DETECTION OF CALCIUM IN THE SKIN LESIONS OF LEPROMATOUS CASES OF LEPROSY BY HISTOCHEMICAL TECHNIQUE

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In 1955 McGee-Russell (5) reported the selective activity of "Kernechtrot," or nuclear fast red (Batch No. 3569 of G. T. Gurr), in staining the soluble inorganic calcium salts in animal tissues. Chowdhury *et al.* (2) showed that calcareous bodies in *Taenia saginata* reacted positively for calcium when subjected to McGee-Russell's technique. In this note, we present some of our findings on the presence of calcium in lepromatous skin as revealed by both nuclear fast red and von Kossa's silver method (4).

METHOD

Biopsy material from lepromatous lesions of the skin were fixed in 10 per cent neutral formalin for 24 hours. Frozen sections were cut 10 microns thick and subjected to treatment with: (1) von Kossa's silver technique, and (2) McGee-Russell's method for calcium. Some sections were treated with citrate buffer solution (pH 4.5-5.0) to remove the soluble portion of the calcium, and were then subjected to these two treatments. As von Kossa's reaction is dependent on the presence of a phosphate or carbonate radical and not on calcium alone, it was felt necessary to employ the technique of Bunting (1) for the identification of carbonate, and the microchemical test for the detection of phosphate in combination with calcium.

OBSERVATION

It was found that the histiocytes and macrophages of foamy nature present in the tissue took up the nuclear fast red very strongly, producing a brilliant red precipitate in and around these cells (Fig. 1). The rest of the tissue either reacted to the dye very faintly, or did not respond at all.

The presence of calcium in these areas was also confirmed by the von Kossa method (Fig. 2). Although the soluble calcium salt was removed from the tissue by treatment with citrate buffer, it nevertheless gave a faintly positive reaction to the silver method. This positive reaction indicated that calcium salts of either carbonate or phosphate were present in the tissue. Bunting's method for the detection of carbonate gave negative results, but the tissue reacted positively to the microchemical test for phosphate. Thus it appeared that calcium was present in the tissues in both soluble and insoluble forms, and that the latter was in combination with a phosphate radical.

It has long been known—most recently discussed by Dharmendra and Mukherjee (3)—that the histiocytes and macrophages of lepromatous lesions contain intracellular lipoid, a product of degeneration of phagocytosed lepra bacilli. It was interesting to note that the areas containing the

intracellular lipoid showed strong affinity for nuclear fast red. The intensity of the stain was found to be more or less proportionate to the concentration of cellular lipoid, as was evidenced from preparations stained by Sudan black and Sudan III and IV (Figs. 3 and 4). It may therefore be concluded that the distribution of calcium as phosphate salt was found in and around the cells containing lipoid, and that the calcium phosphate and lipoid occurred in these histiocytes and macrophages as loosely-bound calcium phosphate-lipoid complex.

RESUMEN

Se analizó piel lepromatosa en cuanto a la presencia de calcio con dos técnicas, la coloración de Kernechtrot o del rojo resistente nuclear y el método argéntico de von Kossa. Los histiocitos y macrófagos de índole espumosa absorbieron intensamente el rojo resistente nuclear, tiñéndose poco o nada el otro tejido. La intensidad de la coloración fué más o menos proporcional a la concentración de los lipoides celulares. Se dedujo que la distribución del calcio en forma de sal fosfática era en las células que contenían lipoide y alrededor de ellas y que el fosfato de calcio y el lipoide se presentaban en estos histiocitos y macrófagos en forma de un complejo de fosfato de calcio-lipoide, fijado sin mayor cohesión.

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DESCRIPTION OF PLATE

PLATE 3

FIG. 1. Calcium in and around the foamy cells of a lepromatous lesion, demonstrated by the Kernechtrot stain.

FIG. 2. Von Kossa's silver section, showing calcium phosphate in foamy cells.

FIG. 3. Intracellular lipoids in foamy cells, Sudan black stain.

FIG. 4. Intracellular lipoids in foamy cells, after Sudan III and IV staining.

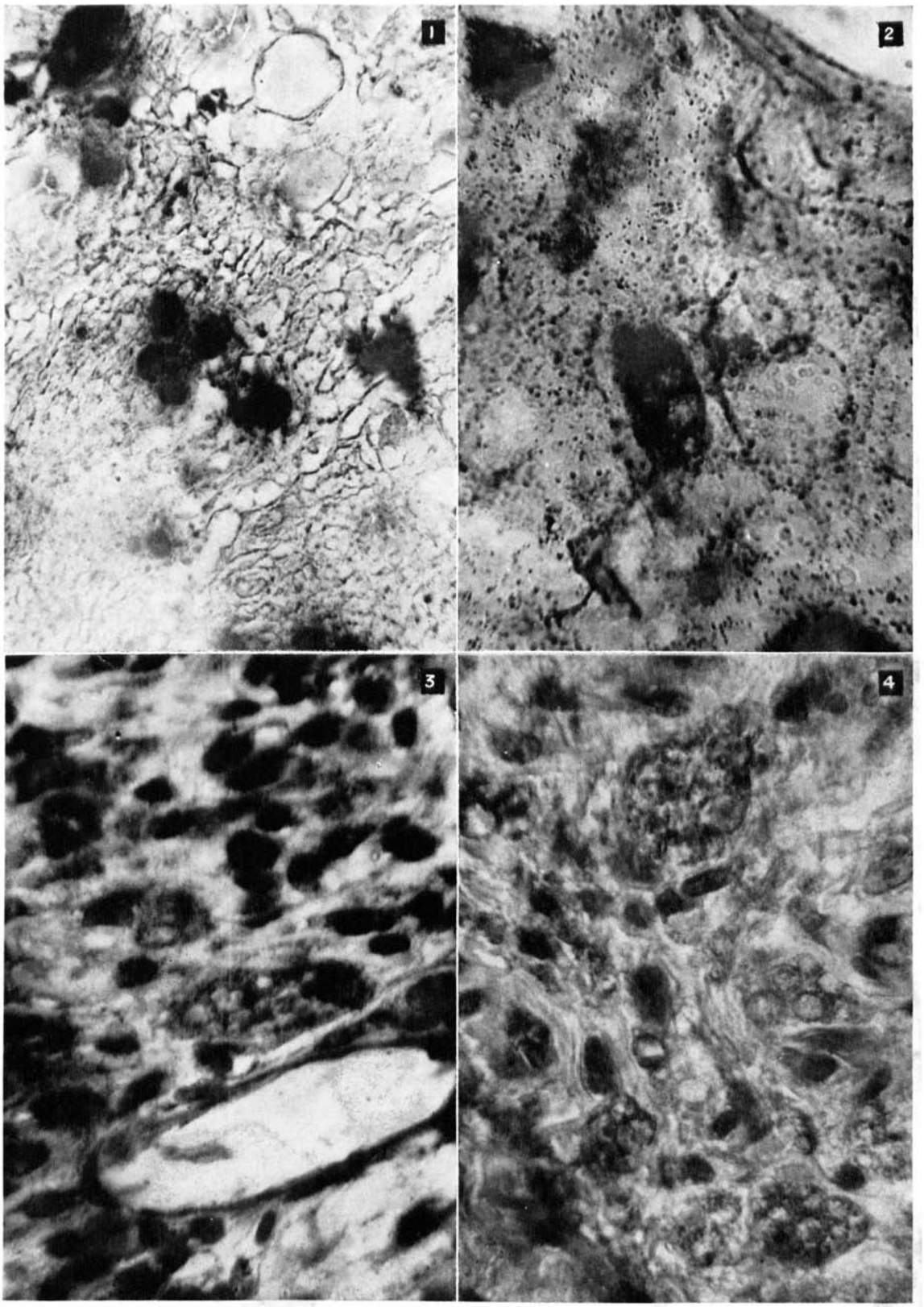

PLATE 3