plasma during pregnancy is believed to be an aminopeptidase acting on the hemicystinyl-tyrosyl bond (*6*), but also capable of acting as a relatively unselective aminopeptidase (*7*). Although the placenta is believed to be the source of the oxytocinase in the plasma of pregnant women, it also contains "tissue oxytocinase," which appears first to cleave the disulfide bond of oxytocin, after which degradation by aminopeptidase takes place (*8*). Both oxytocinases destroy biological activity of oxytocin under the conditions described. The greater effectiveness of tissue oxytocinase over that of plasma oxytocinase in destroying immunologic activity may be due to the production of smaller peptides by tissue oxytocinase as a result of the dual action of disulfide cleavage plus aminopeptidase degradation.

Our investigations are of practical importance in immunoassays. It is often essential to assay plasma or body fluids containing no hormone as control materials with which to detect artifactually elevated hormone concentrations in the particular assay system. Our data suggest that extreme caution must be exercised in using proven biologic inactivators as hormonal inactivators in an immunoassay system. Each inactivator must be evaluated for effectiveness in the particular test system employed, be it biological or immunological. The immunoassay of oxytocin might at times measure degraded and biologically inactive fragments of oxytocin.

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Group A Streptococci: Localization in Rabbits and Guinea Pigs Following Tissue Injury

Abstract. *Rabbits injected intravenously with extracellular products ("toxins") of group A streptococci develop myocardial, muscular, and hepatic lesions. When such animals are then challenged with fluorochrome-labeled group A streptococci or with titanium oxide particles the labeled bacteria or particles localize within phagocytic cells in the tissue lesions caused by the toxins. Similarly, labeled streptococci or titanium oxide particles will also localize within phagocytic cells in skin lesions of guinea pigs that develop delayed hypersensitivity to tuberculin or to bovine gamma globulin. It is proposed that a combined mechanism of injury and localization of bacteria in damaged tissues may be responsible for post-streptococcal sequelae or other chronic inflammatory diseases.*

In a previous study (*1*) it was shown that a single intramyocardial injection of group A streptococci in rabbits induced granulomatous lesions at the site of injection. Neither trauma to the heart with a needle nor the intravenous injection of streptococci alone caused any lesions in the heart. On the other hand, granulomatous lesions developed in the heart at the site of needle trauma when living or dead streptococci were injected intravenously 24 hours after heart puncture. The experiments suggested that mechanical trauma predisposed to the localization of streptococci that could induce a granulomatous response. In addition, extracellular products (SEP) of group A streptococci induced severe myocardial, hepatic, and diaphragmatic lesions in rabbits after intravenous injection (*2, 3*).

This production by a group A streptococcus of a "tissue-damaging toxin" raised the possibility that, by analogy to "needle trauma," such toxic components may prepare the heart for the localization of streptococci. In this report we propose a possible mechanism by which group A streptococci and some of their components localize in tissues of rabbits injured by SEP or by unrelated delayed hypersensitivity reactions.

Myocardial, diaphragmatic, and hepatic lesions were induced in New Zealand white rabbits, weighing 1.5 to 2.0 kg, by the intravenous injection of SEP as described previously (*2, 3*). A fraction containing the tissue-damaging factor (TF) was isolated from SEP by gel filtration through Sephadex G-150 columns and was found to be associated with the high molecular weight material excluded from the column. The TF contained two antigens that reacted with antiserum to SEP and two protein components as revealed by gel electrophoresis. This fraction did not contain any detectable amounts of hyaluronidase, nicotinamide adenine dinucleotide nucleosidase activity, streptokinase, C-polysaccharide, or M-protein, but contained trace amounts of deoxyribonuclease and substantial amounts of streptolysin O, acid phosphatase (*4*), and a cell-sensitizing factor (*5*).

When injected intravenously into rabbits, 1 to 2 mg of TF protein (*6*) regularly caused severe coagulation necrosis in the liver, interstitial myocarditis, and a steep rise in serum levels of glutamic oxaloacetic transaminase and total lipids (*3*). Some of the animals were then injected intravenously or intraperitoneally with 1 ml (10^8 cell/ml) of washed type 4 streptococci that had been labeled directly with fluorescein isothiocyanate (FITC) or with rhodamine isothiocyanate (RITC) according to a method described previously (*7*). Other animals treated with TF were injected with titanium oxide (TiO_2) particles (100 per kilogram of body weight) as a control for nonspecific uptake of particles by the reticuloendothelial system (*8*).

Delayed hypersensitivity was induced in Hartley guinea pigs weighing 300 to 350 g by immunization (footpad injection) with 100 μg of bovine gamma globulin (BGG) in complete Freund's adjuvant that contained 2 mg of *Mycobacterium tuberculosis* (strain H37RV). Thirteen days after the injection of BGG, the animals received an intravenous injection of FITC- or RITC-labeled type 4 streptococci or of TiO_2 as described above. Four hours later several of the animals were challenged intracutaneously with PPD (purified protein derivative) (20 μg per injection site) while others received BGG (40 μg per injection site). A second injection of labeled streptococci or of TiO_2 was given intraperitoneally 8 hours after injection of the antigens. Twenty-four hours after intracutaneous injection of antigen, the animals developed typical delayed hypersensitivity lesions in the skin.

All animals were killed 1 to 7 days

after injections of the label, and the localization of fluorescent streptococci in tissues of the animals was determined by examination of unstained deparaffinized sections by ultraviolet microscopy (*7*). Localization of TiO_2 particles in the tissues was determined on deparaffinized sections, stained with hematoxylin and eosin, with the use of a dark-field condenser (*8*).

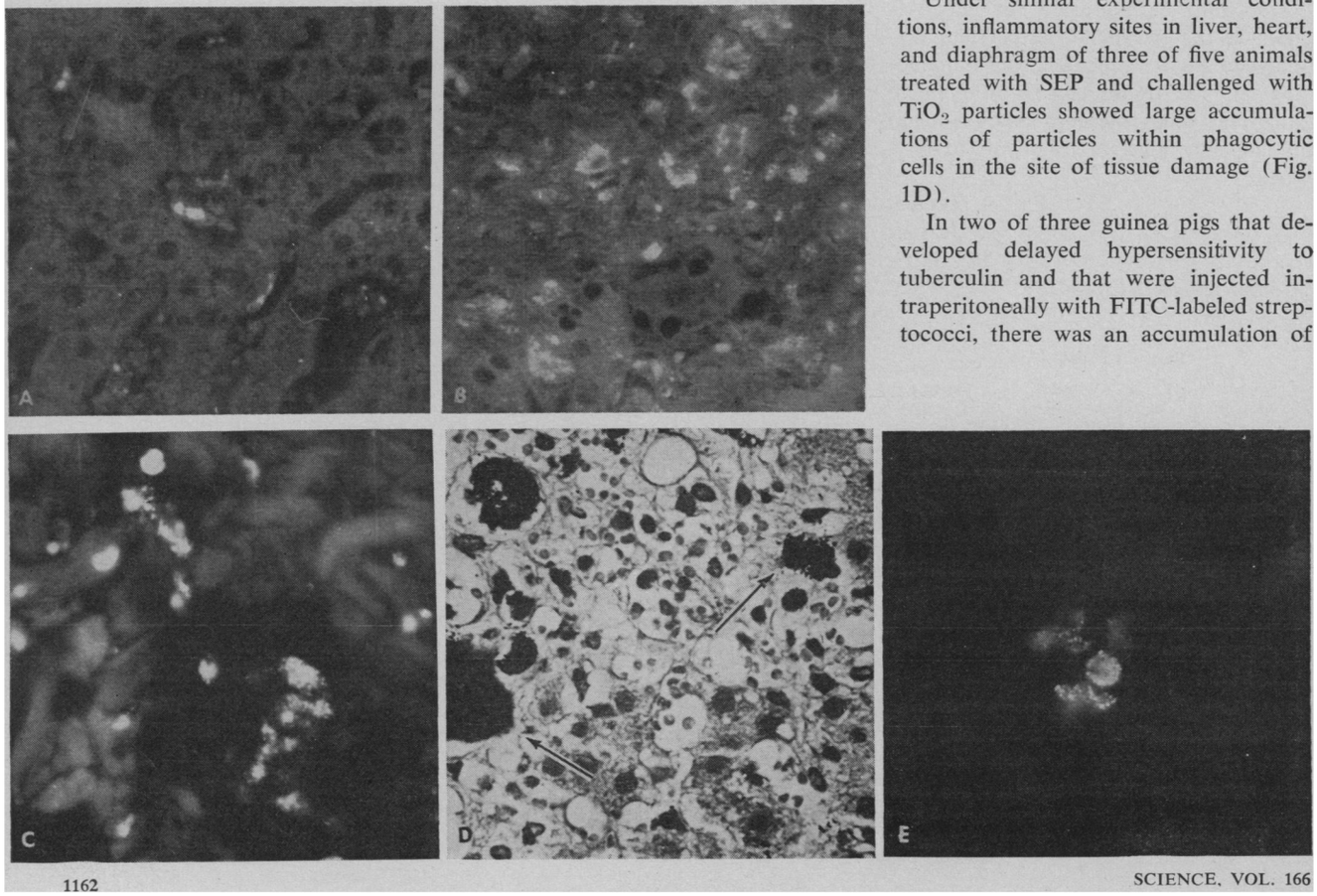
The distribution of labeled streptococci in tissues of normal rabbits and guinea pigs was similar to that of TiO_2. Animals killed 1 to 7 days after injection showed large accumulations of label within phagocytes in the red pulp of the spleen and in Kupffer cells in the liver (Fig. 1A). Occasionally, small numbers of phagocytes loaded with label were seen in the lungs and kidneys. In animals injected intraperitoneally with either streptococci or TiO_2, the peritoneal exudate contained large numbers of polymorphonuclear leukocytes loaded with labeled particles 1 to 3 hours after injection. At 24 hours, the majority of peritoneal cells containing the label were of the monocytic type. Usually the streptococci or titanium particles disappeared from the peritoneal cavity by the 3rd day. Distribution and localization of TiO_2 in the tissues of animals was essentially similar to the localization of colloidal carbon as described by Benacerraf *et al.* (*9*).

Six of eight rabbits injected intravenously with SEP (5 mg) or with TF (1 to 2 mg) and challenged with FITC- or RITC-labeled streptococci had severe necrotic lesions in the liver that developed into giant cell granulomas. Four of eight rabbits had interstitial myocarditis that developed into granulomas in two animals (*2, 3*). Sites of coagulation necrosis and granulomas in the liver were infiltrated with numerous granulocytes and mononuclear cells, many of which contained specifically brightly fluorescent coccoid bodies arranged in short chains (Fig. 1B). On the other hand, liver tissue taken from sites remote from the inflammatory areas showed occasional fluorescence only in the Kupffer cells, as was found in normal controls (Fig. 1A). Areas of interstitial myocarditis and granulomatous inflammation in the heart contained numerous mononuclear cells, many of which were filled with brightly fluorescent coccoid bodies (Fig. 1C). Usually the phagocytes loaded with fluorescent coccoid bodies were concentrated in areas surrounding the granulomas. Also, the cytoplasm of the giant cells forming the center of the granuloma was filled with fluorescent particles (*7*). Tissues of all three animals injected with SEP and challenged with unlabeled streptococci showed the same patterns of lesions but no trace of fluorescence in any of the numerous preparations examined. Gram stains of tissues revealed, however, that many of the phagocytic cells at the site of inflammation contained Gram-positive cocci.

Under similar experimental conditions, inflammatory sites in liver, heart, and diaphragm of three of five animals treated with SEP and challenged with TiO_2 particles showed large accumulations of particles within phagocytic cells in the site of tissue damage (Fig. 1D).

In two of three guinea pigs that developed delayed hypersensitivity to tuberculin and that were injected intraperitoneally with FITC-labeled streptococci, there was an accumulation of

Fig. 1. (A) Section of liver of a rabbit injected intravenously with FITC-labeled type 4 streptococci. Fluorescence is confined to Kupffer cells. Unstained preparation examined with ultraviolet microscope (× 150). (B) Section of liver of a rabbit injected intravenously with tissue-damaging factor followed by the intravenous injection of FITC-labeled streptococci. Note the accumulation of inflammatory cells filled with coccoid bodies at site of coagulation necrosis (× 150). (C) Section of heart from the same animal showing accumulation of fluorescent inflammatory cells in lesions (× 310). (D) Accumulation of TiO_2 particles (arrows) in liver lesion induced by SEP (× 310). (E) Section of skin of a guinea pig with delayed hypersensitivity lesions to tuberculin, injected intraperitoneally with FITC-labeled type 4 streptococci. Note inflammatory cells laden wtih fluorescent cocci (× 310).



numerous mononuclears and granulocytes, many of which contained brightly fluorescent coccoid bodies (Fig. 1E). Gram stains of tissues revealed that such cells contained Gram-positive cocci. Specimens of skin taken from areas remote from the inflammatory lesions showed only very few macrophages, none of which contained any fluorescence. Specimens of skin taken from lesions of delayed hypersensitivity in three of five animals challenged intravenously with RITC-labeled streptococci had large numbers of streptococcal chains in the tissue debris in the dermis as well as within phagocytic cells at the site of inflammation. Two animals that developed delayed hypersensitivity to BGG and were challenged with TiO_2 particles showed accumulation of the particles in the inflammatory sites. Both animals that developed delayed hypersensitivity to BGG and that were challenged intravenously and intraperitoneally with FITC-labeled goat antiserum to BGG showed the regular pattern of macrophage accumulation in the skin at the site of delayed hypersensitivity lesions, but no trace of fluorescence was found in any of the cells. On the other hand, examination of the peritoneal exudates of these animals showed that many of the granulocytes and macrophages had brightly fluorescent amorphous intracellular masses, which indicates that the phagocytes had taken up the labeled protein.

Two rabbits that were injected with TF and challenged with FITC- or RITC-labeled streptococci developed endocardial lesions that consisted of large accumulations of granulocytes and fibrin-like amorphous material. The majority of granulocytes were filled with large numbers of brightly fluorescent cocci. Also two guinea pigs that developed delayed hypersensitivity to BGG and were challenged intraperitoneally with FITC-labeled streptococci had focal lesions in the peritoneal linings in which large numbers of granulocytes containing fluorescent cocci were found.

Results of this preliminary study indicate that streptococci or inert particles circulating in the blood or in the peritoneal cavity later on become localized within phagocytic cells in sites of tissue injury previously produced by a streptococcal "toxin" or by immunological reactions of delayed hypersensitivity in rabbits or guinea pigs, respectively. Chase (*10*) noted a similar phenomenon in which guinea pigs injected with complete Freund's adjuvant developed spontaneous disseminated granulomata. Once dissemination had occurred, various stimuli such as the injection of tuberculin or the application of skin irritants produced new granulomatous lesions in the skin at the challenge sites.

While the uptake and localization of both cocci and titanium particles seem similar, and therefore nonspecific, the importance of the former lies in the demonstrated capacity of some streptococcal components to persist for long periods of time and to produce and perpetuate chronic lesions (*11, 12, 7*). Furthermore, not only the toxic factor used in this study but also other streptococcal products [streptolysin O (*13*), streptolysin S (*2, 14*), proteinase (*15*), and others] can cause cellular damage and produce tissue trauma. It is thus tempting to speculate that the combination of naturally occurring group A streptococcal infection (producing circulating "toxic factors"), when followed by a bacteremia or by circulatory transport of cocci-laden phagocytes, might lead to chronic nonsuppurative sequelae. Clearly, however, the outcome of the streptococcal localization in distant, toxin-traumatized tissues must depend on the length of persistence of some of their products in tissues. Long persistence of streptococcal products in rabbits was found to cause chronic inflammatory lesions in rabbit skin (*11*) and, as demonstrated elsewhere (*7*) and in this study, in other rabbit organs as well.

Also, Mallory and Keefer (*16*) observed lesions of multiple organs in fatal cases of infections caused by hemolytic streptococci. These were late lesions with monocytic elements in tissues of patients with bacteremia who survived more than 10 days. It is intriguing to consider the possibility that the combination of effects seen in these patients could have been caused in a fashion similar to what has been observed in the rabbit model. Finally, it is possible that chronic diseases of obscure etiology may be caused by similar mechanisms involving bacteria other than hemolytic streptococci.

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Herpes Simplex Virus: Dry Mass

Abstract. *Dry mass of herpes simplex virus particles was measured by quantitative electron microscopy after isolation by surface spreading and critical-point drying of infected cells. The core weighed about 2×10^{-16} gram, the empty naked capsid 5×10^{-16} gram, the full naked capsid 7×10^{-16} gram, and the enveloped nucleocapsid 13×10^{-16} gram.*

Herpes simplex virus strain 11140 (*1*) was grown in monolayer cultures of a line of kidney cells derived from LEW-BN rats. Virus particles were isolated by spreading the cells, 20 to 40 hours after infection, on a trough filled with distilled water at *p*H 6.4. For electron microscopy, they were