255

OBSERVATION ON THE BACTERIOPHAGE TYPING

OF MYCOBACTERIUM TUBERCULOSIS ISOLATES

FROM THE SOCIALIST PEOPLE'S LIBYAN

ARAB JAMAHIRIA

For the Degree of

Doctor of Philosophy

(University of London)

by

Mohamed Taher Elghoul

1981
Department of Medical Microbiology.
London School of Hygiene and Tropical Medicine.

TITLE:

Observation on the bacteriophage Typing of M.tuberculosis
isolates from Libya.

A B S T R A C T

Strains of Mycobacterium tuberculosis isolated from
Libyan patients suffering from pulmonary tuberculosis were
studied to determine strain variation. Susceptibility to
mycobacteriophages was chosen as the principal method. Strains
of mycobacteria were collected from a Libyan population in two
batches at an extended interval, isolates from the first batch
being made in 1976-77 and the second batch in 1979. Several
bacteriophage types were recognised in each batch. The first
group of isolates consisted principally of Type A strains of
M.tuberculosis with only a few strains of Type B or Type
Intermediate (I). The second batch, though showing high
susceptibility to bacteriophage Type A (53% of isolates) also
showed quite a high percentage to Type B (21%) and Type I
(25.8%). A considerable change in bacteriophage susceptibility
between the strains of the first and second batch was therefore
demonstrated. The change was clear-cut in Tripoli, Zawia and
Tarhuna, where both Type B and I increased. No correlation was
observed between in-vitro drug resistance of the strains of
bacteriophage types. Strain resistance to isoniazid was
observed amongst all three bacteriophage types. No correlation

was demonstrable between the strains isolated from new and previously-diagnosed cases. A small study of M.tuberculosis strains isolated from patients in the expatriate group in the region under study revealed that bacteriophage Type A and I strains predominate among Algerians, Tunisians and Chadians, while Type I is the commonest among strains from Sudanese and Pakistani expatriates. Seventy-five per cent of the strains isolated from Egyptain expatriates were found to be susceptible to the bacteriophages lytic for Type B strains. It is concluded that bacteriophage Type B is rare in most of North Africa, with the exception of Egypt, while Type A is the most common.

The increase of pulmonary tuberculosis among expatriates is suggested as a possible factor in the exchange of M.tuberculosis strains between the local population and the expatriate groups. This exchange hypothesis, formulated on the basis of the frequency change of different bacteriophage types, is discussed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.   **INTRODUCTION**

1.   INTRODUCTION

Tuberculosis is a communicable bacterial disease that still, in many parts of the world, ranks high as a public health problem. It affects man and animals, all ages and both sexes. Historically the disease was described in the work of Hippocrates, and spinal caries have been observed in the mummies of ancient Egypt. It was known among the Hindoos from 500 B.C. onwards. It has killed millions of people throughout the centuries in different countries of the world, and,with the spread of urbanisation and civilisation, it became more rife (Wilson and Miles, 1975). A steady decline in the mortality rate of tuberculosis was achieved in certain parts of Europe by improvements in hygiene and environmental conditions, by relief from overcrowding, and improvements in the nutritional status.

In the 1950s national control programmes utilised the discovery of effective chemotherapy and the availability of Bacillus Calmette Guerin (BCG) vaccine; and tuberculosis prevalence as well as incidence steeply declined in different parts of the world, especially in the developed countries. At present the exact prevalence and incidence of tuberculosis in the world is unknown, but it has been estimated (Bulla, 1977) that there are about seven million infectious cases, with a yearly incidence of about four million. In countries where national tuberculosis control programmes have been long established the incidence rate has decreased considerably

(Bulla, 1977), whereas the contrary occurred in those countries where tuberculosis control programmes were only recently set up. The increase was expected from the gradual extension of case-finding measures. For the purpose of this thesis a case of tuberculosis is defined by the isolation of M.tuberculosis or M.bovis,although tuberculosis due to M.bovis has become rare in certain parts of the world. Clinically, the differentiation between this species and M.tuberculosis is found unnecessary, but epidemiologically is of considerable importance. During the past twenty-five years it has become evident that tuberculosis-like disease due to mycobacteria other than M.tuberculosis or M.bovis are more frequent than was earlier assumed (Runyan, 1959; Wolinsky, 1979). The acceptance of these opportunistic mycobacteria as the cause of tuberculosis-like disease in man has stimulated several investigators to improve the means of identifying these different species of mycobacteria. Considerable advances have been made in the identification of these mycobacteria in recent years by simple biochemical and other tests (Stanford and Grange, 1974).

The use of certain biochemical tests - e.g. sensitivity to anti-tuberculosis drugs, and virulence tests for species identification of M.tuberculosis - has led to the recognition of subspecies variations which was difficult to distinguish by means of colonial morphology or growth characteristics on certain media.

During the 1960s many investigators used these
biochemical and virulence tests to study the epidemiology of
M.tuberculosis strains isolated from different patients from
different countries. The results of these studies have shown
that M.tuberculosis isolated from patients in Southern India
were generally less virulent than strains isolated from patients
in Britain (Bhatia et al, 1961), and were more susceptible to
the bactericidal action of hydrogen peroxide (Subbiah,
Mitchison and Selkan, 1960), and were more resistant to
thiacetezone and para-amino salicylic acid (PAS) (Joseph et al,
1964), than were British strains. Strains from Hong Kong were
more resistant to thiacetezone than were strains from Great
Britain, but were similar to indigenous British strains in
their virulence for guinea-pigs (Dickinson et al, 1963;
Mitchison and Lloyd, 1964). Since then, many other in-vitro
tests have been tried in an attempt to subdivide M.tuberculosis
strains, examples being lipid analysis by gas chromotography
(Larsson and per-Anders Mardh, 1976), susceptibility to
mycobacteriocins (Takeya and Takiwa, 1974), and susceptibility
to mycobacteriophages.

Gas chromotography was found unsuccessful for the
subspecification of M.tuberculosis (Larsson and per-Anders
Mardh, 1976). Mycobacteriocin typing methods for M.tuberculosis
were reported (Takeya and Tokiwa, 1974), but were criticised
as being impossible to repeat with precision (Grange and Redmond,
1978). The use of mycobacteriophages in species identification
were recognised as valid, but, because of the development of

biochemical techniques which showed more precise characterisation
of mycobacteria, the emphasis on the use of bacteriophage typing
moved towards the subdivision of recognised species, notably
M.tuberculosis, for epidemiological purposes.

The value of mycobacteriophages in epidemiological studies
was demonstrated by Baess (1966), Bates and Fitzhugh (1967) and
Bates and Mitchison (1969). Three bacteriophage types of
M.tuberculosis have been recognised, plus an Intermediate type.
Investigators, (Baess, 1969; Bates and Mitchison, 1969; Grange
et al, 1978; and Rado et al, 1975), have found bacteriophage
typing techniques to be the most consistent and reliable despite
in-vivo changes in drug susceptibility, animal virulence, or
biochemical criteria such as catalase production. Susceptibility
to bacteriophage action appears to be a property of real
consistency and as stable as any other inherited trait.

For these reasons bacteriophage typing was chosen to
study the strain variations of M.tuberculosis isolated from
Libyan patients suffering from pulmonary tuberculosis. The
study will comprise the frequency distribution of the bacterio-
phage types, the stability of that frequency, and the analysis
of the results in relation to certain influences such as the
increase in the rate of incidence of tuberculosis among
expatriates in the last few years, and the role of these ex-
patriates in contributing to strain variations.

2.   <u>SOCIALIST PEOPLE'S LIBYAN ARAB JAMAHIRIA (LIBYA)</u>

2. LIBYA

2.1 THE LAND

2.1.1 SITE: The Country lies on the North Coast of Africa,
along the mediterranean seaboard, between the latitude 20° and
33°N and longitude 10° and 25°E. It is bounded by Tunisia and
Algeria on the west, by Egypt and Sudan on the east and south
east, and on the south by Chad and Niger (Fig.1). No border is
less than 1,000 km. in length (612.4 miles).

2.1.2 AREA: The total area of Libya is 1,759,540 sq.km.
(679,182.44 sq.miles). It is nearly twice as large as Egypt and
fourteen times larger than England. The Western region has an
area of 260,000 sq.km. (100,360 sq.miles).

2.1.3 TOPOGRAPHY: Traditionally the country is divided into
three regions, namely, Western, Eastern and Southern (Fig.1).
Topographically it is divided into a region of Steppe along the
coast, a desert region in the south and a transitional region
in-between. The coast aligned on an east-west course is broken
by the broad gulf of Sirte, which indents the land. One-third
of the Western, and three-fourths of the Eastern regions are
desert. The Southern Sahara region makes up four-fifths of the
total area of the country. The relatively fertile parts are the
coastal strip, the Highlands in the Eastern and Western regions,
and a few oases in the South. There are no permanent rivers,

FIG. 1

MAP OF LIBYA SHOWING ITS LOCATION, THE THREE TRADITIONAL REGIONS, THE MAIN CITIES AND THE TUBERCULOSIS CONTROL CENTRES

KEY

A = The Western Region

B = The Eastern Region

C = The Southern Region

◯ = Main cities

✖ = Regional tuberculosis control centres

• = Peripheral tuberculosis control centres

1 = Tripoli	7 = Zwara
2 = Kohms	8 = Tarhuna
3 = Zliten	9 = Garian
4 = Misurata	10 = Yefrin
5 = Sirte	11 = Nalut
6 = Zawia	12 = Gadamus

1: 11400 00
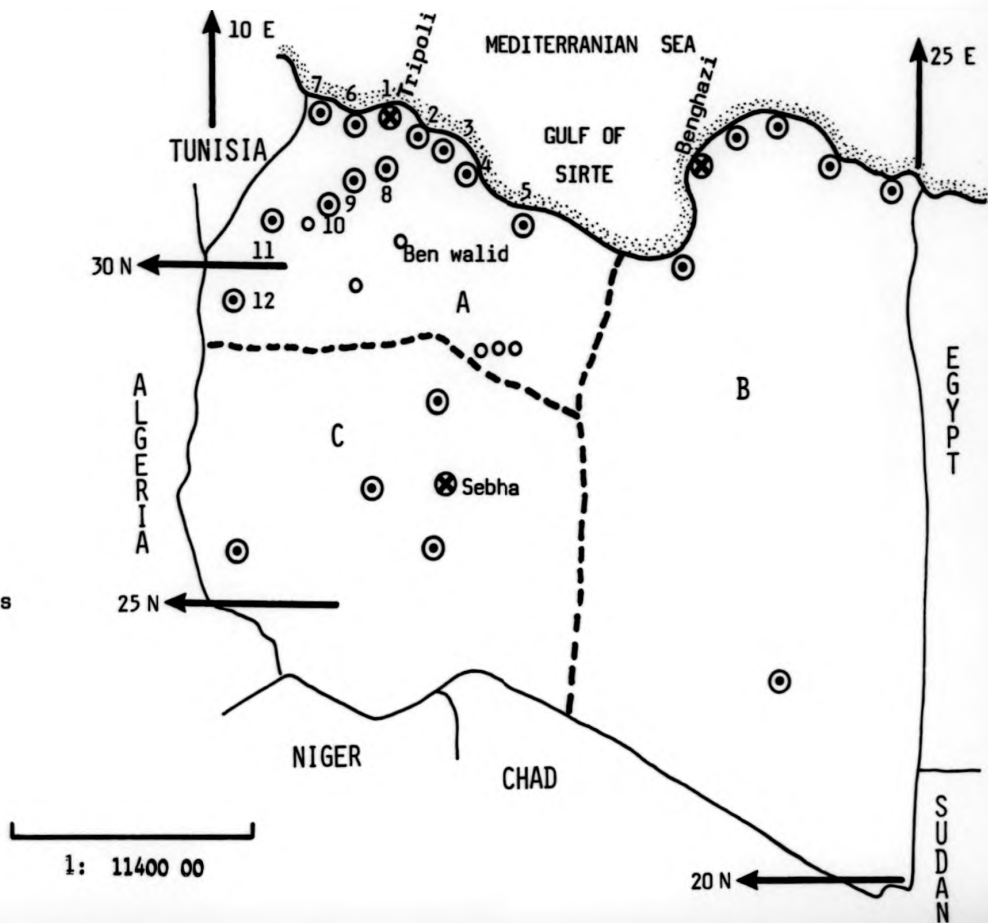
but wadis flood during the rain and remain flooded for a few days. The source of water is the underground water and the rain during the winter.

2.1.4 CLIMATE: The climate in the north is temperate. The average minimum temperature during the month of January (1971-1975) was 4.4^{o}C. The average maximum was 42.7^{o}C in the month of June for the same period as recorded at Tripoli meterological station. The average annual rainfall for the whole country is 285mm (11.115 inches). It occurs sporadically over 30-40 days in the year and mostly during the months of November to January. In the Southern Sahara Region, the temperatures are more extreme than those along the coastal belt, with little or no rainfall. The average relative humidity at midday on the coast is approximately 55% and decreases steadily southwards.

Because of the low rainfall and lack of vegetation, dust and sand storms are not infrequent, and hot, dry sand-laden winds (giblis) are common during the summer months and even occur during the winter. These giblis limit physical activities on account of the extreme conditions of heat and discomfort.

2.1.5 SOCIO-ECONOMIC PATTERN: Prior to the petroleum discovery of 1956 the majority of the people were practising animal husbandry and primitive agriculture. Later especially after the September Revolution in 1969 the involvement of the people in mechanised agriculture, the petroleum industry,

building and construction, public utilities and other small-scale industries increased. According to the 1973 census, the population of ten years of age and over numbered 1,260,075. Thirty per cent of them were economically active; 26% in agriculture, forestry, hunting and fishing; 36% in community, social and personnel services, and less than 2% in mining and quarrying. Several agricultural projects are currently being extended throughout the fertile and semi-fertile areas of the country.

Fifty-one per cent of the national population over ten years of age were illiterate. Ninety-two per cent of the total households were regarded as permanently settled. This was attributed to the widely distributed agricultural and industrial projects. Urbanisation is incomplete and still forty per cent of the population were recorded as rural.

Developments in agriculture and industrialisation have been followed by improvements in other aspects of life, such as housing, education and health services.

Every household is eligible for modern housing and free education up to and including University level.

2.2   POPULATION

2.2.1   POPULATION ORIGIN: Historically the country is central and accessible, and it was on important trade routes. It was successfully invaded by Egyptians, Phoenicians, Greeks, Romans,

Vandals, Byzantines, Normans, Arabs, Spaniards, Knights of
Malta, Turks, Italians and finally was governed under mandate
or treaty by Americans, British and French. The invasions
were largely confined to the fertile coastal strip, and left
little lasting mark except for the eleventh-century invasion
of the Arabs from Arabia. They brought a patriarchal, nomad,
pastoral culture, and the beliefs and moral code of Islam,
which were rapidly adopted by the autochthonous population.
It may be said that modern Libyans are mainly descended from
the aboriginal population and the immigrant Arabs, and that
their life is moulded by Islam (see Wallace, 1976).

2.2.2   POPULATION STATISTICS: There have been three census
years since the country's independence in 1951; namely 1954,
1964 and 1973. The counts were taken on the 31st July. The
total population figures of 1954, 1964 and 1973 were (1,088,889),
(1,564,369) and (2,249,222) respectively. That estimated for
the 31st July, 1976, was (2,500,000), an increase of one-third
between 1964 and 1976. Table 1 demonstrates the increase in
total and Libyan-national population from 1954 to 1975.

The population density per 100 sq.km. (38.6 sq.miles)
was 62 in 1954, 89 in 1964 and 129 in 1973. Of particular
relevance to this study is the fact that the Western region
population increased from 730,000 in 1954, to 1,444,000 in 1973,
while its population density increased from 412 in 1964, to
590 in 1973.

Table 1: Population estimates for Total and Libyan Nationals for the years 1954-1975

	Population estimates as on 31st July			Population estimates as on 31st July	
Year	Total	Libyan National	Year	Total	Libyan National
1954	1,008,889*	1,041,599*	1965	1,628,773	1,567,443
1955	1,129,000	1,082,000	1966	1,695,828	1,621,145
1956	1,171,000	1,142,000	1967	1,765,645	1,676,698
1957	1,214,000	1,168,000	1968	1,838,335	1,743,154
1958	1,259,000	1,213,000	1969	1,914,018	1,793,579
1959	1,305,000	1,260,000	1970	1,992,817	1,855,041
1960	1,353,000	1,308,000	1971	2,974,861	1,918,609
1961	1,403,000	1,359,000	1972	2,160,281	1,984,355
1962	1,455,000	1,412,000	1973	2,249,222	2,052,357*
1963	1,509,000	1,467,000	1974	2,341,818	2,122,683
1964	1,564,369*	1,515,501*	1975	2,438,229	2,195,422

* Census results   NOTE: Population estimates are based on separate growth rates for Libyan
National and for Total Population. Source: Statistical Abstract
in Libya (1975).

According to the 1973 census 20.75% of the national population was under five years of age; more than a half was under fifteen years of age, and more than four-fifths were under fifty years of age (Fig.2).

According to the 1976 estimates the male percentage was 53.7% and the female was 46.29% respectively. The average family size was 5.8 individuals.

The changes of crude birth and death rates from 1966 to 1975 is shown in Table 2. The death rate in the 1976 estimate was seven per thousand and the birth rate 47.7 per thousand. This change is greatly influenced by the improvement of hygiene and sanitary conditions, and improvements in and coverages of the health services which contributed to the reduction in infant mortality.

2.2.3   POPULATION CHANGE: The change in the population (Table 1) has been influenced by many factors. The return of many nationals who left the country during the second world war; the increase of birth rate, and the decrease of the death rate, especially infant mortality rate. Population data is markedly influenced by the increase in number of immigrants, especially labourers, who are attracted by the possibilities of gainful employment in the large developmental projects, begun during the early 1970s. As shown in Table 3, the majority of these immigrants

FIG. 2

A HISTOGRAM OF NATIONAL LIBYAN POPULATION DISTRIBUTED BY AGE ACCORDING TO THE 1973 CENSUS
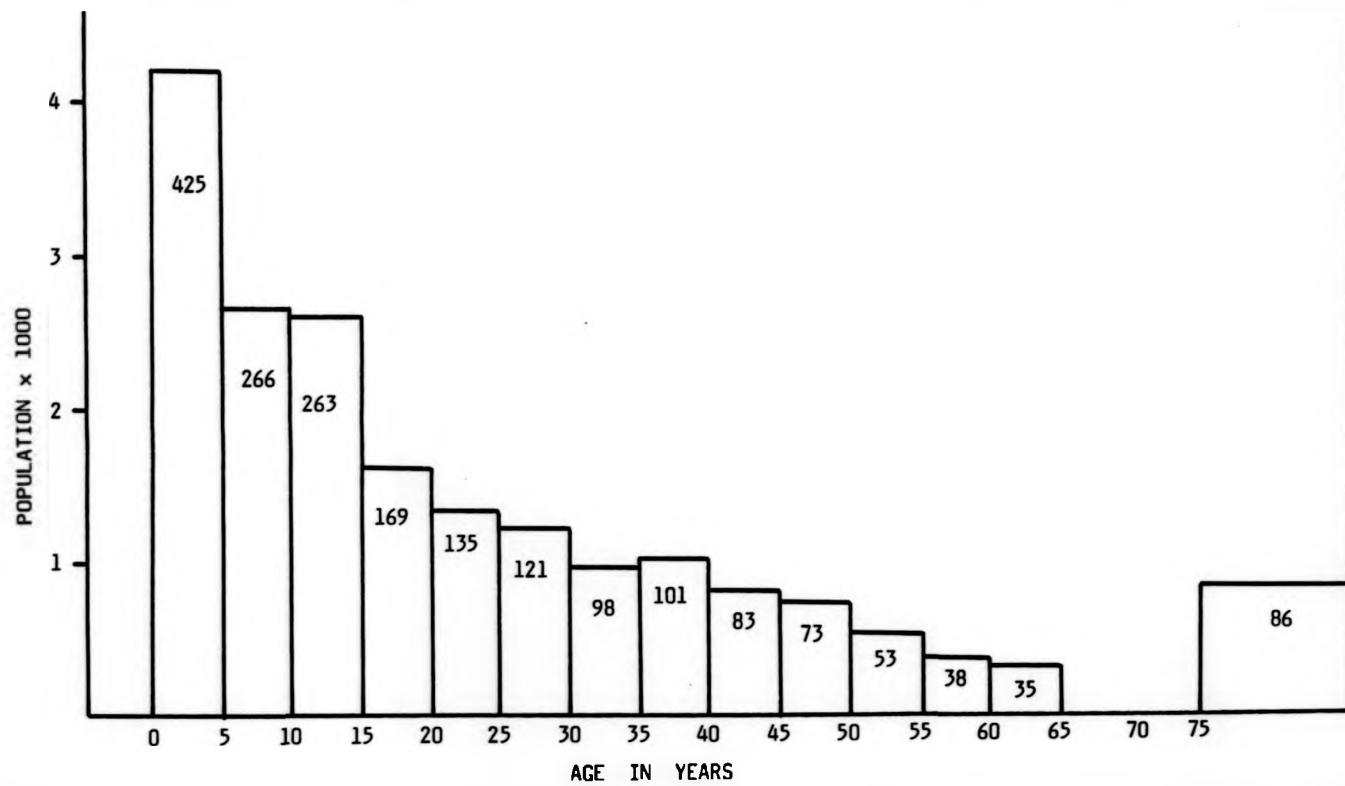
Table 2: Crude birth rate and crude death rate in Libya:
          (1966-1975)

Year	Crude birth rate*	Crude death rate*
1966	34.2	5.6
1967	37.5	6.2
1968	35.3	4.9
1969	42.1	7.2
1970	41.3	7.6
1971	46.5	8.1
1972	46.6	9.1
1973	46.9	8.7
1974	48.1	8.2
1975	47.7	7.O

* per thousand

Source: Statistical Abstract in Libya (1975)

Table 3: Arabs and Asians residing in Libya according to
type of residence and broad nationality groups
up to 20th December 1975.

Nationality Group	Special residence with work	without work	Temporary residence with work	without work	Total
Arab Countries*	152	184	224,635	55,023	279,994
Africa	–	2	569	611	1,182
Asia	10	21	7,910	2,420	10,361
Europe	566	570	24,751	5,158	31,045
U.S.A.	–	2	2,173	848	3,023
Canada	2	–	254	91	347
South Africa	–	–	83	78	161
Oceania**	–	–	83	70	153
U.S.S.R.	–	–	103	76	179
Not Stated	1	2	14	7	24
Total:	731	781	260,575	62,382	362,469

* Arab Countries: Countries where the official language is
Arabic, such as Egypt, Tunisia, Syria, etc.

** Oceania: Australia and New Zealand.

Source: Statistical Abstract in Libya (1975)

were from officially Arabic-speaking countries. They are mainly
male, of low socio-economic class. They live with the national
population, mainly labourers, in over-crowded areas, and probably
contribute to a reduced observance of hygienic standards.

2.3   ADMINISTRATION

The country traditionally is divided into three
regions, the Western, Eastern and Southern regions. Administrat-
ively each region was divided into districts or governorates but
recently the country has been divided into a number of municip-
alities. Each municipality is governed by a local popular
committee or governorate chosen by the people resident within the
boundaries of that municipality. The committee conforms to the
national council decisions in certain functions of service. In
each committee of the municipality there is a representative
member of the Ministry of Health who is responsible for the health
services in the area of the municipality. Coterminosity is
mandatory, in contrast with some other countries (e.g. United
Kingdom) where a national health service exists.

The Ministry of Health has an under-secretary and eight
general departments (see Fig.3). Each department is headed by
a Director General. The Community Health Department is composed
of four sections; one of these being the communicable diseases
section, which consists of epidemic and endemic disease units.

Fig. 3: Organisation of Ministry of Health, Libya.
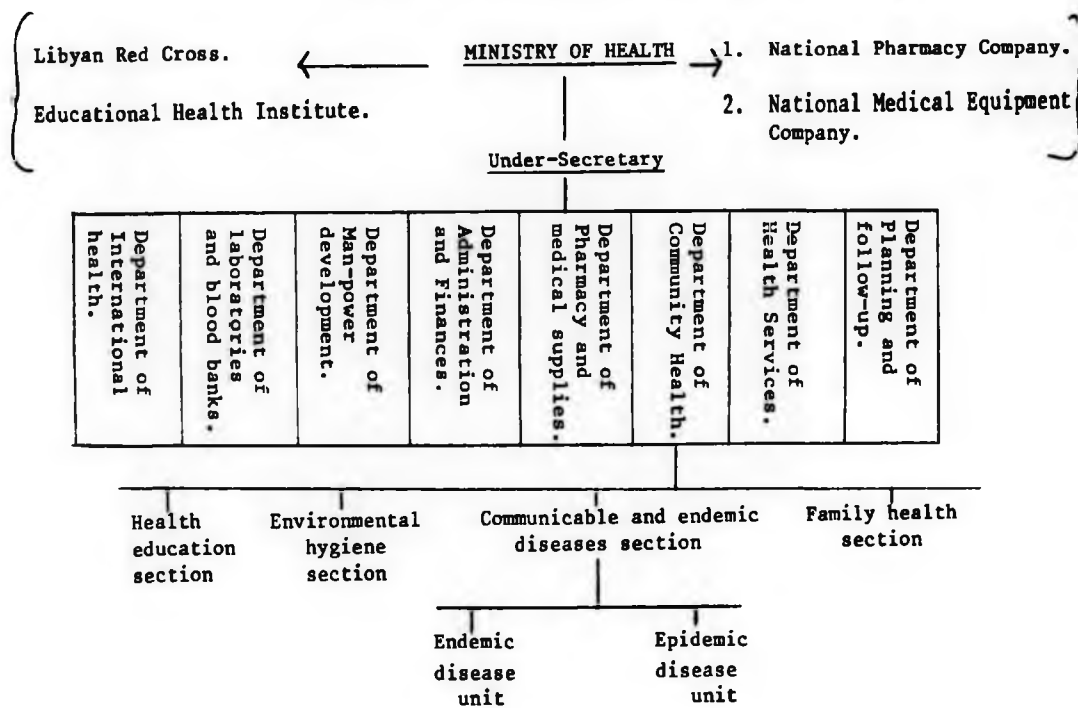
The latter unit is principally concerned with tuberculosis,
leprosy, malaria, schistsomiasis, cutaneous leishmaniasis and
other parasitic diseases.

According to the Ministry of Health Annual Statistical
Report (1978) the number of medical doctors has increased from
1,025 (1972) to 3,838 (1978)(see Table 4), and the ratio of
medical doctors in 1978 was one per 732 of population. There is
also an increase in the number of nursery staff. The ratio of
nurses in 1978 was one per 446 individuals. Libyan nurses and
midwives represent 27% of the total, while Libyan assistant nurses
and health visitors comprise 94% of the total. The total staff
in the health services in 1978 was 16,866, 52% being Libyan
nationals. In Libya in 1978 there were 1,447 treatment units
(Table 5). These include polyclinics, dispensaries and other
health service clinics without beds for in-patients. They are
scattered according to the population density in each district
and municipality branch. The number of hospitals, general and
special, for the same year 1978 was sixty, supplying a total of
13,418 beds. The ratio is 4.7 beds per 1,000 individuals. These
hospitals were furnished with modern medical equipment and
facilities. The regional polyclinic (a large modern out-patients
facility) will be one of the main components of the medical system
in Libya. It is intended that they provide all necessary out-
patients services and will replace the dispensaries and other
health services' clinics except in relatively remote regions of
the country. The Ministry of Health in 1970 announced plans to
educate and train Libyan nationals to take an increased part in

Table 4:   The development of medical staff between 1972
           and 1978 inclusive in Libya.

Years:	Doctors medical	Nurses male and female	Assistant nurses	Health visitors
1972	1,025	2,736	1,820	262
1973	1,736	3,160	3,322	309
1974	2,063	3,615	3,309	333
1975	2,085	4,059	3,972	367
1976	2,558	4,129	4,884	452
1977	3,350	4,705	6,099	553
1978	3,838	4,648	6?436	469

Source: Annual Statistical Report: Ministry of Health,
          Libya (1978).

Table 5: Number of Nursery Schools, Clinics and Hospitals

(1978) in Libya.

Item present in 1978	Numbers	Remarks
Assistant Nursing Schools.	23	Accept primary school graduate. Period of study 18 months. 3,178 have graduated between 1972-78.
Qualified Nursing Schools.	7	577 graduated between 1972-78. Preparatory school graduates accepted.
Polyclinics, Dispensaries, and other Health Services Clinics.	1,447	Without beds for in-patients.
Hospitals		
General	37	Patients attended these
Special	23	60 hospitals in 1978 = 275,551

There are other institutes for graduation and training in health

inspection, laboratory technicians, radiology technicians and

Pharmacy.

the different branches of the health services. Accordingly,
many schools and institutions were constructed to train nurses,
assistant nurses and technicians.   In the Ministry of Health
Annual Statistical Report (1978) it was stated that there are
23 assistant nursery schools, seven nursery schools and other
institutions for technicians already operative (Table 5).   In
addition to nursery and technical schools there are two medical
schools, one in the Eastern region, at Benghazi; and the other
in the Western region at Tripoli.   Graduation from Garyunis
Medical School started in 1976, and from Tripoli Medical School
in 1979.   The total number of graduates from both schools
between 1976-1979 inclusive was approximately 300.   Many of
these graduates are now studying in medical institutes abroad.

3.   <u>**TUBERCULOSIS IN LIBYA**</u>

3.   TUBERCULOSIS IN LIBYA

3.1   GENERAL HISTORY

Tuberculosis was documented (Kanter, 1967) as prevalent
in 1887 in the coastal district of Libya's Western Region, but
it was found hardly to exist in the countryside. Eighty-eight
cases were reported in 1912 in the old city of Tripoli; and a
few cases in other parts of the Western Region. An investigation
in the 1930s in 72 different places in the Southern Region
revealed only two cases and few fibrotic forms.

It may be inferred that tuberculosis in Libya had
little chance of spreading in such a small community scattered
over such a vast territory. However, during and after the
Second World War which ravaged the country, it is thought that
the prevalence of tuberculosis increased much in the same way as
occurred in Scandinavian countries and Holland during the
occupation in the Second World War, or in Britain during both
world wars (Wallace, 1976).

The industrial revolution of the 1960s then disturbed
the free-living nomads. Hitherto socially-stable population
groups, especially from the Southern Region, moved into towns
and cities, particularly into the capital city, Tripoli, where
they lived in unhygienic conditions. As happened elsewhere in
such circumstances, the prevalence of tuberculosis increased
sharply, especially in the Western Region, Tripoli. One national

response was that in 1953 a BCG-vaccination campaign was
conducted throughout the country. Following this the death rate
decreased, but the number of tuberculosis cases increased,
especially among males (Lollini, cited by Kanter, 1967).

3.2   PREVALENCE OF TUBERCULOSIS

The first survey to assess the disease situation was
conducted in 1959 in the Eastern Region of the country (Newman,
1961). The survey was carried out on nine random samples each
of 300 people. The investigations included: tuberculin skin
testing, X-ray examination, and, where there was thought approp-
riate, microscopic smear examination of sputum. The prevalence
of tuberculosis based on finding M.tuberculosis was 1.8%.
This figure applies only to defined groups and cannot properly
be extrapolated and extended to the whole country, especially to
a country as vast as Libya, where conditions and circumstances vary
so widely. Nevertheless, the experience of the Central Anti-
tuberculosis Clinic, in Tripoli, where about 36,000 persons were
examined annually, led to the conclusion that the prevalence of
active tuberculosis amounted to 1.5% (Kissopoules, see Bland et
al, 1967). This conclusion was conceded in 1965 in a meeting in
Tripoli of participant doctors. Kadiki and Ashraf (1972) stated
"during the year 1968, at the start of the Tuberculosis Control
Programme there would have been no less than 20,000 cases even if
we take the lowest estimate of bacillary excretors".

3.3   PREVALENCE OF TUBERCULOSIS INFECTION

An investigation on the prevalence of tuberculosis
infection was carried out in 1954 in Ben Walid, a remote,
isolated and settled community in the Western Region in Libya,
when approximately 1,300 persons were examined (Nyboe, 1960).
The results revealed a high prevalence of tuberculosis infection:
19% amongst the 5-9 years age group, and 80% of those above 20
years of age, reacted to the tuberculin test by a measured
reaction zone of 10mm or more. In the 1959 Eastern Region Survey
the results showed a high level of infection in all age groups.
The proportion of persons reacting to the tuberculin test (10mm
or more) was 22% among the 5-9 years age group, and 73% in
those above 20 years of age.

During 1969-71 tuberculosis infection surveys were
carried out on groups of school children in the cities of
Tripoli and Benghazi. The results gave an indication of lower
prevalence of infection than was previously experienced.
Reactions of 10mm or more were found in only7-8% among the 6-9
years age groups. The results of the three surveys are shown
in Table 6.

3.4   THE TUBERCULOSIS CONTROL PROGRAMME (TCP)

Bland et al (1967) stated that until the early nineteen
sixties, the tuberculosis services in the country comprised
nineteen doctors, and only three tuberculosis clinics at Tripoli,

Table 6: Prevalence of tuberculosis infection in different

age groups observed in surveys 1954, 1959 and

1969-1971 in Libya.

Age Group in years:	1954	1959	1969-1971
	Ben Walid	Eastern Region	School children in Tripoli and Benghazi
5-9	19%	22%	7-8%
Above 20	80%	73%	-

Benghazi and Zawia, in co-operation with two specialised tuberculosis hospitals, one in Tripoli and another in Benghazi, 312 beds in all. No routine attempts were made at case-finding; hospitalized patients commonly left before completion of their treatment, despite the prevailing knowledge that inadequate treatment tends to render M.tuberculosis drug resistant.

In 1963 a tuberculosis control programme was started as a pilot project in the Eastern Region, and extended towards country-wide coverage from 1970 onwards.

3.4.1   THE TCP ORGANISATION AND STAFF: The Community Health Department (Fig.4) has the overall responsibility for the planning and evaluation of the TCP, and the Endemic Diseases Section directly supervises the activities of the TCP. The Directors of the Regional Tuberculosis Control Centres (RTCC) at Tripoli, Benghazi and Sebha, have responsibility for the technical and operational aspects of TCP in the Western, Eastern and Southern regions respectively. The activities of the entire TCP are assessed by a Central Committee, which is headed by the Director General of the Community-Health Department and membered by the three directors of the RTCC and the four directors of the Tuberculosis and Chest Disease Hospitals (TCDH) with the chief of the Endemic Disease section as the Committee's Executive Secretary.

Figure 4:   Organisation of Tuberculosis Control Programme, Libya.

MINISTRY OF HEALTH

Director General of Community Health Department

Control Tuberculosis Unit

Central Tuberculosis Committee ————————→ ←———————— Endemic Disease Section

Regional level

| Director RTC SEBHA | Director RTC Benghazi | Directors Tuberculosis Hospitals | Director RTC Tripoli |

← R Technical Committee   ← R Technical Committee   ← R Technical Committee

District level

| Director TCCS | District Director TCSS | | Director TCSS |

Under SEBHA: Sebha, Murzuk, Brak, Obari, Ghat

Under Benghazi: Benghazi, Kufra, Ajdabia, Almirj, Beida, Derna, Tobruk

Under Tripoli: Tripoli, Gadames, Nalut, Zleiten, Sirte, Misurata, Khoms, Tarhuna, Gharian, Yefren, Zawia, Zawara

RTC = Regional Tuberculosis Centre
TCCS = Tuberculosis Control Centre
Under RTC Benghazi = Tuberculosis hospitals Shahat and Kuwaifia
Under RTC Tripoli = Tuberculosis hospitals Abusitta and Misurata

Three regional committees were established, each with the respective Regional Director as Chairman, and the Directors of the Tuberculosis Control Centres (TCCS) and chest hospitals in the region, as members.

The TCP covers the whole country (Wallace, 1976) by means of a network of twenty-four TCCS: (see Fig.1 and 4). Twelve in the Western Region; seven in the Eastern Region, and five in the Southern Region. Each TCCS employs at least one tuberculosis specialist, a number of bacteriology technicians, X-ray technicians, one or more home visitors and administrative staff. There are 597 beds in four tuberculosis hospitals. The Ministry of Health Annual Statistical Report (1978) showed that 1,935 patients entered the hospitals for an average of 60 days, and that bed occupancy averaged 70% (see Table 7).

Libya has sufficient economic resources for its medical services; however, it has had to rely to the extent of nearly 90% on expatriate medical and paramedical personnel of different nationalities and training, different professional backgrounds and exhibit various degrees of commitment to the TCP (Khalil and Sathianathan, 1978).

3.4.2   THE TCP ACTIVITIES

CASE-FINDING: This was started by indiscriminate mass miniature X-ray (MMR) examination and suspects were examined

Table 7: Tuberculosis Hospital Activities in 1978 (Libya)

Tuberculosis hospital	Number of beds	Number of patients in	Number of patients out	Number of patients died	Percentage died	Percentage beds occupied	Average patients residence day
Abusitta Tripoli	172	476	471	16	3.4	72.7%	80.6%
Kuwaifia Benghazi	150	452	425	5	1.1	70.8%	68.7%
Misurata	105	509	504	12	2.4	91.7%	63.2%
Shahat	170	498	486	6	1.2	46.7%	62.2%
Total:	597	1,935	1,886	39	2%	70%	69%

Source: Annual Statistical Report (1978)

bacteriologically by direct smear followed by culture (Gebreel and Ashraf, 1974b). This indiscriminate MMR was later discontinued (Annual Report of TCP in Libya 1975, 1976) and replaced by X-ray screening of those persons who reported to the TCCS because of their symptoms, those referred by other institutions, and those who were obliged to attend TCCS for health clearance certificates. Subjects showing radiographic abnormality suspected as being tubercular were investigated bacteriologically. Sputum specimens were subjected to both microscopic smear examination and bacteriological culture on Lowenstein-Jensen medium.

TREATMENT: As previously mentioned, 90% of medical and para-medical personnel were of different nationalities. Despite this difficulty, standard treatment was implemented throughout the country in 1971. Treatment regimens recommended were: streptomycin (SM), isoniazid (INH), para-aminosalicylic acid (PAS) as the first line for all cases of tuberculosis; pyrazinamide (PR), cycloserine (CYC) and ethionamide (ET) as the second line, and ethmabutal (EB) and rifampicin (RF) with or without other drugs as the third line. From 1973 onwards, the availability of the anti-tuberculosis drugs was restricted to designated specialized tuberculosis services, and treatment was conducted for 18-24 months by these special tuberculosis services. New regimens were proposed in 1976 (Stylbo, 1976) for considerations as shown in the Table overleaf.

THE PROPOSED NEW REGIMENS

	Initial course of treatment	Follow-up course of treatment
First line drugs:	SM or (ET) + INH + EB or (PAS)	RF + INH
		or
Second line drugs:	RF + PR + Cyc	RF + EB

The second line drugs will be given if the first line drugs have been compromised by previous treatment or in event of failure, the first or second line drugs would be given for three months when the results of sensitivity tests will be known. Thereafter treatment can be continued as in the above table for another 9-12 months according to the results of the sensitivity tests for the first line drugs.

BCG VACCINATION: The stated policy of BCG vaccination is to vaccinate as many people as possible, primarily younger age groups (below 20 years). BCG is given to the new-born (obligatory under a decree issued in April 1972) during the first month of life (Gebreel and Ashraf, 1974a). It is also given to children at school entry, contacts of tuberculosis patients (including staff working in tuberculosis institutions), to those attending TCCS and found to be tuberculin negative, and to contacts of

patients with leprosy (Ashraf, see Wallace, 1976). The 1972 BCG vaccination law has recently been modified so that all children who do not bear a scar at school entry are vaccinated. Further, all school children are vaccinated when they leave school at thirteen years of age. The last provision has been made because the incidence of pulmonary tuberculosis rises sharply in the 15-44 age group. Of patients diagnosed during 1973, 22% were aged 15-24 years, and 49% were aged 25-44 years (Gebreel and Ashraf, 1974b). The vaccination is practised at maternity and child health clinics, TCCS, and on location during special vaccination campaigns.

3.4.3   THE IMPACT OF TCP

INCIDENCE: As shown in Table 8 and expressed diagramatically in Figures 5 and 6, the number of pulmonary tuberculosis cases registered every year from 1973 to 1978 has decreased in the total population from 1,375 cases to 839 cases, and the incidence rate in the total population has declined from 0.6 per thousand in 1973, to 0.3 per thousand in 1978. The incidence rate in the local national population has decreased from 0.50 in 1973, to 0.28 in 1976. Pulmonary tuberculosis cases registered in the whole population during the period 1971-77 were 8,390. The number registered for the same period in the Western Region was 5,246 (Table 9), of which 78% (66% males) were Libyan nationals. The number of cases has been decreasing among nationals from 743 in 1971, to 380 in 1977.

Table 8: The yearly incidence of new cases of pulmonary
tuberculosis in Libya from 1966-1978 inclusive.

| Year | Total Population | | Libyan National |
	No. of cases	Incidence rate*	Incidence rate*
1966	3,482	2.1	-
1967	4,117	2.3	-
1968	3,341	1.8	-
1969	1,905	1.0	-
1970	2,685	1.3	-
1971	1,178	0.6	-
1972	1,246	0.6	-
1973	1,375	0.6	0.5
1974	1,299	0.55	0.4
1975	1,245	0.5	0.3
1976	1,136	0.43	0.28
1977	911	0.33	-
1978	839	0.30	-

Source: Annual Statistical Report: Ministry of Health,
Libya (1978).

* per thousand.

FIG. 5

THE INCIDENCE PER THOUSAND OF PULMONARY TUBERCULOSIS IN LIBYA FROM 1966 - 1979
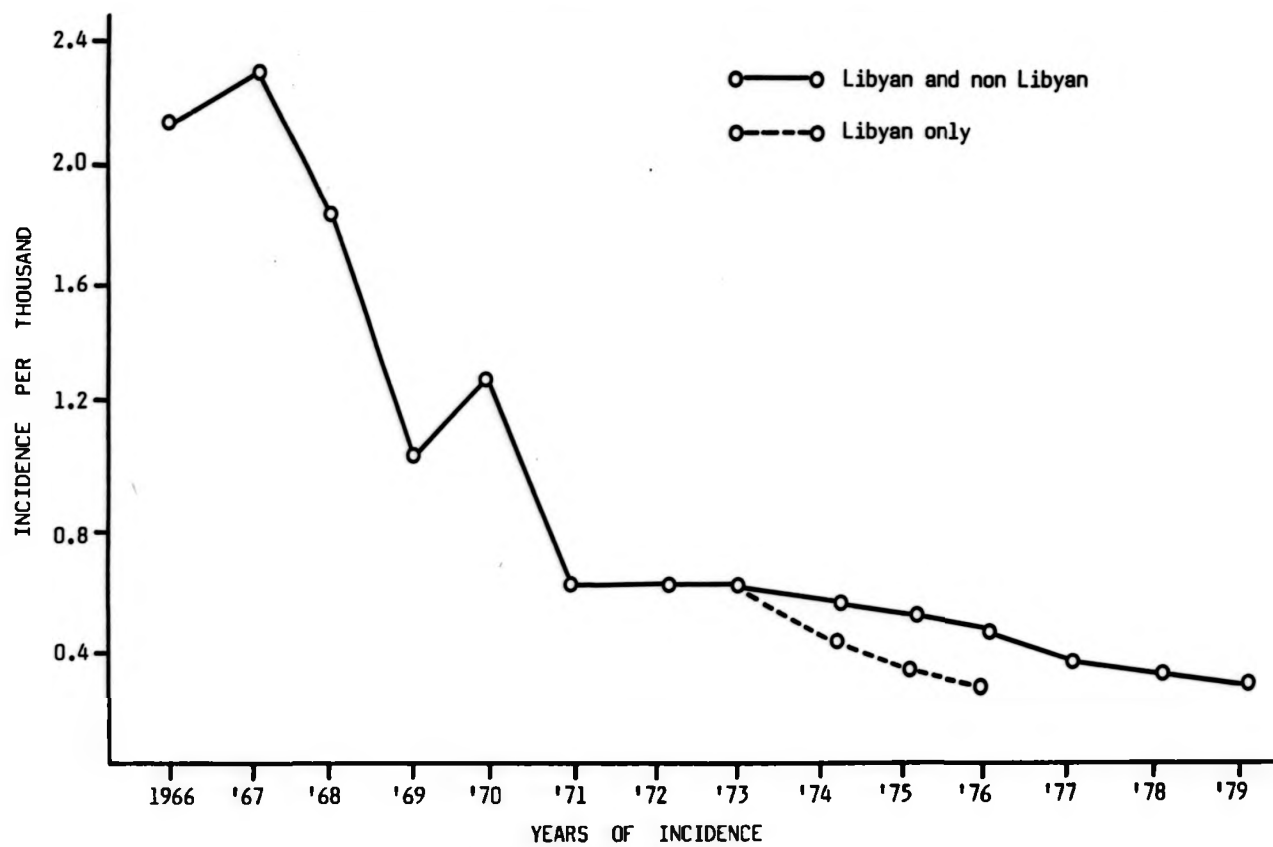
FIG. 6

THE NUMBER OF CASES OF PULMANARY TUBERCULOSIS IN NATIONALS AND EXPATRIATE FROM 1972 - 1978 IN LIBYA
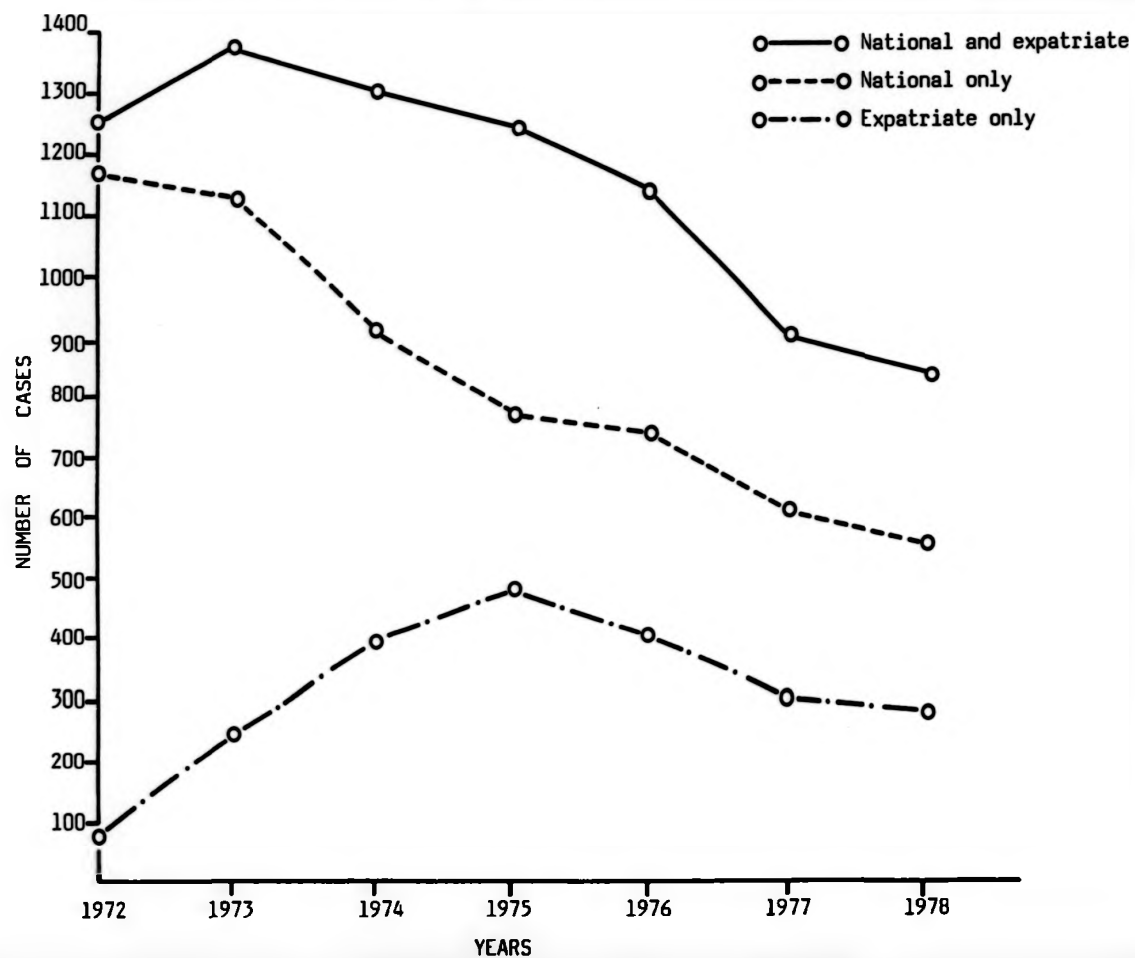
Table 9:   Pulmonary tuberculosis cases registered during
           1971-1977 in the Western Region of Libya.

Year	Local	Expatriate	Total
1971	743	5	748
1972	818	17	835
1973	676	122	798
1974	600	223	823
1975	434	304	738
1976	465	249	714
1977	380	210	590
Total:	4,116 (78%) 97% male	1,130 (22%) 97% female	5,246 (100%)

Source: Rizgalla and Hashmi (1978)

The number of cases registered in Tripoli TCC for the same
period (1971-77) was 1,715. As shown in Figure 7, 68% of these
cases were male, and 32% female; the highest incidence being in
the 20-30 years age group.

PREVALENCE RATE: In 1977, the government, through the
Central Tuberculosis Committee, and with collaboration of WHO,
carried out a prevalence survey on a national scale to assess
the tuberculosis situation and the TCP impact on the population,
(Husain and Thorup, 1978). The results of the survey demonstrated
that tuberculosis in Libya shows a downward trend and has already
reached a low level when compared with other developing countries.
The prevalence of bacteriologically-confirmed cases related to the
general population was approximately 0.9 per thousand. The number
of cases diagnosed by the tuberculosis services, mentioned in the
Incidence, has shown a downward trend in the past few years, reaching
a level of 0.33 per thousand population for 1977. Thus, the above
ratio of 1:3 between the annual number of cases diagnosed, and the
estimated prevalence, indicated a reasonably acceptable degree of
efficiency in the diagnostic services.

The prevalence of the disease in individuals over the
age of ten years has fallen from 1.8% in 1959 to 0.16% in 1977.

The prevalence of tuberculosis infection in 1977 was
28%. A comparison between the results of 1977, 1959 and 1954 surveys
(Table 10 and Figure 8) provide evidence of a decline in tuber-
culosis infection over the last two decades. The proportion of

FIG. 7

THE INCIDENCE OF PULMONARY TUBERCULOSIS DURING THE PERIOD (1971 - 1977)

BY AGE AMONG THE LOCAL POPULATION.FIGURES FROM REGIONAL TUBERCULOSIS CONTROL CENTRE (TRIPOLI)

●——● Male 68%

●----● Female 32%

Total cases are 1715

NUMBER OF PATIENTS

AGE IN YEARS
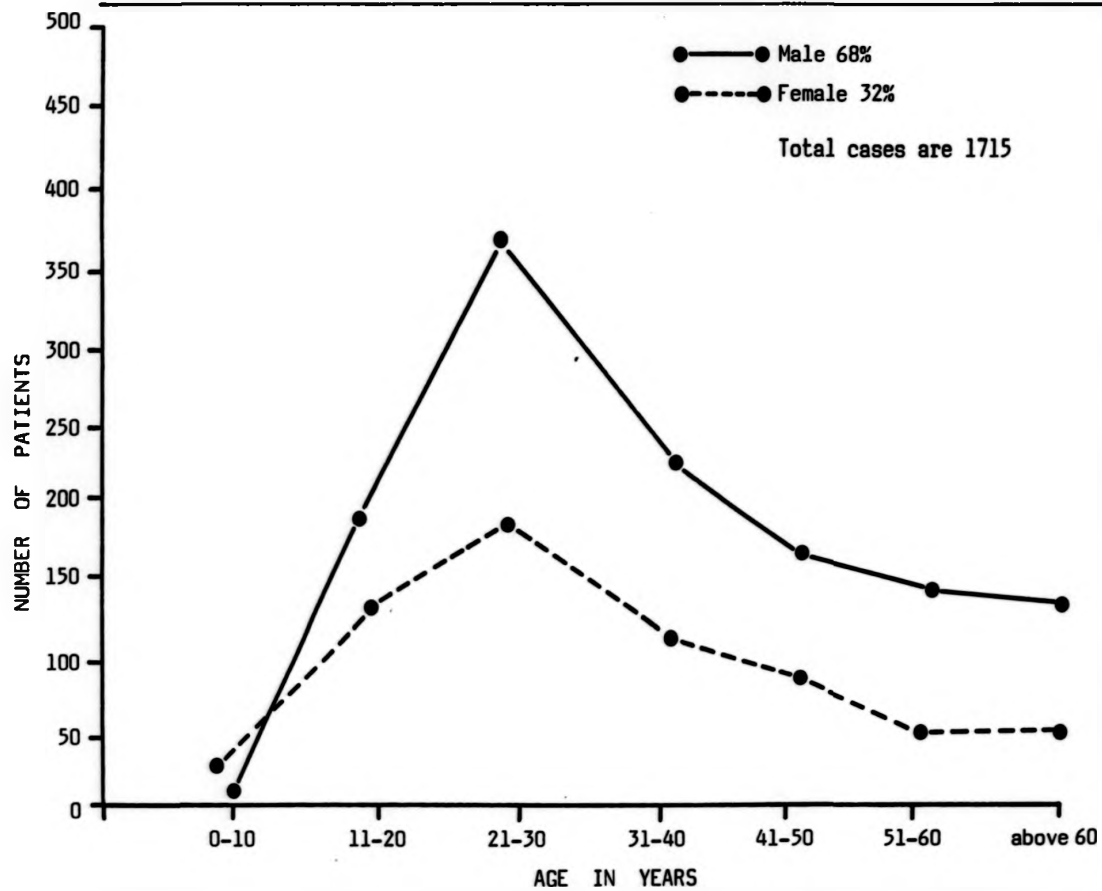
Table 10: Comparison of prevalence of tuberculosis infection
observed in the 1954, 1959 and 1977 surveys.
Proportions of unvaccinated persons showing reaction
to the tuberculin test of 10mm or more, by age.

	1954	1959	1977
Age Group in years:	Ben Walid 5TU	Eastern Region 1TU	Eastern Region 2TU
5-9	19.1%	21.9%	3.2%
10-14	38.4%	39.1%	12.1%
15-19	37.2%	47.1%	18.9%
All ages surveyed	47.5%	48.6%	34.3%

Source: Husain and Thorup (1978)

FIG. 8

PREVALENCE OF TUBERCULOSIS INFECTION IN THREE

SURVEYS 1954,1959 and 1977

unvaccinated persons showing reaction to the tuberculin test
of 10mm or more has decreased in all three age groups surveyed.
The surveys were not directly comparable,however, since the
quantity of tuberculin used in each survey was not identical
(5, 1 and 2 TU), and the surveys were carried out in different
areas.

The risk of tuberculosis infection as shown in Table 11
showed an annual decline of 9% over the past eighteen years among
the 10-14 year age group.

THE BCG VACCINATION PROGRAMME: Assessments by means of
an examination made for the presence of BCG scarring showed that
80% of the school children and approximately 50% of 6-11 month
old infants were vaccinated. BCG vaccination in Libya gives very
weak post-vaccination tuberculin sensitivity, an aspect which has
been reported as requiring further study.

The overall results of treatment are reported to be better
in the Eastern Region, than in the Western Region; in the latter
there were more chronic tuberculosis excretors. In 1975 it was
reported that there were 60 cases of chronic tuberculosis in the
Western Region but none in the Eastern Region. This was explained
by reason of the delay in starting TCP in the Western Region and
the variability of directorial policy (Stylbo, 1976).

unvaccinated persons showing reaction to the tuberculin test
of 10mm or more has decreased in all three age groups surveyed.
The surveys were not directly comparable,however, since the
quantity of tuberculin used in each survey was not identical
(5, 1 and 2 TU), and the surveys were carried out in different
areas.

The risk of tuberculosis infection as shown in Table 11
showed an annual decline of 9% over the past eighteen years among
the 10-14 year age group.

THE BCG VACCINATION PROGRAMME: Assessments by means of
an examination made for the presence of BCG scarring showed that
80% of the school children and approximately 50% of 6-11 month
old infants were vaccinated. BCG vaccination in Libya gives very
weak post-vaccination tuberculin sensitivity, an aspect which has
been reported as requiring further study.

The overall results of treatment are reported to be better
in the Eastern Region, than in the Western Region; in the latter
there were more chronic tuberculosis excretors. In 1975 it was
reported that there were 60 cases of chronic tuberculosis in the
Western Region but none in the Eastern Region. This was explained
by reason of the delay in starting TCP in the Western Region and
the variability of directorial policy (Stylbo, 1976).

Table 11:   Development in annual risk of infection in Libya.

Age group in years:	Annual decrease in risk of infection from previous surveys to 1977	Annual risk of infection				
		1977	1972	1967	1962	1954
5-9	12%	0.17%	0.3%			2.4%
10-14	9%	0.34%		0.83%		2.4%
15-19	4%	0.79%			1.67%	2.5%

Source: Husain and Thorup (1978)

The precise effect of the TCP on prevalence and annual
risk of infection is difficult to assess. However, changes in
standards of living in Libya in terms of improved housing
conditions and nutritional status have been very remarkable and
have undoubtedly contributed to these epidemiological changes.
The decrease in annual infection risk has been approximately
10% in the 5-15 year age group, even were one to accept as valid
for Libya the assertion made for the Netherlands (see Husain and
Thorup, 1978) that not more than 5% decreases were accounted for
by the changes in socio-economic conditions. There are still
reliable grounds for concluding that the TCP has contributed
materially to the reduction of the tuberculosis problem.

3.5   TUBERCULOSIS IN THE EXPATRIATE

In the late 1960s and early 1970s, owing to increasing
national affluence and job opportunities, large numbers of
people from neighbouring countries came to seek employment (see
Table 3). This increased the tuberculosis problem in the
country and several tuberculosis cases came undetected into the
country each year (Khalil and Sathianathan, 1978). This led the
Government to issue a legislative act which was implemented in
1973, to the effect that all foreign and local workers were to
be screened for tuberculosis before seeking employment, or
during the course of employment for those already employed.
The relative and absolute yearly increasing incidence of tuber-
culosis among expatriates is shown in Table 12.

Table 12:   Number of pulmonary tuberculosis cases for the years
1972 to 1978 in the total and expatriate populations,
Libya.

Year	Total number of pulmonary cases	Number of pulmonary cases in the expatriate	Percentage of cases in the expatriate
1972	1,246	80	6%
1973	1,375	245	18%
1974	1,299	385	30%
1975	1,245	471	38%
1976	1,136	394	35%
1977	911	304	33%
1978	839	283	34%

Comparison of yearly cases in the national and
expatriate is made in Figure 6. This figure shows the
continuous decrease of pulmonary tuberculosis cases in the
total and Libyan national populations, and an increase in
the expatriate population up to 1975, where the number of
cases became nearly constant to 1978. This levelling might
suggest a decrease in the number of expatriates in the country
during the period 1975-1978, rather than any other cause. In
the Annual Report of TCP in Libya (1976) it was shown that at
district level the incidence of tuberculosis among expatriates
approached half the total numbers of newly-diagnosed tuberculosis
patients in Tripoli (46%), Zawia (47%) and exceeded half in
Misurata (52%). It also showed that no less than 242 (or 19%)
of cases of pulmonary tuberculosis were diagnosed among labourers.
In a more recent study, Rizgalla and Hashmi (1978) reported that
the number of pulmonary tuberculosis cases in the expatriate
registered during 1971-1977 in the Western Region was 1,130
(97% male). These represent 22% of the total cases in the
region during the same period (see Table 9).

Reliable records are not available to show the occup-
ations of the expatriates concerned, but observation suggests
that the majority are labourers in different branches of
services. The remainder consists of professionals of various
higher social classes, such as teachers and medical staff.

The incidence of pulmonary tuberculosis in the
expatriate adds to the problem of tuberculosis in the country
in several ways. Firstly, the examination of expatriates might
consume the time needed for research investigation of tuberculosis.
Secondly, the high incidence of infectious tuberculosis in foreign
workers might be a possible factor influencing the high prevalence
of primary drug resistance in Libyan nationals of the 25-44 age
groups. Thirdly, those foreigners may bring to the Libyan
community new strains of M.tuberculosis. Fourthly, the continuous
movement of expatriate labourers, their contact with younger
age groups at work, and their tendency to dwell with the lower
class of Libyan, might retard steps taken towards control of the
disease. Finally, there is deflection of costs and effort of TCP
into the examination of expatriates and the treatment of
identified cases.

3.6 DRUG RESISTANCE

The prevalence of primary drug resistance was shown
in Table 13 and 14. Table 13 gives the percentage of primary
resistance among Libyan patients of all ages for the Eastern
Region from 1971 to 1975, and for the Western Region for 1974,
1975 and 1976 when the Libyans began to be recorded separately.
The percentages are confined to Libyan patients only. This is
a particularly important proviso as large numbers of workers in
the country are foreigners, many coming from countries where
the prevalence of resistance to anti-tuberculosis drugs is

Table 13: Primary resistance of <u>M.tuberculosis</u> to anti-
tuberculosis drugs (INH, Sm and PAS) in the Libyan
patients of all ages in the Western and Eastern
Regions of Libya.

Year	Eastern Region	Western Region
1971	16%	-
1971	12%	-
1973	14%	-
1974	11%	11%
1975	11%	9%
1976	10%	10%

(-) No record of nationality in the Western Region before 1974.

Table 14: Primary resistance of <u>M.tuberculosis</u> to anti-
tuberculosis drugs (INH, Sm and PAS) in the Libyan
plus non-Libyan and Libyans only of all ages in the
Eastern Region of Libya.

Year	Libyan + non-Libyan	Libyan only
1971	16%	16%
1972	14%	12%
1973	17%	14%
1974	13%	11%
1975	12%	11%

different and may be relatively high. A record of nationality
has been kept in the Eastern Region since 1971, and the effect
of including foreigners with Libyans is illustrated in Table
14. The addition of non-Libyans increases the percentages.

The primary drug resistance in Libya is 10%; it is
similar to the figures from France (10%) and lower than the
prevalence in Italy (13%), Algeria, Morocco and Tunisia (12%)
(see Wallace, 1976). It was reported (Khalil and Sathianathan,
1978) that in the Libyan national there was a higher prevalence
of resistance in the younger age groups than in the other groups,
and these workers postulated that the observed decline in primary
drug resistance corresponds to the continued fall in the incidence
of new cases. The same authorities reasoned that primary
resistance to a strain of M.tuberculosis arose most probably from
resistance acquired during passage in a host being treated by
chemotherapy and suggested that one possible factor responsible
for the high prevalence of primary drug resistance among Libyan
nationals aged 25-44 years is their greater degree of contact with
tuberculosous foreign workers. This again supports the notion
that tuberculosis might be transmitted from expatriates to the
nationals, i.e. new strains might be introduced.

4. **THE PURPOSE OF THE STUDY**

4. THE PURPOSE OF THE STUDY

        The following epidemiological observations were
mentioned in the National TCP Reports: The high incidence of
infectious pulmonary tuberculosis among expatriates; the high
incidence of tuberculosis among the young Libyan national age
groups in spite of the observed decline in the general population;
the high prevalence of primary drug resistance among M.tuberculosis
isolates in the young age groups of nationals than in the older
groups; and the prevalence of resistance to anti-tuberculosis drugs
may be relatively higher in the expatriate than in the Libyan
nationals. None of these observations led to epidemiological
investigation aimed at establishing the association among these
factors in prospective study, but each observation was treated
separately. In 1973 a law was issued for the compulsory examin-
ation of both national and expatriate labourers, and a change in
the BCG vaccination programme was ordered to include vaccination
of school-leavers with the stated intention of minimising the
incidence of tuberculosis in the young age group. Khalil and
Sathianathan (1978) in a retrospective study postulated that an
association existed between the high prevalence of primary anti-
tuberculosis drug resistance in the Libyan nationals aged 25-44
years on the one hand, and the high incidence of tuberculosis in
the expatriate on the other, and was the result of transmission
of the disease to the former, from the latter group.

On the basis of these observations and others, the
present study was formulated in order to study the epidemiological
connection between the expatriate and the indigenous national.
Mycobacterial cultures were collected with the intention of
exploring possible strain differences among them, and of assessing
the degree of difference among clinical isolates from the same
area of Libya over a given period of time. This study would,
it was hoped, shed light on the hypothesis that tuberculosis among
the young Libyan nationals is influenced by the relatively high
incidence of the disease among expatriates. Complete demographic
information regarding the patients from whom the clinical isolates
were made was unobtainable, so this study will differ from those
epidemiological investigations where strain variations among
M.tuberculosis isolates were sought in patient groups sharing
certain demographic characteristics such as occupation or residency.
In the geographical region selected for the study, it had been
observed that a raised tuberculosis incidence among nationals
might be correlated with the high tuberculosis incidence in the
expatriate, and it might be suggested that the disease was
becoming increasingly limited to the low socio-economic classes
of both expatriate and national groups.

Grange et al (1977) reported that tuberculosis will not
easily spread from immigrants to the native population where
routes of infection extend only infrequently outside a tightly-
knit exclusive immigrant community, but that the speed of spread
might be increased by close and rapid integration of the two
communities. They postulated that in Great Britain little exchange

of M.tuberculosis occurred between Asian communities and the
native British community because of little integration between
the two. In Libya, close and rapid integration of the two
communities is the rule.

As mentioned above (p12), neither cytological nor
biochemical criteria were of great value in the epidemiological
studies on M.tuberculosis. Varied studies designed to explore
the geographical distribution of M.tuberculosis demonstrate that
susceptibility to mycobacteriophage, although still in relatively
early stages of development, is the method of choice for detection
of strain variation of M.tuberculosis in different countries
(reviewed by Grange and Redmond, 1978). In short, the purpose of
the present study was to investigate the epidemiology of
M.tuberculosis and the degree of M.tuberculosis strain interchange
between local and expatriate populations, of a selected region
of Libya. The method chosen was the susceptibility of
M.tuberculosis strains to lytic mycobacteriophages.

5.   **LITERATURE REVIEW**

5. LITERATURE REVIEW

5.1 INTRODUCTION

Since the isolation of bacteriophage lytic for
mycobacteria by Gardner and Weiser (1947), several attempts
have been made to utilise bacteriophage typing as a method of
differentiating and classifying the genus mycobacteria (Froman,
Will and Bogen, 1954; Hnatko, 1956; and Takeya and Yoshimuza,
1957). Although numerous strains of bacteriophage lytic for
mycobacteria were isolated and tested, relatively few were
initially found to lyse the pathogenic and opportunistic strains,
and in particular few, if any, bacteriophages specific for one
strain of mycobacteria were isolated. When it was later shown
that bacteriophage lytic against pathogenic M.tuberculosis could
be isolated, that these bacteriophages could be used for
identifying these important organisms, and that there was a
possibility that M.tuberculosis and M.bovis might be different-
iated by this means (Redmond, Cater and Ward, 1963), interest in
mycobacteriophage typing increased considerably. However, as
improved biochemical and serological techniques developed at
that time, allowing a more precise classification of mycobacteria
(Stanford and Grange, 1974) expectation of, and interest in
utilising bacteriophage typing as a taxonomic tool waned. Thus
the continued emphasis lay on isolating and characterising
bacteriophages that would be useful in subspecies classification
and identification.

5.2 THE VALUE OF MYCOBACTERIOPHAGES IN THE EPIDEMIOLOGICAL STUDY OF M.TUBERCULOSIS

Bacteriophage lytic for M.tuberculosis were first isolated by Froman et al (1954) who also reported that the strains examined exhibited different bacteriophage patterns. Other workers (Murohashi et al. 1963; Takeya et al. 1959) were able to subdivide local Japanese M.tuberculosis strains into different bacteriophage types.

The value of mycobacteriophages in epidemiological studies was illustrated by Baess (1966) after her isolation of mycobacteriophage BKI. By means of this bacteriophage, it was possible to divide M.tuberculosis strains isolated from tuberculosis cases into two bacteriophage groups. Except for a few cases, all strains from patients with presumed epidemiological connections were either bacteriophage-susceptible or bacteriophage -resistant. At that point BKI and Redmond's GS4E (Redmond, 1963) were the only two bacteriophages with accepted potential for subdividing the species M.tuberculosis for epidemiological usage.

In 1967 Bates and Fitzhugh reported that 92 wild strains of M.tuberculosis, isolated from patients in the United States of America (USA), were subdivisible into bacteriophage types A, B and C, using a battery of bacteriophages. Most of these strains (76%) were Type A and were resistant to all but bacteriophage DS6A. Fourteen per cent of strains were highly susceptible to

lysis by the bacteriophages DS6A, GS4E and BGI, isolated by Redmond (1963), and were designated Type B; a mere 10% were lysed by all the bacteriophages, as well as D-34 and were designated Type C (Table 15).

Tokunaga, Maruyama and Murohashi (1968), who used nine bacteriophages for typing M.tuberculosis strains, found results similar to those of Baess (1966). Strains of M.tuberculosis isolated from patients with presumed epidemiological connections were found susceptible to the same bacteriophages, in contrast to strains isolated from patients without proven epidemiological links, where the bacteriophage susceptibility differed markedly. These workers reported that no alteration in bacteriophage type despite changes in drug susceptibility. They also found that seven strains from India showed intermediate degrees of susceptibility to GS4E and BKI.

Bates and Mitchison (1969) used bacteriophage typing of M.tuberculosis as a marker for effecting comparisons with biochemical or virulence characteristics of strains collected from widely separated locations throughout the world. Several items of epidemiological importance emerged from this study. First, an unexpected bacteriophage type of M.tuberculosis designated as 'Intermediate' was detected among isolates made from patients in Southern India. The term 'Intermediate' was given for these strains because they showed a pattern intermediate

Table 15:   Bacteriophage types of M.tuberculosis (Bates and
            Fitzhugh, 1967)

Phage type	DS6A	GS4E	BG1	D-34
A	+	−	−	−
B	+	+	+	−
C	+	+	+	+

+ = lysis at routine test dilution

− = no lysis at routine test dilution

between Type A and B in regard to their susceptibility to lysis
by the bacteriophages GS4E, BGI and BKI. Previously, all strains
tested from other geographical areas had shown consistent patterns
of either total susceptibility or resistance to those three
bacteriophages. Many of the strains from Southern India did not
show this homogeneity and therefore were not clearly Type A
or B. Second, M.tuberculosis strains from different regions
tended to be of different bacteriophage types. Thus, Type B
strains of M.tuberculosis were more common in Britain than in
Hong Kong or Southern India, whereas Type A strains predominated
in Hong Kong, and Type Intermediate strains together with the
Type A strains were both observed in Southern India. Type C
strains were not observed among the strains in this study.
Third, it was found that the bacteriophage type did not change
despite the in-vivo change in drug susceptibility, in animal
virulence or catalase production. Fourth, it was found that
bacteriophage type did not correlate with drug resistance when
isoniazid, streptomycin and para-amino salicylic acid were the
drugs employed in the study. Fifth, it was observed that
the South Indian isoniazid-sensitive strains, of decreased
virulence for guinea-pigs, were randomly distributed between
bacteriophage Type A and the Intermediate type.

Another large epidemiological study was reported by
Baess (1969), in her evaluation of 230 strains of M.tuberculosis
obtained from Danish patients who were linked epidemiologically.

Using bacteriophage BKI, reported to be stable and yielding
highly reproducible results, it was possible to divide 203
isolates of M.tuberculosis into 64 bacteriophage-susceptible,
and 143 bacteriophage-resistant strains. The patients with
a presumed epidemiological relationship were grouped into 88
groups; 22 groups were found to harbour only bacteriophage-
susceptible strains, while 61 groups harboured only bacterio-
phage-resistant organisms. She found only a few strains lysed
by bacteriophage D-34, observed that the plaques produced by
it were turbid, and expressed concern that D-34 would not be a
useful bacteriophage for epidemiological studies. An additional
five bacteriophages were used by Baess, but none served to
subdivide M.tuberculosis into more than the two categories
detectable by means of bacteriophage BKI.

Thereafter, several additional epidemiological studies
were reported by investigators who continued using the spotting
technique developed by Redmond et al (1963). Mankiewicz (1972)
studied the different distributions of bacteriophage types among
various immigrant groups and Eskimos in Canada, and observed that
Asian immigrants had a lower incidence of Type B than immigrants
from Europe. A number of bacteriophages were isolated and included
in the bacteriophage testing, namely bacteriophages DNA 1118,
Clark, Legendre and Sedge, the last three being isolated from
sarcoidosis lesions. These bacteriophages were used to extend
the Bates scheme shown in Table 16 to subdivide bacteriophage
Types A, B, C and Intermediate into subgroups as shown in Table 17.

Table 16: Bacteriophage types of <u>M.tuberculosis</u> according to
the scheme of Bates.

Phage Type	DS6A	GS4E	BG1	BK1	D-34
A	+	−	−	−	−
B	+	+	+	+	−
C	+	+	+	+	+
Intermediate					
I	+	+	−	−	−
II	+	+	+	−	−
III	+	−	+	−	−

Source: Mankiewicz (1972)

Table 17:   Enlarged bacteriophage typing scheme of M.tuberculosis (Bates - Mankiewicz)

Phage Type	DS6A	GS4E	BG1	BK1	D-34		Subgroup	DNA1118	CI	LE	SE
A	+	-	-	-	-		1	+	+	+	+
B	+	+	+	+	-		2	-	-	-	-
C	+	+	+	+	+		3	+	+	+	-
Intermediate							4	-	+	+	+
I	+	+	-	-	-		5	-	+	-	-
II	+	+	+	-	-		6	-	+	+	-
III	+	-	+	-	-		7	-	+	-	+
							8	+	-	-	-
							9	+	+	+	+
							10	-	-	-	+

Abbreviations used: CI - Clark; LE = Legendre; SE = Sedge.

Source: Mankiewicz (1972)

In 1972 Ionesco (see Engel, 1978) detected differences
in bacteriophage type distribution even within France, types in
the West being significantly different from those found in the
East and South West. Clavel (1975) reported that both Type A
and B are common in France.

Kitahara (1973) studied the geographic distribution of
bacteriophage types of M.tuberculosis in Japan and Kenya, and
was able to show that in Japan about 20% of strains were
susceptible to lysis by BKI, but in Kenya only 5% were of this
type. Of considerable interest and importance was the apparently
anomalous observation that 32% of Kenyan strains (7 strains)
were susceptible to lysis by D-34, whereas only very few such
strains were found in Japan. The finding of increased frequency
of D-34 susceptible strains from Kenyan patients coincides with
unpublished data obtained by Bates and Rado, 1970 (see Redmond,
Bates and Engel, 1979), who have found that such strains are also
very frequent among Egyptian and Bolivian patients. In this
same report Kitahara (1973) used bacteriophage typing to study
the intrafamilial spread of tuberculosis in Japan.

Additional geographical studies on bacteriophage types
of M.tuberculosis were reported by Mizuguchi et al (1973), who
studied the bacteriophage susceptibility of strains isolated in
Japan, the Netherlands and Sri Lanka. A number of bacteriophages
not extensively used prior to this report were included in the
bacteriophage testing. The ones isolated by Mankiewicz (1972)

and the "pH" bacteriophage isolated by Sushida and Hirano (1972) were included. The bacteriophage susceptibilities of the study of 54 strains from Sri Lanka were notable in that 87% were susceptible to BKI, 68% to pH and 8% to D-34. The Japanese and Dutch strains were generally more resistant to the bacteriophages than previously reported. This result was confirmed by Murohashi and Mizuguchi (1975), and Engel (1975). Both bacteriophage Type A and B were reported to be common in Czechoslovakia (Sula and Solova, 1975) and in the United States (Jones, 1975b; Ward and Sieg, 1975).

Clavel (1975) reported that Type A strains are more predominant in West and Central Africa, which matched the earlier finding of Mankiewicz (1972), who investigated bacteriophage types among immigrants from East Africa. Grange, Collins and McSwiggan (1976) reported the distribution of bacteriophage types of M.tuberculosis isolated from both European and Asian patients in Britain. The incidence of bacteriophage Type A was found not to differ significantly between the European and Asian patients, but a difference was observed in the distribution of other bacteriophage types, particularly Type B, which accounts for 30% of the strains from Europeans but only 5% of those from Asians. This appeared to indicate that Asian strains were less susceptible to bacteriophage action than European ones. In another study, Grange et al (1977) found that strains from Asian immigrants to Britain retain the bacteriophage pattern charact- eristics of the strain from the Asian continent, suggesting that

there has been little interchange between the Asian community
and the African and British communities alongside whom they lived.
These authors also showed in the same study that the Intermediate
bacteriophage type is a real entity, and postulated that it may
represent a particular variant of M.tuberculosis. The Intermediate
bacteriophage type, as previously mentioned, was observed to be
present among Indian and Asian immigrants.

Baess (1975) has questioned the existence of the
Intermediate bacteriophage type and reported that bacteriophages
which subdivide the bacterial strains into groups A and B
reproduce the same subdivision over a wide range of bacteriophage
concentrations, while those bacteriophages which subdivide
groups A and B showed a new subdivision for each bacteriophage
concentration. This last-named effect was especially noticeable
when confluent lysis as well as the presence of discrete plaques
was taken as a positive result.

Further, when establishing criteria for recording results
of bacteriophage   typing was moved so that any degree of lysis
less than semiconfluence were to be defined as nil, the subdivision
of the two groups of bacteriophages A and B became even more
convincing.

The foregoing studies indicated the usefulness of
bacteriophage typing of M.tuberculosis in the study of
tuberculosis epidemiology, but its use in studying the
pathogenisis of tuberculosis has so far been limited.

Raleigh and Wichelhausen (1973) demonstrated exogeneous reinfection by M.tuberculosis of the bacteriophage type differentfrom that of the original infecting strain in a patient who had recovered from pulmonary tuberculosis.

A more recent work by Mankiewicz and Liivak (1975), who studied individual colonies of M.tuberculosis isolated from Canadian Eskimos and from Canadians of European heritage, demonstrated that concurrent pulmonary infection with more than one bacteriophage type of M.tuberculosis exists among Canadian Eskimos. The Eskimo patients had experienced repeated hospital admissions, multiple drug therapy regimens had been prescribed, and treatment failures were common.

The possibility of infection with two or more bacteriophage types and the impact of this on planning a tuberculosis control programme has been reviewed by Raleigh et al (1975). In a study of 26 patients who had relapsed following initial treatment for pulmonary tuberculosis, these investigators found that in 9 patients the bacteriophage type of the pre-treatment and the last positive isolates prior to the patient's becoming culture negative differed from the bacteriophage type of isolates obtained after the bacterio-logical relapse. It was suggested that both bacteriophage types were significant, and thus selected as the isolates for use in bacteriophage typing. Redmond et al (1979) has written that these two reports require further study and

documentation, but the epidemiological implications are apparent.   Infection by two or more bacteriophage types suggests that the effectiveness of immunity induced by the initial infection may be less than that previously thought. Indeed, the frequency of dual infection may be greater than those studies would suggest even in developed countries where tuberculosis control programmes are taking effect.

Further data and comment regarding dual infection in man has been supplied by Bates, Stead and Rado (1976), who studied isolates of M.tuberculosis obtained from patients infected in two or more anatomic sites.   In this study, single-colony isolates were not evaluated, but instead numerous colonies from each culture were mixed together and subcultured for typing.   This technical point is noteworthy because of the overshadowing effect of resistant bacteria in any study involving resistant and susceptible strains.   Since Type A is resistant to all bacteriophages except DS6A and AG1, it follows that if this strain is present together with M.tuberculosis of any other bacteriophage type, the lytic pattern of the latter will not be apparent because the potential zones of lysis will be overgrown by the Type A bacteriophage-resistant M.tuberculosis present on the medium.   They concluded that, if more than one bacteriophage type is present in a culture, typing methods currently used will fail to detect the type which is more susceptible to bacteriophage lysis.   Despite this technical limitation, the results showed that two of 88 patients with concurrent infection at two or more

sites were infected with M.tuberculosis of different
bacteriophage types. Thus, despite inadequate technique, dual
infection was recognised with unexpected frequency among
patients in the United States of America. These reports
revealed that repeated exogenous infection with M.tuberculosis
does occur in developed countries, and one can speculate that
exogenous reinfection might well be even more frequent among
persons living in locations where tuberculosis continues to be
a public health problem.

The question was raised (Grange and Redmond, 1978) as
to whether the finding of dual infection indicated reinfection
by different strains, initial infection by more than one
strain, or a mutational change in bacteriophage type. Initial
infection by more than one strain may occur in area in which
there is a high incidence of tuberculosis, but no evidence
exists of multiple infection in areas in which tuberculosis was
much less common (Mankiewicz and Liivak, 1975). The possibility
that bacteriophage types might alter under the influence of
treatment may be considered (Grange and Redmond, 1978). However,
the spontaneous mutational loss of susceptibility to a single
bacteriophage is less than 1.3×10^{-8} per generation and is
unaffected by the acquisition of resistance to isoniazid or
rifampicin (Clavel and Clement, 1977).

5.3   BACTERIOPHAGE TYPING METHODOLOGY

Many technical variations have been employed in the
investigation of bacteriophage susceptibility among
M.tuberculosis strains. Early designations for bacteriophages,
bacteriophage types and mycobacterial host strains were
diverse and confusing. To correct this problem, the World
Health Organisation (WHO) gave support to a working group
studying the development of bacteriophage typing for myco-
bacteria. The efforts of this group have been directed towards
formulating a more uniform methodology and nomenclature. An
important study from this group was published in 1973, and
suggested that uniform techniques could be applied world-wide
and that the technology required to achieve uniform results
was within reach of investigators working in all developed
countries (Sula et al, 1973).

This study was followed by another study (Rado et al,
1975) which described in detail a standard methodology for
bacteriophage typing of M.tuberculosis using techniques and
media described by Redmond and Ward (1966). The bacteriophages
used for typing, together with their host strains, were given
an alphabetic and numerical designation. This permitted the
development of a systematic method for assigning nomenclature
to bacteriophage types of M.tuberculosis strains, and allowed
for expansion and modification of the system as required by
the advent of new information. The bacteriophages and host
strain nomenclature are shown in Table 18.

Table 18: Mycobacteriophages used for bacteriophage typing of <u>M.tuberculosis</u>.

Present designation	Original designation & 1x RTD*		Host strains	Source
MTPH 1**	AG1	1×10^5	AT7 <u>M.kansasii</u>	Adapted from mixture of several phages and <u>Mycobacterium</u> strains
MTPH 2	DS6A	1×10^6	<u>M.tuberculosis</u> (H37RV)	Soil
MTPH 3	GS4E	1×10^5	<u>M.tuberculosis</u> (H37RV)	Adapted from GS4 (soil)
MTPH 4	BK1	1×10^6	<u>M.smegmatis</u> (ATcc 607)	Soil
MTPH 5	BG1	4×10^3	<u>M.avium-complex</u> (PJ7)†	Adapted from a non-specific Phage from soil
MTPH 6	D-34	1×10^7	<u>M.not speciated Froman's F130</u>	Soil
MTPH 7	DNA1118	1×10^5	<u>M.tuberculosis</u> (H37RV)	Recombinant of Phage Roy
MTPH 8	x20	1×10^6	<u>M.tuberculosis</u> (H37RV)	Clear plaque variant of DNA 1118
MTPH 9	pH	1×10^5	<u>M.tuberculosis</u> H37RV	Enzyme preparation
MTPH 10	Clark	1×10^6	<u>M.smegmatis</u> ATCC 607	Sarcoidosis lesion
MTPH 11	Sedge	1×10^6	<u>M.smegmatis</u> ATCC 607	Sarcoidosis lesion
MTPH 12	Legendre	1×10^6	<u>M.smegmatis</u> ATCC 607	Sarcoidosis lesion

*   Minimal concentration of bacteriophage (Pfu/ml) that gave almost confluent

      lysis when 0.01ml of stock was spotted on propagating host strain. RTD = routine

      test dilution.

**   MTPH = mycobacterial typing phage, human.

†   This bacteriophage was titrated on ATCC 607

Source: Rado <u>et al</u> (1975)

Another technique was presented by Jones and Greenberg (1978) which showed much clearer differentiation of lytic spots, especially with the auxiliary bacteriophages (MTPH 7 and 10-12). This method utilises an adaptation of the soft-agar overlay technique and was described at the meeting of the Bacteriophage Typing Study Group. It is described in detail by Redmond et al (1979). The soft agar overlay technique was also used by Grange et al (1976), who reported that the molten agar spread on the agar base much more readily than when the earlier broth method was used. (Rado et al, 1975; Redmond and Ward, 1966). They also found the technique in general less hazardous to the worker, since the M.tuberculosis cells fix in the soft agar and the petri dish does not need as much manipulation as in the broth method. The soft agar technique enables the detection of small plaques, which might be obscured when the broth method is employed.

It was reported (Grange et al, 1976) that smoother suspensions of mycobacteria for bacteriophage typing could be obtained by shaking the bacterial suspension with glass beads, provided that old or dried cultures were avoided. The reaggregation of the dispersed bacteria was greatly diminished when bovine serum albumen was added. This method saves operator time and might well be less hazardous than the subculturing and centrifugation techniques used by other workers (Rado et al, 1975).

5.4 BACTERIOPHAGES AND BACTERIOPHAGE TYPES

Twelve typing bacteriophages were available (Table 18).
They fall into three categories shown in Table 19. The general
bacteriophages lyse all M.tuberculosis strains, the subdividing
bacteriophages divided the strains into three major types A,
B and C (Table 20). The subdividing bacteriophages separate
also Type A and B into Intermediate types (see Table 16). The
auxiliary bacteriophages show variable patterns on strains of
Type A (see Table 20), but lyse all strains of Type B and C.
Mankiewicz (1972) classified the bacteriophage groups each into
ten subgroups by the auxiliary bacteriophages (Table 17).

Currently there is no international recognised nomen-
clature for bacteriophage types of M.tuberculosis and the
present grouping is based on one suggested for communication
between members of the World Health Organisation Working Group
on bacteriophage typing mycobacteria (Rado et al, 1975).

5.5 CONSISTENCY OF BACTERIOPHAGE TYPES

The stability of bacteriophage type has been demonstrated
in studies of experimentally infected mice. No change of
bacteriophage type could be demonstrated during nine months of
observation (Clavel and Clement, 1977). Bacteriophage type
did not change despite changes in drug susceptibility during
chemotherapy (Bates and Mitchison, 1969).

Table 19:   The general, sub-dividing and auxiliary mycobacteriophages.

Group I	Group II	Group III
General Phages	Subdividing Phages	Auxiliary Phages
MTPH 1	MTPH 3	MTPH 7
MTPH 2	MTPH 4	MTPH 8
	MTPH 5	MTPH 10
	MTPH 6	MTPH 11
	MTPH 9	MTPH 12

Source: Rado et al (1975)

MTPH = Mycobacterial typing phage human

Table 20:   The major and secondary bacteriophage types of

M.tuberculosis.

Bacteriophage type	Lysis by mycobacterial typing phage human (MTPH)					
	2	3	4	5	6	9
A	+	-	-	-	-	-
B	+	+	+	+	-	+
C	+	+	+	+	+	+

Secondary

subtyping of the major bacteriophage Type A.

$$\left.\begin{array}{l} Ax \\ A2 \end{array}\right\} \ = \ \text{A lysed by} \ \left\{\begin{array}{l} \text{no or one} \\ \text{two or more} \end{array}\right\} \ \begin{array}{l} \text{of bacteriophages} \\ \text{MTPH 7, 8, 10-12.} \end{array}$$

Source: Rado et al (1975)

5.6   SUMMARY

Bacteriophages which lysed M.tuberculosis species
were first described by Froman et al (1954) and further
bacteriophages were isolated by Redmond and Cater (1960),
Redmond et al (1963) and Baess (1966). The application of
bacteriophage typing to the study of the epidemiology of
tuberculosis was first suggested by Baess (1966). In this
and subsequent studies bacteriophage typing was used to
assess presumed epidemiological connections between small
groups of patients.

Following the standardisation of techniques by Redmond
and Ward (1966) and the studies by Baess (1969), who stressed
the need to use standardized bacteriophage suspensions, further
steps in the perfection of techniques were made in international
collaborative studies (Rado et al, 1975).

Three major bacteriophage types were first recognised,
namely A, B and C and subsequently Intermediate types were
described. Their existence was questioned by Baess (1975)
but confirmed later by Grange et al (1977). In addition to
these types, a further subdivision of Types A and Intermediate
may be obtained by use of a group of bacteriophages originally
isolated from sarcoid biopsies. These subdivisions are not
as distinct as the major bacteriophage types. The bacteriophage
typing scheme for subdividing the species M.tuberculosis, although

still in the development stage, has yielded some information of largely geographical significance. The distribution of bacteriophage type B appears to be limited to the North American subcontinent and to Europe, whereas Type Intermediate is common in India. Type A predominates in Japan, Hong Kong and Central, East and West Africa. Differences between indigenous and immigrant populations living in the same area are detectable. Bacteriophage typing can be used in determining dual infection.

6. <u>MATERIALS AND METHOD</u>

6.   MATERIALS AND METHOD

6.1   HOMOGENICEITY OF MYCOBACTERIAL CULTURES

6.1.1   MYCOBACTERIAL CULTURES: Three hundred and forty
M.tuberculosis cultures were obtained from the Regional
Tuberculosis and Chest Disease Centre in Tripoli, Libya.
They were obtained in two batches, and had been isolated from
former and newly-diagnosed patients at two different periods
of time (Table 21).   Petroff's method and Lowenstein-Jensen (LJ)
medium were used for strain isolation. The cultures were
isolated from sputum samples from patients with pulmonary
tuberculosis.   Sensitivity to anti-tuberculosis drugs including
isoniazid were done after isolation to some of the cultures.
All cultures of M.tuberculosis were detected by microscopic
examination of Ziehl-Neelsen-stained smear and by macroscopic
growth characteristics on LJ medium.   No biochemical tests or
other identifying methodology had been performed.   All cultures
received were maintained on LJ slopes and stored at 4°C.

6.1.2   METHODS OF CONFIRMATION: All 340 cultures were screened
and confirmed as M.tuberculosis by using the following tests
(Fig.9):   microscopy for cell morphology and acid-fastness;
growth characteristics and colony morphology on LJ medium at
37°C; growth on LJ medium at 25°C; pigment production in the
dark and light; and other biochemical tests such as the nitrate
reductase production, niacin synthesis; and susceptibility to

Table 21: Number of mycobacterial cultures isolated from new and previously-diagnosed cases of pulmonary tuberculosis in the Western Region of Libya.

Batch	Cultures from newly diagnosed patients	Cultures from previously diagnosed patients	Total	Period of isolation
Batch I*	102	98	200	Sept. 1976 to June 1977
Batch II**	102	38	140	First half of 1979
Total:	204	136	340	-

* This batch was isolated by Prof. Wallace, A., Chief Bacteriologist, RTC, Tripoli, Libya.

** This batch was isolated by Mr. Ekram, Bacteriology Unit, RTC, Tripoli, Libya.

Figure 9: Tests applied to M.tuberculosis isolates from Libya
(Western Region).

thiophene-2 carboxylic acid hydrazide (TCH), (Runyon et al, 1974).

All cultures were subcultured onto LJ slopes which contained glycerol and into Middlebrook 7H9 broth (see Appendix 1). Incubation was continued for four weeks at $37^{o}C$ with the slopes at an inclined position for the first 2-3 days so as to produce even growth. The slopes were examined macroscopically by rotating the slopes in direct light. This was done twice in the first week, then weekly for four weeks.

STRAIN CHARACTERISTICS: A Ziehl-Neelsen-stained smear made on the tenth day from 7H9 broth cultures was examined by light microscopy for shape, size, arrangement and regularity of acid fastness.

The LJ slopes incubated at $37^{o}C$ were examined after two to three weeks' incubation for the colonial morphology and growth characteristics, the rate of growth, eugonic or dysgonic.

The cultures were also observed for their ability to grow on LJ slopes at $25^{o}C$, naked-eye examination being made over a period of five weeks.

One of the LJ slopes incubated at $37^{o}C$ and showing confluent growth (2-3 weeks old culture) was exposed to light

thiophene-2 carboxylic acid hydrazide (TCH), (Runyon et al, 1974).

All cultures were subcultured onto LJ slopes which contained glycerol and into Middlebrook 7H9 broth (see Appendix 1). Incubation was continued for four weeks at 37^oC with the slopes at an inclined position for the first 2-3 days so as to produce even growth. The slopes were examined macroscopically by rotating the slopes in direct light. This was done twice in the first week, then weekly for four weeks.

STRAIN CHARACTERISTICS: A Ziehl-Neelsen-stained smear made on the tenth day from 7H9 broth cultures was examined by light microscopy for shape, size, arrangement and regularity of acid fastness.

The LJ slopes incubated at 37^oC were examined after two to three weeks' incubation for the colonial morphology and growth characteristics, the rate of growth, eugonic or dysgonic.

The cultures were also observed for their ability to grow on LJ slopes at 25^oC, naked-eye examination being made over a period of five weeks.

One of the LJ slopes incubated at 37^oC and showing confluent growth (2-3 weeks old culture) was exposed to light

from a 40 watt lamp at 30cm distance for two hours, then
reincubated with the cap loose for another week in order to
assess the degree of pigmentation.

The synthesis of the nicontinic acid was determined
by paper-strip method for niacin using Niacin Test Strips,
TB (Difco). A 1.5ml of distilled water was added to a four-
week-old LJ slope exhibiting confluent growth. Using a pasteur
pipette, the growth was stabbed to release the niacin from the
medium. The slopes were incubated at an inclined position for
30 minutes to two hours after which 0.6ml was drawn from the
slopes into a 13 x 75mm test tube and with a flamed cooled
forceps a niacin strip was inserted. The tubes were immediately
covered and shaken gently every ten minutes. The colour in
the liquid at the bottom of the tube was observed after thirty
minutes and recorded. The appearance of yellow to canary colour
indicate a niacin positive result; negative cultures were
retested.

The nitrate reductase test was determined by Bacto-
Nitrate-Test Strips (Difco). A 10mg wet weight loopful from
confluent growth on LJ medium was mixed in a 0.5ml distilled
water placed in a centrifuge tube. One strip was placed in
the tube which was then incubated vertically undisturbed for
two hours at 37°C. The tube was then tilted back and forth many
times to wet the entire strip. A change of colour of the top
portion of the strip to blue was taken to indicate nitrate
reduction. The necessary controls were included in each test.

Sensitivity of cultures to TCH was detected by the incubation of two 7H11 agar plates, one containing 10µg/ml TCH and the other without TCH which had been inoculated with a standard inoculum from a barely turbid suspension of a M.tuberculosis strain. After incubation of the plates at 37°C for 3-4 weeks under 5% CO_2, a comparison was made of growth occurring on the two plates. The test was performed on each strain. Strains were considered resistant if the growth on the TCH plate was more than 1% of the growth on the control plate, and sensitive if the growth on the TCH plate represented less than 1% of the growth on the control plate.

6.1.3   DISCUSSION: Various criteria have been used for distinguishing M.tuberculosis cultures from other mycobacteria, including M.bovis. Marks (1976) identified M.tuberculosis by the lack of pigmentation, rate of growth, failure to grow at 25°C and susceptibility to para-nitrobenzoic acid, in addition to organism and colonial morphology. He also made the assertion that M.tuberculosis and M.bovis could be differentiated by the oxgyen preference test in addition to its growth characteristics on glycerol egg medium. On the former, M.bovis and M.tuberculosis strains with high levels of resistance to isoniazid will grow similarly.

Other workers have used different combinations of these
tests. The majority of investigators - especially the ones
who tried to identify strain variants among M.tuberculosis
cultures by means of bacteriophage typing - used the above tests
with the exception(s) of the oxgyen preference criteria and
sensitivity to para-nitrobenzoic acid. They used instead the
niacin production test and the nitrate reductase test with or
without susceptibility to TCH (Grange et al, 1977; Jones, 1975a;
Jones, 1979; Mankiewicz and Liivak, 1975 and Rado et al, 1975).

The microscopic characteristics of the organism were
studied either by growing the organism in liquid medium (Middle-
brook 7H9 or modified Dubos broth) or by using the fluid expressed
from cultures on LJ medium (Marks, 1976).

The paper strip methods for niacin production and nitrate
reduction used in the present study were those recommended by
Runyon et al (1974); the nitrate strip method was used by Grange
et al (1977).

Sensitivity to TCH (10µg/ml of media) were recommended
by Runyon et al (1974) and was used at the same concentration by
Jones (1975a). Rado et al (1975) used 5µg/ml, while Grange et al
(1977) used TCH in serial two fold concentration (0.25 - 32µg/ml
of media). The latter used LJ medium instead of the Dubos agar
recommended by Runyon et al (1974) or the Middlebrook 7H11 used
in the present study.

6.2   BACTERIOPHAGE TYPING

6.2.1   MYCOBACTERIAL CULTURES FOR BACTERIOPHAGE TYPING:

The strains became available on LJ medium following the
identification. All confirmed M.tuberculosis cultures were
subcultured on newly prepared LJ slopes. Since smooth growth
is required for bacteriophage work, rough strains were
subcultured into middlebrook 7H9 broth. Incubation of the LJ
slopes was continued at $37^{o}C$ for 2-3 weeks and the resultant
growths were used for bacteriophage typing.

6.2.2   MEDIA FOR BACTERIOPHAGE TYPING: Media used for

bacteriophage typing are shown in Appendix 1. All procedures
were performed in a safety cabinet class 3.

6.2.3   MYCOBACTERIOPHAGES: Nine bacteriophages were obtained

in calcium glyceral supplemented medium (Appendix 1) with their
propagating strains on LJ slopes. The bacteriophages used
(classified into I, II and III categories), their propagating
strains, their RTD concentrations used in the bacteriophage
typing and the WHO mycobacterial typing bacteriophage human
(MTPH) numbers, all are listed in Table 22.

The bacteriophages MTPH 1, 6 and 8 presented in Table
18 were not included in the present study. Bacteriophage MTPH 1
is known always to show lysis at RTD of those strains also lysed
at RTD by bacteriophage MTPH 2, and therefore was regarded as

Table 22: Mycobacteriophages used for bacteriophage typing of
the Libyan M.tuberculosis strains.

Present designation		Original designation and 1 x RTD*		Propagating strains	Bacterio- phage grouping ***
MTPH**	2	DS6A	1×10^6	M.tuberculosis (H37RV)	I
MTPH	3	GS4E	1×10^4	M.tuberculosis (H37RV)	II
MTPH	4	BK1	1×10^6	M.smegmatis (ATcc 607)	II
MTPH	5	BG1	1×10^5	Mycobacteria (15385)	II
MTPH	7	DNA1118	1×10^5	M.tuberculosis (H37RV)	III
MTPH	9	pH	1×10^4	M.tuberculosis (H37RV)	II
MTPH	10	Clark	1×10^6	M.smegmatis (ATcc 607)	III
MTPH	11	Sedge	1×10^6	M.smegmatis (ATcc 607)	III
MTPH	12	Legendre	1×10^6	M.smegmatis (ATcc 607)	III

*   Minimum concentration of bacteriophage (PFU/ml) that gave
      almost confluent lysis when spotted on propagated host strain.
      RTD = routine test dilution.

**   Mycobacterial typing phage human.

*** I general phage   II subdividing phages   III auxiliary phages

Bacteriophages MTPH 2,3,7 and 9-12 were obtained from Dr. Grange, J.M.,
Cardiothoracic Institute, Brompton Hospital, Fulham Road, London, SW3 6MP,
England. Bacteriophages MTPH 4 and 5 were obtained from Dr. Little, T.,
Central Veterinary Laboratory, Weybridge.

superfluous. The bacteriophage MTPH 8 was found to contribute
no information not provided by the other bacteriophages employed,
and has yet to be the subject of a special study as regards its
possible use in typing by means of mycobacteriophages.

Bacteriophage MTPH 6 was reported to be unstable and
lose its titre more rapidly on storage, even at $4^{o}C$. Many
workers separately studied the susceptibility of M.tuberculosis
to this bacteriophage, using the same strains of M.tuberculosis
and the same methodology and media. Their results showed
serious inconsistencies and far less agreement than when they
used other bacteriophages (Rado et al, 1975). Baess (1975)
reported that bacteriophage MTPH 6 produces turbid plaques,
concluding that the bacteriophage susceptibility results obtained
with this bacteriophage are not easily reproduced and that its
value in epidemiological studies is consequently only limited.
Finally, many workers using bacteriophage MTPH 6 reported that
M.tuberculosis strains susceptible to this bacteriophage were
found to be only rarely isolated.

6.2.4   PROPAGATION OF MYCOBACTERIOPHAGES

A)   Propagation in liquid medium: The method was
adapted by Grange et al (1976) from techniques described by
Jones and White (1968).

A 10mg wet-weight loopful of the propagating host
strains (Table 22) from LJ medium was transferred aseptically
to the bijou bottles containing the bacterial suspension medium
and three glass balls (Appendix 1). The contents were mixed on
a Whirlimixer until an almost milky suspension was produced.
A bottle containing a 50ml amount of calcium glycerol-supplemented
medium was inoculated with a suspension of the host strain and
incubated at $37^{\circ}C$ for 24 hours or three days according to the
rate of growth of the host strain. A volume of 0.5ml of the
stock bacteriophage suspension was added to the host strain
culture. After incubation of the M.smegmatis and MC15385
cultures (three days) and of the H37RV culture (seven days),
the bacteriophage-containing broths were separated from the
bacteria by centrifugation at 3,000 rpm for 15 minutes in a
bench centrifuge followed by passage through a $0.22\mu m$ pore-
size membrane filter (Millipore, Ltd., London). The filtrates
were stored at $4^{\circ}C$ until use.

B)   Propagation by soft agar overlay method

Bacteriophage suspensions: A serial of eight ten-
fold dilution of the stock bacteriophage lysate were prepared
in calcium glycerol medium. A 0.1ml volume of a bacteriophage
suspension was added to 0.9ml of medium, after which a 0.1ml
volume of the diluted suspension was further diluted in 0.9ml

of medium. This process was repeated to obtain such serial
dilutions. Separate pipettes were used for each dilution.

   Bacterial suspensions: The propagating host strains
were cultured on LJ medium until the growth was judged
sufficient. Old or dry cultures were avoided as these proved
difficult to homogenize. A standard loopful of bacteria,
approximately 5mg wet weight, was added to a bijou bottle
containing 1.0ml of the bacterial suspension medium and the
glass balls. A smooth bacterial suspension was obtained by
mixing the contents of the bijou bottle on a Whirlimixer for
30-60 seconds. The soft agar overlay medium (Appendix 1) was
melted and cooled to $50^{o}C$ in a water bath. A 0.5ml volume
of the propagating host strain suspension and 0.1ml of the
stock bacteriophage dilutions were added to the melted soft
agar overlay, mixed well and the whole mixture poured on the
hard basal medium which was prewarmed to $37^{o}C$. The plates
were placed on a leveled surface to solidify, then incubated
at $35^{o}C$ and examined daily. The concentration of each
bacteriophage necessary to produce just complete lysis of
the related host strain in the whole plate was determined.
These concentrations of bacteriophages were then used for
inoculation of several plates, and, after complete lysis of
the host strain in the whole plate as before, the plates
were flooded by 3-4ml of calcium glycerol-supplemented medium
and left overnight in the refrigerator at $4^{o}C$. The fluid

media containing the bacteriophages were then pipetted off and centrifuged, filtered and stored as described earlier (p.99) for the liquid propagating method. The soft agar overlay propagating method yielded a higher titre than the liquid propagating method.

6.2.5   **TITRATION OF BACTERIOPHAGE SUSPENSION:** Petri dishes containing the hard basal medium were prepared and warmed to 37°C before use. The soft agar overlay medium (Appendix 1) was melted and placed in a water bath at 50°C. The bacterial host strain suspensions, and the serial ten-fold dilutions of bacteriophage suspensions, were prepared, as previously described, as for the propagation of bacteriophage by the soft agar method. A 0.5ml volume of the host strain suspension, and 0.1ml of the bacteriophage dilution, were added to the molten soft agar. The agar was then poured onto the hard basal medium and the plates allowed to solidify and incubated at 35°C. Examination was made daily for lytic plaques until their maximal plaque size had been developed. The number of plaque-forming units (pfu) in the bacteriophage filtrate were estimated for the plaque counts. This was accomplished by multiplying the number of plaques in a plate containing approx-imately 100-200 distinct plaques by the dilution factor for each plate.

Undiluted bacteriophage suspensions were stored in 1.0ml vials at 4°C and were diluted appropriately immediately

prior to bacteriophage typing. These suspensions were used to propagate further batches of bacteriophage as required.

6.2.6   DETERMINATION OF ROUTINE TEST DILUTION (RTD) OF BACTERIOPHAGE SUSPENSIONS: The routine test dilution (RTD) has been defined as the highest dilution of bacteriophage suspension that will produce complete lysis of the propagating strain of bacteria (Adams, 1959).

The RTD was estimated by the following procedure: Eight ten-fold dilutions of the titrated bacteriophage suspensions prepared as for the bacteriophage titration method were applied onto the surface of plate cultures of M.smegmatis ATCC 607 after 4-5 hours incubation, and on the surface of M.tuberculosis H37RV after 24 hours incubation, by means of loops similar to those employed in the Lidwell pattern bacterio-phage typing applicator. The plates were left undisturbed till the drops of bacteriophages were absorbed, then reincubated at 35°C and examined daily for three days in case of M.smegmatis ATCC 607 and for seven days in case of M.tuberculosis H37RV. When maximal lysis was attained, the highest dilution of the bacteriophage suspension producing an area of confluent lysis was taken as the routine test dilution (Fig.10). Bacteriophage MTPH 5 was propagated on MC15385, but, for its titration and RTD determination, M.smegmatis ATCC 607 was used.

Figure 10:   Determination of the routine test dilution (RTD)

for five mycobacteriophages used in this study.
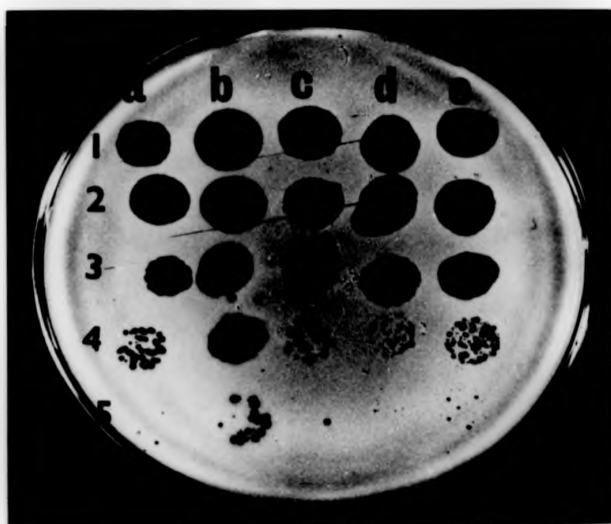
Letters a-e represent bacteriophages MTPH 11,5,4,10 and 12
respectively.

Numbers 1-5 represent the respective bacteriophage dilutions
(10^{-1}-10^{-5}) applied.

The RTD for the bacteriophages MTPH 11,4 and 10 is on row 2 (10^{-2})
dilution.

The RTD for the bacteriophages MTPH 5 and 12 is on row 3 (10^{-3})
dilution.

Figure 10: Determination of the routine test dilution (RTD)

for five mycobacteriophages used in this study.

Letters a-e represent bacteriophages MTPH 11,5,4,10 and 12
respectively.

Numbers 1-5 represent the respective bacteriophage dilutions
(10^{-1}-10^{-5}) applied.

The RTD for the bacteriophages MTPH 11,4 and 10 is on row 2 (10^{-2})
dilution.

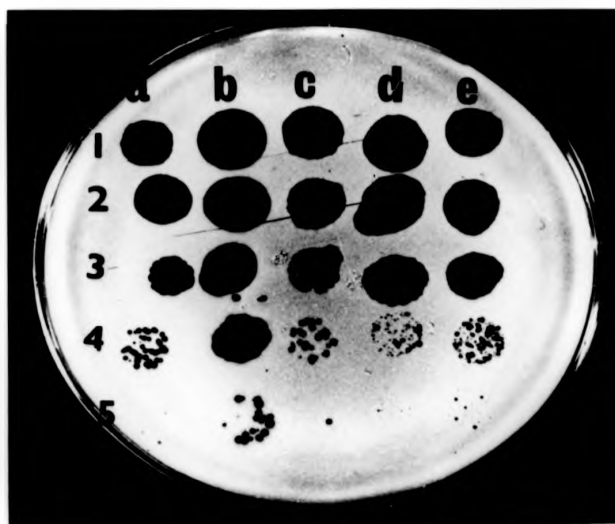The RTD for the bacteriophages MTPH 5 and 12 is on row 3 (10^{-3})
dilution.

6.2.7   BACTERIOPHAGE TYPING OF TEST STRAINS: Suspensions of
each bacteriophage containing 10 RTD, RTD and 0.1 RTD were
prepared freshly immediately before their application.   Test
plates were prepared as for RTD determination, with test strains
incorporated in the soft agar overlays.   The bacteriophage
suspensions (10 RTD, RTD and 0.1 RTD) were applied on the surface
of the plates containing the test strain, after their incubation
at 37°C for 24 hours, by means of Lidwell Pattern Bacteriophage
Typing Applicator (Fig.11).   The plates were reincubated at 35°C
for ten days and examined daily after the first two days.   The
test strains were bacteriophage typed in duplicate and in batches
of 20 strains.   Plates containing host strains (Table 22) were
always included for an assessment of accuracy of the dilutions
made to RTD.   Lysis of the test strains were recorded as
confluent, semiconfluent   single plaques or no lysis, at 10
RTD, RTD and 0.1 RTD (Fig.12).   To allow for fluctuations in the
amount of bacteriophage suspensions delivered by the applicator,
both confluent and semiconfluent lysis at RTD and confluent
lysis at 10 RTD were considered significant.   Rough stains were
typed by pipetting off 1.0ml volume of mycobacterial growth in
7H9 broth after two weeks incubation at 37°C, and subjecting this
to the typing procedure described above.   The concentration of
the inoculum used had a turbidity corresponding to tubes 3-4 on
the McFarland scale.   The inoculum was taken after the culture
was shaken and allowed to stand for five minutes to allow large
clumps to settle.

Figure 11:   Lidwell Pattern Phage Typing Apparatus.
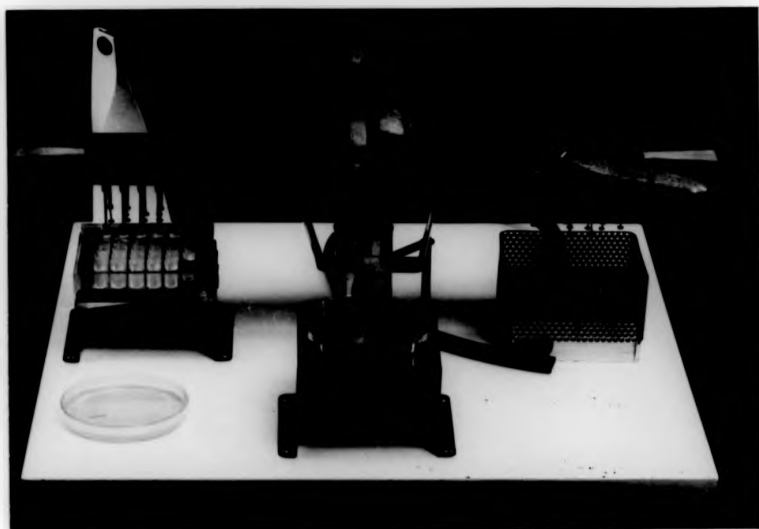
General view taken from the front.

Figure 11:   Lidwell Pattern Phage Typing Apparatus.

General view taken from the front.

Figure 12: Interpretation of bacteriophage lysis results.

Final results	Bacteriophage concentration		
	10 RTD	1xRTD	0.1RTD
+	○	○	⬡
+	○	⬡	∘∘∘
−	⬡	∘∘∘	∘∘
−	∘∘∘	∘∘	no plaques

○ complete lysis   ⬡ semiconfluent lysis

∘∘∘ discrete individual plaques

Source: Rado et al (1975)

After bacteriophage application, and the applied drops had absorbed, the plates were incubated, either stacked on the shelves of the incubator, or, placed in metal containers. Each time the plates were examined the results were recorded, but the readings were considered final when no further change in plaque dimensions could be seen.

6.2.8   DISCUSSION: The soft agar overlay method of Adams (1959) was used in the present study. It has also been used by Baess (1969) and Grange et al (1976); however they had to prepare their media from basic components according to the method described by Redmond and Ward (1966). In the present study commercially-available media was employed, as recommended by Grange (personal communication), described by Jones and Greenberg (1978) and recommended by Bates et al (1976). The soft agar overlay method used in the present study, as described by Grange et al (1976), has definite advantages over the surface lawn method described by Redmond and Ward (1966) and recommended by Rado et al (1975) in that with former: (a) cultures need not be centrifuged and resubcultured to prepare the bacterial surface lawn - procedures which are potentially hazardous to laboratory personnel; (b) the amount of media handling and the time required are reduced; (c) bacterial lawns are smooth and easier to read than the often rough surface lawns.

7.   **RESULTS**

7.   RESULTS

7.1   HOMOGENICEITY OF M.TUBERCULOSIS CULTURES

All strains of both the first and second batch were
found to be bacilliform 2-4μm in length, stained deeply with
the Zeihl-Neelson stain, were arranged in parallel bundles,
grew slowly on LJ medium at 37°C in two to four weeks, and were
incapable of growing at 25°C.

The growth was eugonic and the colonies were raised and
rough (Figure 13), with serpentine arrangements when seen under
the plate microscope. All strains on LJ medium were buff tinted
with no change in pigmentation when grown in darkness, nor after
exposure to light. They were all niacin producers.

Table 23 demonstrates the results of the nitrate
reductase and sensitivity to TCH. Only 5 strains out of the
326 niacin positive were unable to reduce nitrate to nitrite.

Susceptibility to TCH was manifested by only 16 strains,
but the rest were resistant.

Cultures fulfilling the requirements of these tests
were regarded as M.tuberculosis with no exceptions. No attempt
was made to search for M.africanum.

Figure 13: Cultures of <u>M.tuberculosis</u> showing growth characteristics.
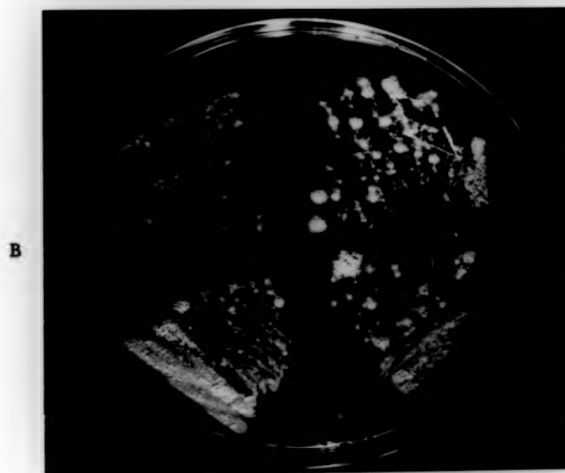
A   Growth characteristics on LJ medium.

B   Growth characteristics on Middlebrook 7H11 agar medium.

Figure 13:   Cultures of M.tuberculosis showing growth characteristics.
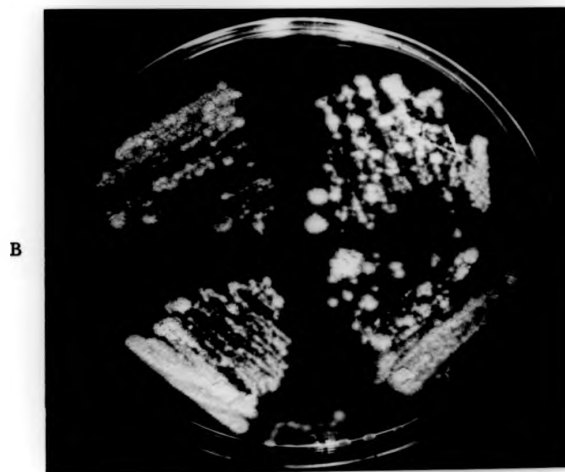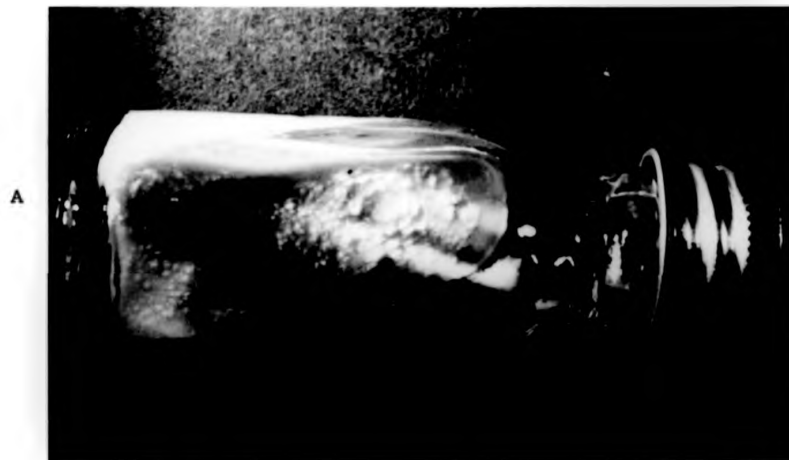
A   Growth characteristics on LJ medium.

B   Growth characteristics on Middlebrook 7H11 agar medium.

Figure 13: Cultures of M.tuberculosis showing growth characteristics.

A Growth characteristics on LJ medium.

B Growth characteristics on Middlebrook 7H11 agar medium.

Table 23: The results of biochemical tests on Libyan strains
of Batch I and II.

Batch Number	Total number of strains tested	Nitrate test		TCH 10ug/ml	
		Positive	Negative	Resistance	Susceptible
I	198	195	3	189	9
II	128	126	2	121	7
Total:	326	321	5	310	16

7.2 DISCUSSION

The results of the present work illustrates the fact that mycobacteria other than M.tuberculosis as a cause of pulmonary tuberculosis, in the geographical area under study, appear only rarely. Cited and reported results (Wallace, 1976) are in conformity with this observation. Wallace (1976) mentions that tuberculosis is rare amongst domestic cattle, and that tuberculosis in man due to M.bovis or M.africanum is of low incidence especially in pulmonary forms of tuberculosis. A prevalence of 0.2% of each species was reported among the strains of mycobacteria. Opportunistic mycobacteria as a cause of tuberculosis also occur only rarely in Libya. Six strains of scotochromogens and five strains of M.fortuitum were isolated only once from each patient among 1,954 strains studied, which represents inadequate evidence for these organisms to be considered as the cause of the disease.

Scotochromogens have been isolated from environmental sources in Eastern and Western regions, and M.fortuitum from the environment in the Eastern region. The alleged rarity of opportunistic mycobacteria in Libya is supported by the work of Stanford et al (1976), who reported that these mycobacteria were much less plentiful in a desert country than in a damp, warm country which bears a heavy cover of vegetation. The few strains which produce niacin but fail to reduce nitrate could be M.africanum. Strains of M.tuberculosis susceptible

to 10µg/ml TCH have been reported (Grange et al, 1977),

7.3   BACTERIOPHAGE TYPING RESULTS

7.3.1   BACTERIOPHAGE SUSCEPTIBILITY PATTERN: At present
there is no internationally recognised nomenclature for the
bacteriophage types of M.tuberculosis.   That used in this study
is based on one suggested for communication by WHO Working Group
on Bacteriophage Typing Mycobacteria and on the one used by
Grange et al (1977).

A.   The first batch classification

On the basis of variation in susceptibility to lysis
by the eleven bacteriophages, 198 strains isolated in 1976-77
were divisible into seven groups.   The pattern obtained and the
number of strains in each group is shown in Table 24.   The
commonest groups were those lysed by MTPH 2 only (60.1%), those
lysed by MTPH 2,3,7 and 9 (8.6%) and those lysed by all eleven
bacteriophages (5.6%); the other three bacteriophage types
contributed only 2.07%.

B.   The second batch classification

The distribution of bacteriophage types in the second
batch - 128 strains isolated in 1979 - is shown in Table 25.

Table 24: Nomenclature of the bacteriophage types and their distribution among 198 patients. Batch I.

Phage type	Group 1	Group II				Group III	Number of strains	%
	MTPH 2	MTPH 3	MTPH 4	MTPH 5	MTPH 9	MTPH 7 * and 10-12		
Ao	+	-	-	-	-	-	119	60.1
Ax	+	-	-	-	-	+	46	23.23
A9	+	-	-	-	+	-	1	.05
A3,9	+	+	-	-	+	-	2	1.01
Ax9	+	-	-	-	+	+	2	1.01
Ax3,9	+	+	-	-	+	+	17	8.6
B	+	+	+	+	+	+	<u>11</u>	5.6

198

* lysis by one or more of the bacteriophages MTPH 7 and 10-12

Table 25: Nomenclature of the bacteriophage types and their distribution among the 128 patients. Batch II.

Phage type	Group 1	Group II				Group III	Number of strains	%
	MTPH 2	MTPH 3	MTPH 4	MTPH 5	MTPH 9	MTPH 7 * and 10-12		
Ao	+	-	-	-	-	-	62	48.4
Ax	+	-	-	-	-	+	6	4.7
A5	+	-	-	+	-	-	15	11.7
A3,5	+	+	-	+	-	-	2	1.6
A3,9	+	+	-	-	+		-	0.8
Ax5	+	-	-	+	-	+	15	11.7
B	+	+	+	+	+	+	27	21.1

128

* lysis by one or more of the bacteriophages MTPH 7 and 10-12

Table 26: Bacteriophage types of strains of <u>M.tuberculosis</u>
(Batch I and II) isolated from patients in the
Western Region of Libya.

Phage Types	Batch I		Batch II		Both Batches	
	No. of strains	%	No. of strains	%	No. of strains	%
Ao	119	60.1	62	48.4	181	55.52
A_x	46	23.23	6	4.7	52	15.95
A_5	–	0	15	11.7	15	4.6
$A_{3,5}$	–	0	2	1.6	2	0.61
$A_{3,9}$	2	1.01	1	0.8	3	0.92
$A_{x,5}$	–	0	15	11.7	15	4.6
$A_{x,3,9}$	17	8.6	–	0	17	5.2
$A_{x.9}$	2	1.01	–	0	2	6.1
A_9	1	.05	–	0	1	0.30
B	11	5.6	27	21.1	38	11.66
Total:	198	100.1	128	100.0	326	

Table 27:   Distribution of bacteriophage types among Batch I
and Batch II strains (Major phage types)

Phage Type	Batch I		Batch II		Both Batches	
	Number	%	Number	%	Number	%
A	165	83.33	68	53.1	233	71.47
I	22	11.07	33	25.8	55	16.87
B	11	5.6	27	21.1	38	11.66
Total:	198	100	128	100	326	100

A = Ao + Ax

I = A5 + A35 + A39 + Ax5 + Ax39 + Ax9 and A9.

These 128 strains could also be divided into seven groups, although some of the groups are different from the division of the distribution of the first batch. The commonest groups were those lysed by MTPH 2 (48.4%) and those lysed by all eleven bacteriophages (21.1%). Those lysed by MTPH 2 and 5 or MTPH 2,5 and 7 each represent 11.7%. The other three groups contributed only 7%.

Strains were considered susceptible to Group III bacteriophages (Table 20) if lysed by one or more of the four bacteriophages (MTPH 7 and 10-12). Ninety percent of strains were shown to be lysed by MTPH 7 with different degrees of lysis from a complete confluent lysis at RTD to a few plaques at 10 RTD. Susceptibility of the strains to other Group III bacteriophages at RTD concentration was only rarely observed. The results of typing with MTPH 2,4 and 5 were clear-cut; the MTPH 3 and 9 produce large plaques with the consequence that 2 or 3 bacteriophage particles could produce a confluent appearance.

Strains susceptible only to bacteriophage MTPH 2 (Ao) and strains show lysis to bacteriophage MTPH 2 and one or more of bacteriophage MTPH 7 and 10-12 (Ax) were grouped into a major bacteriophage Type A.

Strains lysed by MTPH 2 and also by one or more but not all of bacteriophage MTPH 3,4,5 and 9, with or without lysed

by MTPH 7, 1Q-12 were classified as a major Intermediate bacteriophage type (I).

The third major bacteriophage type recognised was Type B which lysed by all used bacteriophages.

In this study, MTPH 4 bacteriophage lysed only strains of Type B; no Intermediate type strains were lysed by this bacteriophage. Table 26 demonstrates the intermediate minor bacteriophage types as they were found to occur in Batches I and II. In the first batch the Intermediate bacteriophage types A5, A3, 5, and Ax5 were not detected, indicating that most strains of this batch were resistant to the bacteriophage MTPH 5. On the other hand, the strains of the second batch show only one strain susceptible to the bacteriophage MTPH 9. Table 27 demonstrates the distribution of the three major bacteriophage types A, B and I.

7.3.2 CHANGE IN FREQUENCY DISTRIBUTION OF BACTERIOPHAGE TYPES:
From an examination of Table 26 and 27, the following conclusions
may be drawn: An increase in bacteriophage Type B strains has
taken place, from 5.6% in the first Batch of strains, to 21% in
the second; there is also an increase in the bacteriophage Type
I from 11.07% to 25.8%, with the decrease in susceptibility to
bacteriophage MTPH 9 and an increase in susceptibility to
bacteriophage MTPH 5. A decrease in bacteriophage Type A from
83.33% to 53.1% was also demonstrated. A significant change in
frequency distribution of M.tuberculosis bacteriophage types
has occurred.

7.3.3 BACTERIOPHAGE TYPES AND LOCALITY: Table 28 demonstrates
the frequency of bacteriophage types distributed according to
district and municipality of strain isolation in 1976-77, and
1979. The change was clear in Tripoli, Zawia, Misurata and
Tarhuna, where both B and I types were increased.

7.3.4 BACTERIOPHAGE TYPES AND DRUG RESISTANCE: No correlation
between drug resistance and bacteriophage types, or strains
resistance to isoniazid, was found among the three bacteriophage
types. Resistance to streptomycin appeared to be divided among
the three major bacteriophage types. Three strains found to be
resistant to PAS were restricted to bacteriophage Type A (Table
29). No correlation was obtained between bacteriophage types
and their in-vitro drug resistance, since the number of strains
from new and previously diagnosed cases were evenly distributed

Table 28: Distribution of phage types of <u>M.tuberculosis</u> strains of both batch I and II in relation to district of isolation.

Phage type	Tp	B	ZA	ZW	MH	MS	Km	Zn	G	TR	St	Ch	Total
A	39	49	8	2	23	20	4	8	1	2	5	1	158
I	4	3	1	2	5	-	1	3	1	1	2		19
B	3	1	1	1	2	1	1	2	1	1	1		10
Total:	46	53	10	5	30	20	4	13	1	2	7	1	187
A	23	17	2	1	7	2	4	2	2	2	-	3	67
I	11	8	1	-	3	3	-	-	2	4	-	-	33
B	12	3	4	-	1	1	1	1	-	2	1	-	26
Total:	46	28	7	1	11	6	5	4	4	10	1	3	126

Tp = Tripoli; B = Busitta; ZA = Zawia; ZW = Zwara; MH = Misurata hospital; MS = Misurata; Km = Kohms;
Zn = Zliten; G = Garian; TR = Tarhuna; St = Sirte; Ch = Chusbat.

Table 29:   Bacteriophage Types and susceptibility to isoniazid,

Streptomycin and PAS of M.tuberculosis strains.

Phage Type	Susceptible to SM INH & PAS	Resistance to SM & INH	Resistance to SM	Resistance to PAS	Total
A	46	2	7	3	68
I	24	5	4	-	33
B	21	2	4	-	27
Total:	91	9	15	3	128

between the three major bacteriophage types. Table 30 demonstrates the bacteriophage type distribution of M.tuberculosis strains isolated from newly-diagnosed cases, in comparison with bacteriophage type distribution of M.tuberculosis strains isolated from previously-diagnosed cases.

7.3.5   BACTERIOPHAGE TYPES IN EXPATRIATES: The bacteriophage-type frequency of M.tuberculosis strains isolated during 1979 from expatriate patients of different nationalities is given in Table 31. Although the number of strains tested was small, results show that Type A and I predominate among Algerians, Tunisian and Chadian strains, while Type I is the commonest in the strains from Asian and Sudanese expatriates. Strains from Egyptian expatriates were mostly (75%) found to be bacteriophage Type B. The general inference from Table 31 is that bacteriophage Type B was rarer in African countries with the exception of Egypt, while Intermediate bacteriophage types are significantly present in those African countries which contribute to the expatriate labour force in Libya.

Several strains from Batch I and II were retested after frequent subculturing in the 7H9 broth without Tween 80. Bacteriophage typing of these strains gave the same bacteriophage types given by the subculturing on LJ medium.

The number of bacteriophage types obtained in the present study resemble the types obtained by Grange et al (1977) who

Table 30: The major Phage Type distribution of <u>M.tuberculosis</u>
strains isolated from new and previously-diagnosed
cases.

Phage Type	New cases	Previously-diagnosed cases
A	84	81
I	12	10
B	6	5

between the three major bacteriophage types. Table 30 demonstrates the bacteriophage type distribution of M.tuberculosis strains isolated from newly-diagnosed cases, in comparison with bacterio- phage type distribution of M.tuberculosis strains isolated from previously-diagnosed cases.

7.3.5   **BACTERIOPHAGE TYPES IN EXPATRIATES**: The bacteriophage- type frequency of M.tuberculosis strains isolated during 1979 from expatriate patients of different nationalities is given in Table 31. Although the number of strains tested was small, results show that Type A and I predominate among Algerians, Tunisian and Chadian strains, while Type I is the commonest in the strains from Asian and Sudanese expatriates. Strains from Egyptian expatriates were mostly (75%) found to be bacteriophage Type B. The general inference from Table 31 is that bacteriophage Type B was rarer in African countries with the exception of Egypt, while Intermediate bacteriophage types are significantly present in those African countries which contribute to the expatriate labour force in Libya.

Several strains from Batch I and II were retested after frequent subculturing in the 7H9 broth without Tween 80. Bacteriophage typing of these strains gave the same bacteriophage types given by the subculturing on LJ medium.

The number of bacteriophage types obtained in the present study resemble the types obtained by Grange et al (1977) who

Table 30: The major Phage Type distribution of <u>M.tuberculosis</u> strains isolated from new and previously-diagnosed cases.

Phage Type	New cases	Previously-diagnosed cases
A	84	81
I	12	10
B	6	5

reported the division of 172 strains into 8 groups; 5 of these groups were called Intermediate. Similar bacteriophage type grouping were used in 1975 by several workers (Engel, 1975; Jones, 1975b; Sula and Solova, 1975).

No attempt was made to correlate the bacteriophage susceptibility and response to treatment or to details of chemotherapy taken from patient records. Thus, the use of the terms 'drug resistant' and 'drug susceptible' are confined entirely to the in-vitro susceptibility tests.

7.4   DISCUSSION ON BACTERIOPHAGE TYPES

The geographical distribution of M.tuberculosis bacterio-phage types in certain parts of the world has been reviewed by Grange and Redmond (1978). Little is known about the bacterio-phage type pattern of M.tuberculosis in North Africa. The nine mycobacteriophages used in the present study divided the strains of both batches into ten bacteriophage types (Table 26). The bacteriophages of Group I and II revealed three major bacterio-phage types: A, B and I types. The Group III bacteriophages provided a further subdivision, but with less accuracy, and their use added little of value to the findings. The distribution of these major bacteriophage types for the first and second batch is set out in Table 27. The results, especially of the first batch, are similar to the reported results obtained from the study

of M.tuberculosis isolates obtained in other parts of Africa
(East, West and Central), and Hong Kong and Japan (reviewed by
Grange and Redmond, 1978). Type A is predominant.

Comparison of the distribution of bacteriophage types of
the Libyan strains with that of other strains isolated from
immigrant or native patients in other countries (Table 32)
revealed that first-batch strains resemble the African (Uganda)
strains, and probably indicate a bacteriophage susceptibility
lying between the British and Asian strains, since the first
batch has less Type B than the British strains, and less Type I
than the Asian strains. On the other hand, the distribution of
bacteriophage types among the second-batch isolates reveals a
proportion of Type B strains similar to British strains and
Type I similar to the Asian, but less bacteriophage Type A than
both the Asian and the British.

One possible explanation is that M.tuberculosis strains
in Libya were subjected to an admixture through importation of
strains along Arab trade routes that have existed for centuries
across the Indian Ocean. The social behaviour and life style
of the Libyan Arab contributes to this admixture, owing to his
traditional ability of integrating easily with other societies.

The results of the present study might indicate that
in a country where immigration is a major feature of life, many

Table 32: Comparison of phage types of <u>M.tuberculosis</u> strain isolated from pulmonary tuberculosis cases in Libya and other countries. (All figures in percentages)

Phage types	India	Hong Kong	British	Asian	African (Uganda)	Batch I	Batch II
	1	2	3	4	5	6	7
A	54	89	68	70	88	84	53
I	43	6	10	25	9	11	25
B	3	5	21	5	3	5	21

1 and 2 Bates and Mitchison (1969)

3,4 and 5 Grange <u>et al</u> (1977)

6 and 7 present study.

bacteriophage types of M.tuberculosis can be found, whereas in more closed societies the prevalence of certain bacteriophage types is striking. Thus the first Batch can be taken to represent the more stable situation; and the second Batch - representing the population becoming increasingly admixed with immigrants - suggests the more unstable situation. Grange (1980) has considered the species M.tuberculosis as being composed of human, bovine and marine variants due to the deletion theory he described. Mankiewicz, on the basis of data in her study of 1972, suggested the possibility that the prevalent bacteriophage type in long-standing reactivated disease or in older patients is different from that found in younger persons or among new-detected cases of tuberculosis. She raised the question of the evolutionary process in mycobacteria. According to the above, then, the multiple bacteriophage types in the present study might well be significantly influenced by the relatively high prevalence of chronic tuberculosis cases in the Western Region.

7.5 DISCUSSION OF THE CHANGE IN BACTERIOPHAGE TYPES DISTRIB-UTION

Comparisons of the respective proportion of bacteriophage types between the strains of the first and second batch studied in the present investigation (Table 26 and 27) revealed that both bacteriophage Types B and I had increased over the interval between the two batches, and that the intermediate subtypes are

different in the two batches. Type I strains of the first batch
are resistant to MTPH 5, and susceptible to MTPH 9 while Type I
strains of the second batch are susceptible to bacteriophage
MTPH 5, and only one strain showed susceptibility to MTPH 9.
The change of bacteriophage type distribution between the two
batches is marked in the districts (Tripoli and Zawia) where
the prevalence of tuberculosis in the expatriate is highest.

On bacteriophage typing, the difference between Type A
and Type B is very marked. Evidence has been offered suggesting
that these two types differ by the presence of endonucleases or
other restriction systems in the Type A strains (Rado, Bates
and Fitzhugh, 1976). Changes in susceptibility to mycobacterio-
phages was found not to be affected by drug therapy or through
mutational changes (Bates and Mitchison, 1969; Clavel and Clement,
1977). Natural lysogency in M.tuberculosis is unknown, and change
in the bacteriophage susceptibility due to the cure of lysogenic
strains has no basis in current theory.

Variation in bacteriophage type distribution between
indigenous and immigrant populations living in the same area
has been reported (Grange et al, 1976; Grange et al, 1977;
Mankiewicz, 1972).

The results of the study of the distribution of bacterio-
phage types of M.tuberculosis isolated from a small sample of

expatriates in the area of study (Table 31) demonstrate that
they approximately resemble the distribution of the second
batch strains, with substantial numbers of Type B (Egyptian)
and Type I (Asians, Sudanese and Tunisian). Grange et al
(1977) reported that little interchange in bacteriophage types
appears to have taken place between the immigrant and
indigenous British populations, who were reported to be different
as regards the bacteriophage patterns of M.tuberculosis isolates.
Proferred reasons include the suggestion that the routes of
infection rarely extended outside the tightly-knit immigrant
(Asian) community, and that tuberculosis consequently will not
easily spread from Asian immigrants to the native British
population. In addition, the high standard of living and the
well-organised health services might be considered as another
explanation. The interchange of M.tuberculosis between the
indigenous and expatriate populations, suggested by Khalil and
Sathianathan (1978), is indirectly confirmed by the change in
bacteriophage types between the first and second batch isolates.
This is contrary to the findings of Grange et al (1977) who
reported little change in bacteriophage types observed among
M.tuberculosis isolates from both British and Asian strains
isolated and tested at intervals of years, (1969, 1975 and 1977).

Possible explanations for the findings of the present
survey derive from the observations that the indigenous Libyan
population is in habitual close contact with the expatriate;

that the latter suffer from a high incidence of infectious
tuberculosis, and that though a high incidence of tuberculosis
is observed among Libyans aged 15-44, they demonstrate a high
incidence of primary resistant M.tuberculosis which were
reported to be high in the expatriate. As well, there is the
high incidence of bacteriophage type B and I in the expatriate;
the high rate of susceptibility of the Egyptian strains to
bacteriophage MTPH 6 (see Bates et al. 1979), and the high rate
of susceptibility of the Tunisian strains to bacteriophage
MTPH 5 (see Sula et al, 1973).

All these explanations collectively might be associated,
and strongly suggest that the change in the bacteriophage
types between the first and second batches studied suggested an
interchange of M.tuberculosis between the expatriate and the
local population. It appears especially to be the case that
the young age group (15-44) is an epidemiological problem
needing particular consideration by the Health Authorities.

8.   <u>GENERAL DISCUSSION</u>

8.   GENERAL DISCUSSION

The high rate of tuberculosis in immigrants is the
most obvious factor contributing to the problem of tuberculosis
in Britain (British Thoracic and Tuberculosis Association, 1973)
and in Canada (Herchfield, 1979), immigrants contributing a
higher rate of infection and their degree of tubercular debility
reflecting the rate of development of active tuberculosis
existing in their home countries. Additionally, increased drug
resistance of imported strains (Hershfield, 1979) and differing
bacteriophage types of M.tuberculosis are other influences which
need to be taken into account (Grange et al, 1977; Mankiewicz,
1972).

The high incidence of tuberculosis, and the high
prevalence of M.tuberculosis drug resistance among expatriates
in Libya observed in this present study and the observed
changes in bacteriophage types, tend to confirm the above
reported statements.

The measures required to meet the challenge of
maintaining the rate of decrease in tuberculosis morbidity rates,
particularly in view of the fact that those rates are affected
by immigration trends, are controversial. The screening of
immigrants for tuberculosis on their arrival at the point of
entry, and attempts made to prevent their entry to the country,
have proved successful in lowering the rate of tuberculosis in

Canada (Enarson, Ashley and Grzybowski, 1979) but in Britain
have not affected the considerably greater incidence of the
disease that develops after arrival in that more industrialised
kingdom (Joint Tuberculosis Committee, 1978; Khogali, 1979).

Follow-up studies of immigrants arriving from areas of
high incidence of tuberculosis is considered necessary (Enarson
et al, 1979), since it is reported as revealing a much greater
incidence of tubercular disease that develops after the admixing
of immigrants with the indigenous population (Joint Tuberculosis
Committee, 1978). In these two host countries of Canada and
Britain, the rate of infection is low and the disease is under
control. Their respective integrated health services are
capable of handling each individual case to its termination. The
immigrant, especially in Britain, is a permanent resident,
having a traceable address and living in tightly-knit communities
with little integration with the indigenous population.

The Libyan situation is, in contrast, one wherein the
majority of the cases among expatriates were initially discovered
by means of the pre-employment medical examination. Screening
for tuberculosis at the point of entry, and follow up after
arrival, are only rarely practised. It was reported (Annual
TCP report, 1976) that insistence upon a clearance medical exam-
ination, made in the country of origin before granting of the
visa to enter Libya, was being taken under consideration, but for
unknown reasons it is a measure currently only incompletely

enforced. This compares unfavourably with the situation in the USA where the measure has been practised with considerable success.

The problem of the expatriate in Libya is more complicated than the problem of immigrants to Britain or Canada. In Libya the majority of the medical and paramedical staff are foreign, of many disparate nationalities, and are continually in a state of change. A country with no clearly defined political or natural boundaries often has no choice, in view of its limited manpower, but to allow many illegal immigrants to move freely across its borders. The expatriate entering Libya can obtain well-paid and employment without a work permit, and may have no reported address where he can be reached for purposes of follow-up. All demonstrate the serious difficulties in attempting control of tuberculosis, especially in view of the continued arrival of immigrants without medical checks being made either in the respective country of origin, or at the point of arrival. A complete solution to the problem cannot be provided by the institution of even the most extensive schemes once immigrants have entered and become distributed among the indigenous population.

Little interchange of M.tuberculosis between the native and immigrant community in Britain was reported (Grange et al, 1977). The results of the present study could be taken as evidence to support the previous observation of Khalil and Sathianathan (1978), to the effect that the influence of the high incidence of tuberculosis in the expatriate is one factor responsible for the

high incidence of the disease in the young age group of the
Libyan population.

In Britain control of the tubercular immigrant was established
by screening at the port of entry in the case of new arrivals,
supplemented by follow-up procedures carried out by the integrated
health services at the place of settlement of both new arrivals and
established immigrants. In Libya, considering the results of the
present work, this policy has to be implemented if tuberculosis
is to be controlled.

A full pre-entry medical examination for immigrants as
practised by the United States and Canada and partially in
Britain could possibly be implemented, and appears all the more
urgent especially when considering that the majority of
immigrants in Libya arrive with some form of active disease.
Undetected cases which might develop after arrival would not
contribute to the greater incidence over and above that
discovered at the pre-entry examination. This step could be
easily practised since the number of immigrants arriving yearly
is not great in comparison with the above-mentioned countries
were staff and equipment supplied in adequate numbers - a financial
burden easily tolerated by a nation such as Libya - the results
of the control measures would be satisfactory.

The BCG vaccination of all immigrants without tuberculin testing has to be considered as an additional adjunctive measure although the benefit would be relatively small and comparatively limited, since most immigrants are adults who are already infected.

In Britain where the medical examination of immigrants at the point of entry is incomplete, it is considered that the essential control activity at ports of entry is to obtain accurate information about the destination of immigrants, and to pass this on to the appropriate local district medical authorities who are responsible for follow-up of those immigrants at their place of settlement. In Libya the comparable step is the pre-employment medical examination, but unfortunately this is seriously hindered by the availability of many jobs where work permits are not required. Follow-up of the established immigrants in order to reduce endogenous exacerbations or exogenous reinfection is not considered a problem in Libya because this group of immigrants is relatively small. It is considered that the risk of infection in the immigrants' environment is relatively high, but this possibly would be reduced in the more ideal situation by BCG vaccination and the earlier diagnosis of new cases. Immigrants need encouragement and education to report symptoms early and medical examinations should be offered on even the slightest suspicion. Annual chest radiography would still be very productive in the high risk group. Improving living conditions and relieving overcrowding are other important adjuncts, and as mentioned earlier (p19), progress is being made in these fields.

Regarding the indigenous and the established immigrant populations the basic policy of case finding among symptomatic patients by self reporting to the tuberculosis centres, and the subsequent domiciliary treatment, plus the policy of BCG vaccination, no change is thought necessary by this reporter. It is essential however that all these activities are kept at the highest level of efficiency. The actual catchment areas of each tuberculosis control centre can be defined, and, wherever settlements beyond these limits are not covered by the tuberculosis control programme, integration of the control programme elements into the general health services then may be considered. Concerning treatment activities, it might be recommended that a permanent system of monitoring and evaluation of activities be established in order to provide regular information on the organisation of treatment, in terms of the regularity of drug intake, defaulter rates and motives, and operational losses between diagnosis and the time of initiating treatment. The largest should be to ensure that all patients diagnosed are initiating treatment and are taking a full course of chemotherapy.

In this connection it is this reporter's conviction that BCG vaccination at birth should be continued inspite of the low rate of infection in the youngest age group, but that the main target must be to vaccinate all children at the time of entering and leaving school. This policy was agreed upon by WHO (1980) in their technical report series number 652 inspite of the disappointing results of tuberculosis prevention

trials in Madras (1979). The annual risk of infection as a
measure of the tuberculosis problem should be monitored by
mantoux testing a sample of unvaccinated children at the time
of entering school.

Further research might comprise the comparison of the
present survey with other projected surveys either on data
from the other two regions of the country or on data from
neighbouring countries especially those representing the
main source of expatriates, using the same methodology.

The bacteriophages currently employed do not differen-
tiate between M.tuberculosis and M.bovis. Further research
in bacteriophage isolation might produce bacteriophages for
use in the rapid laboratory identification of mycobacteria
similar to bacteriophage 33D, which may be used in the identif-
ication of BCG strains in order to distinguish them from both
M.tuberculosis and M.bovis strains. Also new bacteriophages
which will permit a further and reproducible subdivision of the
established bacteriophage types needed to be obtained.

Although the present study revealed the rarity of
M.bovis and other opportunistic mycobacteria in the epidemiology
of tuberculosis, further research might be needed concerning
the prevalence of M.bovis in animals and the role of other
mycobacteria in human disease and their prevalence in nature.
The importance of the latter has been emphasised by Stanford,

Shield and Rook (1981) who hypothesised that the nature and
frequency of exposure to non-tuberculous mycobacteria may
predetermine the protective efficacy of BCG.

Further research areas might include the study of the
prevalence of primary drug resistance of M.tuberculosis
isolates in Libya, and measurement of the tendency for
resistant strains to accumulate within the indigenous and
expatriate communities.

It would also be useful to demonstrate the changes in
the prevalence of resistant strains from that given in previous
reported results (Khalil and Sathianathan 1978; Wallace, 1976),
and to study the contribution made by the expatriate to the
data obtained, since the levels of primary drug resistance
could be used for assessing the amount of M.tuberculosis trans-
mission in the community.

9.   <u>APPENDIX</u>

APPENDIX I

I.   Bacteriophage suspending and propagating medium.

   1.   Oxoid No. 2 broth.

   2.   4% glycerol.

   3.   10 μg/ml Calcium chloride.

Mixed and dispensed in flat bottles of 100ml capacity
in 50ml volumes. Sterilised at 121°C for 15 minutes and
stored at 4°C to be used within the period of a month.   It
will be notified as Calcium glycerol supplemented medium
(CGSM).

II.   Hard basal medium (oleic acid-albumin agar)

   1.   Dubos oleic Agar base (Difco) 4.0g.

   2.   Bacto-Dubos Oleic Albumin Complex (Difco) 20.0ml.

   3.   Distilled water 180.0ml.

The agar base was dissolved in distilled water by
steaming, then sterilised by autoclaving at 121°C for 15
minutes. The medium was cooled to 55°C in a water bath,
then asceptically 20ml of sterile bacto-Dubos oleic albumin
complex (Difco) was added. The content was mixed gently on
the magnetic stirrer where the magnetic bar was placed in
the agar medium before sterilisation. The medium was dispensed
in plastic perti dishes of 85mm diameter, approximately 30ml
of medium per dish. Sterility was checked by three days'

incubation at $37^{\circ}C$ of the whole plate, they were kept at $4^{\circ}C$ and used within a month.

III.   Soft Agar Overlay Medium.

1. Middlebrook 7H9 broth powder (Difco) 6.5g

2. Bacto Casitone (Difco)   5.0g

3. Proteose peptone No. 3 (Difco)   10.0g

4. Glycerol   10.0ml

5. Noble Agar (Difco)   7.5g

6. Distilled water   1000.0ml

The ingredients 1,2,3 and 4 were first dissolved in water, then Noble agar was added. The content was steamed to dissolve the agar. The medium was dispensed in 4.5ml amounts into universal bottles (28ml size) and then sterilised by autoclaving at $121^{\circ}C$ for 15 minutes, stored at $4^{\circ}C$ and used within the period of a month.

IV.   Bacterial Suspension Medium.

1. Nutrient broth   100ml

2. Bovine serum albumen fraction V (Sigma) 2.0g

This was gently mixed on a magnetic stirrer, then sterilised by filtration through 0.22μm membrane filter and asceptically distributed in sterile bijou bottles containing three glass balls, 1.0ml in each bottle, sterility was checked by incubation of the bottles at $37^{\circ}C$ for three days, then stored at $4^{\circ}C$ to be used within the period of a month.

V.   7H9 broth medium.

    1.   Middlebrook 7H9 broth base (Difco)   4.7g

    2.   Tryptone (oxoid)   1.0g

    3.   glycerol   2.0ml

    4.   Distilled water   900.0ml

    5.   Middlebrook OADC enrichment (Difco)   100.0ml

The ingredients 1,2 and 3 were dissolved in water by stirring, then sterilised by autoclaving at $121^{o}C$ for 15 minutes. The medium was cooled and asceptically 100ml sterile Middlebrook OADC was added and dispensed in 5-6ml amounts in sterile, universal bottles (28ml size). Sterility was confirmed by incubation of the bottles at $37^{o}C$ for three days.

VI.   7H11 plate medium.

    1.   Middlebrook 7H11 agar base (Difco)   21.0g

    2.   glycerol   5.0ml

    3.   Distilled water   1000.0ml

    4.   Middlebrook OADC enrichment (Difco)   100.0ml

The ingredients 1 and 2 and 3 were dissolved in water by steaming, then distributed in 180ml amounts in 300 capacity bottles and sterilised at $121^{o}C$ for 15 minutes. To each 180ml sterile medium cooled in a water bath to $55^{o}C$, 20ml of OADC was added under asceptic conditions. The medium was distributed

in plastic petri dishes (85mm diameter) about 30ml per petri
dish. After solidification the plates were dried and incubated
at 37°C for three days for sterility control.

10. <u>**BIBLIOGRAPHY**</u>

1. Adams, M.H. (1959) Bacteriophages. Academic Press Inc. New York.

2. Annual report of tuberculosis control programme in Libya for 1975 (1976). Endemic Disease section, Community Health Department, Ministry of Health, Libya.

3. Annual statistical report (1978). Department of Planning, Ministry of Health, Libya.

4. Baess, I. (1966) A bacteriophage for subdividing the species M.tuberculosis. American Review of Respiratory Disease, 93, 622-623.

5. Baess, I. (1969) Subdivision of M.tuberculosis by means of bacteriophages. Acta Pathologica et Microbiologica Scandinavica, 76, 464-474.

6. Baess, I. (1975) Results of typing of M.tuberculosis with seven known and two adapted phages. Annali Sclavo, 17, 563-567.

7. Bates, J.H. and Fitzhugh, J.K. (1967) Subdivision of the species M.tuberculosis by mycobacteriophage typing. American Review of Respiratory Disease, 96, 7-10.

8. Bates, J.H. and Mitchison, D.A. (1969) Geographic distribution of bacteriophage types of M.tuberculosis. American Review of Respiratory Disease, 100, 189-193.

9. Bates, J.H., Stead, W.W. and Rado, T.A. (1976) Phage types of tubercle bacilli isolated from patients with two or more sites of organ involvement. American Review of Respiratory Disease, 114, 353-358.

10. Bhatia, A.L., Csillag, A., Mitchison, D.A., Selkon, J.B., Somasundaram, P.R. and Subbaiah, T.V. (1961) The virulence in the guinea-pig of tubercle bacilli isolated before treatment from South Indian patients with pulmonary tuberculosis. 2 Comparisons with virulence of tubercle bacilli from British patients. Bulletin of the World Health Organisation, 25, 313-322.

11. Bland, R.H., Teodorovic, B., Verna M. Huffman and Margreitner, K. (1967) Planning of health services of Libya, survey and recommendations, EM/PHA/116, World Health Organisation, Alexandria.

12. British Thoracic and Tuberculosis Association (1973).
    A tuberculosis survey in England and Wales 1971: the
    influence of immigration and country of birth upon
    notifications. Tubercle. 54, 249-260.

13. Bulla, A. (1977) Global review of tuberculosis morbidity
    and mortality in the world. World Health Statistical
    Report. 30, 2-38.

14. Clavel, S. (1975) Lysotypie due bacille tuberculeux dans
    quelques pays d'Afrique tropicale et an Guadeloupe.
    Comparaison avec la France. Rapports avec la classification
    de M.africanum. Annali Sclavo, 17, 571-577.

15. Clavel, S. and Clement, F. (1977) Stability of bacteriophage
    type of M.tuberculosis. Absence of variation caused by
    experimental chemotherapy in mice and analysis of
    spontaneous variation. American Review of Respiratory
    Disease, 115, 443-447.

16. Dickinson, J.M., Lefford, J.M., Lloyd, J. and Mitchison,
    D.A. (1963) The virulence in the guinea-pig of tubercle
    bacilli from patients with pulmonary tuberculosis in
    Hong Kong. Tubercle, 44, 446-451.

17. Enarson, D., Ashley, M.J. and Grzybowski. (1979)
    Tuberculosis in immigrants to Canada. A study of present
    —day patterns in relation to immigration trends and birth
    place. American Review of Respiratory Disease, 119, 11-18.

18. Engel, H.W.B. (1975) Phage typing of strains of
    M.tuberculosis in the Netherlands. Annali Sclavo, 17,
    578-583.

19. Engel, H.W.B. (1978) Mycobacteriophages and phage typing.
    Annales de Microbiologie, (Inst. Pasteur) 129A, 75-90.

20. Froman, S., Will, D.W. and Bogen, E. (1954) Bacteriophages
    active against M.tuberculosis. I, Isolation and activity.
    American Journal of Public Health. 44, 1326-1333.

21. Gardner, G.M. and Weiser, R.S. (1947) Discovery of a
    bacteriophage for M.smegmatis. Journal of Bacteriology,
    54, 272-273.

22. Gebreel, A.O. and Ashraf, M. (1974a) BCG vaccination
    in Libya, Endemic Disease Section, Community Health
    Department, Ministry of Health, Libya.

23. Gebreel, A.Q. and Ashraf, M. (1974b) Tuberculosis Control
     Programme of Libya, Endemic Disease Section, Community
     Health Department, Ministry of Health, Libya.

24. Grange, J.M. (1980) Mycobacterial diseases, Edward Arnold,
     London.

25. Grange, J.M., Aber, V.R., Allen, B.W., Mitchison, D.A.,
     Mikhail, J.R., McSwiggan, D.A. and Collins, C.H. (1977).
     Comparison of strains of M.tuberculosis from British,
     Ugandan and Asian immigrant patients: a study in
     bacteriophage typing, susceptibility to hydrogen peroxide
     and sensitivity to thiophen-2-carbonic acid hydrazide.
     Tubercle, 58, 207-215.

26. Grange, J.M., Aber, V.R., Allen, B.W., Mitchison, D.A.
     and Goren, M.B. (1978) The correlation of bacteriophage
     types of M.tuberculosis with guinea-pig virulence and
     in vitro indicators of virulence. Journal of General
     Microbiology, 108, 1-7.

27. Grange, J.M., Collins, C.H. and McSwiggen, D.A. (1976)
     Bacteriophage typing of M.tuberculosis strains isolated
     in South East England. Tubercle, 57, 59-66.

28. Grange, J.M. and Redmond, W.B. (1978) Host-phage
     relationships in the genus mycobacterium and their clinical
     significance. Tubercle, 59, 203-225.

29. Hershfield, E.S. (1979) Tuberculosis in the World.
     Chest, 76, 805-811.

30. Hnatko, S.I. (1956) The application of bacteriophages to
     the study of acid-fast micro-organisms from tuberculous
     patients. Canadian Journal of Microbiology, 2, 39-44.

31. Husain, S.A. and Thorup, Ib. (1978) Report of Tuberculosis
     prevalence survey (EM/TB/152) World Health Organisation,
     Libya.

32. Joint Tuberculosis Committee (1978) Tuberculosis among
     immigrants in Britain. British Medical Journal, 1,
     1038-1040.

33. Jones, W.D. (1975a) Differentiation of known strains
     of BCG from isolates of M.tuberculosis and M.bovis by
     using mycobacteriophage D33. Journal of Clinical
     Microbiology, 1, 391-392.

34. Jones, W.D. (1975b) Phage typing report of 125 strains
    of M.tuberculosis. Annali Sclavo, 17, 599-604.

35. Jones, W.D. (1979) Further studies of mycobacteriophage 330
    (Warsaw) for differentiation of BCG from M.bovis and
    M.tuberculosis. Tubercle, 60, 55-58.

36. Jones, W.D. and Greenberg, J. (1978) Modification of
    methods used in bacteriophage typing of M.tuberculosis
    isolates. Journal of Clinical Microbiology, 7, 467-469.

37. Jones, W.D. and White, A. (1968) Lysogeny in mycobacteria.
    -I. Conversion of coloney morphology, nitrate reductase
    and Tween 80, hydrolysis of mycobacterium sp. ATCC 607
    associated with lysogeny. Canadian Journal of Microbiology.
    14, 551-555.

38. Joseph, S., Mitchison, D.A., Ramachandran, K., Selkon, J.B.,
    and Subbaiah, T.V. (1964) Virulence in the guinea-pig
    and sensitivity to PAS and thiacetagone of tubercle
    bacilli from South Indian patients with pulmonary
    tuberculosis. Tubercle, 45, 354-359.

39. Kadiki, O. and Ashraf, M. (1972) Tuberculosis in Libya,
    Endemic Disease Section, Community Health Department,
    Ministry of Health, Libya.

40. Kanter, H. (1967) Libya - A Geomedical Monograph, Springer
    - Verlag, Heidelberg, New York.

41. Khalil, A. and Sathianathan, S. (1978) Impact of anti-
    tuberculosis legislation in Libya on the prevalence of
    primary and acquired resistance to the three main drugs
    at a major tuberculosis centre. Tubercle, 59, 1-12.

42. Khogali, M. (1979) Tuberculosis among immigrants in the
    United Kingdom: the role of occupational health services.
    Journal of Epidemiology and Community Health, 33, 134-137.

43. Kitahara, K. (1973) Studies on the identification of
    infection source for pulmonary tuberculosis in family
    using bacteriophage types as a marker. Kekkaku, 48,
    61-69.

44. Larsson, L. and per-Anders Mardh (1976) Gas chromato-
    graphic characterization of mycobacteria: analysis of
    fatty acids and Trifluoroacetylated whole cell methano-
    lysates. Journal of Clinical Microbiology, 3, 81-85.

45. Mankiewicz, E. (1972)   Bacteriophage types of mycobacteria. Canadian Journal of Public Health, 63, 342-354.

46. Mankiewicz, E. and Liivak, M. (1975)   Phage types of M.tuberculosis in cultures isolated from Eskimo patients. American Review of Respiratory Disease, 111, 307-312.

47. Marks, J. (1976)   A system for the examination of tubercle bacilli and other mycobacteria.   Tubercle, 57, 207-225.

48. Mitchison, D.A., and Lloyd, J. (1964)   Comparison of the sensitivity to thiacetazone of tubercle bacilli from patients in Britain, East Africa, South India and Hong Kong.   Tubercle, 45, 360-369.

49. Mizuguchi, Y., Maruyama, Y., Suga, K. and Murohashi, T. (1973)   Comparison of phage susceptibility of M.tuberculosis isolated from patients in Japan, Netherlands and Ceylon. Kekkaku, 48, 219-225.

50. Murohashi, T. and Mizuguchi, Y. (1975)   Phage typing of Japanese strains of M.tuberculosis.   Annali Sclavo, 17, 615-617.

51. Murohashi, T., Tokunaga, T., Mizuguchi, Y. and Maruyama, Y. (1963)   Phage typing of slow growing mycobacteria. American Review of Respiratory Disease, 88, 664-669.

52. Newman, R. (1961)   Report of Tuberculosis prevalence survey (EM/TB/56)   World Health Organisation, Alexandria.

53. Nyboe, J. (1960)   The efficacy of tuberculin test: an analysis based on results from 33 countries.   Bulletin of the World Health Organization, 22, 1-37.

54. Rado, T.A., Bates, J.M., Engel, H.W.B., Mankiewicz, E., Murohashi, T., Mizuguchi, Y. and Sula, L. (1975)   World Health Organisation studies on bacteriophage typing of mycobacteria.   Subdivision of the species M.tuberculosis. American Review of Respiratory Disease, 111, 459-468.

55. Rado, T.A., Bates, J.H. and Fitzhugh, J.K. (1976)   Evidence for host modification and restriction of bacteriophage DNA in M.tuberculosis.   Journal of General Virology, 30, 91-97.

56. Raleigh, J.W. and Wichelhausen, R.H. (1973)   Exogenous reinfection with M.tuberculosis confirmed by phage typing. American Review of Respiratory Disease, 108, 639-642.

57. Raleigh, J.W., Wichelhausen, R.H., Rado, T.A. and Bates,
     J.H. (1975) Evidence for infection by two distinct strains
     of M.tuberculosis in pulmonary tuberculosis. American
     Review of Respiratory Disease. 112, 497-503.

58. Redmond, W.B. (1963) Bacteriophages of the mycobacteria,
     a review. Advances in Tuberculosis Research, 12, 191-229.

59. Redmond, W.B., Bates, J.H. and Engel, H.W.B. (1979)
     Methods for bacteriophage typing of mycobacteria. In
     methods in microbiology. Ed. Bergan, T. and Norris, J.R.
     Vol. 13, Ch.8, Academic Press, London, New York.

60. Redmond, W.B. and Cater, J.C. (1960) A bacteriophage
     specific for M.tuberculosis, varietas hominis and bovis.
     American Review of Respiratory Disease, 82, 781-786.

61. Redmond, W.B., Cater, J.C. and Ward, D.M. (1963) Spotting
     method for phage typing mycobacteria. Typing mycobacteria
     with routine test dilutions. American Review of Respiratory
     Disease, 87, 257-263.

62. Redmond, W.B. and Ward, D.M. (1966) Media and methods for
     phage typing mycobacteria. Bulletin of the World Health
     Organisation, 35, 563-568.

63. Rizgalla, A. and Hashmi, A.A. (1978) Report of tuberculosis
     cases registered during 1971-1977 in the Western Region of
     Libya. Endemic disease Section, Community Health Department,
     Ministry of Health, Libya.

64. Runyon, E.H. (1959) Anonymous mycobacteria in pulmonary
     disease. Medical Clinics of North America, 43, 273-290.

65. Runyon, E.H., Karlson, A.G., Kubica, G.P. and Wayne, L.G.
     (1974) Mycobacterium. In manual of clinical microbiology.
     Ed. Blair, J.E., Lennette, E.H. and Truant, J.P. Ch.16,
     American Society for microbiology, Bethesda, Md.

66. Stanford, J.L. and Grange, J.M. (1974) The meaning and
     structure of species as applied to mycobacteria. Tubercle,
     55, 143-152.

67. Stanford, J.L., Shield, M.J., Paul, R.C., Khalil, A., Tobgi,
     R.S. and Wallace, A. (1976) The effect of desert conditions
     on the reactivity of Libyan school children to a range of
     new tuberculins. Journal of Hygiene, 77, 63-75.

68. Stanford, J.L., Shield, M.J. and Rook, G.A.W. (1981)
    How environmental mycobacteria may predetermine the protective
    efficacy of BCG. Tubercle, 62, 55-62.

69. Statistical abstract of Libya (1975) Vol. 14, Census and
    Statistics Department, Ministry of Planning, Libya.

70. Stylbo, R. (1976) Report on visit to the Endemic Disease
    Section (Tuberculosis programme), Community Health Depart-
    ment, Ministry of Health, Libya.

71. Subbaiah, T.V., Mitchison, D.A. and Selkon, J.B. (1960).
    The susceptibility to hydrogen peroxide of Indian and
    British isoniazid-sensitive and isoniazid-resistant tubercle
    bacilli. Tubercle, 41, 323-333.

72. Sula, L., Redmond, W.B., Coster, J.F., Baess, I., Bates, J.H.,
    Caroli, G., Mankiewicz, E., Murohashi, T. and Vandra, E.
    (1973) World Health Organisation Co-operative studies on
    the phage typing of mycobacteria. I phage lysis of
    Czechoslovak and Italian strains of M.tuberculosis. Bulletin
    of the World Health Organisation, 48, 57-63.

73. Sula, L. and Solova, J. (1975) Phage typification of 100
    strains of M.tuberculosis isolated in Czechoslovakia.
    Annali Sclavo, 17, 623-628.

74. Sushida, K. and Hirano, N. (1972) Isolation from media of
    two bacteriophages, protamylase H37Rv (PH) active against
    M.tuberculosis. American Review of Respiratory Disease,
    106, 269-271.

75. Takeya, K. and Tokiwa, H. (1974) Bacteriocin typing of
    M.tuberculosis. American Review of Respiratory Disease,
    109, 304-305.

76. Takeya, K. and Yoshimura, T. (1957) Isolation of bacterio-
    phage active against all types of M.tuberculosis. Journal
    of Bacteriology, 74, 540-541.

77. Takeya, K., Yoshimura, T., Yamura, K. and Toda, T. (1959)
    Studies on the biologic properties of mycobacteriophage.
    American Review of Respiratory Disease, 80, 543-553.

78. Tokunaga, T., Maruyama, Y. and Murohashi, T. (1968)
    Classification of subtypes of human tubercle bacilli by phage
    susceptibility. American Review of Respiratory Disease, 97,
    469-471.

79. Tuberculosis prevention trial Madras (1979). Trial of BCG
     vaccines in South India for tuberculosis prevention. Indian
     Journal of Medical Research, 70, 349-363.

80. Wallace, A. (1976) The bacteriology of tuberculosis in Libya,
     Baroni press, Tripoli, Libya.

81. Ward, A.J. and Sieg, S.M. (1975) Phage susceptibility pattern
     of M.tuberculosis strains freshly isolated from Virginia
     patients. Annali Sclavo, 17, 629-633.

82. Wilson, G.S. and Miles, A.A. (1975) Topley and Wilson's
     principle of bacteriology, virology and immunity. 6th edition.
     Vol.11, p1724, Edward and Arnold, London.

83. Wolinsky, E. (1979) Non-tuberculous mycobacteria and associated
     diseases. American Review of Respiratory Disease, 119, 107-159.

84. World Health Organisation (1980) BCG vaccination policies.
     Report of a WHO study group. World Health Organisation
     Technical Report Series No. 652.