III. MATERIAL AND METHODS
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Clinical material:

The present study is based on a series of 218 leprosy patients, of them 54 were lepromatous and 164 tuberculoid. Cases belonging to borderline and indeterminate have not been included.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Showing age and sex incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>0-10</td>
</tr>
<tr>
<td>Lepromatous</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Tuberculoid</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
</tr>
</tbody>
</table>

Table I, chart 1 showing age and sex incidence. The maximum age incidence was in the age group over forty showing 42.6% and the minimum was in the first decade showing 5%. The male incidence was 62.9% in cases of lepromatous and 64.6% in tuberculoid. Female incidence was 37% in lepromatous and 35.3% in cases of tuberculoid.
Biochemical investigations:

Blood serum was examined for serum calcium, serum inorganic phosphorus and serum alkaline phosphate in 12 lepromatous, 12 tuberculoid with and without bone changes and 12 normal persons using standard laboratory methods. Of the lepromatous group 2 had bone involvement in the form of destruction of metatarsals in both feet and the tuberculoid group included 3 with absorption of the phalanges of foot. All were from low socio-economic group and were between the age of 20-60 years. Duration of the disease varied from 2-15 years.

Histopathology of blood vessels:

Twenty one cases were selected for histopathology of vessels, of those 5 were lepromatous and 16 tuberculoid. All of them belonged to the age group of 20-30 years; there were 3 females and 18 males. Skin smear was positive in all lepromatous cases and in only 2 of the tuberculoid group. Superior extremity was involved on 7 occasions and the rest 14 were inferior extremity. Skiagrams of the hand and feet showed bony changes in 2 of the superior extremity and 9 in the inferior extremity (Table II). Digital vessels in the interosseous spaces of the hands and the feet were selected for operation. Under local anesthesia about 1 cm...
of the vessels was removed after ligation of the proximal and distal ends. The skin was thoroughly washed to make it bacilli free before operation. The wound was closed with interrupted sutures and healed up nicely supported by antibiotics.

**TABLE II**
Showing number of cases of Histopathology of blood vessels

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of cases</th>
<th>Male</th>
<th>Female</th>
<th>Skin smear</th>
<th>Bone involvement</th>
<th>Superior extremity</th>
<th>Inferior extremity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepromatous</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>++</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Tuberculoid</td>
<td>16</td>
<td>14</td>
<td>2</td>
<td>+</td>
<td>5</td>
<td>11</td>
<td>2 cases</td>
</tr>
</tbody>
</table>

After fixing the specimen in 10% formal saline, it was thoroughly rinsed with running tap water. It was then put into graded alcohol (70%, 90% and then absolute alcohol), subsequently cleared with xylol and put in the paraffin bath (3 to 4 changes). Blocks were then prepared by microtome (size 4 to 5μ). It was stained with Haematoxylin and Eosin stain. It was finally stained for acid fast bacilli (Fite Farraco technique).
Histopathology of nerves:

Five cases were selected for nerve biopsy, of whom 2 were lepromatous and 3 tuberculoid. Four were males and 1 female and all of them belonged to the age group of 30-40 years. The duration of the disease was 5-10 years. All were suffering from ulnar nerve involvement, showing typical features of ulnar palsy. Skin smear was positive in the lepromatous cases only. The skin was thoroughly cleaned with soap and water and other antiseptics to avoid cross infection. The ulnar nerve was exposed under general anaesthesia and 1 cm. of the palmar cutaneous branch was resected about 3 cm. distal to its junction with the ulnar nerve. The wound was closed with interrupted sutures and the wound healed up with antibiotic therapy and dressing. The tissues so collected were fixed and stained in the manner as adopted for blood vessels.

Examination of bone marrow:

Fifty leprosy patients were selected for marrow biopsy of whom 15 were lepromatous and 35 tuberculoid. Of the 15 lepromatous cases 5 had ulcers with bone change, 6 had ulcers without bone involvement and the rest 4 had only neural involvement (Table III, chart 2). Skin smear was positive
in all. All of them were in the age group of 20-30 years. The duration of the disease on an average was 2-10 years. Of the 35 tuberculoid cases, 14 had ulcers with bone involvement, 7 had ulcers with no bone change and 14 had nerve involvement, with anaesthesia. Skin smears were positive in 10 tuberculoid cases. Of these 35 cases 27 were males and 8 females. They were also within the age group of 20-30 years and the duration of the disease was on an average 5-10 years. All cases were X-rayed for evidence of any bone change. Four areas were selected for the purpose of bone punctures, e.g., sternum, ilium, upper end of tibia and calceneus.

The areas selected for puncture were X-rayed specially for evidence of any bone involvement and cases with any change in the bones of the foot, calcaneus was not selected.

**TABLE III**

Bone involvement in cases of bone marrow biopsy

<table>
<thead>
<tr>
<th>Type</th>
<th>Foot</th>
<th>Hand</th>
<th>Involved Hand</th>
<th>Involved Feet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepromatous</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Tuberculoid</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>
BONE INVOLVEMENT IN CASES OF BONE MARROW BIOPSY

- Lepromatous
- Tuberculoid

CHART - 2
Bone involvement was limited to the small bones of the foot only in eleven, five in hands only and 3 had involvement of both extremities. Of the lepromatous cases, 5 had bone involvement, changes were limited to 3 in right foot, 1 in left foot and 1 in the right hand. Of the 14 tuberculoid cases who had bone involvement, 4 had in the right foot, 3 in the left foot, 1 in the left hand, 3 in the right hand and 3 had in both hands and feet.

**TABLE IV**

<table>
<thead>
<tr>
<th>Type</th>
<th>Sternal puncture</th>
<th>Ilium puncture</th>
<th>Calcaneum puncture</th>
<th>Tibial puncture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepromatous</td>
<td>8</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Tuberculoid</td>
<td>11</td>
<td>9</td>
<td>6</td>
<td>9</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>11</td>
<td>9</td>
<td>11</td>
<td>50</td>
</tr>
</tbody>
</table>

Fourteen patients with involvement of bones of the foot were subjected to sternal puncture, 5 with affection of bones of the hand, had tibial biopsy. The remaining 31 cases without bone affection were examined as follows: 11 patients in lower age group had ilium puncture, 5 had sternal puncture, 6 had tibial puncture and the remaining 9 had calcaneal puncture (Table IV). Before the operation, the skin over the site of
the puncture was thoroughly cleaned with soap and water and antiseptic dressings applied. The smear from the skin of the expected puncture area was examined for acid fast bacilli. The puncture was made with a standard sternal puncture needle fitted with a record syringe. Once the needle entered the marrow cavity sucktions were made with the syringe and the material was collected over a slide. Rigid care was taken to avoid cross infection from the skin. The material thus collected was stained for acid-fast bacilli by Zeil Nehlson's method for detection of M. leprae.

Histopathology of bone:

Bone specimens taken from phalanges of leprosy patients were examined for macroscopical and microscopical study of the tissue. Specimens belonged to 4 lepromatous and 9 tuberculoid cases. The patients belonged to the age group of 20-40 years and all were males. Duration of disease varied from 10-15 years. The skin smear was positive in 3 lepromatous cases and none in tuberculoid. The bony tissue was removed with minor surgery as most of them were attached loosely to the part. Of these 13 specimens 4 were sequestra embedded in the soft tissue and all were recovered by minor surgery including the remaining 9. The sequestra were clearly demonstrated by X-rays (in 4 cases) and confirmed by surgery.
Of the rest 9 cases, densities were seen as small bony fragments lying in the soft tissue with loss of definition of the articular ends and/or borders showing a process of absorption and destruction.

The specimens after removing were kept in 10% formal saline. After fixing the bone specimen in 10% formal saline, it was kept in Decasor fluid until completely decalcified. In the next step the bone piece was thoroughly rinsed with running tap water. It was then put into graded alcohol (70%, 90% and then absolute alcohol), subsequently cleared with xylol and put in the paraffin bath (3 to 4 changes). Blocks were then prepared for section by microtome (size 4 to 5μ). Finally these were stained with Haris Haematoxylin Eosin Stain.

Bacteriological examination of sputum:

Sputum was collected from 18 leprosy cases of whom 6 were lepromatous and 12 tuberculoid. All of them had some kind of respiratory distress with copious sputum. All were males. The entire sputum for 24 hours of each case was collected in clear glass containers and concentration done by Pretropffer's method. Multiple smears were made on clear glass slides and fixed with the help of Bunsen flame. Two slides each were made from each sample and stained with Zehul Neholson's Method and Sudan Black and the stain was burnt till a blue flame.
emitted out. These were first washed with water and then with acetone for 3-5 minutes. These were again counterstained with Safranin and finally washed with water. It is considered that with the help of this method the *M. tuberculosis* are seen in preference to *M. leprae*.

Radiology of bones and joints:

Out of a total of 218 cases of leprosy, 170 have been X-rayed and altogether 306 skiagrams have been studied with evidence of bone and/or joint changes in 134 cases. Of these 43 were lepromatous and 91 belonged to tuberculoid variety. There were 96 males and 38 females. The duration of the disease varied from 10-15 years. In almost all the cases two views, postero-anterior and oblique or lateral have been taken in double coated films without intensifying screens. A uniform standard was maintained as regards the quality of film. The X-ray generator used was (15 M.A./60 K.V.P.) or portable plant with self rectification. Attempts were made in all cases to avoid under or over exposures, keeping in mind that any difference in technique might alter the details of the skiagrams to a considerable extent. Fluoroscopic examination was not done on any occasion. The parts examined were 89 feet including the ankle, hands including the wrists 70, and knee joints 11.
Foot:

i) Antero-posterior:

Patient was placed on his back on the table, the knee flexed, with plantar surface of the foot on cassette. The foot was placed in a position so that principal ray was centered over the midportion of foot. The film target distance was 30". Radiographic factors were 40-45 K.V./12 M.A.S.

ii) Oblique view:

Patient lies on the table rolled well into the affected side, the dorsum of the foot resting on the cassette, opposite leg flexed at the knee and drawn upward out of the way. The principal ray was projected at an angle of 15° towards the head so that the principal ray is centered over the midportion of the sole of the foot. The film target distance was 30", and the radiographic factors were 40-45 K.V./10-12 M.A.S.

iii) Lateral view:

Patient was placed on the affected side on the table, outer margin of foot in contact with cassette, foot in lateral position with the principal ray perpendicular to the film. The principal ray was centered over the midtarsal region on
the inner side. The film target distance was 30" and the radiographic factors were 40 K.V./10-12 M.A.S.

Technique for knee joint:

Postero anterior view: The knee was placed on a cassette, the posterior surface of the knee lying in close contact with the cassette. The X-ray tube was tilted 15° towards the head with perpendicular ray centered to the lower margin of the patella. The factors used were 50 K.V./12 M.A.S. and the target to film distance was 30".

Lateral view: The patient was asked to lie on the affected side, knee flexed at right angles while the outer side was in contact with the cassette. The opposite leg was acutely flexed and drawn upward and forward out of the way. The part was shifted into position of film so that the principal ray was centered over the mid portion of the joint. The factors used were 50 K.V./12 M.A.S. and the target to film distance was 30".

Hand:

1) Palmar view:

Patient sat by the table placing his hand, palm up on the cassette, so that central ray was directed through the midportion of the hand on the cassette. The film
target distance was 30" and the radiographic factors were 40 K.V./12 M.A.S.

ii) Oblique view:

Patient sat by the table, ulnar margin of the hand resting a little to one side of a cassette. The hand was held obliquely, each finger uncovering adjoining fingers, the tips of the middle, ring, and little fingers resting on the cassette, the tips of the index finger and the thumb held together. The thumb was held parallel to the film. The central ray was directed at a right angle to the dorsum of the hand, while the midportion of the hand was lying on the centre of the cassette. The film target distance was 30" and the radiographic factors were 40 K.V./10 M.A.S.

Angiography:

Fourteen cases were selected of whom 6 were tuberculoid, 5 lepromatous and 3 as control, not suffering from any appreciable disease. Ulcers were present in 4 of the tuberculoid cases and 3 in lepromatous group. All were males and were suffering from nerve involvement. The site of selection was the brachial artery in the cubital fossa before its bifurcation. Eight cases were done by open method and 6 by blind method. The open method was by a small incision in the cubital fossa and the dye was injected
in the brachial artery where it was exposed. The wound was closed with interrupted sutures and the patient placed on antibiotics for 5 days subsequently. The blind method was rather difficult and 2 failures were recorded and in the open method there were 3 failures. The failures were mostly evident by non-visualisation of the peripheral arteries and when venous phase was only seen. Radio-opaque material used in all cases was 10 ml. Diodone 35\%.

Skiagram of the outstretched palm was taken in the antero-posterior position with the palm looking up. In absence of a serial changer, cassette with exposed films were changed very quickly at 5, 15 and 30 seconds, with the help of a team of personnel and a particular type of work assigned to particular person, e.g., one operating the switch at the exact time, another giving the injection, one lifting the hand, another removing the cassette and the last person putting the exposed film in the lead lined box. The person, who removed the exposed film put another film below the limb. The lead lined box was always kept near the X-ray couch when the injection was given. The target to film distance was 30" with 40 K.V. To keep the time factor to the lowest possible minimum a 200 M.A./80 K.V. set was used with 1/20 second as exposure time giving 10 M.A.S. Almost all the patients complained of severe spasm of the fingers with flexion deformity.
Venography:

Twenty-two cases of tuberculoid leprosy patients were chosen for venography; equal number of cases for lower and upper limbs with ulcers and three volunteers. All the cases belonged to the age group of 20-40 years and out of these 25 cases selected for venography, 3 were control, 19 males and 6 females. The duration of the disease varied from 2-10 years. All the patients had ulcers in hand or foot and 2 of them had in both the extremities. Four of the patients suffering from ulcers of the upper extremity had involvement of both ulnar and median nerve and ulcer in the tip of the ring finger and index finger. Four patients had bi-lateral involvement of the hands. Three had unilateral involvement of the ulnar nerve only with ulcers on the tip of the fingers.

Those selected for venography of the lower extremity had ulcers distributed over the sole of the foot and digits.

A slightly modified Moore's technique (1955) designated as superficial venography was used.

Technique for the lower limb:

Thirteen lower limbs were selected for the experiment, of these two belonged to volunteers. A small incision under
local anaesthesia was made and the long saphenous vein just in front of the medial malleolus was exposed. A 50 ml. syringe fitted with thick bore needle was fully charged with 35% solution of Diodone. The vein was canalised and the foot end of the table tilted down to about 45° degree angle below the horizontal level. 7 ml. of the Diodone solution was injected within 4 to 5 seconds and a lateral radiograph of the leg taken immediately. Another 15 ml. was then injected in a gush taking not more than 7 to 8 seconds and a radiograph of the thigh taken at the end of the injection.

For the leg, the knee was flexed so that the leg was kept at an angle of 45°. In absence of a serial changer, a higher capacity generator was used with an output of 200 M.A./80 K.V.P. to keep down the time. The factors were as follows: 50 K.V.P. - 12 M.A.S. the target to film distance was 30". For the thigh, a bigger cassette was used and the knee kept flexed as before and the film was placed beneath the thigh and a radiograph taken as soon as another 15 ml. was injected in one gush. A light tourniquet was applied to prevent quick return of the dye. Antero-posterior views were not helpful because of super-imposition. The same generator was used as for leg and the factors applied were 30" distance (target to film) 60-65 K.V. and 1/8th second giving 25 M.A.S. The wound was closed with multiple sutures and the patients were kept under antibiotics. All patients had uneventful recovery.
Technique for the upper limb:

Twelve subjects were selected for venography including one control. A small incision was made on the dorsum of the hand, the cephalic vein was exposed while a tourniquet was applied around the elbow. Similarly 30 ml. syringe was filled with 35% Diodone solution and injection made rapidly while the head end of the table was raised 45° degree and the tourniquet loosened. Skiagram of the forearm was taken when 5 ml. was injected. The entire forearm was placed on a 10" x 12" cassette and skiagram taken in both antero-posterior and lateral projections. The central ray passed through the centre of the cassette. For the arm, the tourniquet was applied to the axilla and another 10 ml. of the Diodone solution was injected with a gush and films taken in antero-posterior position as for the forearm. The radiological technique for the forearm as well as for the arm was that of angiography. The patients often complained of pain at the shoulder joint because of the spasm where the cephalic vein pierces the coraco-clavicular fascia.

Lymphangiography:

Lymphangiography was done in 6 lepromatous and 5 tuberculoid leprosy patients having ulcers on the plantar
surface of the foot. Two of these had more than one ulcer, 4 had one ulcer each. The ulcers were situated mostly on the lateral aspect of the plantar surface. Similar X-ray examination (lymphangiogram) was undertaken in five tuberculoid cases without any ulceration. They had only nerve involvement with anaesthetic patch in the leg, 2 in the right and 3 in the left. The healthy limb of 1 male patient was used as control.

The age of the patients varied from 30-50 years, 2 were in the fourth decade and the rest 4 were in the fifth decade. Of 11 patients only 1 was female. The duration of the disease varied from 5-10 years.

A preliminary subcutaneous injection of 11% patent violet blue in distilled water was given distal to the site of exploration. The lymphatics which looked like blue threads after being exposed by incision were injected with 70% Diodone. The injection was made rapidly. After 7 to 8 ml. were injected radiographs were taken immediately and after 2, 5 and 8 minutes to watch the transit of the dye upwards. While investigating the whole of the inferior extremities total 12 ml. of the dye was injected in a lymphatic vessel in front of the medial malleolus and radiographs for leg were taken after 2 minutes and of the thigh and inguinal region after 5 and 8 minutes respectively.
The radiological technique was 40-50 K.V. x 10 M.A.S. with 30" target to film distance.

Lungs:

Altogether, 128 patients, 46 lepromatous and 82 tuberculoid were selected for skiagram of lungs. The age variation was from 20 to 40 years. There were 97 males and 31 females. Duration of the disease varied from 10-30 years. In all the cases routine postero-antero views were taken in double coated film with intensifying screens. A standard technique and standard tube film distance was maintained. The tube was tilted in such a way, so that the principal ray was horizontal to the centre of the cassette. It was supported in upright position by vertical film holder, which was adjusted to the height of the patient. Patients stood erect before the vertical film holder with back to the tube. The chest was held in close approximation to the cassette, hands on hips with thumb held forward, head held high and chin resting on top. The X-ray generator output was 200 M.A./80 K.V.P. Factors varied from 7.5 M.A.S. to 10 M.A.S.; 65-70 K.V.P. and 60" - 72". Attempts were made in all cases to avoid under or over exposure.
Experimental studies

Procedures:

1) **Nerve crushing**: An experimental study was undertaken to produce trophic ulcer of the foot in rabbits by crushing the sciatic nerve. Five male rabbits weighing about 1 kg. each were selected for the experiment, while 3 were kept as control. One died during operation. The sciatic nerve in the mid-thigh was exposed by careful dissection and the nerve trunk was crushed by a Kocher's artery forcep under deep anaesthesia. The animals' hindlegs were X-rayed every 21 days for any bone changes. The radiographic technique was as follows: 40 K.V./20 H.A.S. and 30" target to film distance. Only 1 view i.e. posterior anterior view was taken. X-ray prior to operation was taken to exclude any pre-existing disease of the bones. The generator used was a 80 K.V./200 M.A. plant. Envelope wrapped X-ray films were used to ensure better details of bones.

11) **Animal intubation with sputum**: Fifteen guineapigs were selected for experiments as regards the probability of lung infection by *M. leprae* vis a vis *M. tuberculosis*. They were first X-rayed for lungs to detect any lung lesions and subsequently divided into three groups of five each and kept
in different cages. In the first group, sputum from advanced lepromatous subjects was instilled in the bronchi by means of curved pipette. In the second group, similar instillations were done with sputum from pulmonary tuberculosis patients, which was positive to acid fast bacilli. The instillation was repeated daily for 3 consecutive days. The third group was kept as control in the same way with sputum instilled from healthy persons. The animals were followed for about 6 months and in the meantime no immunity depressing agents were administered and repeated X-rays for lungs in postero anterior position were done every 12 weeks to note any abnormal shadow in the lung fields. The radiographic technique was 40 K.V./7.6 M.A.S. and 72" target to film distance. X-ray generator used was 80 K.V.P. - 500 M.A. rotating anode tube with fine focus.

The animals were sacrificed after 6 months for histological examination of lungs.

The excised lung tissue was fixed in 10% formal saline for 24 hours. It was then cut into several pieces of 4-5 mm each and washed in tap water for half an hour. The pieces were then kept in 70% alcohol for one night and in 90% alcohol for one night and these pieces were subsequently washed in absolute alcohol in 3 stages at an interval of one hour each. These were again washed in xylol in two stages at an interval
of half an hour each until these were transparent to light. Finally, these were bathed in paraffin and blocks prepared by microtome (size 4-5μ) for staining with Haemotoxylin and Eosin and finally stained for acid fast bacilli (Fite Ferraco technique).