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TUBERCULOSIS IN THE CATTLE

Health and welfare of man is closely associated with those of the cattle, more so in a predominantly agrarian state like India where, besides milk supply, cattle is used extensively for agriculture, transport etc. The contact between man and cattle can also be very intimate. In many places they share the same shelter during winter and rains. This increases the chance of spread of communicable diseases from one to the other. The problem of tuberculosis arises in all these contexts, but mainly from milk supply.

In a number of developed countries like U. K. and others about 80 per cent of non-pulmonary tuberculosis in children was caused by bovine bacillus. This was due to contamination of milk from udder tuberculosis of the cattle. Slaughter and segregation of tuberculin reactor cattle eradicated the danger.

In India no case pulmonary or non-pulmonary, has yet been reported which is due to infection by bovine bacillus. This may be due to the habit of drinking boiled milk. Besides, *tuberculous mastitis* in cattle appears to be very rare, as compared to that in the developed countries. A total of about one and half a dozen cases have so far been reported mostly from the Indian Council of Agriculture Research Scheme (Annual Report 1941-42). Anatomy of cattle here and abroad can not differ. The cause for such low prevalence of *tuberculous mastitis* in India remains unknown.

Studies on *tuberculous infection* in cattle have not been extensive enough. Besides a few reports from individual workers there had been one planned survey as reported by Lall et al in their working paper at the National Seminar on Zoonoses in India (Transaction, National Instt. of Communicable Diseases, 1968). The rates of infection recorded in and around four cities were: Punjab—20.4%, Bombay—16.7% Madras—2.1% and Bihar—2.0%. Also the rates of infection in the herds of some organised Farms varied from 0 to 55%. Such a wide difference in the infection rate is unexplainable unless the groups tested were highly selected, specially in relation to the extent of contact with open cases of tuberculosis in the herds. No such information has, however, been made available.

Determination of *true rate of infection* by M. Tuberculosis is difficult, as the reaction to the test has to be differentiated from the false or non-specific one caused by other bacteria, specially the anonymous mycobacteria. Attempts should also be made to distinguish infection by bovine bacillus from that caused by human and avian strains. For this purpose WHO (Joint WHO/FAO Expert Committee Report on Zoonoses. Tech. Rep. Ser., 1967, 378, p. 44) recommends use of standardised test with tuberculin derived from

different types of bacilli mentioned above with a view to draw inferences by comparing qualitative and quantitative reactions. The Report endorses the method used in Great Britain (Paterson et al, J. Hyg., 56, 1, 1958) and suggested **P.P.D** as the tuberculin of choice for all the strains.

Studies on *tuberculous Disease* in cattle in India are not only few, but even the diagnostic criteria used can not be entirely relied upon. The study of Madras Veterinary College published in this issue of the journal appears to be most valuable. The investigation includes histological but not bacteriological examination of 874 consecutive autopsies. The rate of disease detected was about 4.0 per cent. It is, however, difficult to reconcile this rate with the 2.1% infection rate detected in the same area unless the group studied was highly selected in some way.

Positive bacteriological finding is the only sure evidence of tuberculosis. Information in this regard is quite inadequate. Results of two studies only are mentionable. Hundred percent of the 40 cultures made by the Indian Veterinary Research Institute proved the causative organism to be bovine bacillus, whereas 27 percent of 11 cultures studied by the Madras Veterinary College proved to be so and 73 per cent were detected to be of human type. Such a large difference can not be explained on the ground of rural and urban habitation of the herds. The finding of Madras study may be regarded very important on an least two counts. It challenges the current concept that human bacilli can not cause progressive disease in the cattle and that man can not transmit disease to the cattle.

Clearly, more authentic information obtained through epidemiological studies is needed to design a suitable control programme for cattle. As large scale slaughter or even segregation of positive reactors can not be undertaken under Indian conditions the programme should be phased and initially limited to practicability. In this light it is suggested that the infected cattle be segregated and watched carefully without interrupting milk supply from them. On manifestation of disease the cattle must be slaughtered. This programme, in the first phase, should be made applicable only in large organised Farms which supply milk products. Chemoprophylaxis as suggested by WHO should be considered later.

In a programme like this, Veterinary, Agricultural and medical sciences should be equally interested and jointly plan and execute a proper study in suitably located centres in India. Indian Council of Medical Research has already to its credit, the conduct of some valuable co-operative studies. It may take the initiative in this important study also.

BOVINE TUBERCULOSIS DUE TO THE HUMAN STRAIN OF MYCOBACTERIUM TUBERCULOSIS

K. P. CHANDRASEKHARAN AND R. RAMAKRISHNAN
(From Madras Veterinary College, Madras)

An extensive survey on bovine tuberculosis done on the basis of tuberculin testing during the years 1950-51, under the auspices of the Indian Council of Agricultural Research, in and around the cities of Lahore, Ahmedabad, Madras and Patna revealed an incidence of 20.4%, 16.7%, 2.1% and 2.0% among 12,079, 25,142, 27,428 and 20,071 animals tested respectively.

Though there are several reports on tuberculosis in bovines from this country, very scanty information is available regarding the type of the organism involved. Iyer (1946) and Nagarajan (1948) were the early workers to study this aspect of the problem. In a recent report, Lall (1968) stated that all the 40 cultures isolated from cattle and buffaloes during 1942-67 were of the bovine type. The purpose of the present study is to determine the type of organism involved in cases of bovine tuberculosis that were encountered and to discuss this finding from the stand point of epidemiology and control.

Materials and Methods

Tuberculosis of bovines, which died or destroyed in the college clinic, furnished the materials for the present study. Smears were prepared from the gross lesions and examined by Ziehl Neelsen's method. Materials were also collected in 10% formalin for histopathological study. Relevant specimens were forwarded to the Tuberculosis Chemotherapy Centre, Madras for isolation of *Mycobacterium Tuberculosis* and typing. The criteria stated to have been employed for typing the organism were :

- (a) Niacin production.
- (b) Catalase production.
- (c) Sensitivity to PAS, streptomycin and Isoniazid.

Results

The autopsy incidence of tuberculosis among bovines and the distribution of lesions in the different cases along with the typing result where available are set out in Tables I and II.

TABLE I

Year	Total number of bovine autopsies	No. of cases of tuberculosis in bovines encountered
1960	63	2
1961	84	3
1962	63	9
1963	72	11
1964	79	1
1965	91	0
1966	114	1
1967	152	4
1968	156	4
	874	35

Gross appearance

In all cases, except A. 236/63 and A.331/63, the lesions were quite extensive involving various organs and lymph nodes. They ranged from early caseation to extensive cases calcareous alteration in the lymph nodes. Extensive breakdown of the tissues was also noted in some cases e.g., A. 1175/67, A. 1426/67 and A. 1459/67. Even in the 2 cases A. 236/63 and A. 331/63, where lesions were confined to the lymph nodes of mesentery and the mediastinum the alterations were quite advanced as to result in caseation.

The typing results available for 11 materials revealed 8 of them to be of the human strain and 3 only of the bovine strain of *Mycobacterium tuberculosis*.

Discussion

During the 9-year period 1960-68, 35 cases of tuberculosis were seen amongst 874 bovines autopsied. This indicates that the disease is existing in a form sufficiently important to be of public health hazard.

Results of typing were available in respect of 11 cases only. Out of these, the bovine

TABLE II
Showing distribution and the nature of lesion due to human type of *Mycobacterium tuberculosis*

S. No.	Autopsy Number	Description of the animal	Nature of lesions and organs involved	Typing Results	Remarks
1.	A. 834/62	Guernsey Heifer 3 years.	Granulomatous areas in costal pleura; Multiple caseating nodules (3 to 4 mm size) in both lungs; Mediastinal and mesenteric lymph nodes enlarged, the latter showing cases of calcareous areas; Supramammary lymph nodes enlarged with pale yellowish areas.	Human type	Died
2.	A. 236/63	Sahival Cross bull, adult	Caseating foci in mesenteric lymph nodes.	Human type	Destroyed
3.	A. 327/67	Kangeyam Bullock	Calcified and caseous foci in both lungs and mediastinal lymph nodes; Flesh coloured nodules in spleen. Pearl lesions on the pleura; Flesh coloured areas in hepatic lymph node.	Human type	Destroyed
4.	A. 331/63	Kangeyam Bullock	Caseo Calcaerous nodule in middle mediastinal lymph node.	Human type	Destroyed
5.	A. 87/67	Non-descript cow, 5 years	Caseation and calcification of right retro pharyngeal lymph nodes; middle and posterior mediastinal lymph nodes very much enlarged, caseated and calcified; Visceral pleura showed diffuse velvety areas; pea-sized greyish white gritty nodules in both lungs.	Human type	Died
6.	A. 1175/67	Cross-breed cow	Extensive caseo calcareous areas in both lungs; enlargement and calcification of mediastinal lymph nodes; A small calcified nodule and a caseating area 5 cm. in diameter in the liver with calcified areas in hepatic lymph nodes; calcified and caseous area about size of a hen's egg in the anterior pole in both kidneys.	Human type	Destroyed
7.	A. 1426/67	Cross-breed bull-calf 6 months (son of item 8)	Multiple yellowish caseous areas from a pinhead to a marble in size in both lungs; mediastinal submaxillary retro-pharyngeal lymph nodes enlarged and caseated. Mesentery thickened and mesenteric lymph nodes caseated; Two millet sized foci of caseation in the liver; mucosa of jejunum and ileum showed minute white spots.	Human type	Died
8.	A. 1459/67	Non-descript cow 6 years (Dam of the calf A. 1426/67)	Numerous caseating foci from a millet to a marble in size in the lungs; caseation and calcification of the right retro-pharyngeal, mediastinal and mesenteric lymph nodes ulceration of intestinal mucosa; caseo-calcareous foci about 2 cm. in diameter in the kidneys; Endometrium studded with numerous nodules upto 2 cm. in diameter.	Human type	Destroyed

strain was found responsible for the disease in 3 cases and the human strain in the remaining 8 cases. This is a very unexpected finding, for the rarity of bovine infection by the human strain in tuberculosis have been commented upon by several workers. Besides, the non-progressive nature of the lesion in the bovine due to the human type has also been

stressed in literature. Stableforth and Galloway (1959) state that the human and avian type show little capacity to infect ox, goat, cat, horse, rabbit and vole. The W.H.O. in their report (1959) states that infection with human strain does not cause progressive lesions in cattle but often gives rise to a marked tuberculin reaction. Rich (1951) is also of the

opinion the human type tubercle bacillus has a distinctly much less range of pathogenicity than the bovine and avian types—cattle, sheep, goats, rabbits and birds except parrots and canaries being highly resistant. Heaf (1957) recorded the isolation of human tubercle bacillus from naturally infected cattle but only as producing slight lesions and important from the point of sensitising to tuberculosis.

However, Hillermark (1946) in Stockholm, isolated the human type of tubercle bacilli from more than 12 cattle. Although in 7 animals (5 cows and 2 calves) no microscopic lesions were seen, the organisms were recovered by animal inoculation and culture in 5 cases from lung and local lymph node, in one, from the mandibular and parotid lymph node, and in the other, from the mesenteric and mediastinal lymph nodes. In 5 of the cases, the source of the infection was traced to attendants with open tuberculosis. In his opinion, systematic typing of bacilli from local lesions in calves in areas where human TB is common, might show a higher incidence of bovine infection with the human tubercle bacilli than hitherto suspected.

From a perusal of the distribution of the lesion, it is seen that in 6 out of the 8 cases, the lesions were extensive, involving more than one organ and lymph node. Even in the 2 cases, where the lesions were seen in the mesenteric and mediastinal lymph nodes respectively, the disease process had extended to caseation and calcification. Histopathologically, the lesions were those of typical tubercles in all the cases. It was also seen in one case (S. No. 8) that the lesions were of a progressive type with extensive caseation, necrosis and the relative absence of giant cells. In the calf (S. No. 7) again, the lesions were extensive and involved the retropharyngeal lymph node, liver, the latter location a feature of congenital tuberculosis (Thornton). From these observations it is reasonable to conclude that the human type in the series of cases included here caused typical tuberculosis most of which was clinically detectable. This view appears to have been not fully appreciated by majority of workers cited above except Hillermark.

One probable reason for isolation of the large number of human type of bacilli in the present series was that, the study was conducted in an urban area, where there is very close, almost intimate, contact between man and the animals he keeps. It becomes quite possible in such a state of intimacy for the bovines to be repeatedly exposed to the infection from diseased human beings. Animals in the rural

areas however spend a greater part of their time away from human habitation and this may explain the relative scarcity of tuberculosis due to the human type in animals kept under such environment.

It seems therefore reasonable to conclude that under conditions of urbanisation, chances of association between infected human beings and bovine population exist and the possibility of the human type of tuberculosis infection in bovines is rather great.

Summary

During the 9 year period 1960-68, 35 cases of tuberculosis were observed among 874 consecutive bovines autopsied, and this gives an incidence of 4%. Typing of the strain of organism was done in 11 cases. The human strain was found responsible for 8 cases and the bovine strain in the remaining instances. The human type was found to cause extensive lesions in 6 cases and in the other 2, which were destroyed, the lesions were confined to the lymph nodes. The public health significance of isolation of human type of *Mycobacterium tuberculosis* in bovines, most of which were open cases, is discussed.

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SOME OBSERVATIONS ON ATYPICAL MYCOBACTERIA FROM TUBERCULOUS MENINGITIS IN CHILDREN

Part I : Isolation, Cultural, Biochemical and Biological Studies.

K. N. JOISHY AND M. V. SANT
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Among atypical mycobacteria, rapid growers draw a special attention due to the increased variation exhibited by them. These mycobacteria are highly pleomorphic in nature and have been isolated from both human and other extraneous sources. Several cases of infection of both pulmonary and extra-pulmonary origin were reported to be caused by these entities (2, 10, 13, 24). *Mycobacterium fortuitum*, the only known pathogen of this group (Runyon Group IV) was described by Costa cruz in 1938 (5). Besides this, Group IV also consists of other rapid growers of subgroups, A, B, and C, and many saprophytic mycobacteria. This classification is based mainly on their susceptibility to various dyes incorporated in the Yeast Extract Glycerol Agar (17).

Although the Group IV rapid growers were labelled as causative agents of various diseases of human origin, the reports on their isolation from the cases of tuberculous meningitis in children are rare in the literature. However, the incidence of the cases of tuberculous meningitis due to atypical mycobacteria, especially Group I photochromogen could be traced in the literature. Wood *et al.* (31) reported a case of 34-year-old white female with tuberculous meningitis caused by photochromogens. Later, Chan and Pathmanathen (3) also reported a photochromogen to be the aetiologic agent in a case of tuberculous meningitis in 9-month-old female child. Recently, Huempfer (15) isolated both *Mycobacterium tuberculosis* and *Mycobacterium kansasii* from cerebro-spinal-fluid and meninges respectively, from a 2-year-old female with tuberculous meningitis.

In the present study, cerebro-spinal-fluid (C.S.F.) samples collected from a total of 100 cases of tuberculous meningitis in children, were subjected to the bacteriological investigations and 12 strains of rapid growers (Runyon Group IV, here after referred to as C.S.F. isolated) were isolated on culture. All cases investigated were confirmed on clinical grounds and biochemical investigations of C.S.F. samples to be tuberculous meningitis. Unfortunately, the detailed proper species identification of these strains could not be carried out due to the variation in microbiological characteristics exhibited by them.

A comparative study with *Mycobacterium phlei* and with a reference strain of rapid growers (Group IV, T-64), which was kindly supplied by Dr. Runyon, Utha, U.S.A., was carried out. *Mycobacterium phlei* strain was locally isolated and identified in the laboratory. The observations made with these strains are presented here as also in a subsequent paper (31a). This paper deals with the isolation, cultural, biochemical and biological studies.

Materials and Methods

Cerebro-spinal-fluid samples were collected by lumbar puncture (2 to 5 c.c. of sample from each patient) in sterile test tubes. The samples were concentrated by Wayne's Zephiran Concentration Technic (30). Before culturing the concentrated sediment on Lowenstein Jensen Egg medium, acid-fast staining was also carried out. Two sets of media were inoculated with the sediment. One set was incubated at room temperature (28-37°C. and the other at 37°C. Weekly observations were made of the inoculated slants for the presence or absence of growth.

Twelve strains of mycobacteria were isolated, which grew after ten days at room temperature (28-30°C) and seven days of incubation at 37° C on primary isolation. The cultures were examined by gram staining, acid-fast staining and acid-alcohol fast staining.

Cultural characteristics of the isolates were studied on both solid and in liquid media. A loopful (3mm inner diameter) of culture was plated on Lowenstein-Jensen Egg medium, Glycerol agar, Soil extract agar, Nutrient agar and Blood agar. All the inoculated media were incubated at 37° C for 2-4 days and the growth characteristics were noted down.

One tenth ml. of suspension of cultures in sterile normal saline was inoculated into Youmans' liquid medium (9), Dubos Tween 80 broth (7), Glycerol broth, Nutrient broth, Sautons' liquid medium (26), Kirchners' liquid medium (20), and Long and Seiberts' protein-free synthetic medium (22). The growth characteristics were noted down after 2-4 days incubation at 37°C.

The colony characteristics of the strains on Corn Meal Agar were studied as described by Kubica and Jones (21). The results are presented in table 1.

The growth characteristics at different temperatures *viz.*, 4°C, 28-30°C (R.T.), 37°C, and 42° C were studied on L-J egg medium. The presence or absence of growth was recorded after 2-4 days incubation at these temperatures. The results are presented in table 2.

Cord test was done in Dubos Tween Albumin broth by the method of Middlebrook *et al* (23). The results are presented in table 3.

Biochemical studies :

Isolates were subjected to a series of biochemical tests as follows :

Catalase activity (4), niacin test (4), neutral red test (8), nitrate reduction test (29), Arylsulphatase activity (4), urease activity (27), and Tween 80 degradation test (4). The results are presented in table 4.

Carbohydrate fermentation test : The ability of these strains to ferment carbohydrates was studied in Ayer's medium (1) incorporated with 1% glucose, 1% mannose, 1 % mannitol, 1% arabinose, 1 % rhamnose and 1 % inositol as the sources of carbohydrates. The method was described by Gordon and Smith (12). The results are presented in table 5.

Utilisation of sodium nitrate and ammonium sulphate as the sole sources of nitrogen : The organisms were tested for their ability to utilise the above nitrogen sources in Sauton agar containing 0.17% sodium nitrate and 0.264% ammonium sulphate. The method has been described elsewhere by Tsukamura (28). The results were recorded after four weeks at 37° C. The results are presented in table 6.

Dye-susceptibility test: This was carried out in Yeast Extract Glycerol Agar, the respective dye being added to 0.01% concentration (w/v). The dyes used in the test were malachite green (MG), eosin yellow (EY) and pyronin B (PYRB). The method was described by Jones and Kubica (17). The observations were made after 5 and 11 days incubation at 37°C for the presence or absence of growth with any notable change in the colour of the medium. The results are presented in table 7.

MacConkey's agar test : This test was carried out as described by Jones and Kubica (18). The results were recorded on 5th, 10th,

15th and 20th day of incubation at 37°C on the basis of growth or no growth. Any notable change in the colour of the medium was also noted down. The results are presented in table 8.

Results

Morphologically the C.S.F. isolates were longer and nicely beaded than the Group IV rapid grower (T-64). They were bacilli having the dimensions of 3-6;μ X 1.5-2μ, and the long filamentation forms of 90[μ length were also observed. Most of them stained faintly by gram staining and were gram positive bacilli. They were both acid and acid-alcohol fast and exhibited pleomorphism, including a tendency to branch a characteristic feature of Actinomycetes.

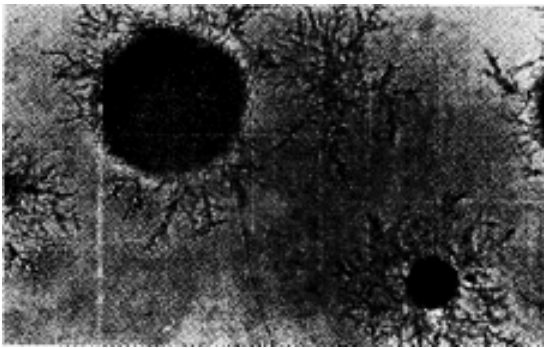
Cultural characteristics of these strains on both solid and in liquid media differed to some extent from that of reference Group IV (T-64) strain. The reference strain (T-64) grew luxuriantly and profusely showing smooth to rough variation (S-R), accompanied by typical rhizoid formation and beige colour on most of the solid media, *viz.*, L-J medium, glycerol agar and Soil extract agar. But, on nutrient agar and blood agar the growth was comparatively poor and no rhizoid formation was observed. A characteristic patchy growth was observed on nutrient agar. The C.S.F. isolates showed typical creamish white luxuriant growth with characteristic frog egg like colonies. The colonies were pearl like, round, raised, convex with entire margin. They were easy to suspend and gave turbid suspension in normal physiological saline. With the exception of one strain, which exhibited both chromogenic and non-chromogenic variation, no special growth characteristics were observed with these isolates on solid media. The organisms grew richly on soil extract agar. The growth characteristics on nutrient agar were similar to that of reference group IV strain (T-64) on this medium. On blood agar the growth was very poor.

Both the reference group IV strain (T-64) and C.S.F. isolates formed thick, wrinkled, greyish white surface pellicle in Youman's liquid medium, Sautons' liquid medium, Glycerol broth, Kirchners' liquid medium and Long and Seibert's liquid medium. A creamish white to yellow coloured, membranous surface pellicle was also observed with few strains. These strains changed the colour of the medium from light yellow to golden yellow when grown in Sautons' broth, Kirchners' medium and Long and Seiberts' protein free synthetic

medium. In nutrient broth the growth was very poor.

Mycobacterium tuberculosis H₃₇R_V and *Mycobacterium phlei* exhibited typical morphological characteristics as described by many workers and hence not dealt with in detail here

As regards the colony characteristics on corn meal agar, both rapid growers and *Mycobacterium phlei* exhibited characteristic colonies on this medium. *Mycobacterium tuberculosis* failed to grow on corn meal agar. *Mycobacterium phlei* exhibited smooth 'T' type of colonies with slightly opaque centre and transparent granular, and undulant periphery. Out of 12 C.S.F. isolates, only four strains exhibited colony characteristic compatible with that exhibited by the reference strain of rapid grower (T-64). The colonies were smooth 'FP' type with opaque centre and filamentous periphery (photograph 1). No prominent growth was observed with rest of the strains on this medium (table 1).



From table 2, it is clear that *Mycobacterium phlei* did exhibit typical serpentine cords in which the bacilli were bundled up in parallel position. The cords were thick and long (Photograph 2). However, with the exception of a few C.S.F. isolates, no strains formed cords in Dubos Tween Albumin Broth. No cord formation was observed with the reference Group IV strain (T-64) (table 2).



Good and luxuriant growth was observed at 28°C, 37°C, and 42°C with C.S.F. isolates. However, at 4°C only 2 strains grew, of which one absorbed the dye malachite green from the medium. *Mycobacterium tuberculosis* failed to grow at both 4°C and 28°C, whereas, good growth was observed at 37°C. At 42°C, the growth was very poor and hence was not taken into consideration. *Mycobacterium phlei* grew rapidly and luxuriantly at 28°C and 37°C. Fairly good growth was observed at 42°C but the same failed to grow at 4°C. The reference strain (T-64) exhibited growth pattern comparable to that of C.S.F. isolates (table 3).

TABLE 1
Colony characteristics of Mycobacteria on corn meal agar.

Strains	Colony characteristics on corn meal agar
<i>M. tuberculosis</i> H ₃₇ R _v	No growth on the medium
<i>M. phlei</i>	Smooth 'P' type colonies with slightly opaque centre with transparent, granular, undulant periphery.
Reference Group IV rapid grower (T-64)	Smooth 'FP' type coloniss with opaque centre and filamentous periphery.
C.S.F. isolates	Smooth 'Ff type colonies with opaque centre and filamentous periphery. (4 strains) No prominent growth on the medium. (8 strains)

Only *Mycobacterium phlei* and atypical mycobacteria (C.S.F. isolates) gave positive catalase test at 68°C (pH 7.0), whereas *Mycobacterium tuberculosis* produced catalase only at room temperature (28-30°C). Cerebrospinal-fluid isolates exhibited increased catalase activity both at R.T. and 68° C, the activity being 10-30 mm. foam column. This was not the case with *Mycobacterium phlei* and the reference Group IV strain (T-64). The catalase activity of these two strains fluctuated from 5 mm and 10-20 mm foam column respectively. *Mycobacterium tuberculosis* produced niacin. Two strains out of 12 C.S.F.

isolates were weakly positive by niacin test, whereas, the reference Group IV strain (T-64) was negative. Both neutral red test and urease activity varied considerably with the present isolates. Nitrate reduction test was positive (3 to 4) with *Mycobacterium tuberculosis*, *Mycobacterium phlei* and* reference strain of Group IV (T-64). However the test was found less significant with the present isolates since only two strains were weakly positive by this test. Arylsulphatase activity (both 3 days and 2 weeks) which is supposed to be the characteristic property of group IV atypical mycobacteria was not shown by most of the

TABLE 2

Cord test of Mycobacteria.

Strains	Cord formation in Dubos Tween Albumin broth.
M. tuberculosis H ₃₇ R _v	Thick, typical, long serpentine cords in which bacilli were arranged parallelly.
M. phlei	Thick, typical cords with bacilli arranged parallelly.
Reference Group IV strain (T-64)	No cords were formed
C.S.F. Isolates	Loose atypical cords (2 strains). No cords formed (10 strains).

TABLE 3

Growth characteristics of Mycobacteria at different temperatures

Strains	Temperatures				
	tested	4°C	28-30°C	37°C	42°C
M. tuberculosis H ₃₇ R _v	no growth	no growth	good growth	good growth	no growth
M. phlei	poor growth	poor growth	good growth	good growth	fairly good growth
Reference Gr. IV strain (T-64)	poor growth	poor growth	good growth	good growth	fairly good growth
C.S.F. Isolates	no growth (10) poor growth (2) absorbed dye(1)	no growth	good growth(12)	good growth(12)	fairly good growth (12)

isolates. Only 3 isolates were found to show weak positive activity by this test. Most of the C.S.F. isolates were positive in Tween 80 degradation test but the time elapsed to exhibit the activity varied with these strains (table 4).

Consistently varying results were obtained in carbohydrate fermentation tests with our strains. Most of the C.S.F. isolates failed to ferment glucose, dextrose, mannose, mannitol, arabinose, rhamnose and inositol. Those strains which have fermented some of these carbohydrates shared the properties of both *Mycobacterium phlei* and reference strain of rapid grower (T-64) in their fermentation reaction. Two strains out of 12 fermented arabinose which has also been fermented by the reference strain (T-64). Mannose and manitol were found to be the differential sugars for *Mycobacterium tuberculosis* and other mycobacteria viz., *Mycobacterium phlei*, reference strain of rapid grower (T-64) and some of the C.S.F. isolates. Arabinose could differentiate *Mycobacterium phlei* from reference strain of Group IV (T-64). Rhamnose and inositol were found to be of less value in the differentiation (table 5).

Mycobacterium phlei, the reference strain of Group IV (T-64) and C.S.F. isolates utilised both sodium nitrate and ammonium sulphate as sole sources of nitrogen in Sauton's Agar. However, *Mycobacterium tuberculosis* failed to utilise sodium nitrate, but, the same strain could utilise to some extent, ammonium sulphate as a sole source of nitrogen in Sauton's Agar (table 6).

Mycobacterium tuberculosis showed partial sensitivity to malachite green and eosin yellow and complete sensitivity to pyronin B in Yeast Extract Glycerol Agar. A characteristic absorption of green colour of malachite green by the colonies of *Mycobacterium tuberculosis* was observed in this medium *Mycobacterium phlei* showed luxuriant growth with discolouration of the medium in malachite green agar and to some extent pyronin B agar after 11 days incubation at 37°C. It was resistant to all the three dyes used in the test. The colonies of *Mycobacterium phlei* also absorbed green colour of malachite green in the medium (MGYEGA). The reference strain of Group IV (T-64) has also exhibited resistance to all the three dyes. This strain can be differentiated from *Mycobacterium phlei* from its growth characteristics in Malachite Green Yeast Extract Glycerol Agar (MGYEGA) and in Eosin Yellow Yeast Extract Glycerol Agar (EYYEGA). The growth of this strain was poorer than that of *Mycobacterium phlei* on these medium.

Unlike *Mycobacterium phlei*, it brought about the discolouration of the medium and absorbed the rosy red colour in EYYEGA. Out of 12 C.S.F. isolates, only 8 were susceptible to malachite green and eosin yellow. Some of them were susceptible to pyronin B too. However, as regards their growth characteristics in these media, they shared the properties of both *Mycobacterium phlei* and the reference Group IV strain (T-64) with comparatively poor growth in these media (table 7).

Mycobacterium tuberculosis failed to grow on MacConkey's agar, whereas, *Mycobacterium phlei* showed rich growth without causing the clearance in the medium. The clearance in the medium (from red colour to yellow colour) was observed only with reference Group IV strain (T-64). Only two C.S.F. isolates exhibited this characteristic whereby they agreed well with characteristics of the reference Group IV strain (T-64). Ten strains of C.S.F. isolates failed to grow in this medium even after 20 days at 37°C (table 8).

Discussion

Although an attempt was made to study the C.S.F. isolates with a view to identify and classify them, the results were not much convincing due to the increased variation in various microbiological properties, exhibited by these strains. Morphologically, the bacilli were longer than both *Mycobacterium phlei* and the reference Group IV strain (T-64). These organisms retained acid-fastness with typical beaded appearance. The most striking characteristic of these strains to form long typical filaments gave rise to a suspicion of them being akin to *Nocardia* or other Actinomycetes. But, unlike *Nocardia* and other Actinomycetes, these organisms were strongly acid fast and no alternate acid-fast and non-acid-fast state was observed. Hence the suspicion of belonging to *Nocardia* or other Actinomycetes species could be ruled out. This was further substantiated from the results of slide culture and paraffin utilisation (not shown here).

Majority of the C.S.F. isolates differed from both *Mycobacterium phlei* and the reference strain of Group IV (T-64) in their growth characteristics on both solid and in liquid media. The typical frog egg shaped or pearl like colonies were observed on solid media with these strains. A few of them showed the rhizoid formation, the topical characteristic of reference Group IV strain (T-64). A mixed growth of both chromogenic and non-chromogenic variety was observed with one strain. However, these varieties were later separated in pure cultures.

TABLE 4
Cytochemical and Biochemical Reactions of mycobacteria

Strains	Number Tested	Catalase activity		Gradation	Niacin Test	Neutral red Test	Nitrate Reduction Test	Urease Test	Arylsulphatase activity		Tween 80 Degradation Test
		R.T.	68°C						3D	2Wks.	
<i>M. tuberculosis</i> H ₃₇ R _v	1	+	—	5mm.	+	+	4+	+(4 hrs.)	—	—	—
<i>M. phlei</i>	1	+	+	5mm.	—	—	3+	+(48 hrs)	—	—	+(5 days)
Reference Group IV strain (T-64)	1	+	+	10-12mm.	—	—	4+	+(72 hrs)	+++	+++	+(5 days)
C.S.F. Isolates	12	+	+	10-30mm. 30mm.	±(2) —(10)	+(1) W.P.(5) —(6)	+(2) W.P.(2) —(8)	+(1) (24hrs) +(1) (5days) —(10)	+(3) —(9)	+(3) —(9)	+(5) (5 days) +(5) (5-10 days) +(1) (10-15 days) —(1)

* Catalase activity was graded on the basis of the height of the foam column produced after the addition of Tween 80—hydrogenperoxide mixture.

W.P.—Weekly Positive.

TABLE 5

Carbohydrate fermentation test of Mycobacteria

Strains	Number tested	Carbohydrates used in the test.						
		Gl.	Dxt.	Man.	Mannitl.	Arab.	Rh.	Inostl.
<i>M. tuberculosis</i> H ₃₇ R _v	1	+	+	—	—	+	—	—
<i>M. phlei</i>	1	+	+	±	+	±	—	—
Reference Group IV (T-64)	1	+	—	+	±	—	—	—
C.S.F. Isolates	12	+(5) -(7)	+(5) -(7)	+(4) -(8)	+(1) ±(2) -(9)	+(2) -(10)	—	—

Gl: Dxt: Glucose + Positive reaction
 Man: Dextrose ± Variable reaction
 Mannitl: Mannose — Negative reaction
 Arb: Mannitol
 Rh: Arabinose
 Inostl: Rhamnose
 Inositol

TABLE 6

Utilisation of Sodium nitrate and Ammonium sulphate by Mycobacteria as sources of nitrogen.

Strains	Growth on Sodium nitrate Sauton Agar	Growth on Ammonium sulphate Sauton Agar
<i>M. tuberculosis</i> H ₃₇ R _v	Very poor	++
<i>M. phlei</i>	+++	+++
Reference Group IV (T-64)	+++	+++
C.S.F. Isolates	++ to +++	+++

++Moderately good growth.
 +++Luxuriant growth.

The C.S.F. isolates were fastidious on some media, whereas, their less fastidious nature on nutrient agar was an interesting observation. On nutrient agar, they grew with patchy colonies. Some of them also grew with greyish white scattered colonies on this medium. The colonies had undulant margin. Similar type of observation has been reported by Hirano and Sushida (16), who grew *Mycobacterium tuberculosis* on nutrient agar. Rich growth was observed with most of the C.S.F. isolates on soil extract agar, with the exception of a few strains which failed to grow well on this

medium, the reason being unknown. An explanation of their natural habitat in the soil might have helped them to grow luxuriantly on this medium. Cerebro-spinal-fluid isolates failed to grow on blood agar, whereas, the reference strain of Group IV (T-64) grew with characteristic greyish white smooth and rough colonies.

In liquid media too, a diverse growth characteristic was observed with C.S.F. isolates. Some grew with thick surface pellicle formation, whereas, some showed very thin mem-

TABLE 7
Dye susceptibility test of *Mycobacteria*

Strains	Growth on Yeast Extract Glycerol Agar Containing					
	Malachite Green		Eosin Yellow		Pyronin B	
	5 days	11 days	5 days	11 days	5 days	11 days
<i>M. tuberculosis</i> H ₃₇ Rv	1+ (no discolouration)	1+ (no discolouration. Colonies have taken up slight green colour.)	1+ (no discolouration. Colonies have taken up slight red colour.)	1+ (no discolouration.)	—	—
<i>M. phlei</i>	3+ (discolouration of the medium. Colonies have taken up slight green colour.)	4+ (discolouration of the medium. Colonies have taken up slight green colour.)	4+ (no discolouration. No colour was taken up by the colonies.)	4+ (no discolouration. No colour was taken up by the colonies.)	1+ (slight discolouration. No colour was taken up by the colonies.)	2+ (discolouration of the medium. No colour was taken up by the colonies.)
Reference Group IV (T-64) strain.	2+ (Slight discolouration. No colour was taken up by the colonies.)	2+ (Slight discolouration. No colour was taken up by the colonies.)	3+ (no discolouration was taken up by the colonies.)	4+ (discolouration of the medium. Rosy red colonies.)	3+ (discolouration slight colour was taken up by the colonies.)	3+ (partial discolouration. Peripheral growth has taken up the colour.)
C.S.F. Isolates	1+ (8) slight 2+ (4) discol. (Slight colour was taken up by the colonies.)	1+ (8) slight 3+ (4) discol. (Slight colour was taken up by the colonies.)	2-3+ (2)-(10) Discolour and pink colonies (2) No discol. (10).	2-3+ (2)-(10) Discolour and pink colonies (2). No discolour (10).	2-3+ (no discol. Slight colour was taken up by the colonies.)	3+ (partial discolouration. Peripheral growth has taken up the colour.)

1+ little growth.
2+ Moderate growth.
3+ Luxuriant growth.

TABLE 8
MacConkey's Agar Test of Mycobacteria

Strains tested	Number	MacConkey's Agar Test							
		5 Days		10 Days		15 Days		20 Days	
		Growth	Clearance	Growth	Clearance	Growth	Clearance	Growth	Clearance
M. tuberculosis H ₃₇ Rv I		-	--	-	-	-	-	-	-
M. phlei	1	++	—	++	—	++	—	+++	—
Reference Group IV Strain (T-64)	1	+++	+	+++	+	+++	+	+++	+
C.S.F. Isolates	12	+++(2)	+(2)	+++(2)	+(2)	+++(2)	+(2)	+++(2)	+(2) -
		(10)	-(10)	-(10)	-(10)	-(10)	-(10)	—(10)	—(10)

+ Little growth
 ++ Moderate growth
 +++ Luxuriant growth
 + Clearance in the medium
 — no clearance in the medium

branous pellicle. Hence, liquid media seem to be of less value in the differentiation of these strains from saprophytic mycobacteria.

Some of the C.S.F. isolates exhibited a marginal tendency to form loose cords in Dubos Tween Albumin Broth. However, *Mycobacterium phlei* formed typical serpentine cords comparable to that of *Mycobacterium tuberculosis*. Hence, the correlation between cord test and virulence may not help much since both *Mycobacterium phlei* and C.S.F. isolates were later found to be non-pathogenic to laboratory animals. The reference Group IV strain (T-64) did not form cords.

Cerebro-spinal-fluid isolates grew at a wide range of temperature i.e. 4°C to 42°C. However, 28-37°C was found to be the optimal temperature for the luxuriant growth of these strains. Not much difference was observed between C.S.F. isolates and a reference Group IV strain (T-64) as regards to their growth at various temperatures. When kept at 4°C, some of the isolates absorbed, malachite green incorporated in L-J egg medium. This did not bring about any drastic change in the medium. A similar observation has been reported by Darzins (6) and Hartwig et al (14). According to them, the dye absorbing property at refrigeration temperature (4°C) is characteristic

of *Mycobacterium fortuitum*. But Rodda (25) stated that the phenomenon is not related to the temperature of incubation and the same is difficult to produce at will. We presume that the absorption of dye at this temperature might have something to do with the respiration of the organism and hence the process might act as a defensive mechanism. *Mycobacterium tuberculosis* grew only at 37°C, which is the optimal temperature for this organism.

Corn meal agar was found to be the best medium for the differentiation of typical mycobacteria from other mycobacteria on the one hand and Group IV atypical mycobacteria from saprophytes on the other. *Mycobacterium tuberculosis* failed to grow on this medium. Since Group IV atypical mycobacteria grew with characteristic colonies with filamentous periphery, we feel that this property could be effectively used in the laboratory, in the differentiation of these strains from saprophytes in general. However, all the strains of C.S.F. isolates did not agree with this property and the only possible reason might be their fastidious nature as regards to nutritional requirements. According to Kubica and Jones (21), the colonial morphology on corn meal agar depends upon various factors such as ingredient of the medium, moisture and oxygen tension. The failure of both *Mycobacterium tuberculosis*

and *Mycobacterium bovis* to grow on corn meal agar was also reported by Fregnan and Smith (11).

Cerebro-spinal-fluid isolates exhibited hypercatalase activity at 68°C (pH 7.0). The catalase activity at this temperature is the characteristic property of atypical mycobacteria in general and Group III and Group IV mycobacteria in particular. The test has been used by many workers in the primary differentiation of atypical strains from typical ones. With respect to this particular characteristic, C.S.F. isolates very well agreed with reference Group IV strain (T-64). The hypercatalase activity of these strains might be due to the presence of the enzyme catalase which is more thermostable than that present in typical mycobacteria.

Some of the test strains also initiated *Mycobacterium tuberculosis* in niacin and nitrate reduction test. Urease activity did not help much in the differentiation of C.S.F. isolates from others. Arylsulphatase activity which is supposed to be the characteristic property of Group IV rapid growers was shown by only a few C.S.F. isolates. Those which produced arylsulphatase after 3 days and 2 weeks, exhibited comparatively less degree of activity.

All the C.S.F. isolates showed wide range of Tween 80 degradation activity. However, they took a pretty long time to produce the enzyme. *Mycobacterium tuberculosis* gave the negative test. Hence the only characteristic biochemical reactions shown by C.S.F. isolates were catalase activity at 68°C (pH 7.0) and Tween 80 degradation test.

Cerebro-spinal-fluid isolates shared the properties of both *Mycobacterium tuberculosis* and *Mycobacterium phlei* in their ability to ferment various carbohydrates. Sugars such as mannose and mannitol differentiated typical mycobacteria from both saprophytic and atypical mycobacteria. Most of the C.S.F. isolates did not ferment sugars and this might again be due to their fastidious nature to grow in Ayer's medium which is not an enriched one. Rhamnose and inositol have much less value in the test.

Almost all C.S.F. isolates utilised both sodium nitrate and ammonium sulphate as the sole sources of nitrogen. This property can be very well used in the laboratory for the differentiation of typical mycobacteria from atypical strains, since the former failed to utilise sodium nitrate as the sole source of nitrogen. However, typical mycobacteria did utilise ammonium sulphate (to some extent) as the sole source of

nitrogen. Not much difference was observed between *Mycobacterium phlei*, C.S.F. isolates and reference Group IV strain (T-64) as regards this property.

Mycobacterium tuberculosis was susceptible to all the dyes, whereas, *Mycobacterium phlei* and reference strain of Group IV (T-64) were resistance to them. *Mycobacterium phlei* can be differentiated from the rapid grower by their growth characteristics on EYYEGA and PYBYEGA. Most of the C.S.F. isolates were susceptible to malachite green and eosin yellow. Few of them also shared the properties of both *Mycobacterium phlei* and the reference strain of Group IV (T-64). When compared with the dye susceptibility pattern of mycobacteria as reported by Jones and Kubica (19) the present isolates appear to belong to subgroup C. This requires further scrutiny since the dye methylvoilet B has not been used in the test. Very few strains also showed the pattern akin to that of *Mycobacterium fortuitum*. This has to be confirmed further.

MacConkey's agar test was found to be helpful only in the differentiation of *Mycobacterium tuberculosis* from other mycobacteria. The test did not give encouraging results with present isolates.

Thus, the overall observations showed that the C.S.F. isolates cannot be boldly classified in Group IV a typical mycobacteria, except for their properties to grow rapidly at 30°C and hypercatalase activity. These strains grew within 7-10 days on primary isolation. At the same time, they cannot be called saprophytes too since they did not agree well with the saprophytes in most of the tests. Hence, they form a sort of biological link between typical mycobacteria and saprophyte on the one hand and saprophytic mycobacteria and Group IV a typical mycobacteria on the other. This can be more precisely explained on the basis of genetics and variation in micro-organisms.

Summary

About 12 strains of rapidly growing (Group IV) typical mycobacteria were isolated from a total of 100 cases of tuberculous meningitis in children, investigated. The strains were subjected to various studies such as morphological, biochemical, and biological tests with a view to identify and classify them. A comparative study with *Mycobacterium tuberculosis*, *Mycobacterium phlei* and a reference strain of Group IV (T-64) atypical mycobacteria was also carried out. It was found to be very difficult to classify these strains either with

Group IV rapid grower or with saprophytic and typical mycobacteria. They formed a biological link between all these strains. The only stable characteristics exhibited by these strains are their rapid growth at 37°C and hypercatalase activity at 68°C (pH. 7.0). These are the typical characteristics of Group IV rapid growers.

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SOME OBSERVATIONS ON ATYPICAL MYCOBACTERIA FROM TUBERCULOUS MENINGITIS IN CHILDREN

Part II: Studies on Drug sensitivity, Animal pathogenicity and Serological inter-relationship

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Introduction

Group IV rapidly growing atypical mycobacteria have been labelled as aetiological agents of various diseases in human beings (1, 3, 5, 6, 8). However, their isolations from the cases of tuberculous meningitis are rarely reported in the literature. The detail introduction of these mycobacteria has already been given in the previous article by the authors (1 la). This paper mainly deals with the observations on drug sensitivity, animal pathogenicity and serological interrelationship of these strains. Twelve strains of rapidly growing atypical mycobacteria were isolated from a total of 100 cases of tuberculous meningitis, in children investigated. The method of isolation, cultural, biochemical and biological characteristics of these strains have been described in the earlier paper by us (1 la).

Materials And Methods

A total of 12 strains of rapid growers were isolated from cerebro-spinal-fluid (C.S.F.) sample collected from children suffering from tuberculous meningitis. The strains (hereafter referred to as C.S.F. isolates) were subjected to the studies as follows :

Drug Sensitivity Test : The strains were tested for their sensitivity to antibiotics like streptomycin (SM) and Kanamycin (KM); drugs like Para-amino-salicylic acid (PAS), Isoniazid (INH), Ethionamide (ETH) and Thiacetazone (THTA) in liquid medium by the method of Doub and Youmans (4). The sterile normal horse serum (without preservatives was added) to the test medium to a final concentration of 10%. All the anti-tuberculous agents were tested in concentrations ranging from 0.1 r to 400 r /ml. The higher concentrations were preferred with a view to assess the ability of these strains to tolerate the drugs. The results are presented in table 1.

Animal Pathogenicity Test: The C.S.F. isolates were tested for their pathogenicity to guinea pigs, mice and hamsters. Guinea pigs : Young, healthy, Haffkine bred male guinea pigs weighing 250 grams approximately were selected for the experiment. Tuberculin test was performed by injecting 0.1 ml. of 1: 20 dilution of old Tuberculin (O.T.) intradermally on a

carefully shaved area on the side of the animal: approximately half way between the axillary and inguinal regions. Tuberculin negative guinea pigs (after 48 hrs. of observation) received a dose of 2-3 ml. of the test culture in Dubos Tween Albumin Broth (suspension adjusted to Optical Density (O.D.) 0.3 on a Leitz Photoelectric Colorimeter) intraperitoneally. One guinea pig was inoculated with each strain. A control animal which received the same amount of the Dubos Tween Albumin Broth or sterile normal saline intraperitoneally was also kept in the test. Mice : Young, healthy, three weeks old male albino mice (NTH strain bred in Haffkine Institute) weighing 20-25 grams approximately were selected for the experiment. Two mice were inoculated with each strain. Each mouse received a dose of 1.0 ml. of the test culture in Dubos Tween Albumin Broth (suspension adjusted to the O.D. 0.3 on Leitz Photoelectric Colorimeter) intraperitoneally. A control group of mice received 1.0 ml. of the medium or sterile normal saline intraperitoneally. Hamsters : Syrian Golden Hamsters (*Cricetus auratus*) weighing 20-30 grams approximately were selected for the test. One hamster was inoculated with one strain. Each hamster received a dose of 2 ml. of inoculum (test culture in Dubos Tween Albumin Broth and adjusted to the O D, 0.3 on a Leitz Photoelectric Colorimeter) interaperitoneally. The control animal received the same amount of the medium or sterile normal saline intraperitoneally.

All the inoculated animals were kept under observation for a minimum period of eight weeks before they are sacrificed. At the end of this period, the animals were killed and the internal organs were observed both macroscopically and microscopically for the presence of any pathological changes in the infected organs. A piece of infected tissue was also cultured for acid-fast bacilli. The histopathological examination of the infected tissues was done after staining the same by Hematoxyline-Eosin (HE) method. The results are presented in table 2 (a) and 2 (b).

Serological Studies : Serological interrelationship between typical mycobacteria, the reference Group IV strain (T-64) and the

TABLE I
Drug sensitivity pattern of mycobacteria

Strains	Number tested	Antituberculosis Agents Used In The Test											
		SM		PAS		INH		ETH		THIA		KM	
		SN	RS	SN	RS	SN	RS	SN	RS	SN	RS	SN	RS
M. phlei	1	1(10)	1(1)	0	1(400)	0	1(400)	0	1(100)	0	1(100)	0	1(1)
Reference Group IV Strain (T-64)	1	0	1(400)	0	1(400)	0	1(10)	0	1(100)	0	1(100)	0	1(10)
C.S.F. isolates	12	0	12(400)	0	4(400) 7(100) 1(10)	0	7(100) 3(10) 2(1)	0	1(100) 8(10) 3(1)	0	5(100) 5(10) 2(1)	3(0.1)	1(100) 4(10) 4(1)

The number given in the brackets is the concentration of the antibiotic or drug in micrograms per ml.

SM=Streptomycin; PAS=Para-amino-salicylic acid; INH= Isoniazid;
ETH=Ethionamide; THIA=Thiacetazone; KM=Kanamycin.

TABLE 2 (a)

Animal pathogenicity test of mycobacteria

Strains	Number tested	Route of Inoculation	Animals used in the experiment					
			Pathogenic To			Non Pathogenic To		
			Guinea pig	Mice	Hamster	Guinea pig	Mice	Hamster
M. Phlei	1	I.P.	0	0	0	1	1	1
Reference group IV strains (T-64)	1	I.P.	0	0	0	1	1	1
C.S.F. Isolates	12	I.P.	1*	2	0	11	10	12
* Atypical changes were observed in the tissues of the organs infected with these strains. I.P. Intraperitoneal.								

TABLE 2 (b)

Animal pathogenicity test of mycobacteria

Strains	Number Tested	Route Of Inoculation	Pathogenic To Mice	Non-Pathogenic To Mice
C.S.F. isolates	12	I.C	3*	9
* Atypical changes were observed in the brain tissue. I.C.=Intracerebral				

C.S.F. isolates was studied by Schaefer's Agglutination Method (9). The detailed method of preparation of antigen, antisera and the performance of agglutination test was described precisely by Schaefer in 1965 (9). In the present investigation, the antigens prepared from C.S.F. isolates were tested against the antisera of the strain of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium avis*, *Mycobacterium phlei* and the reference strains of atypical mycobacteria of all the four groups by tube agglutination test. The results are tabulated in table 3.

Results And Discussion

From the results of drug sensitivity pattern of mycobacteria, it is clear that *Mycobacterium phlei* exhibited the resistance to common chemotherapeutic regimen. However, the same

organism was comparatively less resistant to ethionamide (ETH) and thiacezalone (THIA) and more sensitive to kanamycin (KM). The reference strain (T 64) of Group IV atypical mycobacteria exhibited increased resistance to SM and PAS, whereas, the degree of resistance decreased in the order of ETH, THIA, 1NH and KM. The drug sensitivity pattern of C.S.F. isolates varied considerably. All the 12 strains were resistant to SM and a few to PAS also. As regards the other drugs, a considerable variation was encountered with these strains. The increased drug resistance of these strains to SM and PAS might be due to the frequent use of these drugs in the treatment. Since ETH, THIA and KM are not being used frequently in the therapy, a considerably less resistance to these drugs was shown by these strains." Three strains of C.S.F. isolates were sensitive to KM. From the

TABLE 3
Agglutination test of mycobacteria

Strains	Number tested	Antisera used in the test										No. of strains untypable	
		Anti-H37Rv	Anti-Bovis	Anti-Avis	Anti-phlei	Anti-Ph	Anti-Sc	Anti-Bt	Anti-Rg				
M. phlei	1	0	0	0	1:256	0	0	0	0	0	0	1:32	—
Reference Group IV Strain (T-64)	1	1:16	0	0	1:32	0	0	0	0	0	0	1:512	—
C.S.F. Isolates	12	4(1:16)	0	0	0	0	0	0	0	0	0	3(1:32)	5

Ph = Photochromogen (Gr. I)
Sc = Scotochromogen (Gr. II)

Bt = Battey type (Gr. III)
Rg = Rapid grower (Gr. IV)

concentration of drugs like ETH, THIA and KM tolerated by these strains, it is clear that apart from the acquired resistance a natural tendency to resist the drugs exists in mycobacteria in general and atypical mycobacteria in particular. The factor responsible for this natural resistance might lie in the gene fragment of these organisms and the latter might give a clue that the altered genes as a result of mutation might be responsible for the emergence of so called atypical mycobacteria from typical ones. In the present study, the higher concentrations of the antituberculosis agents were preferred in the test with a view to assess the drug tolerance ability of these strains. It was found that most of the isolates tolerated the antituberculous agents in the range of 10 r/ml. to 400 r/ml. This warrants definite objection in the chemotherapy of tuberculosis ; especially that of mycobacteriosis ; if the emergence of drug resistant strains continues in this fashion (table 1).

Table 2 (a and b) gives the results of pathogenicity test of C.S.F. isolates in comparison with that of *Mycobacterium phlei* and the reference strain (T-64) of Group IV. All the strains were tested by intraperitoneal route to evaluate their pathogenicity in guinea pigs, mice and hamsters. Since the isolation of C.S.F. isolates was done from the cerebrospinal-fluid samples collected from tuberculous meningitis cases ; it was felt to test these strains by intracerebral route in mice. From the table 2 (a), it is clear that both *Mycobacterium phlei* and the reference strain (T-64) of Group IV atypical mycobacteria were non-pathogenic to guinea pigs, mice and hamsters. In fact, the test was carried out by inoculating a high dose (2 ml.) of suspension intraperitoneally. In spite of this heavy challenge, the reference strain (T-64) did not reveal any visible changes both macroscopically and microscopically. However, out of 12 C.S.F. isolates inoculated, one was pathogenic to guinea pig and two were pathogenic to mice, whereas the hamsters did not respond well in evaluating their pathogenicity. In the infected tissues of these animals, the changes observed were purely of atypical type. Only large atypical nodule consisting of mononuclear cells and devoid of caseation was observed on histopathological examination. No definite necrosis or giant cell formation was seen. In guinea pigs, only liver was found to be infected, whereas, the other organs revealed normal histological architecture. In mice, intralobular multiple microabscess was observed, which have, however, no relation to the portal radical in the liver. Here too liver was found to be infected. In both these ani-

mals, the strains showed increased affinity for the liver; the reason being unknown. The reisolation of these strains from the infected liver was successful.

Table 2 (b) presents the results of intracerebral inoculation of C.S.F. isolates in mice. Only 3 strains were pathogenic to mice by this route. Here too, atypical changes were observed in the brain tissue. There was exudate in subarchnoid space followed by the aggregation of lymphocytes in grey matter. Although the typical tuberculous changes were not observed in these animals, the smears and cultures from the infected tissues were positive. This definitely indicates that the strains were capable of multiplying and infecting the animals; but due to some unknown factor might have failed to bring about the typical tuberculous changes resulting in the death of the animal.

The smears prepared from exudate also revealed acid fast bacilli and many macrophages. The bacilli were found to be phagocytosed. Steiner (10) succeeded in producing abscess and typical picture of chronic meningitis in dogs by intracerebral inoculation. He too reported that the macrophages were essential reacting cells. Besides this observation the reports on intracerebral inoculations in other animals are very scant in the literature.

The failure of these strains to infect and bring about pathological changes in the laboratory animals may depend upon many factors. The question as to why these strains are unable to produce tuberculous changes in the laboratory animals is worth to discuss. The observation made by the following authors might explain this. Suzuki *et al.*(11) observed the bacteriostatic effect of guinea pig serum on tubercle bacilli in less than 0.1 percent concentration. Nicewicz *et al.* (7) also observed a considerable lag phase of tubercle bacilli in guinea pig tissues. Besides these observations, some workers have reported about the bacteriostatic action of guinea pig organ emulsions on mycobacteria. These observations are as regards to guinea pigs and typical tubercle bacilli. The same may or may not hold good in case of other animals and atypical mycobacteria. The animal species used in the experimental tuberculosis also plays an important role in the evaluation of pathogenic potentiality of mycobacteria. The individual resistance of the animal towards the infection might retrogress the spread of infection. Bloch (2) observed the increased resistance of Haffkine Institute mice to H₃₇Rv infection. When these animals can withstand the infective dose

of H₃₇Rv; it is but natural that they resist the infection due to less pathogenic atypical mycobacteria.

The results of serological inter-relationship between the C.S.F. isolates, the reference strain (T-64) of Group IV atypical mycobacteria and *Mycobacterium phlei* is presented in table 3. The antigens prepared out of these strains were tested against the antisera of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium avis*, *Mycobacterium phlei* and all the four representatives of four groups of atypical mycobacteria viz. Photochromogen (Group I), Scotochromogen (Group II), Battey type (Group III) and Rapid grower (Group IV) by Schaefer's Tube Agglutination Method (9). From the results, it appears that some antigenic relationship exists between *Mycobacterium phlei*, Group IV reference strain (T-64) and a few strains of C.S.F. isolates. However, the reactive titre was comparatively less. It was surprising to note that some C.S.F. isolates did react with anti-H₃₇Rv serum in 1 : 16 dilution. This indicates that some common antigens are shared by *Mycobacterium tuberculosis* H₃₇Rv and C.S.F. isolates. Five strains did not show convincing agglutination and hence were classified as untypable. Thus, throughout the studies; the C.S.F. isolates shared the properties of the three strains viz; *Mycobacterium phlei*, *Mycobacterium tuberculosis* H₃₇Rv and the reference Group IV strain (T-64). They also exhibited many variations in the other properties like cultural, biochemical and biological characteristics (Part I). Hence, these strains can be considered as mutants of one of the above species; possibly of *Mycobacterium tuberculosis*. However, their parent organism of this mutagenic change is difficult to trace out. They seem to be in the half way; in their mutation process and might shift to one variety either on continuous subculturing under laboratory conditions or continuous passage in the susceptible experimental laboratory animal. It is difficult to explain this phenomenon fully at this stage; since the bacterial variation is unavoidable.

Summary and Conclusions

Twelve strains of rapid growers (Runyon Group IV) were isolated from a total of 100 samples of cerebro-spinal-fluid (C.S.F.); collected from the cases of tuberculosis meningitis in children. The drug sensitivity pattern, animal pathogenicity and serological relationship of these strains were studied in comparison with *Mycobacterium phlei* and the reference Group IV strain (T-64) of atypical myco-

bacteria. All the C.S.F. isolates exhibited high resistance of SM and comparatively less resistance to PAS, INH, ETH, THIA and KM. Three strains were sensitive to KM. A few of these isolates brought about atypical changes in organs of guinea pigs and mice. Intracerebral inoculations of these strains also revealed atypical picture in the brain tissue of mice. No typical tuberculosis changes were observed throughout the experiment. Serological interrelationship existed between C.S.F. isolates, *Mycobacterium tuberculosis* H₃₇Rv and the reference strain (T-64) of Group IV atypical mycobacteria. These organisms were found to share some of the common antigens. Thus, the C.S.F. isolates formed a sort of biological link between typical and atypical mycobacteria.

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SEASONAL VARIATION OF A GROUP OF TOXICITY TO THIOACETAZONE*

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Introduction

It has been established by now that combination of INH and thioacetazone is as effective as INH and PAS. The doses have also been standardized (INH 30 mgs, and thioacetazone 150 mgs, per day). As this combination is most economical, less bulky and most convenient for self administration, we get maximum co-operation from the patients and greater therapeutic response during the course of treatment (2, 3, 4, 5, 8 and 11). This combination however has one disadvantage, i.e. higher toxicity (6) though some reports claim low toxicity for this combination in comparison to INH+PAS (5). Toxicity is thus the only handicap in the use of this combination.

This study is planned to draw attention to the incidence of toxicity and its relationship with the seasons and point out some probable association, so that a detailed and thorough study can be carried out in centres with better facilities for research.

Material and Methods

This study is based on all tuberculous patients treated with INH and thioacetazone in 1965-69 in the Mana Camp, situated at a distance of about seven miles from Raipur city (M.P.). All of them were above 12 years of age, both males and females; and included patients treated both in the hospital and at home. They were in various stages of the disease, from advanced to minimal lesions; and bacteriologically, some were sputum positive and some negative. Most of them were refugees from East Pakistan, some were local from the nearby villages. Almost all of them were from low income group with poor nutrition and were living in congested houses with considerable over-crowding.

Climatically, the year in Mana can broadly be divided into three seasons :

1. *Winter Season* : From the month of

In this preliminary report, the author has adduced evidence to suggest some relationship between thioacetazone toxicity and seasonal variations. This apparent association however could be due to some other factor and this needs thorough investigations, before the association can be accepted. The paper has been whetted by the Editors.

November upto the first half of February.

2. *Summer Season* : From the second half of February upto the month of June or July, depending on the onset of monsoon.
3. *Rainy Season* : From June or July upto the end of the October.

Humidity starts diminishing from November and is at a minimum from February to June, till the onset of the monsoon. Temperature rises gradually from the last half of February and reaches its maximum in the month of June or July. When the rains start, there is a sharp fall of temperature and rise of humidity. This persists upto the month of October, when the humidity starts falling gradually and the temperature also starts going down during winter from November upto the first half of February. The temperature ranges from 85°F to 119°F. Exact range of humidity is not known but is guessed from the dryness of the atmosphere, absence of rains, sensible perspiration, dryness of the soil and lowering of the subsoil water level etc. Efforts were made to get exact data regarding temperature and humidity range, but were not successful.

Findings

Toxic reactions to thioacetazone have been studied in all patients put on INH+thioacetazone treatment since May, 1965. Table 1 shows the number of patients under treatment and the number of patients with toxic manifestations in each particular month. It is obvious from the table that the percentage of patients showing toxic reactions to thioacetazone is proportionately higher during the period February to July in all years. There is almost complete absence of toxic reactions from August to January inspite of the number of persons under treatment remaining more or less the same.

In almost all the above cases the drugs were stopped for a few days and then restarted. There was no recurrence of reaction except in seven cases where the combination had to be withdrawn permanently. Percentage of cases of toxicity where thioacetazone had to be permanently withdrawn is 10.29%.

There was some reduction in the occurrence of toxicity in the year 1967-1968 (in 1969 the

TABLE I

Toxicity to Thioacetazone in proportion to the number of persons getting INH Thioacetazone combination per month

Month	19 55	19 56	19 57	19 68	19 69
	Non Toxic	Toxic	Non Toxic	Toxic	Non Toxic
January	Nil	Nil	28	1	96
February	Nil	Nil	28	3	103
March	Nil	Nil	35	4	114
April	Nil	Nil	48	4	121
May	7	1	52	3	98
June	8		66	6	95
July	5	3	69	6	101
August	9	Nil	105	Nil	96
September	⁹	Nil	98	Nil	99
October	8	Nil	92	1	109
November	8	Nil	104	Nil	106
December	23	1	92	1	102
TOTAL	77	11	817	29	1240

* Absence of toxicity which is found in the period of maximum toxicity is due to occasional pre-monsoon rains in those months which occurs in every year.

study is only upto April) which was probably due to a change in the mode of administration of the drugs. In 1965-66 INH and thioacetazone was given in a single dose in the morning whereas in 1967/68 (and also in 1969) it was given in two divided doses after meals. This is in conformity with the experience of others (6, 10).

For the purposes of this study, the year can be divided into two periods :

1. Period of no or minimum toxicity from August to January.

2. Period of maximum toxicity from February to July.

In the first period, temperature is comparatively low and humidity high in August, September and October and low in November, December and January. In the second period, temperature is high and humidity low. It therefore appears that a combination of high temperature and low humidity is associated with toxic reactions to thioacetazone.

Details of the various toxic reactions is shown in Table 2, in relation to seasonal variation in the following groups :—

1. Reactions having relationship with the season.
2. Reactions having no relationship with the season.

There were two cases of intractable vomiting, of seasonal relationship, for which drugs were withdrawn.

Discussion

From the above findings, there appears to be some relationship between thioacetazone toxicity and atmospheric conditions. It has been observed that most of the toxic reactions are gastrointestinal (1,4,7, 9, 10) and maximum number occur in one particular period of the year viz February to July. Factors which may influence the incidence of toxic reactions may be enumerated as follows :

1. Under-nourished condition of the patient leading to low body resistance.
2. Some kind of minor Electrolyte imbalance in the body, due to high excretion of salt and water through perspiration.
3. Low excretion of the drug through the kidney due to increased perspiration.
4. High concentration of the drug in the liver causing derangement of liver function, which is again due to low excretion of the drug through kidney.
5. Mucosal change in the gastrointestinal system making it more vulnerable to the untoward effects of the drug locally.

Atmospheric temperature and humidity in a particular geographical area are beyond our control. Whether and to what extent they are instrumental in influencing toxic reactions

requires careful study ; especially the inter-relationship, if any, of above mentioned factors with atmospheric data. The object of this preliminary report is to bring this interesting observation to the notice of other workers, with more facilities at their disposal, so that the association, if any between thioacetazone toxicity and seasonal variations could be properly investigated.

Summary

Relationship between toxicity to thioacetazone and atmospheric temperature and humidity has been studied from 1965 to 1969. It is found that 89.70% of common toxic reactions to this drug occur from February to July.

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INTRA-INDIVIDUAL & INTER INDIVIDUAL VARIATIONS IN MICROSCOPY OF SPUTUM SMEARS

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Radiology had, till lately, been the prime diagnostic procedure for pulmonary tuberculosis. Sputum was being relegated to a secondary role, either to provide corroborative evidence or to clinch the diagnosis. Positive sputum, however, being the only authentic diagnostic criterion, sputum examination is being increasingly depended upon in case-finding programmes. Further, the national tuberculosis control programme aims at taking case-finding as far to the periphery as possible, since 80% of the total cases in the country live in areas served by peripheral health institutions, where diagnostic facilities so far have been none at all or only rudimentary. Steps are now being taken to provide facilities for examination of sputum smears in all existing health centres. When the programme gets going, a large number of para-medical personnel with varying degrees of training and proficiency will be engaged in microscopy of sputum smears under varying environments. Dependability of the results of microscopy is, therefore, pertinent at this stage.

Intra-individual and inter-individual differences in the reading of X-ray films are well-known and universally recognized. Whether microscopy of sputum is also subject to such variations and if so, to what extent, is yet to be established. With this object, a study was planned in the New Delhi Tuberculosis Centre to determine the intra and inter-individual variations in microscopy of direct smear sputum slides.

Methods and material

The study was started in June, 1968 with all the sputum smear slides prepared in the laboratory of the Centre during routine work on six days. These numbered 185. The sputum slides had been prepared in the usual way and stained by the Ziehl-Neelsen technique using 0.5% methylene blue solution as counter-stain. After routine microscopy, the slides were passed on to the Statistical Section for the purpose of this special study.

For assessment of inter-individual differences, microscopy of all the slides was carried out by five different persons, one after the other. One of them was the bacteriologist, one a senior technician of 14 years' experience and the remaining three were junior

technicians with experience ranging from 3 to 8 years. Each one of them was given 24 stained slides at a time, once a day, and their microscopic examination had to be completed within 2 hours. All slides were numbered randomly by the statistician and the slide numbers were changed after one microscopist had finished all the slides and before the second microscopist started and so on for each microscopist.

For assessment of intra-individual differences, one of the microscopists examined all the slides once again under changed numbers and the results of the two examinations were compared. The interval between the two examinations was more than 2 months.

The microscopists marked the slides as positive if 10 or more than 10 bacilli could be seen in the smear within 5 minutes. The slide was marked as negative if not even 10 bacilli could be seen after 5 minutes' microscopic search.

As the study progressed, it became obvious that there were considerable differences in the results of microscopy by different persons. If, therefore, the results were to be analysed in respect of 'false positives' and 'false negatives' of any microscopist, it would be essential to determine which slide was to be taken ultimately as positive and which negative. Rao (1969) adopted the criterion of positive culture as a determinant of positivity by microscopy. This, however, would appear to be very unsatisfactory since a certain percentage of sputa, definitely positive by direct microscopy, do give a negative culture. Secondly, if the culture is positive it does not necessarily mean that the bacilli in the sputum were more than 10,000 per ml. If the bacillary content of a sputum specimen is less than this number, the smear result would ordinarily be negative. (Holmes Sellers and Livingston, 1952). Further, whereas a large film or follow-up of the patient may prove whether the reading of the miniature X-ray film was correct or not, similar criteria are not available for assessment of the microscopy result.

It was therefore necessary to set up a definition of a "Positive" slide for purposes of this study. In the absence of adequate objective criteria, it is inevitable that any definition

would be, to some extent, subjective and arbitrary and therefore open to criticism. However, it was thought that a combination of the following categories of slides could be taken to constitute the sum total of positive slides :

- (1) All slides marked positive by *all* the five microscopists;
- (2) slides marked positive by some and negative by other microscopists, but positive by 'umpire' microscopy; and
- (3) slides marked positive by some and negative by other microscopists and negative by 'umpire' but positive by culture.

Categories (1) and (3) do not admit of any controversy except that, by the nature of the study, the number of microscopists (five) itself introduces an element of arbitrariness, albeit slight. Category (2) introduces an obviously subjective element but is nonetheless necessary because, as is well known, a considerable number of patients positive on direct smear fail to produce a positive result on culture. The fact that the 'umpire' was an experienced bacteriologist may, however, be considered an adequate safeguard to reduce to a minimum the element of arbitrariness.

The slides for which the culture and the umpire microscopy were both negative and which, in the initial microscopy, had been marked positive by some and negative by others, were labelled as 'doubtful' positives.

All the slides, thus, were divided into five groups (Table 1). Out of 185 slides, 33 were marked as positive by all the five microscopists (Group I); 122 negative by all (Group V); 30

positive by one to four, out of which 19 were marked positive by umpire (Group II); 5 were not marked positive by umpire but were confirmed by culture (Group III) leaving finally 6 which were confirmed neither by the umpire nor culture (Group IV). For the purposes of subsequent tables, 57 slides (Group I, II & III) were finally taken as positive and 6 (Group IV) as 'doubtfully' positive.

Results

(a) Inter-individual differences

Table 2 shows that only 33 slides (60%) out of the 57 taken as positive were marked as such by all the five microscopists. Six slides were marked positive by four, 7 by three, 8 by two and 9 slides by only one of the microscopists. Whereas 88% of the 33 slides in Group I were confirmed by a positive culture, only 44% were confirmed by culture if only one microscopist had marked the slide as positive. All the slides marked positive by four and three microscopists respectively were subsequently confirmed by umpire and/or culture, but 2 out of the 8 originally marked positive by two microscopists and 4 out of the 9 by one microscopist were neither confirmed by umpire nor culture ('doubtful' positives).

Table 3 shows the 'false positives' and 'false negatives' in respect of each microscopist. The number of slides marked positive by them varied from 44 to 51; the 'false positive's were few (0 to 3) and the false negatives' varied from 8 to 15 but the differences are not significant ($X^2=3.29$ for 4 d.f., $0.50 < P < 0.70$). It could be concluded that, on an average, every microscopist correctly marks only 80% of the positive slides and nearly 20% are missed, the false positives being negligible.

TABLE I

*Classification of slides according to criteria for positivity**

I. Positive by all five microscopists	33
II. Positive by less than five microscopists, but Positive by Umpire	19
III. Positive by less than five microscopists, Negative by Umpire but confirmed Positive by Culture	5
IV. Positive by less than five microscopists, but Negative by Umpire and Culture	6
V. Negative by all five microscopists	122
Total number of slides	185

*For definition of a 'Positive' Slide, see the text

TABLE 2

Results of microscopy of 185 sputum smear slides

	Number of slides	Number of slides confirmed by		
		Umpire	Culture	Umpire and/or Culture
Positive by 5	33(100.0)	33(100.0)	29 (87.9)	33(100.0)
Positive by 4	6 (100.0)	6 (100.0)	(83.3)	6 (100.0)
Positive by 3	7 (100.0)	(71.4)	4 (57.1)	7 (100.0)
Positive by 2	8 (100.0)	5 (62.5)	4 (50.0)	6 (75.0)
Positive by 1	9 (100.0)	3 (33.3)	4 (44.4)	5 (55.6)
Positive by at least one	63 (100.0)	52 (73.0)	46 (82.5)	57(90.5)
Negative by all five	122		9 (7.4)	

Figures in parenthesis indicate percentages.

TABLE 3

Inter-individual variations in microscopy results

Definitely positive slides (I, II, III)	57			
Negative & Doubtfully positive slides (IV, V)	128			
	185			
	Total slides marked Positive	Positive slides correctly marked	Positive slides missed	Negative and doubtfully Pos. slides marked as Positive
Microscopist A	45	42	15	3
B	44	44	13	0
C	50	49	8	1
D	50	47	10	3
E	46	44	13	2
Average	47	45.2 79.3%	11.8 20.7%	1.8 1.4%

The microscopy results were co-related to the final diagnosis of the patients and for tuberculous patients, microscopy results were analysed in respect of extent of disease at the time when smear slide was prepared and whether the patients had any treatment for tuberculosis previously. Table 4 shows that moderately advanced and far advanced cases were in no way more frequent amongst the 33 whose slides were marked positive by all the five microscopists or the remaining 30 which were marked positive by less than five. In 2 cases of positive microscopy, the diagnosis was non-tuberculous. One of these had been marked positive only by one microscopist and the culture and umpire reading were also negative and therefore the slide was included amongst 'doubtfully' positive. The other however had been marked positive by 3 microscopists and the umpire and was therefore included (by definition) amongst the definitely positive even though the culture of this specimen was negative and the clinical and radiological evidence was incontrovertibly against tuberculous diagnosis. It may also be mentioned in passing that only 7% of the 73 sputum specimens from tuberculous patients found negative by all the microscopists were culture positive.

Table 5 shows that there was preponderance of previously untreated cases amongst the 33 whose slides were marked as positive by all, as compared to the other 30. Since the bacillary content of the sputum in previously untreated cases is likely to be higher than in previously treated cases, other things being equal, the degree of bacillarity of the specimen could be responsible for some of the slides being 'false negatives' by microscopy.

Table 6 tends to corroborate this. In as many as 30 slides out of the 33 marked positive

by all five microscopists, the bacillary content* was f-f- or + ++. At the other end of the scale, only 3 of the 17 slides marked positive by one or two microscopists had a bacillary content of + + and none was ++ +. Since frequencies in some cells are too small, certain categories have been combined for a test of significance. This gave $X^2=22.4$ for 1 d.f., $P<0.001$. This confirms what appears plausible even otherwise, viz., that slides with a high bacillary content are likely to be missed by microscopists far less often than those with a low bacillary content.

(b) *Infra-individual differences*

Table 7 shows the intra-individual differences. Whereas the microscopist A had marked 45 slides as positive first time, 48 slides were marked positive by him the second time. Out of these, only 38 slides were common and 7 were marked positive in the first microscopy but negative in the second and 10 vice versa. As expected most of these 'missed' slides were in the categories of low bacillary content of the sputum. The difference in the result of two microscopies by A is more or less of the same order as the differences between A and the other microscopists.

Discussion

The study has shown that there are wide inter and intra-individual variations in the results of microscopy of sputum smears. All microscopists miss some positive slides. This is true irrespective of the yardstick of positivity;

- * Bacillary content of the sputum • was graded as follows by the bacteriologist.
- + 1 bacillus per field on an average.
- ++ 1 to 10 bacilli per field on an average.
- +++ More than 10 bacilli per field on an average.

TABLE 4

Results of microscopy according to diagnosis & radiological extent of disease

	Positive by all five	Positive by 1 to 4	Negative by all five	Total
Minimal	3	3	20	26
Mod. Adv.	15	12	32	59
Far Adv.	15	13	21	49
All TB Cases	33	28	73	134
Non-TB Cases	—	2	49	51
Total	33	30	122	185

TABLE 5

Variations in the results of microscopy in previously treated and untreated cases

	Total	Treated cases	Untreated cases
Positive by all five	33	10	23
Positive by less than five	30	13	17
Total	63	23	40

TABLE 6

Variations in the results of microscopy in relation to the bacillary content of sputum

	Bacillary content of sputum*				Total
	+++	++	+	Less than 10 bacilli	
Positive by five	13	17	3	—	33
Positive by four	1	2	3	—	6
Positive by three	—	3	3	1	7
Positive by two	—	2	2	4	8
Positive by one	—	1	3	5	9
Total	14	25	14	10	63

*For gradation of bacillary content, see the text.

TABLE 7

Infra-individual differences in microscopy of sputum smears

First microscopy	Second microscopy		
	Positive	Negative	Total
Positive	38	7	45
Negative	10	130	140
Total	48	137	185

whether the yardstick is all slides marked positive by even one microscopist or an arbitrarily arrived at number as in the present study.

The number of positive slides missed by the various microscopists in this study, ranged from 1/4th to 1/6th of the total positive slides. On an average only about 80% of the slides are likely to be correctly marked as positive. Nearly 20% positive slides are liable to be missed, but the false positives are negligible.

The differences in microscopy could be due to one or more of the following

- (a) Proficiency of the microscopists.
- (b) Time taken for microscopy of each slide.
- (c) Bacillary content of the sputum specimen.

The differences noticed in this study are not likely to be due to variation in the proficiency of microscopists to any appreciable extent. Even though their experience ranged from 14 years to 3 years, the degree of variation between the microscopists is of the same order. Furthermore, the differences between the two results of the same microscopist (who was asked to see the slides twice to determine intra-individual differences) are also of the same order. Had the proficiency of the microscopist been a significant factor, the differences between the two results of the microscopist A would have been negligible. Rao (1969) compared the result between N.T.I, technicians and laboratory trainees and Nagpaul et al (1968) compared the result of microscopy carried out by N.T.I, technicians" and the technicians in the primary health institutions at the periphery. They found that the consistency of the results even in these groups with considerable difference in the standard of proficiency was of a fairly high order.

Similarly, the time factor could also be absolved from being the likely reason. All the microscopists were required to see the slides in batches of 24 at a time, and 5 minutes' time was allotted for each slide. This leaves bacillary content of the sputum as the likely main cause.

Table 6 has shown that the bacillary content of most of the positive specimens missed by some microscopists was very low and where the bacillary content was high, almost all microscopists had marked the slide as positive. It appears logical to presume that where bacillary content of the sputum is high, large number of bacilli are present in practically all fields, and the slide is, consequently, likely to

be marked positive by all microscopists. However, if the number of bacilli is not much and (therefore) the bacilli are not evenly distributed on the slides, the slide could be marked as negative if the microscopist happens to see, by chance, only those fields which did not contain any tubercle bacilli, since no microscopist can cover the entire slide in 5 minutes. This also explains why the results of microscopy of the same person can be different for the same slide seen a second time, if the bacillary content of the sputum is low and also why a second microscopy may help to retrieve some of the 'false negatives' of the first microscopy.

In short, failure to find tubercle bacilli by direct microscopy does not necessarily mean absence of tubercle bacilli in the slide. An average microscopist will miss nearly 1/5th of the positive slides. This has a fairly serious significance for centres where culture and x-ray facilities are not available and the diagnosis depends upon direct microscopy. It may, however, be pointed out that of the 15 positive slides that the microscopist A missed in the first microscopy (Table 3), 10 were correctly marked by him as positive the second time i.e. if culture facilities are not available, second microscopy will help to retrieve nearly 2/3rd of the slides missed in the first microscopy and the number missed by both microscopic examinations is very small indeed. Thus it is possible, theoretically, to retrieve all the 'misses' of one microscopy by increasing the number of microscopic examinations but this would be uneconomical.

In conclusion, the results of microscopy of direct smears are no less subject to inter- and intra-individual variations than the reading of miniature x-ray films. The variations are inherent in the nature of the examinations and given a reasonable standard of training and diligent search for the stipulated five minutes, inter-individual variations seem to depend, by and large, on the bacillary content of the sputum rather than the proficiency of the microscopist. In previously untreated, fairly advanced cases where the bacillary content of sputum is likely to be very high, many positives are not likely to be missed. Further, many of the 'misses' could easily be retrieved by a second microscopy.

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PATTERNS OF TUBERCULAR LESIONS IN RESECTED SPECIMEN OF LUNG*

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With the advent of chemotherapy for the treatment of tuberculosis numerous studies have been undertaken by various workers to study its effects on the pattern of tubercular lesions in the lung. It is generally believed that adequate chemotherapy increases the healing rate of the lesions and claims have also been made that chronic cavities close on adequate chemotherapy. However, opinion in literature is divided and others think that the individual pathological lesions are not significantly different in cases treated by anti tubercular drugs and otherwise.

In view of this controversy we have studied the pattern of tubercular lesions in resected specimens of lungs from 71 cases removed over a period of ten years. In this paper we have tried to correlate the type of lesion seen on histopathology with that of efficacy of chemotherapy received (Cotter, Foreman and Seal 1958).

Material and Method

The observations are based on 71 cases of pulmonary tuberculosis treated by resection at the Gandhi Memorial and Associated Hospitals, Lucknow, during a period of ten years. Only those cases who had complete documentation and follow-up have been included.

The resected specimens of lung were inflated with air and formalin after ligation of bronchus and were then placed in 10 per cent formalin for 48 hours. The fixed specimen was studied for gross pathology, the external appearances, state of pleura and the consistency of lung. The lung specimen was cut into thin slices and studied for evidence of cavities or solid caseous nodules or calcified areas and the state of bronchi. The cavities were studied in detail for their lining and communications. Multiple sections were taken from different sites for histology including the site of bronchial division. The sections were stained with Haematoxylin and Eosin stain, Van Gieson's stain for collagen, Verhoeff stain for elastic tissues and Zeihl Neelsen's stain for A.F.B. An average of 10 sections were studied in each case.

* Paper read at the XII All India Thoracic Surgeons Conference held at Lucknow.

Observations

Age and Sex

The age of 71 cases ranged from 3 to 54 years. There were 46 males and 25 females. The maximum number of cases 57.7 per cent were in 21 to 30 years age group (Table 1).

TABLE 1

Age incidence

Age in years	No. of cases	Percent
0—10	2	2.8
11—20	20	28.1
21—30	41	57.7
31—40	5	7.1
41 and above	3	4.2

Symptoms and their duration

The symptoms on admission are given in Table II. All the patients had cough and most had expectoration and pyrexia. The duration of symptoms (Table III) has been classified into three subgroups : upto 12 months (24 cases, 33.8 per cent), 12 to 24 months (26 cases, 36.6 per cent) and more than 24 months (21 cases, 29.5 per cent).

Sputum status

In all the cases sputum smears were examined for A.F.B. repeatedly (Table IV).

TABLE 2

Symptoms on admission

Symptom	No. of cases	Percent
Cough	71	100
Expectoration	63	88.7
Pyrexia	43	60.5
Pain in Chest	27	38
Haemoptysis	20	28.1
Loss of weight	32	45
Dyspnoea	4	5.6

TABLE 3

Duration of symptoms

Duration	No. of cases	Percent
Upto 12 months	24	33.8
12—24 months	26	36.6
Over 24 months	21	29.5

TABLE 4

Sputum status

A.F.B. Status	On admission	Pre-operatively
A.F.B. — ve	47 (69.5%)	64 (90.1%)
A.F.B. +ve	24 (33.8%)	7 (9.8%)

Sputum culture and sensitivity to anti-biotics was also done in personally studied cases. In our series 24 (33.8 per cent) cases had A.F.B. positive sputum on admission. They were converted to A.F.B. negative status prior to operation except 7 (9.8 per cent) cases which remained positive. The bacilli in these cases were found to be resistant to usual chemotherapeutic drugs and the patients were given operative coverage by other drugs like Viomycin, Terramycin and Unithiazide etc.

Radiological findings

Cavitation : Radiological evidence of cavity was found in 31 cases but examination of the resected lungs revealed cavities in only 23 cases. On the other hand, there were 6 cases where radiologically cavities were absent, while examination of the lung revealed cavities. The lesion in the lung in the former cases consisted of large encapsulated caseous nodules with necrotic centres, which radiologically appeared as cavities. Similar observations have been reported by Robert Mayock (1955). It is common knowledge that cavities may not be seen on X-ray examination and yet may be present in resected specimens. This explains the absence of cavities on radiological examination in 6 of our cases.

Bronchiectasis

Bronchogram were done in 36 cases and bronchiectasis was found in 25. This was confirmed histopathologically subsequently.

Chemotherapy

The cases have been classified according to the adequacy of chemotherapy they received

on the lines of Cotter, Foreman and Sae (1958) as follows :

- (a) *Type A* : Initial continuous chemotherapy in recognised dosage for minimum period of eight months (32 cases, 45 per cent).
- (b) *Type B* : Acceptable regimes continuously for 5 to 8 months or for a total of more than 5 months with interruptions (17 cases, 23.9 per cent).
- (c) *Type C* : Ineffective regimes which do not prevent emergence of resistant organisms (14 cases, 19.7 per cent).
- (d) *Type D* : Good regimes, but of less than 5 months duration (8 cases, 11.2 per cent).

Surgical procedure employed

The various types of surgical procedures carried out are given in Table V. 39 (54.9 per cent) cases had lobectomies or lobectomy+segmentectomy. Segmentectomy alone was done in 14 (19.7 per cent) cases. There were 18 (25.2 per cent) cases of pneumonectomy or pleuro-pneumonectomies.

TABLE 5

Surgical procedures performed

Operation	Right	Left	Total	Percent
Segmentectomy	9	5	14	19.7
Lobectomy+ Segmentectomy	31	3	39	54.9
Pneumonectomy	5	6	11	15.4
Pleuro- pneumonectomy	2	5	7	9.8
Total	47 (66.1%)	24 (33.8%)	71	100

47 (66.1 per cent) resections were done on the right side and 24 (33.8 per cent) on the left side. Highest incidence of involvement was right upper lobe 18 (25.3 per cent) cases, next was whole of the left lung 12 (16.9 per cent) cases and then the right lung 8 (11.2 per cent) cases. The involvement of dorsal segments was seen in 8 (11.2 per cent) cases i.e., apical and posterior segments of upper lobe and superior segment of lower lobe.

Post operative complications and mortality

The commonest post-operative complication in our series was broncho-pleural fistula in 11 (15.4 per cent) cases. In 6 of these, there was infection of pleural space as well. There were 3 cases of contralateral spread and 2 cases of recurrence (Table VI).

TABLE 6

<i>Post-operative complications</i>		
Complication	No. of cases	Percent
Broncho-pleural Fistula	11	15.4
Contra-lateral spread	3	4.2
Wound rupture and infection	2	2.8
Recurrence	2	2.8

Over all mortality occurred in 10(14.0 per cent) cases. There were 6 cases of early deaths within first six weeks and 4 of delayed deaths

occurring more than 6 weeks after the operation. In the early group, 2 died of cardiac arrest, 2 of post operative shock, one of hyperpyrexia and one had a fatal pulmonary embolus. In the delayed death group, one died of post-pneumonectomy empyema, one of B.P.E. and one each of post-operative shock following space obliterating thoracoplasty and of contralateral spread (Table VII).

Morbid anatomy and histology of tubercular lesion

For descriptive purposes the tubercular lesions in the resected lungs have been broadly classified into two groups (Table VIII).

Group I: Localised lesions : In these cases the lung showed one large lesion, either a solid caseous nodule or a cavity with small satellite tubercular foci. The disease in these cases was either confined to one segment or to a lobe of the lung. There were 5 (7.0 per cent) such cases. In 3, the lesion consisted of solid

TABLE 7

<i>Mortality</i>			
Early deaths (Before 6 weeks)	No. of cases	Late deaths (After 6 weeks)	No. of cases
Cardiac Arrest	2	Post-pneumonectomy Empyema	1
Post-operative shock	2	Broncho-pleural Fistula	1
Hyperpyrexia	1	Shock (Thoracoplasty)	1
Pulmonary Embolism	1	Contra-lateral spread	1
Total	6(8.4%)		4(5.6%)

TABLE 8

<i>Patterns of lesions</i>		
Lesions	No. of cases	Percent
Localised Lesions (7.0%) :		
Solitary Caseous Nodule	3	4.2
Solitary cavity	2	2.8
Wide spread or Multiple lesions (93.0%)		
Caseous nodules	32	45.0
Cavities	4	5.6
Caseous nodule and cavities	18	25.3
Extensively destroyed Lung	12	16.9

large encapsulated caseous nodules, while in 2 cases there were cavities measuring 3 cm. and 5 cms. in diameter, their inner lining being smooth. Microscopically, the caseous nodules presented minimal inflammatory cellular aggregates but collagenisation was intense which led to encapsulation of the nodule or cavity. The inner surface of the cavities was lined by tuberculous granulation tissue.

Group II: Multiple or wide spread lesions : The lesions in these cases were widely scattered in the lung parenchyma. The lesions have been further subdivided into four sub-groups mainly on the basis of their predominant pattern as shown in Table VIII. (i) Thus in 32 (45.0 per cent) cases the tubercular lesion appeared as numerous widely scattered caseous nodules varying from pin-head to over 5 cm. in diameter. The caseous nodules in these cases were surrounded by fibrocollagenous tissue, except in one case where there was little peripheral collagenisation. This case had positive sputum at the time of operation, (ii) in 4 cases the predominant pattern was one of multiple cavities, tubercles being fewer and inconspicuous, (iii) in 18 (25.3 per cent) instances both caseous nodules and multiple cavities were found, (iv) while in 12 (16.9 per cent) cases the disease had extensively destroyed lung parenchyma with confluent tubercular foci, multiple cavities, areas of fibrosis, atelectasis and extensive bronchial changes. Out of the 18 pneumonectomies, 12 belonged to this group and the remaining 6 cases had lesions like multiple nodules and cavities.

The cavities in majority of the cases of this group varied from 2 cms to 5 cms in diameter. There were a few small sized cavities 0.5 cm

to 2 cm. In one case, there was a large hydatid cyst associated with the tubercular lesion. Multiple cavities were seen in 27 cases. The common site of cavities was in the apicoposterior segment of the upper lobe (44 per cent). The wall of the cavities (Table XII) were thick in 22 and thin in 7 cases. Their inner surface was smooth in 11, but irregular and ragged in 18 cases. Microscopically, only in one case the cavity presented epithelisation with healed tubercular process. In the remaining cases the inner surface of the cavity was covered with caseous necrotic debris, the wall of the cavity showed epitheloid cells, Langhan's giant cells and lymphocytes while there was evidence of collagenisation at the periphery. Acid Fast bacilli could be demonstrated in the caseous material in 2 cases.

Associated Pathological lesions in the lung

The lung specimens besides the tubercular lesions described above presented pleural thickening, changes in the bronchi, interstitial pulmonary fibrosis, lymphoid hyperplasia and endarteritis (Table IX).

Bronchial Pathology : Two types of changes have been observed, Bronchitis and Bronchiectasis. The involvement of bronchi was seen only in widespread or multiple lesions and was associated with intensive interstitial scarring, atelectasis and blocking by viscid secretion.

Tuberculous bronchitis and bronchiectasis was found in 14 (19.7 per cent) and 9 (12.6 per cent) cases respectively, while nonspecific chronic bronchitis and bronchiectasis was found in 15 (21.6 per cent) and 16 (21.0 per cent) cases respectively.

TABLE 9

Associated pathology in lungs

Associated pathology		No. of cases	Percent
Pleural-thickening		29	54.9
Pulmonary Fibrosis		41	57.7
Lymphoid Hyperplasia		7	9.8
Endarteritis		19	26.7
Bronchitis Endo-Bronchial	Non-tubercular	15	21.0
	Tubercular	14	19.7
disease Bronchiectasis	Non-tubercular	169	21.0
	Tubercular		12.6

Interstitial pulmonary fibrosis : Interstitial fibrosis was found in 41 (57.7 percent) cases, this change was observed both in the localised and the multiple or diffuse type of lesions. Out of the 41 cases, 22 had received Type A, 10 Type B, 8 Type C and 1 case Type D therapy. This again shows that adequate chemotherapy promotes fibrosis and collagenisation in the parenchyma adjoining the lesion.

Lymphoid Hyperplasia, Calcification & Endarteritis : These changes were commonly found in case with multiple lesions and in cases who had received Type A or B chemotherapy. Pans Parelli (1956) found that with prolonged anti-tubercular treatment there was tendency for periarteritis and periphelbitis. Endarteritis seems to be a common sequelae of natural

arrest of tubercular lesions. Whether hypertrophy of the lymphoid tissue is an indication of the healing process is not yet decided but this type of change has been observed by other workers in the successfully treated cases.

Discussion

A correlation between the type of tubercular lesion in the lung and the duration of clinical symptoms is shown in Table X. If the duration of symptoms could be roughly taken as duration of disease then the analysis of data shows that there is no relationship between the duration and the type and the extent of lesion in the lung.

Relationship of type of lesions and the chemotherapy is given in Table XI. It shows

TABLE 10
Correlation of pattern with duration of symptomatology

Lesions	Total No. of cases	Upto 12 months	From 12-24 months	More than 24 months
Localised :				
Solitary Nodule	3	1	2	—
Solitary Cavity	2	1	—	1
Multiple :				
Caseous Nodules	32	13	11	8
Multiple Cavities	4	1	1	1
Nodules and Cavities	18	5	6	7
Extensively destroyed lung	12	3	6	3

TABLE 11
Correlation of pattern with chemotherapy

Lesions	No. of cases	Type A*	Type B**	Type C+	Type D++
Localised :					
Solitary Nodule	3	1	—	—	2
Solitary Cavity	2	2	—	—	1
Multiple ;					
Caseous Nodules	32	16	8	7	—
Multiple Cavities	4	1	1	2	—
Nodules and Cavities	18	7	6	1	4
Extensively destroyed lung	12	5	2	4	1

* Adequate chemotherapy for a minimum period of 8 months

** Acceptable chemotherapeutic regimes for less than 8 months but more than 5 months.

+ Ineffective regimes

++ Good chemotherapeutic regimes for less than 5 months.

that a fair number of patients (29 out of 66 cases) who received 'Type A' therapy had wide spread tuberculous lesions in the lungs; whereas 2 out of 5 patients with localised lesions had received 'D-Type' of therapy. It suggests that besides chemotherapy the host resistance is an important factor in influencing the pattern of lesion in the lung. This also demonstrates that administration of intensive and adequate chemotherapy (Type A) may not succeed in eradicating all tuberculous lesions. One of the striking changes in these cases was evidence of fibrous encapsulation around the caseous nodules, which was found in all except one.

The cavities were also surrounded by a fibrous capsule in all the 29 cases. It is significant that out of these 29 cases showing cavities, the naked eye appearance showed smooth lining in 11 cases (Table XII). However, the histological examination of the cavity wall showed extensive fibrocollagenous reaction with caseation in all the cavities and presence of tubercle bacilli in 2 cases. One case showed epithelisation with absence of tubercular granulation tissue. Our observation suggests that chemotherapy is not capable of completely eradicating the tubercular lesion or the cavity but certainly limits them by encouraging encapsulation. These findings are not in conformity with the observations of Stewart, Turnbull, Macgregor (1956). They found that with prolonged and effective chemotherapy there is an increasing tendency for the cavity to clean up and get lined by collagen tissue. Table XII shows that out of 22 thick walled cavities 13 were found to have received 'Type A' chemotherapy suggesting a response towards collagenisation. This is in conformity with the findings of workers like Figuerdo and Paola (1955), Cotter et al (1958), Stewart, Turnbull and Macgregor (1956).

In our series bronchial disease has been an important factor in the post-operative complication of broncho-pleural-fistula which was seen in 11 (15.4 percent) cases (Table VI). In all these cases bronchial disease had been histologically demonstrated; bronchitis in 8 cases, bronchiectasis in 7 cases. In four cases the lesions co-existed. This is supported by the finding of other workers as well like Medlar (1955). Contra-lateral spread of disease occurred in 3 cases. In one of these cases the caseous nodules did not present fibrous encapsulation, while in the other two cases the disease was extensive and the patient had the complication of B.P.F. Recurrence of disease was seen in two cases. They had multiple type of extensive disease. All these cases did not respond to chemotherapy (Table XIII).

Summary

1. A study of tubercular lesions in 71 cases of resected lung has been carried out and the pattern of these lesions has been described with a view to establish correlation with 'type of chemotherapy' and post-operative complications in these cases.
2. Localised type of lesion was noticed in 5 cases while multiple type of lesions were found in 66 cases.
3. It was found that besides chemotherapy the host resistance is an important factor in influencing the pattern of lesions in the lung.
4. Even the most satisfactory chemotherapeutic regime may not succeed in eradicating all tuberculous lesions, but helps in their encapsulation.

TABLE 12
Effect of chemotherapy on cavity wall and lining

Cavities	No. of cases	Type of Chemotherapy			
		A	B	C	D
<i>Wall :</i>					
Thick walled	22	13	2	3	4
Thin walled	7	2	2	2	1
<i>Lining :</i>					
Smooth	11	7	2	2	—
Ragged	18	10	2	3	3

TABLE 13

Correlation of pattern with complications

Lesions	No. of cases	B.P.F.	Contralateral spread	Recurrence
<i>Solitary :</i>				
Caseous Nodule	3			
Cavity	2			
<i>Multiple :</i>				
Caseous Nodules	32	3	1	
Cavities	4			
Nodules and Cavities	18	5		
Extensively destroyed lung	12	3	2	2

5. The post operative complications of B.P.F. which occurred in 11 cases could be explained on the basis of endobronchial disease.

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CHRONIC APICAL PNEUMONIA

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There are very few conditions which have to be considered in the differential diagnosis of pulmonary tuberculosis. This is especially true when the disease localises itself at the extreme apex or subapical areas of lungs. These conditions include coccidioidomycosis, blastomycosis, chronic Freidlander's bacillus pneumonia and apical bronchiectasis. Sometimes conditions like apical infraction or apical carcinoma in its early stage can also be considered. However, apical pneumonia as such has not found any mention in recent monographs on chest diseases and tuberculosis. An article published recently on the subject (Langen and Elst Verhoeff, 1967) deals with acute pneumonia of upper lobe. The present article deals with a collection of seven cases suffering from Chronic (or unresolving) apical pneumonia who had been diagnosed and treated as pulmonary tuberculosis before their admission to the hospital. These cases on clinical, bacteriological and radiological examinations turned out to be suffering from chronic pneumonic consolidations of upper lobes.

Case Reports

Case No. 1:

B., male, aged 32 years, farmer, general condition: fair, complaints : cough with expectoration, low grade fever and pain in right upper chest. Previous treatment taken : some injections (administered intramuscularly) and some tablets (names not known), clubbing present. Chest X-ray ; Pneumonic consolidation of right upper lobe with small central cavity. No infiltrations. Sputum for AFB (Smear examination) : negative. Mantoux Test (ITUPPD RT23 with Tween 80) : negative (induration 3 mm in diameter).

In view of the history of having had fever with rigor initially and of bringing out large amount of sputum, a tentative diagnosis of chronic pneumonic consolidation of right upper lobe was made. Culture of sputum for secondary organisms revealed the presence of *Neisseria Catarrhalis* and *Streptococcus Viridans* : The patient was put on Crystalline Penicillin 1 million units by IMI twice a day. The pneumonic consolidation cleared off subsequently during next 3 months leaving behind small residual cavity with few fibrotic bands. The patient was then advised to get right upper lobectomy done which he declined, He has

been followed up for nearly six years and last chest X-ray taken revealed the presence of only a residual cavity in right infra clavicular area.

Case No. 2:

NR, male, aged 30 years, was admitted in March, 1961 to our hospital with similar complaints as case 1. The clinical examinations of the patient revealed the presence of cubbing of finger nails. The patient had received 30 injections of Streptomycin and some tablets prior to his admission. Chest X-ray (Fig. 1)

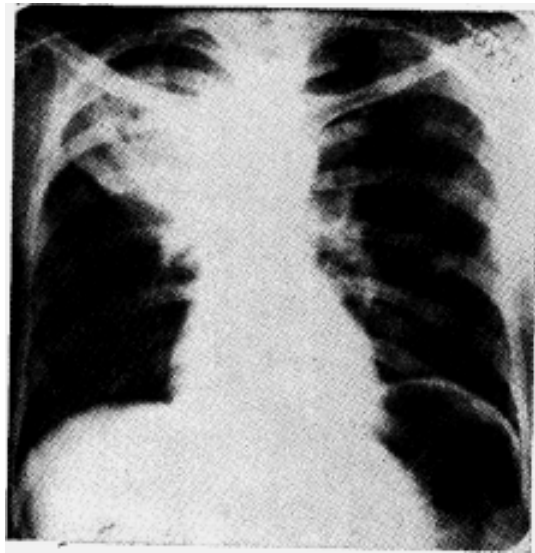


FIG. I

Chest X-ray demonstrating Pneumonia with Cavities in right upper zone (N.R.)

revealed the presence of pneumonic consolidation of right upper lobe. Mantoux Test and Sputum examination for AFB, were negative. Culture of sputum for secondary organisms revealed the growth of only *Neisseria Catarrhalis* and *Streptococcus viridans*. A tentative diagnosis of apical pneumonia was made and the patient was put on crystalline-Penicillin ; one million units twice a day. One month treatment with the drug did not produce any clinical or radiological change in the condition of the patient. It was then hypothesised that the consolidation could be tuberculous in nature. Since the sputum was repeatedly found to be negative for AFB, and attempt was made to clinch the diagnosis by performing a lung biopsy, which, however, failed and resulted in

mild haemoptysis. Thereupon, right sided scalence node biopsy was performed which again was unrevealing (chronic lymphadenitis). Bronchoscopic examination and blind bronchial biopsy were found to be normal. It was, therefore, decided to put the patient on anti-tuberculosis drug (Streptomycin 1 Gm. I MI once a day and Isoniazid 400 mgms per day) along with prednisolone 20 mgms per day (5 mgms four times a day orally). At the same time sputum was sent for culture for fungal organisms. 6 weeks therapy with these drugs did not produce any material change in the condition of the patient. Sputum did not grow any fungii. Thereafter, it was considered that the patient could have been suffering from apical pneumonia due to secondary organisms resistant to penicillin (and streptomycin). He was, therefore, given Tetracyclin (250 mgms 4 times a day). The patient's clinical and radiological (Fig. 2) condition improved. After 3 months

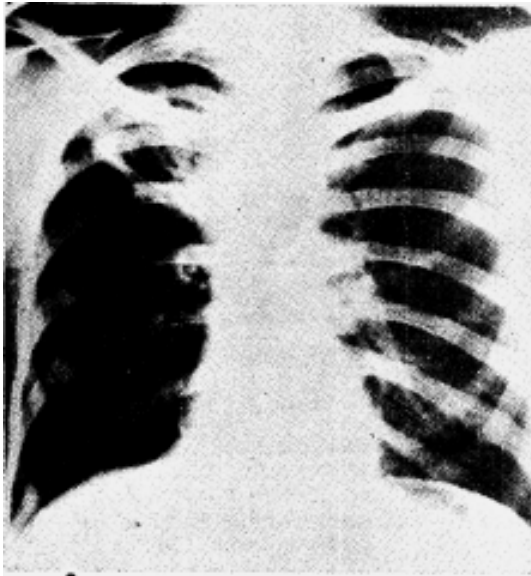


FIG. II

Chest X-ray demonstrating Cavities in right upper zone. Kindly note the Complete clearance of Pneumonia (N.R.)

of therapy, he had improved to an extent that right upper lobectomy was done to remove the residual necrotic lobe (Fig. 3). Histological examination of the specimen revealed the presence of chronic inflammation and multiple necrotic abscess cavities. The patient was last seen on August 5, 1963 when he was well and the Chest X-ray : normal.

Case No. 3

SL, male, aged 40 years, farmer, attended our

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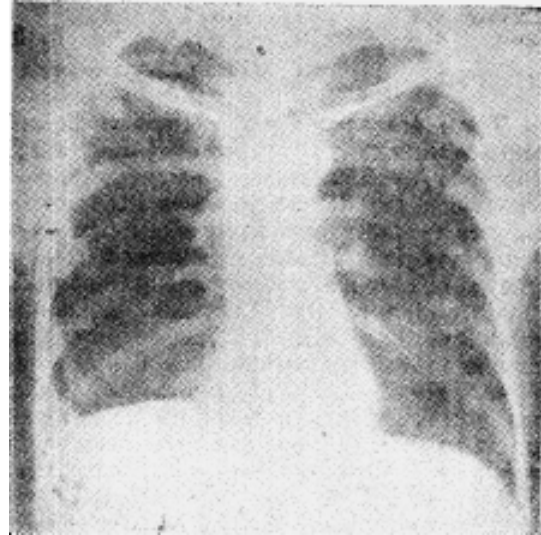


FIG. III

Chest X-ray following right upper lobectomy (N.R.)

OPD on March 27, 1964 with complaints of cough, excessive foul smelling expectoration, occasional bouts of haemoptysis and low grade fever for the past 6 months. Clubbing was present. The patient had been irregularly treated with anti-tuberculosis drugs at his home without deriving any benefit. Mantoux test was negative (after 72 hours). Sputum : negative for AFB (on smear examination). Chest X-ray revealed the presence of pneumonic condition in right upper lobe and a pneumonic patch in right parahilar area. He was prescribed anti-tuberculosis drugs (PAS 10G/day—INH 400 mgms/day). It was presumed that sputum was negative for AFB because the patient had received chemotherapy irregularly in the past. However, his sputum was sent for culture for T.B. The patient turned up two months later (16.5.64) without obtaining any benefit. Chest X-ray revealed increase in size of the pneumonic consolidation in the right parahilar area. A tentative diagnosis of apical and parahilar pneumonia was made and tetracyclin 250 mgms 4 times a day was started. The patient registered recovery. On 16.7.64, the chest X-ray revealed only the presence of fibrotic strands in right upper and middle zones. The patient was discharged from the hospital with the advice to continue taking Tetracyclin at his home. He, however, did not pursue the therapy. He was last seen on 15.2.66 when the chest X-ray was found to be almost completely normal.

Case No. 4

RA, male, aged 28 years was admitted 'to

the hospital on 5.10.1966 with complaints of cough, excessive expectoration, fever, loss of weight and loss of appetite for 6 months. He was treated at home as a case 'of pulmonary tuberculosis with Streptomycin and isoniazid irregularly, without having derived any benefit. In the hospital investigations e.g., Mantoux test and sputum examination for AFB were found to be negative. He was diagnosed to be suffering from chronic apical pneumonia of left lung on the basis of radiological findings which included the presence of pneumonia with a cavity in left upper and middle zones (Fig. 4).

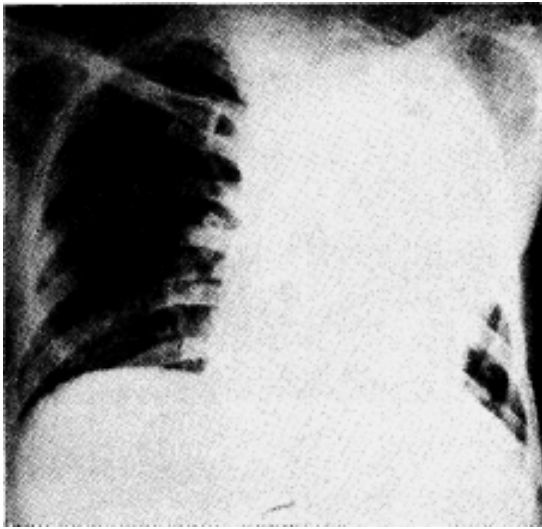


FIG. IV

Chest X-ray demonstrating pneumonia in left upper and middle zones (R.A.)

Doubtful evidences of clubbing were also present. The patient's sputum culture for T.B. and fungus organisms were found to be sterile. Culture for secondary organisms revealed the presence of *Kl. Pneumoniae*, *Str. Viridans* and *N. Catarrhalis*. He was administered Tetracyclin 250 mgms 4 times a day. Subsequent radiograph taken on 6.2.67 revealed clearance of pneumonic shadows and there was only a small residual cavity (Fig. 5). The patient refused to undergo resectional surgical treatment. He was discharged from the hospital with the advice to take Tetracyclin at his home. He has not turned up thereafter.

Case No. 5 :

J.S., a young man aged 35 years, farmer, was seen by the author on 28.11 67 with the history of cough, fever and expectoration, pain in right side of chest and occasional breathlessness for the past one year. At his home he had consulted 3 doctors, all of

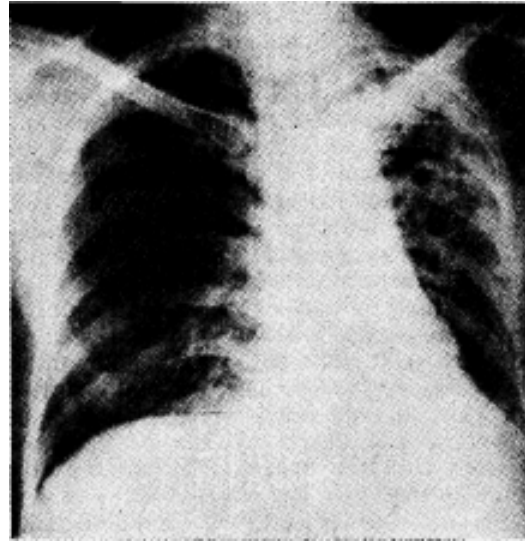


FIG. V

Chest X-ray demonstrating clearance of pneumonia in left lung. Cavities can be seen in the areas previously consolidated (R.A.)

whom treated him for pulmonary tuberculosis with Streptomycin, PAS and INH. He had, however, taken the treatment irregularly. The presence of clubbing, leucocytosis (12000/cumm) with 76 per cent Polymorphs and the presence of pneumonic consolidation in right upper zone on chest X-ray led us to diagnose him as suffering from apical pneumonia. The Mantoux test revealed an induration of 15 mm (diameter) after 72 hours. Sputum was cultured for fungal organisms and T.B. In both instances it was found to be sterile. Only *M. Catarrhalis* and *Str. Viridans* were cultured from the sputum. He was put on Tetracyclin 250 mgms 4 times a day. Subsequent chest X-ray taken on 13.1.1968 was normal. The patient was discharged in a fit condition.

Case No. 6 :

Apical pneumonia complicating pulmonary tuberculosis. G., young man, aged 32 years, was admitted to our hospital with complaints of cough, fever, excessive expectoration, haemoptysis (recurrent) and breathlessness for 8 months. He had not taken any treatment at his home. In the hospital, the investigations revealed following positive findings; sputum +ve for AFB (smear examination), Mantoux test+ve (20 mm diameter of induration), and chest X-ray (PA view) and area of consolidation in right upper and middle zones with scattered infiltrations. He was administered Streptomycin (1 Gm. IMI OD) PAS (10 G/day) and INH (400 mgms/

day). The patient continued to have swinging temperature, and his sputum remained positive for AFB despite 4 months of adequate chemotherapy in the hospital. In fact radiologically the area of pneumonitis tended to increase. Bronchoscopy revealed "purulent pus coming out of right upper lobe bronchus". The pus was sent for culture for fungal organisms. It was sterile. Culture and sensitivity on T.B. revealed that the organisms were resistant to PAS. Therefore, he was administered Streptomycin, Thioacetazone and INH in usual dosage. In view of purulent secretions (and sputum) from the right lobe bronchus, it was decided to add Tetracyclin 250 mgms 4 times a day to his therapeutic regimen. The patient started improving, the quantum of sputum and pus diminished and ultimately the patient was subjected to right upper lobectomy (after 4 months of therapy). The resected lobe confirmed the presence of tuberculous lesion with extensive necrosis.

Case No. 7 :

Apical pneumonia complicating Tropical Pulmonary Eosinophilia, B. K., a young boy aged 15 years, attended the hospital on 17.5.67 with complaints of cough (dry), fever and breathlessness for 5 days. Important clinical and investigational findings included : presence of broncho-spasm in both lungs, with diminished air entry at right apex. Mantoux test : negative, differential leucocyte count revealed 42 per cent Eosinophils. The sputum was negative for AFB (smear examination) absolute eosinophil count 3000/cmm and chest X-ray revealed the presence of pneumonitis in right upper zone. He was advised to take 3000 mgms of Diethylcarbamazine per day for 2 weeks. The patient turned up after 2 weeks of therapy without having obtained much symptomatic relief. The chest X-ray taken again, revealed the same picture. Eosinophil counts were still 34 per cent (absolute count 2900/cumm). The patient was then suggested to take the same dose of Diethylcarbamazine along with Tetracyclin 250 mgms 4 times a day for next 2 weeks. On 17th June, 1967 the chest X-ray was clear and eosinophil counts had come down to 15 per cent.

Case No. 8 :

L. D., a young man aged 30 years was admitted to our hospital with complaints of cough, fever, expectoration (foul smelling and 4 ounces per day) and occasional bouts of haemoptysis. Clinical examination revealed presence of parrot beak clubbing of

fingers and coarse crepitations in right Supra-scapular area. Mantoux test (with ITUPPD-23 with Tween 80) was negative, sputum negative for AFB (smear examination) and no fungal or mycobacterial organisms could be cultured from sputum. Culture of sputum for secondary organisms revealed the growth of only *Neisseria catarrhalis* and *Streptococcus viridans*. Chest X-ray (figure 6) revealed

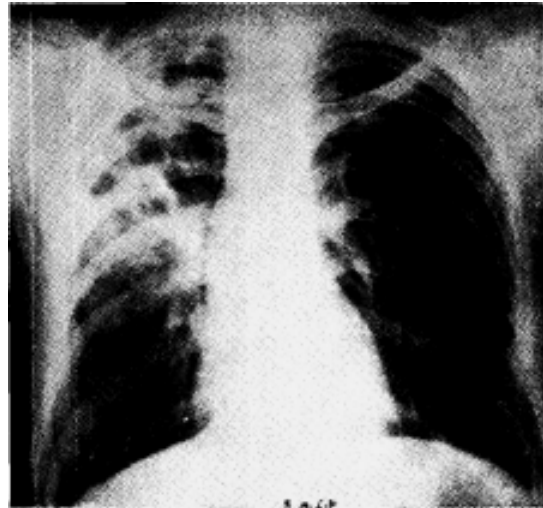


FIG. VI

Chest X-ray demonstrating pneumonia with multiple cavities in right upper- and middle zone (L.D.)

pneumonia in right upper and middle zones : with multiple cavitation. In view of typical history and suggestive clinical and radiological picture a tentative diagnosis of apical pneumonia was made and the patient started on oxy-tetracyclin (250 mgms 4 times a day) and postural drainage. The purulent nature of sputum rapidly changed to frothy in nature. The patient rapidly improved and became afebrile. After 1 month of therapy, when the quantity of expectoration had reduced considerably, the bronchogram revealed bronchiectatic changes in right upper zone (figure 7).

Discussion

Seven cases presented above can broadly be classified in 2 groups :

- Group I :* Apical pneumonia presenting as such without any complicating or concurrent lung disease (case No. 1 to 5).
- Group II :* Pneumonia complicating bronchial or pulmonary diseases e.g., pulmonary tuberculosis (case No. 6)

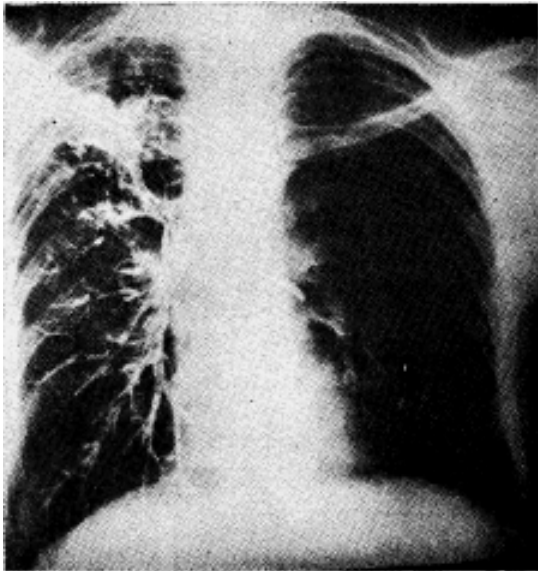


FIG. VII

Bronchogram demonstrating clearance of pneumonia and bronchiectatic changes in right upper lobe bronchi (L.D.)

tropical pulmonary eosinophilia, (case No. 7) apical bronchiectasis (case No. 8) bronchogenic carcinoma and bronchial adenoma.

The distinctive diagnostic features of these cases are being discussed in detail because such cases are very often confused for other pulmonary diseases characterised by their apical localisation. According to the author's personal observations these features are as follows :

Clinical

The syndrome seems to involve the same age-group of persons as those in which pulmonary tuberculosis is more common. Further, it appears to involve the males predominantly. The patient is not as a rule toxic, nor is he emaciated. He might give a history of fever with rigor, bringing out large amount of sputum at the onset of his illness. Hectic fever and excessive sputum may still be present at the time of hospitalisation provided he has not received any treatment previously including even anti-tuberculosis therapy (because Streptomycin being an anti-biotic inhibits secondary organisms also). Clubbing is as a rule present, usually of parrot beak type.

Laboratory Investigations

These investigations are directed towards

excluding the presence of tuberculosis (Mantoux test, sputum examination for AFB and culture for T.B.) fungus infections (culture and smear examination of sputum for fungal organisms) and establishing the presence of suppuration in the lung (leucocytosis mainly polymorphonuclear and isolation of offending organisms from sputum by culture examination). The bacteriological proof of pulmonary suppuration was not established in this series of cases. This was mainly because of the irregular treatment which the patients had taken previously. Indiscriminate use of antibiotics, a common practice in this country, is bound to mask these evidences and distort the clinical presentation. Bronchoscopy must be done in every case atleast to exclude the presence of any obstructing pathology in the upper lobe bronchi (carcinoma or adenoma).

Radiological Investigations

These are the most important diagnostic evidences. Presence of pneumonic consolidation of a lobe or segment without infiltrations in the remaining lung, with or without a small cavity in the centre of the pneumonic area should be regarded as pathognomic of pneumonia. However, radiological findings must be, as far as possible, supported by bacteriological observations.

Therapeutic Observations

These observations are assuming increasing importance because it is not always possible to obtain a bacteriological confirmation of the lesion. Further, the radiological picture may not always be diagnostic particularly when pneumonia complicates or coexists with pulmonary diseases (Group II). In absence of a bacteriological confirmation of the disease, the reliance had to be placed on broad spectrum antibiotics (Tetracyclins) therapy although in one case crystalline penicillin alone was successfully used. The response was uniformly satisfactory. It might be argued that these cases suffered from tuberculous pneumonia and that Tetracyclins being a tuberculostatic led to their resolution. However, it must be emphasised that 1 G of Tetracyclin administered alone once a day does not exert significant tuberculostatic action (The tuberculostatic dose of Tetracyclin is 2-4 G. per day).

The problem is different when pneumonia complicates pre-existing disease in the lungs or bronchi e.g., pulmonary tuberculosis (case no. 6). The presence of pneumonia in such cases should be suspected because the patient's sputum is persistently +ve for AFB (on smear

examination or for T.B. on culture) and at the same time he presents with cardinal evidences of apical pneumonia. In such cases the response to therapy of basic disease might be highly unsatisfactory unless the therapy for both the disorders are instituted simultaneously. This is equally true for pneumonia secondary to carcinoma obstructing the upper lobe bronchus. The pneumonitis produced in these cases may seem to resolve under chemotherapy, but is likely to recur at the same site after the withdrawal of the drugs (unless the obstruction has been relieved). Recurrent pneumonia for that reason in the same lobe or segment should lead to intensive search for any underlying obstructive pathology in the bronchi.

The duration of therapy in these cases should be decided on the merits of the case if self. It shall be guided by the speed of clinical response and of radiological clearance. Normally, the therapy should be continued till the pneumonic focii have cleared off completely. Surgical interference- should be done for

residual cavitory focii. An average case will require 3 to 4 months of therapy. None of the five cases who have been followed up so far (period ranging from 6 years to 6 months) have relapsed.

Summary

Seven cases suffering from chronic unresolved apical pneumonia have been presented. Five cases did not have any pre existing lung disease. In one case the pneumonia was superimposed on pre-existing pulmonary tuberculosis and in another on tropical pulmonary eosinophilia. Diagnostic evidences of chronic apical pneumonia have been summarised.

REFERENCES

Langen, C. de. and Elst Verhoeff, H.E. Van. Pneumonia of the Upper Lobe.

Selected Papers, The Royal Netherland Association, The Hague, Hoiland, 1967 ; 10 ; 61.

MESSAGES

for
20th TB SEAL SALE CAMPAIGN



*From Shri V. V. Gin, President of India and Patron,
 Tuberculosis Association of India*

I am very happy to give my support to the activities of the TB Associations of India which are engaged in a relentless campaign against one of mankind's worst enemies—tuberculosis. The efforts made by any organisation or any individual to alleviate the human suffering and to lessen the distress caused by tuberculosis deserve the help and encouragement of every one of us.

The advent of specific anti-tuberculosis drugs during the past twenty five years has made possible the treatment of patients in their own homes. We also have much better means of detecting and thus preventing tuberculosis—and prevention is always better than cure. We must teach people how to avoid getting tuberculosis. We must dispel the fear that it is incurable and that getting it is a death warrant. We must encourage people to come forward for early diagnosis and treatment. All this can most appropriately be undertaken by the Tuberculosis Associations. To help them the Seal Sale Campaign is organised every year, beginning from the 2nd of October, that is Mahatma Gandhi's birthday. This year the Campaign has a special significance as it coincides with the Centenary celebrations of Mahatmaji. In the name of that great and compassionate soul, I appeal to every citizen of India to respond generously to this national effort.

* * * * *

*From Dr. S. Chandrasekhar, Union Minister of State for Health,
 Family Planning and Urban Development, and President, Tuberculosis
 Association of India*

The Tuberculosis Association of India of which I have the privilege of being the President will be inaugurating the TB Seal Sale Campaign on October 2, the birthday of Mahatma Gandhiji. I take this opportunity to send

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my greetings and good wishes to our Tuberculosis Associations, Tuberculosis Workers and the people of India.

May I appeal to everyone of you kindly to support the anti-TB campaign and help those who are afflicted with tuberculosis ? People contract tuberculosis for no fault of theirs, and therefore society has a responsibility to look after them. Our TB Control programmes provide direct services to the people, but these can become effective only with the full cooperation of the general public. Such cooperation should be canalised through our TB Associations which should have their own resources in men and money. The Seal Sale Campaign is the one central programme which can draw the various sections of the community to strengthen the hands of the Government in implementing the programmes and bring in funds to support voluntary work. Most of our Associations have undertaken programmes to supplement Government effort. Sisters and brothers, in whatever walks of life you may be, the TB Seal Sale Campaign offers you a golden opportunity to associate yourselves with the anti-tuberculosis movement and to serve those unfortunate people who suffer from this disease. Please buy TB Seals and affix them on your letters, gift packets and greeting cards specially during the festivities you are going to celebrate during the campaign period. Please make the forthcoming Seal Campaign a great success and help make your TB Associations strong and useful organisations.

NEWS AND NOTES

TB Seal Sale Campaign

The 20th TB Seal Sale Campaign commenced, as usual, on October 2, 1969. The Railway Board, All India Radio, Rotary and Lions Clubs and other welfare organisations have been requested to help the campaign. The campaign is in progress.

25th National Conference

The Twenty-fifth National Conference on Tuberculosis and Chest Diseases will be held in Patiala from 28th to 31st January, 1970. Dr. M. Umesh Rao of Mangalore will preside over the Conference. The Conference programme includes Panel Discussions on "Urban TB Programme", "Integration of TB Services", "TB of Bones and Joints", "Prevalence and incidence of TB among contacts", "Pyogenic Disease of the Chest". There will also be a session on "Chemotherapy" besides large number of assorted papers.

Health Visitor's Course

The Tuberculosis Health Visitor's Course which this Association conducts every year from the month of January will commence from 1970 in July. There will be an examination in the first week of December and Interneeship will last from 1st January to March. Full details of the revised course will be announced shortly.

NEWS FROM STATES

Andhra Pradesh

The Andhra Pradesh TB Association organised a "Tuberculosis Week" from 6th to 11th October. In Hyderabad meetings were held in 9 domiciliary centres and 3 TB clinics. In all 7 meetings were held and over 250 patients were given clothes worth about Rs. 3,000. During the week a waiting-shed was constructed at a cost of Rs. 3,500 at the Nompally Domiciliary TB Centre and there are schemes to construct 4 more sheds at the various domiciliary centres in the city.

The IV Andhra Pradesh TB and Chest Diseases Workers' Conference was organised at the Niloufer Hospital, Red Hills, Hyderabad on 2nd and 3rd October. Shri Khandubhai Desai, Governor of Andhra Pradesh, inaugurated the Conference and Dr. K.N. Rao, former Director General of Health Services presided. About 150 delegates attended the conference.

The 20th Seal Sale Campaign was inaugurated in the State by the Governor of Andhra Pradesh. Smt. Roda Mistry, Minister for Women's Welfare and Tourism, distributed prizes to the winners of the 19th Seal Sale Campaign. Shields were awarded to districts of Krishna and Khamam for best Seal Sale Collections while individual prizes were given to the Medical Officer, Primary Health Centre, Aganampudi and Medical Officer, Government Hospital, Kamareddy.

The Association has also instituted from this year two Trophies for best District collections.

Goa, Daman and Diu

The Association's Diversional occupation Therapy Scheme at Margao has become very popular. There was a profit of over Rs. 300 from the sale of items produced at this Centre. The Association had also launched a programme of enrolling Life Members and Ordinary Members and collected a sum of Rs. 1,354 therefrom.

The Association also organised a charity film show to raise funds for the Association. The film was offered free of charge and exempted from entertainment tax. The net amount collected from the charity show was Rs. 1,921.60.

Kerala

Dr. S. Sivaraman, Supervising Medical Officer, BCG Campaign, has taken over as the Honorary Secretary of the Kerala TB Association from Dr. K.G. Menon, Director, TB Centre, Trivandrum who retired recently.

The 20th TB Seal Sale Campaign was inaugurated in the State with a sheet of TB Seal autographed by the President and the City Mayor and put to auction. Smt. Padma Ramachandran, I. A. S., Secretary to the Government for Health, bought the same for Rs. 50.

A new building constructed in the compound of the State TB Association in Trivandrum is functioning from 2nd October with 3 full time employees.

Maharashtra

On the occasion of the inauguration of 20th Seal Sale Campaign, Maharashtra Association

held a "Brain Trust" in which many leading TB specialists took part. Questions were asked on various aspects of tuberculosis and were answered by the panel of specialists.

The Maharashtra State Anti-Tuberculosis Association runs a clinic where all investigations and treatment (including costlier second line drugs) are carried out completely free of charge. They also undertake X-Ray survey of organised groups and BCG vaccination of school children etc. During the last year they carried out 12,484 X-rays and 267 screening examinations to detect tuberculosis at an early stage in various 'contact', 'non-contact' and 'vocational groups' and treated 732 patients on domiciliary lines. The clinic takes part in cooperative clinical trials of Indian Council of Medical Research and other research trials also.

They do not limit the activities to the city of Bombay but visit all the districts of Maharashtra State and hold Anti-TB Camps in rural areas. During the four camps held last year, they examined 1964 patients out of which 674 suspected cases were screened and 133 active cases discovered. Free drugs were provided to all these patients and their follow up treatment entrusted to local centres. During these camps they also carried out 2,026 BCG vaccinations and gave oral polio vaccine to 431 children under the age of 5 years. There are altogether 20 peripheral centres in the rural areas regularly visited by them and which are supplied with free drugs.

The Association also organised a charity premier show of the James Bond film, "You Live Only Twice" donated by United Artists to raise funds for the organised Home Treatment. Shri K.K. Shah, Minister for Health and Family Planning, inaugurated the Charity Show at Regal Theatre on 23rd October.

Mysore

The Mysore State TB Association conducted a Refresher Course at Bangalore in August in association with the Bangalore branch of Indian Medical Association. About 40 doctors who attended the course also received lectures and demonstrations at S.D.S. Sanatorium and National TB Institute.

The District TB Association of Chickmagalur have raised a donation of Rs. 60,001 from Shachandanmal Hirachand and Co. for anti-TB work in the district.

The Hassan district Association organised

a TB case finding week in Hassan town for 6 days. 337 persons were X-rayed.

Tamilnadu

The TB Association of Tamilnadu has decided to award a Trophy to District Association which sells a minimum of 2,00,000 Seals.

West Bengal

The Bengal Tuberculosis Association has instituted Trophies to be awarded for highest Seal Sale collections. The Shields to be awarded include (1) O.K. Khemka Challenge Shield for highest collection, (2) Tarini Charan Law Smriti Trophy for second highest collection, (3) Rani Birla Challenge Shield for highest collections among the affiliated Associations or clinics and (4) Naba Kishore De Memorial Challenge Shield for highest collections among educational institutions. Besides these a Silver Plaque will also be awarded to those who collect Rs. 300 or above by the sale of TB Seals.

Classification of Tuberculosis

The Tuberculosis Association of India has revised the "Classification of Pulmonary Tuberculosis" which was published by it in July, 1940. Copies of the revised classification can be had from the office of the T.A.I., 3, Red Cross Road, New Delhi-1.

International TB Conference in Moscow

The Council of the International Union have decided to hold the next International TB Conference in Moscow during September 1971. The Council also elected Prof. B.V. Chebanov of the USSR as President of the Union vice Dr. James E. Perkins.

The New York Conference

The 20th International Tuberculosis Conference was held in New York from 2nd to 6th September. Dr. S. Chandrasekhar, Union Minister of State for Health and Family Planning, Drs. N.K. Menon, S.P. Tripathy, P. Benjamin and Shri B.M. Cariappa attended this Conference from India. The general impression was that nothing new or very sensational was presented, but extremely useful information was made available. The consensus was that today tuberculosis control work is more organisational, managerial and financial than medical and scientific, and that National TB Associations should be strengthened particularly in the developing countries,

Eastern Regional Conference

The Eastern Regional meeting held in New York in September 1969 decided to hold its seventh Conference in Taipei, Taiwan, in November 1970.

Lectures on Tuberculosis for Public Health Nurses and Health Visitors

A hand book on Tuberculosis by Dr. S.P. Pamra, Director, New Delhi TB Centre, has been published by the Tuberculosis Association of India. It covers, in simple language, information that para-medical students should know about tuberculosis such as the clinical features of this disease, the present philosophy of tuberculosis control based on finding as many unknown cases and as quickly as possible and treating them effectively and near their houses as possible. This book has been commended in a foreword by Dr. P.K. Duraiswami, Director-General of Health Services, Government of India and Chairman, Tuberculosis Association of India. A copy costs Rs. 3 only.

Gift of Books

The Chest and Heart Association, London, has offered to donate to the Association the following books for its library :

1. Modern Drug Treatment in Tuberculosis (4th Edition).
2. Transactions of the International Chest and Heart Conference.
3. Tuberculosis—Prevention and Control.

4. Transactions of the Nigerian TB Conference.
5. Pneumoconiosis—Modern Trends.
6. The Protection of the Nurses.
7. Social Work in Tuberculosis.

Indian Council of Medical Research

Applications and/or Nominations are invited for the National Award of the value of Rs. 5,000 to an Indian National for outstanding research work in Biomedicine in the field 'of Family Planning done in an Indian Institution and published in Indian/Foreign Journals in 1967, 1968 and 1969. The last date for the submission of the same is 15th December, 1969. For full details write to the Director-General, Indian Council of Medical Research, P.O. Box No. 4508, New Delhi.

Indian Academy of Medical Sciences

The Indian Academy of Medical Sciences has been conducting post-graduate examinations in different disciplines of medical sciences on an All India basis with a view to admit candidates to the Membership of the Academy. The examination will be held in two parts during January to July, 1970 in New Delhi.

Full particulars about the examinations and application forms can be obtained from the Executive Director, Indian Academy of Medical Sciences, C-II/2, Medical Institute Campus, Ansari Nagar, New Delhi-16 on payment of Rs. 2.

The Indian Journal of Tuberculosis

ABSTRACTS

Vol. XVI

October 1969

Abst. No. 4

Pulmonary Resection in Bullous Disease

Vincent Lopez Majano, Richard F. Kieffer Jr., David N. Marine, Domingo A. Garcia and Henry N., Wanger Jr. Amer Rev. Res. Disease, Vol. 99, No. 4, April 1969.

Of the 179 patients with bullous disease, 18 underwent 22 thoracotomies in order to decrease physiologic dead space, improve ventilation and correct imbalance between ventilation and perfusion.

The indications for resection were large and compressed pulmonary tissue to produce ventilatory insufficiency.

Of these 18 patients, bullae were excised in all except one in whom pulmonary resection was done. In three besides excision of bullae lobectomy was done and one had vagotomy.

Of the 18 patients, 15 had other complicating bullous disease, the most frequent were obstructive emphysema and chronic bronchitis.

In 2 patients, results of surgery were excellent with return of pulmonary function tests to normal and resumption of normal life good in 9 patients with marked improvement in pulmonary function and return to normal activities, fair in 3 patients with symptomatic improvement but only slight change on objective evaluation of pulmonary function and continued limitation of activity and inability to work and poor in 4 patients with 3 deaths caused by respiratory insufficiency produced by chronic lung disease.

Thus the presence of severe obstruction airway disease or of parenchymal fibrosis of pulmonary tissue markedly reduced the likelihood of beneficial results from surgical treatment.

Regional function studies appeared to be of considerable value in selection of patients for surgery.

H B.D.

The Ethionamide Sensitivity of East African Strains of Mycobacterium Tuberculosis Resistant to Thiacetazone

M. J. Lefford, Tuberc, Land, (1969), 50, 7.

Pretreatment cultures which are naturally

sensitive Or naturally resistant to Thioacetazone did not differ appreciably in their sensitivity to Ethionamide. Pair of cultures obtained before and after six months treatment with Isoniazid plus Thioacetazone were found to differ significantly in their sensitivity to Ethionamide. Although none of the pretreatment cultures were resistant to Ethionamide, Six of 21 post-treatment cultures were definitely resistant. Natural resistance to Thioacetazone is not accompanied by resistance to Ethionamide whereas among cultures with acquired resistance to Thioacetazone approximately 30 per cent are resistant to Ethionamide.

H.B.D.

Observations on Infection with Myco Human bacterium Bovis

Y.P. Kataria. Tub., Land., (1969), 50, 14.

In series of 41 cases of Pulmonary infection due to Mycobacterium bovis, 26 had strains primarily resistant to PAS, Isoniazid or both, although only partially or to low degrees, one strain was resistant to Streptomycin and all the strains were highly resistant to Pyrazinamide. Although the time for conversion to negative cultures was related to sensitivity results, the final results of treatment were favourable inspite of frequent apparently in appropriate use of major drugs. It is concluded that only minor adjustment to normal regimens are needed to off set the mild natural resistance of Mycobacterium bovis.

H.B.D.

The Sensitivity to Thioacetazone and Para-Amino Salicylic Acid and the Virulence in the Guinea Pigs of East African Strains of Mycobacterium Tuberculosis:

M. J. Lefford, Jean M. Dickinson and D.A. Mitchison. Tub., Lond., (1969), 50, 1.

The virulence in the guinea pig and the sensitivity to PAS of pretreatment, Thioacetazone-sensitive and Thioacetazone-resistant strains were less virulent and yielded more colonies on 4 mg/ml Sodium PAS than

Thioacetazone-sensitive strains. The results suggest that low virulence in guinea pigs and an abnormal sensitivity pattern to Thioacetazone and PAS are characteristics common to some strains of Miliary Tuberculosis prevalent in East Africa and in South India.

H.B.D.

Ethambutol in re-treatment of pulmonary tuberculosis

United States Public Health Service Tuberculosis Therapy Trial. Amer. Rev. Res. Dis. ; 1968, 98, 825.

INH, Ethambutol and 7 other anti-tuberculous drugs were used in a re-treatment study of 277 patients from 23 tuberculosis hospitals participating in the tuberculosis therapy trials of the U.S. Public Health Service. The patients were predominantly white males more than 50 years of age, with far advanced cavitory disease and INH resistant bacilli. All patients were treated for 16 weeks with INH plus two other drugs not administered, previously. All but one regimen contained ethambutol, in a dose of 25 mg. per kg. for the first 8 weeks followed by 15 mg per kg. for the last 8 weeks. Cycloserine, ethambutol and capreomycin were the most frequently used companion drugs.

At the end of 16 weeks about 3/4th of the patients were converted. In those converted patients who were followed subsequently, sputum reversion occurred in 1/4th of the patients. Kanamycin and viomycin were less effective in patients with streptomycin resistant organisms. Otherwise all regimens appeared equally effective in eliminating tubercle bacilli from the sputum, although the number of patients in each regimen was small.

The 25 mg per kg. dose of ethambutol was no more toxic than the lower 15 mg dose. Neither dose produced an unequivocal decrease in visual acuity. Ethambutol had to be discontinued because of intolerance in 3% of the patients. No adverse reaction occurred amongst patients treated with capreomycin.

The authors conclude that a regimen consisting of INH, ethambutol and capreomycin emerged as the regimen of choice from the stand point of both toxicity and reversal of infectiousness.

S.P.P.

Anti-tuberculous activity of rifampin in vitro and in vivo and the concentrations attained in human blood.

L. Verbist and A. Gyselen Amer. Rev. Resp. Dis.: 1968, 98, 923.

Three hundred and seven strains of myco-

bacteria isolated from human beings, either sensitive or resistant to one or more anti-tuberculous drugs, were found uniformly susceptible to rifampin, 5 per ml. The mean count of live bacilli with 10 mg per kg, and 20 mg per kg. rifampin for established tuberculosis infection in mice, decreased from 10^8 to 10^{1-2} per lung. In 3 of the 4 mice sacrificed at various intervals, the lungs were completely sterilized.

Rifampin resistant strains were isolated from the lungs of mice treated with that drug alone. Addition of a low dose of INH (2 mg per kg.) prevented the emergence of bacilli resistant to rifampin.

With 450 mg and 600 mg of rifampin, administered in a single dose on an empty stomach in humans, mean peak concentrations of rifampin in the blood of about 7 per ml was obtained 1½ hours after drug administration. The drug remained detectable in the blood after 9 hours in all subjects and in 4 out of 12 subjects after 12 hours as well.

S.P.P.

Rifampin and ethambutol in the re-treatment of advanced pulmonary tuberculosis

A. Gyselen, L. Verbist, J. Cosemans, L.M. Lacqet and E. Vandenbergh. Amer. Rev. Resp. Dis. ; 1968, 98, 933.

The anti-tuberculous effect of ethambutol and rifampin was compared in 52 patients with advanced pulmonary tuberculosis who had been treated and re-treated for several years previously with other anti-tuberculous drugs. In 14 patients ethambutol was given in addition to previously used drugs and in 7 patients rifampin was used. Nine of the 14 patients on ethambutol were converted but 4 subsequently reverted. Reversal of infectiousness occurred in all 7 patients on rifampin with subsequent reversal in 2 patients. In another group of 12 patients both ethambutol and rifampin were administered simultaneously along with one of the other 4 companion drugs. Of these, 11 were converted with one reversal. Neither ethambutol nor rifampin provoked any evidence of intolerance or drug toxicity.

S.P.P.

The Oto-toxic effect of streptomycin and Dihydrostreptomycin on the foetus

F. Rasmussen Scand. J. Resp. Dis ; 1969, 50, 61.

Thirty-six children, aged 2 to 15 years, whose mothers had received streptomycin (SM), dihydrostreptomycin (DHS) or both during

pregnancy were examined with respect to indications of oto-toxic damage. In one child was found slight (30 dB) unilateral sensory-neural high-tone hearing loss* All children were found to have normal vestibular function. In 8 of 33 mothers there was found loss of hearing which apparently could be attributed to DBS treatment. In one mother the vestibular function was reduced. The present material tends to show that the danger of oto-toxic damage to the foetus of the mother under treatment with DHS or SM during pregnancy is slight.

S.P.P.

Streptomycin & Dihydrostreptomycin medication during pregnancy and their effect on the child's inner ear.

Erkki Varpela, Jaakko Hietalahti & Matti J.T. Aro. Scand. J. Resp. Dis.; 1969, 50, 101.

It is considered theoretically feasible, and likely in practice, that streptomycin and dihydrostreptomycin may jeopardize the inner ear of the foetus. Apart from accidental over dosage, excessive drug concentration may be caused by defects in the mother's excretory and detoxication functions. The magnitude of the risk is not so far known. Fifty children whose mothers had received streptomycin or dihydrostreptomycin in various stages of pregnancy and in various amounts were studied. No sensory-neural hearing loss exceeding 30 dB occurred in the speech frequency range in any of these children. Forty children, 5 years of age or older were subjected to complete pure-tone audiometric examination and a single case with perceptive hearing loss higher than 15 dB was detected.

The results of the study suggest that inner ear defects induced in the foetus by streptomycin or dihydrostreptomycin during pregnancy do not seem to be very common.

S.P.P.

Isoniazid prophylaxis in a slum area

Sami A. Khoury, Andrew Theodore and Vivian J. Platte Amer. Rev. Resp. Dis. ; 1969, 99, 345.

Approximately 1,400 reactors identified in a population of about 9,000 in one census tract of the district of Columbia department of Public Health were given chemoprophylaxis for 34 weeks. Only about 18% took the entire course as advised ; 19% did not take any pills ; 27% took more than 50% pills and the remaining 36% took pills less than 50%. Pill taking also varied with age. Fifty percent of those less than 20 years and 54% of those 50 years or above took at least one half of the pills and

they were the best pill-takers. The authors conclude from this study that chemoprophylaxis among reactors is not feasible as a city-wide-policy in the district of Columbia.

S.P.P.

A ten year experience with the treatment of forcibly detained male tuberculous patients in New York city.

Robert Glass Amer. Rev. Resp. Dis.; 1968 98, 883.

A total of 172 recalcitrant male patients with active pulmonary tuberculosis were admitted in a hospital where the conditions amounted almost to forcible detention. During the period of detention conventional treatment for tuberculosis with multiple drugs was carried out.

Nearly 58% of the patients were eventually returned to the community with inactive disease to continue their treatment/supervision from the out-patient clinics. Nineteen patients died and 30 were still in the hospital with active disease at the end of 10 years. Twenty three patients (13.4%) absconded from other hospitals to which they were referred from the detention service for various conditions and were therefore lost to further supervision and treatment.

S.P.P.

A continuing study of patients with "Open Negative" status at Battey State Hospital

R.F. Corpe and F.A. Blalock, Amer. Rev. Resp. Dis. ; 1968, 98, 954.

The relapse rate in patients discharged from hospital during a 12-year period after availability of INH (1-1-1953 to 31-12-1964) have been reviewed. All patients treated during the last 6 years of the study period (when chemotherapeutic regimens may be taken to have been more effective) the relapse rate among patients with "Open Negative" status was only 2.3% compared to 6.2% relapse rate for those treated during the first 6 years to the study period. The overall relapse rate in "Open Negative" patients was 4%.

Relapse was more frequent in patients with cavities of less than 2 cm. in diameter than in those with larger cavities. The relapse was also more in patients with thick walled cavities than in those with thin walled cavities. Those treated with streptomycin, INH and PAS daily experienced fewer relapses than those treated only with INH and PAS. The failure of patients to take drugs after hospitalization increased the risk of relapse. The relapse was lowest in patients who received a regimen of

daily streptomycin and pyrazinamide alternating monthly with daily INH and PAS.

Non-infectious status was retained in 98.6% of the "Open Negative" after surgical treatment. The authors conclude that multiple drug regimens and long term drug therapy were the important factors in preventing relapse.

S.P.P.

BCG-induced allergy and immunity in guinea-pigs during the first year after vaccination.

K. Tolderlund, M. Weis Bentzon, K. Bunch-kristensen, B Mackeorang, J. Guld & H. Waakr. Bull. Wld. Hlth. Org.; 1967, 36, 747-758.

It has been traditional practice in many countries to let re-vaccination with BCG depend on the outcome of periodically repeated tuberculin testing. Acquired resistance to tuberculosis and tuberculin skin sensitivity are concomitant results of BCG vaccination, and it is apparently inferred that a waning resistance will be faithfully reflected in a waning tuberculin sensitivity. However, it has been demonstrated in recent years, both in man and in animals, that the waning of tuberculin sensitivity may be prevented merely by a repetition of the tuberculin test, while data presented suggest that tuberculin testing has no such influence on the course of BCG-induced resistance to tuberculosis.

S.P.P.

Duration of allergy and immunity in BCG-vaccinated guinea-pigs

K. Toledrlund, K. Bunch-Christensen & J. Guld. Bull. Wld. Hlth. O'g. ; 1967, 36, 759-769.

The guinea pig's response to BCG is very different from that of man. Tuberculin sensitivity and acquired resistance to infection is independent of the dose of BCG in a guinea pig whereas in man, there is quite a predictable dose-response relationship for tuberculin sensitivity. Nevertheless, the qualitative pattern of tuberculin sensitivity is strikingly similar in man and guinea pig. In both, it wanes after a certain time and in both it can be maintained at its original level by repeated tuberculin injections for at least 5 years (possibly even longer in man). In contrast, the acquired resistance to tuberculosis in guinea pigs vaccinated several years previously is of intermediate strength, inferior to that of the newly vaccinated and is not restored (apparently not influenced at all) by the injection of tuberculin. It is thus not

ABSTRACTS

possible to follow the course and eventual waning of resistance by means of repeated tuberculin testing, and the very common practice of timing re-vaccination of the individual according to the outcome of such testing must therefore be considered to be without scientific basis.

S.P.P.

Pulmonary disease due to inhalation of derivatives of Bacillus Subtilis containing proteo-lytic enzyme

M.L.H. Flindt; The Lancet; 1969 i, 1177.

Allergic reactions of the lungs to enzymes of Bacillus Subtilis

J. Pepys, et al.; Ibid 1969, i, 1181.

Organic Dusts and Allergic Lung Diseases

Leading Article ; Ibid; 1969, i, 1195.

An investigation was made of chest illness among certain workers in a factory using a preparation containing proteolytic enzyme derived from *Bacillus Subtilis* in the manufacture of detergent products. Although primary irritant effects may have occurred, the severe and sometimes prolonged breathlessness in most of those investigated was thought to have been due to allergic mechanisms. Supporting evidence of allergy to the enzyme was obtained from immediate and late reactions to inhalation and skin-prick tests. Serum precipitins were present in some of these affected individuals and also in unexposed controls. The findings indicate that, in addition to causing acute illness, inhalation of this organic dust may lead to irreversible impairment of lung function (which is usually of the obstructive type), and that insidious lung damage could occur without episodes of overt illness.

Two types of reactions are suggested. If the subject is basically atopic i.e. having a constitutional tendency to be readily sensitized to the commoner allergens, immediate type-I allergic reaction is likely to occur. The atopic subject may however also develop precipitins and then manifest Arthus type III as well as type-I allergic reactions. In non-atopic subjects exposure, probably more intensive, to a particular allergen results in the appearance of precipitins and of type-III allergenic reactions. These immuno-pathological responses underlie the different clinical manifestations and are also a guide to the investigation of effects of organic dusts.

S.P.P.