guanine to xanthine for 3 experiments by the formula of Rakovsky, Zimmerman & Beck(10):

$$\frac{\text{Ex(initial)} - \text{Ex(final)}}{0.9} \times 100 = \%\ \text{conversion}$$

$$\text{Ex} = \frac{\text{D(250 m}\mu\text{)}}{\text{D(270 m}\mu\text{)}}$$

a 22% conversion of guanine (10 μg) for 15 minutes of incubation was obtained. Table I shows results of some incubation experiments with hypoxanthine and xanthine as substrates; the uric acid produced was relatively low. Homogenates and cell suspensions when incubated for 30 minutes with uric acid showed no uricase activity. A blank not incubated was run simultaneously.

Some experiments in which guanine was added to ciliate suspensions and the μl O_2/mg measured in the Warburg respirometer showed that guanine in the concentration of 75 μM stimulates respiration slightly; the same was observed with iso-guanine, whereas higher doses (150 μM) are partially inhibitory. Azaguanine does not stimulate but inhibits strongly on the same molar basis.

Summary. Cell suspensions and homogenates of *Tetrahymena pyriformis* contain dehydrogenases which reduce triphenyltetrazolium chloride in anaerobiosis to formazan in the presence of some purine substrates (guanine, iso-guanine, hypoxanthine, xanthine). The dehydrogenase activity was also revealed in oxygen consumption experiments using methylene blue as hydrogen acceptor. Homogenates of *Tetrahymena* oxidized purine substrates in the air only weakly and were shown to be devoid of uricase activity. Guanine in lower concentration corresponding to the amount required for growth stimulated slightly the respiration of washed cells, but higher concentrations inhibited markedly the O_2 consumption. The instability of purine dehydrogenases and inequality between oxidase and dehydrogenase activities may account for some discrepancies in the literature.

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Absence of Cellular Localization of Antigen in Delayed Hypersensitivity. (29986)

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Although it is now well established that delayed hypersensitivity is mediated by cells and not by conventional circulating antibody (1), the relationship between the cells involved in the reaction and the eliciting antigen is not known. One suggestion has been that the antigen interacts directly with specific mononuclear cells by combining with a cell bound antibody or with substances near the cell surface(2). The existence of such a reaction remains speculative since it has not been demonstrated that the antigen is local-

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ized on or within the cells which comprise the inflammatory exudate.

In the present study delayed tissue reactions to ferritin and vaccinia virus were examined with the electron microscope in an effort to identify specifically these antigens at the site of a delayed hypersensitive response and to determine whether they were localized on or within cells. We were not able to identify these antigens on or within cells or parenchymal tissue of delayed reactions elicited in skeletal muscle. It thus seems that frequent direct contacts between antigen and sensitized cells do not occur in delayed hypersensitivity.

Materials and methods. 1. *Immunization to ferritin.* Hartley male guinea pigs were immunized by foot pad inoculation of 300 μg of horse spleen ferritin (Nutritional Biochemicals, Cleveland, Ohio) administered in an emulsion with complete Freund's adjuvant.

2. *Infection with vaccinia virus.* Vaccinia virus was propagated in monolayer cell cultures of guinea pig kidney(3) or on allantoic membranes of embryonated chick eggs. Virus titers were determined by plaque assay on monkey kidney cells(4). Hartley male guinea pigs were infected by intradermal injection of 0.1 ml containing $10^{6.8}$ plaque forming units of the chick vaccinia virus.

3. *Production of delayed hypersensitivity reactions.* Guinea pigs sensitized to ferritin were challenged on the ninth day after immunization by intradermal injection of 150 μg of ferritin in saline. Delayed skin reactions thus elicited measured an average of 24 $\times$ 22 mm after 24 hours. Vaccinia virus grown in guinea pig kidney cells was prepared for use as a skin test antigen by heating at 60°C for 20 minutes to inactivate the virus. Two weeks after infection of the animals the heated virus was injected intradermally in a volume of 0.1 ml; the delayed skin reactions produced measured an average of 20 $\times$ 15 mm. The same methods were used to produce delayed reactions to ferritin and vaccinia in the skeletal muscle of sensitized guinea pigs. In addition, delayed reactions in skin and muscle were produced in guinea pigs previously sensitized to tuberculin.

4. *Electron microscopy of delayed tissue reactions.* Delayed hypersensitivity reactions, elicited in skeletal muscle, to ferritin, vaccinia virus and tuberculin were biopsied for light and electron microscopy 24 hours after injection of the antigen. Unsensitized control animals were also injected with these antigens and the muscle biopsied at the injection site 24 hours later. Guinea pigs sensitized to either vaccinia or tuberculin were challenged with a combination of both antigens and the site of tissue reaction biopsied for electron microscopy after 24 hours. In addition, typical delayed skin reactions were produced and these, as well as reactions in skeletal muscle, were fixed in 10% buffered formalin for light microscopy. The tissues for electron microscopy were fixed for 3 hours in Palade's osmium tetroxide buffered fixative(5). The tissue was then dehydrated in a graded series of alcohols and embedded in 1:5 methyl-butyl methacrylate. Sections were cut on a Porter-Blum ultramicrotome, mounted on formvar-coated copper mesh grids and examined with an RCA EMU 3G electron microscope.

Results. 1. *Light microscopy of delayed reactions.* The histological characteristics of the skin reactions produced with ferritin were quite typical of delayed hypersensitivity reactions(6). The lesion was characterized by subepidermal infiltrates of mononuclear cells which were most prominent in the adventitial layer of small blood vessels. At 24 hours there was a modest polymorphonuclear component to the inflammatory exudate but the lesions were not accompanied by epithelial necrosis or hemorrhage.

The ferritin lesions were similar histologically to those produced with vaccinia or tuberculin; however, these antigens often elicited severe reactions which had central necrosis at 24 hours. The delayed reactions in muscle had similar histological features, again being characterized by predominant perivascular mononuclear cell infiltrate. There was only a minimal polymorphonuclear exudate and no evidence of fibrinoid necrosis of small vessels. Thus, on the basis of the specificity, the histologic features and the temporal development of the reactions the lesions in skeletal muscle were considered true delayed hypersensitivity tissue reactions.

2. *Electron microscopy of delayed reac-*

tions. a. *Delayed reactions to ferritin.* Examination of ultrathin sections from the delayed hypersensitivity lesions in muscle failed to disclose ferritin molecules within the mononuclear cells which composed the main cell population of the inflammatory exudate. The histiocytes were identified most readily near small blood vessels and in this location occurred in large aggregates. The blood vessels appeared normal; in contrast to the Arthus reaction(7) ferritin molecules were not localized within the walls of small blood vessels. Ferritin was not visualized within polymorphonuclear leukocytes, skeletal muscle fibers or other parenchymal elements. The mononuclear cells were oval or fusiform in shape and in some instances their intracytoplasmic organelles were swollen, vesiculated, or fragmented. Occasional cells were disrupted but these were the only tissue elements which were necrotic. Control biopsies from non-sensitized animals challenged with ferritin showed a mild polymorphonuclear cell infiltrate and, as in the delayed lesion, the antigen could not be identified within the tissue. The absence of ferritin in the delayed tissue reaction was in marked contrast to its deposition, as antigen-antibody complexes, in immediate reactions of the Arthus type(7). The failure to identify ferritin within the characteristic cell of the delayed reaction suggested that a union between mononuclear cells and the eliciting antigen might not occur; however, to elucidate further these observations, delayed reactions to another antigen which could be specifically identified with the electron microscope were studied.

b. *Delayed reaction to vaccinia virus.* Electron microscopy of delayed reactions to vaccinia virus disclosed a more profuse mononuclear cell infiltrate than that observed with ferritin. The mononuclear cells occurred in aggregates of varying size and were most often encountered near blood vessels. In some instances mononuclear cells were in direct contact with the outer wall of capillaries or venules. There was more evidence of cellular degeneration and fragmentation in the vaccinia virus lesion than in the ferritin response; the perivascular cellular infiltrates often contained a heterogeneous mixture of intact cells, degenerating cells, and nuclear and cytoplasmic fragments. In no instance was it possible to identify vaccinia virus within the area of tissue reaction. The fragmentation of cells and cytoplasmic debris made it difficult to be sure about the exact identification of many small particles which superficially resembled viral particles; however, vaccinia virus had a characteristic ultrastructure and this was not observed in the many sections in which intact mononuclear cells were examined. Vaccinia virus was not observed in sections of muscle taken from non-infected control animals injected with the heat-killed virus. There was no appreciable cellular response to the injected virus in the control biopsies.

Vaccinia virus was readily identified, with the electron microscope, in a pellet prepared from the test virus. Thus our inability to observe the virus within tissues was not related to an alteration in morphology produced by heat inactivation.

c. *Delayed hypersensitivity to tuberculin.* Delayed reactions in tuberculin sensitive animals were similar to those previously described for ferritin and vaccinia. The tuberculin reactions had a more extensive mononuclear cell response than the others and the cells showed less evidence of degeneration and fragmentation. In this instance the antigen (tuberculin) could not be used as a label for electron microscopy and the histological features alone were studied. In some tuberculin sensitive animals a challenging injection of tuberculin mixed with heat inactivated vaccinia virus was administered. The histological features of these lesions did not differ from those obtained with tuberculin alone; the cellular fragmentation seen in vaccinia lesions was not observed and the virus could not be identified within the mononuclear cells which comprised the tuberculin reaction. Similarly the injection of tuberculin along with heat inactivated vaccinia virus into guinea pigs previously sensitized to vaccinia virus did not alter the appearance of the lesion.

Discussion. These observations concerning delayed hypersensitivity are in striking contrast to those in ferritin-induced immediate hypersensitivity reactions. In immediate re-

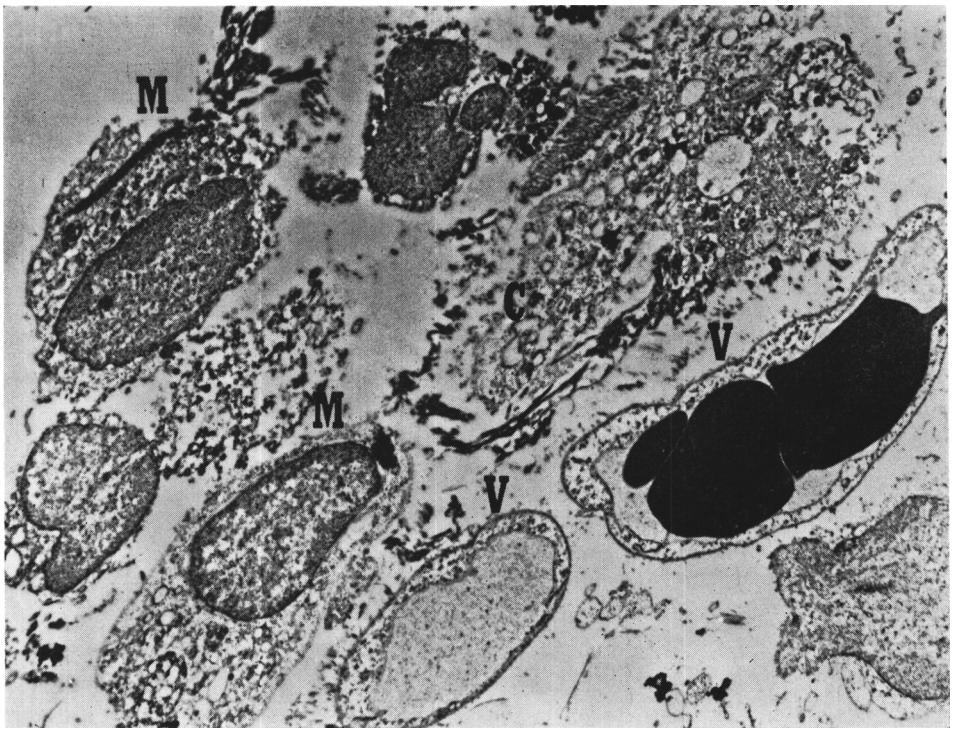

FIG. 1. Electron micrograph of delayed hypersensitivity reaction in guinea pig skeletal muscle, 16 hr after challenge with vaccinia virus. Mononuclear cells (M) are seen in the vicinity of 2 small blood vessels (V). Free nuclei and vesiculated cytoplasmic debris are present in interstitial space. Vaccinia virus cannot be identified on or within mononuclear cells or in association with any parenchymal elements. Magnification ×5080.

actions the injected ferritin combines readily with precipitating antibody and the resultant antigen-antibody complexes become localized about small blood vessels where they can be easily visualized with the electron microscope.

The findings in the present study indicate that the antigens which elicit delayed tissue hypersensitivity do not localize on or within the mononuclear cells which are the important histological component of the reaction since neither ferritin nor vaccinia virus was seen within the cells or parenchymal elements of delayed reactions produced with these antigens.

It has been demonstrated that delayed hypersensitivity can be transferred in animals with cells, but not with serum(8); and, that the transferred cells actually participate in the delayed tissue reactions. Thus, Najarian and Feldman(9) have shown that transferred lymphoid cells, labelled with tritiated thymidine, appear at the site of delayed tissue hypersensitivity when the recipient animals are challenged with the sensitizing antigen. It has thus seemed reasonable to suppose that in some fashion the sensitized cells are attracted to and interact with the injected antigen. Although the present observations do not exclude some chemical or humoral interaction between the sensitized cells and antigen they do suggest that specific antigen-cell complexes occur infrequently if at all.

It may be, however, that infrequent antigen-cell contacts would suffice to initiate the reaction. Such contacts could remain undetected by morphological examination.

The present observations with ferritin are similar to those previously reported by Goldberg *et al*(10). These workers only very rarely identified ferritin molecules within membrane-bounded vacuoles of histiocytes in delayed skin reactions; furthermore, ferritin molecules were also observed within histiocytes when animals sensitized to a different antigen were challenged with a mixture

of ferritin and specific antigen. Thus it seemed unlikely that the presence of ferritin within mononuclear cells, in the ferritin lesions, represented a specific localization of this antigen.

Summary. Delayed hypersensitivity reactions were produced in the skin and striated muscle of guinea pigs sensitized to ferritin, vaccinia virus, or tuberculin. Biopsies of the lesions were examined with the electron microscope in an effort to determine the localization of these antigens at the site of tissue reactivity. Neither ferritin nor vaccinia virus, both of which have a characteristic appearance in the electron microscope, could be identified within the mononuclear cells which form the predominant and characteristic cell of the delayed reaction. Although these cells often showed degenerative changes in the ferritin and vaccinia lesions, such alterations were rare in the tuberculin lesions and there was no association of antigen with the degenerating cells. These observations suggest that frequent direct anatomical interactions between specific antigen and sensitized cell do not occur in delayed hypersensitivity.

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Alterations in Protein-Bound Carbohydrate Levels of Rat Serum During Food Restriction and Refeeding.* (29987)

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Previous reports have demonstrated that protein depletion and inanition cause pronounced decreases in protein-bound hexose levels of serum(1). In the current study, other nutritional parameters, food restriction and refeeding were investigated, and the chemical analyses were extended to include the estimation of protein-bound sialic acid and hexosamine.

Experimental. Adult, male rats of the Sprague-Dawley strain weighing approximately 400 g were used. The animals were caged singly in suspended wire cages with wire bottoms, and were maintained with a diet of commercial laboratory chow† and tap water. Blood samples were collected from lightly anesthetized rats by cardiac puncture. Each animal was bled only once. The age and weight control group (A2) was fed 22 g/day, an amount adequate for weight maintenance, for 34 days. The other experimental groups (B, C, D) were fed one-half this amount until weight losses of 12, 26, and 33%, respectively, were obtained. At this time part of the rats were bled (B1, C1, D1) and the remainder were fed the stock diet *ad libitum*. Blood samples were collected when each of the groups (B2, C2, D2) had attained its original weight. The number of rats per group varied from 11 in Group D1 (33% depleted) to 20 in the normal control group, A1.

Protein-bound hexose, seromucoid protein,

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† Purina Laboratory Chow, Ralston Purina Co., St. Louis.