# Formation of Acid Glycoproteins in Serum.* (25200)

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The perchloric acid-soluble protein fraction in serum, often designated as the seromucoid or the mucoprotein fraction(1,2), is a protein fraction of high carbohydrate content. In humans it comprises less than 1.5% of the total proteins, but about 10% of the total protein-bound carbohydrate. The major component of this fraction is identical with the acid $\alpha_1$-glycoprotein isolated and characterized by Weimer, Mehl and Winzler(3) and by Schmid(4). Characteristic of this glycoprotein is the high sialic acid content(5), which is responsible for the low isoelectric point. The small-molecular $\alpha_2$-glycoproteins of Schmid(6) are probably also contained in this fraction. The acid glycoprotein fraction is of clinical interest, since it has been shown that its concentration increases in serum in a variety of neoplastic and infectious conditions (1). Therefore it has often been assumed that the increase in this fraction is in some way associated either with tissue destruction or with cell proliferation. The possibility that the liver is involved in production of acid glycoproteins is suggested by the observations by Greenspan *et al.*(7,8) that content of this fraction in serum decreases in uncomplicated parenchymatous liver diseases. In rheumatoid arthritis there is a marked increase in acid glycoproteins, serum level being significantly correlated to activity of the disease(9). We found(10) remarkably high concentrations of acid glycoproteins also in synovial fluid of patients with active rheumatoid arthritis. The values were almost as high as in serum in spite of the much lower total protein concentration in the synovial fluid. These data suggest the possibility that in arthritis there might also be a localized production of acid glycoproteins in the inflamed synovial tissue. It seemed of interest, therefore, to study the rôle of the liver in formation of acid glycoproteins both under normal conditions and in experimental arthritis. The effect of carbon tetrachloride-induced damage to the liver was therefore studied. Since the extensive liver injury produced by this agent also could be expected to affect the reticulo-endothelial system (RES) in the liver, control experiments with the RES blocking agent Thorotrast were included. To estimate extent of liver damage, serum GPT (glutamic puruvic transaminase) content was determined. The effect on the function of the RES was followed by determinations of prothrombin and proconvertin. Recent results by Slätis(11) strongly indicate that these coagulation factors are synthesized in the RES.

*Materials and methods.* All experiments were carried out on white male rabbits weighing 2.5 - 3.5 kg. Blood was collected from the marginal vein of the ear by piercing the vein with a lancet after the ear had been rubbed with xylene for about a minute. Care was taken not to rub the site of incision in order to avoid hemolysis. Usually about 6-8 ml blood was collected. In view of the findings of Werner(12) that serum hexosamine in rabbits increases after repeated bleedings control experiments were done in order to find out whether the withdrawal of blood might influence the acid glycoprotein content of serum. Four rabbits were bled 30-40 ml daily for 2 days and determinations of total protein and acid glycoproteins were made 24 hours after the second bleeding. No significant change in the acid glycoproteins was found (average change + 2%) whereas total protein content decreased on an average 15%, ($P<0.01$). Carbon tetrachloride was administered through a stomach tube in doses of 2 ml per kg body weight. Thorotrast (Testagar & Co., Inc., Detroit, Mich.) was injected into the marginal vein of the ear using 4 ml per kg body weight. Arthritis was produced by intra-articular injection (left knee joint) of 0.8-1.2 ml of a suspension containing about $10^9$ living bacteria per ml. Experiments were performed with a strain of *staphylococcus*

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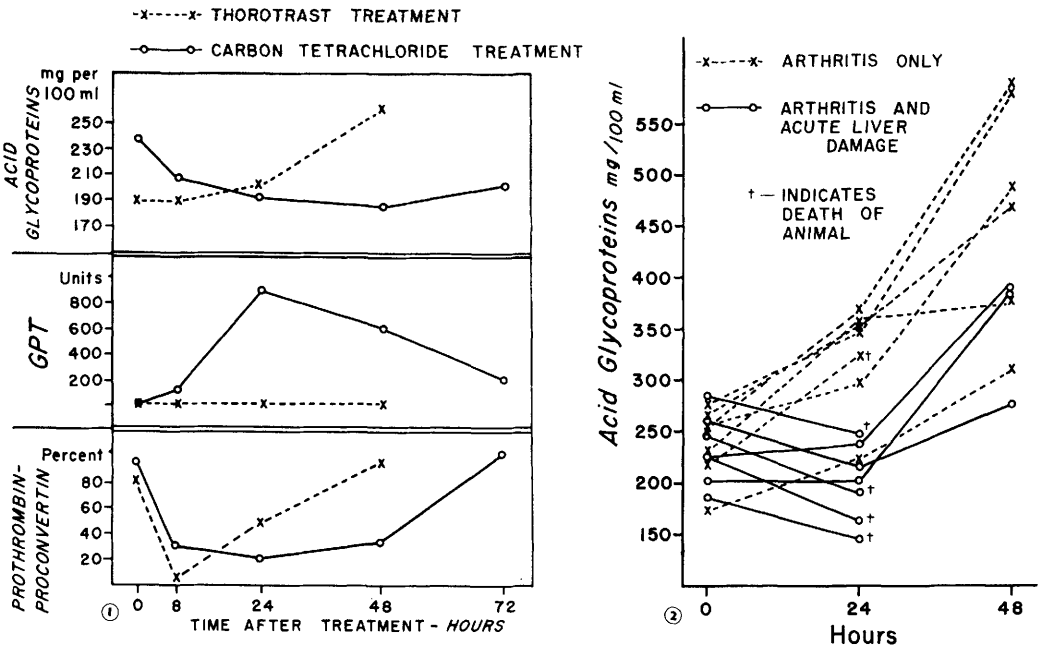


FIG. 1. Changes in acid glycoproteins, GPT and prothrombin-proconvertin following RES blockade (Thorotrast) and induced liver damage (carbon tetrachloride). Three rabbits in each group; points represent average values. Thorotrast (4 ml/body weight) was administered intravenously and carbon tetrachloride (2 ml/kg body weight) through stomach tube.

FIG. 2. Effect of acute liver damage on acid glycoprotein response in experimental arthritis. Arthritis produced by intra-articular injection of suspensions of living bacteria. At the same time carbon tetrachloride was given to 7 animals. Average percentage change in acid glycoprotein concentration after 24 hr: Arthritis only ($n = 7$) $+ 34.4\%$; $t = 7.56$; $P < .001$. Arthritis $+ CCl_4$ ($n = 7$) $-13.1\%$; $t = 3.17$; $P < .05$. Difference between groups: $47.5 \pm 6.1$ ($P < .001$).

*aureus* and with a strain of pneumococcus (Type IV). With both bacterial suspensions signs of arthritis, limping and impaired mobility, were present one day after injection. Acid glycoproteins were determined according to a modification(13) of the method of Winzler *et al.*(14) and are expressed in mg/100 ml serum. For 20 normal rabbits an average value of 245 ± 10 mg/100 ml serum (standard deviation : 43) was obtained. Prothrombin and proconvertin (P & P) were determined by a micro modification of Owren's method(15) and expressed as % of average value found for normal rabbits. Glutamic puruvic transaminase (GPT) was determined according to Frankel and Reitman(16) and expressed in Karmen units.

*Results.* Fig. 1 shows a comparison between the effect of RES blockade and acute liver damage in 2 groups each comprising 3 animals. The response was similar for all animals within each group. The marked rapid decrease in the P & P values following administration of Thorotrast is similar to the effect obtained by Slätis on rats(11). The coagulation factors almost disappeared within 8 hours and later rose almost as rapidly to values slightly above the initial ones. The absence of increase in transaminase values suggests that Thorotrast had no significant toxic effect on the liver. There was no significant change in acid glycoprotein concentration of the serum during the first 24 hours but after 2 days there was a moderate increase. The concentration returned to normal 2 weeks after Thorotrast injection.

With carbon tetrachloride the P & P values also decreased, although not to the same extent as with Thorotrast. The change paralleled the increase in GPT rather closely. The acid glycoproteins also decreased slowly reaching a minimum after 48 hours. The decrease at this time was found to be statistically significant (average decrease for 6 ani-

mals = 17.3%; t = 5.20; $P<0.01$).

Fig. 2 shows the marked increase, 90% after 48 hours, in acid glycoproteins following intra-articular injection of suspensions of pneumococcus (Type IV) or *Staphylococcus aureus*. The results were essentially similar with both types of bacteria. When carbon tetrachloride was administered simultaneously with injection of bacteria, acid glycoprotein concentration decreased after 24 hours in 6 of the 7 cases. The difference between acid glycoprotein concentration of the serum in the 2 groups is highly significant.

All animals survived the arthritis with the exception of one, which died 2 days after injection. Bacterial injection combined with carbon tetrachloride poisoning caused the death of 4 of the 7 animals on the second day. Histological examination of the liver showed very extensive necrotic changes. It is also evident from Fig. 2 that in the surviving animals acid glycoprotein concentration increased rapidly.

*Discussion.* Administration of carbon tetrachloride to rabbits produced acute liver damage manifested by rapidly increasing serum transaminase values. Since the RES of the liver may be affected by this treatment the function of this system may also be impaired. Evidence for this was obtained by a decrease in the prothrombin-proconvertin test values following carbon tetrachloride. It was nearly as marked as that obtained by RES blockade with Thorotrast. On the other hand Thorotrast produced no increase in GPT.

Since carbon tetrachloride, in contrast to Thorotrast, produced a significant decrease in the acid glycoproteins, it seems likely that this effect should be attributed to liver cell injury and not to the effect on the RES.

Experimental arthritis produced a rapid increase in the perchloric acid-soluble protein fraction of a degree similar to that observed in arthritis in man. The results (Fig. 2) clearly indicate that the initial rise in this protein fraction following infection should be ascribed to increased production in the liver. However, it was found that, in those animals which survived the massive dose of carbon tetrachloride for more than one day, acid glycoproteins started to rise again on the second day. The most likely explanation for this observation is that a fast restoration of some liver functions occurs. That some localized production of acid glycoproteins may also have occurred can, of course, not be excluded by the experiments reported here.

The results support the view gained from previous clinical studies that the liver is the site of formation of the acid glycoproteins in serum.

The increase in acid glycoprotein production as a response to infection is remarkably rapid and serum concentration may rise to more than twice the initial value in 48 hours. The recent results of Boström *et al.*(17) also indicate that the turnover of the acid glycoproteins is considerably higher than that of other plasma proteins.

The physiological rôle of the acid glycoproteins is still unknown. Werner(12) observed an increase in serum hexosamine, $\alpha$- and $\beta$-globulins after repeated large bleedings in rabbits and suggested that the synthesis of carbohydrate-rich proteins is associated with increased plasma protein synthesis. In similar bleeding experiments we found however no significant change in the acid glycoprotein concentration in spite of a significant decrease in total protein concentration.

In clinical studies it has on the other hand been demonstrated that changes in the acid glycoproteins are regularly associated with other changes in the serum protein pattern (18). Notable is the negative correlation between acid glycoprotein and albumin content which is especially marked in rheumatoid arthritis. These observations may indicate that the acid glycoproteins of the serum are in some way associated with albumin synthesis.

*Summary.* Acid glycoproteins of serum have been studied in experimental arthritis, after carbon tetrachloride poisoning and after RES blockade with Thorotrast, in rabbits. The results indicate that liver cells are the site of formation of the acid glycoproteins.

1. Winzler, R. J., in: *Methods of Biochemical Analysis II*, Interscience, N. Y., 1955, p279.

2. ———, in: *Chemistry and Biology of Mucopolysaccharides*, Churchill, London, 1958, p245.

3. Weimer, H. E., Mehl, J. W., Winzler, R. J., *J. Biol. Chem.*, 1950, v185, 561.

4. Schmid, K., *J. Am. Chem. Soc.,* 1953, v75, 60.
5. Odin, L., Werner, I., *Acta Soc. Med. Upsal.,* 1952, v57, 227.
6. Schmid, K., *J. Am. Chem. Soc.,* 1955, v77, 742.
7. Greenspan, E. M., Tepper, B., Terry, L. L., Schoenbach, E. B., *J. Lab. Clin. Med.,* 1952, v39, 44.
8. Greenspan, E. M., Dreiling, D. A., *Arch. Int. Med.,* 1953, v91, 474.
9. Shetlar, M. R., Payne, R. W., *J. Lab. Clin. Med.,* 1958, v51, 588.
10. Nettelbladt, E., Sundblad, L., *Arthritis and Rheumatism,* 1959, v2, 144.
11. Slätis, P., *Scand. J. Clin. Lab. Invest.,* 1958, v10, suppl. 33.
12. Werner, I., *Acta Phys. Scand.,* 1949, v19, 27.
13. Goa, J., *Scand. J. Clin. Lab. Invest.,* 1955, v7, suppl. 22.
14. Winzler, R. J., Devor, A. W., Mehl, J. W., Smyth, I. M., *ibid.,* 1948, v27, 609.
15. Sundblad, L., in: *Kliniska Laborationsmetoder,* Ed. II, Del V, Esselte, Stockholm, 1955, p271.
16. Reitman, S., Frankel, S., *Amer. J. Clin. Path.,* 1957, v28, 56.
17. Beström, H., Rodén, L., Yamashina, I., *J. Biol. Chem.,* 1958, v230, 381.
18. Björnesjö, K. B., *Nord. Med.,* 1958, v60, 1312.

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## Temperature Control During Homogenization.* (25201)

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Length of time for which biological materials can be subjected to high speed homogenization is frequently limited by sharp rises in temperature which accompany such treatment. These temperature increases are particularly severe with fibrous tissues, and have also been observed during disruption of microorganisms by agitation with fine glass beads in the Waring Blendor(1). Such temperatures not only destroy labile cellular constituents, but constitute a safety hazard when volatile, flammable solvents are used. Temperature control is particularly difficult in homogenizers whose drive unit is located beneath the container, since the container can not be packed in ice or cooled in a water bath. Procedures commonly used for controlling homogenate temperatures in instruments such as the Waring Blendor include pre-cooling the container, working in the cold room, alternately homogenizing and chilling the container, and dropping ice into the homogenate. These measures frequently are not only either slow or inconvenient, but are ineffective when prolonged homogenization is required. With apparatus modifications and procedures described below, it is possible to maintain low temperatures during extended homogenization. The necessary equipment modifications may be made on standard homogenizer containers; these alterations are simple, inexpensive, and require only materials and tools available to most laboratory shops. Although methods and results described below are for the Waring Blendor, it should be feasible to adapt them to other makes of homogenizers. Evidence for the effectiveness of apparatus and procedures is presented in this report.

*Methods. Temperature control in glass Waring Blendor containers* is readily obtained by use of an internal cooling coil of the type shown in Fig. 1. During construction, care must be taken (a) not to collapse the tubing when winding the coil and (b) to position the coil so that it clears the blades by a safe distance. When coil is in use, water, a few degrees cooler than the temperature desired in the homogenate, is circulated through it by a small centrifugal pump. *Temperature control in semi-micro containers.* While the internal cooling coils described above are suitable for large glass and metal homogenizer containers, they cannot be used in smaller semi-micro units. Temperature control in these containers, however, is easily obtained by installation of water jackets. Fig. 2 shows a metal semi-micro container for the Waring Blendor fitted with cooling jacket made from 3-inch diame-

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