INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is “Missing Page(s)”. If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in “sectioning” the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.

University Microfilms International
300 N. ZEEB ROAD, ANN ARBOR, MI 48106
18 BEDFORD ROW, LONDON WC1R 4EJ, ENGLAND
TUOZZO, CARL

IMMUNOLOGICAL ENHANCEMENT OF INDUCED TISSUE DAMAGE

The University of Arizona

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106
IMMUNOLOGICAL ENHANCEMENT
OF INDUCED TISSUE DAMAGE

by

Carl Tuozzo

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF GENERAL BIOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILosophY
WITH A MAJOR IN GENERAL BIOLOGY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1980
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Carl Tuozzo entitled Immunological Enhancement of Induced Tissue Damage and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.
STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of the scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: [Signature]
ACKNOWLEDGMENTS

I wish to thank professors Milos Chvapil, Ivan M. Lytle, and Wayburn Jeter. I would also like to thank the staff of the Department of Surgical Biology at the Arizona Medical Center for their assistance and Jo Overturf for her assistance in preparation of this manuscript.
# TABLE OF CONTENTS

| LIST OF TABLES | .................................................. | v  |
| ABSTRACT       | .................................................. | vi |
| INTRODUCTION   | .................................................. | 1  |
| MATERIALS AND METHODS | .................................................. | 12 |

- Sperm Antigen Preparation and Sensitization Method ........................................ 12
- Testis Antigen Preparation and Sensitization Method ....................................... 12
- Procedures for Challenging .................................................................................. 13
- Skin Testing ......................................................................................................... 13
- Sperm Immobilization ............................................................................................. 13
- Schultz-Dale Test ................................................................................................... 14
- Skin Wounds - Breaking Strength .......................................................................... 14
- Lung Fibrosis ........................................................................................................ 15
- Liver Fibrosis ......................................................................................................... 16
- Implanted Ivalon Sponges ...................................................................................... 16
- Histology ............................................................................................................... 17
- Statistical Analysis ............................................................................................... 17

| RESULTS                      | .................................................. | 18  |
| DISCUSSION                  | .................................................. | 33  |
| LITERATURE CITED            | .................................................. | 47  |
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Comparison of average wound breaking strengths - (500 g scale) in immunized versus non-immunized animals</td>
<td>23</td>
</tr>
<tr>
<td>2. Lung wet weight analysis</td>
<td>24</td>
</tr>
<tr>
<td>3a. Comparison of the determination of the mg of hydroxyproline per gram of silica induced fibrotic lung tissue between sensitized and challenged animals versus non-sensitized animals</td>
<td>25</td>
</tr>
<tr>
<td>3b. Comparison of the determination of the mg of hydroxyproline per fibrotic lung</td>
<td>26</td>
</tr>
<tr>
<td>4. Comparison of liver weights</td>
<td>27</td>
</tr>
<tr>
<td>5a. Comparison of the mg of triglycerides per gram of injured liver in sensitized and challenged animals versus non-sensitized animals</td>
<td>28</td>
</tr>
<tr>
<td>5b. Comparison of mg at triglycerides per whole injured liver</td>
<td>29</td>
</tr>
<tr>
<td>6. Comparison of the mg of hydroxyproline per gram of liver as determined from the ultrafiltrates of homogenized liver samples in sensitized and challenged animals versus non-sensitized animals</td>
<td>30</td>
</tr>
<tr>
<td>7. Comparison of the weights of explanted Ivalon sponges in repeatedly immunized versus non-immunized animals</td>
<td>31</td>
</tr>
<tr>
<td>8. Determination of mg of hydroxyproline per whole sponge</td>
<td>32</td>
</tr>
</tbody>
</table>
ABSTRACT

Skin incision wounds, experimental silicosis, carbon tetrachlor­
ide poisoning and implanted Ivalon sponges (granulation tissue) are all
forms of induced tissue damage with resultant fibro productive inflam­
mation. In each case, fibrosis will eventually result. The aim of this
study was to examine the effect of a stimulated immune system concurrent
with the ongoing fibro-proliferative processes associated with the
various forms of induced tissue damage.

Immune stimulation took the form of initial sensitization and
repeated immunization of out bred guinea pigs with homologous epididymal
sperm on testis in complete Freund's adjuvant. The animals remained
stimulated during the response to injury. Proof of immunization was
demonstrated via skin testing, determination of sperm immobilization
titres, and Schultz-Dale responses. The amount of collagen formed was
measured by the determination of hydroxyproline.

Animals receiving the compounded regimen of skin incision
wounds and immune insult demonstrated a higher wound tensile breaking
strength than those receiving skin incision wounds alone, thereby
demonstrating more collagen formation. Immune stimulated animals,
receiving intra-tracheal injections of silica, demonstrated more colla­
gen per lung than those receiving the silica alone. Similarly, those
stimulated animals implanted with Ivalon sponges beneath their skin
demonstrated more collagen (more encapsulation) in and around the
implanted sponges.
In the case of carbon tetrachloride induced hepatotoxicity, there was no significant increase in liver fibrosis. However, higher triglyceride levels and collagenase activity was observed in the test animals. Histological examination was consistent with the biochemical determination.
INTRODUCTION

The immune system is one of the most important systems for the maintenance of homeostasis. A stimulated immune system whether at a local or systemic level involves the activation and differentiation of numerous cell types, the release of pharmacologically active substances and chemical mediators. One end result of any fibro-productive inflammatory condition is production of collagen. Will the interaction of a fibor-productive inflammatory state with the components of a stimulated immune system be a major factor in determining the final amount of collagen produced? Studies to date indicate that the degree and type of non-specific immuno-stimulation present may be a major determining factor since many specific components of the cell mediated and humoral response have been found to effect the fibrotic process. One of the major consequences of immunity is the production of pharmacologically active substances or lymphokines. It has been demonstrated that lymphokine rich supernates from phytohemagglutinin (PHA) stimulated human male peripheral blood mononuclear cells caused increased collagen accumulation by human male embryonic lung fibroblasts (WI-38), as measured by the hydroxyproline content of fibroblast monolayers, and C14 proline incorporation into soluble collagen (1). This is the first described lymphoid cell derived activity capable of increasing collagen production. Fibroblast stimulating activity may be implicated in the abnormal fibrosis seen in association with the chronic inflammation in a variety of disease states. Chronic mononuclear infiltrates are seen in association
with fibrosis in conditions such as chronic hepatitis, tuberculosis, sarcoidosis and polymyositis. In each case, there is good evidence that a cell mediated immune mechanism may be implicated in the evolution of the disease (2). The appearance of increased collagenous tissue in concurrence with mononuclear infiltrates in retrobulbar connective tissue and muscle in Grave's disease (3) and in the liver in portal, post-necrotic and biliary cirrhosis has been documented (4-6). A connective tissue activating peptide in normal and stimulated leukocytes as well as inflammatory lesions has also been found to increase the metabolic activity of human fibroblasts (7). Lymphokines regulate the immune response but their production is not limited to lymphoid cells. Production extends to cell types such as fibroblasts, kidney cells and other cell lines. Lymphokines may interact with the metabolic machinery that regulates the growth and division in non-lymphoid tissues, and as such interact with such molecules as epidermal growth factor, nerve growth factor, and fibroblast activation factor. The first observation that non-lymphoid cells were capable of lymphokine production was in 1972 when it was reported that supernatants from cultures of fibroblasts (WI-38, 3T3) caused inhibition of macrophage migration. The greatest inhibition came from supernatants of cultures of growing cells (8). It was also demonstrated that supernatant fluids of fibroblast cultures from skin biopsy specimens from children with severe combined immuno-deficiency, contained a substance inhibiting the migration of cultured human lymphoid cells. These results suggest that macrophage inhibitory factor (MIF) is not exclusively produced by
lymphocytes but many activated cell types. The role of MIF may not only be concerned with the cell mediated immune responses, but other processes such as wound healing, cell differentiation and organogenesis.

Recently a lymphokine found in both antigen and mitogen stimulated cultures has been characterized and may explain the arrival of this cell type at inflammed sites (9). This mediator is known as lymphocyte derived chemotactic factor for fibroblasts (LDCF-F) and serves as a chemotactic factor for fibroblasts.

The macrophage plays a role in the process of wound repair as is evident when one considers the effects of hydrocortisone and antimacrophage serum (10). In the repair process, the principle cell type responsible for wound debridement is the macrophage, but this cell may also be required to stimulate fibroblast proliferation in some unknown way. Inflammation follows wounding and persists throughout the duration of repair. Elimination of polymorphonuclear cells and lymphocytes does not effect primary repair, but if macrophages are eliminated fibroplasia is reduced (11), while on the other hand wounds that become further infected, with, for example, a bacterial inoculum, demonstrate large numbers of activated macrophages and a subsequent higher tensile breaking strength (12).

Pneumoconiosis induced by inhalation of silica provides the best example of an inflammatory lesion in which fibroblasts and macrophages are highly activated. The lesions are characterized by extensive fibrosis and it is plausible that the stimulus to collagen synthesis is derived from macrophages (13). Macrophage cultures exposed to silica
particles have also been shown to release a fibrogenic factor (14). Lung connective tissue antibodies also stimulate macrophages to release this same collagen stimulating factor for fibroblast target cells. In sub-cytotoxic amounts, this antibody had not such an effect directly on fibroblasts (15). Immune adherence reactivity of rat alveolar macrophages following inhalation of asbestos may reflect an in vivo antigen-antibody-complement interaction on the surface of the alveolar macrophage from animals which have inhaled asbestos dust (16). In any type of fibrotic lung disease histopathology demonstrates the derangement of parenchymal collagen and infiltration of the parenchyma with chronic inflammatory cells. The cellular reaction may be associated with autoimmunity to a constituent to the alveolar interstitium since these collagen induced cell mediated phenomena are obviated with human T-lymphocyte antiserum implicating T-lymphocytes and lung collagen in the pathogenesis of human fibrotic lung disease (17). This would partly explain the presence of circulating antibodies to lung connective tissue in a number of persons with chronic lung disease (18).

If extensive fibrosis replaces the parenchyma of organs functional impairment will ensue. Hepatic fibrosis following liver cell damage and inflammation is one of the main factors in circulatory disturbances of the liver (19). Disturbed hepatic circulation elicits further fibrogenesis by inducing hepatocellular injury and enhanced subsequent inflammation, which in turn stimulates fibroblasts further (20). The most effective factors interfering with blood flow in the sinusoids results from marked swelling of liver cells and obliteration of the
sinusoids by macrophage proliferation (21).

The liver has both a fundamental and central role in immune responses as is partially evident by the fact that the hepatocyte is both an antigen handling and antibody forming cell (22). Antigen containing Kupffer cells are found to elicit no antibody related response. It has been suggested that these macrophages differ from other components of the reticuloendothelial system (RES) in their treatment of antigen and may be mainly concerned with its breakdown and disposal rather than providing a stimulus for the initiation of antibody synthesis (23). The stimulus of collagen biosynthesis in liver disease may be due to the release of factors which stimulate liver fibroblasts to increase biosynthetic activity (24). Similar factors seem to be present in the healing of wounds, but are absent from normal skin (25).

An ongoing process of immunization may regulate the events associated with the repair process and have an effect on the amount of connective tissue produced. Evidence that the end-products of a stimulated immune system may regulate the cells involved in fibro-productive inflammation were investigated in terms of the effect of non-specific immunization on fibroblast activity in reactive granuloma tissue. This study demonstrated enhanced fibroblast activity and subsequent collagen biosynthesis during the immunization process (26). As a result of immunization, antigen triggered lymphocytes may contribute to the control of fibroblast function as is evident by the fact that antigen interaction with T-dependent lymphocytes causes the release of non-dialyzable factors which increase DNA synthesis of dermal fibroblasts (27). It is
possible that fibroplasia, consisting of increased numbers of fibroblasts and increased collagen deposition, associated with chronic inflammatory diseases may be a consequence of specific antigenic challenge. The formation of antigen-antibody complexes is a possible consequence of active immunization. These immune complexes can act as pathological agents capable of interacting with serum factors and/or cells leading to inflammatory and degenerative changes. All active complexes have an affinity for complement and interact with complement. The activity of the complex depends on the properties of the antibody not antigen. Rabbits, humans and guinea pigs all form active complexes (28). The complexing of antigen and antibody in the circulation and tissues will cause host mediation systems to be activated. The humoral and cellular factors will then combine to produce all aspects of the inflammatory process (28). Immunofluorescence indicates that circulating immune complexes may be pathogenic in the presence of granular deposits of immunoglobulin and complement in lesions. Such deposits may be accompanied by an inflammatory infiltrate and fibrinoid necrosis may result (29). Circulating complexes may be either phagosytized by the RES or deposited in blood vessels and other tissues. Saturation of the RES will lead to an impairment of clearance. Local factors are important in that complexes will localize at a site of arterial damage or tissue injury. Agents which decrease vascular permeability of liberate vasoactive amines facilitate vascular deposition of circulating complexes, while antagonists of these amines prevent the deposition of immune complexes (30). Complement dependent direct action of immune
complexes on platelets causes the release of a large quantity of mediators of inflammation and tissue injury. The anaphylactoid type reaction will cause the further release of vasoactive ammiones through the mediation of platelet activating factor. Platelet-immune complex interaction with subsequent release of platelet-factors play a role in stimulating cell proliferation, in vivo, particularly in relation to the pathogenesis of the proliferative lesions of atherosclerosis (30). Large quantities of immune complexes seem to be related to the symptoms of some diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, diabetes mellitus, and melanoma (31). There seems to be a high incidence of infarcts in coronary vessels and widespread fibrosis in some persons with SLE (32, 33). Circulating immune complexes are also present in most patients with chronic liver disease. Liver biopsies from patients with circulating immune complexes together with various collagen diseases, showed immunoglobulin types G and M binding to the hepatocyte membrane possibly indicating the liver in the removal of immune complexes during RES saturation (34). That a localized immune response may be a direct cause of enhanced fibrosis, was seemingly deduced in an interesting experiment demonstrating the extractability of IgG from keloid tissue (35, 36). If immune complexes that accompany many infections and immunological diseases of man are capable of inducing severe vascular diseases, they may well play a role in degenerative arterial disease. Vasectomy is a unique and simple surgical procedure which may elicit an immunological response to self antigens (37, 38). The occurrence of circulating antibodies to spermatic antigens in
complexes on platelets causes the release of a large quantity of mediators of inflammation and tissue injury. The anaphylactoid type reaction will cause the further release of vasoactive ammines through the mediation of platelet activating factor. Platelet-immune complex interaction with subsequent release of platelet-factors play a role in stimulating cell proliferation, in vivo, particularly in relation to the pathogenesis of the proliferative lesions of atherosclerosis (30). Large quantities of immune complexes seem to be related to the symptoms of some diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, diabetes melitus, and melanoma (31). There seems to be a high incidence of infarcts in coronary vessels and widespread fibrosis in some persons with SLE (32, 33). Circulating immune complexes are also present in most patients with chronic liver disease. Liver biopsies from patients with circulating immune complexes together with various collagen diseases, showed immunoglobulin types G and M binding to the hepatocyte membrane possibly indicating the liver in the removal of immune complexes during RES saturation (34). That a localized immune response may be a direct cause of enhanced fibrosis, was seemingly deduced in an interesting experiment demonstrating the extractability of IgG from keloid tissue (35, 36). If immune complexes that accompany many infections and immunological diseases of man are capable of inducing severe vascular diseases, they may well play a role in degenerative arterial disease. Vasectomy is a unique and simple surgical procedure which may elicit an immunological response to self antigens (37, 38). The occurrence of circulating antibodies to spermatic antigens in
vasectomized and infertile men, has been reported for years.

A cellular radioimmunoassay assay has been developed and used to quantitate antibodies to a group of surface antigens on guinea pig sperm and testicular cells. These antigens are called testicular cell-sperm differentiation antigens or TSDA. They are specific for testicular cells and sperm and are present on all testicular cells and epididymal spermatozoa. TSDA includes all immunogenic surface antigens which can withstand collagenase-trypsin treatment. A significant anti-TSDA antibody response has been demonstrated in guinea pigs of several strains (39, 40). Inbred strains of guinea pigs differ in the development of an immune response to spermatic antigens after vasectomy (41). Genetic differences may be responsible for the fact that not all vasectomized individuals respond with anti-sperm antibodies (40). Other causes may be the quantity of antigen or degree of complexing of antigen with antibody. In man, the incidence and intensity of sperm immobilizing antibody increases with increasing pre-operative history of autoimmune disease (42). Studies demonstrate immune complexes in vasectomized and immunized rabbits. Data suggest that these immune complexes involve sperm antigens and occur in the presence of antogeneemia and absence of free circulating antibody (43). As was discussed previously, these circulating immune complexes could well play a role in a variety of potential systemic effects including glomerular nephritis and atherosclerotic changes. Rabbits immunized with guinea pig testicular antigen demonstrate immune complex testicular pathology concurrent with a mononuclear infiltrate (40), while rabbits immunized
with homologous rabbit testicular antigen rarely had anti-sperm antibodies and developed a pathology similar to experimental allergic orchitis as it occurs in the guinea pig without immune complex deposition (40).

In the guinea pig, pathology, like experimental allergic orchitis, is seen after vasectomy. Inflammatory lesions are characterized by the invasion of boundary tissue of the semineferous tubules by clusters of macrophages and lymphocytes. Patchy areas of aspermatogenesis are present—no immune complexes have been detected. Pathological features seen in the testis of long term vasectomized guinea pigs differ from those of immune complex mediated orchitis in vasectomized rabbits but are similar to those of experimental allergic orchitis as it occurs in the guinea pig (40, 44). Guinea pigs immunized with sperm in CFA develop orchitis, make high titres of complement fixing antibodies and demonstrate lymphocytes which can transfer orchitis when inoculated into normal recipients and produce in vitro migration inhibitory factors.

During the past few years, there has been much interest in the exacerbation of atherosclerosis by immunologic injury. The combination of serum sickness and hyperlipoproteinemia has been shown in rabbits to result in more extensive atherosclerosis than hyperlipoproteinemia alone (45, 46). Immunologic injury was also demonstrated to enhance the development of atherosclerosis in baboons (47). Alexander and Clarkson found a striking exacerbation of atherosclerosis in a small group of vasectomized cynomolgus monkeys maintained on a high cholesterol diet (48). It remains to be learned whether this is applicable to other
species or to those maintained on a normal diet. More extensive atherosclerosis was noted among those monkeys that were found to lack demonstrable free circulating antibody. It has been suggested that these antisperm antibodies that form after vasectomy may result in circulating immune complexes that exacerbate atherosclerosis even further (49).

The aim of this study is to further examine the consequences of immunity - specifically autoimmunity - on the cellular events contributing to fibrosis in various forms of induced tissue damage and in some way generalize an immuno-fibrotic effect.
MATERIALS AND METHODS

Sperm Antigen Preparation and Sensitization Method

The epididymis from out-bred adult male guinea pigs were dissected away from the testes and blood vessels. Spermatozoa were obtained by either mincing the epididymis in a small volume of .9% saline and collecting the fluid by withdrawal with a Pasteur pipet or by flushing out the tubes of an intact organ with .9% saline. In this case, a 25-gauge needle was inserted in the vas deferens and approximately 2 ml. of saline forced through by means of a syringe after a small incision had been made in the distal end of the epididymis. The sperm suspension was centrifuged for 5 minutes at 2500 rev./min., resuspended in 3 vol. of saline and emulsified with 4 vol. of Freund's complete adjuvant (CFA). Each guinea pig was injected intra-dermally in the nuchal region in a number of sites with not more than .1 cc of injected material given per site. An equivalent of approximately 50 mg. fresh sperm was injected into each pig.

Testis Antigen Preparation and Sensitization Method

Testis from out-bred adult male guinea pigs were homogenized with an equal volume of .9% saline. The homogenate was then strained, freeze-thawed and emulsified with an equal volume of Freund's complete adjuvant. Each guinea pig received approximately 1.0 cc of freshly prepared emulsion corresponding to 100 mg of wet tissue.
NOTE: Control animals received equal volumes of CFA and physiologic saline.

Procedures for Challenging

All animals were challenged 5, 6, and 7 weeks after the initial sensitization with the corresponding antigen preparation. Sperm antigen: prepared as above except each guinea pig received an emulsion corresponding to 25 mg of fresh sperm. Testis antigen: prepared as above except each guinea pig received an emulsion corresponding to 50 mg of wet tissue.

Skin Testing

All guinea pigs were skin tested approximately 7 weeks after initial sensitization with an intra-dermal injection of .1 cc of an antigenic solution containing 1 mg of the appropriate antigenic material which had been heated to 56°C for 30 minutes. The intensity and area of reaction were observed at 15 minutes, 4 hours, 24 hours, and 48 hours.

Sperm Immobilization

For the study of motility of sperm, a binocular dissecting microscope at 400 x 800 x magnification was used. To .1 ml of serial dilutions of serum heated at 56°C for 30 minutes from a sensitized guinea pig, was added .01 ml of fresh guinea pig serum followed by .1 ml of a sperm suspension containing approximately 20 million sperm/ml. After incubation for 15 minutes at 37°C, the greatest serial dilution giving near complete immobilization was determined. Sperm
Immobilization titres were determined from blood samples taken at the time of sacrifice.

**Schultz-Dale Test**

Ileal segments were tested in the following manner. Ileae were fixed to a glass rod inferiorly and to a muscle liner superiorly. The entire ileum was bathed in Tyrode's solution kept at 37°C while oxygen was bubbled through constantly. Contractions which were maintained for more than 5, 15 or 30 minutes after antigenic challenge, were given values of 1, 2, and 3 respectively. When no such effect was observed, the rating was 0. Contractures were recorded on a physiograph.

**Skin Wounds - Breaking Strength**

 Twelve Hartley adult male guinea pigs (400-600g), after being sensitized and challenged with sperm antigen along with ten controls, were given skin incision wounds as the first form of induced tissue damage. The animals were anesthetized with Innovar Vet (Pittman Moore 5002) by injection of .25 cc per 500 g body weight i.m. with supplemental dosages as indicated. Animals were shaved and prepped with iodine. A 4.5 to 5.0 cm incision was made along the mid-line dorsal lumbar surface through the dermis to the deep fascia. Wounds were closed with 3 0 proline non-resorbable sutures (Ethicon, Inc., N.Y.) with five interrupted stitches. Animals were allowed to recover and maintained as usual. After 11 days, the animals were sacrificed by cardiac air emboli under deep anesthesia (innovar). The wounds were then excised in block, sutures removed and pinned upside down on a
wooden board under even tension. Both fascia and penicules carrosus were removed. Three 0.5 cm wide tangential strips per wound were cut with a bladed caliber. Breaking strengths were measured on an Instrom Model TM tensiometer with a loading rate of .5 cm per second.

Lung Fibrosis

Two Hartley adult male guinea pigs (600-800g), after being sensitized and challenged with sperm antigen along with eight controls, were given lung fibrosis as the second form of induced tissue damage. Animals were anesthetized with nembutal (Abbot) - .1 cc per 100 g body weight i.p.. The ventral neck surface was shaved and prepped. A 2 cm long incision was made across the sub-maxillary gland which was blunt dissected to expose the tracheal muscle sheath. This structure was also blunt dissected to expose the trachea. An injection of 1 cc of .9% saline containing 25 mg of silica particles DQ12 (99% pure SiO₂, size < 5μ) were infused through a 20 gauge needle intra-tracheally. The skin was closed with 3 0 proline (Ethicon Inc., N.Y.). Animals were allowed to recover and maintained as susal. After six days, surviving animals were euthanized by cervical dislocation. The lungs were removed and separated from the bronchi and weighed. Lung samples from the same lobe of each animal were hydrolysed in 6N HCl for 18 hours at 105°C in capped tubes after which time determination of hydroxyproline were made on each sample according to Stegemann (50).
Liver Fibrosis

Ten Hartley adult male guinea pigs along with ten controls (600-800g) were sensitized and challenged with sperm antigen and given liver fibrosis as the third form of induced tissue damage. The animals were injected with 0.2 cc per kg. body weight of a 20% solution of CCl₄ in mineral oil i.p. once a week during the sixth, seventh and eighth week during the immunization schedule. Surviving animals were sacrificed 3 days after the last carbon tetrachloride injection by cervical dislocation and the livers were removed and weighed. Determinations of hydroxyproline were made on similar whole samples and ultrafiltrated homogenized samples using an amicon well filtration device. (Plasma membrane: 30,000). Other liver samples were also homogenized and extracted with a 2:1 chloroform-methanol solution for the determination of triglycerides according to Levy (51).

Implanted Ivalon Sponges

Nine Hartley adult male guinea pigs (600-800g) along with nine controls, were sensitized and challenged with testis antigen after which time they were implanted with Ivalon sponges as the fourth form of tissue damage. The animals were anesthetized with innovar and two 1.5 cm contralateral long incisions were made over the shoulders through which a kelly 5 inch curved hemostat was utilized to form two subcutaneous sockets in which were placed a 1 cm (diameter) 4 cm length polyvinyl alcohol cylindrical sponge. The incisions were closed with 3 0 proline sutures (Ethicon, Inc., N.Y.). Before implantation, a
large rectangular sponge was washed for four hours in running tap water to remove anti-bacterial formaldehyde. It was then frozen and lyophilized after which time cylindrical sponges were cut from the main sponge with core boring equipment. The cut sponges were then boiled in double distilled water and allowed to cool before being inserted. After twelve days, the animals were euthanized and the sponges removed and weighed. Determinations of hydroxyproline were made.

**Histology**

Histology was performed on samples from lungs, livers and sponges. After standard fixation, 5 μm sections were made and stained according to Massons Trichrome Modified Method and with Hematoxylin and Eosin.

**Statistical Analysis**

Student T-tests were performed on all grouped data.
RESULTS

Fibrosis is a major form of most forms of tissue damage. The amount of collagen produced in response to injury varies with the degree of injury. To examine the possibility of immune mediated enhanced fibrosis, proof of immunization must be established. The amount of hydroxyproline corresponding to the degree of fibrosis can then be measured by various biochemical techniques.

All auto-sensitized animals used in the study demonstrated positive skin testing. The skin reactions were rather similar in appearance and duration. One characteristic reaction may be described in detail:

At 15 minutes after - ill defined faint redness and soft swelling, 25 by 25 mm, slightly raised; center light purple, 10 mm by 10 mm.
At 4 hours after - well defined redness and soft swelling 40 by 30 mm, raised 7 mm; center purple, 10 by 10 mm.
At 24 hours after - well defined redness and well defined soft swelling, 35 by 30 mm, raised 4 mm; center pale, 3 by 3 mm.
At 48 hours after - ill defined redness and ill defined soft swelling, 25 by 20 mm, raised 3 mm; center white, 2 by 2 mm.
At 72 hours after - ill defined faint redness, 6 by 6 mm, no swelling; center white, 2 by 2 mm.
Immunized animals also demonstrated a sperm immobilization titre. Titres from all sensitized animals ranged from 16 to 256. Controls were negative.

Schultz-Dale reactions were observed on a few random guinea pig ileum. Contractions were observed. The strength of contractions were essentially uniform whereas the duration varied from 5 to over 30 minutes. Controls were negative.

By these facts, we established that in all experiments reported below, the animals were sensitized to sperm or testis antigen.

For tissue damage in the form of skin incision wounds, determination of the wound breaking strength provides the most direct measurement for determining the degree of healing by collagen deposition. It is namely the collagenous structure which is the major determinant of the mechanical characteristics of the skin incision wounds. Results demonstrating the average wound breaking strength for animals receiving skin incision wounds is given in Table I.

Statistical analysis using the Student T-test demonstrated a significant difference between these two groups. The wound healing was significantly faster at P<.001 in group A animals.

Intra-tracheal injections of silica which induce lung fibrosis (experimental silicosis) provide a typical form of fibro-proliferative tissue damage. Biochemical determination of hydroxyproline will provide a measure of the degree of fibrosis. Simple analysis of lung weight is another parameter which is considered typical for the evaluation of lung damage. In fact, several authors have shown a
significant correlation between the amount of collagenous hydroxyproline and lung weight (52). Lung weight analysis (Table 2) shows a significant increase of lung weight in the sensitized and challenged group at p < .001. Results on the determination of hydroxyproline are given in Table 3a and 3b.

These results demonstrate no statistically valid difference when comparing the amount of collagen per gram of lung but a significant difference (p < .001) is apparent when one considers the entire organ. It seems that in immune stimulated animals the whole tissue responds to a noxious agent more uniformly than in the non-stimulated. As all lung compartments are stimulated in a proportional manner, the collagen (density of the collagenous frame of the lung tissue) remains the same and serves as an adequate supporting structure. Still, as the whole lung in the immunized animals are significantly larger, the collagen in the total lung is also in proportion significantly degraded. Similar findings have been reported with respect to the response of lung tissue to environmental pollutants, toxic or fibrogenic agents.

Injection of CCl₄ provides another form of induced tissue damage in the form of centrolobular necrosis. An analysis of the liver weights is given in Table 4.

Here again, an adaptive growth response is seen. Similar responses have been reported elsewhere - see discussion.

Comparison of the amount of hydroxyproline per gram and per whole injured liver in immunized as opposed to non-immunized animals demonstrated no valid difference. A characteristic feature of CCl₄
hepatotoxicity is the accumulation of lipids inside the hepatocytes. Among the lipids they are mainly the triglycerides, which are not exported out of the cell because of the lack of a proteinaceous carrier. Fatty liver is typical of CCl₄ hepatotoxicity.

In response to CCl₄, determination of triglycerides were made. Results are given in Table 5a and 5b.

A significant difference (p< .01) similar to those observed in the fibrotic lungs were seen only after one considers the whole organ. For most injured tissues the turnover of various tissue components is enhanced. Thus, in the case of collagen, both collagen synthesis and degradation will be increased. The rate of collagen synthesis was not measured but attention was focused on the determination of the degree of collagen degradation. It is known that some collagen is degraded by collagenases - small peptides containing hydroxyproline are present in higher amounts in the affected tissues.

Indeed, the results (See Table 6) show that in the repeatedly immunized animals, the amount of dialyzable hydroxyproline is twice as high as in the control group. Collagen degradation was apparently stimulated and possibly also collagen synthesis. This would explain the finding of no net excessive deposition of collagen in liver tissue injured by CCl₄.

In view of the hydroxyproline results from the injured livers, hydroxyproline determinations were made on the filtrates from the ultrafiltration of a few random homogenized liver samples from each group to test for collagenase activity.
The implantation of Ivalon sponges allowed for the formation of granulomatous tissue with corresponding fibroblastic activity. The amount of collagen in and around the Ivalon sponges was measured by hydroxyproline determination on whole sponges. A comparison of the weights of the explanted sponges and the determination of the hydroxyproline per sponges are given in Table 7 and Table 8.

Histological examination was consistent with the results obtained from the biochemical determination.

Examination of liver cross sections showed more of a fatty acid content in the test animals as was evidenced by the appearance of more and larger lipid vacuoles as opposed to the controls.

Examination of lung cross sections demonstrated a higher degree of parenchymal derangement and disorganization in the test animals.

Cross sections of the implanted Ivalon sponges in the sensitized and challenged animals demonstrated a higher degree of cellular infiltration (more encapsulation) and organization compared with the sponges of the control animals, which were less encapsulated and showed diffuse cellular infiltration.
Table 1. Comparison of average wound breaking strengths - (500 g scale) in immunized versus non-immunized animals.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>167</td>
<td>91</td>
</tr>
<tr>
<td>175</td>
<td>100</td>
</tr>
<tr>
<td>213</td>
<td>125</td>
</tr>
<tr>
<td>183</td>
<td>75</td>
</tr>
<tr>
<td>280</td>
<td>55</td>
</tr>
<tr>
<td>134</td>
<td>33</td>
</tr>
<tr>
<td>125</td>
<td>88</td>
</tr>
<tr>
<td>167</td>
<td>112</td>
</tr>
<tr>
<td>185</td>
<td>113</td>
</tr>
<tr>
<td>234</td>
<td>125</td>
</tr>
<tr>
<td>92</td>
<td>173</td>
</tr>
<tr>
<td>242</td>
<td></td>
</tr>
</tbody>
</table>

Average = 183  99
St. Dev. =  53  37

a. All Group A animals were sensitized to homologous epididymal sperm, challenged and given skin incision wounds.

b. All Group B animals received only skin incision wounds.
Table 2. Lung wet weight analysis.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung wet weight</td>
<td>Liver wet weight</td>
</tr>
<tr>
<td></td>
<td>(grams) - % body weight</td>
<td>(grams) - % body weight</td>
</tr>
<tr>
<td></td>
<td>14.43 - 2.2</td>
<td>5.40 - .8</td>
</tr>
<tr>
<td></td>
<td>11.36 - 1.7</td>
<td>6.43 - 1.0</td>
</tr>
<tr>
<td></td>
<td>8.94 - 1.3</td>
<td>6.54 - .9</td>
</tr>
<tr>
<td></td>
<td>8.37 - 1.2</td>
<td>5.80 - .9</td>
</tr>
<tr>
<td></td>
<td>13.68 - 2.1</td>
<td>6.20 - .9</td>
</tr>
<tr>
<td></td>
<td>14.21 - 2.2</td>
<td>6.22 - .9</td>
</tr>
<tr>
<td></td>
<td>13.03 - 2.1</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>12.12g (1.8%)</td>
<td>6.08g (.9%)</td>
</tr>
<tr>
<td>St. Dev.</td>
<td>2.58 (.4%)</td>
<td>.41 (.06%)</td>
</tr>
</tbody>
</table>

a. Group A animals were sensitized and challenged with homologous epididymal sperm and given intra-tracheal silica injection.

b. Group B animals received only silica injections.
Table 3a. Comparison of the determination of the mg of hydroxyproline per gram of silica induced fibrotic lung tissue between sensitized and challenged animals versus non-sensitized animals.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.02</td>
<td>1.44</td>
</tr>
<tr>
<td>1.02</td>
<td>1.48</td>
</tr>
<tr>
<td>2.31</td>
<td>1.21</td>
</tr>
<tr>
<td>2.02</td>
<td>1.10</td>
</tr>
<tr>
<td>1.02</td>
<td>1.28</td>
</tr>
<tr>
<td>1.09</td>
<td>1.44</td>
</tr>
<tr>
<td>1.24</td>
<td></td>
</tr>
</tbody>
</table>

Average = 1.52 1.33  
St. Dev. = .55 .15

a. See Table 2.  
b. See Table 2.
Table 3b. Comparison of the determination of the mg of hydroxyproline per fibrotic lung.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.68</td>
<td>7.77</td>
</tr>
<tr>
<td>26.12</td>
<td>9.00</td>
</tr>
<tr>
<td>17.96</td>
<td>7.87</td>
</tr>
<tr>
<td>15.90</td>
<td>6.32</td>
</tr>
<tr>
<td>13.95</td>
<td>7.93</td>
</tr>
<tr>
<td>15.45</td>
<td>8.95</td>
</tr>
<tr>
<td>16.71</td>
<td></td>
</tr>
</tbody>
</table>

Average = 17.25  
St. Dev. = 4.12  

a. See Table 2.

b. See Table 2.
Table 4. Comparison of liver weights.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung wet weight (grams) - % body weight</td>
<td>Liver wet weight (grams) - % body weight</td>
</tr>
<tr>
<td>14.14 - 6.1</td>
<td>24.02 - 3.8</td>
</tr>
<tr>
<td>38.33 - 6.2</td>
<td>25.61 - 3.4</td>
</tr>
<tr>
<td>31.51 - 4.7</td>
<td>22.92 - 3.6</td>
</tr>
<tr>
<td>33.11 - 5.0</td>
<td>22.65 - 3.3</td>
</tr>
<tr>
<td>33.96 - 6.0</td>
<td>22.10 - 3.4</td>
</tr>
<tr>
<td>29.33 - 5.2</td>
<td></td>
</tr>
</tbody>
</table>

Average = 34.39g - 5.5%  | 23.46g - 3.5%
St. Dev. = 4.10 - .64%   | 1.39 - .2%

Liver weights p < .001
% body weights p < .001

a. Group A animals were given CCl₄ during the immunization schedule. The antigen used was homologous epididymal sperm.

b. Group B animals were given CCl₄ injections alone.
Table 5a. Comparison of the mg of triglycerides per gram of injured liver in sensitized and challenged animals versus non-sensitized animals.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.10</td>
<td>21.47</td>
</tr>
<tr>
<td>18.14</td>
<td>19.10</td>
</tr>
<tr>
<td>16.01</td>
<td>19.42</td>
</tr>
<tr>
<td>17.87</td>
<td>20.94</td>
</tr>
<tr>
<td>17.65</td>
<td>20.18</td>
</tr>
<tr>
<td>18.44</td>
<td></td>
</tr>
</tbody>
</table>

Average = 17.78  
St. Dev. = .80  

No difference at p = .05

a. See Table 4.

b. See Table 4.
Table 5b. Comparison of mg at triglycerides per whole injured liver.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>726.97</td>
<td>491.88</td>
</tr>
<tr>
<td>694.87</td>
<td>487.10</td>
</tr>
<tr>
<td>504.72</td>
<td>445.12</td>
</tr>
<tr>
<td>591.70</td>
<td>474.29</td>
</tr>
<tr>
<td>599.46</td>
<td>446.97</td>
</tr>
<tr>
<td>540.96</td>
<td></td>
</tr>
</tbody>
</table>

Average = 609.78  469.02
St. Dev. = 86.23  21.94

a. See Table 4.
b. See Table 4.
Table 6. Comparison of the mg of hydroxyproline per gram of liver as determined from the ultrafiltrates of homogenized liver samples in sensitized and challenged animals versus non-sensitized animals.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>.10</td>
<td>.03</td>
</tr>
<tr>
<td>.09</td>
<td>.05</td>
</tr>
</tbody>
</table>

a. See Table 4.
b. See Table 4.
Table 7. Comparison of the weights of explanted Ivalon sponges in repeatedly immunized versus non-immunized animals.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.74g</td>
<td>1.53</td>
</tr>
<tr>
<td>3.41</td>
<td>1.34</td>
</tr>
<tr>
<td>1.95</td>
<td>1.36</td>
</tr>
<tr>
<td>2.21</td>
<td>1.29</td>
</tr>
<tr>
<td>1.84</td>
<td>1.33</td>
</tr>
<tr>
<td>1.62</td>
<td>1.41</td>
</tr>
<tr>
<td>1.63</td>
<td>1.43</td>
</tr>
<tr>
<td>1.68</td>
<td>1.42</td>
</tr>
<tr>
<td>1.52</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>1.41</td>
</tr>
</tbody>
</table>

Average = 1.95  
St. Dev. = .58  
p < .05

a. Group A animals were repeatedly immunized with testis antigen and implanted with Ivalon sponges.

b. Group B animals only received the implanted sponges.
Table 8. Determination of mg of hydroxyproline per whole sponge.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.01</td>
<td>1.72</td>
</tr>
<tr>
<td>4.02</td>
<td>1.52</td>
</tr>
<tr>
<td>2.23</td>
<td>1.54</td>
</tr>
<tr>
<td>2.58</td>
<td>1.32</td>
</tr>
<tr>
<td>1.93</td>
<td>1.60</td>
</tr>
<tr>
<td>1.94</td>
<td>1.75</td>
</tr>
<tr>
<td>1.90</td>
<td>1.58</td>
</tr>
<tr>
<td>2.11</td>
<td>1.63</td>
</tr>
<tr>
<td>1.72</td>
<td>1.69</td>
</tr>
</tbody>
</table>

Average = 2.26
St. Dev. = .7

a. See Table 7.
b. See Table 7.
DISCUSSION

Spermatic and related self antigens can readily elicit an autoimmune response in an individual as they first appear at puberty long after tolerance to self antigens has been established. Sperm specific antigens do not normally come into contact with the immune system as they are sequestered by the Sertoli cell barrier of the testis and by epithelial cell barrier elsewhere in the male reproductive tract. Spillage of sperm outside its normal compartment will result in a vigorous granulomatous reaction (49). The most convincing evidence for the lack of immunological tolerance to sperm is provided by the following findings: a high incidence of anti-sperm antibodies is found in pre-pubertal boys; antisperm antibodies have an age related incidence that best matches with antibody responses to foreign and not self antigens; antisperm antibodies frequently develop after vasoligation when patients are most likely to be stimulated by their own sperm and sperm antigen reactive T-lymphocytes exist (53). The consequences of autoimmunity to sperm antigens vary considerably from species to species and even within a given species and may lead to a number of pathobiological sequences. One classical example is the experimental induction of allergic orchitis in the guinea pig following immunization with testicular or sperm antigens in complete Freund's adjuvant.

In EAO (experimental allergic orchitis), immunization with aspermatogenic antigens in CFA elicits a strong immune response, which includes an active and early T-lymphocyte component (54). CFA may
temporarily disrupt the blood-testis barrier allowing antibodies to reach the inner compartments where the spermatids and sperm reside. Various immunological levels of activity are involved in diseases mediated by sperm immunity. In EAO, reactions involving humoral antibodies and T-lymphocytes produce the different histopathological lesions of EAO (55, 56). Anti-sperm antibodies can react with cell surface antigens and disrupt the plasma membrane of sperm in the presence of complement in vitro (57). This may be related to immune infertility vis-a-vis the reaction of anti-sperm antibody with seminal sperm. In EAO, antibody may react with soluble sperm antigens to form immune complexes in the rete, ductus efferentes, and epididymis to bring about lesions with heavy polymorphonuclear infiltration (58). Adaptive transfer experiments provide data for a requirement of T-lymphocytes but the exact roles of the T-lymphocyte in the induction of the morphological lesions of EAO has not been defined. The T-lymphocytes are the antigen reactive cells, as such. They can function as killer cells and mediators of delayed hypersensitivity reaction or as helper cells for the production of humoral anti-sperm antibodies by the antigen reactive B-lymphocyte from the host.

Humoral immunity to sperm antigens will readily develop as circulating anti-sperm antibodies have been detected in every mammalian species where sperm antigens have come into contact with the immune system. If humoral immunity can develop, it is most likely that the cellular branch of the immune system can also become stimulated by sperm antigens. Reports of lymphocytic infiltration of the testis and
epididymis after vasectomy in guinea pigs, rabbits and rhesous monkeys provide indirect evidence that sperm specific cell mediated immunity is elicited in some species (59, 60). Mumford, Barsales, Ball and Gordon, (1971) were the first to use an in vitro lymphocyte transformation assay for the detection of auto-immunity to sperm antigens in men. They demonstrated that lymphocytes from a large percentage of oligospermic men reacted with autologous semen whereas lymphocytes from fertile men were unresponsive (61). Nagarkatti and Rao used this same assay to test for CMI in vasectomized men and found increasing reactivity to sperm antigens with time after vasectomy (62). Assays for the detection of anti-sperm CMI are rarely attempted as purified sperm antigens are difficult to obtain and some major problems can be associated with using whole semen or sperm extracts. Seminal plasma, for instance, contains powerful immuno-suppressive factors and sperm extracts may be cytotoxic for lymphocytes (63, 64). Anti-sperm immunity may give rise to an immunopathology of a systemic nature. Some investigators have considered the possibility that such immunity may give rise to the production of non-specific (sperm) auto-antibodies that cross react with other tissues of the body. Matthews, Skegg, Vessey, Konice and Holborrow (1976), did report on weak antibody reactions to auto-antigens other than sperm (65). The development of auto-antibodies to a connective tissue antigen in men vasectomized five years earlier has been reported (66). The formation of immune complexes provides another example of an immunopathology. Tung provided direct evidence of circulating immune complexes in rabbits immunized with sperm antigens (67). These
circulating immune complexes may become attached to blood vessel walls particularly at points of bifurcation. Vasoactive factors such as histamine and serotonin play an important role of increasing permeability in rabbits with immune complex disease (68). Local injury to the endothelial surface may result from the binding of certain types of immune complexes to lytic enzymes present in the blood (69). The chemotactic complement components will attract polymorphonuclear cells to the site. The further release of lysozomal enzymes will also damage the blood vessel endothelium. An inflammatory site concurrent with platelet accumulation often occurs. Animals given repeated injections of a foreign protein will develop arteritis as a result of circulating immune complexes (69). When such immunological insult is coupled with hyperlipoproteinemia, extensive atherosclerosis results (46). Rabbits fed a lipid rich diet concurrent with immunologic insult will develop atherosclerotic plaques that histologically resemble those found in man (45).

The guinea pigs in this study were repeatedly immunized with homologous testes or sperm antigens in CFA. They developed the following manifestations of hypersensitivity: (a) Schultz-Dale reactions, (b) antibodies capable of immobilizing sperm in the presence of complements and (c) skin reactions with an immediate and a delayed component.

With any type of tissue injury, a complex series of events occurs that in a healthy individual, will lead to repair and eventual scar formation. In a skin wound, for instance, cells will be disrupted, platelets and collagen will interact, the complement cascade will be
activated, injured vessels will thrombose, venules will dilate, platelets and white cells will stick to the endothelial lining and leukocytes will migrate between the endothelial cells and injured area, the edge of which will become infiltrated with macrophages and granulocytes. Within a short while, fibroblasts will become apparent. The fibroblast is one of the major cells involved in the reparative stage of all types of tissue injuries. They will replace most of the white cells in the injured area. As this replacement takes place, the amount of collagen that is synthesized will increase. An injury, such as a cut to the skin, will begin to gain strength through an increase in the amount of collagenous links that will be formed. The biochemical-biophysical signals that will give rise to fibroblastic proliferation and enhanced activity are not well defined at this time. Regardless of the signal, the evidence is convincing that the majority of the total fibroblast population originates in the injured area where they will synthesize and deposit collagen, the principle structural protein of the body (70). Collagen synthesis will remain rapid for many months after injury. The amount of collagen that is finally amassed will be determined by the fact that even as collagen is being synthesized, it is being degraded in a very delicate balance that in the normal-healthy individual is highly regulated.

In this study, skin incision wounds provided the first form of induced tissue injury. The breaking strengths of such wounds is proportional to the amount of collagen that is synthesized by the stimulated fibroblasts in the wound and the resultant amount of
collagenous cross links that will be formed in the repairative stage of the healing process. The breaking strength of the skin incision wounds of the immunized animals, that is, animals whose healing processes were concurrent with the specific ongoing immune response associated with EAO, demonstrated a significantly higher breaking strength than the non-immunized animals.

About ten years ago, it was demonstrated that an immune response could be elicited at lympho-epithelial surfaces in a manner independent of systemic immunity (71). Since the respiratory tract has associated lymphoid tissue (72, 73), studies of the immune responsiveness of the respiratory tract were a natural outgrowth. Antibody production in the respiratory tract is similar to the immune response at other mucosal surfaces. Cell mediated immune responses in the respiratory tract are a difficult matter to ascertain. It was demonstrated that guinea pigs sensitized to ovalbumin by intra-muscular administration of antigen in CFA, were shown to be sensitized to further antigen administration by aerosol (74). This antigenic challenge led to an inflammatory response in the alveoli and peri-bronchial tissues showing a histological resemblance to the delayed hypersensitivity skin test reactions (74). Mayamoto, Kafe, Noda, Kobayashi and Miura transferred tuberculin skin test sensitivity to normal guinea pigs by immune spleen cells, then successfully challenged these animals to aerosilized purified protein derivative (75). Yamamoto, Anacker and Ribi found that following aerosol vaccination of mice with bacillus Calmette-Guerin, lung cells produce lymphokines in the presence of antigen (76). Local CMI
responses are probably very important to the lungs protection. As stated previously, lymphoid tissue is found throughout the entire respiratory tract associated with the walls of the airway, pulmonary vessels and also related to the lymphatic channels (77). Immunoglobulin producing plasma cells or probably their B cell precursors are present in the peribronchial lymphoid tissue of the upper respiratory tract. If antigen can get into the proper places, the peribronchial and peri-bronchiolar lymphoid tissue contain or recruit T and B cells and phagocytes, which are fully competent at least to some antigens (78). Preformed circulating antibody enhances the uptake of antigens by the RES (79, 80). This enhancement phenomena by specific antibody results in a marked increase in the uptake of intra-venously administered antigens by the lung (79). The mobilization of leukocytes is central to normal pulmonary defense against infections, tumours and environmental factors such as coal, silica, and asbestos. In addition to plasma or serum derived chemotactic factors, granulocytic and monocytic cellular products are secreted as a result of exposure to chemotactic stimuli (81), phagocytosis (82), or antigen processing (83, 84), to further amplify the inflammatory-immune response, by either being themselves chemotactic, generating chemotactic activity, or immobilizing leukocytes and preventing immigration. During antigen processing and expression of CMI, a number of immunologically specific and nonspecific processes occur that are analogous to the events in the inflammatory response and serve to amplify the inflammatory immune response. Exposure of human mononuclear cells to specific antigen will cause the
release of chemotactic lymphokines. These lymphokines are chemotactic for mononuclear cells, neutrophils and lymphocytes (84). MIF is released from lymphocytes upon stimulation with antigen (85) and serves to inhibit the migration of pulmonary macrophages (86). The reaction of lymphokines with antigen specific immune complexes will yield Eosinophil Chemotactic Factor (ECF) (87). Four different effector moieties specifically attractive or stimulatory for eosinophils have been delineated which are sufficient to provide explanations for the mechanisms underlying the eosinophilia that accompanies disease resulting from all types of immune injury (88). The enormous and complex vascular network, the heavy concentration of vasoactive cells as well as the unique relationship in the respiratory tract between the external and internal environment, establish the lung as an organ of prime importance in hypersensitivity diseases in general and vasculitis in particular (89). Circulating antigen antibody complexes, antigen, or sensitized lymphocytes can reach the pulmonary vasculature through the circulation (90), resulting in inflammation of blood vessels (91) and possible acute fibrinoid necrosis. Discussion of immunologic and infectious reactions in the lung is quite complicated. As stated, the lung is unique in its relationship to the external environment and as such the object of a large number of different insults of which progressive and fatal fibrosis is the result of many. The extent to which a noxious agent is dangerous depends on how it is handled by the host. The cytotoxic properties of silica allow for incomplete elimination and ineffective macrophage handling, which, in turn, allow for continuing
circuits of inflammation with progressive organization and ultimate fibrosis (92).

Intra-tracheal injections of silica giving rise to lung fibrosis provide the second form of induced tissue injury. Both groups of animals were subjected to this form of tissue injury with the experimental group receiving the regimen of silica injections combined with that of immune insult in the form of repeated auto-immunizations. The animals receiving the combined injury, demonstrated more of a fibrotic lung than those receiving silica alone. This valid difference is noted when one compares the amount of collagen per lung rather than per gram. This same type of difference is also seen when silicotic lungs are compared with normal lungs (93). The normal lung may contain more collagen per gram than the silicotic lung. This injury induced growth in size is an attempt to function normally but compensate for the damage and ongoing pathology that is occurring and is one prime example of an organ attempting to maintain homeostasis by a growth compensatory mechanism.

The liver, like any other organ, is subject to damage. As stated, there are a number of ways by which tissue can be damaged by immune mechanisms. One of the major mechanisms is that mediated by the release of pharmacologically active substances from mast cells or basophils following reaction between antigen and IgE fixed to the mast cell. Evidence has been presented that repeated anaphylactic shock results in hepatic necrosis and scarring (94). Cytotoxic antibodies have not been described in liver disease and it is unlikely this
mechanism can be implicated in any type of liver disease including necrosis of the liver (94). Antibodies can damage in another way. The various antoantibodies described in liver diseases might be indirectly implicated via immune complex formation. Immune complexes have rarely been detected in the liver. They have been sought after in various chronic liver diseases and only rarely have immunoglobulin and complement been localized. It must be remembered that immune complexes are preferentially deposited in damaged tissues and there is mounting evidence that damaged liver tissue has a specific attraction for circulating complexes (94).

There is compelling evidence for the involvement of immune mechanisms in various forms of liver damage. In vitro lymphocyte migration inhibition is seen in patients with alcoholic hepatitis (95). It is also seen in patients with primary biliary cirrhosis. When alcoholic hyalin is the transforming agent, the specific transfer factor has been found (96) as well as an activity that promotes the incorporation of tritiated hydroxyproline into liver collagen (97) - a fibrogenic promoting factor. Cytotoxicity with peripheral blood lymphocytes is found in patients with chronic active hepatitis using rabbit liver cells as targets (98), in patients with alcoholic hepatitis using human embryonic liver cells as targets (99), and in patients with various types of chronic liver diseases (100). In the livers of patients with alcoholic hepatitis, there is evidence of infiltration with immunologically competent cells, the nature of which have been found to be of the T-type (101). The liver is the site of infiltrating lymphocytes which will be
transforming in response to various antigens and releasing lymphokines (101, 102). In vitro, these lymphokines stimulate fibrogenesis. The role and importance of immune mechanisms in the pathology of toxic liver injury are still not fully understood but appear to be implicated in liver pathology whatever the etiological agent. Specific hepatic factors inducing fibroblasts to increase synthetic activity, have been isolated from the livers of rats treated with CC14 and from humans with alcoholic cirrhosis (103). These factors have also been isolated from healing wounds and are neither species nor tissue specific (104). The activity of these factors might be mediated through prostaglandin sensitive adenyl cyclases evolved from inflammatory and immunological processes. Other factors may be lymphokines with chemotactic potential for macrophages and possibly for fibroblasts (9).

The amount of fibrous connective tissue in the liver is a result of a dynamic equilibrium between the synthetic and degradative processes. Decreased degradation rather than increased synthesis may be a more important reaction in some forms of hepatic fibrosis. Active collagenases are present in leukocytic granules and are also synthesized by RES cells including macrophages and Kupfer cells (105). Proteolytic enzymes, as well as heparin and lymphokines, produced by lymphocytes may all activate collagenase.

Another well documented consequence of CC14 administration is the induction of fatty liver. Covalent binding and lipid peroxidation represent the most important chemical consequences of CC14 metabolism and play the major role in liver damage. The production of
chloromethyl free radicals from CCl$_4$ has been shown in several ways. Livers of animals given CCl$_4$ develop large signals for free radicals in electron spin resonance devices (106). The production to free radicals can be detected by applying electron capture detectors to gas chromatographic separation columns analyzing extracts from the livers of treated rats (107). Another proof is given by the detection in treated livers of covalent binding of chloromethyl groups to lipids and proteins (108). Many lines of evidence have also been given that demonstrate increased lipid peroxidation during CCl$_4$ metabolism. There is an increased production of malonyl-dialdehyde from peroxidizing lipids that takes place in higher amounts when homogenates from normal liver are incubated in vitro in the presence of CCl$_4$ and NADP (109), and when they are isolated from CCl$_4$ treated rats and then incubated in the same way (110). Furthermore, an increased content in malonylaldehyde has been found to occur in the liver after CCl$_4$ treatment (109). It is now certain that metabolism of CCl$_4$ is needed to get damage by CCl$_4$. Various lines of evidence have been offered. Newborn rats which have very low activity in the SER oxidizing system, resist high doses of CCl$_4$ (111). Adult rats fed a protein free diet, have a decreased efficiency of their drug metabolizing system and decreased toxicity of CCl$_4$ (112). Both covalent binding and lipid peroxidation may produce irreversible functional damage to lipids and proteins. Proteins that interact with lipoperoxides show a loss of function and denaturation (113). Nucleotides are also destroyed. When hepatocytes are incubated in the presence of small concentrations of CCl$_4$, there is still a block in lipoprotein
secretion but the cells remain viable and develop triglyceride (TG) infiltration (114). After the discovery that heavy mitochondrial damage occurs after treatment with $\text{CCl}_4$ (115), it was proposed that decreased fatty acid oxidation may be regarded as responsible for fat accumulation. Experiments showed that TG accumulation starts much before the decrease in fatty acid oxidation (116). This mechanism can only contribute to maintaining high TG levels in the late stages of poisoning and cannot be responsible for the onset. Recknagle and Lombardi (1961), showed that a block in lipoprotein secretion occurs shortly after $\text{CCl}_4$ administration and a chronological coincidence between this block and the onset of TG accumulation exists (117). The block of protein synthesis is probably the most important mechanism giving rise to the decrease in lipoprotein secretion after $\text{CCl}_4$ treatment. The lipoperoxidative disintegration of SER membranes might be another possible cause for early TG accumulation as this is probably the site of assembly of the different lipoprotein constituents (114). An increase synthesis in TG as a consequence of decreased fatty acid oxidation in mitochondria may contribute to maintaining high TG levels in the latter stages of poisoning (114).

The liver can adapt to enhanced functional load or to certain types of injury by an increase or decrease of certain specific enzymes, by multiplication of specific organelles and by growth of the whole organ (118, 119). Liver enlargement produced by some form of stress indicates a true growth response as evidenced by the fact that the proportion of main cell constituents after growth remains the same.
(120, 121), the bulk of enzymes increases in proportion with liver size (121, 122), and the multiplication of DNA along with cellular proliferation is apparent (121, 123, 124). The response does not necessarily have to be associated with structural signs of damage but is if the dose is high enough (118, 121).

This study demonstrated no statistically valid difference in the amount of fibrosis between the experimental and control groups (CCl₄ treatment plus immune insult vs CCl₄ treatment alone). Ultrafiltration studies demonstrated more collagen degradation in the experimental group. The activation of degradative enzymes is consistent with the functional capacity of the liver as an antigen handling and processing organ vis-a-vis the hepatocyte and Kupfer cell. Determination of TG revealed a greater amount of TG in the experimental group again in terms of whole organ size. This is consistent with the adaptive growth response of the organ receiving the compounded injury. Immunological enhancement of the generalized fibrotic response to injury is still valid in view of the fact that all species of lipids increase previous to fibrosis in lungs and livers subject to tissue damage (93).

The last form of induced tissue injury was in the form of implanted Ivalon sponges. Here the role of non-specific auto-sensitization on fibroblast function in reactive granuloma tissue, was investigated. The results demonstrated enhanced collagen synthesis in and around the sponges of the auto-sensitized and repeatedly challenged animals.
LITERATURE CITED


47


