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.

R. I. LEININGER, C. W. COOPER
R. D. FALB, G. A. GRODE
Battelle Memorial Institute, Columbus, Ohio

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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

band given by red protein (well *B*); the other, complete fusion with the single band given by lactoperoxidase (well *C*).

Immunodiffusion analysis of extract of bovine lacrimal gland revealed the presence of components immunologically indistinguishable from red protein and lactoperoxidase isolated from cow's milk. As shown in Fig. 1 (top left and bottom right), lacrimal-gland extract (wells *A*) gave a single band of precipitation with antiserum to red protein (wells *1*), which fuses completely with the purified reference red protein placed in wells labeled *B*. Lactoperoxidase (wells *C*) showed no reaction with the antiserum to red protein. Similarly, the antiserum to lactoperoxidase revealed the presence of an antigen in the lacrimal-gland extract, indistinguishable from purified lactoperoxidase from milk (Fig. 1, top right). Extracts of the harderian gland gave similar results.

Presence of lactoperoxidase and the red protein in the lacrimal gland was further confirmed by their isolation. As we have said, when the gland extracts were treated with the ion-exchange resin, both lactoperoxidase and red, nonheme, iron proteins were adsorbed to the resin; they were separated from each other almost quantitatively by the elution procedure. The eluted fractions were analyzed for enzyme activity by the guaiacol assay procedure (*2, 3, 4*). More than 95 percent of the peroxidase activity was contained in the fraction eluted with 0.5*M* sodium acetate; the lactoperoxidase was, however, contaminated with other hemoproteins such as cytochrome *c* and hemoglobin. On a molar basis, almost twice as much lactoperoxidase as cytochrome *c* was extracted by the procedure employed.

Although the proteins and enzyme activity in tears and lacrimal glands have been variously studied (*8, 9*), this is the first report, to our knowledge, of the presence of a peroxidase. The occurrence of both lactoperoxidase and red protein in the salivary and lacrimal glands of steers as well as cows shows that these proteins are not only products of the mammary gland.

While human γ_A immunoglobulins of parotid secretions and colostrum resemble one another, they differ significantly in their immunological properties from γ_A found in human serum (*9*). It is noteworthy that lactoperoxidase obtained from lacrimal and salivary glands is immunologically indistinguishable from that obtained from the mammary gland; similarly, the red proteins appear to be immunochemically identical.

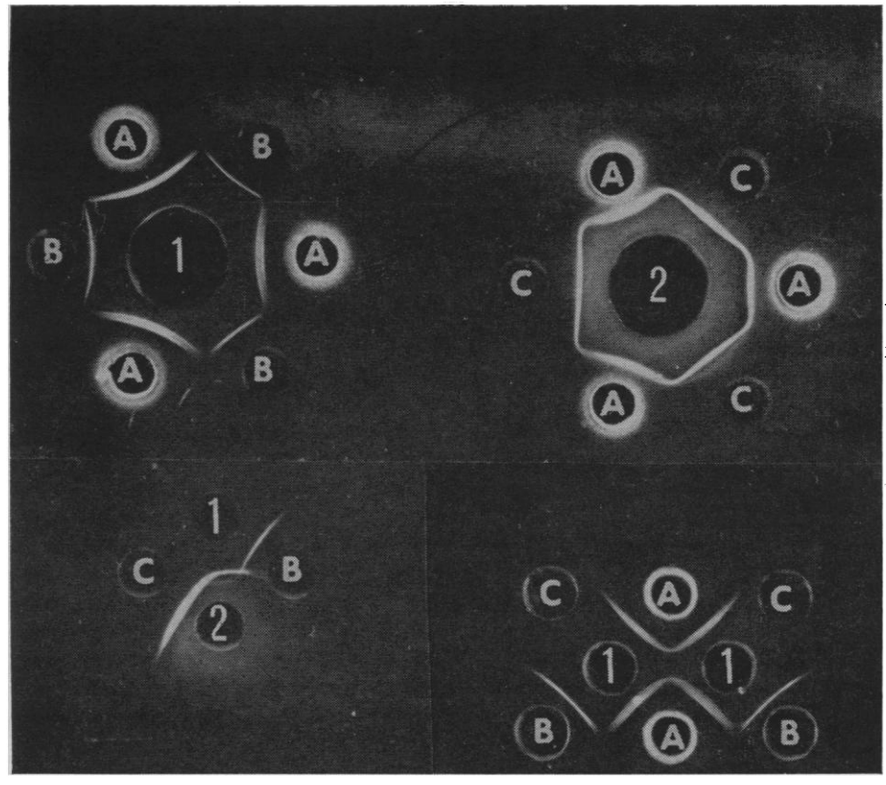

Fig. 1. Immunodiffusion analysis of bovine lacrimal-gland extract. Wells labeled *1* contain rabbit antiserum (R7879 abs.) prepared against red protein from cow's milk. Wells labeled *2* contain rabbit antiserum (R7377 abs) to lactoperoxidase from cow's milk. Bovine lacrimal-gland extract in wells *A*, purified red protein in wells *B*, and purified lactoperoxidase in wells *C*.

The presence of lactoperoxidase in the four exocrine glands of ectodermal origin suggests a common function. The role of the enzyme is still a matter for speculation, although there is evidence that it may be protective (*10*); our studies have shown that it can inhibit certain bacteria under aerobic conditions, so that it may serve to control bacterial flora of the oral, nasal, and ocular area. Interestingly the enzyme lysozyme also has been found in milk (*11*), saliva (*12*), and tears (*13, 14*), and is believed to be an antibacterial agent.

Immunoelectrophoretic studies (*14*) have shown that under mild trauma human tears contain the nonheme iron protein transferrin. Red nonheme, iron proteins isolated from milk have been referred to as a transferrin or lactotransferrin (*15*). This nomenclature is used primarily because the spectral properties of this iron protein resemble those of serum transferrin. However, the iron protein in the lacrimal gland is not identical with serum transferrin in electrophoretic, chromatographic, or immunochemical properties; moreover, it has not been detected in any tissue other than salivary, harderian, lacrimal, and mammary glands. The name lactotransferrin may be misleading since it implies that the protein has a role as a milk-specific iron-transfer protein; evaluation of its role may reveal another function. Recent work on nonheme iron proteins has shown their importance in biological oxidation systems.

MARTIN MORRISON
Department of Biochemistry, City of Hope Medical Center, Duarte, California

PETER Z. ALLEN
Department of Microbiology, University of Rochester Medical Center, Rochester, New York

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Electrophoretic Heterogeneity of the Polypeptide Chains of Human G-Myeloma Proteins

Abstract. *The light and heavy polypeptide chains derived from human G-myeloma proteins are electrophoretically heterogeneous as judged by disc electrophoresis of the polypeptide chains in urea-acrylamide gels. Individual myeloma proteins contained as many as eight light-chain and nine heavy-chain components.*

Normal human serum immunoglobulin G (IgG) consists of a group of molecules that are heterogeneous when judged by immunochemical and electrophoretic criteria (*1*). Human G-myeloma proteins, by contrast, have been considered homogeneous (*2*). It has become increasingly apparent, however, that myeloma proteins are only relatively homogeneous by electrophoretic criteria.

Electrophoretic heterogeneity in the starch-gel patterns of intact G-myeloma proteins has been demonstrated by Askonas, Fahey, and others (*3*) using myeloma proteins from both man and mouse. The constituent light and heavy polypeptide chains of human myeloma proteins are also electrophoretically heterogeneous. Cohen and Porter (*4*) demonstrated two bands in alkaline urea starch-gel analyses of light chains from most partially reduced and alkylated G-myeloma proteins. Heavy polypeptide chains (γ-chains) yielded a diffuse electrophoretic zone that did not resolve into bands. Poulik (*5*) reported even greater heterogeneity; he found at least five components in some myeloma light chains analyzed by starch-gel electrophoresis at alkaline *p*H. His studies in acid urea starch gel also suggested the presence of multiple components in myeloma protein γ-chains.

Myeloma proteins are the most homogeneous preparations of immunoglobulin molecules now available. For this reason they have served as models for structural analyses of immunoglobulin molecules. The high resolving power of disc electrophoresis in acrylamide gels prompted us to reexamine the electrophoretic homogeneity of polypeptide chains isolated from myeloma proteins.

A G-myeloma protein molecule is constructed of two light polypeptide chains that are antigenically either of type K (κ-chains) or type L (λ-chains) (*6*) and two heavy polypeptide chains of one of the four antigenic subclasses (γ_{2a}, γ_{2b}, γ_{2c}, γ_{2d}) (*7*). Four human G-myeloma proteins were selected to represent the four heavy chain subclasses and both types of light chains. These proteins were isolated (*8*) and found by immunochemical means to be relatively uncontaminated (*9*).

In previous electrophoretic studies of myeloma polypeptide chains (*4, 5*) the chains were obtained by partial reduction of the myeloma proteins, under conditions that resulted in the breaking predominantly of interpolypeptide-chain disulfide bonds. To minimize the possibility that folding of polypeptide chains (caused by intrachain disulfide bridges) might contribute to electrophoretic heterogeneity, the isolated proteins were extensively reduced in 7*M* guanidine hydrochloride (*10*) with 0.1*M* dithioerythritol, and then alkylated in 5.2*M* guanidine hydrochloride with 0.22*M* iodoacetamide at *p*H 8.2. Light and heavy polypeptide chains were isolated by gel filtration on Sephadex G-200 in 5*M* guanidine hydrochloride (*10*). Since it is not possible to perform electrophoresis in the presence of high concentrations of charged molecules (such as guanidine hydrochloride), the polypeptide chains were dialyzed against 8.5*M* urea and then analyzed by disc electrophoresis on 4 percent polyacrylamide gels at *p*H 9.4 in the presence of 8.5*M* deionized urea (*11*).

The acrylamide-gel patterns of heavy-chain preparations from myeloma proteins representing the four antigenic subclasses of IgG all showed from six to nine discrete bands (Fig. 1, upper). Light polypeptide chains isolated from these same myeloma proteins showed from three to eight discrete components (Fig. 1, lower). Light chains from pooled normal serum IgG showed seven to eight components (Fig. 1, lower). The intensity of staining of light-chain bands of most myeloma preparations differed markedly from the "gaussian distribution" usually seen in patterns of normal light chains (*4*).

These gel patterns indicate considerable degrees of electrophoretic heterogeneity of both the light and heavy chains derived from G-myeloma proteins. The following experiments were performed to examine several possible causes for the multiple electrophoretic components. A single molecular species might produce multiple bands as the result of some unknown process during electrophoresis. In that instance, reelectrophoresis of one isolated band should lead again to the generation of multiple bands. In an experiment performed on the κ-chains from protein Sp, two identical gels were subjected to electrophoresis simultaneously at *p*H 9.4. The multiple bands were

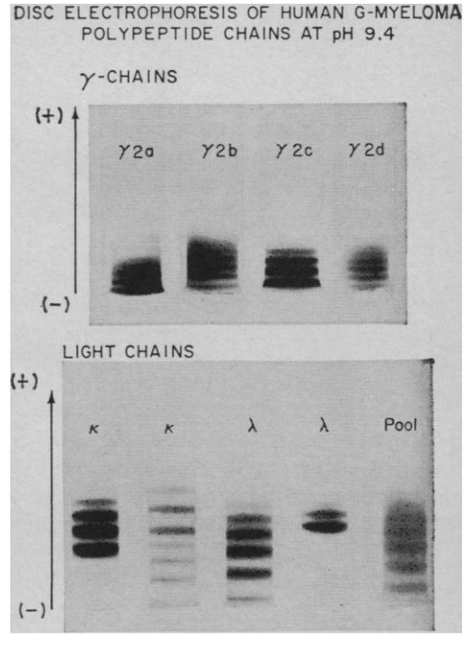

Fig. 1. Electrophoretic patterns of reduced and alkylated G-myeloma polypeptide chains. Upper: heavy chains, type γ_{2a} from Sa; γ_{2b} from Sp; γ_{2c} from Ta; γ_{2d} from Me. Lower: light chains, type κ from Sa; κ from Sp; λ from Ta; λ from Me; and light chains from pooled normal IgG. Disc electrophoresis in direction of arrow in 4 percent acrylamide gel, *p*H 9.4 tris-HCl buffer, 8.5*M* urea.