A COLLABORATIVE STUDY OF THE PATHOLOGY AND BACTERIOLOGY
OF HUMAN AND ANIMAL TUBERCULOSIS IN THE SUDAN

By

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The Scope of the Study

Despite the fact that tuberculosis is a fairly common disease in the Subanu there is very little recorded information about the various aspects of the disease in both man and animals. It is felt therefore that a detailed study of the prevalence and pattern of the disease in man and animals is a top priority in a country like the Suban. The general concept that glandular and bone tuberculosis in the Suban may be epizootic needs scientific documentation. Such a concept emerged from the well known close association between man and his domestic animals in this country. The well known habit of eating raw meat (barrem) and of drinking fresh un-boiled milk and the common occurrence of lymph nodes and bone tuberculosis have further lent support to the possible prevalence of bovine tuberculosis in man. The main themes of the study is as follows:

1. To throw light on the prevalence of the disease as judged on the results of prospective and retrospective analysis of material from animals and patients.
2. To study in detail the pathology of the disease and to compare it with the pattern seen in other tropical parts of the world particularly Africa.

3. To identify and classify the mycobacteria isolated from infected cattle and man.
Abstract

The material used for this study was obtained from both human and animal sources.

46 specimens mainly of bone, joint, lymph nodes and ascitic fluid were collected from patients at Khartoum, Khartoum North, Omdurman and Wau hospitals. Chest radiography and Mantoux test were carried out on some of them.

In a retrospective study for the period 1959/70, 669 tuberculous biopsies were analysed. Affected organs consisted of 384 lymph nodes, 145 bones and joints, 23 endometrium, 22 testis and 75 various organs. The tuberculous nature of these specimens were confirmed histopathologically.

A consecutive study of 895 autopsies in the period 1957/70 yielded pathological evidence of tuberculosis in 121 cases, 28 being confirmed histologically. These cases were divided into 38 extra-pulmonary and 83 pulmonary tuberculosis, the latter was classified into isolated pulmonary tuberculosis
in 16, lung lesions with mediastinal glandular involvement in 6
and pulmonary lesions with dissemination in 51 cases.

50 bovine specimens collected from Wau and Onuruman
Central Abattoir were examined. These were classified according
to the distribution of the lesions into 5 cases of extra-pulmonary
tuberculosis and 45 pulmonary ones - 8 of which were of the
disseminated type.

The comparative intradermal tuberculin test was carried
out in 760 selected cattle in Bulgrewin and Elbeid Government
dairies.

63 mycobacterica from lesions were primarily isolated in
Lowenstein-Jensen medium; 53 strains of these were typed - 51
being mycobacterium tuberculosis and two mycobacterium bovis. 43
of these strains were found fully sensitive to streptomycin,
Isoniazid and para-aminosalicylic acid; one of the two myco-
bacterium bovis strains being fully sensitive and thus included.
The rest of the strains were variable resistant to one or two of
these drugs.
33 mycobacteria from cattle were also isolated in
Lavenstein-Jensen medium and only 19 were typed as mycobacterium
bovis. One of these particularly V2b was atypical, rough and
agonalic. All these strains were sensitive to streptomycin,
Isomycin and para-amine sulphonamide.

Some of the human and bovine strains were subjected to
the virulence tests as measured by spleen and mental indices.
These strains were virulent for guinea pigs. Some of the bovine
strains were inoculated into rabbits and proved to be virulent
for them.

These mycobacteria were isolated from two bovine and one
marine lysogenic strains. This procedure entailed no usage of
an inducing agent - neither chemical nor physical. The ultraviolet
irradiation trials for liberation of mycobacteria from these
strains proved to be a failure.
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Tuberculosis has been recognized since ancient times as one of the greatest scourges of man. It has also been recognized among domestic and wild animals. That the disease was known to ancient civilizations, is indicated in the code of Hammurabi written before 2000 B.C. Further Hippocrates in the fifth century B.C. described "Phthisis" as the most prevalent disease. The disease was known under several names such as "Phthisis, Scrofula, Tuberculosis, Inflammation of the lungs, Gastric fever, Lupus, Consumption, Great white plague and wasting disease." Sydenham (1624 - 1689) described two types of tuberculosis namely "Phthisis" - (a severely progressive type of tuberculosis affecting young and adults) - and a more chronic type known as "Winter cough." Lartet (1698) considered the term "Phthisis" or "Consumption" to indicate wasting of the body from any cause, and Huxley (1722) who advanced "The Germ Theory" stated that "Phthisis" or any other wasting disease...
was caused by minute living creatures, which is called Animalcula (little animals or little vegetables), Villemain (1665) demonstrated haemolysis as a specific affliction caused by an in-
curable agent or germ; the latter being transmissible from man or
cow to rabbits or guinea pigs and could also be transmitted from
one infected animal to another in continuous series. His work
suggested a difference between the behaviour of human and bovine
tuberculosis. The final proof of the infectious and bacterial
nature of tuberculosis was provided by Robert Koch who announced
his discovery in 1882 that tubercle bacilli are constantly present
in the lesions from which they could be isolated in pure culture.
He showed that infection of animals with these cultures produced
typical lesions of tuberculosis. The literature on this subject
is extensive and voluminous and is difficult to cover fully, but
interested readers are referred to the reviews by Cummins
(Tuberculosis in history, 1940) and Mackan (The conquest of
Tuberculosis, 1962).

1. Classification and Types

Classification of the tubercle bacilli is based on various

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characters such as cultural characteristics, colonial morphology and animal pathogenicity. The tubercle bacilli are also divided into *Mycobacterium* (human and bovine) and *Avian* mycobacteria (Paterson 1959, Soltyš 1963). Topley and Wilson (1964) suggested a classification into *Mycobacterium*, *Avian* and *Atypical* types.

The terms "Atypical", "Anonymous" or "Unclassified" mycobacteria were originally suggested by Rungon (1952) who further subdivided this type into four groups, namely: I. Photochromogen, II. Heterochromogen, III. Non-photochromogen and IV. Rapid grower. Xalabarder (1961) reviewed the literature on atypical mycobacteria. The global occurrence of the latter was reported on by several authors such as Beck and Associates (1963) from England and Urabe et al. (1961), Corps, Rungon and Lester (1963) from Japan. Bauer and Davies (1965) isolated 170 anonymous mycobacteria out of 2852 strains examined in Nigeria. Moreover Cavanagh (1965) reported one *Anonymous* mycobacterium among 522 strains isolated from Sudanese patients, while Grande (1957) characterized three atypical strains after examining 693 strains.
The bovine type of the bacillus has been frequently isolated from extra-pulmonary lesions such as joints, lymph nodes (Degarmo, 1942 and Habib and Vartanian, 1966). Cases of pulmonary tuberculosis due to the bovine type were also occasionally found (Burns et al., 1962 and Magnus, 1967). In 1962 Karib reported one bovine type out of 15 strains isolated from Sudanese patients. This was duplicated by Kahn in 1970. The latter claimed that the strain was the first of its type to be isolated from man in the Sudan.

Recently a new strain intermediate in characteristics between mycobacterium tuberculosis and mycobacterium bovis was reported from West and Central Africa (Riss, 1970). This strain is known as mycobacterium africanum and was entered under this name in the American type culture collection (ATCC). In 1974, Cast et al. worked on 360 strains isolated from pulmonary lesions in the Cameroons; they found that 60% of those isolated from the southern area were of the mycobacterium africanum type while all the strains from the North were characterised as mycobacterium
tuberculosis. There work showed that pulmonary tuberculosis due to \textit{mycobacterium africanum} was indistinguishable clinically from that due to \textit{mycobacterium tuberculosis}.

\textit{Mycobacterium avium} was included in the \textit{Rugyn Third group} (Bojali, 1961) as it is very difficult to differentiate between this \textit{mycobacterium} and members of the \textit{Rugyn Third group}. It is possible however to differentiate between them by aero-typing as described by Schnafer (1965). Human infection by the avian type of \textit{mycobacteria} was reported by Jahn (1959) as a pulmonary disease and by Kubin et al. (1966) as an extra-pulmonary tuberculosis. In Kubin's and Natukova's work in 1968, the study increase of the disease in man caused by \textit{mycobacterium avium} was referred to as a deep change in the ecology of the acid-fast microorganism. Kubin and Nikova (1971) isolated \textit{mycobacterium avium} from pulmonary lesions and from cervical and generalized tuberculous lymphadenitis.

In the Sudan, one single case of avian tuberculosis was reported in a duck (Annual Report of the Sudan Veterinary Services, 1966) but no attempts were made to culture it.

2. Bacterial morphology and staining

The tubercle bacilli appear as slender acid-fast rods.
They may be straight, slightly curved, comma shaped or angular with rounded or slightly flattened ends. The size varies considerably, with a length ranging between 1 and 10 microns (average 4 microns) and a breadth of 0.2 to 0.6 microns. This size variation depends on the media of growth. Thus bacilli grown on solid media tend to be shorter than those grown in liquid media. Human bacilli are long, thin, curved and show granular staining whereas bovine bacilli are short, straight, thick and show uniform staining (Paterson 1929, Soltyso 1963, Topley and Wilson 1964, Page and Associates 1964).

The Ziehl-Neelsen technique of staining has stood the test of time and is still the most commonly used method. The tubercle bacilli can also be stained by fluorescent dyes such as auramine which resists acid-alcohol decolourisation with the organism standing out brightly in the darkened field of the fluorescence microscope.

3. Colony morphology

On artificial culture media the colonies of Mycobacterium
tuberculosis are rough and euronic. Typical bovine colonies are smooth, glistening, and euronic, *Mycobacterium africanum* has both rough and euronic colonies. The colony morphology of atypical mycobacteria varies from smooth to rough (Petersen 1959, Soluyi 1961).

4. Virulence and pathogenicity

*Mycobacterium tuberculosis* causes disease in man, monkey, pigs and occasionally dogs and porcine. The bovine type affects cattle, pigs, horses, men and occasionally dogs, cats and sheep. The Avian type infects birds and occasionally man, pigs, cattle and cattle (Topley and Nilson, 1964).

The virulence of the tubercle bacillus is subject to variation. *Mycobacterium tuberculosis* is virulent in guinea pigs but much less pathogenic for rabbits, *Mycobacterium bovis* is virulent in both guinea pigs and rabbits while the virulence in chickens is produced by the avian type (Page and associates 1964). Variation is also recorded in the virulence of *Africanum* and *Aeuropermum mycobacteria*. In 1960, Hitchison et al. confirmed

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used the splenic and osmental indices for measuring the degree of
virulence of Czechoslovakian, African and Asian strains.

Mitchison et al. (1960) introduced the root index of virulence.

Guinea pigs were inoculated with 0.1 - 1 mc (mouse weight of
tubercle bacilli) intramuscularly. One was killed 6 weeks and the
other 12 weeks after inoculation. At post-mortem the extent of
the tuberculous infection in the animal was assessed by a score of
0 to 100. The virulence index was obtained by dividing the score
by the survival time in days for each animal. The square roots of
the 6 and 12 weeks indices were calculated and termed the 6 weeks
and 12 weeks root index respectively. The mean of the root indic-
ies for all the animals infected with the culture was termed "the
root-index of virulence". Rabita et al. (1961) used the root-
index of virulence for comparing the degree of virulence of
tubercle bacilli isolated from British patients with that from
Indian patients. In a different set of experiments, Basuki et al.
(1965) employed the root-index of virulence by injecting 0.1 mc,
subcutaneously in guinea pigs and sacrificing all animals 8 weeks

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Later, Ieki, Nakah and Ghaza (1968) demonstrated the usefulness of measuring the virulence of tubercule bacilli in guinea pigs by using the root spleen index as follows:

\[
\text{Root spleen index} = \frac{\text{Spleen weight (group)}}{\text{Body weight (group)}} \times 100
\]

The results were comparable to those obtained by Mitchell (1964) using the mean of the root index of virulence at 6 and 12 weeks.

5. Identification of Mycobacteria

Page et al. (1964) grouped the identification list under growth at various temperatures, pigment formation, growth in different culture media, drug sensitivity patterns and biochemical and enzymatic reactions.

A. Growth at various temperatures

Mycobacterial growth optimally at 37°C, avian mycobacteria at 42°C, and camelid mycobacteria at a temperature ranging from 25°C to 42°C.
B. Pigment formation

Pigmentation of the colony depends on the age of the culture and exposure to light. The colour observed varies from pale to deep orange. Fresh subcultures should be used for observing such phenomena. Photochromogenicity is tested by exposing young cultures on egg media for a brief period to light and the pigmentation observed after re-incubation in the dark.

C. Growth on different culture media

The inocula are prepared through two processes namely: homogenization and concentration. Pagel et al. (1962) stated that homogenization is achieved by physical or chemical treatment which is as well decontaminant for bacterial flora. For such purposes the classical Petroff’s method employing 4% sodium hydroxide is now widely used. (Thomas et al., 1961, Grande 1967, and Covington 1968, Kublan et al. 1965) claimed that more ammobilized and detectable colonies could be provided by the addition of alkaline N-acetyl cysteine which is as well responsible for rapid digestion. This was not confirmed by Selkon et al. (1965). The method used
was different in some aspects from that of Kubio et al. (1963).  
5% oxalic acid was also used by Stewart et al. (1970).

Lovenstein-Jensen medium with or without starch is the 
most reliable and the most widely used for growing zoospores of 
liquid Lovenstein-Jensen medium in an attempt to minimize the adherence of sediments to the walls of the centrifuge tube.

Other media such as Stone-Brink's, Agana's and Lesslie's are also used for the isolation of zoospores. Egg media containing 
glycerol acid neutralized with sodium hydroxide, potato starch and 
a CM agar are used by Savoian and Roche (1969) for isolation 
of the Cryptomonas strain. Liquid media have been used by Dubois and 
Middlebrook (1967) and Sura (1968). In his experiment to compare 
Sura's liquid medium, Lovenstein-Jensen medium and Agana's medium, 
Sura (1968) found that better results were given after 8 weeks 
incubation in the liquid media whereas the best results in 
Lovenstein-Jensen medium required 8 weeks of incubation.
Several factors affect the growth of tubercle bacilli. Increasing the surface tension affects it adversely (Alexander and Soltys 1946). The addition of trace elements such as zinc and cobalt to synthetic media stimulates the growth of Mycobacterium avium (Petersen 1960). Storage and transport even in optimal conditions (e.g., by refrigeration) decrease the cultivability of bacterial suspensions (Sula et al. 1960).

D. Drug sensitivity patterns

In spite of the differences of opinion regarding the techniques and interpretation of the results, the pattern of sensitivity to drugs can be considered as a method for the classification of mycobacteria. No accepted criteria of drug resistance to tubercle bacilli exist (Pazgal et al. 1964). The drug susceptibility test may be performed by either direct or indirect methods. The direct method is performed on a specimen in which large numbers of acid-fast bacilli can be demonstrated on a smear prepared directly or indirectly by concentration. The indirect method is performed by using a subculture from a primary culture as the inoculum.
The methods now used as described by Cassetti et al. (1965) and (1960) are as follows:

a. The absolute concentration method

The medium used is Lavanstein-Jensen medium. Each set of media containing drugs is inoculated with either the test strain or a laboratory sensitive human strain (H37Rv) as a control. Control drug-free medium should be used in each test. Findings of less than 20 isolated colonies in the test are referred to as effective inhibition, provided that growth is profuse in the control. For each drug, it is necessary to determine the lowest concentration at which the tubercle bacilli may no longer be considered susceptible i.e., critical concentration. The strain is considered resistant if growth at critical concentration provides more than 20 colonies.

b. Assay ratio method

The control and the test strains are handled and inoculated in the media as in the above test. The growth is defined as the presence of 20 or more colonies. The resistance ratio (RR) is the
minimal concentration of the drug inhibiting the growth of the
test strain divided by the minimal concentration inhibiting the
growth of the standard sensitive strain (H37Rv) in the same set
of tests. The strain is sensitive if the resistance ratio is 2
or less while it is resistant if the ratio is 8 or more; 4 is
considered doubtful.

c. Proportion method

In this method the laboratory sensitive human strain
(H37Rv) is used as control. The method is based on the fact that
every wild strain of tubercle bacilli contains some mutants re-
sistant to anti-tuberculous drugs, when 2% or more of organisms
which grow on the control are observed to grow on a particular
drug, then that particular drug is not or soon will not be valuable
for treatment. The proportion of resistant organisms is calculated
from the following formula:

\[
\text{Number of colonies on the drug} \times 100 = \% \text{ resistance}
\text{Number of colonies on the control}
\]

Suki and Suvareva (1963) modified the absolute concentra-

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tion method of Croatti et al. (1965) by using a different concentration of drugs and Sula liquid medium for streptomycin instead of Lowenstein-Jensen medium.

E. Biochemical and enzymatic reactions

Several such methods are used for the differentiation of the various types of mycobacteria. Of these the important are:

a. Mincin test (Koune 1956, Rajajli 1951, Sykov et al 1967): The test is based on the fact that Mycobacterium tuberculosis produces a larger amount of nicotinic acid than other mycobacteria.

b. Catalase test: This was described by Middlebrook (1954) and Sykov et al. (1967). Sula and Longorova (1963) introduced the simultaneous catalase peroxidase test. Catalase is detected by the liberation of oxygen bubbles and peroxidase by a dark brown coloration of the colonies, after a freshly prepared mixture of equal parts of 3% hydrogen peroxide and 0.2% pyrocatechol solution was poured over the lower half of the culture grown on egg medium.

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2. Nitrate test: the nitrate test was described by Virtanen (1960) and modified by Sytareva (1964). The pronounced reducing effect of Mycobacterium tuberculosis on nitrate is indicated by pink colouration. Reduction is either very slight or absent in case of Mycobacterium bovis, BCG and other atypical strains.

4. Oxidase 2-carboxylic acid: Hydrated (TH)
was described by Vostal and Huben (1967). Mycobacterium tuberculosis grows in media containing TH up to 10 mg/ml, which is completely inhibitory to Mycobacterium bovis; strains resistant to iodoacetamide (IAM) are exceptions.

6. Mycobacteriophages

Despite the fact that the phage classification of some pathogenic organisms such as salmonella and Escherichia, is in routine use, no major advances have yet been achieved in the field of mycobacteria (Julio, Badamoni1969). Mathia (1969) in India isolated two phages designated CRI-3 and CRI-9 from soil and stock of
tuberculous patients. He claimed that both were highly specific phages which could differentiate human from bovine mycobacteria. Phage 16 and 41A have been isolated from ecological dung soil by Breba, et al. (1959). These were capable of lysing laboratory human strains and few wild ones. In the fifth symposium on isolation, classification and world-wide distribution of mycobacteria in 1969 in U.S.A., it was revealed that only the phages designated 16-4, AG1 and 20-1 can produce lysis of European, African, Asian and Latin American human mycobacteria regardless of the geographical sources of origin.

Russel, et al. (1969) reported lysogenicity in mycobacteria. Using ultraviolet irradiation as an inducing agent, Bunczewskia, et al. (1969) were able to detect 5 mycobacteria from lysogenic strains (2 avian, 2 Bovituberculosis and one non-pigmented atypical strain). Sala and Bulov (1970) detected spontaneous lysogenic in thin sections of mycobacterium bovis (Argentin strain); those resembled phage-lytic plaques. The phages were firstly isolated from these strains without the use of any inducing agents. This has lead Sala (1971) to formulate a hypothesis stating that
"mycobacterium bovis BCG strain was originally myytypical inorganic bovine strain which had lost its pyogenic due to successive passage carried out since 1905 on bile aborted guinea. This was accompanied by modification of growth character from pyogenic to organized type of growth, together with attenuation of virulence.

II. TUBERCULOSIS IN THE TROPICS

1. Epidemiology of tuberculosis in the tropics

Before the advent of chemotherapy, tuberculosis was a widely prevalent disease and a common cause of death throughout the world. Now it has decreased in most parts of the world as a result of advances achieved in hygiene and therapeutics. Nevertheless, it remains one of the big problems in developing countries.

It has long been known that the pattern of tuberculosis in the tropics differs considerably from that seen in temperate climates. The reasons underlying the differences in the rapidity and the extent of tuberculous lesions in the coloured and white races are poorly understood. The subject aroused much debate for a
very long time, and differences of opinion still exist. As stated by Cullen and Koffman (1912) the controversy went to the extent of attributing the differences in disease pattern to differences in skin colour.

The two races differ greatly in their susceptibility to tuberculosis. The negro race exhibits a characteristic form of tuberculosis, namely an acuto, generalised markedly fatal type that differs from the chronic pulmonary disease observed in white subjects.

Rogers (1920/21) assumed that there were no essential differences between American negroes and whites, dying from tuberculosis as far as the types and extent of the disease are concerned. However he found that generalised tuberculosis was more common in the negroes than in their white counterparts. Corson (1920/21) reported that the death rate from tuberculosis among the negroes was four times that in white subjects. Byde (1923/25) emphasised the fact that miliary tuberculosis among the coloured subjects was at least four and half times as common as in the whites; the acute form being the commonest among the negroes. These findings...
were supported by Carter (1926) and Murrill (1929). Cajalan in 1931 stated that diverse races such as Jews and negroes differ in their innate susceptibility to tuberculosis in the same manner as the rat and guinea pig, etc. Brook and Black (1951) reported that the acute exudative or fulminating tuberculosis as seen in the negroes is rarely seen among the whites. Minor and Kasper (1932) have shown that the death rate from tuberculosis among the American negroes of age 10 years or older was 32.8% compared to a rate of 7.1% among the corresponding white population. In the same group the average death rate from miliary tuberculosis was 37.7% in the negroes and 15.6% in whites. The same authors went to the extent of stating that "while it was not possible to diagnose from the external organs of a patient, dead of tuberculosis, that the patient was white, it was in a high percentage of cases possible to state that he was coloured. In his report on autopsies, Macartt (1933) concluded that tuberculosis evidently pursued a much more acute course in blacks than in whites. Similar results were reported by Cullen et al. (1942) and Averbach (1950). Mojiboye and Dahlstroem (1945) found that the incidence of active
Indians to the disease was less than that shown by whites; the resistance of American negroes as judged from morbidity and mortality rates was even less.

The disease was successively reported as a progressive type in Australian aborigines (Clark, 1911/12), in the negroes of Panaman Canal Zones (Clark, 1915/16) and in India (Powell, 1922). In Brazil, Lambert and De Castro (1925) revealed, that dark mulatto showed a higher percentage of lethal tuberculosis than the negroes; compared to the whites, these two races showed a higher incidence of generalised tuberculosis between the age of 18 and 30 years. In Jamaica attention was drawn to the prevalence of an acute fatal form of tuberculosis among Jamaican negroes with greatest incidence in early adult life (Opie, 1930a, Opie and James, 1930 and Opie, 1951b).

The subject of tuberculosis in native races in Africa has for years attracted attention. Norman (1920) observed an acute highly fatal form of tuberculosis among Senegalese troops in France. The same was reported in Kenya by Wilson (1927/28), in Eritrea-Salem by Wilson (1928), in Uganda by Stones (1929/30).
and in East Africa by Vint (1926/29). These findings were supported by the work done by the South African Medical Research Council (1932), by Sterne (1932/33) in Uganda and by the work done by Hillooks in East Africa in the years (1932/33, 1933/34 and 1934/35). However Hillooks (1933) investigating tuberculosis in Tanganyika reported that, a few cases were acute but the majority seemed to be intermediate in severity between rapidly progressive cases and chronic cases. Matthews (1935) adds that tuberculosis among natives of Zanziber was less acute than in Senegalaise.

Scott (1935) refuted the idea that tuberculosis was rare in Rhodesia, Uganda, Kenya and Tanganyika and stated that on the contrary, the natives were very susceptible to the disease. Campion (1938) in Uganda and Davies (1947) in East Africa reported that tuberculosis tended to spread more rapidly in Africans than in Europeans. In his investigation on tuberculosis in West Africa, Holf (1953) found that in Gambia, Sierra Leone and Gold Coast, tuberculosis was of acute and chronic types and that in Lagos 7.02% of all deaths were attributed to tuberculosis.
As far as the Sudan is concerned, von Becker (1924) drew attention to the intense susceptibility of the Sudanese resident in Egypt to the disease. These findings were supported by Cousins (1911/12) who observed the same phenomenon in Sudanese resident in Egypt as compared to the indigenous Egyptian population. The study made by Dhoqali (1970) in the Gezira irrigated area revealed a high prevalence of tuberculosis in that part of the Sudan.

Several surveys of tuberculin testing were carried out. Burrows (1935) tested 3,662 Dinka with the Intradermal tuberculin test (0.2 ml dilution 1:1500 dose 0.1 cc). 112% i.e. 32.7% in the different age groups were positive and positively increased with age as in Table No. 1. As quoted by Burrows (1935), Salalga obtained similar results in Dinka living near Rumbel and out of 1,487, 501 i.e. 34% were positive. A brief review of tuberculin surveys in Sudan was given by Raboeb (1954) who revealed a positivity of 34% in school children in Khartoum province.

As far as the sensitivity pattern, very little work has been done on the disease. Cavanagh (1955) tested Sudanese strains against the first nine drugs (Streptomycin, para-aminosalicylic...
Table 1. The results of the intradermal tuberculin test performed by Surveys (1935) in Dinka tribe, Sudan.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Tested</th>
<th>Reacted</th>
<th>Number</th>
<th>%</th>
<th>Raiser</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 5</td>
<td>426</td>
<td>11.6</td>
<td>32</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - 10</td>
<td>616</td>
<td>23.1</td>
<td>166</td>
<td>10.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 - 25</td>
<td>1148</td>
<td>39.5</td>
<td>523</td>
<td>36.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over 25</td>
<td>942</td>
<td>23.7</td>
<td>478</td>
<td>50.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3662</td>
<td>100</td>
<td>1199</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Or. % 32.7
acid and isolated). The results revealed a primary drug resistance to one of more of these drugs in 28% of a group of 181 strains and a secondary drug resistance in 83% of a second group of 180 strains. On the other hand Grande (1967) reported primary drug resistance in only 16% of 277 strains tested and secondary drug resistance in 76% of 294 strains tested.

2. The Pathology of Tuberculosis in the Tropics

To clarify the situation of the disease in tropical countries, it is necessary to review the pathology of the disease in these countries. Numerous workers have performed series of autopsies and biopsies which have thrown some light on why and how the different races are affected.

A. Tuberculosis in the American Negroes

Flinner and Mayer (1932) reporting on autopsies in 303 negroes and 219 whites dying of tuberculosis, pointed out that miliary tuberculosis was more prevalent in the negroes than the whites. Chronic pulmonary tuberculosis was recorded in 47.9% of white subjects and 2.8% of negroes. Cavitations surrounded with thick fibrous walls were seen four times as frequent in the whites.
as in the negroes. Tuberculosis in adult negroes was characterized by, massive lymph node excitation; lesions being exsudative in type, with generalized nodular fuel occurring in different organs of the body.

Deveret (1933) in a report on 402 autopsies found that 65 patients died of tuberculosis. 44 of these i.e., 67.7% were colorved subjects while 21 i.e., 32.3% were whites. Out of 22 negroes with adult-ood type of tuberculosis, 14 showed excitation with sharply defined walls and 8 revealed cavities with rugged walls. In the group of 22 negroes showing child-ood type of tuberculosis, 18 had cavities of which 11 presented organized walls while smooth walls were only seen in the other seven. Acute xiliary tuberculosis occurred in only two negroes of the whole series. Tuberculosis meningitis was seen twice and both cases were white adults. The adult-ood type of infection was found in 19 of 21 white people, all white subjects had excitation but in only one case were the walls necrotic.

Armstrong (1944) analyzed 1,596 consecutive autopsies done on tuberculous persons. He found acute generalized miliary tuber-
tuberculosis in 297 i.e. 17.3% cases, 131 of these showed varying
degrees of chronic pulmonary tuberculosis complicated by miliary
dissemination. 106 had miliary tuberculosis alone, 31 of these
were less than 20 years of age and 19 cases older than 40 years.
In the 131 patients that showed chronic pulmonary tuberculosis
with generalized miliary spread, there were 21 patients who were
less than 20 years of age and 75 cases who were more than 40 years.
Out of the 106 patients with miliary tuberculosis alone there were
72 negroes and 34 white subjects while 67 of the negroes, 122 of
the whites and 2 of the yellow races belonged to the 131 patients
with chronic pulmonary tuberculosis and acute generalized seeding.
Miliary tuberculosis was seen in 46 i.e. 45.7% of the cases that show-
ed acute generalized miliary tuberculosis; it occurred chiefly in
the younger age group. Male genital and urinary tract tuberculosis
was seen in 44 cases i.e. 59.7%, and occurred in the older age
group dying of chronic pulmonary disease with acute miliary
tuberculosis. Miliary tuberculosis affected the lungs and spleen
in all cases, the kidneys in 205 i.e. 65% of the autopsies, the
intestinal glands in 66 i.e. 24.3%, the brain in 31 i.e. 10.4%, the
pancreas in 14 i.e. 4.7% and the thyroid in 10 i.e. 3.4% of the autopsies. The author observed that the progressive involvement of the skeletal, urinary and genital tracts was associated with caseous tuberculous lymphadenitis. This observation led to the concept that development of extra-pulmonary tuberculosis was the result of drainage of tubercle bacilli from an active extrapulmonary focus or a primary one (usually progressive) into the lymphatic system; the latter in turn spreading into the venous system.

Medlar (1947) described the postmortem findings in 100 autopsies, 27 of whom were coloured and 73 white subjects. Calcified lesions were found in the thoracic lymph nodes in 50.1% of the whites and 14.8% of the coloured subjects; 95.5% of the latter showed casesation while this was only seen in 24.9% whites.

Spain (1947) performed 503 postmortems for tuberculous patients, 450 of whom were whites and 153 negroes. Generalized disseminated lesions were revealed in 231 cases, 132 i.e. 21.7% of whom were without pulmonary involvement. Those with pulmonary lesions were represented by 56 whites and 45 negroes. In those

37/
100 cases, 51 had cavities and 39 had miliary hilar lymph nodes. In 10 cases the only chronic miliary foci were extra-pulmonary, indicating that miliary foci in the lungs are not an important dissemination source for miliary extrapulmonary tuberculosis.

In their series of 215 autopsies, Horror and Purves (1947) found that moderately or far advanced lesions were more in the coloured subjects rather than in the whites. The frequency of dissemination was 12% in the whites and 30% in the non-whites.

The most common sites of dissemination were the lymph nodes, the peritoneum, the bones and the genito-urinary system. There were only two cases of miliary spread into the lungs, one of whom also had extra-pulmonary miliary spread.

In extra-pulmonary tuberculosis, Horta and Sar (1943) reported ulcerative tuberculosis of the stomach in coloured, with involvement of the lungs, mesenteric lymph nodes and kidneys. In 144 cases, Johns and Dooley (1946) recorded tuberculous pleurisy with effusion in the absence of the tuberculous lesions anywhere in the body; of these, 106 cases (73.6%) were negroes and 38 (26.4%) whites. Tuberculous pleurisy was associated with tuberculous meningitis in 13 cases. The disease was confirmed in 29 cases by needle aspiration of the pleural fluid.
tuberculosis elsewhere in 37 patients of whom 35 were negroes and 2

whites. Kreuzin and Cooley (1943) reported 9 cases of tuberculosis

with tuberculous pericarditis. Six of these were negroes.

Amerbach (1930) reporting on 2,333 cases of tuberculosis, found

tuberculosis of the serous surfaces in 209 cases (8.9%). 139 of

these (66, 50) were negroes, 68 (32.8%) whites and 2 (0.9%) Asians.

In the majority of cases specifically 160 (76.8%), the involvement

was limited to one serous cavity. The sites involved were,

peritonitis in 90 (43.3%), pericarditis in 15 (7.2%), pleuritis in 55

(26.3%), peritonitis and pleuritis in 35 (16.9%), pleuritis and pericardi-

tis in 6 (3.8%), peritonitis and pericarditis in 1 (0.4%), and

pleuritis, peritonitis and pericarditis in 5 (2.3%). Extra-pulmonary

tuberculosis occurred in 153 patients. 114 showed skeletal

lesions and the urogenital tract was affected in the remaining 39

patients.

3. Tuberculosis in tropical countries other than Africa.

Chalmers (1921/12) described the occurrence of tuberculosis in two

Australian Aborigines with miliary generalized tuberculosis affect-

41/
in the mediastinal glands, lungs, liver, kidneys and spleen. The parotids and peritonaeum were involved in one of them. Competing on the records of 441 autopsies performed in negroes dying of tuberculosis in the Panama Canal Zone, Cl. M. (1915/16) stated that acute miliary tuberculosis was not infrequently seen; the commonest lesion in fatal cases being pulmonary. Abscession and cavity formation were observed but fibrosis was less frequent than in whites. The lesions in the spleen, liver and kidneys were usually of the acute or chronic miliary type. In many cases the spleen showed large caseous nodules. Tuberculosis of the bones and joints and primary tuberculosis of genital-urinary systems were not infrequently encountered. A large number of the cases showed tuberculous ulcerations in the intestine. Tuberculosis of the epididymis and testes was of common occurrence. Adrenal tuberculosis was frequent.

Lambert and DeCotto (1925) classified their findings on 420 autopsies performed in Brazil, into, lethal, subsidiary, healed and doubtful tuberculosis. Healed lesions were found in 5.9% of the whites and 6.2% of the negroes. Lethal tuberculosis was in
27.3% of the Negroes and 15.3% of the whites. The highest incidence of lethal tuberculosis (59.5%) was in the age group of 20 - 39 years. The main sites of involvement were the thoracic lymph nodes in 76.2%, the cervical lymph nodes in 6%, the meninges in 10.7%, and the pericardium in 1.8%. No lesions in the myocardium or endocardium were observed. Gipio (1936) in 9 autopsies performed in Negroes in Jamaica noted that 8 adults had childhood tuberculosis. Only one case showed lesions similar to those seen in the whites. Gipio and James (1936) reported that in Jamaican types of tuberculosis other than pulmonary were seldom reported. The average duration of fatal illness in Jamaican young adults was 9 months compared to 2 years in North America.

C. Tuberculosis in Africa

Barrel (1920) was a pioneer on his pathological studies in Senegalese troops dying of tuberculosis. He divided tuberculosis in the Senegalese into two stages, a lymphatic stage in which the lymph nodes only were involved. This was later followed by a generalized stage. The supraventricular glands were involved in
70% of cases while the tracheo-bronchial ones were involved in 8%. Caseous pneumonia was noted in 30% and caseous pneumonia with miliary spread in the lung in 20%. Miliary tubercles were recorded in the lungs, liver, spleen and mediastinum in 25%. Haemorrhage was beautifully described by Borch's sentence, "it seizes as tubercle". Chronic pulmonary tuberculosis as seen in Europeans was only encountered in 5% admissions were rarely seen. Pleuro-pericardium involvement occurred in 10% of autopsies and abdominal tuberculosis was uncommon. Surgical tuberculosis was also rare. Wilson (1927/28) reported 34 out of 37 cases of a chronic form and hence his statement that in natives of Kenya the chronic form of the disease was the rule rather than the exception. The mortality rate due to tuberculosis was 10.3% which is in agreement with the results obtained by Vint (1934/35). The letter analysed 176 autopsies in Kenyans and recorded tuberculosis as the cause of death in 16 i.e. 9%. healed tuberculosis was rarely seen and it was uncommon to find fibrous tissue formation around active lesions. Acute miliary tuberculosis was the commonest form of the disease, 50% of cases had cavities as well and all organs in the
abdominal cavity except the pancreas were affected. No macroscopic lesions were seen in either the brain or kidneys, even in generalized cases of the disease. In Ber-es-Salah, Filoocke (1935) worked on 178 tuberculous autopsies comprised of 7 Europeans, 36 Asians and 135 Africans. No healed tubercles were found in Africans dying of diseases other than tuberculosis. Cavity formations were only revealed in few cases. The results obtained by Filoocke (1935) showed that tuberculosis was the cause of death in 11 out of 71 autopsies performed on natives of Tanganyika, six of whom had hilar tuberculosis. Fibrous tissue formation and calcification were rarely seen. The report published by the South African Medical Research Council (1932) indicated that out of 600 autopsies performed on tuberculous natives, 358 died of acute tuberculosis, 200 of chronic tuberculosis associated with silicosis and 62 of causes other than tuberculosis. In all of the acute cases the lesions were mainly thoracic while in the remaining 97 they were mainly extra thoracic. Chronic pulmonary tuberculosis was seen in more than 50% of cases, lung cavitation and were rarely fibrocaseous. In the natives
cervical glands were rarely affected in comparison to the thoracic and abdominal lymph nodes. Involvement of the spleen and liver was much more common in the natives. Tuberculous peritonitis and involvement of other abdominal organs are also common in the natives and so is tuberculous pericarditis.

In Uganda the pulmonary form of tuberculosis exceeds the glandular, bone and peritoneal types (Stokes 1932/33). Two autopsy series were performed by Davies (1937) on indigenous Ugandans. In series I there were 55, tuberculous cases out of 2294 autopsies i.e. 11.8% while in series II, 75 out of 1600 were recorded i.e. 18%.

These cases were divided into two main groups:

1. Tuberculosis with pulmonary involvement: this was represented by 297 cases in series I i.e. 86.8% and 65 in series II i.e. 40.7%.

In 162 out of the 297 subjects (series I) there was no involvement of the lymph nodes, and the main organs affected were the spleen, liver, peritoneum, pleura, pericardium and kidneys. The usual pathology was of the acute type. Fibroid tuberculosis was extremely rare and only occurred in 0.7% in series I and 1.6% in series II.
The general pathological pattern in this group was the presence of "abscesses" with necrotic walls; cavitation. Fibrosis and calcification were very rare. Tuberculosis was mostly of the childhood type which leads to dissemination and death. Intestinal tuberculosis and ulceration occurred nearly in every case at autopsy.

Hilar tuberculosis was common. Adrenal tuberculosis was extremely rare. Bones, joints and renal involvements were uncommon. A relatively high unusual incidence of angiomylar tuberculosis was observed in this group.

11. Generalized tuberculosis without pulmonary involvement: this occurred in 57 (15.2%) in series I and 10 (13.5%) in series II. This was further subgrouped as follows:

1. Gheldicular tuberculosis in 35 out of 57 cases in series I and in 7 out of 10 cases in series II.

2. Non-glandular tuberculosis in the remaining 23 cases of series I. It affected the sinus, liver, kidney, spleen and other organs; bone and joint tuberculosis being rare.

Hallers (1935) assumed that pulmonary tuberculosis was the most common form in children, 0.6% of 275 tuberculous cases,
269 (97.8%) were pulmonary. The rest were represented by 4 in the cervical lymph nodes, one in the peritoneum and one in the spine. Cases of tuberculous peritonitis and meningitis were few. The condition was less acute than that in the Sowetakos.

Special reference to extra-pulmonary tuberculosis in Africa is rather important. In East Africa extra-pulmonary tuberculosis was frequently seen but almost entirely due to lobar infection (Heer 1953). The same was recorded in East Africa. In 57 specimens of cervical lymph nodes collected throughout Kenya, all strains isolated were typed as Mycobacterium tuberculosis (Juk, et al. 1960).

Yilekosha (1932/33) in East Africa reported 50 (8.2%) out of 612 tuberculous cases as extra-pulmonary. Harris (1919) recorded two cases of tuberculous meningitis in adult Africans, no other lesions in any other part of the body were encountered and hence his suggestion that in adult Africans, tuberculous meningitis, as an isolated lesion, is rather common. Heer (1953) stated that tuberculous meningitis was rarely seen in eastern African children. In 200 autopsies performed, Selman (1948) found
tuberculosis of the brain in 6 (0.3%) cases. He concluded that in contrast to Europeans, tuberculosis of the brain was commonly encountered in Africans. Stone (1928/29) found that peritoneal tuberculosis in African infants and young children was very rare and in contrast to Europeans where the incidence is high, only one case was reported during 25 years.

Vint (1922) reported that tuberculous pericarditis in East Africa was not uncommon and occurred by extension from the lungs and/or small tuberculous nodules on the endocardium. The pericardium however was very rarely affected. Tuberculosis of the aorta was infrequent; the highest incidence being in the age group of 15 to 30 years (Vilcock 1932/33). In 20 autopsies of East African tuberculous children, only 3 cases i.e. 1.5% of spinal tuberculosis were found by Bryen (1940).

Smith (1950) reported facial tuberculosis in a Nigerian girl. The lesion involved the whole upper lip. Among the indigenous people of East Africa, purely cutaneous tuberculosis appeared to be rare (Piers and Wright 1946).
In Nairobi, tuberculosis of the spleen as a part of miliary spread was commonly seen in Africans. Tuberculosis in Africans was reported by Omer (1961) in 20 out of 357 tuberculous cases. Only 20% of them were children, 5 were bilateral and 15 unilateral.

D. Tuberculosis in the Sudan

As far as the Sudan is concerned, accurate information about tuberculosis and its pathology is still lacking. Most of the literature available on this subject deals with the public health aspects of the disease. The earliest observations on tuberculosis were made by Schweinfurth (1871) and Junker (1883) who found no case of tuberculosis among the Dinka tribe. This was confirmed by Gumsa (1907) who found no evidence of the disease among natives, domestic or wild animals. Contrary to Egyptians, the Sudanese resident in Nigeria were highly susceptible to tuberculosis as stressed by Von Beske (1904). In 1957 Simba reported several cases of chronic pulmonary and non-pulmonary tuberculosis among the Dinka tribe. The peculiarities of such a disease in the Sudanese
lungs, liver, and spleen. Symptoms such as diarrhea, fever and dysentery were as well attributed to tuberculosis. Nevertheless, the disease was reported by Archibald (1922) as being very rare among the desert tribes and the secluded tribes in the South. The author found that the commonest forms of tuberculosis in succession were lymphadenitis, pneumonia and pleurisy; Pott's disease being exceedingly rare. He diagnosed the first case of breast tuberculosis in a 40 years old Sudanese woman; the lesions were confined to the breast and the regional lymph nodes. Burrows (1935) reported 173 cases of tuberculosis amongst the Dinka of Eastern Districts, 149 of which were of the pulmonary type. The remaining non-pulmonary cases were comprised of 6 in the cervical lymph nodes, 2 in the peritonium, 19 in the bones and one in the epididymis. Children of the desert under 12 years of age were found free. In 1942 the Province Medical
Table 2. The geographical distribution of diagnosed tuberculosis patients during the period 1951 - 1958.

<table>
<thead>
<tr>
<th>Province</th>
<th>Cases in 1951 to 1958</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khartoum</td>
<td>11,091</td>
</tr>
<tr>
<td>Vasala</td>
<td>8,403</td>
</tr>
<tr>
<td>Sennile</td>
<td>8,435</td>
</tr>
<tr>
<td>Northern</td>
<td>4,582</td>
</tr>
<tr>
<td>Upper Nile</td>
<td>3,728</td>
</tr>
<tr>
<td>Kordofan</td>
<td>3,964</td>
</tr>
<tr>
<td>Bahr el Ghazal</td>
<td>2,036</td>
</tr>
<tr>
<td>Equatoria</td>
<td>1,691</td>
</tr>
<tr>
<td>Darfur</td>
<td>1,491</td>
</tr>
<tr>
<td>Grand total</td>
<td>45,691</td>
</tr>
</tbody>
</table>

*The above figures are taken from Sudan Medical Service Reports 1951 - 1958.*
Table 3. The age distribution of pulmonary and non-pulmonary tuberculosis in 5690 patients seen during the year 1963/64.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Pulmonary No. of cases</th>
<th>% of Pulmonary</th>
<th>Non-Pulmonary No. of cases</th>
<th>% of Non-Pulmonary</th>
<th>Total No. of cases</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1</td>
<td>7</td>
<td>0.12</td>
<td>15</td>
<td>0.26</td>
<td>22</td>
<td>0.39</td>
</tr>
<tr>
<td>2 - 5</td>
<td>32</td>
<td>0.56</td>
<td>35</td>
<td>0.61</td>
<td>67</td>
<td>1.18</td>
</tr>
<tr>
<td>5 - 15</td>
<td>205</td>
<td>3.50</td>
<td>135</td>
<td>2.37</td>
<td>340</td>
<td>5.97</td>
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<tr>
<td>16 - 25</td>
<td>710</td>
<td>12.68</td>
<td>24</td>
<td>4.39</td>
<td>994</td>
<td>17.47</td>
</tr>
<tr>
<td>26 - 35</td>
<td>1330</td>
<td>23.37</td>
<td>266</td>
<td>4.67</td>
<td>1596</td>
<td>28.05</td>
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<tr>
<td>36 - 45</td>
<td>1236</td>
<td>21.72</td>
<td>221</td>
<td>3.88</td>
<td>1457</td>
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<td>46 - 65</td>
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<td>12.37</td>
<td>197</td>
<td>3.46</td>
<td>895</td>
<td>15.73</td>
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<tr>
<td>Over 65</td>
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<td>0.07</td>
<td>9</td>
<td>0.16</td>
<td>13</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Grand total: 4456 - 78.30 - 1234 - 21.67 - 5690 - 100

* Data taken from the Sudan Medical Services Report 1963/64.
literally stated that "the primitive tribes are highly susceptible to tuberculosis because in the absence of the tubercle bacillus they have never been obliged to protect themselves against that organism and that civilised people are highly protected against tuberculosis, because having been in contact with the tubercle bacillus they have elaborated protective substances against it". This was put forward to explain the high susceptibility of the primitive tribes of the Sudan, whenever they were brought into contact with civilization. The high susceptibility of monkeys in captivity to tuberculosis was made use of to support such a theory. Barrow (1920) considered the Geneese as "in terrain virosa" i.e. a virgin soil to tuberculosis; the disease being highly fatal with a tuberculin positivity of origin—5% in Prague camp as compared to a range of 60 to 90% in the Parisians. In support to the virgin soil theory Filock (1928) stated that no healed tubercle was found in Africans dying of diseases other than tuberculosis; thus refuting previous exposure to the tubercle bacillus. Opie (1930) stated that the virgin soil theory could not explain the case of the American negroes who despite the fact that they were
in contact with the whites for three centuries and thus equally exposed to infection, yet they reacted differently to the tubercle bacillus. This is shown by the much higher incidence of the acute fatal type of tuberculosis among them than their fellow whites. Malnutrition, overcrowding and lack of elementary sanitary principles among the American negroes may play a rôle in increasing their susceptibility to tuberculosis. Scott (1935) declined the theory of virgin soil as a complete answer to the higher susceptibility of people in the tropics to tuberculosis. He argued that negroes, originally of an African ancestry, after spending hundreds of years in another continent with continuous exposure to tubercle bacilli, still possess a high susceptibility to the disease, is a proof that tuberculosis in Africans is a special type depending on racial peculiarities. Contrary to Scott, Ansbach and Behlstrom (1942) believed that racial or genetic characteristics as genotypic traits are not liable to make people susceptible to the disease. On the other hand lack of exposure of any group of people, regardless of race, produced an extremely high incidence of acute tuberculosis once it is introduced in the
community. Investigations carried out by Grady (1924/25) revealed that the average coloured child was considerably heavier, taller and less susceptible to fatigue than the average white child of the same age group. Nevertheless, observations made by the same author showed that a big strong negro with a moderate infection deteriorates and dies in spite of treatment while a weak Jew with a great deal more trouble becomes well. He concluded that the only possible explanation for this is the racial susceptibility.

Lambert and De Castro (1925) refuted the idea that high susceptibility to tuberculosis is the peculiarity of negroes living in the tropics as this phenomenon was observed in a very different race (Indian Terra de Fogo) living in a cold climate. They added that the high incidence among the American negroes may be partly related to their lower standard of living and greater ignorance in hygiene.

Brook and Black (1931) claimed that the ordinary exposure of the white races to the sun rays up to adult life, may be responsible for a certain energy which produces a cumulative protective change perhaps in the lymphocyte forming tissues and increases
the immunity to tuberculosis, more so than in the negro, who is unable to absorb and utilize properly the sun rays on the account of his more pigmented skin. However the comparative lack of continuous infection of the negro race with tubercle bacilli may be a definite factor. Primer and Kasper (1932) analysed and criticized different theories and hypothesis on the basis of the evidence available. While the environmental factors were considered a probable explanation of the excessive case incidence and mortality from tuberculosis among the negroes, the authors doubted that similar explanation could be given to the pathological differences between the two races. In support to this, they mentioned that during the late years of the war in Germany, the incidence of tuberculosis among the Germans rose markedly due to poor environmental factors e.g. overcrowding and starvation, but the character of the disease remained essentially the same. Therefore, in an attempt to explain the peculiarities of the disease in the negroes, support was given to the hypothesis of racial susceptibility on the basis of existence of genotypic anatomical and physiochemical differences. These were concluded to be the part played by skin pigmentation.
(Brook and Black, 1931) and the observation made by Loos (1926). The latter showed that the average size of the spleen in the adult negro (106 gm) was below that in the corresponding white subject (145 gm). This was true for all age groups, in either sex and in various pathological conditions. In tuberculosis the splenic ratio remained the same - 112 gm for negroes and 160 gm for whites. It was, thus, presumed by Pummer and Kesper that these differences played a big role in supporting the hypothesis of racial susceptibility in spite of the lack of a conclusive proof.

Other explanations were given by Powell (1922) and Harrell (1929). The former related the outcomes of human tuberculosis in India to the absence of the bovine tuberculosis bacillus while the latter assumed an interrelationship between low blood calcium in negroes and the rapid development of the disease.

Niles (1933/34) put forward three possible explanations. These could safely be concluded in a greater virulence of bacilli in Africans, a lesser resistance of natives and a possibility of native customs being conducive to more massive infection. In 1935 the author referred the rapid deterioration in East African
patients to the fact that natives did not come to hospital for treatment until the disease had obtained a firm hold and the threshold had been passed. Beck and Hasson (1938) added that the negro has a lower sensitivity to pain, and that since tuberculosis is a relatively painless disease, it can progress to a far advanced stage before the negro is aware of it. This was refuted by Cullen and Hoffman (1942) on the ground that pain is necessarily an evil associated with illness. They thought that the lower resistance of the negro to tuberculosis was due to some extrinsic factors such as the physical, environmental and mental ones in addition to lack of medical care; the genotypic racial factors being minor.

b. Animal Tuberculosis in Africa

The view, widely held in the past, that bovine tuberculosis is unknown in Africa, is rapidly undergoing modification. Stone (1928/29) assumed that cattle in Uganda were not prone to tuberculosis. However the study made by Carriacu (1935) in Uganda revealed that cattle tuberculosis while not entirely absent, was
Infrequent. Tuberculous lesions were found in 259 (0.8%) out of 33627 Zebu cattle inoculated and in another group of 176 cattle, only one reacted to tuberculin. Generalized cases of tuberculosis were occasionally reported; the lesions were mostly in the thoracic glands with a caseous diaphragmatic empyema, oedema, and calcification. Nevertheless, the results obtained by Carmichael in Zebu cattle were different from those in Ankole. Of 1021 (178) out of 5908 Ankole carcasses revealed tuberculosis while in another series of 585 animals, 233 i.e., 34% were also tuberculous. A high percentage of tuberculin reactors amounting to 52% was revealed in a group of 560 tuberculin-tested animals. 12% of these animals showed generalized tuberculosis with 50% of the lesions being confined to the thoracic cavity. The remaining lesions were found in the abdominal cavity and peripheral lymph nodes. This indicates that Beem are more resistant to infection than Ankole cattle. This was further supported by Pieters and Wright (1945) who revealed a higher incidence of tuberculosis in Ankole cattle. Nicks (1933) claimed that bovine tuberculosis was a rare phenomenon in Tanganyika which may account for the rarity of
non-pulmonary tuberculosis in Tanzania. The findings of Cornell (1934) revealed an extremely low incidence of tuberculosis among Zebu cattle in Tananyika. The lesions were confined to the glands and were of the calcified non-progressive type. In animals cattle of Uganda the incidence was high while in Zanzibar, bovine tuberculosis was very rare in spite of the occasional incidence in animals imported from Europe.

In West Africa, the greatest incidence of tuberculosis in cattle occurred in the Cameroon and decreased westwards to a point of extreme rarity in the Gold Coast and Sierra Leone (Dear 1953).

In Sudan, animal tuberculosis is a hazard to human health. Apart from the close association between the man and his animals particularly in the South, there are other dangerous habits. Large numbers of Sudanese eat raw meat in the form of 'basmah' (liver, kidney, lungs, brains and maw) and very especially in the rural communities drink raw milk. The review of the available literature on animal tuberculosis reveals a peculiarity of material. Animal tuberculosis was thought to be a rare disease in the Sudan (Cummins 1902). The first case of animal tuberculosis was reported...
in a turkey, but no attempts were made to isolate the causal agent (second Report of Welsome Research Laboratory, 1966). Archibald (1911) detected caseous nodules — the size of millet seeds — and were scattered throughout the surface of a canal’s lung; smears revealed acid-fast bacilli. In Sudanese cattle, tuberculosis was first reported from Egypt (annual Report of the Sudan Veterinary Services, 1915). In spite of Archibald’s claim in 1922 that tuberculosis in cows, goats and camels was unknown, occasional cases were revealed in the Annual Reports of the Sudan Veterinary Services, 1916-1954. Burrows (1935) assumed that tuberculosis among Dinka cattle was rare.

In Khartoum abattoir, tuberculosis in Zebu cattle amounted to 0.02% of the total carcasses slaughtered (Annual Report of the Sudan Veterinary Services, 1945).

The true picture of the prevalence of the disease in different parts of the country is still not clear. However, Karib (1962), Mustafa and Pauli (1960), El Naasti (1966) and Mustafa (1970) concluded that the incidence was high in the humid South where close herding is practiced and low in the dry zones where...
the nomadic cattle of Western Provinces, the semi-nomadic cattle of Eastern and Central Sudan are located, close the less precise data are still lacking.

The disease is epizootic in the three southern provinces, particularly in Behr-al-Ghazal Province where the spread of the disease is enhanced by the method of sheltering the animals (Annual Report of the Ministry of Animal Resources, 1957/58). It was also reported in Darfur, Kordofan, Blue Nile and in the Northern Province (Annual Report of the Ministry of Animal Resources, 1963/64). With regard to the pathology of the disease, Korir (1962) admitted that the disease takes several forms, generalized with pulmonary involvement, glandular, hepatic and/or mediastinal and bronchial.

Tuberculin testing was carried out by several workers. Barrois (1935) tested 56 cattle in Behr-al-Ghazal by the intradermal method (0.1) and found that only 2 were positive, but of 30 cattle tested in Nau Government Dairy 23% were positive and 13% doubtful (Annual Report of the Ministry of Animal Resources, 1955/56). Tuberculin testing was extended to include dairy's from different parts of the country (Annual Report of the Ministry of Animal Resources, 1956/57 and 1957/58). The results are shown in table 4.
Table 4. Tuberculin test in different parts of Sudan

<table>
<thead>
<tr>
<th>Province</th>
<th>Herd</th>
<th>Cattle Examined</th>
<th>Positive</th>
<th>Doubt</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue Nile</td>
<td>Wad Medani (1956/57)</td>
<td>124</td>
<td>4</td>
<td>18</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Wad Medani (1957/58)</td>
<td>198</td>
<td>11</td>
<td>17</td>
<td>170</td>
</tr>
<tr>
<td>Khartoum</td>
<td>Belgrade (1956/57)</td>
<td>374</td>
<td></td>
<td>-</td>
<td>374</td>
</tr>
<tr>
<td></td>
<td>Shambat (1957/58)</td>
<td>88</td>
<td></td>
<td>-</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>Kur notes (1956/57)</td>
<td>34</td>
<td>7</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>El Obeid (1956/57)</td>
<td>33</td>
<td>4</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>El Obeid (1957/58)</td>
<td>53</td>
<td>1</td>
<td>-</td>
<td>52</td>
</tr>
<tr>
<td>Northern</td>
<td>Aflah (1956/57)</td>
<td>288</td>
<td>5</td>
<td>20</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>Aflah (1957/58)</td>
<td>155</td>
<td></td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Malakal Arab I (1956/57)</td>
<td>117</td>
<td>13</td>
<td>35</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Malakal Arab II (1956/57)</td>
<td>205</td>
<td>26</td>
<td>33</td>
<td>146</td>
</tr>
<tr>
<td>Upper Nile</td>
<td>Malakal Government</td>
<td>90</td>
<td>18</td>
<td>20</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Fort (1956/57)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malakal Official</td>
<td>25</td>
<td>4</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Malakal (1957/58)</td>
<td>39</td>
<td>4</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1653</td>
<td>97</td>
<td>187</td>
<td>1362</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td></td>
<td>5.6%</td>
<td>11.3%</td>
<td>82.8%</td>
</tr>
</tbody>
</table>

*Results compiled from the Annual Report of the Ministry of Animal Resources 1956/57 and 1957/58*
MATERIALS AND METHODS

I. MATERIALS

The material used in this study was obtained from both human and animal sources.

A. Human material

1. Specimens from hospitalized patients: These were collected from 18 patients at Chestnut, Chestnut North, Ordman, and New hospitals. 13 of these patients were tuberculin-tested and 16 showed a past history of drinking raw milk. Reports on chest radiography were only available for 18 of them. The specimens included 10 lymph nodes, 18 biopsies from bones, 46 sputum specimens from all patients, one breast and two endometrial biopsies. The lymph nodes and sputum samples were collected in advance before any treatment with anti-tuberculous drugs. Specimens from bones and joints were obtained one month after treatment with streptomycin, isoniazid, and para-aminosalicylic acid. These specimens were submitted for pathological and bacteriological examinations.

2. Analysis of biopsy records: This series included the analysis of the records on 640 biopsies submitted to the Pathology.
Relevant, Faculty of Medicine, Khartoum for histopathological examination between the period 1959 - 1970. 384 were from lymph nodes 143 from bones, 23 from the endometrium, 22 from testis and 75 from miscellaneous sites. A firm diagnosis of tuberculosis based on the well known histological picture of the disease was made on each biopsy.

3. Autopsies:

a. Retrospective study: A study of the autopsy records (1957-67) in the Pathology Department, Faculty of Medicine, Khartoum was made. This revealed 348 autopsies with their complete histopathological picture. 49 of these cases were histologically shown to be tuberculous.

b. Prospective study: In the period 1967-70, 547 consecutive autopsies were performed in the pathology Department, Faculty of Medicine, Khartoum; 72 cases of these showed typical tuberculous lesions. All the cases were subjected to routine histopathological examination. Bacteriological examination was only carried out on 26 out of the 72 tuberculous cases.
B. Animal material:

1. Biopsies from slaughter houses: 50 specimens with typical tuberculous lesions were collected from cattle slaughtered at Qahran, Mahalch and El Abattoirs. The specimens were prepared for bacteriological and pathological examinations as described below.

2. Tuberculin test in dairy farms: Using the comparative intradermal tuberculin test, 528 cattle in Telgawia and 232 cattle in El Osaid dairy farms were tested for tuberculosis.

II. TUBERCULIN TEST

Mantoux test: The tuberculin used was imported from the Tuberculin lab, Cairo. It was obtained from El Sheib Hospital at Khartoum. The (PPD) was of a concentration of 1 I.U. per 0.1 ml. and a dilution of 1:1,000. 0.1 ml. was injected intradermally in the middle of the left forearm. A reaction of 5 mm. or more after 24 and 72 hours was considered as positive.

The comparative intradermal test: The mammalian and avian tuberculins produced by Sybridge Laboratory, England were used. The former had a concentration of 2.04 mcg. PPD/1 ml. and was

\[ \frac{1 \text{ mcg. PPD}}{1 \text{ ml.}} = 50,000 \text{ i.u./ml.} \]

PPD = purified protein derivative.
Inoculated intradermally in the middle third of the left side of the neck 20 cm. below the crest. The latter showed a concentration of 0.5 mg. FP/1 ml. and was intradermally injected in the upper site 10 cm. below the crest. The skin at the site of inoculation was clipped and cleansed with cotton soaked in alcohol, skin fold being measured and 0.1 ml. of each tuberculin was then injected intradermally in the appropriate site. The reaction was read and registered after 48 and 72 hours as described by Ritchie (1959).

III. LABORATORY METHODS

1. Histopathological examination: Material for histology was fixed in 10% formal-saline, processed and embedded in paraffin as usual. Sections were cut and stained with either Heidenhain and/or hematoxylin and eosin as described by Gullier (1955).

2. Direct examination: Human and animal specimens were treated similarly. The specimen was macerated in a mortar after the addition of 4 ml. sterile saline and was further softened by grinding in a Griffith tube. Two slides were then prepared from each specimen and stained - one with Gram's method and the other
with Zeihl-Neelsen using 25% sulphuric acid as a decolorizing agent. The remainder of the material was used for cultivation in the appropriate media (viz. below).

3. Cultivation: As a control, human specimens were directly inoculated in blood agar and MacConkey's media for determination of contaminates. Both human and animal specimens were similarly digested and decontaminated in sterile MacConkey screw-capped bottles by adding an equal quantity of 1% sodium hydroxide. The material was then shaken for ten minutes on an electric shaker, incubated at 37°C for 30 minutes and the bottles centrifuged at 3,000 r.p.m. for 15 minutes. The supernatant fluid was then decanted and the sediment washed twice in sterile distilled water.

Part of the material was inoculated on two slopes of Lowenstein-Jensen medium (potato starch being incorporated) and incubated at 37°C for up to 12 weeks in negative cases. Two slides were also prepared from the rest of the sediment and stained with Zeihl-Neelsen method. Cultures that yielded tubercle bacilli were subcultured and subjected for further investigations. Sula liquid medium, Ogawa and 1%ar media were also used. The growth of human
and review strains were tested in Lebch semi-liquid medium; H37RV strain being used as a control.

4. Pigment formation (Krylov, Boust et al., 1967):

Each specimen was subcultured in two slopes of Lowenstein-Jensen medium and incubated at 37°C; one being wrapped in a black paper. Then sufficient growth occurred in the un-wrapped slope, the colour of the colonies in each tube was recorded. Both tubes were then exposed to day light for 5 to 8 hours and returned to the incubator. The following day any change in colour was recorded and the cultures were accordingly identified as non-pigmented, photochromogenic or otochromogenic.

5. Sensitivity test: The sensitivity of the strains under test and that of the standard human strain (H37RV) was a strain control was tested in a series of media containing the appropriate concentration of drugs as seen below. Similar media free of drugs were also used as a medium control. The inoculum used consisted of 0.1 ml of a good growth in Sulb liquid medium of either H37RV or the strain under consideration. The cultures were incubated at 37°C for 2 to 4 weeks. The absolute concentration method of Sulb et al. (1963) was followed and the following concentrations were used:

*Obtained from Tuberculosis Research Institute, Prague.
a. Streptomycin: 100 mcg/ml, 10 mcg/ml, 3 mcg/ml, and 1 mcg/ml, in Saga liquid medium.

b. Para-aminosalicylic acid (PAS): 100 mcg/ml, 10 mcg/ml, 3 mcg/ml, and 1 mcg/ml, respectively, in Lowenstein-Jensen medium (without potato starch).

c.isoniazid (INH): 100 mcg/ml, 10 mcg/ml, 1 mcg/ml, 0.2 mcg/ml, and 0.1 mcg/ml, respectively, in Lowenstein-Jensen medium (without potato starch).

6. Thio-glauca-2-carboxylic acid hydrazide (TCH): A concentration of 10 mcg/ml in Lowenstein Jensen medium (without potato starch) was used for differentiation between bovine and human strains. All strains including the 3372W were tested against the above concentration of TCH. Mycobacterium tuberculosis grew at this concentration while the bovine strains did not.

7. Catalase test: The reagents used consisted of solutions A and B (Zykove et al., 1967):

Solution (A): 30% hydrogen peroxide in distilled water.

Solution (B): This is formed of 10% Tween 80 in distilled water. Tween 80 was dissolved in a minimal amount of warm distilled water. Distilled water was then added to make up the requisite
volume and the solution stored at 4°C. Fresh preparations of solutions A and B were made by mixing equal parts of each. The formed peroxides Tween solution was distributed in 1 ml quantities in Khan test tubes. Instead of the usual platinum loop, sterile glass loops made from Pasteur pipettes were used. One third of a loopful of growth was scrapped up and placed in a Khan test tube containing the mixed solution. The reaction was then immediately observed and the results recorded as follows:

<table>
<thead>
<tr>
<th>Bubbles</th>
<th>Few bubbles</th>
<th>Fast mixing</th>
<th>Marked frothing</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>I</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

8. Simultaneous catalase and peroxidase test (Suh and Langova, 1963): A freshly prepared mixture of equal parts of 3% hydrogen peroxide and 0.12% pyrocatechol was poured over the lower half of cultures grown on Lowenstein-Jensen medium. A catalase-positive reaction was indicated by rise of oxygen bubbles within five minutes while a peroxidase-positive reaction imparted a dark brown coloration to colonies in 1 to 24 hours.

Personal communication with Dr. N. S. Al Faiq of the Pathology Department, Faculty of Medicine, Anbar University, 1981.
9. Nitrate test modified by Sytaxova (1964): The strains under consideration and the N37RV were grown in Sula liquid medium containing 0.02% sodium nitrate (Na NO₃). After sufficient growth was obtained, the test was carried out as described by Vitanen (1960). Positive reactions were manifested by a pink or purple discoloration of culture media.

10. Guinea pig inoculation: Guinea pigs were inoculated for determination of the spleen and omental indices along with the viable count.

a. Spleen and omental indices were calculated according to the technique set by Sula et al. (1964). Strains grown on Sula liquid media were used for the inoculation. For each strain two guinea pigs were used - one given 0.5 ml, subcutaneously in the inguinal region and the other an intraperitoneal injection of 0.25 ml. The animals were weighed before inoculation and just after sacrifice. The guinea pigs inoculated intraperitoneally were sacrificed 4 weeks later and the macroscopic lesions in the internal organs, i.e., omentum, spleen, lungs and liver were evaluated. Those inoculated subcutaneously were sacrificed 8 weeks.
after inoculation, the macroscopic lesions in the inguinal lymph nodes, spleen, lungs and liver were also examined. The spleen and mesentery were fixed for 24 days in 10% formal saline, washed in running water over night and then dehydrated in alcohol for 24 hours. They were then weighed and the spleen and mesental indices determined according to the following formula:

Spleen index = \( \frac{\text{Weight of spleen (gram)}}{\text{Weight of the body (gram)}} \times 100 \)

Mesentery index = \( \frac{\text{Weight of the mesentery (gram)}}{\text{Weight of the body (gram)}} \times 100 \)

b. The viable counts were determined by using seven, tenfold serial dilutions of the original bacterial suspension used for animal inoculation; sterile saline being used for the purpose. 0.2 ml. from each dilution was inoculated into 4 petri dishes, two containing Lowenstein-Jensen medium, and two Ogawa medium. All inoculated dishes were put into polyvinyl-chloride bags and incubated at 37°C for 3 - 5 weeks; those of the bovine strains were previously sealed with plaster. Colonies were then counted taking as a base the dilution with the best count of colonies. The number of organisms inoculated in each guinea-pig, were thus easily calculated.
11. Rabbit inoculation: 5 bovine strains were used for intravenous inoculation in the liver of a maximum number of rabbits. The inoculum consisted of 1 ml. of the strain concerned in 5 ml. liquid medium. The rabbits were weighed before incoculation and immediately after death. Viable counts being determined as before. The macroscopic lesions were observed and weight of the internal organs, the spleen, liver, lungs and kidneys were recorded.

12. Ultraviolet irradiation of lysogenic strains: In an attempt to liberate phages, ultraviolet irradiation was used as an inducing agent for the five lysogenic bovine strains that showed spontaneous lysis of colonies in agar medium; the latter was kept in refrigerator for 26 - 30 weeks.

For preparation of the cells for irradiation, several tubes of RBS liquid medium (Redmond and Yard, 1962) were each inoculated with approximately 0.5 ml. of good bacterial suspension (grown on liquid medium). Cultures were incubated at 37°C for 6 - 10 days and were shaken daily in order to achieve a homogenous suspension. These were then pooled, centrifuged at 3,000 r.p.m.
for 20 minutes and the supernatant fluid discarded. The cells were then suspended in 14.4 ml of phosphate buffer pH 7.5.

For every strain the 14.4 ml of suspended cells were distributed in 4 petri dishes containing 3.6 ml each. These were successively subjected to ultraviolet irradiation at a distance of 70 cm, for 10 seconds, 20 second, 40 seconds and 60 seconds. The irradiated cells were then resuspended in RYE liquid medium and incubated at 37°C for 40 - 48 hours. A series of test tubes were prepared; each contained 0.5 ml of a ten fold dilution of the irradiated cells. To each 0.5 ml of a single strain of the following was added. The strains were ATCC 607, N3787, mycobacterium smitite, Japanese 2C6, 2CG725, Danish 2C0 and Murine (H263) strain. The rapid growers e. g. ATCC 607 and mycobacterium smitite were pipetted on the surface of agar Nut infus and synthetic agar media (2-1). The rest of the strains were slow growers and were all seeded on RVA agar media. The inoculum in all cases was evenly spread to cover the entire surface of media concerned.

The petri dishes were tilted and the excess fluid allowed to drain.

American type culture collection ATCC 607 strain.
to one side and then removed. They were then left to dry and in-
subated at 37°C for 1 - 2 days for rapid growers and 2 - 3 weeks
for slow growers. The petri dishes were examined daily for lytic
plaques. 7 Sudanese bovine strains (strains numbers 530/69, 75,
V15, V21, V44, V65 and V49) were used in this study.

13. Isolation of mycoplasmas from lysogenic Sudanese
bovine mycoplasma: A quantity of approximately 3 - 5 ml. of L2
medium was poured over the surface of a culture on haeuclstein-
Jensen medium which had been kept for 25 - 30 weeks in the refig-
erator and some of the colonies were suspended using a pipette.
The suspension was left over night in the refrigerator.

3 agar plates were prepared; one containing simple synthet-
ic agar medium (I), the second simple synthetic agar medium +
albumin and the third agar medium (I-2). These were insonated
with mycoplasma magnatia i.e. 4905/67 grown in Sula liquid
medium for 24 hours. After spreading the suspension so as to cover
the whole surface, the excess fluid was removed and the plates
were allowed to dry for 4 hours in the incubator at 37°C. Areas to
be spotted were marked by a grease pencil.
A drop of the above overnight suspension was poured on each of the marked areas. Plates were then incubated at 37°C for 24 hours and examined later for the presence of lytic plaques.

a. Multiplication of mycoplasmas: 0.5 ml suspension of ATCC 607 grown on Sibra liquid medium for 24 hours was used for inoculation of several tubes of EMB liquid medium. These tubes were incubated at 37°C for 4 hours, received 0.5 ml of the mycoplasma suspension each and reincubated at 37°C for 24 hours more. The presence of mycoplasmas was then tested as described before.

b. Determination of the Routine Test Dilution (RTT):

i. Eight ten-fold serial dilutions of the stock phage suspension was prepared.

ii. Three tryptic soy agar plates are covered with bacterial suspension of ATCC 607 (grown on Sibra liquid medium for 24 hours). Each plate was rotated to give an even distribution of the bacteria, the superfluous liquid removed with pipette and the plates were placed in the incubator to dry for 2-3 hours. The areas in which the phage suspension was to be applied were marked as before.
iii. Using Pasteur pipettes and beginning with the original undiluted suspension, drops of a ten fold phage dilution in series was placed in the marked areas. The plates were allowed to dry, incubated at 37°C and examined for clear spots at 24 hours intervals. RTD was considered as the highest dilution of the myco phage which produced complete clearance of the host strain.

c. Titration of mycoplasma: The number of active phage particles per unit volume (millilitre) was determined according to the work of Redmond and Wards (1966). Five ten fold dilutions of the phage suspension were prepared as previously described. Several tubes of IMH medium were inoculated with bacterial suspension of ATCC 607 and incubated at 37°C for 24 hours. A mixture of 0.1 ml. phage suspension to 1.0 ml. of the above bacterial suspension was prepared and pipetted on the surface of an IMH plate; spreading so as to cover the entire surface was effected to insure uniformity. These excess liquid was removed and the plates were incubated at 37°C for a period ranging between 24 and 72 hours. The plates were examined daily for lytic plaques. The number of the plaques on the most representative plate was counted and hence 80/
Lymph nodes were the most commonly affected. The histology of the lesions was characterized by a granuloma composed of multinucleated giant cells and epithelioid cells with or without caseation. Bacteriologically all isolated mycobacteria were of the human type.

In a retrospective study for the period 1959/70, 649 tuberculous biopsies were reviewed and the relevant data are listed in Table 6. Affected organs consisted of 584 lymph nodes (163 being cervical and 221 various), 145 bones and joints, 23 endometrium, 22 testes and 75 various organs. Bacteriological examinations were not carried out but the tuberculous nature of the lesions was confirmed histopathologically.

As seen from the Table, an increase in the prevalence of the disease is shown in the age group 20 to 39 followed by an immediate decrease in the age group 40 to 49. Organs accounting to less than 20 in number were not taken into consideration for being less informative.

b. Autopsy

A consecutive study of 895 autopsies in the period 1971/70 yielded pathological evidence of tuberculosis in 121 cases i.e. 13.5%, 26 being confirmed bacteriologically. These cases were divided into 38 extra-pulmonary and 53 pulmonary
<table>
<thead>
<tr>
<th>Tissue/organ</th>
<th>(0-9:10-19:20-29:30-40:49)</th>
<th>10-19</th>
<th>20-29</th>
<th>30-40</th>
<th>40+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical lymph nodes</td>
<td>15</td>
<td>30</td>
<td>34</td>
<td>32</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Various lymph nodes</td>
<td>13</td>
<td>29</td>
<td>54</td>
<td>49</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>Bones and joints</td>
<td>5</td>
<td>24</td>
<td>52</td>
<td>51</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Endometrium</td>
<td>1</td>
<td>12</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Uterus</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>3</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Lungs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Peritonium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
</tr>
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... Appendices, epididymis-crural, fallopian tube, kidneys, maxillary sinus, rectum, stomach, uterus, vulva (2 each)

Epididymis, eyes, heart, lung, lymph nodes, spleen and prostate (2 each)

Grand total | 649 |
tuberculosis. The latter included 36 cases with lung cavitation; 19 of these were studied with reference to the presence or absence of fibrosis around the cavities. Fibrosis was revealed in 8 patients (5 Northerners, 2 Southerners and one un-identified) while in the remaining 11 patients, this feature of fibrosis was completely absent. Localities concerned were represented by a Northerner, 8 Southerners and two unknown patients.

1. Pulmonary tuberculosis

According to the distribution of the lesions the 83 pulmonary cases were further subdivided into three categories as follows (vis. Table 7):

1. Isolated pulmonary lesions only: In 16 out of the 83 cases i.e. 19.3%, the infection was confined to the lungs and the mediastinal lymph nodes were not affected. 5 patients had bronchopneumonic tuberculosis with cavitation, 9 showed cavitation only and the remaining two showed caseous nodules. Renal amyloidosis was seen in one patient. All patients were above 20 years of age.

ii. Lung lesions with mediastinal glandular involvement:
this was revealed in 6 cases i.e. 7.2%. Four had lung lesions in
the right lower lobe with mediastinal involvement. The remaining
two cases revealed primary complex with caseating nodules in the
prophraxis of the lung: the mediastinal lymph nodes were considerably
enlarged and showed caseation. All these autopsies were in the age
group 20 to 39.

iii. Pulmonary lesions with dissemination: this group
consisted of 61 cases i.e. 73.2%: 51 of which were males and 10
females. Apart from pulmonary lesions, the group was characterized
by involvement of other organs and/or lymph nodes. The disease was
mostly common in the age group 30 to 39 years, in both Southerners
and Northerners with extensive hilarry tuberculosis being observed
in 41 cases. Cavitation was seen in 20 cases and bronchopneumonia
in 10. Generalized tuberculous lym hemositis was revealed in 36
autopsies. The mediastinal lymph nodes were involved in 36 cases,
the cervical in 11 only while involvement of the abdominal lymph
nodes (non-spécified) was shown in 11 autopsies, mesenteric in 31
cases, para-aortic in 23, lymph nodes around the esophageal axis in
18 and portahepatis lymph nodes in 5 cases. In 12 of those
subject the abdominal lymph nodes were greatly enlarged and matted
together in a mass of up to 32 cm. in its greatest diameter. In-
testinal ulcerations were seen in the large intestine in 7 cases,
in the small intestine in 3 and in both small and large intestine
in 4 patients. The liver and spleen were both involved in 42
autopsies. The liver alone was involved in three cases and
the spleen in two. The hepatic lesions were represented by
miliary tuberculos\(\text{sis}\) in 25 cases, nodular tuberculosis in 14
cases and by combined miliary and nodular tuberculosis in 6
cases (viz. Figs. 1 - 3). Similar lesions were seen in the spleen;
these being consecutively represented by 23, 19 and 2 cases (viz.
Fig. 4). Involvement of the kidney was seen in 23 cases (viz.
Fig. 5). Spinal tuberculosis was observed in 12 autopsies (Fig. 6).
In 10 instances tubercles were detected in the heart; 3 being in
the myocardium and 7 confined to the pericardium (Fig. 7). In one
patient the anterior mediastinal lymph nodes and the base of the
heart were completely fused in a big caseous mass communicating
with both atria. Osophageal lesions in the form of nodules were
seen in three cases with additional ulcers being revealed in one
Fig. 2. Section of the same liver showing large coalescent nodules with partial central cavitation and occasional bile-stained contents.
Fig. 3. Human liver from H37Rv showing few miliary tubercles (1) and large caseous nodules (2) (viz. appendix)
Fig. 4. Section of spleen from R15/70 showing cortical nodules.
Fig. 5. Section of right kidney from 1953/70 revealing two osseous nodules.
Fig. 6. Section of the lumbar vertebrae from T10, T11, T12, showing variation and a recent dislocation of the second lumbar vertebrae.
Fig. 7. The pericardium (200/70) prepared with milky tubercles.
of them (Fig. 5). The brain and meninges were affected in 8 sub-
jects, adrenal in 9, pancreas in 6, thyroid in 4 cases only and
male genital organs in 8 patients. Only in two cases were the
urinary bladder affected.

2. Extra-pulmonary tuberculosis
This group consisted of 38 cases with no evidence of pul-
monary involvement. The disease occurred mostly in the age group
20 to 39 and over 50 years (viz. Table 7). According to the probable
route of infection, these were categorized as 30 cases of intestina-
l tuberculosis and 8 various cases; the infection being through the
colimentary tract in the former with the abdominal organs and lymph
nodes and other organs being also involved. Simultaneous involve-
ment of organs and lymph nodes occurred in 10, lymph nodes alone
in 10 and organs in 2 instances. The abdominal lymph nodes were
the most frequently affected; they included the mesenteric lymph
nodes, those around the celiac axis and the parotid group. The
cervical, portal, inguinal and axillary lymph nodes were less fre-
quently affected. In one case, the lymph nodes alone, the thoracic
duct were seen to be involved (Fig. 4). The thoracic duct was
also affected and showed small tuberculous nodules. The affected
90/-
Fig. 6. Tuberculous nodules in the esophageal wall (H/E5/40).
Fig. 9. Storing the thoracic duct with a probe inside (1) and enlarged cervical lymph nodes in the mediastinum (2) (Vis. T1153/59).
lymph nodes were mostly enlarged, cæsated and matted together.
The liver was the organ most frequently involved, with the spleen
being next in frequency. The hepatic lesions were either miliary
or nodular and formed clusters of a comparatively large size; these
occurred chiefly in the periportal tissue. Tuberculosis of the spleen
was characterised by a miliary type and/or tubercles of various
sizes. In few instances the gastric and pancreatic peritonium was
prepared with miliary nodules. The lesions in the small and large
intestines were in the form of subseral tuberculous nodules;
tuberculosis being rare among these cases. Tuberculosis of the
urogenital tract was rather rare, only two cases were reported.
Apart from involvement of the peritonium andomentum, secondary
lesions were also revealed in the pericardium, spine, thyroid,
brain and meninges.

In the second group where infection occurred through disre-
tensible routes, solitary affection of the spinal vertebrae was
observed in two cases only, three other cases revealed localized
tuberculous lymphadenitis in the submaxillary, inguinal or cervical
lymph nodes while in one case, only bilateral affection of the
dural plates was seen. Another case showed involvement of the

---
adrenal gland along with that of the spine, brain and meninges.

In our cases lesions were confined to the mediastinal, pelvic and inguinal lymph nodes. In these eight cases, it was rather difficult to assess with certainty the exact portal of entry.

B. Animal material

50 positive bovine specimens were collected from Wax and Okkunen Central Abattoirs. These were classified according to the distribution of lesions as follows:

a. Pulmonary tuberculosis
   
   This group consisted of 37 cases i.e. 74% of all cases examined. Bronchial and mediastinal lymph nodes were both affected in 16 instances, bronchial alone in 14 and mediastinal alone in 7 cases. These lymph nodes were constantly enlarged and caseated while the lung lesions were in the form of caseous nodules. There was no evidence of cavitation, (Fig. 16)

b. Pulmonary tuberculosis with dissemination
   
   In this group, apart from lung lesions, other organs and glands were also involved. The group consisted of 8 animals i.e. 16%. The liver was involved in 3 cases and the spleen in one; the
Fig. 10. Lung of cattle showing caseous-calcified nodules in the bronchial lymph node and in the right diaphragmatic lobe. The posterior mediastinal lymph node is also involved.
Pl. 11. Postel's disease in the gums of cattle
lesions being in the form of caseous nodules in both organs. In one case the pleura was affected (24, 11), two animals showed generalised lymphadenopathy. The bronchial and mediastinal lymph nodes were involved in 5 cases, the abdominal in 2, retropharyngeal in 1 and the inguinal in 2. No intestinal ulcers were detected.

1. Extra-pulmonary tuberculosis

This was revealed in 5 cases (10%) only. 4 animals had affection of the retropharyngeal lymph nodes and in one the para-aortic lymph node was involved. The primary focus was not detected in these cases.

2. Tuberculosis test in dairy farms

Out of 526 cattle tested in Bulgravia Dairy Farm, only one animal was tuberculin-positive, 21 doubtful and 505 negative. Similar tests carried out in 332 cows in Bl Obock Government Dairy; 42 animals were doubtful and 190 negative.

2. Histopathology

The microscopic picture displayed by both human and animal specimens was in no way different from the usual histological pattern seen in tuberculosis, while caseation was a common
Fig. 12. Human liver X 100 (H & E)
typical tuberculoid caseous-granulomatous lesions
with clear giant cell formation; no calcification
is seen.
feature in both specimens, calcification was rather rare in nbn.

In many it was prominent in cattle particularly (Fig. 12). On the contrary it was relatively in affected lymph nodes, (Fig. 15).

In a number of affected human lymph nodes, the lesions were marked by proliferation of epithelioid cells and fibrous encapsulation. In another group, the general feature was that of lymphatic hyperplasia, marked reticular proliferation and formation of numerous small tuberculous follicles; few of the latter revealed recent caseous necrosis. This picture was less marked in other group of lymph nodes with minute caseous foci. In cold abscesses, fibrous encapsulation was fairly common.

In the bovine material, the affected lymph nodes were seen to be soft and caseous but mostly gritty, firm, tumorous and fibrous with reddening yellowish streaks; the most prominent microscopic feature being calcification coalescence and presence of epithelioid and giant cells formation. This is surrounded by round cells infiltration and fibrous tissue, (Fig. 14)

3. Experimental inoculation

a. Guinea pig's inoculation

Subcutaneous inoculation to guinea pigs was tested by using
Fig. 13, Cattle: Bronchial lymph node X 100 (X & J). Tuberculous lymphadenitis with pronounced calcification.
Fig. 14. Bovine bronchial lymph node X 100 (II & III)
Tuberculous granuloma in a starting supplicative stage showing clear fibrous encapsulation and a typical horseshoe giant cell.
12 human and 10 bovine strains. Except for one human strain (H17),
both types were all successful in producing progressive lesions of
a similar form in different organs. These were confined to the site
of inoculation, the regional lymph nodes, spleen, liver and lungs.
The precrural and lumbar lymph nodes were enlarged and showed varying
degrees of caseation; the site of inoculation being the right
flank in all animals. The spleen was considerably enlarged and re-
vealed confluent tubercules. The liver contained minute irregular
gray-yellowish foci. The lung lesions were slight and showed few
miliary foci. Other lesions were seen in the tracheo-bronchial
lymph nodes. These were moderately enlarged, hard and fibrous;
caseation being revealed on sectioning. The lesions in general were
comparatively more severe after inoculation with the bovine rather
than with the human strain viz. Table 3. All the guinea pigs,
which were inoculated with the bovine strains, including that of a
human source, died in a period of 36 to 90 days after inoculations.
With the exception of two animals that died in 34 and 43 days
successfully, all the other animals which were inoculated with the
human strains were sacrificed 8 weeks after inoculation. The histo-
logical picture was characterized by extensive caseation, irregular
| Duration | Microscopic | Macroscope | No. % of | After | Spleen | Lungs | Liver | Days | Gross | Gross | |----------|-------------|-----------|---------|-------|-------|-------|-------|------|-------|-------|
| 25 | 58 (exc.) | 570 | 1.25 | 0.38 | +++ | +++ | ++ | 33,500 |
| 32 | 58 (exc.) | 530 | 0.57 | 0.18 | +++ | ++ | + | 15,000 |
| 47 | 58 (exc.) | 530 | 0.60 | 0.11 | - | - | - | 9,500 |
| 39 | 58 (exc.) | 580 | 1.46 | 0.28 | +++ | + | + | 10,500 |
| 276 | 58 (exc.) | 380 | 3.40 | 0.89 | +++ | ++ | + | 4,200 |
| 56/60 | 58 (exc.) | 430 | 2.20 | 0.51 | +++ | +++ | + | 25,500 |
| 60/76 | 58 (exc.) | 570 | 2.20 | 0.51 | +++ | ++ | + | 15,500 |
| 60/76 | 58 (exc.) | 550 | 1.85 | 0.35 | +++ | + | + | 18,600 |
| 55/70 | 58 (exc.) | 500 | 3.25 | 0.35 | +++ | + | + | 30,250 |
| 55/70 | 58 (exc.) | 450 | 4.25 | 0.35 | +++ | + | + | 35,000 |
| 54 (exc.) | 340 | 0.75 | 0.22 | + | ++ | + | 20,100 |
| 76/85 | 43 (exc.) | 450 | 2.75 | 0.61 | +++ | + | + | 25,000 |
| 85/86 | 10 (exc.) | 400 | 1.85 | 0.46 | +++ | + | + | 10,000 |
| 10 | 46 (exc.) | 260 | 1.60 | 0.57 | ++ | + | + | 25,000 |
| 20 | 46 (exc.) | 280 | 2.11 | 2.18 | +++ | ++ | +++ | 25,000 |
| 26 | 46 (exc.) | 260 | 4.71 | 2.81 | +++ | +++ | + | 26,500 |
| 24 | 46 (exc.) | 340 | 4.20 | 1.24 | +++ | ++ | + | 75,000 |
| 30 | 46 (exc.) | 270 | 7.75 | 3.39 | +++ | +++ | + | 75,000 |
| 39 | 47 (exc.) | 490 | 2.75 | 1.53 | + | ++ | + | 21,000 |
| 0 | 34 (exc.) | 270 | 4.10 | 1.62 | +++ | +++ | + | 10,500 |
| 0 | 54 (exc.) | 350 | 5.85 | 1.57 | +++ | +++ | + | 32,500 |

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+++ = more than 30 feet
++ = more than 10 and less than 30 feet
+ = less than 10 feet

exc. = sacrificed

No. = number of organisms inoculated as evaluated by the visible count.

+ = 2 to 10 feet

* = one or two feet
giant cells formation and slight fibrosis; the latter being more prominent in suppurative granulomas than in AFB nodules.

14 human and 6 bovine strains were used for intraperitoneal inoculation into guinea pigs. All animals were sacrificed 4 weeks after inoculation, vis. table 9. The lesions revealed were in both cases represented by milky tuberculasis in caecum, peritoneum, mesenteric lymph nodes, spleen, liver and lungs. The caecum was thickened and studded with small nodules. The spleen was moderately enlarged and revealed sub-capsular nodularity. The liver was peppered with minute irregular greyish nodules while only few opaque ones were seen in the lungs.

The pathological picture was that of necrotic necrosis, caseousoid and giant cells formation; fibrosis being absent.

2. Rabbits inoculation

Intravenous inoculation of mycobacterium bovis into rabbits was experimentally tried and 8 bovine bovis strains were used. With the exception of two rabbits that successively died in 46 and 54 days, the survival periods of all others ranged between 18 to 33 days vis. table 10. The lesions produced were in the form of milky tuberculasis in different organs; the lungs being severely affected.
They were extensively showered with uncountable minute foci. Similar or grayish irregular lesions were present in both liver and spleen. Only few coccous foci were revealed in the kidneys; these were confined to the cortex. Casual lesions were reported in other organs such as joints. The lesions were characterized by extensive caseous necrosis and partly absence of fibrous encapsulation. The histological picture was in no way different from the usual tuberculous pattern.

II. Pathology

1. Isolation

In a collection of 72 various human specimens, Ziehl Neelsen method revealed acid-alcohol fast mycobacteria in 60 specimens. This was attained either directly or after using the concentration method. 52 of these were primarily isolated in Lovenstein Jensen media; growth being obtained in 11 to 21 days. In further subculture, 9 strains failed to grow (See Table 5). The remaining 53 strains were typed as seen below, 51 as mycobacterium tuberculosis and two i.e. 7390/G8 and 73/72 as mycobacterium bovis.
Sweats stained with Ziehl-Neelsen's method were positive for rhodobacteria in 50 bovine specimens collected from different abattoirs. Although 33 of these were isolated in Koenenstein Jensen medium in 28 to 28 days, only 19 were positive to starch. In subcultures and hence being typed as rhodobacteria bovis (Fig. Table 9).

4% sodium hydroxide was effective in suppressing contaminants in both human and animal material; however the digestibility of the latter was rather cumbersome since a comparatively large amount of residue was left.

2. Culture characteristics and pigmentation

Typical granular growth in liquid medium was shown by all isolated strains, whether human or animal. In semi-liquid Lebac medium, all human strains yielded surface growth, while the bovine strains were irregularly characterized by deep ring or deep and surface type of growth (Fig. Table 1). Rhodobacteria tuberculosis usually d organic, rough and non-pigmented colonies in Koenenstein Jensen, Agar and bovine serum agar media (Fig. 15). All except one of the bovine strains were dorganic, smooth and non-pigmented in Koenenstein Jensen, Agar and bovine serum agar media. The
Fig. 15. Typical mycotic rough colonies (Mycobacterium tuberculosis, 2/3/63) grown on bovine serum agar for four weeks.

Fig. 16. Mycotic rough colonies (Mycobacterium bovis V24) grown on bovine serum agar for four weeks.
remaining bovine strains yielded eugenic colonies in all media; these were rough and non-pigmented in Lowenstein Jensen and bovine serum media (Fig. 16). The colonies in the latter were mostly surrounded by a characteristic wrinkled formation (Fig. 17). On the contrary, the colonies were smooth and yellowish in agar media (Fig. 18) and the growth was comparatively rich.

3. Morphology and staining

The isolated strains of mycobacterium tuberculosis and bovis were acid-fast and non-branching acid and alcohol fast. They resisted decolourisation by 2% sulphuric acid and 3% HCL in 70% alcohol. However, the stain of mycobacterium tuberculosis was invariably pleomorphic. The granules were necrophilic and evenly distributed throughout the length of the bacilli while in shorter bacilli, this was limited to a bipolar presence. The feature was predominantly seen in young cultures. The bovine bacilli were comparatively shorter and thicker than their corresponding human types.

4. Drug sensitivity pattern

43 strains isolated from man were found fully sensitive to streptomycin, isoniazid and to para-aminosalicylic acid. The group consisted of 19 strains from biopsy lesions of bones, joints and...
Fig. 17. Salmonella typhimurium bact. (V24) showing
epidemic rough colonies surrounded by a character-
istic wrinkle formation, grown on brain serum
agar for four weeks.
Pl. 10. Subnarcissus pseudonarcissus (L.) albosin, en site, gallois. Meul'telcolis, ron en
A scem neum fur fuei note.
lymph nodes, 7 from sputum specimens, one from endometrial biopsy and 20 from variable post mortem lesions. The bones and joints cases were previously subjected to treatment with three drugs for a period of a month before the biopsy performance. Similar treatment for a week was revealed in the records of a post mortem case. The history of the remaining post mortem cases was not reportable. The 7 patients with sensitive sputum bacilli were newly admitted at the time.

In another group of six sputum strains, one was fully resistant to streptomycin, a second one to IM (200 mcg/mL) and to PAS (10 mcg/mL). The remaining four sputum strains were resistant to IM (one to 1 mcg/mL, one to 0.2 mcg/mL, and one to 10 mcg/mL). They were new admissions and apparently not subjected to previous treatment.

An additional strain isolated from a bone lesion was only sensitive to streptomycin and resistant to IM (1 mcg/mL) and PAS (3 mcg/mL). The patient had already been subjected to one month treatment with the three drugs. Three various post mortem cases yielded strains resistant to IM (two to 0.2 mcg/mL and one to 10 mcg/mL). The latter was as well fully resistant to streptomycin and represented a case of generalized tuberculosis and bone involvement.
All the bovine strains isolated from slaughter-house material were found sensitive to EN, PAP, and streptomycin.

5. Biological characteristics
With the exception of two strains, typed later on as yersiniae bovis, all other 51 human strains were TCH-resistant and nitrate-positive. 43 of these strains were peroxidase and catalase-positive. The two bovis strains gave similar results. One strain was doubtful for both peroxidase and catalase test. Another strain was peroxidase-positive and catalase-negative. On the contrary, the remaining six human strains were peroxidase-negative and catalase-positive.

The 15 bovine strains isolated from slaughter-house specimens were all TCH-sensitive, Nitrate-negative and catalase-positive. With the exception of four strains, all the strains were peroxidase-positive. The four were represented by two peroxidase-negative and two doubtful strains (via table 5).

(6) Virulence test
A. Spleen index
The spleen index of 12 human strains and 10 bovine strains selected at random were determined. With the exception of strains No. 417, all these strains were found virulent for guinea pigs.
(vis Table 8). The splenic index of the isolated human strains varied from 0.11 to 0.95 while that of the bovine strains ranged between 0.67 and 2.18. One bovine strain isolated from human gave a splenic index of 0.46. All guinea pigs inoculated with the bovine strains (including that of a human source) died in a period of 36 to 49 days after subcutaneous inoculation. With the exception of two guinea pigs that died in 34 and 43 days respectively, all other ones were sacrificed 6 weeks after inoculation. Tuberculous lesions were produced in all guinea pigs except the one inoculated with the human strain H17.

3. Omental index

Omental indices for 24 human and 3 bovine strains were also calculated. Omental index of human strains ranged between 0.19 to 1.07 while a higher range of 1.13 to 2.50 was given by the bovine strains (vis Table 9). Although an increase in the animal weight was seen in 20 out of the 22 inoculated guinea pigs at the time of death or sacrifice, no correlation was seen to exist between this increase and the corresponding omental index. It is also noteworthy that strains K17 proved to be virulent through the intra-peritoneal route.
C. Rabbit inoculation

9 rabbits were inoculated with 8 bovine strains of which mainly V24 yielded two types of colonies, hence each type was inoculated in a separate animal. All inoculated rabbits died within a period of 18 to 54 days (viz. Table 10). A considerable increase in the weight of all animals was noticed at the time of death and a generalized type of lesions was produced. Although different strains were used for inoculation, the virulence was invariably proportional to the increase in the viable count.

The rough colonies of strain V24 proved to be more virulent than the smooth.

III. Myrcoplasmas

The ultra-violet irradiation trials for liberation of mycoplasmas proved to be a failure. No lytic plaques were detected in all tested lyogenic strains i.e. R209/69, V5, V15, V26, V44, V45 and V49. For this purpose different host strains and media were used at different times of exposure.

11 Isolation

As seen in the methodology the successful procedure for isolation of mycoplasmas entailed no usage of an inducing agent but

Using the RTO method, the lytic effect on the above plaques was studied in comparison with that of a known control i.e. plaque 654*.  

*supplied by the Tuberculosis Research Institute, Prague.
Fig. 10. *Salmonella typhimurium bovis* (53/36)
colonies. Agarose streak plates on bovine
serum agar; lysed colonies indicated by
arrows.
Fig. 20. *Sulmonae mycobacterium bovis* (V34) showing eugonic rough colonies on bovine serum agar. Lysis of colonies indicated by arrows.
Fig. 21. Sharing petri dish (A) containing RV11 medium, (B) RV11-broth medium, (C) RV1 medium and (D) containing R2 medium; NSC 697 being as a host strain. Lysis only seen in case of 2 i.e. *rickettsia* V24, 5 i.e. *rickettsia* RV2/69 and 6 i.e. *rickettsia* R2/73. No effect was produced in case of (1) i.e. *rickettsia* V5, (3) i.e. *rickettsia* V10, (4) i.e. *rickettsia* V15, (7) i.e. *rickettsia* R60 and (8) i.e. *rickettsia* R21.
In a group of 12 strains of rapidly growing mycobacteria, the four myco-geuses were equally lytic for four strains (521, 121, CRCA and 607) and equally non-lytic for strains 605 and phleg.

Table 11 reveals that myco-geuses V26 and MP272 are comparatively similar in their lytic effect. They could easily be differentiated from myco-geuses MSG/69 and the control A61. The latter showed some similarity in their lytic effect as well.

Myco-geuses V26 and MP272 could easily be differentiated through the lytic effect on strain Magnesia. While no lytic effect on Magnesia was produced by MP272, countable and isolated lytic plagues were affected by V26. Apart from the generally poor lytic effect of MSG/69 particularly on mycobacterium magnesia, myco-geuses MSG/69 and the control A61 were rather similar.

The lytic effect of the above myco-geuses was also studied on another group of human, bovine, avian and canine strains. They included 15 Sudanese human strains, 9 Czechoslovakian human strains, one human laboratory control strains (1371W), 5 Sudanese bovine strains, 2 avian and one marine strain. All the human strains, irrespective of their source of origin, were lysed by myco-geuses MSG/69 and A61 and showed resistance to myco-geuses V26 and MP272.
One of the Sudanese human strains i.e. HM152/69 was excluded for partly being negative in growth or mostly contaminated. Seven strains of the same Sudanese group showed occasional contamination in some of the plates but still the lytic effect was rendered possible to read from the remaining ones. The three Sudanese bovine strains shown in Table 12 are members of a group of 15 strains, twelve of which failed repeatedly to grow. Those three were also sensitive to phage types 2789/69 and 262 and resistant to V24 and NF272; their growth was rather sparse and meagre and not even enhanced by the richness of Redmond medium (RVM). Contrary to strain V24 the lysoytic bovine strain HF90/68 was not immune to the lysozyme it produced. The two avian strains grew well in all the plates and showed no lytic effect whatsoever, thus proving their resistance to the four phage types. Although a lytic effect was shown on the marine strain K265 by phages K265/69 and 262, this was only revealed in two media (Ulelukun and K265/14).

In another experiment, 13 000 strains from different sources were subjected to the four phage types. A similar lytic pattern to that of the human and bovine ones was revealed. They
were sensitive to mycophages MP10/69 and AC1 and resistant to VP2
and LP272; the Armenian 206 strain being slightly different. It
showed partial lysis by mycophages VP2 in T1 medium. The English,
French and the Japanese 126 strains were distinguished from
growth was either negative or contaminated (Table 13).
1. Pathology of human tuberculosis

The relative high frequency of tuberculosis (11.3%) among autopsies analysed in this study agrees with the findings of previous workers in other underdeveloped countries. Comparable studies revealed the following: Everett (1933) in the United States reported an incidence of 16.2% in coloured and white subjects, Wilcockson (1935) found 15.8% among natives of Tanganyika, Auerbach (1944) in America recorded an incidence of 17.0% and Davies (1947) findings in two autopsy series done in Kampala showed an incidence of 11.3% and 13% respectively. Tuberculosis was frequent (10.9%) among postmortems done on natives of Kasa (Wilson, 1927/28), Vint (1928/29) pointed out that tuberculosis accounted for 2.2% deaths among the natives of Kasa. In West Africa (1891) 7.9% of all deaths were due to tuberculosis (Neal, 1953).

The autopsy findings in the present study indicate that tuberculosis in this country manifests unusual pathological features. These lesions were classified according to the system suggested by Davies (1947) who studied a series of autopsies performed on natives at Kampala.
Different forms of tuberculosis were encountered. Large number of autopsies showed primary intestinal tuberculosis with massively enlarged abdominal lymph nodes. 95% of cases were of primary pulmonary tuberculosis. All autopsies of primary pulmonary tuberculosis were in adults (20 – 30 years). When primary infection takes place in an adult it indicates a low degree of natural resistance and will usually lead to fatal consummation (Papel and associates, 1954). Childhood tuberculosis in adults was reported before by Everett (1915) who noted this form in 50% of American negroes and Opie (1930b) who found that 8 out of 9 negroes in Jamaica were affected with childhood tuberculosis. Adulthood type of tuberculosis was less frequently encountered (13.2%). All these patients were above 20 years of age. Adulthood tuberculosis was rare (1%) among G nepalese (Saraf 1926). Pinor and Kazpor (1932) reported phthisis in 27.7% of whites and 2.4% of the negro subjects. Davies (1947) found that between 0.7% and 2.6% of the natives at Mysore were affected with chronic pulmonary tuberculosis.

Pulmonary tuberculosis was more common in patients from Northern Sudan than from Southern part. This interesting
contrast may in part be related to the lower resistance of the Southerner who is unable to localize his tuberculous lesions. The rarity of fibrosis among southern races is similar to the findings of Clear (1917) in natives of the Panama Canal Zone and Vint (1929/30) among the natives of Tanganyika. Pinner and Rosser (1932) mentioned that cavitation with thick fibrous walls was seen four times more frequently in the whites than in the negroes. Everett (1933) reported that fibrosis in the negroes was less common than in the whites. Wilcock (1935) pointed out that in the natives of Tanganyika fibrous tissue formations and calcification were rarely seen and Davies (1947) reported that in natives of Nyasaland fibrosis was rarely seen and calcification was unusual, the usual picture was purulent infection without cavitation and with formation of ragged thin-walled abscesses filled with semi-liquid caseous pus.

The disseminated form of the disease among our autopsies was common. This is in agreement with the findings of Vint (1929/30) in natives of Tanganyika, the report of the South African Medical Research Council (1932) among the natives, Wilcock (1935) among-
est natives of Tanzania and Davis (1947) in natives of Kapsulka. Raymond (1920/21) findings indicated that, generalized tuberculosis was more common in the negroes than in the whites. Lambert and DeGastro (1925) reported that lethal tuberculosis was found in 27.3% of the negroes and 15.3% of the whites and Harrow and Purves (1947) said that the frequency of disseminated tuberculosis was 10% and 12% in non-whites and whites respectively. This is in contrast with the results obtained by Clark (1915) who found that acute miliary tuberculosis occurred in 10% of the negroes in the Fiume Canal Zone. Ellis (1927/28) also reported that, among natives of Pemba, the chronic form of the disease was the rule and not the exception.

Disseminated tuberculosis with pulmonary involvement was the most common (80.4%) form encountered in the autopsy material reported in the present study. The peak incidence occurred in the age group 30 - 39 years. Miliary tuberculosis with or without nodular tuberculosis was recorded in most of the cases. Lymph nodes, liver and spleen were affected in most of autopsies. Other organ involvement included the kidneys, pancreas, brain, bone, thyroid,
adrenal and peritoneum. Male and female genital organs were uncommonly affected in disseminated tuberculosis. The most striking feature of this form was the nodular nature of the tuberculous lesions in the different organs particularly the liver and the spleen. Generalized tuberculosis with nodules in the lungs, liver and spleen in Sudanese was reported before (Cleland, Tropical Research Laboratory, Fourth Report 1921). Similar findings were also described in Australian Aborigines (Cleland 1912). Piiper and Kasper (1932) mentioned that tuberculosis of the adult negro was characterized by massive lymphatic involvement with generalized nodular foci in different organs of the body. Barron and Purves (1947) found that in the coloured, the most common sites of dissemination were, lung nodes, peritoneum, bone and genito-urinary system.

In the disseminated form of the disease it is of course difficult to say where the disease had originally started, but from the marked involvement of the lymphatic tissue it is possible that most of the cases were of the progressive primary tuberculosis with lymphatic dissemination. Haeomogenous dissemination of
Tuberculosis may occur through the involvement of blood vessels directly or through the drainage of tubercle bacilli into the lymphatic and venous systems.

The findings of several workers indicated that abdominal tuberculosis was more common in the negroes than in the whites. Clark (1915) said that 50% of the negroes showed involvement of some abdominal organs in generalized disseminated tuberculosis.

The lesions in the spleen and liver were usually of acute and chronic miliary type. Barret (1916) reported that abdominal tuberculosis was common among Senegalese and that miliary tuberculosis in the liver and spleen was found in 25% of cases. Vint (1926/29) found that in the natives of Kongo all organs in the abdomen except the pancreas were affected. Tinner and Kasper (1932) comparing between whites and negroes in the degree of involvement of abdominal organs found, that the liver was affected in 21.5% of the negroes and 11.5% of the whites. The spleen was involved in 25% and 10.8% in the negro and white respectively.

Abdominal lymph nodes tuberculosis was seen in 39.5% of the negroes and 7.6% of the whites. The South African Medical Research
Council Report (1932) indicated that in the natives, the involve-
ment of the spleen (70% in acute and 40.7% in chronic) and liver
(62% in acute and 37% in chronic) was common and in the spleen,
specially the degree of involvement was much greater. The intestine
was affected in 21% in acute and 20% in chronic cases. American
findings (1944) showed that miliary tuberculosis in the spleen
was reported in all cases and the mesenteric was involved in 4.7% of
the younger and whites. Wright (1945) admitted that tuberculosis of
the spleen, as a part of miliary spread, was commonly seen in
autopsies performed at Byrd on africans dying of tuberculosis.
Our findings are in agreement with the previous findings of Clark
1915, Dorrel 1920, Raven 1914, and Wright 1945, that
abdominal tuberculosis was common among patients. It occurred
in 73.7% of cases pulmonarly tuberculosis and in 27.3% of dis-
sminated tuberculosis with pulmonarv involvement. Affection of
the liver and spleen usually occurred together. The liver showed
tuberculous lesions in 73.8% of the group labelled as disseminat-
ed tuberculosis with pulmonarv involvement. The liver usually
showed only miliary tubercles but some times large caseous nodules were also seen (viz Figs 1 and 3) and in one case (viz appendix B:15/67) the nodules broke down leading to the formation of bile stained cavities. In this interesting case three hepatic lesions were seen namely miliary tubercles, caseous nodules and cavities. The primary infection was probably through the intestinal tract from where the disease spread to the liver forming tuberculous nodules. At a later stage miliary spread occurred via the systemic circulation involving the liver again, that the primary focus in this case was probably in the intestine rather than the lung is supported by the presence of miliary lesions only in the lungs.

Another case (viz appendix B:70) also showed nodular and miliary tubercules in the liver, but this was associated with nodules and miliary tubercules in the lungs. The primary infection in this case might have been through the respiratory tract. The spleen was affected in 72.1% of the group in question, both caseous nodules and/miliary tubercles were seen. Less commonly affected abdominal organs were the pancreas, omentum and the stomach. Abdominal lymph nodes were affected in 57.3% of this group.
tuberculosis with dissemination) and in 75.7% of extra pulmonary tuberculosis.

Involvement of the liver from abdominal lymph node is easy to understand, and it may conceivably occur via the portal circulation. Splenic affection in the absence of lung involvement is more difficult to explain. Probable mechanisms are as follows:

1. The lung lesions are minimal and are therefore missed.
2. The spleen is involved through the erosion of a node through its arterial blood supply and bacilli are filtered off in the mesopharyngeal system.
3. Furthermore, why is the reticuloendothelial system in these patients so extensively involved. There is no clear cut answer to this question at present but the possibility of diminished cellular immunity could be an underlying cause.

17 strains were isolated from patients with abdominal tuberculosis (age between 18 - 55 years). 16 of these were Mycobacterium tuberculosis and one was Mycobacterium bovis. This indicates that the intestinal infection of the adult with the human type is much commoner than by the bovine strain. So satisfactory
explanation can be offered as to how the human tubercle bacilli reach the intestine without primary lesions in the lungs. However, the possibility that tubercle bacilli may reach the intestine through contaminated food cannot be excluded. One may recall the practice of churning milk in the country side, the milk is poured in a win bag (kin) which is only half filled. The bag is then distended with air blown through the mouth and churning is done. This practice may well play a role in the transmission of infection. Abdominal tuberculosis is often missed clinically.

Although abdominal tuberculosis was common yet intestinal ulcers were uncommon, seen in 2% of pulmonary tuberculosis and in 2% of non-pulmonary tuberculosis. In primary tuberculosis ulcers are small, ulcers in cases associated with lung cavitation are larger on the other hand and are due to the swallowed sputum. It was observed that in the absence of lung lesions, intestinal involvement from ulcerating lymph nodes is usually high up in the digestive tract e.g., in the esophagus, duodenum or stomach.

A relatively high incidence of renal tuberculosis complicating miliary dissemination was reported in autopsy series done in
meses of neoplastic origin (Clark: 1933; Pimner and Kasper, 1932; South African Medical Research Council Report, 1932 and 1946). Our findings showed that the kidney was involved in 37.7% of pulmonary tuberculosis with dissemination. The urinary bladder was rarely affected in our autopsy material, only two cases were found and in both the kidneys were also involved, which indicates that the bladder lesion had resulted from extension of the renal infection. Isolated renal tuberculosis was not encountered in autopsies. It was rarely seen in biopsies.

Bone tuberculosis was found to be more frequent in the negroes than in the whites (Pimner and Kasper, 1932). Tuberculosis of the spine was frequent in West Africa, the heaviest incidence being in the age group 15 - 30 years (Milneka 1932/33). Dreyer (1940) reporting on tuberculosis in West African children found, that 15% of affected children had tuberculosis of the spine. A lower incidence (8.7%) of bone tuberculosis among the Bantu of Eastern Districts was reported by Burrows (1935). In contrast to previous findings, Archibald (1922) noted that bone tuberculosis among Sudanese was exceedingly rare. The South African Medical
at Kampala was uncommonly seen at autopsy, though it was seen in
the wards. Our results showed that spine tuberculosis occurred in
14.3% of the whole autopsy series. In biopsy material bone and
joint tuberculosis was seen in 22.3% of cases and both young and
old were affected but the majority of cases were encountered with-
in the age group 20 - 39 years. In contrast to our findings
Bennett (1964) noted that bone tuberculosis occurred chiefly in
the young age group.

The view that tuberculosis lymphadenitis in cases among
coloured races is supported by several workers, Darrold (1920)
reported that in Senegalese sub-aorticular lymph nodes were in-
volved in 70% of cases and tracheobronchial lymph nodes in 80% of
autopsies. Archibald (1922) found that adenitis was the commonest
form of tuberculosis among the Sudanese. Dubert and Benazet
(1923) emphasized the fact that tuberculous lymphadenitis was
common among the negroes in Brazil. Filmer and Linford (1932) re-
corded that thoracic lymph nodes were involved in 5% of the negro-
es and 13.3% of the whites. Davies (1947) mentioned that mediastin-
el lymph nodes were affected in 3% of autopsies. The provine
Medical Officer, Dr. Nazmi (1942 unpublished data) admitted that
tuberculous lymphadenitis was common among the people of Western
Sudan who had migrated to the Gambia. Several reports, however,
did not agree with these findings, Surtees (1935) had reported
3.5% of tuberculous lymphadenitis cases. Many of Eastern Districts,
Matthews (1935) found 1.3% of tuberculous lymphadenitis in
Zanzibar. Our findings support the former findings as it was found
that tuberculous lymphadenitis was common in autopsies and
biopsies. In our autopsy series, the abdominal lymph nodes are
the most commonly affected. The high frequency of tuberculosis
of the cervical lymph nodes in our autopsy material is explained by
their easier accessibility.

Cervical tuberculosis is reported with variable frequencies
in the literature. Clark (1939) found that tuberculosis of the
epididymis and testis was of common occurrence, both in autopsy
and surgical specimens. Finner and Kasper (1952) reported genital tuberculosis in 11.8% of the negroes and 7% of the whites.\[...\] recorded male genital tuberculosis in 59.7% of patients with chronic pulmonary tuberculosis with miliary spread in an old age group. Our findings indicate that male and female genital organs tuberculosis are rare in autopsies and biopsies. Endometrial tuberculosis was seen in 1.4% of biopsies performed in patients below 20 years of age while those above 40 are almost free of infection.

Previous findings by Andreati and DeCastro (1923), Vint (1935) and Aschebach (1950) showed that tuberculous pericarditis was common. Tuberculosis of the pericardium and myocardium was more common in the negroes than in the whites (Finner and Kasper 1952). Davies (1947) reported tuberculous pericarditis in 10.5% of patients in both series and also a relatively high incidence of myocardial tuberculosis. He assumed that this is a feature of tuberculosis in the African elsewhere, our results showed 5.8% of tuberculous pericarditis in autopsies and myocardial tuberculosis was found in 2.3% of cases, all these had pulmonary tuberculosis with dissemination.
Corval (1920) and Davies (1947) showed that brain and meningeal tuberculosis were uncommon. These were not frequently seen even in generalized tuberculosis (Lambert and Halstro, 1925; Vint 1929 and Auerbach 1944). They were also reported to be rare in South Africa (South African Medical Research Council Report, 1932).

Zenzibar (Matthews, 1935). Miner and Kasper (1932) found tuberculous meningitis in 17.6% and 7% in the negroes and whites respectively. Brain lesions were found in 5.3% of negro and 1.3% of white subjects. In this investigation brain and meningeal tuberculosis was seen in 8.5% in the whole autopsy series. It appears that disseminated tuberculosis is not always accompanied by meningeal or brain tuberculosis.

Adrenal tuberculosis was reported to be a rare disease among Africans (Davies 1947 and Omer 1931). Auerbach (1944) however, reported involvement of the adrenals in 28.3% of negro and white patients who had miliary tuberculosis. Miner and Kasper (1932) found that the negro was more frequently (6.8%) affected than the white (1.4%). In our series adrenal tuberculosis was seen in 5.3% of autopsies which is in agreement with the findings.
of Osser (1933). All cases but one were unilateral. In the single case which had bilateral adrenal disease, the tuberculosis was confined to the adrenals.

Thyroid tuberculosis was reported to be a rare disease (Clark 1915 and Knottch 1944). In the present series thyroid tuberculosis was seen in 4.2% of cases, all of whom had disseminated tuberculosis.

The oesophagus is rarely affected and so far no case was reported in the literature. 2.9% of our autopsies showed oesophageal tuberculosis.

Breast tuberculosis appears to be rare, both in autopsies and biopsies. One case of breast tuberculosis in a Sudanese woman was reported before (Archibald 1922); the lesions were confined to the breast and regional lymph nodes. We found a frequency of 1.2% in the present study of biopsies.

From our results and hospital records, both pulmonary and non-pulmonary tuberculosis seems to be prevalent throughout the country, but the degree of prevalence could not be accurately assessed due to the fact that the hospital records are not accurate.
enough. Further most of these post mortems are usually performed on medicolegal and unclaimed bodies, and are not therefore truly representative of the hospital population.

It is obvious from these results that tuberculosis in the Sudan is frequently an acute severe generalised disease. Possible factors that may account for this are several and include continuous lack of exposure to the tubercle bacilli in isolated communities, malnutrition and poor hygiene, failure to report to hospital early in the course of the disease and depressed immune response in the host.

2. Pathology of Animal Tuberculosis

Limited studies were made on the pathology of animal tuberculosis in Africa including the Sudan. As mentioned in the introduction tuberculosis was noted in cattle from Tamanyika (Cornell 1934) and Uganda (Cormichael 1930). In both countries the infection of Zebu cattle is of the non-progressive type. On the other hand in animal cattle generalised tuberculosis was more frequent. In agreement with previous findings the present study shows that the most common form encountered (MG) was the primary
pulmonary focus with the involvement of the mediastinal and/or bronchial lymph nodes (primary complex). Pulmonary tuberculosis with dissemination was less frequently seen occurring in 10% of cases. Even with dissemination abdominal organs were rarely affected though the abdominal lymph nodes were involved in 50% of cases. Chronic pulmonary tuberculosis and cavitation is not seen. Extra-pulmonary tuberculosis was rarely seen (10%) and was confined to the lymph nodes. The most common lesions were caseous nodules. It appears that, the most common route of infection is the respiratory route. In contrast to humans, abdominal organs and lymph nodes were rarely affected. A more detailed investigation is needed before any definite conclusions regarding the pathology can be drawn.

As far as the incidence of tuberculosis in different parts of the country is concerned, returns from veterinarians can not furnish an accurate information about the incidence of the disease since it is difficult, if not impossible, to differentiate between tuberculosis and bovine fever or myocarditis (caused by Bacoloid or Actinomycosis bovineus) except by laboratory investigation.
which is not practised in a battery. Furthermore there is an anti-social selection through which an unknown number of tuberculous animals are scattered.

It seems that tuberculin testing is the only reliable method available in this country for the detection of tuberculosis in cattle. Testing with tuberculin, however, has not been carried out systematically. From the tuberculin testing carried out previously (Bennon, 1955, Annual Report Sudan Veterinary Services, 1955 - 1956, Annual Report Sudan Veterinary Services, 1956 - 1957, Annual Report Sudan Veterinary Services, 1957 - 1958) and our tuberculin testing, it appears that the incidence of the disease is low in the North and comparatively high in the South. This may be accounted for by environmental factors and husbandry methods as crowded sheltering of animals favour the spread of the disease in the South. Tuberculosis among nomadic cattle is rare (Nurul 1962). Nevertheless the situation can not be fully evaluated unless tuberculin testing is carried more systematically and on a wide scale.

The danger of animal tuberculosis as a public health
hazard is greatly reduced in urban areas because of the modern cheese production, proper pasteurization, and regular tuberculin testing of cattle supplying milk to these areas. In rural communities, large numbers of people eat raw meat (barsa) mainly of sheep origin. Fortunately, tuberculosis in this species is not known in this country. However, infection through drinking infected raw cow milk can occur, but it seems that bovine infection in humans is not that common.

2. Bacteriology

Human material

Information on the types of mycobacteria causing tuberculosis, particularly extra-pulmonary tuberculosis in the Sudan is still limited.

In the present study, it has been shown that only 4 specimens from patients with bone and joint specimens failed to grow on Lowenstein-Jensen medium. This may be due to the effect of anti-tuberculous drugs which were administered a month before surgical intervention. This shows that Rutell's method (LJ medium
hydroxide) is a good method for digestion and decontamination, which agrees with the findings of previous workers, Thomas et al. (1961), Grande (1967) and Cavanagh (1968).

LJ medium without starch is regarded to be the most reliable medium for the isolation of the tubercle bacillus (Hitchison et al. 1960, Sula et al. 1960, Bojáll, 1961, Thomas et al. 1961, Kubica et al. 1963, Grande, 1967, Vylkov et al. 1967, Sula, 1968 and Steward et al. 1970). In the present study LJ medium with starch was used and was found to be equally reliable. Cavanagh and Kayes (1968) used egg medium containing pyruvic acid neutralized with sodium hydroxide with potato starch but without asparagin for the isolation of the Subtubercous strains. Then our strain was mailed to Prague, 9 i.e. 47.2% could not be maintained on subculture. It is possible that these were adversely affected by transport (Sula et al. 1960).

In the present study it has been shown that 10.4%, 18.3% of the strains isolated were resistant to one or two drugs. Earlier Cavanagh (1965) found that primary and secondary drug resistance in Subtubercous strains were in 22% and 68% respectively, while Grande (1967) reported primary drug resistance in 16% and secondary drug resistance in 72% of the strains isolated from Subtubercous
patients. However our findings could not be compared with those of
Cavanagh and Grant for two reasons. First the number of the
strains tested in our case were small in comparison to those re-
results. Secondly history of drug administration in cases other than
surgical cases was not available and perhaps many did not receive
any treatment. Only one strain isolated from bone, proved to have
secondary drug resistance to EN and ES.

It is noted that out of 11 strains isolated from bone
and joints 10 were sensitive to three drugs (EN, ES, SM). This
shows that in some cases, sensitive strains could be isolated even
after one month administration of drugs.

Judging by the limited material studied it appears that
infection of humans by bovine mycobacteria is not a major problem.
Only the bovine strains were isolated out of 53 strains classified.
This is the second report for the isolation of bovine strain from
human material in the country, Karib (1958) isolated the first
bovine strain from a Sudanese patient. Khan (1970) also stated
to have isolated the first case. It seems that only one bovine
strain was in fact isolated from humans before, but it was reported
twice (Carl 1962 and Khan 1970). Our bovine strains were isolated from a post-mortem case via, appendix B90/6 and pulmonary ones.

All 17 cases of extra-pulmonary tuberculosis in the bigay material were due to Mycobacterium tuberculosis. This is in agreement with the results obtained by Bula et al., (1950) who did not report bovine infection in the 41 strains isolated from cervical glands of East African patients. All were Mycobacterium tuberculosis resembling those isolated from Europeans. They thought that this lymph node involvement was a result of haematogenous and lymphogenous dissemination from lesions in the lungs or hilar lymph nodes. This is supported by absence of primary lesions on the tonsils and by the high proportion of pulmonary tuberculosis in this group. However our patients did not show hilar lymph nodes or pulmonary lesions. Our findings are also in agreement with the findings of Henk (1933) in East Africa where extra pulmonary tuberculosis was frequently seen but was almost entirely due to human infection.

The material presented in this study, though small, is perhaps striking in that no atypical strains was isolated. Cavanagh
reported the isolation of one anonymous mycobacterium out of 322 strains isolated from Sudanese patients and Grant (1967) isolated three anonymous strains from Sudanese patients out of 693 strains isolated. In Nigeria Bees and Davies (1965) had isolated 170 anonymous mycobacterium out of 2652 strains isolated.

This study shows that all mycobacterium strains tested (human and bovine) were of normal virulence for guinea pigs. For example strain No. 31 (Table 4) produced generalized lesions in the spleen, liver and lungs. In contrast the strains isolated from South Indian patients were of low virulence to guinea pigs. (Mitchison et al., 1962) and some strains isolated from Japanese patients were of moderate or low virulence to guinea pigs (Iwanz et al., 1963).

Animal materials

17.12. (34%) of the specimens did not grow on Lowenstein-Jensen medium (in contrast to the specimens of human origin), although all of them were positive for acid-fast bacilli by direct smear and all of them histologically showed a reaction indistinguishable from that of tuberculosis. There are at least
two possible explanations for this. First it may be due to the adverse effect of 4% sodium hydroxide on the viability of the organism. Secondly Loevenstein Jensen medium may not be the best medium for the growth of these organisms.

Bovine strains seem to be affected more adversely by transport than the human strains as 42.4% could not be maintained in subculture as against 14.2% for human strains.

All isolated mycobacteria were classified as bovine strains. All of these tested were virulent for guinea pigs and rabbits. One strain (V2a) showed eucinic rough colonies, resembling mycobacterium tuberculosis (Plgs. 15 and 14). However it produced progressive fatal disease in rabbits and guinea pigs and biochemically was a typical bovine strain. This strain was isolated from a slaughterhouse from an ox killed with generalized disease. Strain morphologically similar to this was reported by Jamal (1970) who isolated it from mediastinal lymph nodes of a bullock slaughtered in Dharmashala. This strain caused progressive fatal disease in rabbits but not guinea pigs.

Avian mycobacteria was claimed (Annual Report of the Swiss...
Veterinary Services 1964) to have been found in a dark, but this claim was not substantiated, as no attempts were made to isolate the organism.

The results obtained by the method of Zykov et al. (1967) El-Feky's modification (1968) for catalase test and simultaneous catalase peroxidase test (Hui and Konjarova 1963) were the same, although the latter method is easier. Moreover both catalase and peroxidase could be carried out in one test. Comparing El-Feky's modification (1968) to the original method of Zykov et al. (1967), the former is less expensive as loops made from Pasteur pipettes is used instead of a platinum loop which is used only once. Its disadvantage lies in the difficulty of counting Syngonic smooth colonies.

_Syngonic Smooth_

During our study it was observed that some of the Sudanese bovine strains that were cultured in bovine serum agar and kept in the refrigerator for 25 to 30 days or more displayed spontaneous lyses of colonies, a phenomenon which was never encountered in fresh cultures. Lysogenicity in _Syngonic_ was
reported before by Russel et al. (1960), Bult and Sulava (1969) detected sharply cut lined vacuoles resembling phage lytic plaques in thin section of mycobacterium bovis (Argentin strain No. 247).

Ultra-violet irradiation did not induce the liberation of any phage, although successful induction was observed by Bumaasvaha et al. (1969), who were able to detect 5 mycoplanes from lysogenic strains (2 avian, 2 succohromogen and one none pilmented atypical strain).

Mycoplasma were isolated by a very simple method without previous exposure to ultra violet irradiation, physical or chemical agents. This agrees with the finding of Sula and Sulava (1970), who were able, for the first time, to isolate mycoplasma from lysogenic bovine strain (Argentin strain No. 247).

The present study has shown that no substantial differences were detected between phase (NYC/63) and phase Ag 1 as both are active against rapidly growing strains, slow bovine strains, ECG bovine strain of different geographical origin, laboratory human strain (137 KY) and Sudanese human mycobacteria. A phase of similar lytic pattern was reported by Brodie et al. (1969).
Phages L5 and X64 isolated from zoological King Lyne Laboratory human strain and some wild human strains, Redmond et al. (1969) also found that phages B9-5, 60-1 and 26-1 produce lysins of European, African, Asian and Latin American human mycobacteria regardless of the geographical source of origin.

Unfortunately, none of these discovered phage proved to be highly specific for human or bovine strains. On the other hand, phages CR L-3 and CR L-10 isolated from soil and stool of tuberculous patients, were claimed to be highly specific phages which could differentiate human from bovine mycobacteria (Mabie, 1959).

There is no information in the literature which will permit a complete comparison with the present results. It is of interest to note that mycobacterial BCG/69 isolated from bovine strain (mycobacterial smooth colonies) of human origin and mycobacterial 724 isolated from bovine strain (mycobacterial rough colonies) of animal origin differ markedly in their lytic pattern. A further point of interest is that phage 724, unlike phage BCG/69, does not lyse the strain from which it was originally isolated.
The polyvalence of phage B50/60 may render it unsuitable
for phage classification. Few numbers of bovine strains were sub-
jected to the action of plaque because the growth of bovine strain
was very sparse even on Richard medium. Some questions remain to be
answered. What is the effect of storage on the bovine strain?

Does storage act as an inducing agent for lysogenic bovine strain?

There is no apparent explanation for this phenomenon and further
investigation will have to be carried out to reach an adequate
explanation. He ever Sula (1971) formulated a hypothesis stating
that B50 strains was originally an atypical lysogenic bovine
strain which had lost its polyvalence due to successive passage
carried out since 1966 on bole potato agar. This loss has been
accompanied by modification of its growth character from lysogenic
type of growth to lysogenic one together with the attenuation of its
virulence. Could the new hypothesis explain the similar phenomenon
observed with the lysogenic bovine strain (Sula sees. 1971)?

This hypothesis needs further substantiation.
SUMMARY

In this study it was shown that tuberculosis of the lymph node, bone and joint are the most common form of tuberculosis seen in biopsies.

Human autopsy material revealed a rarity of chronic pulmonary tuberculosis. Primary infection in adult occurs most often in the lungs and intestine and frequently proceeds to generalized progressive fatal type; it is characterized by nodular tuberculosis in different organs particularly the liver and spleen. Abdominal tuberculosis was more common in man than in cattle and was mainly due to Mycobacterium tuberculosis. The usual feature of tuberculosis in cattle was the respiratory type affecting the lungs and corresponding lymph nodes.

A major feature of tuberculosis in the Southerners was the lack of fibrous around pulmonary cavities. This may be due to lowered resistance in the Southerners as compared to the Northerners.

There was little evidence of bovine infection to human in
the Sudan as only two bovine strains were isolated out of 53
strains typed. Strains recovered from cattle were mycobacterium
bovis, except one that showed atypical colony morphology. Some of
the human strains were resistant to one or two drugs, while all
mycobacterium bovis strains except one were sensitive to the three
drugs (isoniazid, para-aminosalicylic acid and streptomycin).

Ultraviolet irradiation failed to induce the liberation
of mycophage from lysogenic bovine strains. Mycophages were
successfully isolated from lysogenic bovine and murine strains
without previous exposure to ultraviolet irradiation, physical or
chemical agents. The mycophage H39/69 isolated from bovine strains
of human origin differ greatly from that isolated from bovine
strain (V2L) of animal origin but resemble mycophage AS1.
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APPENDIX

TM20/65

Southern male 28 years

Clinical history

He was brought to hospital with a history of diarrhoea. Intestinal hemorrhage was suspected but examination of stools was negative for bilharzia.

Postmortem report

The body was that of a poorly nourished man. There was calcified primary complex in the left lower lobe and regional lymph node. Cervical and mainly abdominal lymph nodes were markedly enlarged, the largest measured about 6 x 4 x 4 cm. The parenchyma was soft, with numerous irregular shaped yellow areas surrounded by pink tissue and here and there with white stripes. The liver showed several small miliary tubercles and a few areas of caseous necrosis surrounded by fibrous tissue.

Histology

The spleen showed slight degree of fibrosis and occasionally minute foci of recent coagulation.
B.41/55

Northern male 40 years.

Clinical Diagnosis

Hepa spleno magnally, bilirrasisis, x-rays bilateral miliary maving.

Postmortem Report

The body was that of emaciated man. The lung showed bilateral numerous miliary tubercles, no shufi focus was found. The megrinal lymph nodes showed focal anthraxosis and sell areas of caseous necrosis. Calcification was not seen. The spleen was very enlarged, congested and showed yellow miliary follicles and few hemorrhages on the cut surface. All abdominal lymph nodes were enlarged up to 4 cm. in diameter. In mesenteric and pelvic lymph nodes there were large areas of yellow caseous necrosis. In the cervical lymph nodes apart from areas of caseous necrosis there were numerous follicle of pin head size. The liver was enlarged and firm in consistency with numerous fibrous stripes and multiple grey and yellowish nodules of pin head size. Kidneys were congested with few miliary tubercles.
Histological Report

Cervical, thoracic and mainly abdominal lymph nodes were enlarged and showed a picture of tuberculous lymphadenitis. In some of the mesenteric lymph nodes there were several bilharzial ova of schistosome haematobium type. Bilharzial ova was found in the liver, miliary tubercles were found in nearly all examined organs, kidneys, myocardium and thyroid.

R110/66

Male Jinka about 40 - 50 years old

Clinical history

The patient was admitted to the hospital very ill with high temperature and diarrhoea. Liver biopsy was performed, bilharzial ova and granuloma was found. Bilharzial ova was seen in the stool.

Postmortem report

There were few miliary tubercles in the thickened leptomeninges of chiasmatic system. Plexus chorioidus was swollen, hyperemic with numerous miliary tubercles. Lungs were enlarged, edematous with numerous miliary tubercles in all lobes. Primary complex

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was not detected and no old fibro-caseous lesions were found in the lung tissue. Thoracic and mainly abdominal lymph nodes were enlarged, soft with small areas of yellow caseous necrosis. There were numerous peritoneal adhesions among loops of the intestine. In the liver tissue there were small areas of caseous necrosis with softening. Small and large intestine were without ulcers. Left adrenal was enlarged with irregular swollen areas of caseous necrosis.

**Histology**

Tuberculosis of the cervical, abdominal and thoracic lymph nodes. Liver hilar abscess and tuberculosis were seen. Spleen showed massive miliary tuberculosis with irregular areas of necrosis.

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**Hist/67**

Male Northern 55 years of age

**Clinical history**

No history of pulmonary tuberculosis. Pain in the back, swelling in the lumber and in the right loin region. X-ray showed pott's disease with destruction of 6th cervical vertebra.

164/-
Postmortem Report

There were mililiary tubercles in the plexus chorioides and caseous destruction of the D6 and C7 vertebrae. There was a large cold abscess along the right side of the spine and around right paraspinal muscle, penetrating under the ligamentum inquidale. There were massive mililiary dissemination and a few fibrous caseous nodules in the upper lobes of the lungs. Primary complex was not found. Thoracic lymph nodes showed a few fibrous caseous nodules. Cervical and abdominal lymph nodes were only a little enlarged with few mililiary tubercles on the cut surfaces. Liver showed a few caseous nodules and multiple small mililiary tubercles. Few mililiary tubercles were seen in the kidneys.

Histology

Amyloidosis in the abdominal lymph nodes. Liver showed moderate degree of amyloidosis, Spleen showed follicular amyloidosis and dispersed mililiary tubercles. Few bilateral granulomas were seen in the large intestine. Culture yielded a growth of tubercle bacilli of human type.

169/-
Postmortem Report

Intra-pulmonary lymph nodes were enlarged with areas of recent caseation. Primary complex was not found. Cervical and intrathoracic lymph nodes were moderately enlarged, soft with yellow areas of caseation. Mesenteric and mainly pericolic abdominal lymph nodes were huge forming large masses around the aorta (10 x 6 x 6 cms.). On the cut surface there were large areas of caseation with recent softening. Spleen showed several nodes of caseation 1 - 2 cms. in diameter. The liver was deep brown in colour, with several subcapsular nodes of caseation 1 - 3 cms. in diameter.

Histology

Bone marrow showed small areas of necrosis and calcification and destroyed bone trabeculae. The kidney had amyloidosis of the wall of arterioles and isolated ossuous necromas. Tubercle bacilli of human type was isolated.
Male southern (Maur) 35 years old.

**Clinical History**

The patient was admitted to hospital in semi-coma. He was jaundiced and his urine contains bile. He died one day after.

**Postmortem Report**

Bilateral multiple mililiary tubercles of the lungs. The mediastinal lymph nodes showed very early focal caseation. Liver showed mililiary tubercles 1 - 2 cm. in diameter under the capsule and on the cut surface. The lumen of the common bile duct was almost completely occluded by an enlarged lymph node (3 cm. in its greatest diameter) which showed early focal caseous necrosis. The peritoneum covering the under surface of the diaphragm showed a few mililiary tubercles.

The spleen showed mililiary tubercles, in addition there were caseous nodules in the middle of the spleen (4 - 6 cm. in diameter). The lymph nodes around the coeliac axis and porta hepatis were enlarged (2 - 3 cm. in diameter) soft and showed local early caseous necrosis. The paraortic lymph nodes were slightly enlarged.
(up to 1.5 cm). Bilateral miliary tubercles of the kidneys. The left adrenal showed confluent tubercles filling the medulla. Mycobacterium tuberculosis was isolated.

R23/62
Male Northern from Yrd Ibadan 30 years of age

Clinical History

Bloody diarrhoea for 5 months, swelling of testicles. Suspected pulmonary tuberculosis? Bilharziosis? T.B epididymitis? He died two days after.

Postmortem Report

Caseous mediastinal lymph node was adherent to the pericardium. Heart was dilated and right auricle was penetrated by lymph nodes. There was caseous necrosis of the pole of suprarenal. The left atrium was perforated by caseous mediastinal lymph node. The pleura was thickened. The lungs were enlarged and containing numerous confluent miliary tubercles in all lobes surrounded by coagulated lung tissue. No primary complex, fibrosis or cavitation were seen. Spleen was congested and containing small irregular...
areas of caseation and dispersed miliary tubercles. Caseous nodules were seen in the stomach. Liver showed several large caseous nodules and one large tuberculous abscess 5 cm. in diameter. There were acute ulcers in the duodenum. All lymph nodes were enlarged soft with large areas of caseation. Kidneys were swollen and containing nodules 1 - 2 cm. Large caseous areas were detected in the prostate. Both testicles and epididymis were enlarged with large areas of caseation. Caseous tuberculosis of ductus deferens and vesiculae on both sides. There were two caseous nodules in the isthmus of thyroid.

**Histology**

Confluent miliary caseous pneumonia in the lung. Cervical thoracic and abdominal lymph nodes were tuberculous. Biliharziasis of the urinary bladder, a weld of the kidney. The exsenterate was soft with miliary tubercules and in one place Biliharzial ova was seen in a center of caseous necrosis. Culture yielded *lycobacterium tuberculosi*. 

17/
Clinical History

She was found dead by police and brought to hospital.

Post-mortem Report

The liver showed that looked like grossly abscesses centered irregularly in the parenchyma. The greenish pus they contain was positive for acid and alcohol fast bacilli. They measured between 1 - 2 cm, in diameter and some were surrounded by dark brown parenchyma that simulated Zahn's infarcta. The pancreas contained caseous materials. The paraaortic lymph nodes and those around the coeliac axis were enlarged and caseous. They measured between 1 - 3 cm, in diameter. Lymph nodes at the porta-hepatis were also caseous.

Mycobacterium tuberculosis was isolated.

Clinical History

The patient was found semi-conscious by police and was brought to hospital.
hospital. The patient stayed for a few hours only, deteriorated rather rapidly and died.

Postmortem Report

The body was that of a very emaciated and anaemic with generalised lymph nodes enlargement. The amentus and mesentary showed numerous small whitish nodules. There was generalised lymph adenopathy, every group of lymph nodes being involved. Some lymph nodes were soft and caseous, while most lymph nodes were firm and fibrous with some yellowish streaks. The mesenteric and para aortic lymph nodes were very much enlarged producing abdominal distension.

Tuberculous epididymo orchitis of the right side. The lower dorsal vertebrae showed greenish pus formation. The thyroid showed small caseous nodules.

Neobacterium tuberculosis was isolated.

8/9/49

Male Southerner 55 years of age

Clinical History

The patient was admitted to hospital with the history of diarrhoea
and oedema of the lower limbs of unknown duration. The diagnosis of malnutrition and acute dysentery was made.

Postmortem Report

The lungs were edematous and under the pleura there were a few scattered miliary tubercles. The heart was small and both layers of pericardium were covered with scattered miliary tubercles. The lymph nodes along the thoracic duct were enlarged up to 3 cm in their greatest diameter and caseous. The hilar and both supra-clavicular lymph nodes were enlarged and caseous. The mucosa of the small intestine was covered by a few scattered nodules (3 mm in diameter). The outer surface of the intestine showed scattered tubercles unrelated to the mucosal nodules. Large intestine showed oedema of mucosa and scattered shallow ulcers. The peritoneal cavity contained about two litres of straw coloured fluid. The peritoneum contained scattered tubercles. The para-aortic lymph nodes were enlarged, caseous and united together forming a mass measuring 5 x 3 x 2 cm. The celiac group of nodes were also enlarged and caseous forming a mass measuring 4 x 3 x 3 cm. Spleen showed caseous nodules.

Mycobacterium tuberculosis was isolated.
Male Northern 35 years old

Clinical History

No history was available.

Postmortem Report

Bilateral hilar tubercles were seen in the lungs. The mediastinal lymph nodes were enlarged but not caseated. The esophagus showed large caseous nodules in its wall, but no mucosal ulceration.

The liver and spleen were studied with hilar tubercles and showed numerous large caseous nodules. Mesenteric lymph nodes were greatly enlarged, caseated and matted together. Ulcers were not detected in both small and large intestine. The left kidney showed a tiny tuberculous focus in the cortex.

Nocardia asteroides was isolated.

Male southern (Nolokai) 35 years old

Clinical History

The patient was referred to Khartoum Hospital with the following complaint.
- Abdominal pain
- Fever (maximally)
- Cough with hemicrystal and chest pain
- Loss of weight and anorexia
- Poor appetite

**Provisional diagnosis of**
- Abscess liver abscesses
- Pulmonary Kosh and hydatid disease were put forward

**Investigation**
- SG 8 gm., 54%
- ESR 112 cm./hour
- Mantoux test: negative

Investigation showed a negative mantoux and the liver soon showed that there were areas of liver not taking the isotope. The diagnosis of aseptic liver abscess was put forward and treatment with emetine started but the patient did not show much improvement. Tuberculosis was suspected when a cervical gland biopsy was taken and found to be abscesses. Anti-tuberculous treatment was started immediately after the biopsy was taken but unfortunately the patient died on the 2nd day of the start of the specific treatment.
The autopsyed body was very emaciated and anaemic but not jaundiced. His cervical lymph nodes were palpable. Bilateral milky tuberculosis of the lungs. The oesophagus showed large multiple oesoeus nodules in its wall. There were no unusual ulceration and the oesoeus areas were not connected to any oesoeus centre outside the oesophagus. The wall of the stomach showed multiple discreet tubercules at the lesser curvature. The large intestine showed multiple irregular ulcers in its entire length extending down to the rectum. The liver surface showed very large multiple yellow nodules. The cut surface showed multiple areas of congestion with sheathing in the centre of some oesoeus nodules giving an unlicated appearance. The intervening areas of the liver were studied with milky pin-point tubercules. The left kidney showed large areas of oesoeus necrosis. The cervical, mediastinal, lummary, portal, mesenteric, psoa aertic and pelvic lymph nodes were enlarged and oesoeus and united together. The spleen showed milky tubercules.

*Kyobacterium tuberculosis* was isolated.
Nelso Soutermont 21 years of age.

Clinical History:

No history was available.

Postmortem Report

The observed body was that of a very thin emaciated and anaemic
but not emaciated. Both lungs were peppered with nodules of miliary
tuberculosis. There was no large coalescing centre in either lung.
The surface of the heart showed miliary tuberculosis. The liver
showed large multiple caseous nodules and in between small nodules
of miliary tuberculosis. The cut surface of the nodules showed me-
ble staining in the centres. The large intestine showed scattered
nodules in the wall but the mucosa was intact. Multiple small
nodules on the great omentum. The mesenteric lymph nodes were
moderately enlarged and their cut surface were fleshy. A few para-
enteric lymph nodes were coalesced. The spleen cut surface showed
multiple areas of caseation (1 - 2 cm.) The kidney cut surface
showed multiple caseous nodules 1 - 2 in each. The 2nd, lumbar
vertebrae was affected by caseation and there was small peri-vertebra-
al abscess which dissected upwards to thoracic but did not pant.

Mycobacterium tuberculosis was isolated.