tion of marrow cells which displaced the fat globules normally occupying the femoral marrow cavity. The triglyceride content decreased to half of its original level while the relative proportion of PL and cholesterol, on a dry, fat-free basis, remained unchanged. The proportion of linoleic acid in the remaining portion of TG decreased. Fractionation of the PL showed changes in their composition, particularly a decrease of the less polar components. The relationship of these changes in marrow to the changes observed in the peripheral blood requires further study.

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Received June 8, 1966. P.S.E.B.M., 1966, v123.

Changes in Serum Proteins and Glycoproteins in an Acute Controlled Infection. (31449)

IRVING GRAY*
(With the technical assistance of James Dillworth)
United States Army Medical Unit, Fort Detrick, Frederick, Md.

Changes in serum proteins following infection have been noted for some time. Leutscher(1) in reviewing the medical application of moving boundary electrophoresis noted that a characteristic of several acute infections was a decrease in the serum albumin and an increase in the serum alpha protein. This reviewer stated that these changes were part of a nonspecific response to injury or infection. Jencks *et al*(2), reporting on 1,516 general admissions to a large general hospital, demonstrated an albumin decrease and α_2-globulin increase in infectious disease. However, similar changes occurred in other illnesses as well. Graham *et al* (3) cited several others and reported their own results which were similar to those already noted. The most recent review was that of Peterman(4). Although the cited data were in accord as to the changes found, they were obtained from patients who had presented themselves to the hospital when infection was well established. When do changes in serum proteins appear and what is their relation to the febrile period? These questions are the subject of this research which involves subjects studied in a prospective manner.

Methods. As part of a larger program to develop and test vaccines, it became possible to study serum protein changes in a group of 22 male volunteers. These men, all members of the Seventh Day Adventist Church, were all in excellent health as evidenced by repeated normal physical and laboratory findings. Exposures to the organism, *Pasturella tularensis*, were carried out by aerosol methods routinely used in this unit. The men received a total infective dose of 2.2×10^3 or 2.2×10^4 viable organisms. Two men were

* Present address: Dept. of Biology, Georgetown University, Washington, D.C.

TABLE I. Changes in Serum Protein Fractions Following Infection with *Pasturella tularensis.**

Day post exposure	Control	1	2	3	4f	5f	6	7	8	9	10
No. of samples	23	10	9	10	10	10	10	10	10	9	5
Albumin	67.8	68.2	68.4	68.3	67.8	65.0	66.5	64.6	65.8	65.2	64.5
α_1	3.8	3.7	3.5	3.7	3.8	4.4	4.6	4.4	4.7	3.9	4.3
α_2	7.0	6.9	6.4	7.6	7.8	8.5 (.001)	8.5 (.001)	9.2 (.001)	8.8 (.02)	8.9	9.5
β	8.7	8.1	8.6	8.1	8.5	8.9	8.2	9.1	9.4	9.5	8.9
γ	12.6	13.2	13.0	12.3	12.2	13.3	12.7	12.8	11.3	12.0	12.6

* Values are relative percent. Numbers in parentheses indicate probability values ($P <$). f indicates days of maximum fever.

TABLE II. Changes in Lipoprotein Fractions of Serum Following Infection with *Pasturella tularensis.**

Day post exposure	Control	1	2	3	4f	5f	6	7	8	9	10
No. of samples	23	10	9	10	10	10	10	10	10	9	5
α	20.5	20.3	23.6	21.2	22.1	21.1	23.1	20.4	17.9	16.9	16.6
β	75.5	79.7	76.4	78.9	76.9	78.9	76.9	79.5	82.1	80.9	83.3

* Legend as for Table I.

sham exposed. Fasting blood samples were taken for 2 or 3 days prior to exposure and daily thereafter. The serum was removed and a portion frozen until used for paper electrophoresis.

Electrophoresis was carried out in the Beckman Model R paper electrophoresis system. Strips were stained for serum proteins, lipoproteins and glycoproteins as described in the operating manual of the instrument. The stained paper strips were quantitated by means of an "Analytrol" (Beckman, Model RB) and reported as relative percent.

Tests of significance were carried out by means of the "student's t-test" for unpaired experiments.

Results. Although the volunteers were exposed to 2 concentrations of organisms, there was no relation between the concentrations and the severity of the illness. The patients, based on their clinical response to the infection, were grouped as "typical" or "mild." Inasmuch as there were no significant protein changes in the sham exposed or "mild" groups, nothing further will be reported for them.

The criteria for the "typical" classification was a temperature of more than 101.5°F and the administration of antibiotic therapy. The studies were terminated after 10 days at which time all patients were asymptomatic.

In Table I are shown the changes in the serum proteins resulting from the "typical" reaction to *P. tularensis*. There were no changes in the α_1-, β-, or γ-globulins during the period studied. There was a slight decrease in the albumin, a minimum value on day 7 following exposure, 2-3 days after maximum fever. There was a more marked change in the α_2-globulin. In this fraction, there was a steady increase in the relative percent, reaching significantly increased values on day 5 following exposure, 1-2 days after maximum fever. Thus, it appeared that changes in the serum proteins (of hospital patients) previously reported(1-4) may have occurred shortly after the appearance of symptoms.

Table II summarizes the results of the analysis of the lipoproteins of the serum. No significant changes were measured.

In Table III, the results of the changes in the glycoproteins have been summarized. The changes in these fractions appeared to be more pronounced than in the serum proteins. The decrease in the "glycoalbumin" fraction

TABLE III. Changes in the Glycoprotein Fractions of Serum Following Infections with *Pasturella tularensis.**

Day post exposure	Control	1	2	3	4f	5f	6	7	8	9	10
No. of samples	23	10	9	10	10	10	10	10	10	9	5
"Albumin"	11.3	11.5	11.0	8.8	10.9	9.1	10.0	8.7 (.05)	8.7 (.02)	8.7	9.9
"α_1"	15.5	16.9	15.3	17.4	17.3	20.9 (.001)	20.7 (.001)	19.6 (.01)	19.9 (.001)	17.4	19.1 (.05)
"α_2"	25.4	26.3	27.3	27.0	28.1	29.9	30.5 (.02)	31.2 (.05)	33.1 (.01)	31.7 (.05)	34.1 (.02)
"β"	23.1	22.8	21.5	22.5	19.4	19.2	18.7 (.05)	19.7 (.05)	16.7 (.02)	20.3 (.05)	18.9 (.05)
"γ"	24.7	22.5	24.8	23.1	24.1	22.1	21.3 (.05)	20.5 (.05)	21.8	22.6	19.7 (.02)

* Legend as for Table I.

also occurred on the seventh day, but to a greater extent, 34% decrease in "glycoalbumin" as compared to 5% in serum albumin. It should be pointed out that, as early as the third day post-infection, the average "glycoalbumin" had decreased by 34%, although variability within the group kept the level of significance between 0.10 and 0.05. The carbohydrate-containing globulins were equally noteworthy in their changes. Unlike the α_1-globulin, highly significant increases in the α_1-glycoprotein were seen on day 5 following infection. The α_2-glycoprotein also showed an increase about equal in magnitude to the α_1-glycoprotein. However, the remaining glycoprotein fractions decreased. Each of these changes occurred on the 6th day post-infection. It should be noted that the increase in α_1-glycoprotein showed a level of significance greater than 0.001 earlier than any other fraction. On day 3 post exposure, one day before maximum fever, there was an increase in this fraction with a level of significance better than 0.1 but not 0.05. It is possible that with additional patients these data would become statistically significant.

Fig. 1 illustrates the dynamic changes in the fractions with the major changes. It appears that the glycoproteins (particularly α_2) began increasing in concentration almost immediately after exposure and remained elevated even at the conclusion of the study. The α_2-globulin had a short delay period before it began to rise. The difference in degree of change is readily apparent from the

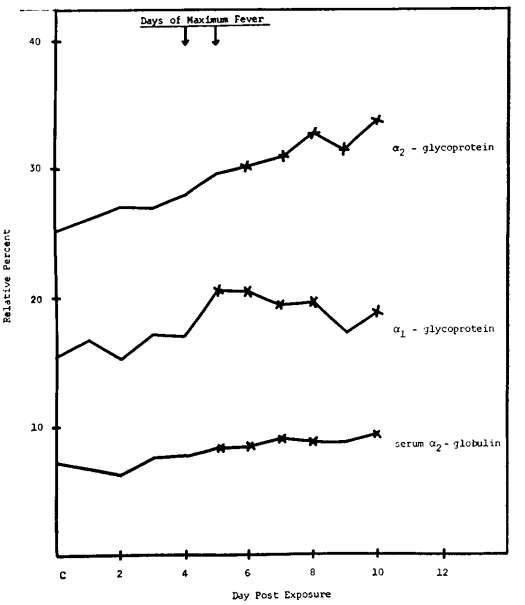

FIG. 1. Changes in serum protein fractions after exposure to *Pasturella tularensis*. X indicates points which are significantly different from control values.

Figure. Limited data reported by Ferris and Budd,(5), indicated similar changes in serum mucoproteins in a variety of diseases including infection. Thus, it appears that changes in the serum glycoproteins may be a more sensitive measure of reaction to a bacterial infection than those of the serum proteins.

Summary. Volunteers have been infected with *P. tularensis*. Maximum fever was seen 4 or 5 days after exposure. The changes in several protein fractions were observed fol-

lowing the infection. A decrease in serum albumin and an increase in α_2-globulin occurred 6-7 days after infection. More pronounced changes were seen in the glycoproteins. The "glycoalbumin" showed a 34% decrease as compared to 5% for serum albumin. "α_1-glycoprotein" had a significant increase on day 5. Both the "glycoalbumin" and "α_1-glycoprotein" were almost at an 0.05 level of significance by day 3 post exposure, prior to the onset of fever, and reached a significant level of 0.001 on the day of maximum fever.

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Received June 9, 1966. P.S.E.B.M., 1966, v123.

Detection of a Kinin Substance in the Testis of Guinea Pigs After Induced Allergic Orchitis.* (31450)

R. E. MANCINI, H. HUIDOBRO, E. FERNÁNDEZ COLLAZO, AND R. MONASTIRSKY
(Introduced by N. R. Rose)
Departamento de Histología, Facultad de Medicina and Departamento de Farmacología Experimental Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Arg.

It is generally assumed that in heterologous induced allergic processes liberation or activation of certain amines and/or polypeptides (plasmakinins) apparently implicated in the development of inflammatory signs and anaphylactic symptoms takes place(1,2).

Lack of data about the presence of similar substances in the male gonad of guinea pigs, carrying an induced allergic orchitis(3,4) prompted us to investigate the problem in these homo- or autosensitized animals. Correlation between the appearance of these substances and the development of testicular lesions and of circulating antispermatic antibodies was also attempted.

Material and methods. A total of 104 healthy adult male guinea pigs weighing 400 to 500 g divided into 4 groups was used. Group I comprised normal control animals. Group II included animals intradermally sensitized in the dorsum with one dose of the antigen consisting of homologous testicular homogenate plus complete Freund adjuvant (Difco 0638), as recommended by Freund *et al*(3). Groups III and IV also used as controls were injected with testicular homogenate or with adjuvant alone in doses similar to those administered in Group II (Table I). After sensitization animals from all groups were killed at intervals of time between 3 and 40 days (Table II). Blood samples were taken by cardiac puncture and circulating antispermatic antibodies determined by complement fixing test (50% hemolysis), using the supernatant of testicular homogenate at an optimal dilution of 1/250. A portion of the gonad was fixed in Bouin's liquid for routine histological examination. Lesions were estimated as vascular changes, accumulation of mononuclear cells, cytolysis and sloughing of the germinative epithelium. The remainder of testicular tissue was used for detection of kinin substances. After dissecting out the albuginea, the mass of tubules and intertubular connective tissue structures were immediately homogenized in buffered saline at pH 7.5 for 5 minutes. Proteins were then precipitated by boiling in a water bath at 100°C for 3 minutes and, after centrifugation, 0.2 ml of the supernatant was submitted to standard biological tests to verify the existence of spasmogenic substances in the testicular tissue. For the biological tests 3 kinds of organ preparation were used: normal guinea pig ileum and rat duodenum, suspended in 2 ml Tyrode solution bath at 34°C and 30°C,

* This work was supported by Grant M-65.22 from Population Council Inc., New York.