Changes in Plasma Protein-Bound Carbohydrates and Glycoprotein Patterns During Infection, Inflammation and Starvation[1] (37179)

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Numerous studies have demonstrated changes in concentrations of serum glycoproteins in man and laboratory animals during various infectious and inflammatory processes (1–4). Other studies have attempted, with somewhat conflicting results, to determine the importance of an adequate nutritional intake as a requisite in these alterations (5–8). However, no definitive mechanism has yet been demonstrated *in vivo* to explain these changes in circulating glycoproteins.

The present study was designed to elucidate patterns of host response to disease under several dietary regimens and thereby help delineate possible mechanisms responsible for such alterations. The effect of a localized, nonfatal inflammatory reaction elicited by turpentine, and an acute, systemic, fatal infection induced by *Diplococcus pneumoniae* were compared during mild and severe food restriction in the rat. Parameters monitored included protein-bound carbohydrates and glycoprotein and protein electrophoretic patterns.

Recent investigations have demonstrated the existence of an endogenous humoral factor(s) which mediates certain facets of host response. These endogenous mediators, released from phagocytizing cells, have been shown to produce a flux of amino acids from muscle tissue to liver (9), alterations in Zn and Fe metabolism (10) and the synthesis and/or release of specific acute phase glycoglobulins in the rat (11, 12). To ascertain whether such humoral factors might also mediate changes in protein-bound carbohydrates and glycoprotein patterns the effect of an extract from stimulated leukocytes on the above parameters was studied.

Materials and Methods. Animals. Adult, male, 250–300 g Fisher-Dunning rats obtained from a commercial source (Microbiological Associates, Walkersville, MD) were placed into 1 of 3 nutritional groups. One group received food *ad libitum*, a second group was fasted through the experiment, and a third group was starved until a 25% weight loss occurred (approx 10 days) prior to the initiation of the experiment and then maintained on a starvation diet throughout the experiment. Next, a group of rats from each nutritional category was injected subcutaneously (sc) with 10^7 organisms *D. pneumoniae*, type 1, strain A5, in a volume of 1 ml. A second group of rats from each nutritional category was injected sc with 1 ml of steam-distilled turpentine. Dietary controls in both groups were given 1 ml sc sterile normal saline. Pneumococcal infected rats and their controls were killed 36 hr, and turpentine-inflamed rats and their controls 48 hr postinoculation. Rats were anesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane (Halothane) and bled by severing the posterior vena cava in the thoracic cavity. Blood was collected using EDTA as an anticoagulant; plasma was separated after

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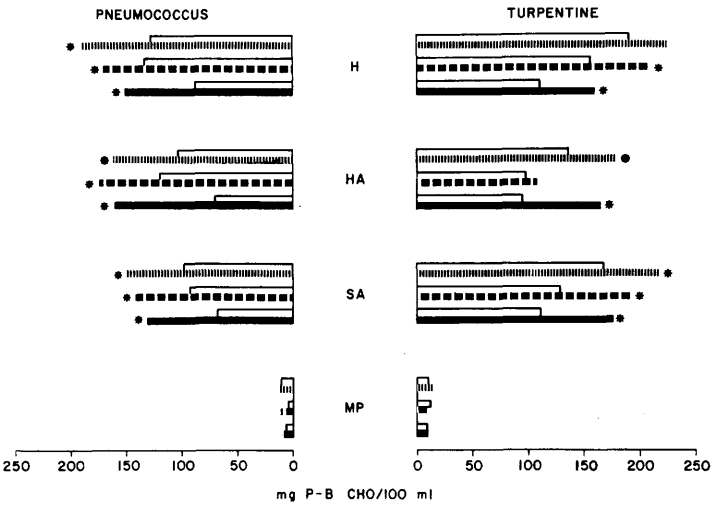

FIG. 1. Effect of infection and inflammation on plasma protein-bound carbohydrates. (light broken bar) Fed group; (heavy broken bar) 36–48-hr-fasted group; (solid bar) 25%-weight-loss group; (*) $p < 0.01$.

centrifugation. In the experiment using secretions obtained from stimulated leukocytes (13), furnished by Dr. R. S. Pekarek, a group of rats were injected intraperitoneally (ip) with 1 ml of fresh extract. Controls received an ip injection of 1 ml of heat-inactivated extract (96° for 30 min). Food was removed at the time of the injection. All rats were killed 16 hr later and blood was collected as described above.

Analytical procedures. Cellulose acetate electrophoresis was performed according to the procedure of Klainer, Beisel and Atkins (14) with minor modifications. Protein-bound hexose was determined by the orcinol method, hexosamines by the method of Elson and Morgan and methylpentoses according to the method of Dische and Shettles. These methods have been summarized by Winzler (15). Sialic acids were determined by the thiobarbituric acid assay of Warren (16).

Results and Discussion. When pneumococcal infected and turpentine-inflamed rats were compared, similar increases were produced in protein-bound hexose (H), hexosamine (HA) and sialic acid (SA) in the fed, fasted and 25% weight loss dietary groups over their respective controls (Fig. 1). Differences in control values between the pneumococcal and turpentine models can be attributed to the fact that these two experiments were not run simultaneously.

Total plasma protein-bound carbohydrate (TP-PBCHO), represented as the sum of the foregoing protein-bound carbohydrates, together with cellulose acetate electrophoresis of plasma and subsequent reaction with periodic acid-Schiff reagent gave plasma glycoprotein distribution in mg/100 ml as illustrated in Fig. 2. Total plasma protein-bound carbohydrate decreased with decreasing nutritional intake, as demonstrated by lowered values in fasted and starved control rats compared to fed control rats. Significant increases in total protein-bound carbohydrate concentration occurred in both pneumococcus and turpentine-stressed rats regardless of the dietary regimen. In the absolute distribution of plasma glycoprotein, both agents produced significant elevations in the α_2 and β glycoglobulin fractions, which were not suppressed by nutritional restriction. The α_1 fraction was increased in pneumococcal infected rats which were fed and in those which were fasted during the experiment. Similar elevated values were noted in all three dietary groups of turpentine-inflamed rats, however, unexplained increases in the α_1 fraction of control rats in the fed and fasted dietary groups masked the significance of alterations

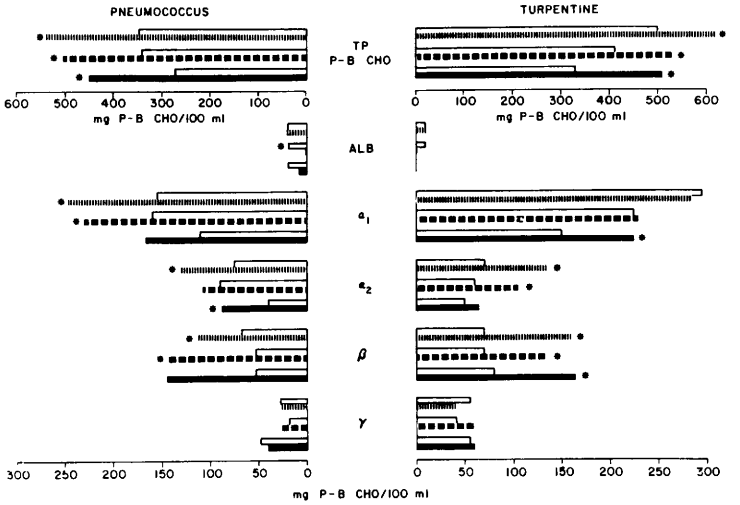

FIG. 2. Effect of infection and inflammation on plasma glycoprotein distribution. (light broken bar) Fed group; (heavy broken bar) 36–48-hr-fasted group; (solid bar) 25%-weight-loss group; (*) $p < 0.01$.

in the respective experimentally inflamed rats. Further work in progress indicates that 48-hr-fasted, turpentine-inflamed rats indeed show an elevated α_1 glycoglobulin fraction, thereby substantiating the significantly increased levels seen in the starved group of inflamed rats in this experiment. The small albumin fractions reflect the scarcity of carbohydrate covalently attached to the proteins which migrate with albumin.

Similar electrophoretic procedures using a general protein stain gave an electrophoretic distribution which was again similar between pneumococcal and turpentine-stressed rats. Absolute decreases occurred in albumin, whereas the α_2 and β globulins were elevated, although not as markedly as in the glycoglobulin fractions. Again, the elevations were not suppressed by decreasing nutritional intake.

These data support the conclusion of Heiskell *et al.* (1) that serum glycoprotein concentrations are elevated nonspecifically with regard to the inciting agent. However, the fact that host response is similar to two diverse phlogogenic agents, one producing a systemic, fatal infection and the other a localized, nonfatal inflammatory reaction, suggests that the alterations caused by each agent may have come about through similar mechanisms. Despite the apparent diversity in these agents, the polymorphonuclear cell response is prominent in both diplococcus infection (17) and the sterile turpentine abscess (18). It therefore seemed pertinent to test the effect of the leukocytic endogenous mediator (LEM) of Pekarek and Beisel (13), on parameters which we had monitored in the previous two experiments. Patterns and directions of alterations in response to LEM were identical to those seen in pneumococcal infected or turpentine-inflamed rats (Fig. 3). Protein-bound hexose, hexosamine and sialic acid were all increased, though not significantly, in rats given LEM over those given heat-inactivated material. A separate group of control rats was given 1 ml sterile saline sc, but since no difference was seen between these and controls given heat-inactivated LEM, only the latter are represented in Fig. 3. Since methylpentose contributes such a small percentage to total protein-bound carbohydrate its determination was not included in this study. As shown in Fig. 3, total plasma protein-bound carbohydrate was significantly increased. Similarly, increases in the α_1, α_2 and β glycoglobulin fractions were observed; the β fraction was significant at the $p < 0.01$ level. The fact that significant changes were not demonstrated in all parameters may

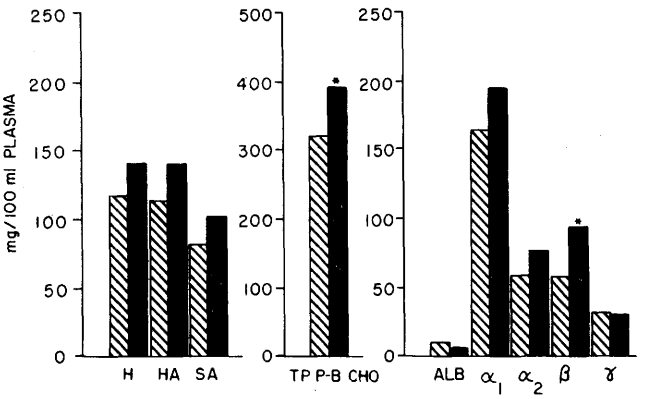

FIG. 3. Effect of LEM on plasma protein-bound carbohydrates and glycoproteins. (solid bar) LEM; (shaded bar) heat-inactivated LEM; (*) $p < 0.01$.

be explained by the single exposure to an exogenous source of mediator in this experiment as contrasted to a continued release of endogenously produced mediator in the turpentine or pneumococcal stressed rats. Preliminary results in our laboratory indicate that repeated injections of LEM even more closely mimic the changes produced by infectious or inflammatory agents (19).

Pneumococcal sepsis has been demonstrated to produce a flux of amino acids (20), Zn and Fe into the liver (13); all being necessary precursors and/or cofactors for protein synthesis (21–23). Recent evidence indicates that similar changes are seen in turpentine-inflamed rats (personal communication, M. C. Powanda). Since LEM has been postulated to be an intermediate factor in stimulating amino acid and trace metal metabolism during infection (10), the data presented in this study suggests that the alterations produced in plasma protein-bound carbohydrates and glycoprotein patterns may also be mediated by a similar endogenous humoral factor(s).

The failure of severe nutritional deprivation to suppress the changes produced by pneumococcus or turpentine suggests that these changes occupy a high priority in the host response. This conclusion is in agreement with experimental findings in rats (24) and in naturally occurring cases of protein-calorie deficiency diseases in Egyptian children (7). In both of these studies the investigators were able to demonstrate the persistence in sera of unusually high concentrations of several specific glycoproteins despite severe malnutrition. If these alterations are due to increased synthesis of acute phase globulins, as data from other workers seem to indicate (25), then the host must be drawing upon some sources of precursor, other than those derived from nutritional intake, to produce the changes noted. The previously cited flow of amino acids from muscle to liver during several disease processes may in part explain the continued anabolic capability of the nutritionally deprived host.

Summary. Localized inflammation and acute systemic infection in the rat produced similar alterations in plasma protein-bound carbohydrates and glycoproteins, characterized by: (a) increased total plasma protein-bound carbohydrates, including protein-bound hexose, hexosamine and sialic acid; (b) increased α_1, α_2 and β glycoglobulin fractions. Moreover, these changes occur despite severe starvation; therefore, they appear to be independent of nutritional intake. Similar changes were produced when an extract from stimulated leukocytes was administered to normal rats. This suggests that changes in protein-bound carbohydrates and glycoproteins during inflammation and infection may have been mediated by an endogenous humoral factor(s).

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