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Nonthrombogenic Plastic Surfaces

Abstract. *Heparin has been chemically combined with a number of plastic surfaces rendering them nonthrombogenic as judged by Lee-White coagulation tests in vitro with human blood. Addition of quaternary ammonium groups to the plastic permitted formation of insoluble complexes with heparin. These heparinized surfaces were essentially nonthrombogenic and adsorb blood proteins to a significantly smaller degree from dilute solution than do the unmodified plastic surfaces. The affinity of the formed blood elements for these modified surfaces is much less than for the unmodified surfaces.*

The thrombogenicity of plastics is the major drawback to their use in artificial organs in contact with blood. Although plastics are used in heart-lung machines, kidney machines, and artificial heart valves and patches, the tendency of these materials to cause coagulation necessitates the use of anticoagulants such as heparin. A few plastics such as polytetrafluoroethylene and the silicone rubbers are more compatible with blood than most plastics, yet they still show thrombogenic characteristics.

The GBH (graphite-benzalkonium-chloride-heparin) surfaces prepared by Gott represented the first real advance in preparation of nonthrombogenic surfaces. These surfaces on plastics, prepared by treating a graphited surface first with Zephiran and then with heparin, have been shown to be nonthrombogenic in dogs over periods of more than 2 years. These surfaces, however, can be applied only to rigid plastics, and the need continues for both flexible and elastomeric plastics that can be used in artificial organs.

Because of the success of this physical coating system, we undertook to bond heparin chemically to plastic surfaces, on the grounds that chemically bonded heparin would, by analogy with the Gott surface, produce blood compatibility. Such a surface, too, would be more like that of the natural vascular system, which is known to contain heparin and other sulfated polysaccharides that have heparin-like activity (*1*).

The initial step in chemical heparinization of a number of plastics has been the formation of quaternary ammonium sites on the plastic surface by several methods including (i) chloromethylation followed by quaternization with dimethyl aniline, (ii) radiation grafting vinyl pyridine to the base plastic followed by quaternization as with methyl iodide or benzyl chloride, and (iii) incorporation of a quaternizable monomer such as vinyl pyridine into a copolymer. After the surfaces were quaternized, heparinization was accomplished by simple contact with a heparin solution. Coagulation times with several treated and untreated plastics are shown in Table 1.

Table 1. Coagulation times with treated and untreated plastics.

Base polymer	Untreated (min)	Treated (hr)
Polystyrene	9–11	>20
Polyethylene	9–12	>20
Silicone rubber	15–20	>20
Polyvinyl chloride	12–16	>1
Nylon 610	8–14	>20
Gott surface		>20

Table 2. Change of ζ-potential during treatment.

Time of test	ζ-potential (mv)	
	Polyethylene	Polystyrene
Initial	−15.2	−16.8
After irradiation	−14.7	−12.6
After graft with 4-vinylpyridine	−19.8	−18.8
After methyl iodide	+11.5	+15.3
After heparinization	−8.4	−14.1

Table 3. Decay of ζ-potential of heparinized polystyrene surfaces in Ringer solution.

Hours in Ringer	ζ-potential (mv)
Vinyl pyridine–methyl iodide	
24	−14.9
48	−11.8
192	−10.9
746	−3.3
1394	−3.3
Chloromethylation–dimethyl aniline	
24	−11.5
48	−7.3
696	−6.8
1344	−5.7

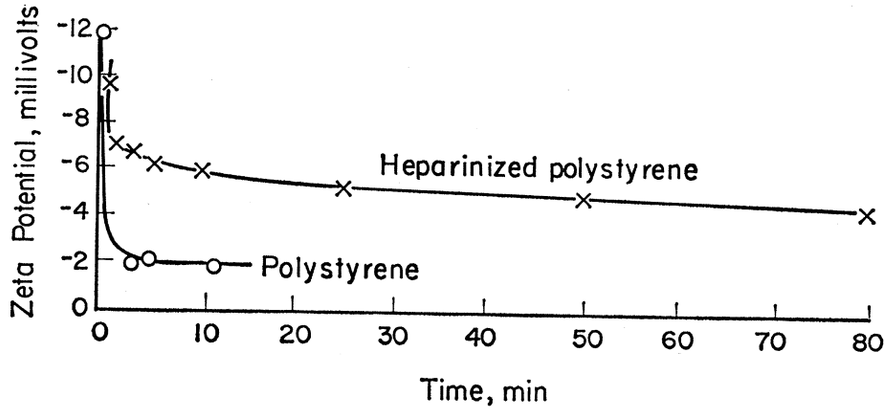

Fig. 1. Adsorption of fibrinogen on polystyrene and heparinized polystyrene. The concentration of fibrinogen in Ringer solution was one-thousandth that usually found in blood.

The quaternization and heparinization treatments were followed by measurements of the ζ-potential of the surface in contact with Ringer solution. Most plastics show a negative ζ-potential. After quaternization the ζ-potential generally swings to a positive value because of the positive ammonium groups, and after heparinization the ζ-potential again becomes negative. The magnitude of the changes is an indication of the extent of the reaction (Table 2). Permanence of the heparinization is necessary if these surfaces are to be used for long-term implants. An indication of these stabilities was gained by following the ζ-potential of heparinized surfaces in contact with Ringer solution over relatively long periods. If the heparin is lost, the ζ-potential would be expected to become positive. Table 3 shows the decay of ζ potentials of some heparinized surfaces. There is some indication from work by Gott (*2*)

that the heparin on his surfaces is in equilibrium with endogenous heparin in the blood and that the Gott surfaces will remain heparinized for periods of more than 2 years.

It has been found (*3*) that unmodified plastics strongly adsorb blood proteins. Changes in ζ-potential (with time) of treated and untreated polystyrene surfaces in contact with Ringer solutions containing fibrinogen, γ-globulin, or albumin at one-thousandth the usual physiological concentrations have been recorded, and Fig. 1 is typical of the result. This change in ζ-potential is attributed to the adsorption of proteins on the plastic surfaces. As a result of this adsorption, the solution no longer "sees" the plastic surface, but rather sees the adsorbed protein layer. Similar results were obtained with other treated and untreated plastic surfaces. In the case of albumin a relatively small difference was found which may be due to the fact that albumin is more negatively charged than the other proteins.

The nonthrombogenicity of the heparinized surfaces as well as that of the Gott surfaces may be related to the reduced adsorption of blood proteins and perhaps other blood components on the heparinized surfaces. It has also been thought that thrombus formation may be initiated by the "sticking" of formed elements such as leucocytes and platelets to a surface (*4*). In some experiments formed elements of the blood adhered to chemically heparinized surfaces to a significantly smaller degree than to the unmodified surfaces.

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Lactoperoxidase: Identification and Isolation from Harderian and Lacrimal Glands

Abstract. *Investigation of bovine lacrimal and harderian glands revealed the presence of the enzyme lactoperoxidase, which was isolated and purified. A nonheme, iron-containing protein was identified at the same time. Both proteins are present in milk, mammary glands, and salivary glands. Their roles are discussed: The lactoperoxidase may be important in controlling bacterial flora.*

An extensive survey of bovine tissues and fluids, using highly specific immunochemical methods, found the enzyme lactoperoxidase only in the salivary and mammary glands (*1*). Extension of this investigation showed for the first time that lactoperoxidase is also present in the harderian and lacrimal glands, which are also exocrine glands of ectodermal origin. A second protein, the nonheme, iron-containing "red protein," which had been isolated with lactoperoxidase from raw bovine milk (*2, 3, 4*), was also found there. The crude preparation of lactoperoxidase obtained by treating raw skim milk with ion-exchange resin (*3, 4*) also contains the red, nonheme, iron protein. Both the lactoperoxidase (*3, 4*) and the red protein (*5*) were further purified by ion-exchange chromatography on IRC-50.

Lacrimal and harderian glands of both cow and steer were excised at the slaughterhouse and packed in ice; the tissue was freed of extraneous fat and connective tissue as soon as possible. Each gram was homogenized at 4°C with 1 ml of 0.1M phosphate buffer containing 1 percent cholate, *p*H 7.4; the homogenate was centrifuged for 30 minutes at 20,000*g*. The soluble extracts thus obtained were examined directly by immunodiffusion analysis.

Essentially the same procedure as for the salivary gland (*1*) was used to isolate the enzyme from the lacrimal gland. The gland was similarly extracted, except that a second extraction was made of the insoluble residue and the combined extracts were dialyzed. To each 100 ml of the extract, 2.7 g of the sodium form of IRC-50 resin was added, and the *p*H of the solution was adjusted to 7.0; the suspension was stirred for 30 minutes. Then, after removal of the resin by filtration or centrifugation, the extract was treated with resin a second time. The resins were combined and washed free of material absorbing light at 280 mμ with distilled water on a sintered-glass funnel, and then washed into a glass tube 2 cm in diameter. The proteins adsorbed to the resin were first eluted with a solution of 0.5*M* sodium acetate, the eluate being collected on an automatic fraction collector.

After a greenish-colored crude-enzyme fraction was obtained, the eluting solution was changed to a solution that was 0.5*M* with respect to sodium acetate and 0.5*M* as to sodium chloride. Collection of the eluate on the automatic fraction collector continued until another fraction red in color was obtained. The contents of the tubes containing the green-colored crude enzyme were combined and dialyzed against distilled water. The crude lactoperoxidase could be freed of contaminating hemoproteins (*6*) such as cytochrome *c* by passage down a Sephadex G-75 or G-100 column (*3, 4*). Alternatively, the crude dialyzed enzyme could be further purified by chromatography on IRC-50, 200 to 400 mesh (*3, 4, 6*).

The contents of the tubes containing the red-colored fraction were combined; their color resulted primarily from a nonheme iron protein that could be further purified by passage down a Sephadex G-100 column or by ion-exchange chromatography (*5*).

Rabbit antiserums were prepared against partially purified lactoperoxidase and crude red protein (*4, 6, 7*); the immunization procedure has been detailed (*4, 6, 7*). Antiserums obtained from two rabbits (R78 and R79) during the course of hyperimmunization with red protein were pooled and serially absorbed by small additions of purified lactoperoxidase, whole bovine serum, lactalbumin, and lactoglobulin to provide a reagent (R7879 abs.) specific for red protein. A pool of antiserum to lactoperoxidase was serially absorbed by small additions of purified red protein to provide a reagent (R7377) specific for lactoperoxidase. Absorbed antiserums gave only a single band of precipitation when examined by immunodiffusion or immunoelectrophoresis employing crude antigen or skim milk.

The presence in each antiserum of an excess of the antigen used in absorption was evident from immunodiffusion findings. As shown in Fig. 1 (lower left), diffusion of antiserum to red protein (well *1*) against antiserum to lactoperoxidase (well *2*) gave two bands of precipitation: one band showed complete fusion with the single