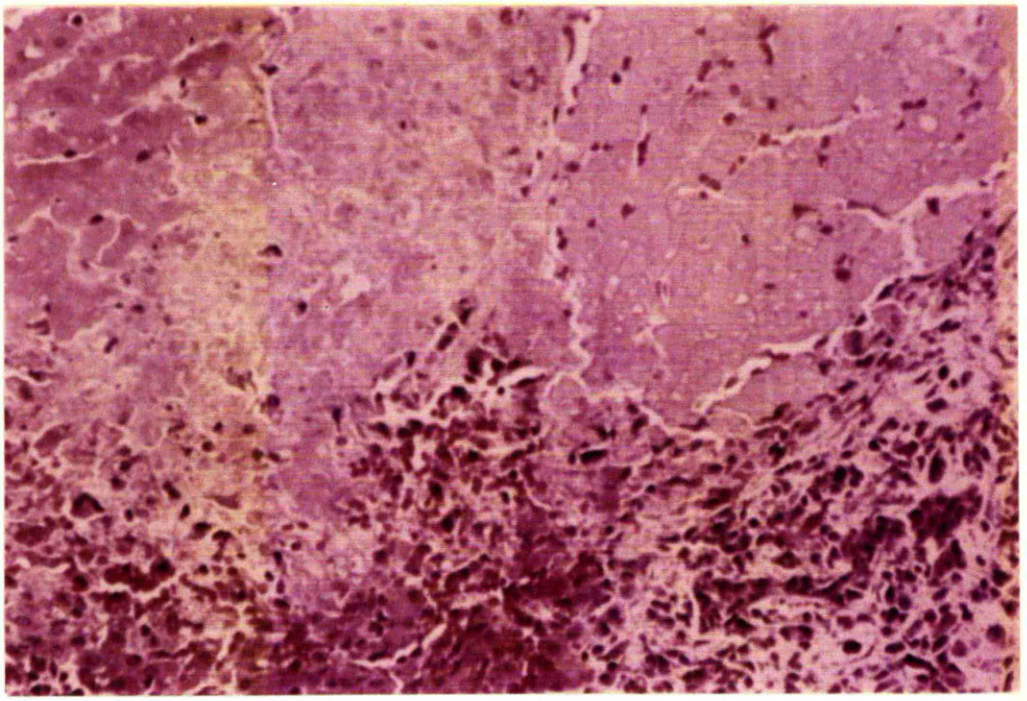

Fig. 18. Liver, H.& E. x 160.

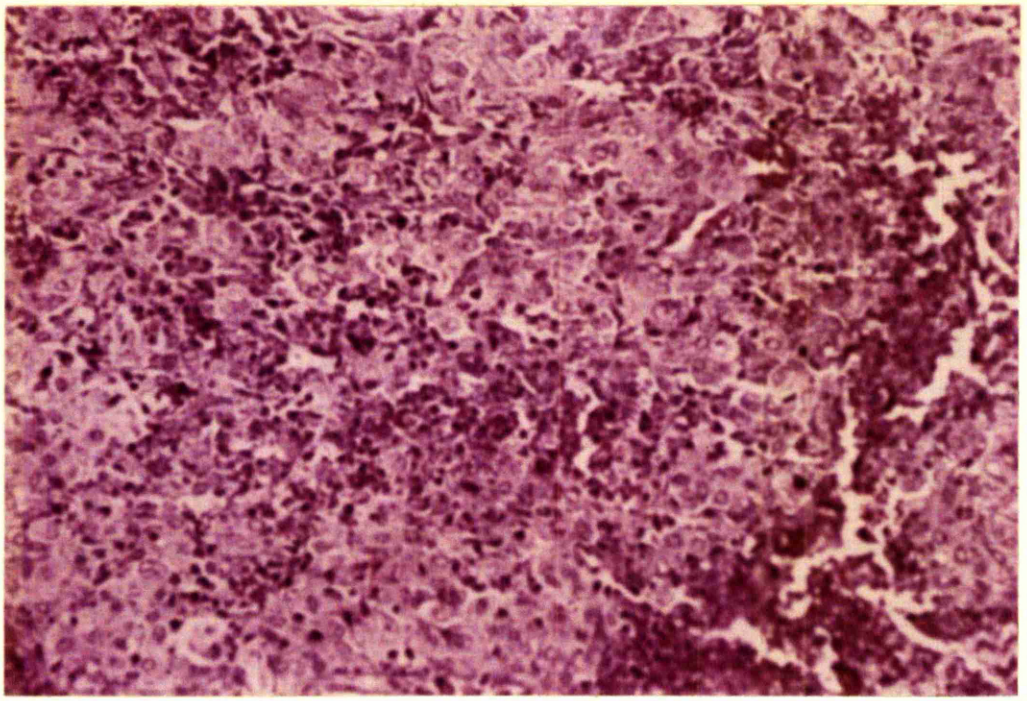

Fig. 19 Spleen, H.& E. x 160

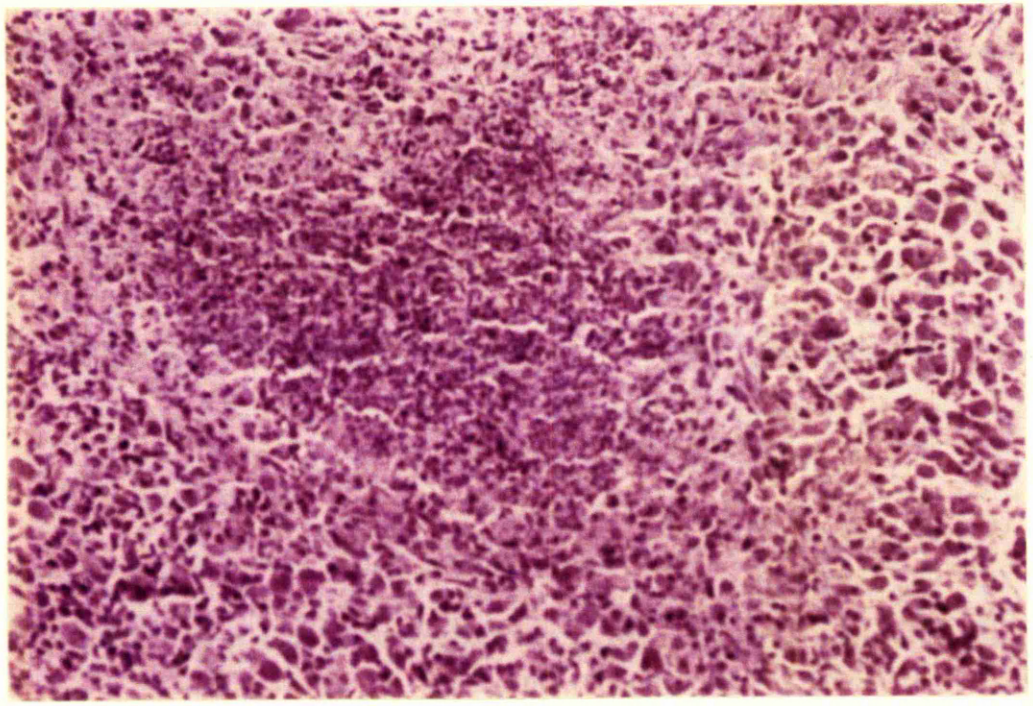

Fig. 20 Gland, H.& E. x 160

/ The first four photomicrographs (Figs. 17 - 20) illustrate the lesions produced in a "control" guinea-pig by inoculation of a drug-sensitive strain of M. tuberculosis in an identical manner to that described for the test strains. The hepatic lesions varied from clearly defined areas of epithelioid cell proliferation around the portal tract (Fig. 17) to extensive destruction and frank caseation (Fig. 18). Changes as severe as the latter were never found in the test animals. The intense epithelioid cell proliferation in the spleen is shown in Fig. 19, and the acute abscess formation (not recognisable per se as of tuberculous aetiology) which occurred in the inguinal glands is seen in Fig. 20.

The next three illustrations are of the type of lesion classed as Group 3. Histological examination of macroscopically normal tissue revealed small tubercles scattered throughout. In the liver (Figs. 21, 22) they tended to be demarcated from the surrounding normal cells by a ring of lymphocytes, often incomplete, while in the spleen epithelioid cell proliferation and giant cell formation were evident in follicles which were less clearly defined (Fig. 23). Group 4 lesions are shown in Figs. 24 - 26; the first two sections are from the liver and spleen of the same guinea-pig, and points to be noted are the diffuseness of the lesion and the paucity or complete absence of giant cells and of any cellular reaction on the part of the host. Similar changes are evident in the liver of a second guinea-pig in this group /

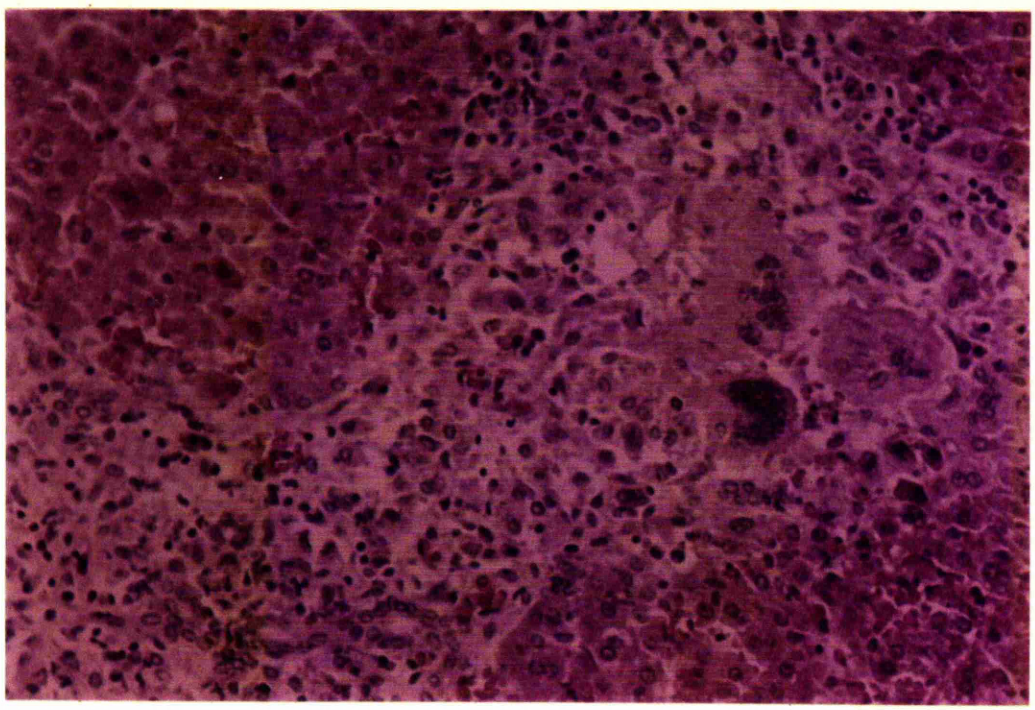

Fig. 21. Liver, H.& E., x 160.

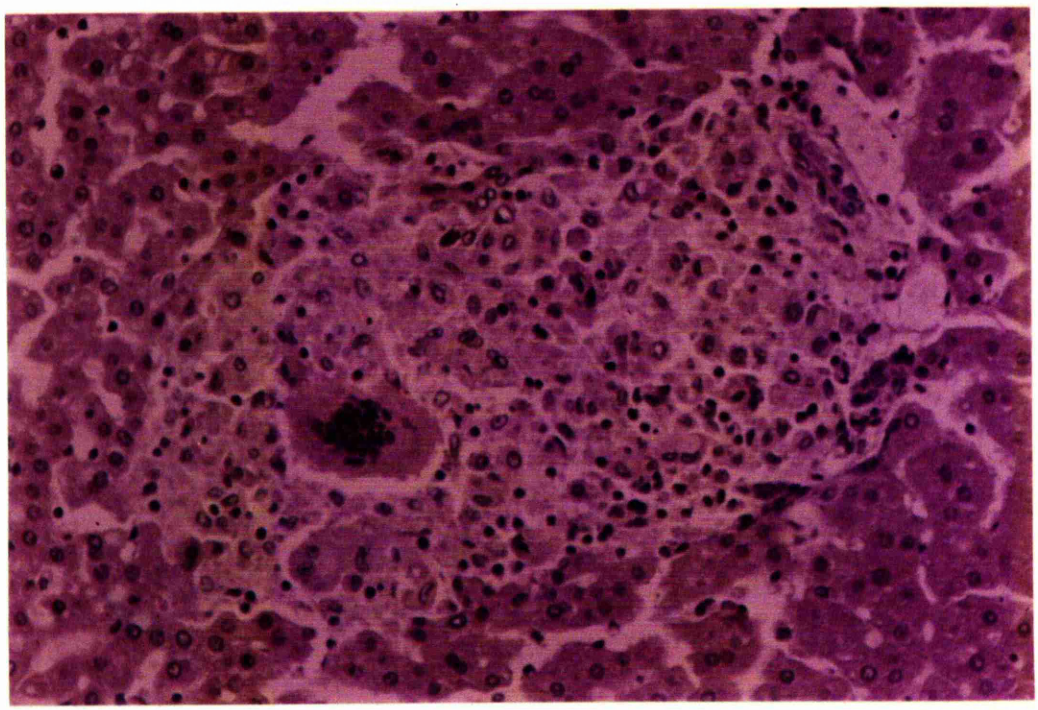

Fig. 22. Liver, H.& E., x 160

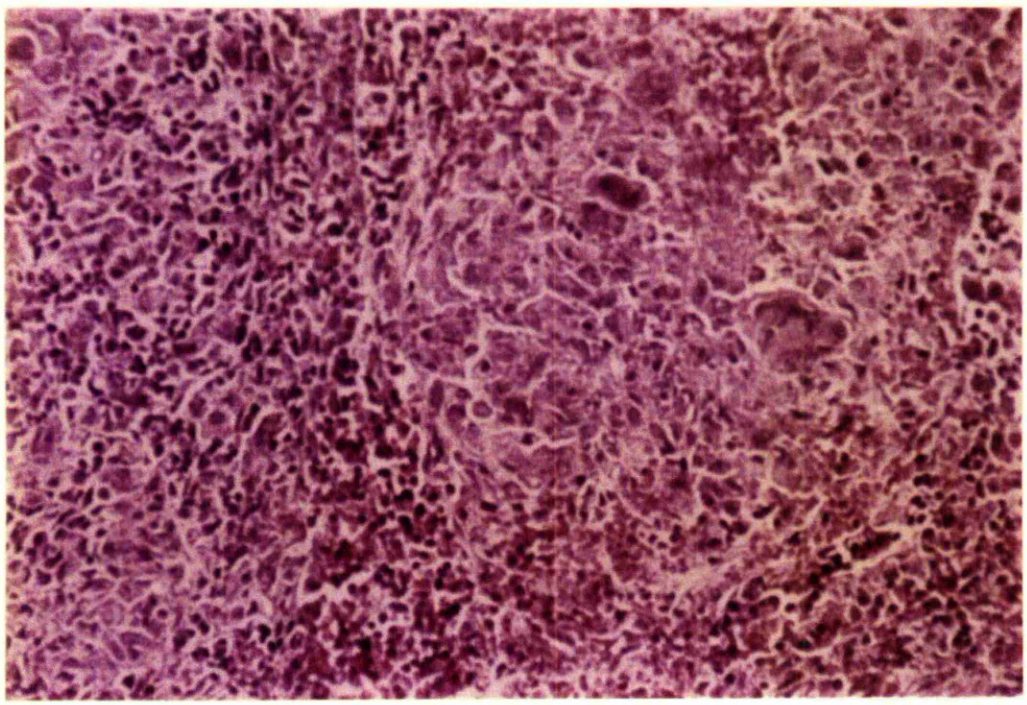

Fig. 23. Spleen, H.& E. x 160.

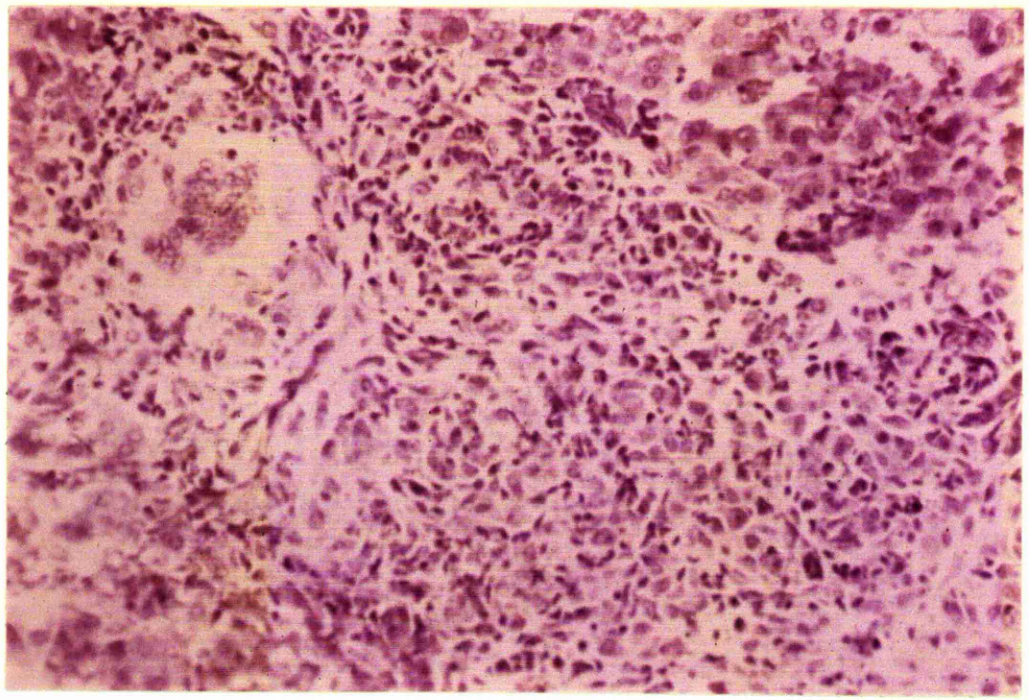

Fig. 24. Liver, H.& E., x 160

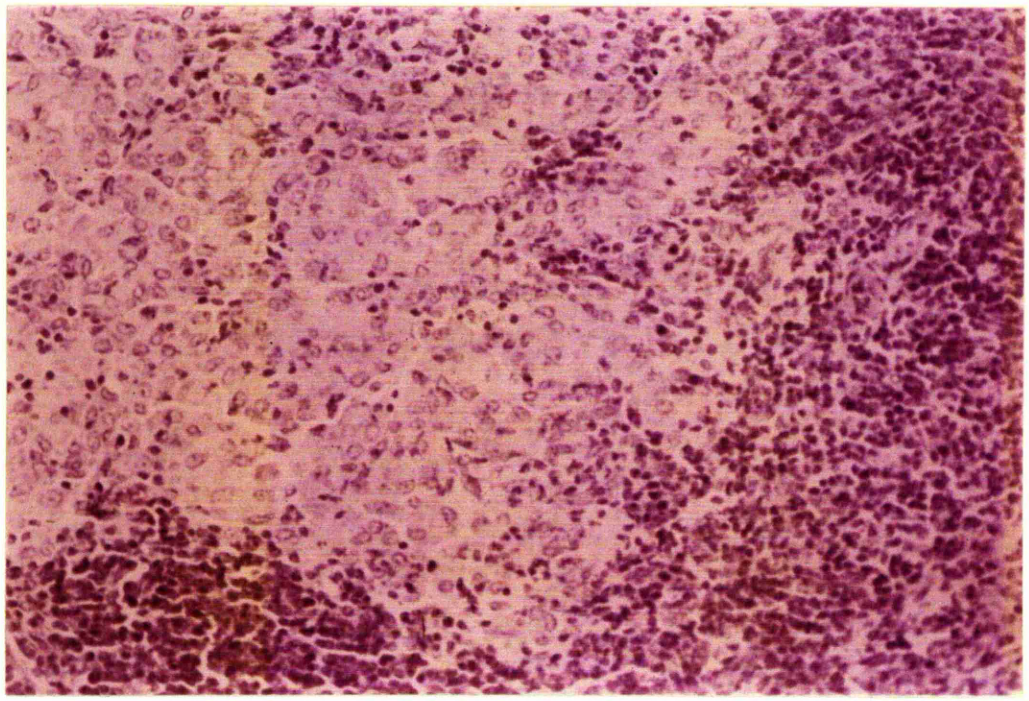

Fig. 25. Spleen, H.& E. x 160.

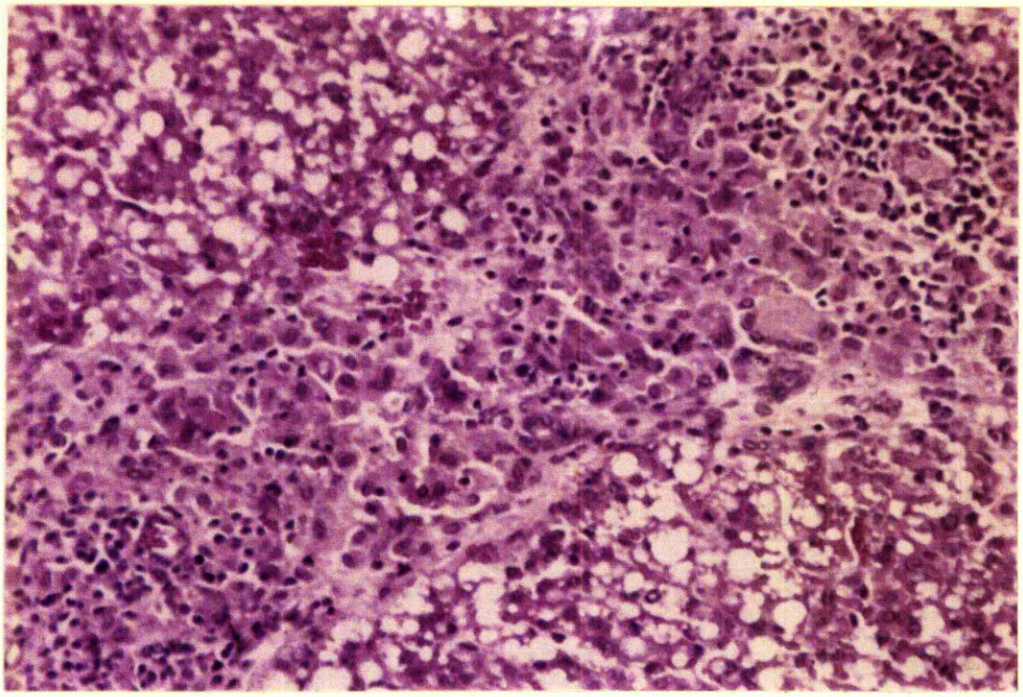

Fig. 26. Liver, H.& E., x 160.

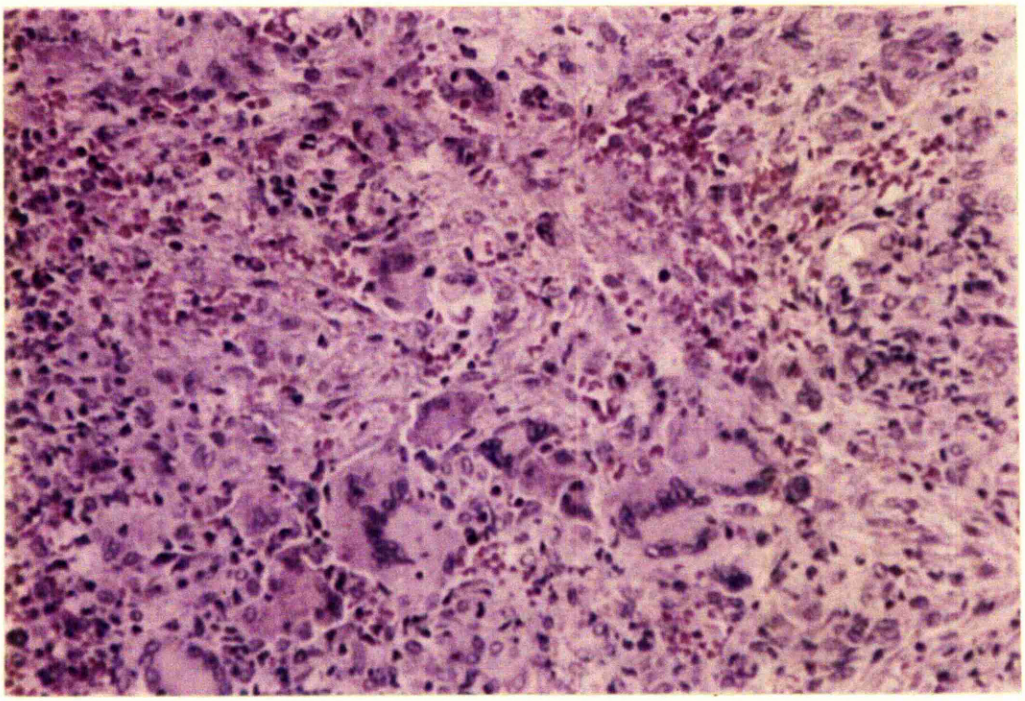

Fig. 27. Liver, H.& E.,x 160.

/group (Fig. 26).

A typical field (Fig. 27) from the liver of one of the animals in which extensive lesions were found at autopsy shows that in this Group the destructive process is more advanced, epithelioid cell proliferation is diffuse and giant cell formation is frequent.

The variation in animal susceptibility is further complicated by a case such as that illustrated in Figs. 28 and 29, where the microscopic appearance are consistent with a Group 4 classification. These sections were prepared from the spleens of two guinea-pigs which appeared healthy before routine sacrifice 12 weeks after inoculation of successive gastric lavages from a child showing clinical and radiological evidence of pulmonary tuberculosis. Confluent growth was obtained on Lowenstein-Jensen medium in 21 days further examination did not suggest that the strain was atypical in any way, and it was sensitive to all the drugs tested. Yet the gross and the histological appearances suggested a degree of virulence much less than that of the control strain.

The classification outlined is not intended to be of diagnostic or prognostic value but rather to show that, when histological studies replace naked-eye impressions, it is possible to recognise several stages of a single disease process all of which are broadly consistent with a diagnosis of "tuberculosis". As long ago as 1954 Mitchison, having compared the animal lesions caused by 8 strains of M. tuberculosis sensitive to isoniazid with those resulting from 8 resistant strains, /

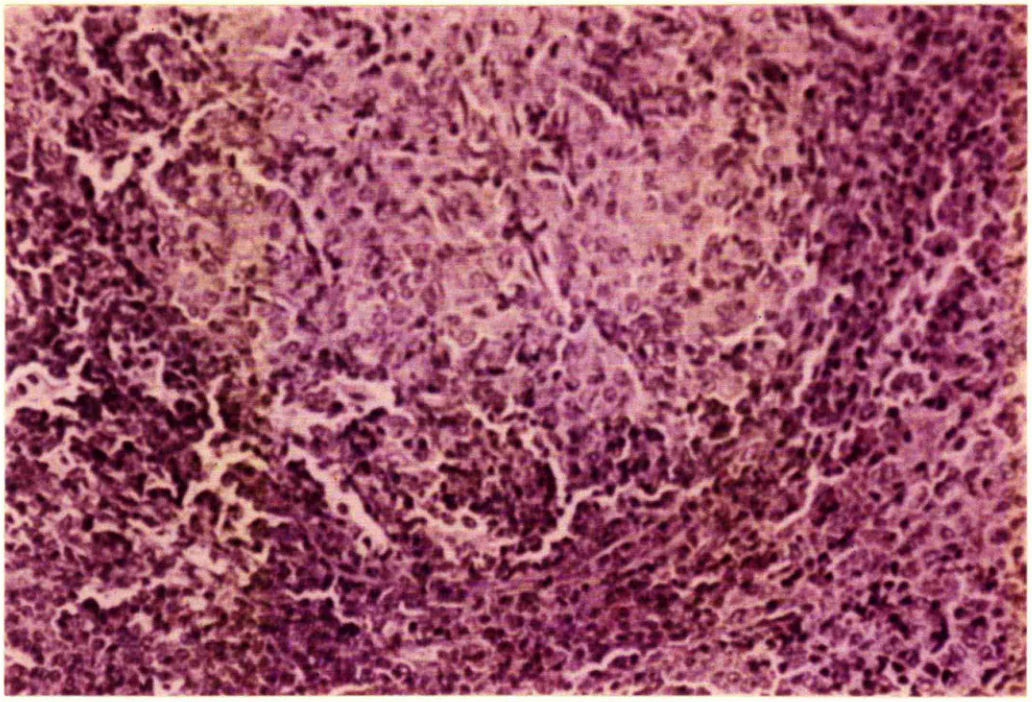

Fig. 28. Spleen, H.& E. x 160.

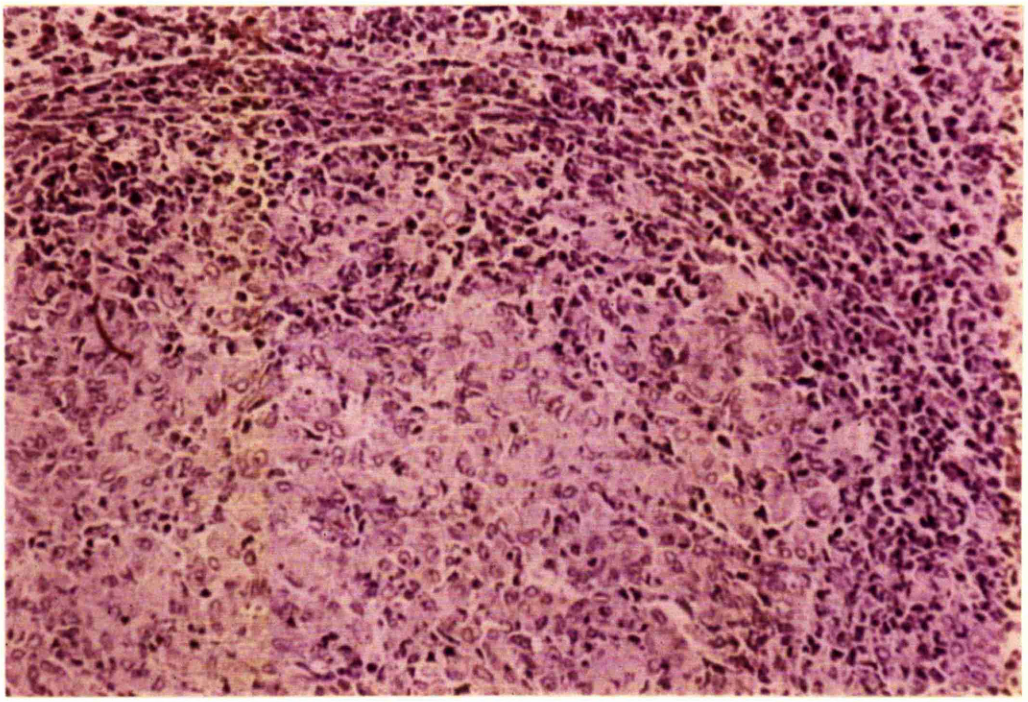

Fig. 29. Spleen, H.& E. x 160.

/ strains, commented that even the most highly resistant set up large abscesses at the inoculation site and produced minimal regressive lesions (in the form of microscopic tubercles) in apparently normal liver, spleen and lungs. This description corresponds closely to that of the well-documented Yersin type of disease.

It seems that the variation in the results of animal experiments are of less significance than was originally thought, and there is no doubt that the present clinico-bacteriological study has shown all strains highly resistant to isoniazid to be virulent in the patients concerned, regardless of their effect on experimental animals.

CHAPTER IX.

Drug-resistant tuberculosis in the Elderly.

The rapid fall in the incidence of tuberculosis has been shared fairly evenly by both sexes (Figs. 30 and 31, England and Wales), but has been much less uniform at different ages (Figs. 32 and 33) for in contrast to the situation prevailing at the turn of the century the greatest mortality rate is now in the over-45 and especially in the 60 - 70 age group, with the figures for men about four times those for women :

Respiratory Tuberculosis deaths by Sex and Age, 1962. (England and Wales).

	Males.	Females.
0 - 14	3	2
15 - 24	7	5
25 - 44	176	148
45 and over	1,915	518
Total	2,101	673
All persons	2,774.	

Anxiety about this position prompted the Joint Tuberculosis Council (1962) to make a special study of tuberculosis in the elderly,/

RESPIRATORY AND NON RESPIRATORY TUBERCULOSIS : NOTIFICATION RATES PER 100,000 POPULATION BY SEX : ALL AGES _ MALES

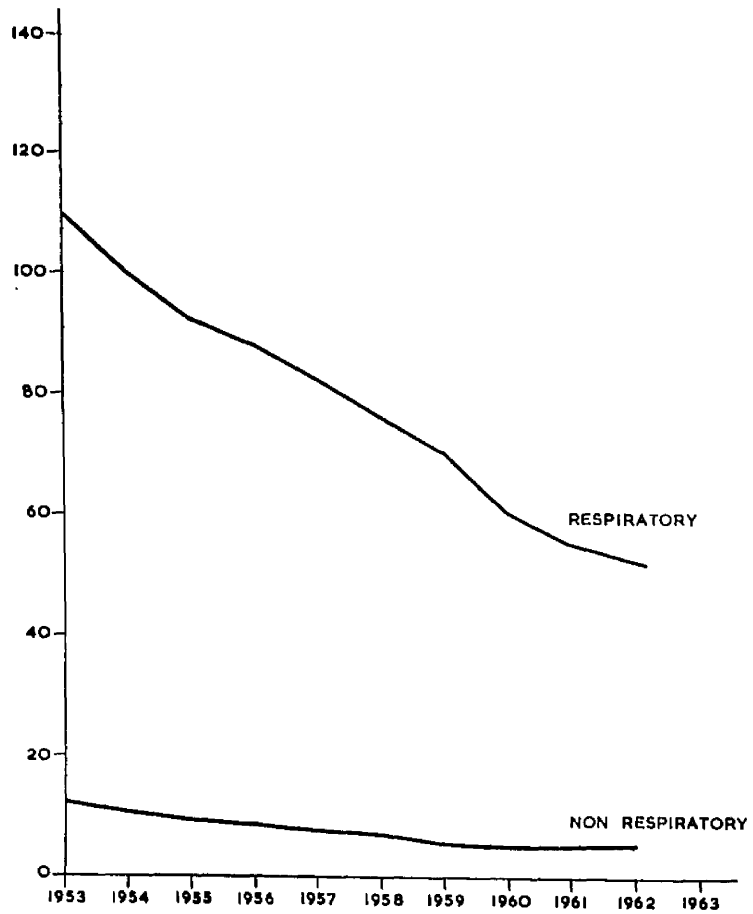

Fig. 30.

RESPIRATORY AND NON RESPIRATORY TUBERCULOSIS: NOTIFICATION RATES PER 100,000 POPULATION, BY SEX : ALL AGES_ FEMALES.

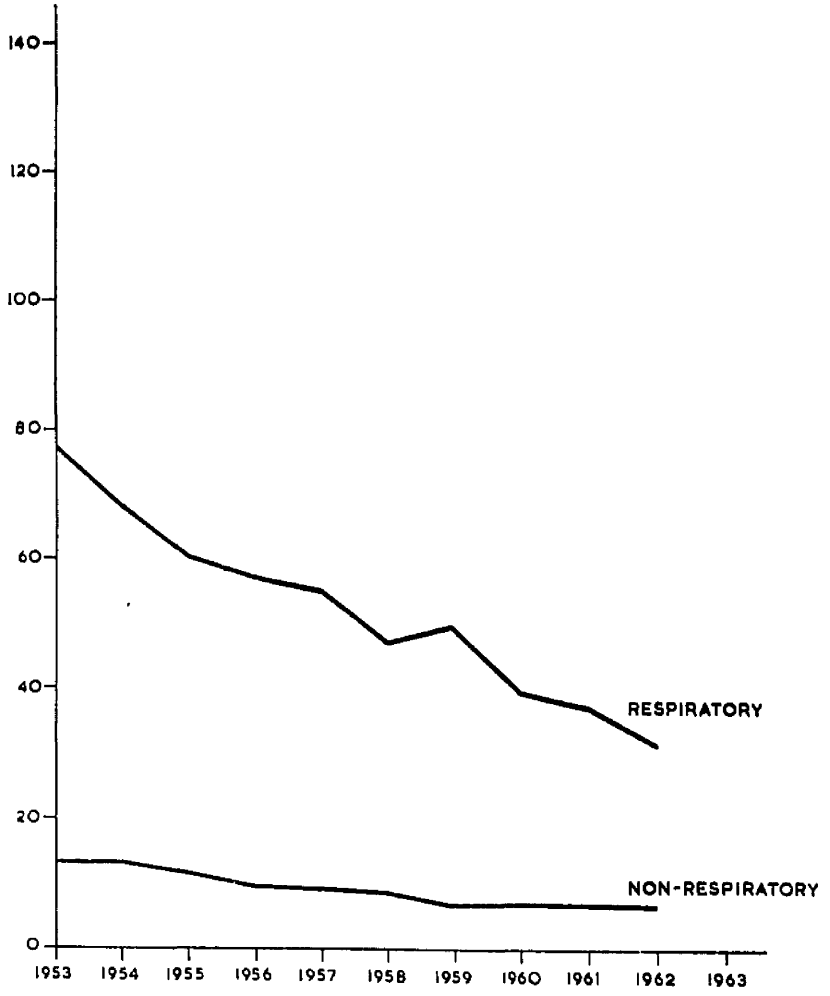

Fig. 31.

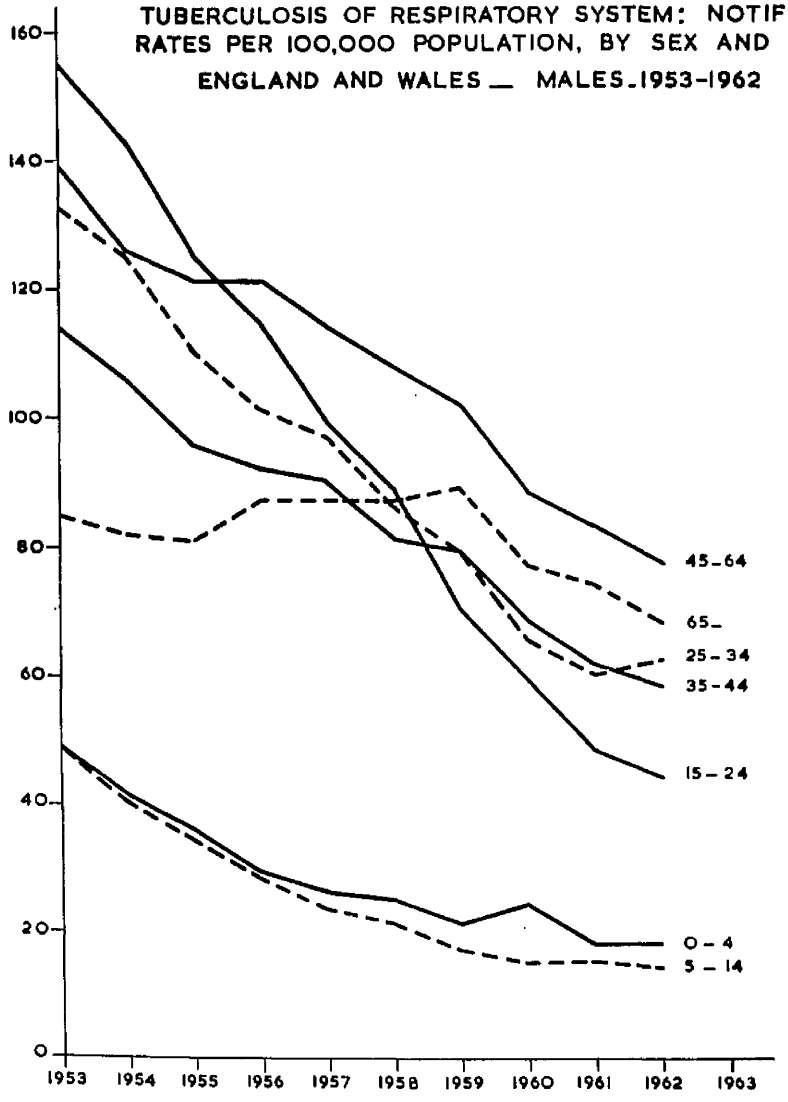
TUBERCULOSIS OF RESPIRATORY SYSTEM: NOTIFICATION RATES PER 100,000 POPULATION, BY SEX AND AGE.
ENGLAND AND WALES — MALES-1953-1962
160
140
120
100
80
60
40
20
0
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
45-64
65-
25-34
35-44
15-24
0-4
5-14

Fig. 32.

TUBERCULOSIS OF RESPIRATORY SYSTEM : NOTIFICATION RATES PER 100,000 POPULATION, BY SEX AND AGE . ENGLAND AND WALES

FEMALES _ 1953 - 1962

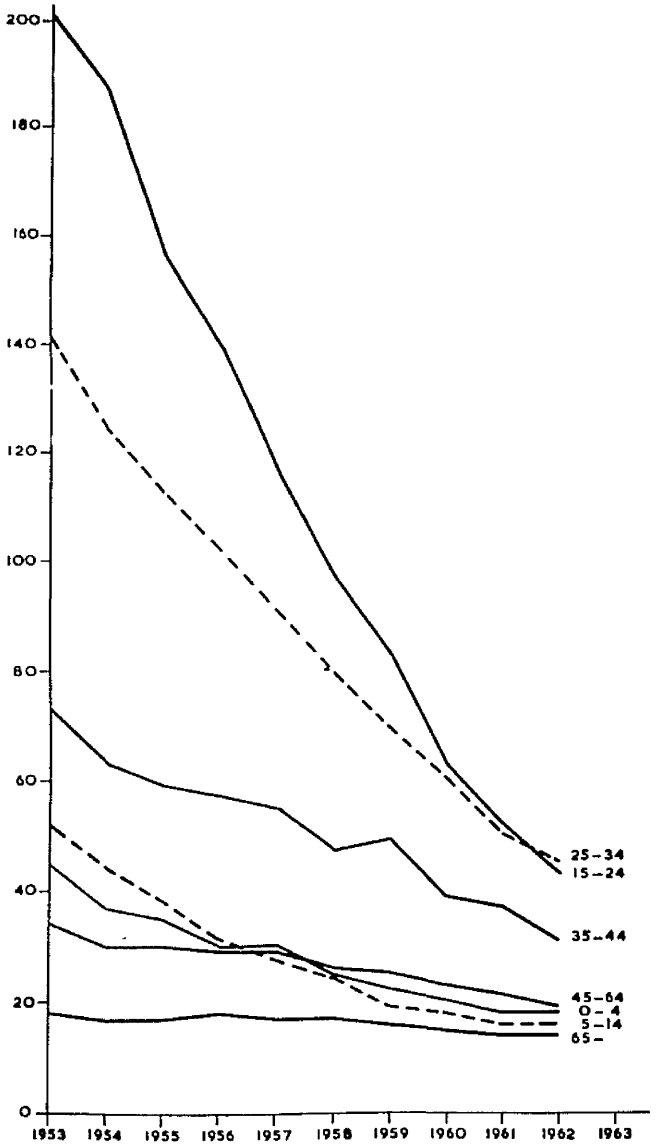

Fig. 33.

/ elderly, and from a detailed survey of 2,307 patients it was confirmed that the disease was still a serious threat in later life, especially to males. Death rates for older men have shown no real improvement, and the notification rate for those over 45 has fallen more slowly than for any other group.

This problem is not peculiar to the United Kingdom; a similar trend is evident in Chicago where Thomson (1960) analysed the case histories of 569 males and 232 females and showed that whereas in 1950 the majority of tuberculosis patients were between 20 and 50 years of age by 1959 the maximum incidence had shifted to the 40 - 80 years group. Even in India, where the expectation of life is considerably less than in Western countries, Agarwal et al. (1963) found that among 3,814 new patients attending their tuberculosis clinic no fewer than 8.8% (294 men, 42 women) were over 50 years of age. Another feature of this study which should cause concern to all those anxious about the development of drug resistance was that in this group as a whole attendances were irregular and drug-taking was erratic.

As long ago as 1948 Medlar et al. declared "tuberculosis among elderly people is the disregarded seedbed of the tubercle bacillus which must be eradicated if tuberculosis is to be eradicated" but although geriatric problems are receiving more attention than ever before the relative failure to improve this situation is still incompletely understood. It is well recognised that the clinical course of /

/ of tuberculosis is not the same in the elderly as in the young, for Myers (1962) has pointed out that older people may have extensive disease without much symptomatology, and many of Thompson's (1960) cases were discovered quite fortuitously. McDonald (1952) postulated that the higher incidence among men resulted from re-infection while at work in crowded conditions. Springett (1952), in contrast, believed that active disease in the elderly followed the breakdown of healed lesions acquired much earlier. Both these theories could be explained by a decline of host resistance which might well be part of the natural ageing process; the first would depend on failure to combat an infection from an external source, the second on failure to maintain a state of equilibrium with a dormant infection. But not all active tuberculosis among elderly people can be attributed to relapse or re-infection for a significant contribution to the total comes from the chronic sputum-positive cases who have survived on long-term chemotherapy to an age which only rarely would have been achieved before the advent of anti-tuberculosis drugs (four-fifths of those who die of tuberculosis are over 45 years of age compared with rather more than two-fifths ten years ago). It is in this group that one might expect to find a substantial number of drug-resistant patients, rendered so by inadquate drug regimes in earlier years or by their own inability (perhaps for reasons other than tuberculosis incapacity) to attend clinics regularly for proper supervision.

With/

/ With these factors in mind a special interest has been taken in the figures for drug-resistant tuberculosis among older people in Glasgow. Many public health reports specify the age-groups 45 - 64 and over 65 but as only 44 cases fall into the latter group in the present survey the analysis is based on the total of those born before 1919. In Table 17 is shown the overall resistance pattern related to age by decades.

TABLE 17.

Year of Birth	Sex	S.	P.	I.	S + P.	S + I.	P + I.	S + P + I.	Total	All Cases.
1870	M	1	-	-	-	-	-	-	1	1
-79	F	0	-	-	-	-	-	-	0	
1880	M	-	-	3	-	-	-	4	7	8
-89	F	-	-	0	-	-	-	1	1	
1890	M	3	1	7	-	5	7	7	30	35
-99	F	1	0	0	-	2	2	0	5	
1900	M	9	1	7	-	21	9	29	76	84
-09	F	1	1	1	-	1	0	4	8	
1910	M	9	-	6	2	21	6	29	73	104
-19	F	5	-	2	2	8	1	13	31	
1920	M	12	2	2	2	10	1	19	48	95
-29	F	3	0	3	0	12	2	27	47	
1930	M	2	1	1	-	2	0	4	10	34
-39	F	5	0	2	-	6	1	10	24	
1940	M	1	-	-	-	1	1	0	3	7
-49	F	1	-	-	-	1	0	2	4	
1950	M	1	-	-	-	2	-	-	3	4
-59	F	1	-	-	-	0	-	-	1	
Total	M	38	5	26	4	62	24	92	251	372
	F	17	1	8	2	30	6	57	121	
All Cases		55	6	34	6	92	30	149	372	

It is probably justifiable to draw only general conclusions about the incidence of drug resistance in the sexes by comparing the /

/ the 1963 resistance figures (Table 2) with the 1962 cumulative total of 1925 (Table 1), made up of 1,132 males and 793 females. Bearing in mind this qualification the figures suggest that drug resistance among men (22.1%) is considerably more common than among women (15.7%). It was hoped to discover whether this finding held good at all ages, but the Glasgow public health returns do not permit a classification of the cumulative total into over-and-under-45 age groups.

The distribution of resistance to single and multiple drugs in the older and younger age-groups is shown in Table 18.

TABLE 18.

BORN	RESISTANT TO									
	1 drug		2 drugs		3 drugs		Total		Total persons	All cases
	M.	F.	M.	F.	M.	F.	M.	F.		
Before 1919	47	11	71	16	69	18	187	45	232	372
% of total	12.7	3	19.1	4.3	18.5	4.8	50.3	12.1	62.4	100%
After 1920	22	15	19	22	23	39	64	76	140	
% of total	5.9	4.6	5.1	5.9	6.1	10.0	17.1	20.5	37.6	

The ratio of resistant strains in men born before 1919 to those born after is almost 3 : 1. Corresponding ratios for resistance to one, two and three drugs are 2 : 1, 3.7 : 1 and 3 : 1 respectively, indicating that a higher proportion of cases resistant to one drug exists among younger men, and a higher proportion resistant to two drugs among older men. The overall ratio for women is 0.6 : 1, and for resistance to one, two and three drugs 0.65 : 1, 0.73 : 1 and 0.48 : 1 respectively, again showing a higher proportion of cases resistant to two drugs among /

/ among older women but also indicating a higher proportion resistant to all three drugs among younger women. In a comparison of the two age groups in their resistance to one, two and three drugs, it can be seen that in each case there is a considerable excess of older over younger men, whereas the reverse is true for women; in fact, the occurrence of multiple-drug resistance is commoner among women under 44 than among men of this age group. Part of the explanation for this undoubtedly lies in the fact that the maximum incidence of the disease occurs in different age groups in each sex, but it raises an important question as to the attitude to be taken to the whole problem of the further treatment of drug-resistant patients. Some workers believe (privately at least) that the complete cure of certain chronic sputum-positive and drug-resistant cases simply cannot be achieved and that the only measures to be adopted are those which will render the patient non-infectious until death terminates his role as a potential danger to the public health. This contention, however unpalatable, may well have to be accepted for males in the upper age brackets; but what of the female cases, among whom the majority of drug-resistant patients are under 45 years? Many of these women are at the centre of family groups and it is inevitable that speculation should arise as to their part in the spread of organisms which will re-appear in later years, in cases of primary resistance. A special effort to ensure permanent cure among this group might well pay dividends in reducing the future incidence of drug-resistant disease, and it might be that appropriate social services would have to be made available to encourage these patients to enter sanatoria for a spell of adequate nursing care as well as for strict medical supervision and bacteriological control.

CHAPTER X.

Drug-resistant Tuberculosis in Immigrants.

Provided that the spread of drug-resistent strains is limited by careful chemotherapy and epidemiological studies the only apparent threat to the continuing downward trend of tuberculosis is the importation of fresh disease to this country. Within the past four or five years increasing disquiet has been felt in the United Kingdom about the effect on the public health of large-scale immigration of African and Asian people, and it is probably no exaggeration to regard this disquiet as one reason for the Commonwealth Immigrants Act of 1962. Objective assessment of the whole problem has been made more difficult by the political and emotional factors which surround and obscure it. There can, however, be no doubt that in localities where immigrants settled in large numbers the incidence of tuberculosis increased. Springett (1958) recorded active tuberculosis in 1,223 immigrants - 391 from India and Pakistan, 74 from the West Indies, 131 from various European, American and African countries and 627 from Ireland. Within a year of arrival in the United Kingdom 3% of the West Indians and 11% of the Asians had recognisable tuberculosis. Aspin (1962) in Wolverhampton noted a steady rise from 2 to 20 new cases of tuberculosis among Indians between the years 1954 and 1960, with a corresponding rise in the proportion of Indians in the total of new adult cases from 0.8% to 11.0%. He also noted a primary resistance rate of 9.3%. Stevenson /

/ Stevenson (1962), dealing exclusively with Pakistanis in Bradford, estimated that among them tuberculosis was 30 times commoner than in the British population, and that among Pakistani contacts the annual incidence was as high as 40 per 1,000.

It has been thought that a certain number of immigrants brought active disease from their homeland to this country and that others acquired tuberculosis here; particularly susceptible were many of the younger people who were probably tuberculin negative on arrival and were suddenly exposed to infection while living in adverse conditions and working in an uncongenial climate. The small epidemic described by Corbett (1961) in which 10 of 22 persons living in three houses contracted some form of tuberculosis illustrated this state of affairs only too well.

From the foregoing it appears that in England at any rate the figures for "imported" tuberculosis are probably large enough to justify the pressure being put on the authorities to institute more thorough checks on the state of health of all persons coming to stay in the United Kingdom - a move which would only bring Great Britain into line with the policies pursued by several other highly developed countries.

It may be, however, that efficient safeguards could be derived from a less obtrusive and expensive scheme which would operate in selected cases. There seems to be good reason to believe that Asian immigrants, especially Pakistanis, constitute a source of tuberculous disease out of /

/ of proportion to their numbers; but the same is not true of the other large group of immigrants to this country, the West Indians. Rerrie (1960) recorded the development in Jamaica within a decade of a well-equipped anti-tuberculosis organisation supported by a co-operative public. This has worked satisfactorily towards the control of the disease and in 1962 Miall and Rerrie were able to report an incidence of active tuberculosis in Jamaica much lower than in comparable tropical and subtropical areas of Africa, India and Asia.

The argument for the relaxation of immigration regulations with regard to tuberculosis has been put forward by Schou (1961). He points to the likelihood of cure with modern chemotherapy and balances the cost of such treatment against the later productivity of the rehabilitated worker, especially if skilled; his contentions are based on the success that Austria and the Scandinavian countries had in coping with tuberculous Hungarian refugees from the uprisings of 1956.

All these arguments should be taken into account before any scheme designed to reject all sufferers from tuberculosis is instituted; it is perhaps salutary to remember that Edwards (1963) considered that British immigrants to Australia showed relatively high rates of tuberculosis.

Glasgow, as a seaport and a large industrial centre, attracts its share of immigrants, and it seemed a reasonable assumption at the outset of this investigation that the incidence of tuberculosis among /

/ among these immigrants would be similar to that recorded in other large towns - even worse figures would not have been surprising in view of the bigger pool of infection already present in the city. As it became evident that the absolute numbers of immigrants harbouring resistant organisms were very small indeed the whole matter was investigated a little more fully.

It is difficult to give an accurate estimate of the numbers of Asian immigrants now in the City, though it has been suggested that they form a smaller proportion of the population here than in the Midlands of England where employment prospects have been brighter over the past few years. Nor is it possible to assess the prevalence of tuberculosis among immigrants because the official notification certificate does not record nationality. With the readily available general practitioner and specialised chest clinic services in the city, however, it seems very unlikely that disease among new immigrants should be undetected, and it is felt that the figures given below are a true measure of the extent of pulmonary tuberculosis among these people.

Only 7 Pakisteni and 5 Indian patients' cultures were received by the Laboratory, and none showed drug resistance; 6 European (other than British) patients' cultures were also examined.

/ Cont'd.

NATIONALITY	SENSITIVE.			RESISTANT.		
	Male	Female	Total.	Male	Female	Total.
Indian	5	0	5	-	-	-
Pakistani	5	2	7	-	-	-
Others	4	1	5	1	0	1*
	14	3	17	1	0	1

* The only case of drug-resistance was that of a Polish male whose organisms were resistant to streptomycin, P.A.S. and isoniazid.

It can therefore be concluded that in Glasgow at present active pulmonary tuberculosis is less frequent among immigrants than might have been expected, and that drug resistance is very much an indigenous problem. Nevertheless, it would be wise to learn from the experience of the English Midlands and to try to prevent the relatively large pool of drug-resistant infection already in the city spreading to these people, and, in particular, to try to prevent their living in overcrowded conditions under which rapid cross-infection would be almost inevitable.

CHAPTER XI.

Drug resistant tuberculosis among homeless men.

In view of the prevalence of tuberculosis among older men and the occurrence of the disease in conditions of overcrowding, neglect and inadquate nutrition particular attention should be paid to the role of habitues of common lodging-houses in the maintenance and spread of tuberculous infection. Seldom can these men be persuaded to remain in hospital while culture and sensitivity procedures are completed and the intermittent therapy to which inevitably they are subjected is very likely to give rise to drug-resistant strains. The prospects of follow-up of these men at out-patient clinics are equally poor, and it is hoped that one of the benefits of the drug-resistence register will be that the Reference Laboratory, by following a patient's progress through different regional laboratories, will be able to give a reasonably up-to-date account of the infecting organism's sensitivity pattern as soon as the patient comes under medical care for any reason at all.

There is a good deal of evidence for believing that tuberculosis is rife among this group. Stoloff (1962) in Philadelphia investigated the district with the highest active disease rate (2.5% per 1,000) and found one hotel which had supplied nearly 10% of the new cases of tuberculosis in 1960 and 1961. In New York in 1960 Chaves et al surveyed 9,000 homeless men and found an incidence of /

/ of 16 per 1,000 of previously undiagnosed active pulmonary disease. Elwood (1961) described a lodging house in Belfast in which, over a period of 10 years, the notification rate stood at 9.8 per 1,000 compared with 1.7 per 1,000 for the surrounding district. All these authors stressed the difficulty of giving satisfactory treatment to these men and an interesting proposal to combat this was made by Hurford (1962). He suggested the establishment of "night sanatoria" to which the tuberculous vagrant would be attracted by better conditions and cheaper rates than those of strictly commercial premises and in which a condition of residence would be the acceptance of regular (night and morning) therapy.

In none of these earlier surveys have the drug-susceptibility patterns of the infecting organisms been systematically examined and it is this aspect on which attention is now focussed. Glasgow in 1962 had 8 registered lodging houses with a total capacity of 2,087; in discussion with the Chief Sanitary Inspector for the City it was ascertained that the total vagrant population of Glasgow was likely to be rather less than this figure, as most of these men move from one lodging house to another within the city boundaries, and newcomers to this "closed circuit" are relatively few. The City's Public Health Department co-operated with the Mass Radiography Unit in an X-ray survey of model lodging-houses in 1962 and 15 new cases of pulmonary tuberculosis were discovered among the 579 residents who submitted to examination (a rate of 26 per 1,000).

During 1963 no less than 36 cases of pulmonary tuberculosis were referred to the Laboratory from this group of men, representing a rate of 17 per 1,000. In view of the findings recorded in the previous paragraph this figure can be regarded as conservative for it is not unlikely that further cases of pulmonary tuberculosis remain undetected in this vagrant population; nevertheless, these levels are far greater than that prevailing for the general population of the city (0.89 per 1,000 in 1962). Twelve of these cases (33%) harboured drug-resistant organisms; fuller details are summarised in Table 19.

TABLE 19.

Age	Years since first notified	Drug sensitivity pattern			Additional Comments.
		Strep.	P.A.S.	I.N.A.H.	
55	3	R	S	S	Resistant also to Cycloserine.
57	12	R	R	R	" " to Viomycin.
43	9	R	R	R	
72	13	S	R	S	
53	7	R	R	R	
54	4	S	S	R	
62	5	S	S	R	
58	5	R	R	R	Resistant also to Viomycin and Cycloserine.
47	6	R	S	R	
63	-	R	S	S	Primary resistance.
58	7	R	R	R	

R = resistant, S = sensitive.

/Cont'd.

Generally, older men were involved, the greatest number being in the sixth decade. The numbers are too small to allow firm conclusions, but it can be seen that nearly half the cases were producing organisms resistant to all three drugs, and a quarter were producing organisms resistant to one or more of the newer or "secondary" drugs; the incidence of primary resistance (8.3%) was a little greater than that for the population as a whole.

These findings show that a determined effort is still required to control, let alone eradicate, tuberculosis in this section of the community.

CHAPTER XII.

Drug-resistance in non-pulmonary tuberculosis.

The success of the drive against the obviously infective pulmonary form of tuberculosis has tended to minimise the importance of non-respiratory disease. Yet non-pulmonary tuberculosis accounted for the deaths of 314 people in England and Wales in 1962 (just over 10% of all tuberculosis deaths), and in the same year in Glasgow 856 patients were known to be suffering from various non-respiratory lesions (5.7% of all tuberculosis cases). Furthermore, there has been no dramatic decline in the incidence of non-pulmonary disease to match that of respiratory tuberculosis (Figs. 30 and 31). These findings indicate a continuing need for care in the diagnosis and skill and persistence in the treatment of non-pulmonary tuberculosis. One regrettable feature discovered in this survey was the failure to notify many of these cases to the public health authorities, a damaging omission from any comprehensive case-finding scheme.

Cultures from 111 patients suffering from non-respiratory forms of the disease were received by the Reference Laboratory in 1963, and 9 of these strains were resistant (8.2%). The types of lesion involved are detailed in Table 20.

/Cont'd.

TABLE 20.

SITE	Sensitive strains			Resistant strains			All Cases	Percentage of resistant strains all cases.
	Male	Female	Total	Male	Female	Total		
Urogenital	18	24	42	3	2	5	47	10.6%
Cervical Adenitis	10	20	30	-	-	-	30	0.0%
Bone and Joint	11	7	18	1	1	2	20	10.0%
Meningeal	3	1	4	1	0	1	5	20.0%
Alimentary	1	4	5	-	-	-	5	0.0%
Miscellaneous	2	1	3	1	0	1	4	25.0%
	45	57	102	6	3	9	111	8.2%

Fuller particulars of the drug-resistant strains are given in Table 21.

TABLE 21.

SITE	Sex	Age	Years since first not'fd.	Drug sensitivity pattern.						Additional notes.
				S.	P.	I.	V.	C.	E.	
Urogenital	F	32	10	R	S	S	S	S	S	-
"	M	26	0	S	R	R	S	R	S	Anonymous strain : not regarded as primary resistance.
"	M	14	10	R	S	S	S	S	S	-
"	M	40	9	R	R	R	S	S	S	-
"	F	37	0	R	S	R	S	S	S	Primary resistance.
Bone & Joint	M	27	0	S	R	S	S	S	S	Primary resistance - Indian patient.
"	F	10	0	R	S	S	S	S	S	Primary resistance.
Meningeal	M	8	0	R	S	R	S	S	S	Primary resistance.
Misdllaneous	M	14	0	R	S	S	S	S	S	Primary resistance - isolated from excised spleen

Although females outnumber males by 1.2 : 1 in all forms of non-respiratory tuberculosis the ratio of resistant strains among females compared with males is only 0.5 : 1. In non-respiratory lesions resistance to streptomycin is shown by 78% of the strains, to P.A.S. by /

/ by 33% and to isoniazid by 44%; corresponding figures for pulmonary strains are 81%, 51% and 82% respectively. Resistance to one drug is found in 5% of non-pulmonary strains, to two drugs in 3% and to all three drugs in 1.2%; corresponding figures for pulmonary strains are 26%, 31%, and 40% respectively. The primary resistance rate (4.5%) is only a little lower for non-pulmonary than for pulmonary isolates, but the overall resistance rate (8.2%) is very much less.

The total number of cases presented here is admittedly small, but the figures suggest that drug-resistance is not a major problem in the treatment of non-respiratory tuberculosis; it occurs less frequently than in pulmonary tuberculosis, and resistance to multiple drugs is relatively rare.

Resistance to the secondary drugs has not been encountered among true M. tuberculosis strains in this series.

Bovine strains were isolated from two cases of cervical adenitis, but neither was drug-resistant. No bovine strain was found among all the cultures from pulmonary lesions in Glasgow patients.

CHAPTER XIII.

Summary and Conclusions.

A deliberate effort has been made in Chapters IV - XII simply to state the facts relevant to Glasgow in the light of experience elsewhere, with little commentary on each aspect examined. It is now necessary to review the subject as a whole, to examine the inter-relationships of its numerous facets, and, at the risk of a certain amount of repetition, to set down briefly the principal conclusions reached.

The project was commissioned to find out the incidence and pattern of drug-resistant tuberculosis in Glasgow and the bare facts of the answer are given in Table 22. The occurrence of anonymous strains is so rare as to have little effect on the overall resistance figures, and no resistant strain of M. tuberculosis var. bovis was found in 1963.

TABLE 22.

	No. of cases	S.	P.	I.	One drug	Two drugs	Three drugs
MALE.	251 100%	196 78%	125 50%	204 81%	69 27.5%	90 35.8%	92 36.7%
FEMALE.	121 100%	106 87%	66 55%	101 83%	26 21.5%	38 31.4%	57 47.1%
ALL CASES.	372 100%	302 81%	191 51%	305 82%	95 26%	128 34%	149 40%

This/

/ This is the first detailed survey of drug-resistant tuberculosis in the City. Indeed, the British Tuberculosis Association's 1960-61 national investigation provided the first accurate estimate of the prevalence of acquired resistance in this or any other country, and a Leading Article (1963) admitted that at that time it was not known whether the number of cases was increasing or not. If drug-sensitive cases are excluded from the Association's figures the following comparison can be made with those of Glasgow.

TABLE 23.

SURVEY.	S.	P.	I.	One drug	Two drugs	Three drugs
Present: Glasgow 1963	81%	51%	82%	26%	34%	40%
B.T.A. : National, 1960-61	77%	65%	90%	18%	32%	50%

These results indicate that in Glasgow neither the pattern of drug-resistance nor the incidence of multiple drug resistance are so different from those in other parts of the country as to suggest any failure in diagnosis or therapy. Presumably the fact that the number of resistant cases now in existence is about five times the national average is simply the legacy of the unusually high incidence of tuberculosis in Glasgow in past years. It is felt that these facts should be more widely known, not to spread alarm or despondency about the situation, but rather to make all concerned with the disease realise that Glasgow still has much to do to achieve parity with the rest of the country in this respect. It is suggested that in future greater /

/ greater emphasis could well be placed on four points:

1). Recognition of patients in "danger groups".

2). Pursuit of a complete case-finding programme.

3). Extension of medico-social services.

4). Use of laboratory facilities.

1). "Danger groups" Even after allowing for the accepted variation in the incidence of the disease between the sexes and at different ages certain groups of patients show an unduly high rate of drug-resistance. It is, of course, true that any new patient is a potential case of drug-resistance but the evidence suggests that men in the over-45 age group, women in the under-45 age group, and vagrants are at particular risk. Now that the numbers of new cases are declining it should not be impossible for any patient from the first two groups to make more frequent visits to the clinic for follow-up and sputum examination, and for the clinician and health visitor to pay even greater heed to social or personal circumstances which might interfere with the patient's treatment. These optimistic plans can hardly be expected to apply to the vagrant, and one cannot but wonder whether the time is not now ripe, in the interest of the great majority, for the available legal sanctions to be used more frequently in dealing with uncc-operative patients of this type. The establishment of the "night sanatoria" described should be seriously considered.

It /

/ It is probably worth emphasising in this connection that at present there is no evidence that immigrants are a significant source of drug-resistant strains, but it is equally important to prevent drug-resistance spreading to this group - failure to do so might set back anti-tuberculosis campaigns by some years.

2). Case-finding and prophylaxis. In compiling this register numerous instances of failure to notify the appropriate authority of new cases of tuberculosis have been uncovered, a state of affairs which must nullify much of the public health department's energetic case-finding work. The time would appear to be ripe for some official re-statement of the statutory obligation which all medical practitioners have to notify the disease. In contrast, it has emerged that so many patients' specimens are examined by different laboratories that a simple summation of drug-resistant cases known to each laboratory would greatly exceed the true number of such patients. This finding has stressed the importance of maintaining a comprehensive register in one central laboratory.

Prophylaxis has to be considered both as B.C.G. vaccination for children and as the use of drugs for patient-contacts of any age. Many physicians have seen pulmonary tuberculosis develop in subjects who have not been vaccinated in view of what appeared to be a positive Mantoux test, and now that anonymous mycobacteria have been shown to occur in Scotland it would seem a logical procedure to try to discover /

/ discover whether skin-sensitivity to these organisms occurs on a significant scale even although actual disease is rare.

Drug prophylaxis is as yet practised less in this country than in the United States, and it is difficult to accept that the administration to this end of isoniazid alone is a justifiable procedure in view of the conclusions reached in Chapter VIII.

3). Medico-social. Compared with many other diseases nowadays the treatment and rehabilitation of tuberculosis is a lengthy process, often ill-understood by patients and relatives alike. Yet adequate therapy is of the utmost importance not only directly for the patient but also indirectly to contacts who might be infected were he incompletely treated, and there would appear to be justification for urging strongly a period of in-patient care, if possible until sputum-conversion is obtained. Sanatorium accommodation exists at present (though it seems to be in danger of conversion to other uses even in areas where this can only be regarded as premature) and no expensive new equipment is required. Greater publicity could be given to the special allowances and grants available to those whose earnings would be interrupted by treatment, for it would surely be a sound investment for the country if the chain of infection could finally be broken in this way.

4). Laboratory services. Laboratory tests are playing a steadily increasing part not only in the diagnosis but also in the treatment of /

/ of disease, and tuberculosis is no exception. Fox (1964) may be right in maintaining that drug-sensitivity testing is a luxury which the developing countries cannot afford, but the same argument should not apply in the United Kingdom. Yet the contribution to be made by the laboratory has not always been fully appreciated, for although figures for drug-resistance in Birmingham fell to less than two-thirds of that expected within five years of the establishment of a specialised laboratory other areas have been slow to take up Thompson's (1961) suggestion that drug-resistance registers based on laboratory findings would be of considerable value in different centres.

Under the existing arrangements in Glasgow it would be impossible for one central laboratory to carry out all the sensitivity testing required, and it may well be that complete centralisation is neither necessary nor desirable. Unnecessary, because the test results obtained by the majority of the regional laboratories compared well with those of the Reference Laboratory (the poorest correlation was for P.A.S., but even this variation of approximately 10% was much more satisfactory than the British Tuberculosis Association's corresponding finding of 30%); undesirable, because consultation between clinician and bacteriologist would inevitably become much more difficult.

Experience has shown that the aims set out in Chapter I can be achieved even when the Reference Laboratory deals with only selected cultures :-

/ Cont'd.

(1) All cultures from newly diagnosed patients. Of great epidemiological value, this allows calculation of the incidence of open cases among new notifications, gives an accurate figure for primary resistance, assists in the planning of correct initial chemotherapy and recognises anonymous and bovine strains at the earliest opportunity.

(2) All cultures showing a suspected or proved change of sensitivity pattern. A true picture of acquired resistance will become available, and by testing the strain's susceptibility to a wide range of drugs the laboratory should be of assistance in planning future chemotherapy. This is the type of investigation which, properly documented, is likely to be of value in the future to developing countries where it seems probable (from current trends) that primary resistance rates will be high.

(3) All cultures showing drug-resistance, whether from newly diagnosed, chronic, or relapse cases. This permits assessment of the total pool of drug-resistance in the community, the incidence of single and multiple drug-resistance and the prevailing drug pattern. It is to be hoped that as far as possible the use of the secondary drugs will be based on laboratory findings, for it is already obvious that mycobacterial strains whose susceptibility to these agents is reduced are not infrequent.

These functions can be adequately served by the existing scheme in Glasgow, and already much valuable information has been /

/ been gathered and many minor deficiencies uncovered and corrected. It is earnestly hoped that by maintaining a close liaison with the regional bacteriologists and a flexible attitude to the needs of the clinicians the laboratory may prove to be a very useful ally in their efforts to rid Glasgow of tuberculosis.

REFERENCES.

ABRAHAMS, E.W. and SILVERSTONE, H. Epidemiological evidence of the presence of non-tuberculous sensitivity to tuberculin in Queensland. Tubercle (1961) 42,487.

AGARWAL, R.L., KHANIJO, S.K. and AGARWAL, V.A. Clinical study of pulmonary tuberculosis in the aged. J. Indian Med. Ass., (1963) 40,449.

AILAN, G.W., LEES, A.W. and ROBERTS, G.B.S. A trial of ethionemide. Brit. J. Dis. Chest (1961) 55,91.

Annual Report of the Chief Medical Officer of the Ministry of Health of for the Year 1962. The State of the Public Health. H.M.S.O., London.

ARONOVITCH, M., GROSZMAN, M., BERKER, I.O. and HARLAND, W.A. Some clinical and experimental studies with viomycin.

ASPIN, J. Tuberculosis among Indian immigrants to a Midland industrial area. Brit. Med. J. (1962), 1,1386.

BARNETT, Margaret, BUSHBY, S.R.M., and MITCHISON, D.A. Isoniazid-resistant strains of tubercle bacilli. Their development and stability. Lancet (1953) 1,314.

BARRY, V.C., CONALTY, M.L., GADNEY, Ethna. Isoniazid resistant strains of Mycobacterium tuberculosis. Lancet (1953) 1, 978.

BATTAGLIA, B., KAUFMAN, I., LYONS, H.A. and MARSH, W. Toxicity of cycloserine combined with isoniazid in the treatment of tuberculosis in children. Amer. Rev. Respiratory Diseases. (1961) 83, 751.

BECK, A. Serological investigations of atypical acid-fast bacilli. J. Path. & Bact. (1961) 82, 45.

BECK, A., KEEPING, J.A. and ZORAB, P.A. Anonymous mycobacteria in man and wife. Tubercle (1963) 44, 378.

BIALKIN, G., POLLACK, Ann and WEIL, A.J. Pulmonary infection with mycobacterium kansasii. Amer. J. Dis. Children (1961) 101, 739.

BLAKE, D.H. The treatment of chronic cavitating pulmonary tuberculosis by long term chemotherapy. Tubercle, (1961) 42,438.

BÖNICKE, R. Bull. Int. Un. Tuberc. (1957), 27,151. Not consulted.
On the occurrence of acylamidase in mycobacteria. Zentralbl. Bakt. Orig. (1960), 179, 209.

BRANCH, A. A study of acidfast organisms other than mammalian tubercle bacilli isolated from disease in man. Tubercle, 1933, p. 337.

British Tuberculosis Assn. Relapse in pulmonary tuberculosis : an analysis of the fate of patients notified in 1947, 1951 and 1954. Tubercle (1961) 42, 178.

British Tuberculosis Association. An investigation of the value of ethionamide with pyrazinamide or cycloserine in the treatment of chronic pulmonary tuberculosis. A report from the Research Committee. Tubercle (1961) 42, 269.

British Tuberculosis Association. A Report from the Research Committee. Acquired drug-resistance in patients with pulmonary tuberculosis in Great Britain - a national survey, 1960-61. Tubercle, (1963) 44, 1.

British Tuberculosis Association. A report from the Research Committee. Sensitivity to human and avian tuberculin among school children in England and Wales. Tubercle (1963) 44, 119.

BROCK, J.M., KENNEDY, C.B. & CLARK, W.H., Jr. Cutaneous infection with atypical mycobacterium. Report of a case. Arch. Dermat. (1960) 82, 918.

BROWN, C.D., and CLARK, Mary E. Study of unclassified acid fast bacilli and strains of Mycobacterium tuberculosis by inoculation of the cisterna magna of guinea-pigs. J. Bacteriology, (1962), 83, 688.

BROWN, L.R., MAY, C.D., & WILLIAMS, S.E. A non-tuberculous granuloma in cats. N.Z. vet. J., (1962), 10, 7.

BUHLER, V.B. & POLLACK, Ann. Human infection with atypical acid fast organism. Amer. J. Clin. Path. (1953) 23, 363.

CANETTI, G. The eradication of tuberculosis : theoretical problems and practical solutions. Tubercle (1962) 43, 301.

CHAN, K.E. and PATHMANATHAN, T. Meningitis due to atypical mycobacteria. Med. J. Malaya (1961), 15, 113.

CHAPMAN, J.S. and GUI, L.R. Scrofula caused by atypical mycobacteria. Pediatrics, 23, 323, (1959).

CHAPMAN, J.S., DEWIETT, H.J. and POTTS, W.E. Cutaneous reactions to unclassified mycobacterial antigens. A study of children in household contact with patients who excrete unclassified mycobacteria. Amer. Rev. Respiratory Dis. (1962), 86, 547.

CHAVES, A.D., ROBINS, A.B. and ABELES, H. Tuberculosis case-finding among homeless men in New York City. Amer. Rev. Respiratory Dis. (1961) 84, 900.

CHRISTIANSON, L.C. and DEWLETT, H.J. Pulmonary disease in adults associated with unclassified mycobacteria. Amer. J. Med., (1960) 29, 980.

CLARK, Mary E. and DRISEBOIS, Phyllis R. The effect of streptomycin in vitro on cultures of Mycobacterium tuberculosis. Amer. Rev. Resp. Dis. (1962) 85, 586.

CLARKE, G.B.M. and O'REA, A.J. Chronic pulmonary tuberculosis. Treatment with ethionamide combined with cycloserine or oxytetracycline. Brit. Med. J. (1961) (1) 636.

COHN, M.L., KOVITZ, C., ODA, U. and MIDDLEBROOK, G. The growth requirements, catalase activities and pathogenic properties of isoniazid - resistant mutants. Amer. Rev. Tubero., (1954), 70, 641.

COLLINS, C.H. The classification of "anonymous" acid fast bacilli for human sources. Tubercle, (1962), 43, 292.

CORBETT, J.E. Tuberculosis amongst a small group of Indian immigrants. J. Coll. Gen. Pract. (1961) 4, 332.

CORPE, R.F. and STERGUS, I. Is the histopathology of nonphotochromogenic mycobacterial infections distinguishable from that caused by Mycobacterium tuberculosis? Amer. Rev. Resp. Dis., (1963), 87, 289.

CORPER, H.J. Virulence of tubercle bacilli isolated from the sputum. J. Infect. Dis. (1918), p. 493.

CRAIG, J.W., WILLIAMS, H. and ALSTON, J.M. Cycloserine treatment of out-patients with chronic drug resistant pulmonary tuberculosis. Tubercle (1961) 42, 7.

CROFTON, J. Tuberculosis undefeated. Brit. Med. J. (1960), 2, 679.

CROW, H.E., KING, T.C., SMITH, C.E., CORPE, R.F. and STERGUS, I. A limited clinical, pathologic, and epidemiologic study of patients with pulmonary lesions associated with atypical acid-fast bacilli in the sputum. Amer. Rev. Tubero., (1957), 75, 199.

DAVIS, S.D. and COMSTOCK, G.W. Mycobacterial cervical adenitis in children. J. Pediatrics (1961) 58, 771.

DESBORDES, J., FOURNIER, E. and ALIX, D.
Action of basic dyes on mycobacteria : effect of pH.
Ann. Inst. Pasteur (1955) 88, 120.
_____ Effect of preliminary washing with different fat solvents.
Ibid., p. 240.

DUBOS, R.J. and MIDDLEBROOK, G. Cytochemical reaction of virulent tubercle bacilli. Amer. Rev. Tubero. (1948), 58, 698.

EAGLE, H. and SAZ, A.K. "Antibiotics" Annu. Rev. Microbiology (1955), 9, 173.

EDWARDS, F.G.B. Tuberculosis incidence in the non-Australian-born. Med. J. Australia (1963) 1, 501.

ELWOOD, P.C. Tuberculosis in a common lodging-house. Brit. J. Prev. Soc. Med., (1961), 15, 89.

ENGBAEK, H.C. Pathogenicity and virulence of atypical mycobacteria for experimental animals with particular reference to pathological processes in the tendon sheaths of rabbits. Acta. Tubero. Scan., (1961) 40, 35.

ENGBAEK, H.C. and MAGNUSSON, M. A bacteriological study of the pathogenic significance of atypical mycobacteria isolated from eleven patients. Acta Tubero. Scan., (1961) 40, 1.

FEILMAN, W.H., DAVIES, R., MOSES, H.E. and ANDBERG, W. An unusual mycobacterium isolated from sputum of man suffering from pulmonary disease of long duration. Amer. Rev. Tubero., (1943), 48, 82.

FERREBEE, Shirley H. and MOUNT, F.W. Tuberculosis morbidity in a controlled trial of the prophylactic use of isoniazid among household contacts. Amer. Rev. Resp. Dis., (1962), 85, 490.

FOREMAN, H.M. King Edward VII Sanatorium Conference. Tubercle, (1962), 43, supp.

FOX, W. The chemotherapy and epidemiology of tuberculosis. Some findings of general applicability from the Tuberculosis Chemotherapy Centre, Madras. Lancet, (1962), 2, 413.

FOX, W. Realistic chemotherapeutic policies for tuberculosis in the developing countries. Brit. Med. J. (1964) 1, 135.

FOX, W., WIENER, A., MITCHISON, D.A., SELKON, J.B. and SUTHERLAND, I. The prevalence of drug-resistant tubercle bacilli in untreated patients with pulmonary tuberculosis : a national survey, 1955-56. Tubercle, (1957), 38, 71.

GERNEZ - RIEUX, C., TACQUET, A., VOISIN, C., and FABRE, M. Virulence for the guinea-pig, mouse and hamster of bacilli resistant to isoniazid; clinical observations of ill carriers of bacilli of attenuated virulence. Rev. Tubero. (1955), 19, 1.

GRIFFITH, A.H., MARKS, J. and RICHARDS, M. Low-grade sensitivity to tuberculin in school-children. Tubercle, (1963), 44, 135.

HAAPANEN, J., GILL, R., RUSSELL, W.F., Jr. and KASS, I.
Re-treatment of pulmonary tuberculosis. Experiences with various combinations of pyrazinamide, cycloserine, and kanamycin in patients excreting tubercle bacilli resistant to both streptomycin and isoniazid.
Amer. Rev. Resp. Dis., (1960), 82, 843.

Health and Welfare Services in Scotland: Report for 1962.
Scottish Home and Health Department. H.M.S.O.

HERBERT, F.J. Pulmonary tuberculosis in the old.
Lancet, (1948) 2, 247.

HEDGECOCK, L.W. and FAUCHER, I.O. Isolation of increased numbers of unclassified mycobacteria following decontamination of sputum with malachite green and crystal violet.
Amer. Rev. Respiratory Dis. (1961) 84, 710.

HOBBY, Gladys L. Primary drug resistance in tuberculosis. A review.
Amer. Rev. Resp. Dis., (1962), 86, 839.

_____ and LEWERT, T.F. Resistence to isonicotinic-acid-hydrazide.
Amer. Rev. Tubero., (1952), 65, 771.

HOLM, J. Address at XVth. conference of International Union against Tuberculosis. (1959). Quoted by CROFTON, J. (1960).

HOSTY, T.S. : personal communication, quoted by Runyon, E.H. (1959).

HUNTFORD, J.V. The "homeless" male with pulmonary tuberculosis.
Tubercle, (1962), 43, 192.

HSU, Katharine H.K., Nontuberculosis mycobacterial infections in children. A preliminary clinical and epidemiologic study.
J. Pediatrics, (1962), 60, 705.

HSU, Katharine, H.K., and JENKINS, D.E. The significance of low-grade tuberculin sensitivity. Canadian J. Pub. Health (1962). 53, 313.

Indian Council of Medical Research. Tuberculosis in India: A sample survey, 1955-58. Spec. Rep. Ser., (1959) No. 34. New Delhi. Not consulted.

Joint Tuberculosis Council. Tuberculosis in the elderly. A report.
Brit. J. Dis. Chest (1962) 56, 101.

KAMAT, S.R., ROSSITER, C.E. and GILSON, J.C. A retrospective clinical study of pulmonary disease due to "anonymous mycobacteria" in Wales.
Thorax (1961) 16, 297.

KATZ, J., KINOFSKY, S., DAMIJONAITIS, V., LAFLEUR, A. and CARON, Theresa. Effect of isoniazid upon the reactivation of inactive tuberculosis. Amer. Rev. Respiratory Dis., (1962), 86, 8.

KENDIG, E.L., Jr. Unclassified mycobacteria in children. Correlation of skin tests and gastric cultures. Amer. J. Dis. Children, (1961), 101, 749.

_____ Unclassified mycobacteria as a causative agent in the positive tuberculin reaction. Pediatrics, (1962), 30, 221.

_____ Unclassified mycobacteria. Incidence of infection and cause of a false-positive tuberculin reaction. New England J. Med., (1963), 268, 1001.

KLIGH, G.A. and PRATT, P.C. Experimental immunisation of guinea-pigs with photochromogenic acid-fast bacilli. Amer. Rev. Respiratory Dis. (1962) 85, 78.

KNOX, J.M., GEVER, S.G., FREEMAN, R.G. and WHITCOMB, Frances. Atypical acid-fast organism infection of the skin. Arch. Dermat. (1961) 84, 386.

KNOX, R., MEADOW, P.M., and WORSSAM, A.R.M. Relationship between catalase activity, hydrogen peroxide sensitivity, and isoniazid resistance of mycobacteria. Amer. Rev. Tubere. (1956) 73, 726.

KONNO, K., KURTZMAN, R., BIRD, K.T. and SHARRA, A. Differentiation of human tubercle bacilli from atypical acid-fast bacilli. Niacin production of human tubercle bacilli. Am. Rev. Tubere. (1958) 77 : 669.

Leading Article. Resistant Tuberculosis in Great Britain. Lancet (1963), 1, 1410.

Leading Article. Indices of tuberculosis. Tubercle, (1964) 45, 66.

LEWIS, A.G., Jr., LASCHE, E.M., ARMSTRONG, A.L. and DUNBAR, F.P. A clinical study of the chronic lung disease due to nonphotochromogenic acid-fast bacilli. Ann. int. Med. (1960) 53, 273.

LOGAN, W.P.D. and BENJAMIN, B. Tuberculosis statistics for England and Wales, 1938 to 1955. Studies on medical and population studies. General Register Office (1957) No. 10. H.M.S.O. London.

MACKIE and McCARTNEY. Handbook of Bacteriology, (1960), p.212. E. & S. Livingstone, Edinburgh and London.

McCUSKER, J.J. and GREEN, R.A. Generalised non-tuberculous mycobacteriosis. Report of two cases. Amer. Rev. Respiratory Dis. (1962) 86, 405.

McCUISTION, C.F. and HUDGINS, P.C. Serum electrophoresis in sarcoidosis, tuberculosis, and disease due to unclassified mycobacteria. Amer. Rev. Resp. Dis., (1960) 82, 59.

McDERMOTT, W. The chemotherapy of tuberculosis. Amer. Rev. Resp. Dis. (1962) 86, 323.

McDONALD, J.C. Factors influencing sex differences in mortality from respiratory tuberculosis in England and Wales. Brit. J. soc. Med., (1952), 6, 259.

MARKS, J. and RICHARDS, M. Classification of the anonymous mycobacteria as a guide to their significance. Monthly Bull. Ministry of Health and Pub. Health Lab. Service (1962), 21, 200.

MARKS, J. and TROTTOPE, D.R. A study of "anonymous" mycobacteria. I. Tubercle, (1960), 41, 51.

——————————— A study of "anonymous" mycobacteria. III. Ibid., 41, 133.

MARSDEN, H.B. and HYDE, W.A. Anonymous mycobacteria in cervical adenitis. Lancet (1962) (1) 249.

Medical Research Council. Long-term chemotherapy in the treatment of chronic pulmonary tuberculosis with cavitation. Tubercle (1962), 43, 201.

MEDLAR, E.M., SPAIN, D.M., and HOLLIDAY, R.W. Disregarded seedbed of the tubercle bacillus. Arch. int. Med., (1948), 81, 501.

MELLMAN, W.J. and BARNESS, L.A. Unclassified mycobacteria. A cause of non-specific tuberculin reactions? Amer. J. Dis. Children, (1962), 104, 21.

MIALL, W.E. and REERIE, J.I. The prevalence of pulmonary tuberculosis in a rural population in Jamaica. West Indian Med. J., (1962), 11, 145.

MIDDLEBROOK, G. and COHN, M.L. Some observations on the pathogenicity of isoniazid-resistant variants of tubercle bacilli. Science, (1953), 118, 297.

MITCHISON, D.A. Tubercle bacilli resistant to isoniazid. Virulence and response to treatment in guinea pigs. Brit. med. J., (1954), 1, 128.

MITCHISON, D.A. In "The treatment of pulmonary tuberculosis" Tubercle (1962), 43, Supp.

MORSE, W.C., WEISER, O.I., KUHNS, D.M., FUSILLO, M., DAIL, M.C. and EVANS, J.R. Study of the virulence of isoniazid resistant tubercle bacilli in guinea pigs and mice. Amer. Rev. Tubero. (1954) 69, 464.

MULDER, R.J. Resistance and virulence of tubercle bacilli. The clinical significance of the loss of virulence of isoniazid resistant tubercle bacilli. Proc. Tub. Res. Council. (1957) 44, 51.

MULDER, R.J. (Royal Netherlands Tub. Assoc^{n}.) Considerations on the results of chemotherapeutic treatment of pulmonary tuberculosis. Proc. Tubero. Res. Council (1960), 47, 49.

MULDER - DE JONG, MARTJE T. Resistance and virulence of tubercle bacilli. Viability and virulence of tubercle bacilli found in resected tuberculous pulmonary tissue. Proc. Tub. Res. Council, (1957) 44, 62.

MYERS, J.A. The natural history of tuberculosis in the human body. Behaviour of tuberculosis among elderly people. Amer. Rev. Respiratory Dis. (1962), 85, 232.

MYERS, J.A. Tuberculosis lurks among the aged. Geriatrics (1952) 7, 324.

NASSAU, E. and HAMILTON, G.M. Atypical mycobacteria in human pulmonary disease. Tubercle, (1957), 38, 387.

OESTREICHER, R., KUSSLER, S.M., RUSSELL, W.F. Jr., GROW, J.B. and MIDDLEBROOK, G. Observations on the pathogenicity of isoniazid resistant mutents of tubercle bacilli for tuberculous patients. Amer. Rev. Tubero. (1955) 71, 390.

OSCARSSON, P.N. Investigation of catalase and peroxidase activity of Lowenstein cultures. Relationship between enzyme activity, isoniazid susceptibility and guinea-pig tests. Acta. Tubero. Scan. (1961) 40, 51.

PAINE, T.F., Jr. and CLARK, L.S. The Effect of streptomycin on oxygen uptake and viability of resting suspensions of Escherichia coli. Science, (1953), 118, 73.

PEIZER, Lenore R., CHAVES, A.D., and WIDELOCK, D. Characteristics of mycobacterial populations found in sputum of tuberculous patients after prolonged isoniazid therapy. Amer. Rev. Resp. Dis., (1960), 82, 568.

PEIZER, L.R., MINKIN, A. and WIDELOCK, D. A further study of virulence in guinea pigs of isoniazid-resistant tubercle bacilli isolated from clinical material. Amer. Rev. Tubero., (1954), 70, 728.

PEIZER, L.R., WIDELOCK, D. and KLEIN, S. Virulence in guinea pigs of isoniazid resistant cultures isolated from clinical specimens. Amer. Rev. Tubero. (1954), 69, 1022.

PETTY, T.L. and MITCHELL, R.S. Successful treatment of advanced isoniazid- and streptomycin - resistant pulmonary tuberculosis with ethionamide, pyrazinamide and isoniazid. Amer. Rev. Respiratory Dis., (1962) 86, 503.

PINES, A. The fate of patients with drug-resistant tubercle bacilli and pulmonary tuberculosis. Brit. J. Dis. Chest, (1962), 56, 163.

PINNER, M. Atypical acid-fast micro-organism. Amer. Rev. Tubero., (1935), 32, 424.

PRISSICK, F.H. and MASSON, A.M. Cervical lymphadenitis in children caused by chromogenic mycobacteria. Canadian med. ass. J., (1956), 75, 798.

Public Health Laboratory Service. Drug resistance in untreated pulmonary tuberculosis in England and Wales during 1960. Tubercle, (1961), 42, 308.

_____ Anonymous mycobacteria in England and Wales. Prevalence and methods of identification. Ibid., 43, 432.

PUGSLEY, H.E., ALLEN, E.A., CHEUNG, O.T., COULTHARD, H.S. and GALE, G.L. Results of the treatment of tuberculosis before and since the introduction of chemotherapy. Canadian med. ass. J., (1960) 83, 424.

REIKES, H. and WASHINGTON, W. Comparative pathology of lesions produced by atypical mycobacteria. Amer. J. Clin. Path. (1962) 38, 244.

RERRIE, I.J. Review of adult tuberculosis in Jamaica. Caribbean Med. J., (1960), 22, 64.

RIST, N., GRUMBACH, F. AND LIEBERMANN, D. Experiments on the antituberculous activity of alpha-ethyl-thioisonicotinamide. Amer. Rev. Tubero. (1959) 79, 1.

Royal Netherlands Tuberculosis Association. Results in the treatment of tuberculosis in cases of resistance to INAH., streptomycin and PAS. Selected papers (1963), 5, 52.

RUNYON, E.H. The recognition and characterization of pulmonary mycobacterial pathogens other than tubercle bacilli. Bull. Un. int. Tubero. (1959), 29, 396.

RUNYON, E.H. Problems posed by unclassified mycobacteria. Amer. Rev. Resp. Dis., (1961), 84, 103.

SALIBA, A. and BEATTY, O.A. Retreatment of tuberculous patients : an experience with the combined use of high doses of isoniazid, pyrazinamide and cycloserine. Southern Med. J. (1961) 54, 10.

SCAMMON, L.A., PICKETT, M.J., ERUMAN, S., WILL, D.W. Nonchromogenic acid-fast bacilli isolated from tuberculous swine. Amer. Rev. Resp. Dis., (1963), 87, 97.

SCHIFF, J. and TARSHIS, M.S. Streptomycin in tuberculous meningitis; report of case with recovery, observed over 3 years, with unusual bacteriologic findings. Northwest Med., (1950), 49, 451.

SCHOU, C. Tuberculosis and immigration. Tubercle (1961) 42, 227.

SINGER, E. AND RODDA, Gwenda M.J. Non-specific sensitization to Old Tuberculin : the ubiquity of acid-fast organisms. Tubercle (1961) 42, 325.

SINGER, J. and CUSNER, E. Urease activity in Mycobacteriaceae. Amer. Rev. Tubere. (1952) 65, 779.

SLAVIN, P. Ethionamide in re-treatment of eleven patients with pulmonary tuberculosis. Amer. Rev. Resp. Dis. (1962) 85, 745.

SMITH, J. How chronic is pulmonary tuberculosis in the elderly? Brit. Med. J. (1959), 1, 1448.

SOMNER, A.R. and BRACE, A.A. Ethionamide, pyrazinamide and cycloserine used successfully in the treatment of chronic pulmonary tuberculosis. Tubercle, (1962), 43, 345.

SPRINGETT, V.H. Pulmonary tuberculosis - the last stages? Roy. Soc. Health J. (1960), 80, 487.

SPRINGETT, V.H., ADAMS, J.C.S., D'COSTA, T.B. and HEMMING, M. Tuberculosis in immigrants in Birmingham, 1956-57. Brit. J. prev. soc. Med. (1958), 12, 135.

STEVENSON, D.K. Tuberculosis in Pakistanis in Bradford. Brit. med. J., (1962), 1, 1382.

STEWART, Sheila M., HALL, Eileen, RIDDELL, R.W. and SOMNER, A.R. Bacteriological aspects of the use of ethionamide, pyrazinamide and cycloserine in the treatment of chronic pulmonary tuberculosis. Tubercle, (1962), 43, 417.

STIEF, M., JENNE, J. and HALL, W.H.
Not consulted.
Trans. 17th Confer. on Chemother. of Tubere. VA - Armed Forces (1958) 17, 279.

STOLOFF, I.L. Skid-row sanatorium? Arch. environ. Health, (1962), 5, 555.

THOMAS, H.E., FORBES, D.E.P., LUNTZ, G.R.W.N., ROSS, H.J.T., SMITH, J.M. and SPRINGETT, V.H. 100% Sputum-conversion in newly diagnosed pulmonary tuberculosis. Lancet, (1960) 2, 1185.

THOMAS, H.E. A tuberculosis drug-resistance register.
Tubercle, (1961), 42, 1.
_____ The incidence and fate of tuberculosis patients with drug-resistant organisms. Proc. Tub. Res. Council, (1961), 48, 1.
_____ A tuberculosis drug-resistance register : 1956-61.
Tubercle (1963) 44, 27.

THOMPSON, J.R. Pulmonary tuberculosis in the aged.
Amer. Rev. Resp. Dis., (1960), 82, 682.

TSUKAMURA, M., YAMAMOTO, M., HAYASHI, M., NODA, Y., TORII, F.
Further studies on cross resistance in Mycobacterium tuberculosis, with special reference to streptomycin-, kanamycin-, and viomycin-resistance. Amer. Rev. Resp. Dis., (1962), 85, 427.

TSUKAMURA, M. Differentiation of Mycobacterium tuberculosis from other mycobacteria by sodium salicylate susceptibility.
Amer. Rev. Resp. Dis., (1962), 86, 81.
_____ and TSUKAMURA, S. Effect of isoniazid on protein synthesis of mycobacteria. Amer. Rev. Resp. Dis., (1964) 89, 572.

UMBREIT, W.W., SMITH, P.H. and OSINSKY, E.L. The action of streptomycin.
J. Bact. (1951) 61, 595.

VAN DIJK, D. Resistance and virulence of tubercle bacilli. Resistance, catalase activity and virulence of tubercle bacilli in the laboratory.
Proc. Tub. Res. Council (1957) 44, 43.

WEED, L.A. Recurring migratory chronic osteomyelitis associated with saprophytic acid-fast bacilli : report of a case of 10 years' duration apparently cured by surgery.
Proc. Staff Meet. Mayo Clinic (1956) 31, 238.

WHITEHEAD, J.E.M., WILDY, P., and ENGBAEK, H.C.
Arylsulphatase activity of mycobacteria.
J. Path. Bac. (1953), 65, 451.

WILKINSON, G.T. A non-tuberculous granuloma of the cat associated with an acid-fast bacillus. Vet. Rec., (1964) 76, 777.

XALABARDER, C. The so-called problem of unclassified mycobacteria.
Amer. Rev. Resp. Dis., (1961), 83, 1.

YAKOVAC, W.C., BAKER, R., SWEIGERT, C. and HOPE, J.W. Fatal disseminated osteomyelitis due to an anonymous mycobacterium. J. Pediatrics (1961)59, 909.

YOUNG, R.D. Pulmonary Disease simulating tuberculosis and caused by chromogenic acid-fast bacilli. Lancet, (1955), 2, 750.