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A Function of the Eosinophil: Phagocytosis of Antigen-Antibody Complexes. (28134)

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Although it is known that eosinophilia can be readily produced by repeated injections of foreign protein(1) and can also be mediated by injection of immune complexes(2), the function of these cells in hypersensitivity states has heretofore not been demonstrated.

Previous studies have shown that antigen-antibody complexes can be visualized with the electron microscope when the electron dense protein ferritin is used as antigen(3). This technic was employed in the present investigation in an attempt to elucidate the function of eosinophils obtained in peritoneal exudates induced by injection of foreign proteins in previously sensitized animals.

Materials and methods. 1. Method of immunization. Horse spleen ferritin, Nutritional Biochemicals, Cleveland, Ohio, was used to sensitize 2-month-old Hartley strain male guinea pigs. Equal volumes of 0.1% ferritin in normal saline solution and Complete Adjuvant (Freund) Difco Labs, Detroit, Mich., were emulsified. The guinea pigs were immunized by injecting 1 cc of the emulsion into multiple sites in the skin and toe pads. Two weeks later the animals received similar injections with 1 cc of an emulsion of Incomplete Adjuvant (Freund) and ferritin.

2. Specificity of anti-sera for ferritin. This schedule of immunization results in formation of antibodies which are immunoelectrophoretically specific for ferritin(4). The anti-sera form complexes with ferritin which have a characteristic electron microscopic appearance. The adequacy of the present immunization was determined by the production of Arthus lesions in the skin of sensitized animals 2 weeks after injection of the Incomplete Adjuvant-Ferritin emulsion. Animals so tested were challenged by intradermal injection of 0.1 cc 1% ferritin solution. Classical Arthus reactions developed in all sensitized animals within 6-8 hours. Anti-sera obtained at the time of challenge were tested for precipitating antibody by layering 0.3 cc ferritin over 0.3 cc anti-sera in a narrow tube. In all of the tubes a precipitate formed at the interface between the antigen and anti-sera.

3. Production of peritoneal exudates. One week after the demonstration of Arthus sensitivity, 5 mg of ferritin in 1 cc normal saline solution was slowly injected into the peritoneal cavity with a 21 gauge needle. Twenty-four hours later the animals were rapidly killed with ether, their peritoneal cavities exposed by a mid-line incision in the anterior

abdominal wall, and all of the peritoneal exudate aspirated with a 16 gauge needle.

4. Preparation of peritoneal exudate for electron microscopy. The peritoneal fluid was centrifuged at 6,000 rpm in a Sorvall SS1 centrifuge for 30 minutes, the supernatant aspirated and the sedimented cells fixed immediately for electron microscopy by adding Palade's buffered 1% osmium tetroxide fixative to the centrifuge tube. The pellet was fixed for 30 minutes, dehydrated in a graded series of alcohols and embedded in 1:5 methyl-butyl methacrylate. Ultra thin sections were cut on a Porter-Blum microtome, mounted on collodion coated copper mesh grids and examined with an RCA EMU 3F Electron Microscope.

Results. 1. Gross appearance of exudates. Cloudy blood-tinged exudates, measuring 5-7 cc were present in the peritoneal cavities of sensitized guinea pigs 24 hours after the injection of ferritin. The visceral peritoneum appeared diffusely erythematous and indurated; however, necrotic lesions of the Arthus type were not found in the peritoneum or in the intra-abdominal organs. Exudates were not obtained when ferritin was injected into non-sensitized guinea pigs.

2. Electron microscopy of peritoneal exudates. Examination of ultra thin sections of the centrifuged pellet disclosed mostly cells of the granulocytic series. Mature neutrophilic polymorphonuclear leukocytes and their precursors predominated; however, eosinophilic granulocytes comprised approximately 10-15% of the cells. The eosinophils were easily identified by virtue of their specific intracytoplasmic granules. The eosinophilic granules are much larger than those of neutrophils, vary considerably in size and contain one or more electron-dense crystalloid rods (Fig. 1, 2).

Aggregated ferritin molecules were observed within the cytoplasm of eosinophils (Fig. 1, 2) and of other polymorphonuclear leukocytes. The aggregates were composed of varying numbers of densely packed ferritin molecules which were associated with an amorphous material of lesser electron density probably representing antibody and complement. Such aggregates were comparable in appearance to *in vitro* ferritin-antibody complexes and were identical in appearance to the immune complexes observed in Arthus lesions(5). The complexes varied considerably in size; some were smaller than the eosinophil granules while others occupied a large part of the cytoplasm and were as much as two-thirds the size of a nucleus. Multiple aggregates, usually of small size, were sometimes observed. There was no ultrastructural evidence that the complexes exerted a toxic effect upon the cells. Even those cells containing large amounts of ferritin-antibody complex were structurally intact and showed no signs of degeneration.

Discussion. The presence of immune complexes within the cytoplasm of eosinophils provides evidence at a molecular level that at least one function of the eosinophil in allergic reactions is the phagocytosis of antigen-antibody complexes.

Litt(2) has shown that peritoneal eosinophilia can be elicited in guinea pigs by the passive transfer of antigen-antibody complexes, or by administration of antigen prior to the passive transfer of specific anti-sera. Eosinophilia was not produced when antibodies were absorbed from the sera prior to passive transfer. These experiments thus demonstrated that immune complexes are capable of mediating eosinophilia and therefore provided an explanation for an often repeated observation that eosinophilia is readily induced by repeated injections of foreign protein.

The observations in the present investigation reaffirm the eosinophilotactic properties of immune complexes and provide visual evidence that eosinophils possess the ability to phagocytose antigen-antibody complexes when attracted to the site of an immune reaction.

These studies seem to indicate that eosinophils are probably not an important source of antibody production. It has been proposed that antibody synthesis may be dependent upon eosinophilic response to antigenic stimulation(6,7). Since high titers of circulating antiferritin antibody were already present at time of challenge and immune complexes were identified within eosinophils and other granulocytes, it seems reasonable to assume that

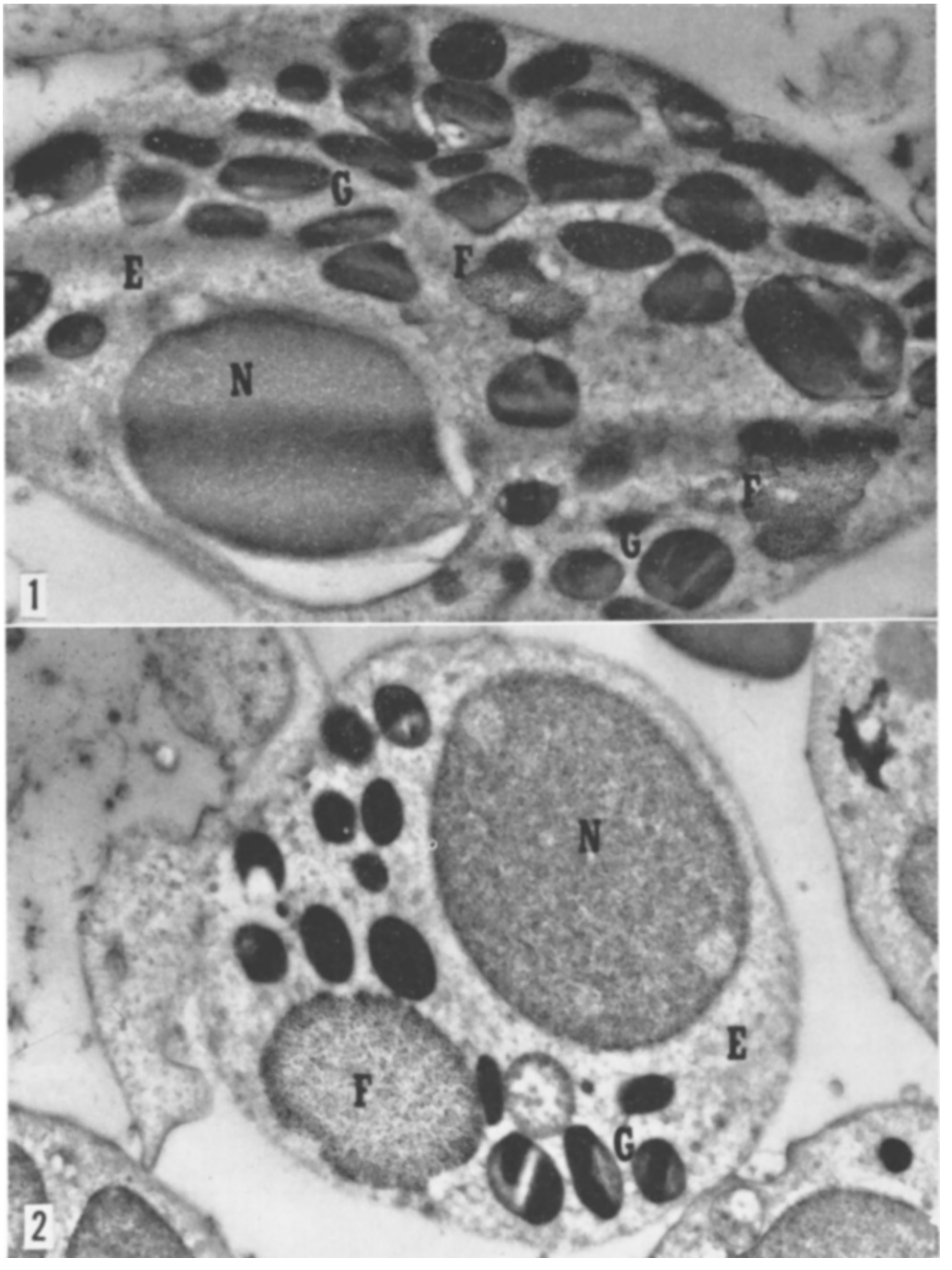

FIG. 1. Electron micrograph of eosinophil (E) obtained in peritoneal exudate produced by injecting sensitized guinea pigs with ferritin. The eosinophil is characterized by the presence of numerous specific intracytoplasmic granules (G) which contain electron-dense crystalloid rods. Phagocytosed ferritin-antibody complexes (F) are present within the cytoplasm. Nucleus (N). Magnification × 21,014.

FIG. 2. Electron micrograph of eosinophil (E) obtained in a peritoneal exudate containing a large ferritin-antibody complex (F). The complex is composed of many densely packed ferritin molecules which are associated with an amorphous material of lesser density, probably antibody and complement. Ferritin has a characteristic electron microscopic appearance. Each electron dense particle in the photograph is a single ferritin molecule. Magnification × 15,466.

eosinophilia is a phenomenon which occurs after formation of antigen-antibody complexes. Whether immune complexes are capable of eliciting further antibody production once they have been phagocytosed by cells remains a matter of speculation. The presence of eosinophils may merely reflect their specific attraction by immune complexes and does not necessarily indicate that they are implicated in production of antibody or in mediation of the hypersensitive state.

Summary. Peritoneal exudates were produced in guinea pigs by intraperitoneal injection of ferritin in previously immunized animals. The centrifuged cells of the exudate examined with the electron microscope were found to contain 10-15% eosinophils. Ferritin-antibody complexes, characteristic of *in vitro* immune complexes were observed within the cytoplasm of eosinophils and other granulocytes. This observation indicates that eosinophilia is mediated by immune complexes and provides evidence that at least one function of the eosinophil is the phagocytosis of antigen-antibody complexes. Our results showed no evidence that eosinophils are implicated in the formation of antibody.

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Effect of Food Fats on Concentration of Ketone Bodies and Citric Acid Level in Blood and Tissues.* (28135)

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Blood ketone concentration in fasting or diabetic animals rises because of the stimulation of fat breakdown. Deuel *et al.*(1) observed increased ketonuria in human subjects maintained on high fat-high protein diet. Roberts and Samuels(2,3) reported higher blood ketones and enhanced ketone body excretion in fat diet adapted rats as compared to carbohydrate fed rats. Tepperman and Tepperman(4) also noticed similar ketonemia in fat fed rats in the fed state.

Recently it has been shown that high fat (groundnut oil) feeding increases acetoacetate production by liver slices and utilization by kidney slices, but the utilization falls considerably on prolonged feeding of same high fat diet for 12 weeks(5).

This report concerns a study of the comparative effect of prolonged feeding of different kinds of food fat on concentration of ketone bodies in plasma and tissues.

Materials and methods. Forty-eight male albino rats averaging 125 g in body weight were divided into 6 equal groups. Group I was kept on normal (carbohydrate) diet whereas all other 5 groups were given high fat and high casein diet containing different kinds of food fat.

The normal diet consisted of casein 20%, sucrose 50%, wheat flour 16%, groundnut oil 9%, cod liver oil 1%, salt mixture 4% (6). High fat diet included casein 30%, wheat flour 30%, fat 35%, cod liver oil 1%, salt mixture 4%. The water soluble vitamins added per 100 g of the diet were thiamine .4 mg, riboflavin .4 mg, pyridoxine .3 mg, nicotinic acid 3 mg, calcium pantothenate 2 mg, folic acid .2 mg, inositol 30 mg, biotin 1 mg, B_{12} 15 μg, choline chloride 500 mg.

Groups II, III, IV, V and VI received groundnut oil, sesame oil, butter fat, hydrogenated groundnut oil and coconut oil respectively.

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