(2) the same buffer was used throughout all our studies, (3) the diet of the feeding animals (Laboratory Chow), did not have an unusually high or low phosphate content (.96%), (4) phosphate could not be responsible for the heat lability of the intestinal factor.

It is clear that iron absorption can take place without either gastric(2) or intestinal contents(4), but it may be difficult for the animal in the face of greater increased demand for iron to increase absorption without these factors. Recently Koepke and Stewart(3) have suggested that there is a factor in the gastric juice of anemic dogs which potentiates the absorption of iron, and this is supported by the findings of Whitehead and Bannerman(9) that the rats following total gastrectomy cannot increase their uptake of Fe^{59} in the face of anemia. It is possible that the intestinal absorption, as distinct from duodenal absorption, is a mechanism for increasing iron absorption when the demands are high. Ordinarily, sufficient iron for daily metabolic needs is absorbed in the duodenum, but under demanding circumstances iron may be absorbed along the whole length of the gut providing that the necessary factors for promoting absorption are present. Our findings are strictly *in vitro* observations, and as such they cannot be interpreted as what happens in the living animal. It is interesting to speculate, however, that a similar mechanism may operate *in vivo*.

Summary. Our observations suggest that there is a factor in the intestinal contents of feeding rats not present in fasting rats, which potentiates the uptake of $Ferrous^{59}$ by everted gut sacs. This factor can be destroyed by heat. There is also a factor in fasting gastric juice other than acid which has a similar effect.

1. Brown, E., Justus, B. W., Am. J. Physiol., 1958, v194, 319.

2. Brummer, P., Acta Med. Scand., 1950, v137, 43.

3. Koepke, J. A., Stewart, W. B., Proc. Soc. Exp. Biol. & Med., 1964, v115, 927.

4. Murray, M. J., Delaney, J. P., Stein, N., Am. J. Dig. Dis., 1964, v9, 684.

5. Murray, M. J., Stein, N., Gastroenterol., 1966, v51, 694.

6. ———, Proc. Soc. Exp. Biol. & Med., 1965, v120, 816.

7. Ohkawara, Y., Bamba, M., Nakai, I., Kinka, S., Masuda, M., Gastroenterol., 1963, v44, 611.

8. Vahlquist, B., Neander, G., Neander, E., Acta Paed., 1944-45, v32, 768.

9. Whitehead, J. S. W., Bannerman, R. M., Gut, 1964, v5, 38.

Received February 14, 1967. P.S.E.B.M., 1967, v125.

Experimental Eosinophilia XI. Cell Responses to Particles of Delineated Size. (32107)

STEPHEN T. KOSTAGE, ANGELO P. RIZZO, AND SHELDON G. COHEN
Research Section of the Medical Service, Veterans Administration Hospital, and Department of Biology, Wilkes College, Wilkes-Barre, Pa.

Eosinophilia associated with immune reactions and hypersensitivity states may be explained in some species by the nature of cell responses to antigen-antibody complexes(1-4) and to some antigens(5-7). In specific instances our earlier findings(3,8) suggested relationships between eosinophil appearance and the molecular aggregated state of the protein antigen. Reported phagocytic functions(9,10) of eosinophils for immune reactants and products of antigen-antibody union may be germane to a consideration of a possible unifying concept of responsible eosinotactic mechanisms. We have pursued this question further through the study of eosinophil leukocyte responses to inflammatory challenge with particles of delineated size and character in the guinea pig.

Materials and methods. Experimental procedures were based upon the development

of eosinophil cell infiltrations within lymph nodes regional to subcutaneous sites of induced inflammation. Details of the foot pad-popliteal lymph node model in guinea pigs have been described previously(3,4).

Monodisperse polystyrene latex particles of uniform diameter* were utilized in unchanged forms and as niduses for effecting molecular aggregates of bovine gamma globulin (BGG)† and of bovine ribonuclease (BRN).‡ Proteins were adsorbed to the particle surface according to a modification of the procedure described by Oreskes and Singer(11). 0.15 M saline was employed throughout the procedure instead of pH 9.01 phosphate buffer in order to bypass the irritant effect of alkaline solution on injected subcutaneous tissue. *In vivo* administration of coated particles immediately after preparation avoided the possibility of non-specific clumping observed in stock solutions. Reagent quality control of the particle suspensions free of contaminating protein in supernatant portions included: (1) filtration through #42 Whatman paper, (2) microscopic examination for absence of clumping, (3) specific agglutination reactions with corresponding rabbit antiserum for efficacy of protein coating, and (4) spectrophotometric determination of particle concentrations in all dilutions made from stock suspensions in preparing dosages.

Guinea pigs weighing 375 to 500 g received subcutaneous injections of (a) 7.6 $\times$ 10^6 polystyrene latex particles of uniform size and (b) an equal number of protein coated particles of corresponding size; each in 0.1 ml suspension into opposite hind foot pads. Experimental series of 12 animals each were based upon receipt of one of 6 particle systems: 0.09, 0.26, and 0.8 μ diameter size and surface coating with BGG or BRN. Prior to their administration blood samples were taken and the sera studied to rule out the possible presence of corresponding or cross-reactive preformed circulating antibody. In each series popliteal lymph nodes were removed from 6 animals 6 hours after the injections and at 24 hours from the remaining 6. The procedures followed for histologic study and for quantitation of infiltrating eosinophil leukocytes identified within lymph node sections were those described elsewhere(3,12).

Identically treated groups of 2 guinea pigs each served to demonstrate the presence of protein coated polystyrene latex particles in affected tissues. Frozen sections were prepared from foot pads and regional lymph nodes and studied by immunofluorescent techniques. Tissues were reacted with: A. fluorochrome labeled (Fl) anti-BGG rabbit globulin, and B. sequentially with (1) rabbit antisera to BGG and (2) Fl anti-RGG sheep globulin, for respective direct and indirect tagging of the particle surface and microscopic identification.

Results. Eosinophil leukocytes were seen within all popliteal lymph nodes regional to foot pads injected with each of the 3 sizes of polystyrene latex particles in both untreated and surface absorbed BGG or BRN states. BGG and BRN coated particles stained with hematoxylin could be identified externally at the surface and within cytoplasmic locations of some eosinophils (Fig. 1). The pattern of cell infiltrations in the cortical and medullary sinuses was typical of involvement of affected nodes described for this model(3). Quantitated findings are summarized in Table I. Analysis of variance at the 95% level of confidence did not establish significant differences among groups for particle size, protein surface coating, or the specific molecular aggregated BGG or BRN.

By both direct and indirect immunofluorescent histologic techniques large numbers of labeled reacting particles were found diffusely distributed throughout the sinuses of lymph node sections while only few were visualized in foot pad sections taken 6 hours after injection. Technical difficulties related to the nonspecificity of fluorochromes for staining eosinophil leukocyte granules(4,13, 14) prevented unequivocal identification of intracellular localized labeled particles.

Discussion. Surface adsorption of protein to uniform size polystyrene latex particles offered the means of obtaining molecular ag-

* Courtesy of Dow Chemical Co., Midland, Mich.

† Armour Laboratories, Kankakee, Ill.

‡ Nutritional Biochemicals, Cleveland, Ohio.

TABLE I. Sampling of Eosinophil Leukocyte Responses within Sections of Popliteal Lymph Nodes Regional to Subcutaneous Sites Injected with 7.6 x 10^6 Monodisperse Polystyrene Latex Particles of Uniform Diameter Size as: a. Untreated Particles (P), and b. Particles with Surface Coatings of a Foreign Protein (CP), 6 and 24 Hours after Their Administration.*

Particle Size (μ)	Coating	Time (Hours)	Eosinophil index in lymph node sections $\frac{t^\dagger}{\frac{w}{l}n^2}$ (Mean ± S.D.) Foot pad treatment P	CP
.09	BRN	6	33.1 ± 31	33 ± 26.8
"	BGG	"	8.3 ± 6.4	13.1 ± 37.3
.26	BRN	"	10.1 ± 5.6	16.3 ± 12.6
"	BGG	"	16.1 ± 10.1	17.6 ± 20.6
.8	BRN	"	5.9 ± 5.4	26.4 ± 23.1
"	BGG	"	16.1 ± 15.1	17.7 ± 13.5
.09	BRN	24	25.3 ± 13.3	30.9 ± 13.1
"	BGG	"	12.1 ± 14.3	15.1 ± 8.6
.26	BRN	"	13.6 ± 23	13.1 ± 5.6
"	BGG	"	13.9 ± 8.9	20.5 ± 14.1
.8	BRN	"	10.1 ± 5.8	12.2 ± 8.8
"	BGG	"	13.9 ± 8.1	20.3 ± 6.1

Eosinophil index in unmanipulated guinea pigs: 0.2 ± 0.1 (references 3, 12).

* Groups of 6 identically treated guinea pigs; P and CP given into opposite hind foot pads.

† t—total no. of cells; w—width and l—length of each section; n—no. of cross fields required for coverage of entire cross sectional area of a lymph node at x470 microscopic study (derivation of formula for cell quantitation given in reference 12).

gregates of homogenous character. By utilizing these preparations in an experimental model of cellular inflammation it was our purpose to study questions arising out of earlier investigation concerned with eosinotactic mechanisms. BRN was selected as a representative protein of low molecular weight (17,000) for comparison of effect with that of the BGG system (mol wt 160,000) given under similar controlled conditions of aggregate size and numbers. Also, it was not clearly understood whether the eosinotactic influence of BRN was a function of specific reagent quality or whether an enhanced inflammatory stimulus was presented by larger numbers of smaller molecules than provided in an equal weight dosage of challenging BGG(4). Not only did this data fail to detect significant differences in the *in vivo* effects of BRN and BGG preparations, but equally revealing was the nonspecificity of their suggested roles. The finding that polystyrene latex particles *per se* can effect eosinophil leukocyte responses favors our previous experimental interpretations(3,8,12) that eosinotactic quality of a protein may be one of physicochemical molecular character shared by other materials, *e.g.*, a colloid system. In turn this would not be totally dependent upon reaction with

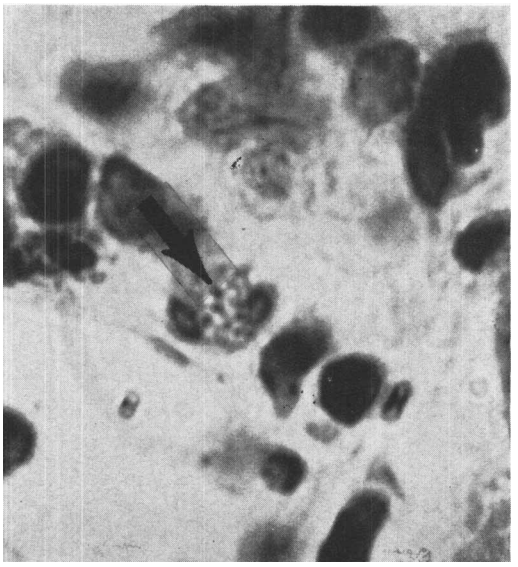

FIG. 1. Section of popliteal lymph node taken from a guinea pig 6 hrs after foot pad injection of BGG coated 0.8 polystyrene latex particles. Two basophilic stained particles are seen within the cytoplasm of an infiltrating eosinophil leukocyte. ×970 (hematoxylin and eosin).

corresponding antibody. Unlike the requirement for antigen adjuvant effect(15) the eosinophil response to polystyrene latex particles can occur apart from alteration in configuration of surface adsorbed proteins.

There were no significant differences in the eosinophilia associated with the administration of either untreated or protein carrier latex particles of 0.09-0.8 μ diameter. Phagocytosis of polystyrene particles by polymorphonuclear neutrophilic leukocytes has been reported(16) and the presumptive evidence here is that eosinophils may function in like capacity without regard to specific immune reactivity. Such bicellular function may resemble the situation for small microorganisms (mycoplasma, 0.015 μ)(17). It is of related interest that some bacterial organisms fall within the upper limits of this particulate size range (*e.g.*, hemophilus 0.5 μ, streptococcus 0.8-1 μ) yet evoke polymorphonuclear neutrophilic leukocytosis and phagocytosis. Whether a matter of size, physical character or chemical specificity of the evoking agent, or chemotactic influence of an intermediate mediator, the exact determinant of eosinophil and/or polymorphonuclear leukocyte response requires further definition and is the subject of our continuing investigation.

Summary. Molecular aggregates of bovine ribonuclease (BRN) and bovine gamma globulin (BGG) were formed by surface adsorption to monodisperse polystyrene latex particles of uniform size: 0.09, 0.29 and 0.8 μ. Homogenous suspension of 7.6×10^6 untreated particles (P) and corresponding size BRN or BGG coated particles (CP) were given into opposite hind foot pads of guinea pigs. Eosinophil granular leukocyte infiltrations were demonstrated within microscopic sections prepared from regional popliteal lymph nodes removed at 6 and at 24 hour intervals after P or CP injections. Stained CP could be identified within the cytoplasm of eosinophils suggesting the occurrence of phagocytosis. Quantitated cell responses to P and CP in groups of 6 identically treated animals did not show significant differences for particle size within the 0.09-0.8 μ range, surface character, or specificity of the protein carried. It is suggested that the eosinotactic effects of proteins in non-sensitized animals may be a function of their physicochemical character as represented by the molecular aggregated state.

1. Litt, M., J. Allergy, 1962, v33, 532.
2. Cohen, S. G., Sapp, T. M., Gallia, A. R., Proc. Soc. Exp. Biol. & Med., 1963, v133, 29.
3. Cohen, S. G., Sapp, T. M., Rizzo, A. P., Kostage, S. T., J. Allergy, 1964, v35, 346.
4. Cohen, S. G., Sapp, T. M., Proc. Soc. Exp. Biol. & Med., 1967, v124, 1034.
5. Litt, M., Am. J. Path., 1963, v42, 529.
6. Cohen, S., Vassali, P., McCluskey, R. T., Benacerraf, B., Fed. Proc., 1965, v24, 696.
7. Roberts, A. M., Nature, 1966, v210, 266.
8. Cohen, S. G., Sapp, T. M., J. Allergy, 1965, v36, 415.
9. Sabesin, S. M., Proc. Soc. Exp. Biol. & Med., 1963, v112, 670.
10. Archer, G. S., Hirsch, J. G., J. Exp. Med., 1963, v118, 287.
11. Oreskes, I., Singer, M., J. Immunol., 1961, v86, 338.
12. Cohen, S. G., Kostage, S. T., Rizzo, A. P., J. Allergy, 1967, v39, 129.
13. Mellors, R. C., Analytical Cytology, ed. 2, McGraw-Hill Book Co., N. Y., 1959, p16.
14. Nairn, R. C., Fluorescent Protein Tracing, E. & S. Livingston, Ltd., Edinburgh, 1962, p120.
15. Litwin, S. D., Singer, S. M., J. Immunol., 1965, v95, 1147.
16. Sbarra, A. J., Karnovsky, M. L., J. Biol. Chem., 1959, v234, 1355.
17. Zucker-Franklin, D., Davidson, M., Thomas, L., J. Exp. Med., 1964, v120, 569.

Received February 14, 1967. P.S.E.B.M., 1967, v125.