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Fine structure and histochemistry of epithelioid cells in sarcoidosis

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James, E. M. Valerie and Jones Williams, W. (1974). *Thorax*, **29**, 115–120. **Fine structure and histochemistry of epithelioid cells in sarcoidosis.** In this fine structure study of epithelioid cells in the granulomata of sarcoidosis we confirm our previous findings that they appear to be synthesizing rather than phagocytosing cells. The numerous characteristic intracytoplasmic vesicles, 0·5 μ in size, are shown to contain mucoglycoprotein and only rarely acid phosphatase activity. Epithelioid cells show varying quantities of extravesicular acid phosphatase activity which is sometimes on the outer surface of the protein-containing vesicles. The possible role of secretory products of epithelioid cells in the formation and persistence of granulomata is discussed. It is suggested that epithelioid cells may be forming lymphokines.

We have previously demonstrated that the fine structure of granulomata in sarcoidosis and tuberculosis (Jones Williams, Erasmus, James, and Davies, 1970), in the Kveim test lesion (Jones Williams, 1972), and in chronic beryllium disease (Jones Williams, Fry, and James, 1972a) are similar. The constituent epithelioid and giant cells show numerous intracytoplasmic vesicles, varying in size from 0·4 to 0·7 μ in diameter, and contain amorphous lightly granular material (Fig. 1). Evidence of active or previous phagocytosis in epithelioid cells is uncommon, and we consider that the cells are essentially biosynthetic rather than phagocytic.

Our present histochemical study demonstrates that the majority of the vesicles contain mucoglycoproteins but not acid phosphatase. The significance of these findings and the possible role of secretory substances in granuloma formation are discussed.

MATERIAL AND METHODS

ACID PHOSPHATASE Tissue blocks of three sarcoid lymph nodes and one sarcoid skin biopsy were fixed in 3% glutaraldehyde in 0·067 M cacodylate buffer, pH 7·4 for two to four hours. After being washed overnight in 0·1 M cacodylate buffer the tissues were incubated for 15 to 30 minutes in the acid phosphatase medium as either minute tissue blocks or 50 μ Sorvall sections. The acid phosphatase technique used was that of Ericsson and Trump (1965) using the lead nitrate Gomori technique. Substrate-free and sodium fluoride control blocks were also prepared. After being washed in acetate buffer solutions the tissues were post-fixed in Millonig's osmium tetroxide, dehydrated, and embedded in Araldite. Sections were cut on an LKB ultramicrotome and 0·5 μ sections were stained with toluidine blue for light microscopy identification of epithelioid cell granulomata. Ultrathin sections were examined, either unstained or stained with uranyl acetate only.

In order to assess the methods used we examined and demonstrated intravesicular acid phosphatase activity in macrophages from a number of tissues, including inflammatory lymph nodes, hyperplastic spleen, and biochemically proven lysosomal fractions from another hyperplastic spleen.

MUCOGLYCOPROTEINS Tissue blocks from two mediastinal sarcoid lymph nodes and one positive Kveim biopsy were fixed in 3% glutaraldehyde in 0·1 M phosphate buffer pH 7·4 for two to four hours. After being washed overnight in 0·1 M phosphate buffer the tissue was dehydrated, without postfixation in osmium tetroxide, and embedded in Araldite. Ultrathin sections of granulomatous areas were mounted on uncoated gold grids and were stained for the detection of 1:2 glycol groups using the periodic acid, chromic acid silver methenamine (PA-CrA-silver) technique of Rambourg (1967) and Rambourg, Hernandez, and Leblond (1969). Control grids included those (*a*) unoxidized, and (*b*) blocked for four hours with a 2:3 solution of acetic anhydride and pyridine before oxidation.

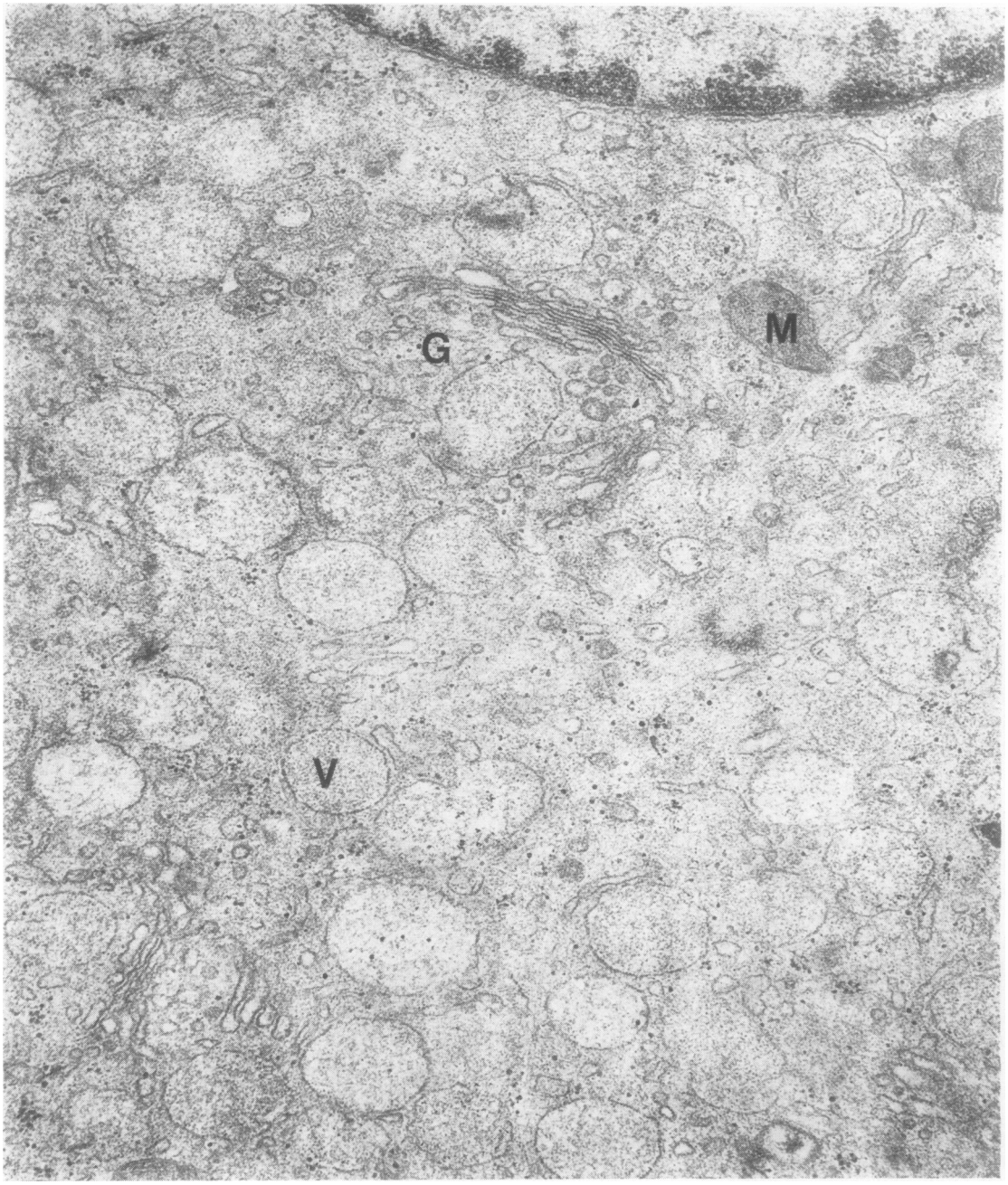

FIG. 1. *Epithelioid cell showing (V) abundant lightly stained vesicles, (G) large and numerous Golgi complexes, and (M) mitochondria. Electron micrograph (EM)* ×35,350.

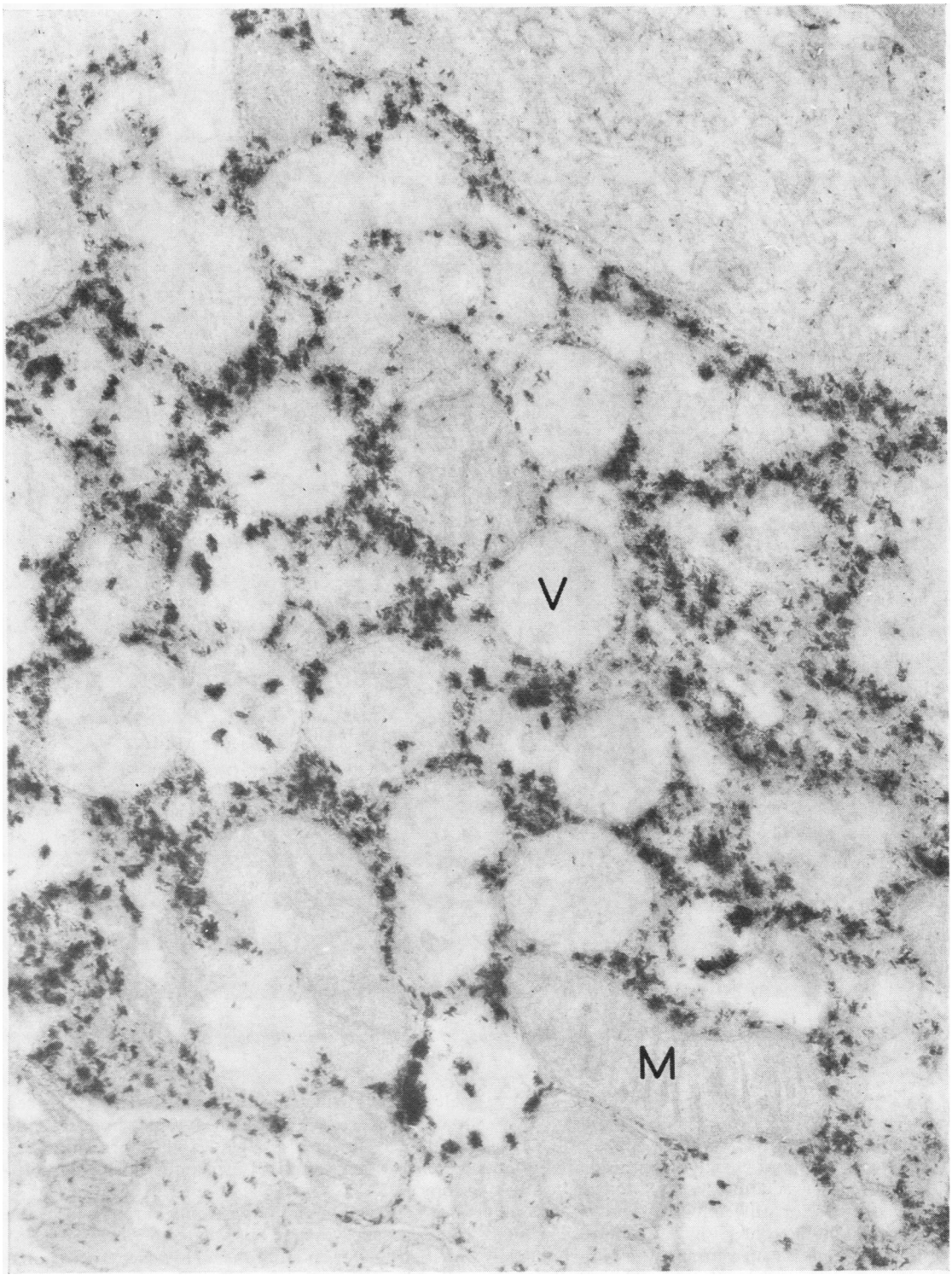

FIG. 2. *Extravesicular acid phosphatase activity in epithelioid cell. Modified Gomori technique. EM* ×35,650.

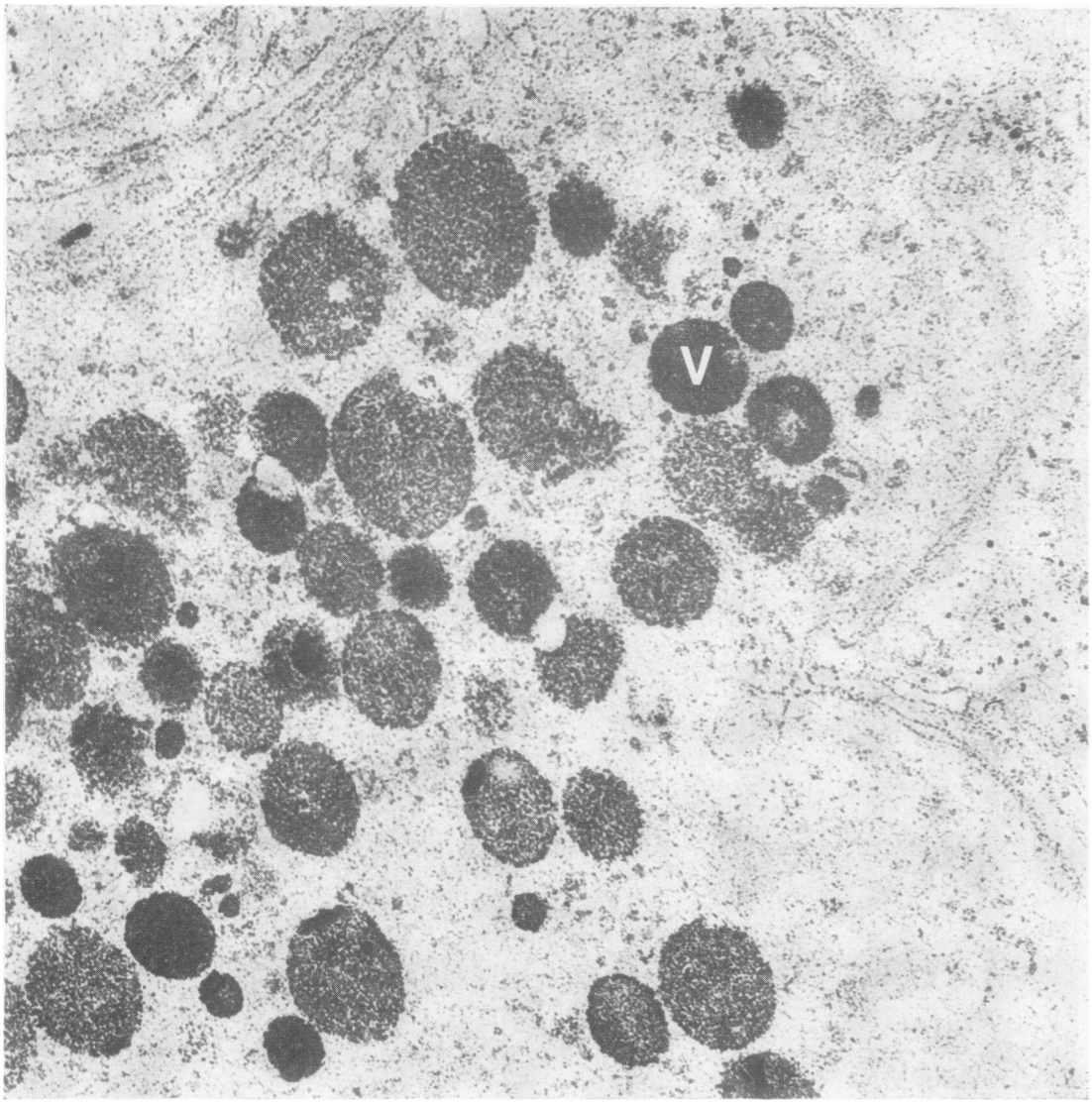

FIG. 3. *PA-CrA-silver stained vesicles illustrating the presence of* 1: 2 *glycol groups in an epithelioid cell. EM* × 31,185.

RESULTS

ACID PHOSPHATASE The localization of acid phosphatase, sodium β-glycerophosphatase in epithelioid cells was similar when studied in 50 μ Sorvall sections or tiny blocks. The epithelioid cells showed slight to heavy granular lead deposits free in the cytoplasm surrounding the mitochondria and characteristic vesicles (Fig. 2). Those cells with heavy enzyme staining contained abundant, large, pale vesicles which on light microscopy appeared foamy. Those with less heavy enzyme staining contained fewer, smaller, and more granular vesicles which were inconspicuous on light microscopy.

The enzyme staining was only rarely within the characteristic vesicles but was sometimes attached to their outer membrane. However, occasional cells showed a few small isolated vesicles with heavy enzyme staining. Lymphocytes and non-epi-

thelioid mononuclear cells also showed occasional stained vesicles but no generalized cytoplasmic staining.

The majority of cell nuclei showed a varying density of lead staining but this could not be correlated with the cell type.

Both the substrate-free and sodium fluoride control technique resulted in completely negative acid phosphatase staining in all epithelioid cells.

MUCOGLYCOPROTEINS The epithelioid and giant cell vesicles gave a strong positive reaction with PA-CrA-silver technique (Fig. 3). The larger vesicles gave a stronger reaction than the smaller vesicles and the reaction product was most conspicuous on the inner aspect of the vesicle membrane. Many of the vesicles showed a central globular area of negative staining. Unoxidized and blocking controls resulted in negative staining. These results demonstrate the presence of 1:2 glycol-groups in the vesicles.

Both epithelioid and giant cells also show varying numbers of electron dense, 222–296 Å diameter, particles. These are randomly distributed throughout the cytoplasm and are occasionally aggregated within membrane-bound vesicles. These particles are considered to be glycogen as they do not stain with the silver methenamine technique after periodic oxidation alone but are stained after treatment with both periodic and chronic acids. This result is similar to that obtained in staining glycogen in liver. The particles are not ribosomes, as ribosomes are difficult to visualize in unstained sections and measure only 148–178 Å in diameter.

In addition, the carbohydrate cell coat, occasional small Golgi vesicles, and collagen were also stained and considered to be 1:2 glycol groups, as the reaction was abolished in unoxidized and blocking controls. Ribosomes and nuclear chromatin remained positive in the controls and therefore were regarded as being non-specifically stained.

DISCUSSION

Our present report highlights the problem as to the exact nature and role of epithelioid cells in sarcoid-type granulomata; in particular, whether they are primarily phagocytic (macrophages) or synthesizing cells. The problem is complicated in that the two functions are not necessarily mutually exclusive and may change with time.

We consider that epithelioid cells in mature human granulomata do not show the features of phagocytic (macrophage type) cells. They rarely contain foreign material, residual bodies, phagocytic vesicles or acid phosphatase containing vesicles. Our results also show that they do contain numerous mucoglycoprotein-containing vesicles, Golgi complexes, and mitochondria. We cannot accept that the so-called 'epithelioid cells' produced under experimental conditions and developing from monocytes/macrophages (Papadimitriou and Spector, 1971 and 1972) are comparable to human epithelioid cells. These experimental cells are rarely in closely packed foci, as in the human situation, are often described as containing lysosomes, lipid droplets, and sometimes phagocytosed debris, and do not contain the characteristic 0·5 μ diameter vesicles of human epithelioid cells.

On light microscopy we have previously demonstrated (Williams, Jones Williams, and Williams, 1969) with the Gomori technique the presence of acid phosphatase in epithelioid cells and interpreted the findings as indicating the presence of lysosomes. However, our present ultrastructural results do not confirm the above findings as the enzyme activity when present was predominantly extravesicular and sometimes attached to the outer membrane of the vesicles. We cannot explain the difference merely on technique as within the same tissue we could show intravesicular staining in non-epithelioid cells and in macrophages in inflammatory lymph nodes. Further, we think it unlikely that intravesicular enzyme has leaked into the cytoplasm because the dense activity which would have been expected on the inner surface of the vesicles was inconspicuous. We, therefore, conclude that we may have misinterpreted the granular staining on light microscopy as membrane-bound when in fact it was extravesicular and on the outside of the membrane.

The occasional close apposition of acid phosphatase activity around secretory granules parallels that found by Smith and Farquhar (1966), who in a study of hormone secretory granules in pituitary glands showed an increase of enzyme activity around secretory vesicles commensurate with their increase in number. They interpreted these findings as indicating a protective feed-back process in cells with excessive hormone production. A similar explanation may apply to excessive secretion in epithelioid cells.

Our description of the fine structure of epithelioid cells agrees with that of other workers (Wanstrup and Christensen, 1966; Epstein, 1971) and we have now confirmed Gusek's (1965) suggestion that epithelioid cells might contain mucoglycoproteins. This latter feature, together with the prominent Golgi complexes, suggests

that the cells are actively engaged in biosynthesis.

It is interesting to speculate on the possible role of secretory products of epithelioid cells. It has been suggested that they form para-amyloid (Wanstrup and Christensen, 1966), amyloid (Gusek, 1968), and a granuloma-inciting factor (Epstein, 1971). Jones Williams *et al.* (1970) suggested that the product may stimulate antigenic committed lymphocytes to develop into epithelioid cells. It has recently been shown (Becker, Krull, Deicher, and Kalden, 1972; Jones Williams, Pioli, Jones, and Dighero, 1972b) that lymphocytes from sarcoid patients, when stimulated *in vitro* by Kveim antigen, produce a macrophage migration inhibition factor which Remold and David (1971) on chemical analysis consider to be a glycoprotein.

In conclusion, we consider that epithelioid cells show both morphological and histochemical evidence of a synthesizing cell. The product appears to be a mucoglycoprotein which, if epithelioid cells develop from lymphocytes, may be a lymphokine (Dumonde, 1970) and thus play a part in both the formation and persistence of granulomata.

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