# THE ORGANIZED EPITHELIOID CELL GRANULOMA: DIFFERENTIATION OF ALLERGIC (ZIRCONIUM) FROM COLLOIDAL (SILICA) TYPES

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The present study deals with a specific type of granuloma, namely, the sarcoid or tuberculoid reaction, produced experimentally in the skin of man by injections of certain metals.

The term granulomatous inflammation is difficult to define precisely; it is used by clinicians and pathologists as a wastebasket for patently dissimilar reaction patterns. To add to the confusion, many investigators fail to designate clearly the type of granuloma under study. This frequently creates a block to thinking and communication. Fortunately, the sarcoid or tuberculoid granuloma is pathologically distinct. In it one characteristically sees epithelioid and giant cells organized to form tubercles or nodules.[1-3]

In 1961, Shelley and Hurley proposed that the sarcoid reaction arose either as a hypersensitivity response to a delayed allergen or as a nonallergic foreign body reaction to a colloidal substance.[4] Their concept derived from two singular observations: (1) Intradermal injection of small quantities of zirconium salts in hypersensitive persons evoked an epithelioid cell response[5,6]; and (2) injections of larger amounts of colloidal silica caused granulomatous reactions in the skin of everyone.[7] We have repeated this work in man and, in general, have confirmed their observations. We wish, however, to extend the interpretation of these findings.

## MATERIAL AND METHODS

Twenty-eight healthy white men were given 2 to 8 intradermal injections of 1 to 4 mg. Ludox colloidal silica in saline. Ludox HS and Ludox LS, 30 per cent colloidal silica with an approximate particle diameter of 15 mμ., were obtained from E. I. DuPont de Nemours and Company, Wilmington, Delaware. Biopsies were performed at intervals of 1 day to 6 months later, fixed in 10 per cent neutral formalin or frozen at −75° C., and prepared for histologic examination, microincineration and fluorescence microscopy.

Nine subjects who had been experimentally sensitized to zirconium[8] were given

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repeated intradermal injections of 0.01 to 1.0 mg. of various zirconium salts and the sites removed for biopsy and prepared for study 1 day to 13 months later; over 100 biopsy specimens of this type were examined.

The sections were stained with hematoxylin and eosin and by a variety of histochemical methods. Paraffin-embedded tissues were cut in serial section at 5 $\mu$, fixed on slides and microincinerated at 650° C. for 90 minutes by Dr. Donald Opdyke (the Procter and Gamble Company, Cincinnati, Ohio). The slides were washed in acid (0.1 N HCl at 37° C.) for 3 hours to extract all minerals except silica and certain metals.[9] The tissues for fluorescence microscopy were prepared after the technique of Coons[10] as used in our laboratory.[11] We were especially interested in autofluorescence of injected silica and zirconium and in localizing gamma globulin in the tissues. As indicated in a prior report,[8] radioactive Zr-Nb$^{95}$ was used to identify the anatomic location of zirconium in tissue.

The histologic sections were examined by light, phase, dark field and fluorescence microscopy.

## Results

The sequence of events occurring after injection of zirconium salts has been described[8]; our findings with colloidal silica confirmed those of Shelley and Hurley[7] and need not be repeated. The significant observation was that the responses to these two agents differed markedly in their mode of development and in the final histologic appearance.

To make our point as clear as possible and to prevent confusion requires a definition of the term "epithelioid cell." Unfortunately, with indiscriminate usage the term has become so vague as to be almost meaningless. We accept the restricted view which holds that these cells have definite structure and an organization simulating epithelial cells.[1,12] The epithelioid cell is large and irregular, with poorly defined cytoplasmic margins joining imperceptibly with its neighbor. The abundant cytoplasm is eosinophilic and amorphous or finely granular. The nucleus appears large, ovoid and palely stained, with a sharp, delicate nuclear membrane. The chromatin is finely clumped, and there often is a centrosome (Fig. 1). Most important, the cells tend to organize into tubercles. This cellular pattern characterizes conditions such as sarcoidosis, tuberculosis and tuberculoid leprosy. It occurs in other diseases but is usually less definitive. Strict adherence to the histologic criteria outlined has allowed the Nickerson-Kveim antigen to become an accurate diagnostic tool in sarcoidosis.[13,14]

With this definition in mind, we may consider the distinguishing features of zirconium and silica granulomas. The cellular reaction following intradermal injection of zirconium in hypersensitive subjects showed a prolonged sequence of events leading to the appearance of epithelioid cells. Focal collections of lymphocytes and monocytes appeared after 2 weeks. By 3 to 4 weeks odd clusters of large monocytes developed within these foci. At this time they were not true epithelioid cells; their

nuclei were vesicular, with clumps of chromatin, and the cytoplasm was vaguely foamy. Not until 6 or 8 weeks had elapsed did true epithelioid cells appear. These cells formed distinct tubercles (Fig. 2). They persisted for months or years (Figs. 3 and 4). This response was vastly different from the usual foreign body reaction to injected zirconium in nonsensitive persons.[15]

In our experimentally produced skin lesions of silicosis, on the other hand, the cells in question appeared *de novo* very early. The cell was recognizable within 7 days (Fig. 6); Shelley and Hurley have described it within 3 days.[7] It remained morphologically distinct without changing for months. The cells clearly invested blood vessels and did not organize into tubercles (Fig. 5). In fact, closer examination disclosed that they were not epithelioid cells as we have defined them (Figs. 6 and 7). The nuclei often were ovoid, but they were small and dark-staining and the chromatin was coarsely clumped; the cytoplasm contained a loosely arranged, eosinophilic, fibrillar substance. The cells did not relate to each other in epithelial pattern. They tended to parallel small blood vessels.

What sort of cells are they? We have been told they represent silica-containing macrophages,[4,7,9] and this we confirmed. The acid-extracted and microincinerated preparations revealed large amounts of refractile material, presumably silica,[9] in the region of the phagocytic cells (Fig. 8). The method, however, did not allow accurate localization of silica within cells (Figs. 9 and 10). This was accomplished by the use of histochemical methods. Within the cytoplasm of these cells we saw fairly large, smooth, metachromatic granules (Fig. 11); they were also PAS-positive and diastase-resistant, and gave a positive colloidal iron reaction. Their structure and staining qualities differed from mast cell granules. Under fluorescence microscopy the silica granules gave a bluish-white autofluorescence; mast cell granules in our preparations appeared golden-orange. Finally, phase microscopy of stained and unstained sections disclosed a brilliant refractivity of the granules (Fig. 12); mast cell granules were not visualized by this method.

In the epithelioid cells of zirconium-induced lesions, no intracytoplasmic inclusions were demonstrated. Microincineration revealed refractile material *only* in the area of macrophages which contained radioactive zirconium ($Zr\text{-}Nb^{95}$)[8] and a PAS-positive substance[15] (Fig. 12). No metallic substance could be demonstrated in epithelioid cells (Fig. 13). This would indicate that epithelioid cells do not contain zirconium.[8]

The search for gamma globulin with fluorescence techniques disclosed a normal distribution in vascular lumens in both silica- and zirconium-induced lesions. This finding runs counter to the supposed increase of gamma globulin in silicotic lungs.[16,17]

## Discussion

Our findings indicate that intradermal injections of colloidal silica cause granulomatous inflammation in 100 per cent of subjects. It develops rapidly, within a few days, and persists for months. The main cell in the infiltrate is a phagocyte containing small aggregates of silica and a metachromatic substance, presumably a mucopolysaccharide. Curiously, a somewhat similar material coats phagocytized zirconium.[15] The role mucopolysaccharides play in phagocytosis of metals remains to be determined. It should be noted that phase microscopy was the best method for visualizing the very small particles of silica.

The primary result of this work, however, was to raise the question whether the silica-induced reaction qualified as an organized, tuberculoid-type of epithelioid cell granuloma. Historically, silicosis has been classed as a variety of tuberculoid reaction,[18,19] but our histologic observations of clinical lesions in pulmonary and cutaneous silicosis have raised doubts about the verity of this view. Typical epithelioid cell nodules were not seen; the reaction was primarily phagocytic and fibrotic. By our definition it was merely a particular type of foreign-body response to a fibrogenic agent. Experimental cutaneous silicosis in man followed the same pattern. The lesion was not characterized by epithelioid cell tubercles. The cells contained silica; they developed *de novo* as phagocytes. In zirconium-induced lesions, on the other hand, epithelioid cells developed slowly from mononuclear cells; they tended to organize into tubercles and did not phagocytize zirconium. Zirconium-laden phagocytes occurred in the lesion, but they remained distinct from the epithelioid cell response; no intermediate forms were seen.[8] The cell type, pattern, and sequence of evolution closely paralleled the response to injections of Kveim antigen[13,14] in patients with sarcoidosis.

These disparate findings with two distinct models of granulomatous inflammation in man provide speculative insight into possible mechanisms of the sarcoid or tuberculoid reaction. The etiology of sarcoidosis has been explained in numerous ways.[19] At present most authors view the disease as a reaction pattern to different etiologic agents; even the idiopathic variety has many causes. Shelley and Hurley[4] support this notion, but they narrow the inciting agents to two main classes; (a) allergic, and (b) colloidal stimuli. Our observations lead us to believe there is only one mechanism for the sarcoid reaction, namely, through an allergic or hypersensitivity response. The classical epithelioid cell is not a phagocyte in the usual sense. It develops slowly and does not contain the inciting agent in the case of zirconium[8] and probably also not in tuberculosis or leprosy.[1,19] Phagocytic epithelioid-like cells should not be confused with the genuine article.

Our concept implies a single mechanism but not a single cause for the sarcoid reaction. Organized epithelioid cell granulomas occur regularly in sarcoidosis, tuberculosis, tertiary syphilis and tuberculoid leprosy. They may be seen in leishmaniasis,[20] berylliosis[21] and tattoos.[22,23] We have observed this response to the green pigment (chromate) in a tattoo. No doubt other inciting agents exist, but the complete tuberculoid response with its epithelioid cells, giant cells and tubercle formation do not usually occur in deep fungus infections or after injections of Freund's adjuvant[24–26] or other foreign substances. It should be stressed that most tattoo reactions do not show the pattern of granulomatous hypersensitivity.[23] Berylliosis probably also fits in this category; only one case of proven granulomatous hypersensitivity is reported,[21] and the experimental model in animals does not contain organized epithelioid cell tubercles.[27]

To elicit the organized epithelioid cell reaction requires the intervention of a unique type of delayed hypersensitivity about which next to nothing is known.

## Summary

The techniques of histochemistry, microincineration, radioautography and fluorescence microscopy were used to compare and contrast allergic (zirconium) and colloidal (silica) granulomas produced experimentally in the skin of appropriate human volunteers.

Striking differences were detected. Colloidal silica was handled primarily as a foreign body with the formation of silica-laden phagocytes that collected perivascularly and imitated epithelioid cells. Zirconium, on the other hand, induced the formation of true epithelioid cells that organized into characteristic tubercles and did not phagocytize zirconium.

These findings indicate that colloidal and allergic granulomas can be distinguished when specific histologic criteria are applied. In addition, special techniques will reveal that the colloidal reaction is essentially phagocytic while the allergic response goes beyond that. Our evidence is consistent with the idea that all organized epithelioid granulomas, namely, tuberculosis, sarcoidosis, and tuberculoid leprosy, etc., are based on a mechanism of hypersensitivity.

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[ *Illustrations follow* ]

## Legends for Figures

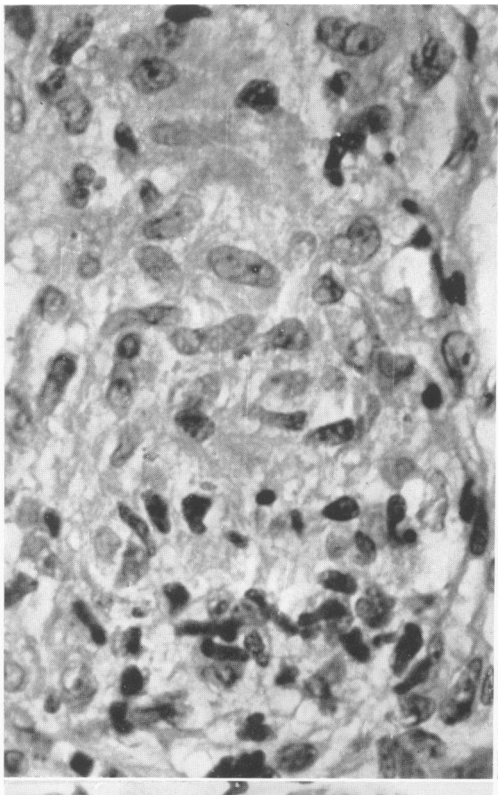
Except where indicated, photomicrographs were prepared from sections stained with hematoxylin and eosin.

Fig. 1. A typical epithelioid cell tubercle in a case of sarcoidosis. The epithelioid cells have poorly defined cytoplasmic borders with an amorphous eosinophilic cytoplasm. The nucleus is large and ovoid and stains palely. The nuclear membrane is distinct; chromatin is finely clumped. × 520.

Fig. 2. Twelve weeks after injection of 0.1 mg. zirconium lactate in a sensitive person reveals tuberculoid nodules throughout the corium. × 67.

Fig. 3. Details of an epithelioid cell nodule in a zirconium granuloma. More inflammatory cells are seen than in sarcoidosis; compare with Figure 1. × 520.

Fig. 4. A higher power view of a zirconium granuloma emphasizes the structure of epithelioid cell nuclei. Note the delicate chromatin pattern. × 1,300.



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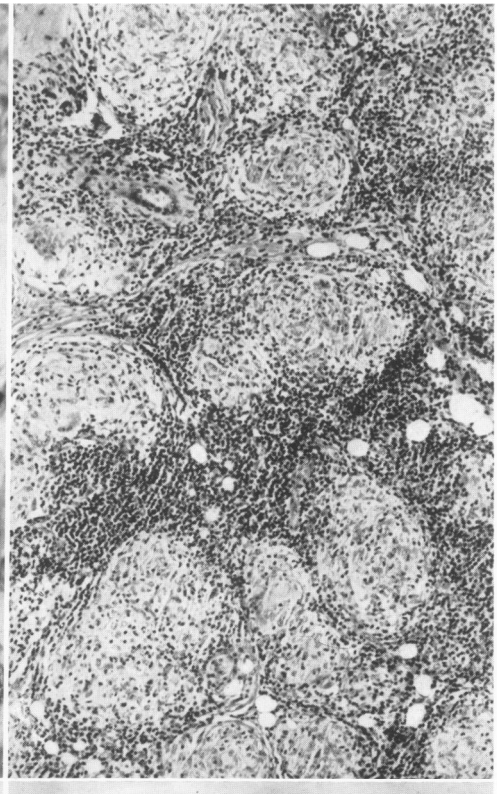

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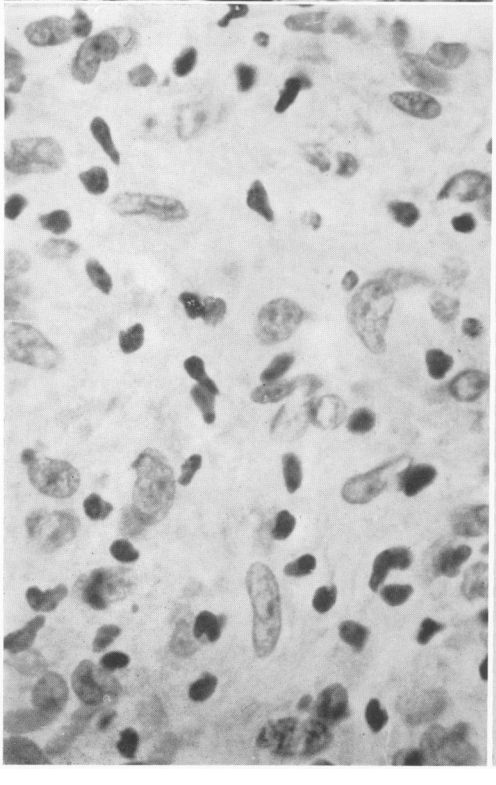

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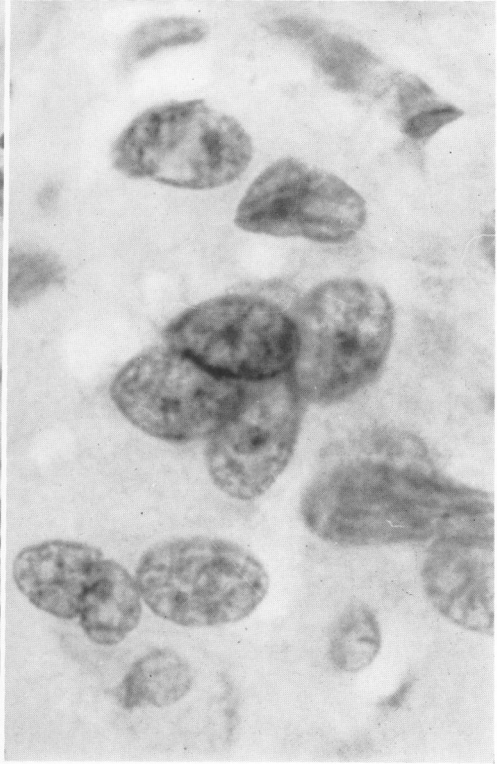

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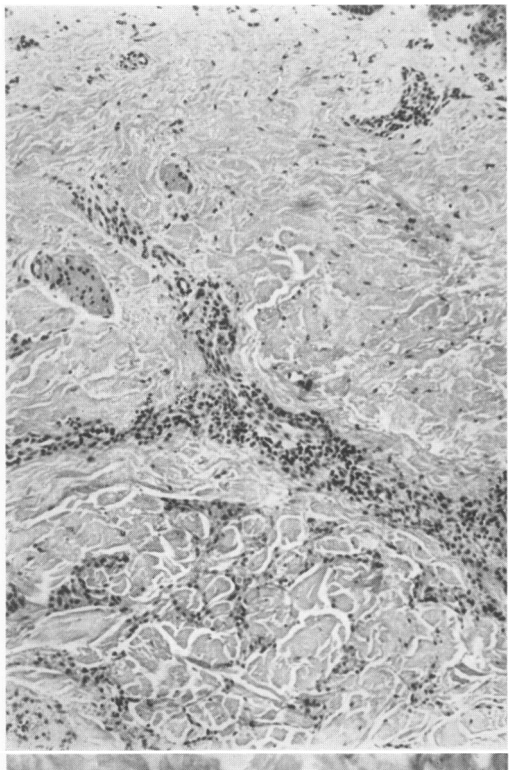
FIG. 5. Ten weeks after the injection of 1 mg. of colloidal silica. The inflammatory cells have a perivascular distribution, and there is a relative paucity of reaction. Compare to Figure 2. × 67.

FIG. 6. One week after 1 mg. of colloidal silica. A few cells with enlarged nuclei and vague cytoplasm appear about a small blood vessel. The nuclear chromatin is intensely stained. × 325.

FIG. 7. Fifteen weeks after 4 mg. of colloidal silica. The foamy cytoplasm and darkly clumped chromatin show well. The cells are not truly epithelioid. Compare to Figures 1 and 3. × 520.

FIG. 8. Dark field of microincinerated and acid-washed preparation from a silica-induced lesion indicates the perivascular concentration of brightly refractile material. × 130.



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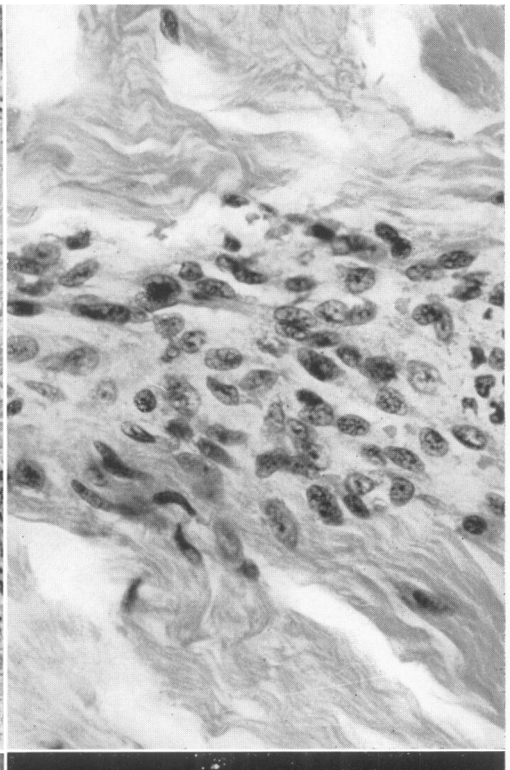

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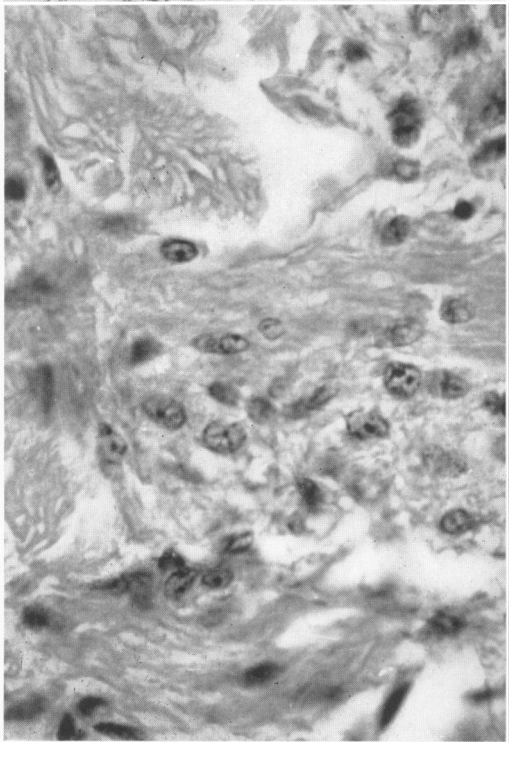

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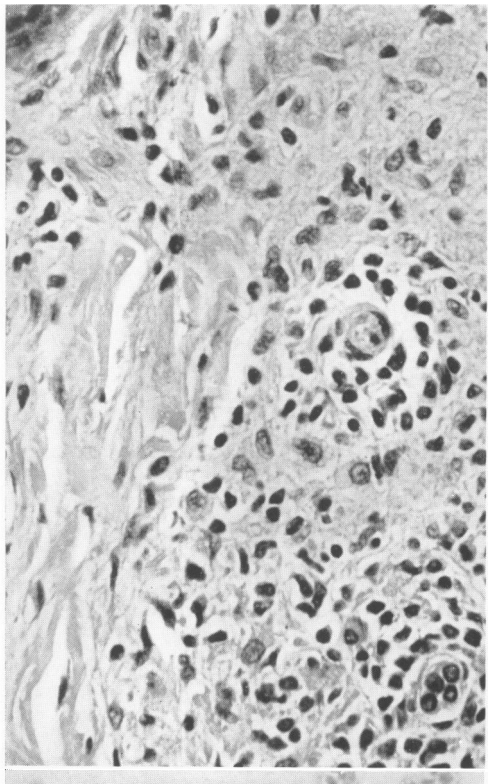
FIG. 9. Serial section of Figure 10. For orientation of silica-induced lesion. Epithelioid-like cells are in perivascular location. × 325.

FIG. 10. Microincineration and phase microscopy. Refractile granules, presumably silica, appear in the region of the perivascular cells. × 325.

FIG. 11. Silica-induced lesion, biopsied and frozen at −75° C., cut, and stained with toluidine blue (pH 7.2). The intracellular granules are metachromatic. × 1,300.

FIG. 12. A similar preparation viewed by phase microscopy. The refractile granules stand out in all lightly stained or unstained preparations. × 1,300.



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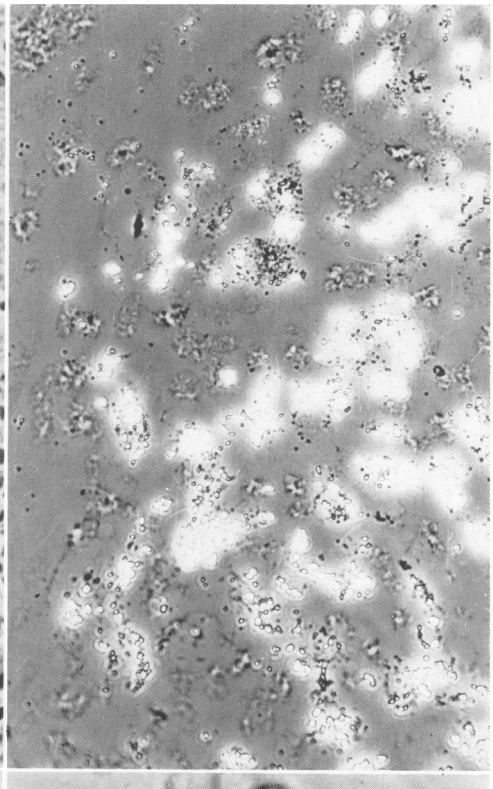

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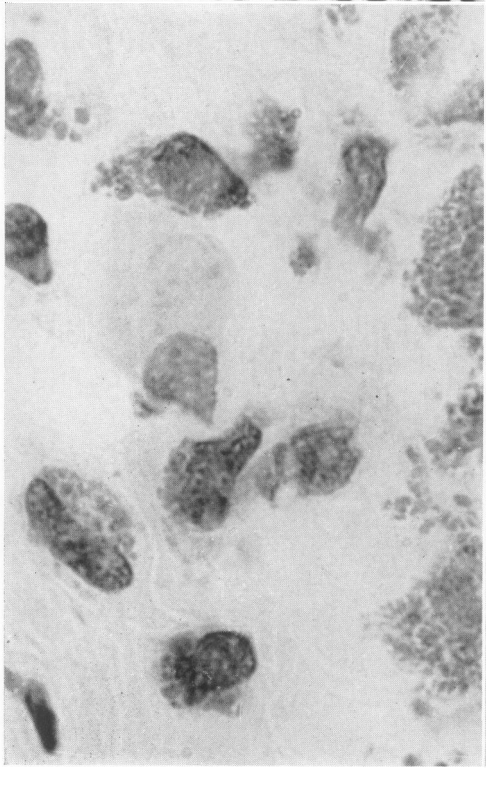

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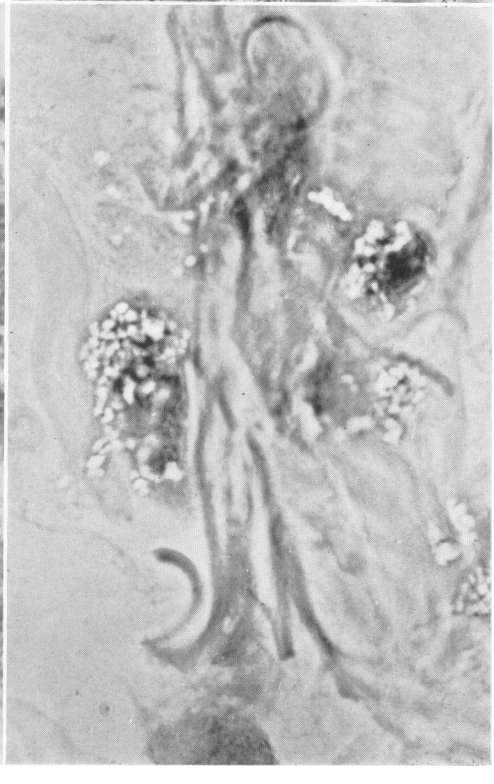

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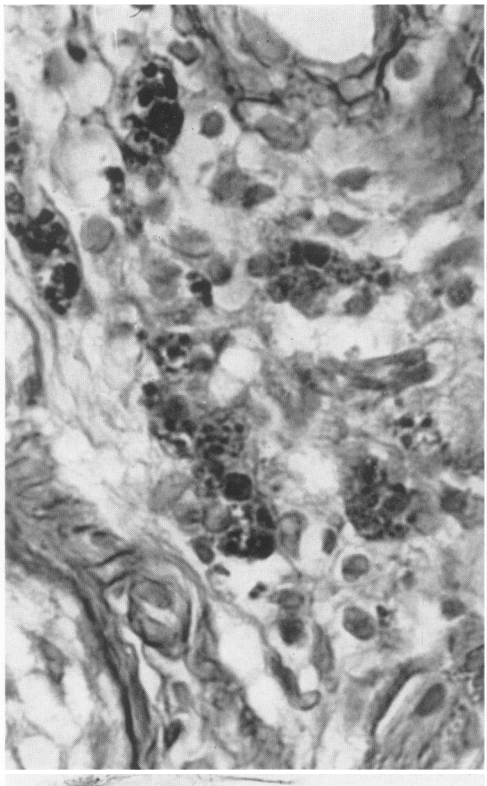
FIG. 13. Zirconium lactate injected into a nonsensitive person. Intracytoplasmic granules of variable size are strongly PAS-positive. A sebaceous gland in left lower corner serves as a landmark. Periodic acid-Schiff stain, diastase digestion. × 666.

FIG. 14. Microincineration and phase microscopy. A serial section of Figure 13 shows refractile material (zirconium) in the region of phagocytes. × 520.

FIG. 15. A typical epithelioid cell tubercle with a giant cell from a zirconium granuloma for comparison with Figure 16. × 166.

FIG. 16. Microincineration and phase microscopy of a serial section reveals the lack of refractile substances in the epithelioid cells. × 208.



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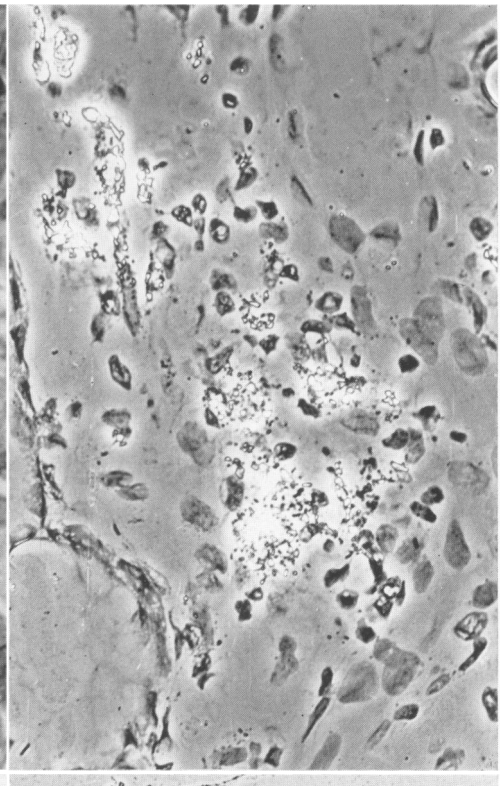

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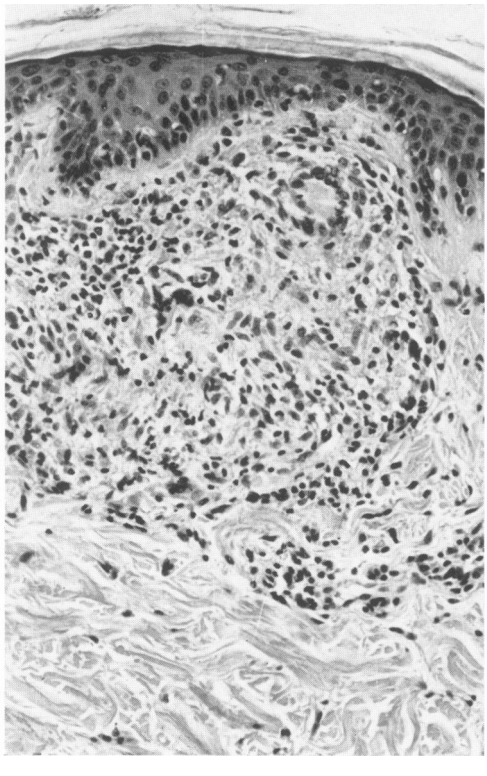

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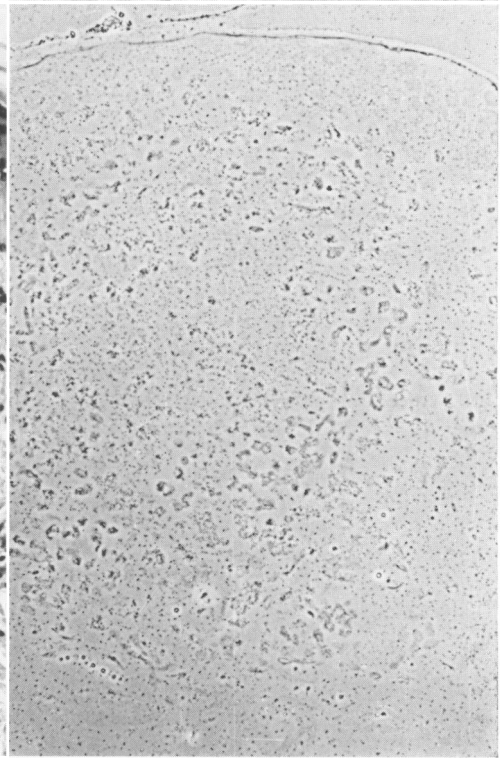

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