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.

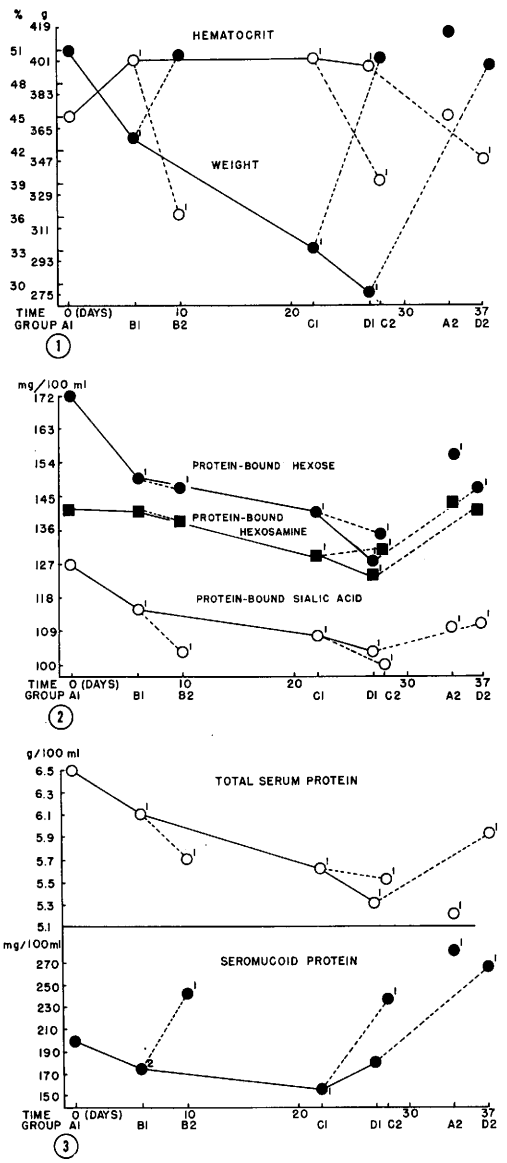

FIG. 1. Effects of food restriction (solid line) and refeeding (dashed line) on body wt and blood hematocrit. Key: ● = weight; ○ = hematocrit. Statistically significant differences from normal values (group A1) are indicated: superscript 1, P = <0.01.

FIG. 2. Effects of food restriction (solid line) and refeeding (dashed line) on protein-bound carbohydrate concentrations of rat serum. Key: ● = protein-bound hexose; ■ = protein-bound hexosamine; ○ = protein-bound sialic acid. Statistically significant differences from normal values (group A1) are indicated: superscript 1, P = <0.01.

FIG. 3. Effects of food restriction (solid line) and refeeding (dashed line) on total serum and seromucoid protein concentrations. Key: ○ = total serum protein; ● = seromucoid protein. Statistically significant differences from normal values (group A1) are indicated: superscript 1, P = <0.01; superscript 2, P = <0.05 >0.01.

total protein, and hematocrit analyses were carried out by procedures described previously(2). Protein-bound sialic acid was determined by the thiobarbituric method of Warren(3), and protein-bound hexosamine by the method of Boas(4). Analysis of the data was made by standard statistical procedures, employing the *t* test(5).

Results and discussion. Fig. 1 depicts the alterations in group weights and hematocrits. An inverse relationship was observed between hematocrit levels and group weights. Hemoconcentration occurred when the restricted diet was fed and hemodilution was evident following weight repletion.

The results of the protein-bound carbohydrate analyses are summarized in Fig. 2. A highly significant decrease in concentration of protein-bound hexose (HE) occurred following 12% depletion (B1). Greater weight losses caused further reductions in the values (C1, D1). Refeeding elicited no conspicuous alterations in HE levels of groups B2 or C2. The observed changes, as was the case in all repletion groups, were partially masked by hemodilution. A significant increase over depletion levels occurred in Group D2. The group mean, however, was subnormal. The response of protein-bound sialic acid (SA) to the dietary regimens was similar to that of HE with the exception that the decline during depletion was not as precipitous. The protein-bound hexosamine (HA) of rat serum was more resistant to the effects of food restriction in the early stage than HE or SA. A highly significant decrease in HA did not occur until a weight loss of 26% was attained. The HA response to realimentation diverged from that of HE or SA. Concentrations either remained in the normal range (B2), increased slightly (C2), or returned to normal (D2). Fucose comprises such a small percentage of the total protein-bound carbohydrates of rat serum (5-7%) that its determination was not deemed essential in the current study.

Data for total serum and seromucoid protein are shown in Fig. 3. Food restriction caused a progressive decrease in total serum

protein concentrations. Significant increases over depletion levels following weight repletion were found only in Group D2. Seromucoid values fluctuated when the restricted diet was fed; first declining (B1, C1), then returning to the normal range (D1). A similar pattern for the fraction has been observed during protein depletion(1). In marked contrast to the behavior of the other serum components, the seromucoid fraction not only returned to normal, but was significantly increased in all groups when the diet was fed *ad lib*. The significant increases in the carbohydrate-rich seromucoid fraction after repletion, in marked contrast to the response of the total protein-bound carbohydrates, is consistent with the findings of Sarcione(6). He observed in perfusion experiments that the synthesis of seromucoid by the liver was considerably more rapid than that of the other serum glycoproteins.

With the notable exception of HA, there was a marked similarity in the curves for total protein and the protein-bound carbohydrates during depletion and repletion. Carbohydrate:Protein ratios (*e.g.*, SA ÷ Protein × 100) involving HE or SA were normal for groups B, C, and D with the exception of the HE:Protein ratio for the severely depleted group, D1, which was subnormal. It may be inferred that the changes in HE and SA reflected the general alterations in the serum proteins. Conversely, HA:Protein ratios were significantly elevated for all groups but B1, suggesting a conservation of HA.

The pronounced serum alterations observed in the age and weight control group (A2) were unexpected in view of the normal hematocrit value and the maintenance of constant weight. Only HA was normal while HE, SA, and total protein values were significantly decreased and the seromucoid fraction conspicuously elevated.

The cause of the substantially greater increase in all serum values for Group D2 in comparison with other repleted groups (B2, C2) despite a greater weight loss is unknown. It was, apparently, not related either to the rate of depletion or of repletion (Fig. 1).

The depressed levels of HE and SA in the depleted and repleted groups, the return to normal of HA values in Group D2 following weight restoration, the elevated HA:Protein ratios and the significant increases in the carbohydrate-rich seromucoid fraction in the repleted groups provide evidence that the various glycoproteins present in rat serum do not respond in a uniform manner to the diet fed.

Summary. The effects of food restriction and refeeding on total protein-bound hexose (HE), hexosamine (HA), sialic acid (SA), and the carbohydrate-rich seromucoid fraction (SM) were investigated. Groups of rats, fed 50% of the food required for weight maintenance were bled following weight losses of 12, 26, and 33%. Other groups were fed *ad lib* following depletion and serum samples were obtained when their initial weight was regained. HE and SA were significantly decreased following a 12% weight loss and declined further as depletion progressed. Refeeding caused a marked increase in the 33% depleted group, but the final level was subnormal. HA was more resistant to moderate depletion and a highly significant decline did not occur until a 26% weight loss was attained. HA levels were normal in the 12 and 33% depleted groups following weight repletion. SM values fluctuated during depletion, first decreasing then returning to normal. SM values were significantly elevated in all repleted groups. It was concluded that the protein-bound carbohydrates of rat serum do not respond in a uniform manner to food restriction and refeeding.

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