findings are discussed in connection with titration of residual virus in heat-treated virus preparations.

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Biochemical Significance of Serum Glycoproteins.* I. Changes in Rat Serum Following Injury. (26809)

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A wide variety of non-specific traumatic conditions(2) are known to cause alterations in the proportions of serum proteins. Such changes in human serum have been shown by electrophoretic studies to involve a reduction in the albumin level accompanied by elevations in α_1 and α_2 globulins(2-8). In a number of diseases increased proportions of hexosamines were also observed to migrate electrophoretically as the α_1 and α_2 globulins(7). Increases have been reported in the seromucoid fraction and in total protein-bound carbohydrates(8). These observations show that the glycoproteins are important factors in the alterations of the serum proteins in the diseased state.

Changes are also known to occur in the serum proteins of laboratory animals after trauma(9-11), thus experimental injury in rats results in elevations of α_2 and β globulins(12). The total serum hexosamine level reaches a maximum value 2 to 4 days after trauma(13). According to Boas and Peterman(13) this response to injury is eliminated if the animals are kept on a starvation diet. Werner(14) prevented the elevation of total serum hexosamine in rabbits by chemically destroying the liver tissues. The elevated total serum hexosamine levels probably reflect the increased α_2 and β globulins, much as described by Bollet for various pathological conditions in humans(7). No direct observations, however, of the distribution of hexosamines following experimental injury have been reported. In this paper are described the changes in electrophoretic distribution of the hexosamine-containing glycoproteins in the serum of rats subjected to 2 standardized injuries.

Methods. Male rats (Yale, Wistar, and Holtzman strains) weighing between 200 and 300 g were injured for the "open wound" study by removing a 2.5 cm circle of skin just caudal to the scapulae (ether anesthesia) or for the "sponge" study by implanting sub-

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cutaneously a 200 mg piece of Ivalon surgical sponge‡ in the same area. The 2 cm long incision was closed with a single skin clip. All of the rats were fed *ad libitum* throughout these studies. At the end of 1, 2, 4, 8 and 15 days the rats were anesthetized and a blood sample was removed by cardiac puncture. A small portion of blood was mixed with ammonium oxalate for determination of the red cell volume (hematocrit) while the remainder was allowed to clot and then centrifuged to yield the serum samples. To avoid complications due to storage, the electrophoretic studies were performed on the same day the samples of serum were obtained. Control rats were pair-fed and bled on comparable days with the experimental ones.

Samples of serum were used for determination of total protein(15) and total hexosamine(16). Proteins were separated electrophoretically§ (0.008 ml of serum) on Whatman 3 MM paper using 140 volts for 15 hours. The strips were then dried at 110°C for 30 minutes, rinsed in methanol, and stained 30 minutes in a 0.1% solution of bromphenol blue in methanol. After washing successively in 2 changes of 5% acetic acid, and once in acetate buffer (9 g sodium acetate · $3H_2O$ in 1 liter of 10% acetic acid), the strips were dried for 15 minutes at 110°C. The color was intensified by exposure to ammonia vapors and the strips were then scanned using the Analytrol automatic integrating densitometer.

For the electrophoretic distribution of hexosamine-containing glycoproteins, 0.05 ml samples of serum were applied to Whatman 3 MM paper. After electrophoretic separation was complete, the strips were dried at 110°C and stained for several minutes in the bromphenol blue solution. The heavily stained strips were washed in a 5% solution of acetic acid and then dried. Four areas were cut from the paper strips, albumin plus α_1, α_2, β, and γ globulins. The 4 portions of paper were cut into small pieces and hydrolyzed at 100°C for 6 hours in 1 ml of 2 N HCl in screw-capped test tubes. The partial hydrolysates were quantitatively filtered through Whatman No. 1 paper and applied directly to a column of Dowex 50, prepared as described by Boas(17). The filter papers and columns were thoroughly washed with water until no evidence of bromphenol blue persisted in the effluents. The hexosamines were then eluted with 2 N HCl and 5 ml of the eluates were collected in screw-capped tubes. After neutralizing these eluates (phenolphthalein) with NaOH, 1 ml of acetylacetone reagent and sufficient water were added to bring the final volume to 8 ml. The acetylacetone solution was prepared immediately before use by adding 0.6 ml of acetylacetone to 10 ml of a pH 10.2 ± 0.1 carbonate buffer (3.0 g $NaHCO_3$ and 17.2 g Na_2CO_3 in 100 ml of solution). The mixtures of sample and acetylacetone reagent were heated and extracted with isoamyl alcohol as previously described(16). To 4 ml of each of the isoamyl alcohol extracts, 1 ml of Ehrlich's reagent was added (0.6 g p-dimethylaminobenzaldehyde dissolved in 2.25 ml of concentrated HCl and 20.25 ml of isoamyl alcohol). The optical density was measured after 15 minutes in a spectrophotometer at 530 mμ. A standard curve was prepared using 3 to 40 μg of glucosamine dissolved in 7 ml of 7.25% saline.

Results. The hematocrit and total protein values were not significantly altered by the injuries inflicted (Table I). However, total serum hexosamine level (Table I), in agreement with published reports(9,13), was significantly elevated 1 to 2 days after both the open wound and sponge implantation ($P = <0.01$).

Fig. 1 shows that the relative distributions of α_2 and β globulins increased to maximum levels 2 to 4 days after injury and thereafter returned to normal. Individual variation in response was considerable. However, the concentrations (g %) of protein migrating electrophoretically as α_2 and β globulins were significantly elevated ($P = <0.01$) above control values 2 days after both injuries, (Table II). During the first 4 days the albumin decreased to a minimum for both open wound and sponge implantation studies.

‡ Polyvinyl alcohol surgical sponge. 200 mg pieces of sponge were boiled in several changes of distilled water and then were heated at 110°C for 30 min.

§ Spinco apparatus.

TABLE I. Total Serum Hexosamine and Protein Levels of Injured Rats. Forty control rats were used; 8-10 rats were used for each time period following injury. Yale, Wistar, and Holtzman rats were used throughout. No significant differences between strains were observed. All values are reported as mean ± stand. dev.

Injury	Days after injury 0	1	2	4	8	15
Open						
Hematocrit (%)	47 ± 4	47 ± 2	47 ± 2	46 ± 3	46 ± 2	46 ± 3
Total protein (g/100 ml)	6.6 ± .4	6.7 ± .4	6.6 ± .3	6.3 ± .2	6.3 ± .3	6.5 ± .4
Total hexosamine (mg/100 ml)	105 ±11	121 ±13	117 ±18	118 ±17	112 ±16	114 ± 9
Sponge						
Hematocrit (%)	47 ± 4	48 ± 3	43 ± 3	46 ± 3	47 ± 3	45 ± 2
Total protein (g/100 ml)	6.6 ± .4	6.1 ± 1.0	6.5 ± .7	6.3 ± .8	6.9 ± .5	6.7 ± .0
Total hexosamine (mg/100 ml)	105 ±11	113 ±12	129 ±17	112 ±14	105 ± 7	106 ±14

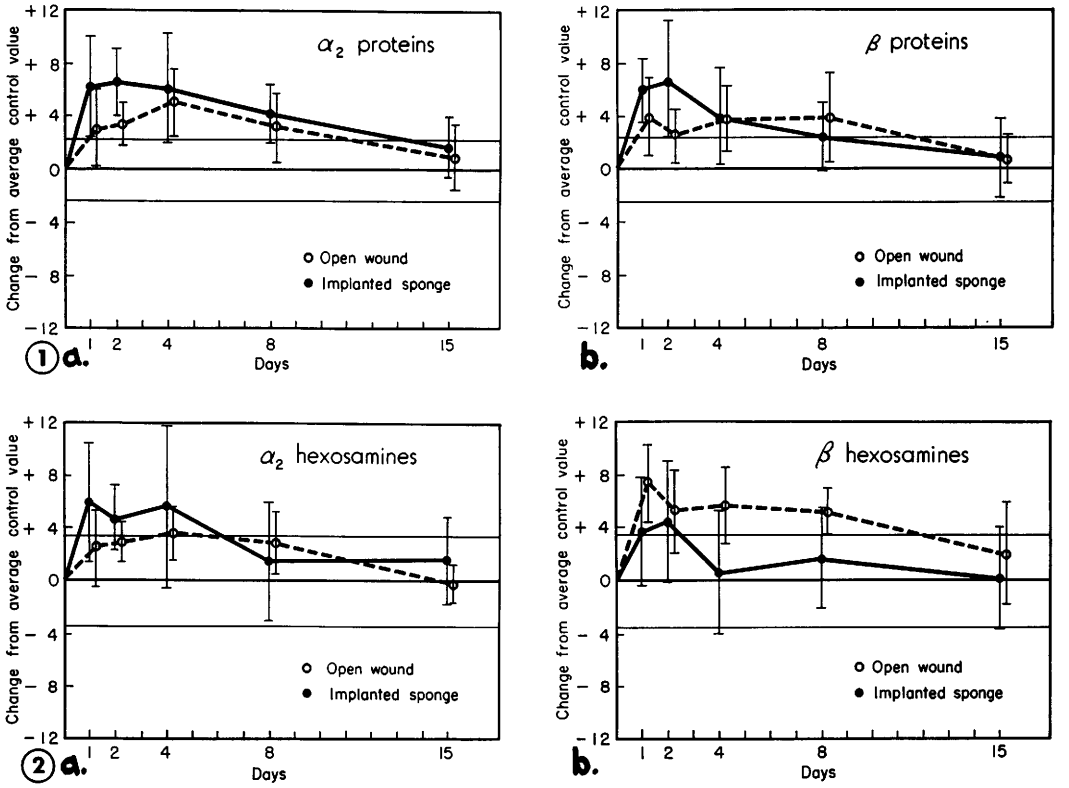

FIG. 1. Increases in serum α_2 and β globulins elicited by injury. Ordinate values represent increases above mean control value. Stand. deviations for the control are given by the lines parallel to the 0. Stand. deviations for each time period are also given. Abscissa values are days after injury.

FIG. 2. Changes in α_2 and β hexosamine-containing glycoproteins caused by injury.

TABLE II. Change in Electrophoretic Distribution of Serum Proteins and Hexosamines in Rats 2 Days Following Injury.

Injury	No. of rats	Proteins (g/100 ml)					Hexosamines (mg/100 ml)			
		Albumin	α_1	α_2	β	γ	Albumin + α_1	α_2	β	γ
None*	40	2.51 ± .34	1.15 ± .30	.83 ± .14	1.19 ± .18	.85 ± .22	56.0 ± 8.3	20.7 ± 4.0	18.6 ± 3.4	9.4 ± 2.7
Open wound	12	2.17 ± .36	1.06 ± .15	1.09 ± .13	1.36 ± .14	.94 ± .22	55.0 ± 10.0	26.0 ± 3.3	26.5 ± 6.0	9.7 ± 2.5
P†		<.05	—	<.01	<.01	—	—	<.01	<.01	—
Sponge	7	1.57 ± .29	1.24 ± .27	1.33 ± .20	1.59 ± .18	.80 ± .17	59.6 ± 11.0	30.7 ± 6.3	28.0 ± 6.5	7.0 ± 2.4
P†		<.01	—	<.01	<.01	—	—	<.01	<.01	—

* Control samples taken at 1, 2, 4, 8 and 15 days during experiment. Values did not differ and were, therefore, pooled.
† Probability.

There were no alterations in serum α_1 and γ globulin zones.

The relative distributions of α_2 and β globulins, measured as hexosamines, increased to maximum levels in 2 to 4 days (Fig. 2). Here also considerable individual variation in response was observed. At the same time the concentration of total serum hexosamine (Table I) increased to a maximum level. As a result the actual concentrations of hexosamines migrating electrophoretically as α_2 and β globulins (mg %) were significantly elevated ($P = <0.01$) 2 days after injury (Table II). The amount of hexosamines in the albumin + α_1 zone and the γ globulin region remained unchanged.

These data demonstrate a qualitatively parallel response to injury in the serum hexosamines and proteins which are classified electrophoretically as α_2 and β globulins. The increase in protein and hexosamine components of the α_2 globulins were of similar magnitude since the percentage of hexosamines in the protein was the same after injury as before (2.3% to 2.4% after injury compared with 2.5% in the control). In the β globulin fraction, however, the elevation of hexosamines after injury was greater than of the protein (1.8% to 2.0% after injury compared with 1.6% in the control). These results are explained by an increased production of serum glycoproteins in response to injury.

It is concluded from this study that the elevated total serum hexosamine levels elicited in rats by injury are caused by increases in the amounts of hexosamine-containing glycoproteins associated with the α_2 and β globulin fractions.

Summary. Changes produced in distribution of serum proteins of rats by injury were studied using paper electrophoresis. The electrophoretic distribution of glycoproteins was determined by measuring the concentrations of hexosamine in 4 zones, albumin + α_1, α_2, β, and γ globulins. Parallel elevations of serum hexosamines and proteins which are classified as α_2 and β globulins were observed in response to 2 kinds of experimental injury. These increases reached maximum proportions 2 to 4 days after injury.

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Nature of Polysaccharides Obtained from Endotoxins by Hydroxylaminolysis. (26810)

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Although endotoxins have been investigated extensively, the exact relationship between chemical nature and biologic reactions is still unknown. All endotoxins contain polysaccharide, lipid, certain amino compounds(1) and phosphoric acid and are known as lipopolysaccharides (LPSP). In the present paper endotoxins from 3 different species of bacteria and their lipid-free polysaccharides are subjected to a comparative study.

Materials and methods. The preparation of endotoxin has been previously described (1). The lipid moiety of the lipopolysaccharides was removed by hydroxylaminolysis (2). The separated polysaccharides were removed from the reaction mixture by centrifugation for 10 minutes at 1.700 g. The precipitates were washed once with alcohol and once with acetone and dissolved in water. The resultant solutions were dialyzed for 24 hours. After addition of 1 mg NaCl per ml the polysaccharides were precipitated with 2 volumes of acetone. The precipitates were washed with acetone and dried over sodium hydroxide under reduced pressure. The yield of polysaccharide was 50-60% on the basis of LPSP employed. Saccharides were determined by the anthrone method(3) with galactose as the standard. Amino sugars were determined by Dische's method(4) after deacetylation. The LD_{50} values(5) of endotoxins varied from 0.25 to 0.30 mg per 18-20 g mouse. The polysaccharides were non-toxic at 5 mg. Materials used in this study were those from *Neisseria gonorrhoeae* 97, *Escherichia coli* 0117:H27 and *Salmonella abortus equi.*

Results and discussion. Analytical data concerning 3 endotoxins and their polysaccharides are summarized in Table I. The nitrogen content indicates that amino compounds have been retained in the polysaccharides and the high phosphorus content points to a high degree of phosphorylation in all materials. The polysaccharides of *E. coli* endotoxin and of *S. abortus equi* endotoxin were free of lipid and in the *N. gonorrhoeae* polysaccharide the lipid content (stearin equivalent) dropped from 23.2% to 1.0%

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