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HISTOPATHOLOGY OF AN AUTOIMMUNE DISEASE IN
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AND ANTIRIBOSOME SERUM

DISSERTATION
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Paul Howard Aldenderfer, B. S., M. Sc.

****

The Ohio State University
1963

Approved by

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Adviser
Department of Microbiology
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INTRODUCTION

For centuries, the belief has been widely held that once an individual contracted certain diseases and recovered, he could not contract them again in his lifetime. Jenner was the first actually to prove this observation by inoculating humans with the living virus of cowpox so that a solid state of immunity towards smallpox was established. Louis Pasteur laid the foundations of modern immunology by discovering methods of immunization against a variety of infectious diseases.

Sixty years ago, immunity was considered to be solely a protective function of the host, which Metchnikoff believed was mediated solely through the phagocytic properties of macrophages, while Buchner and Nuttall considered the bactericidal properties of serum as the most important factor. As is known today, both were correct. It became evident, though, from the observations of Rich and Portier that innocuous substances provoked severe local and systemic reactions in certain individuals. From these considerations and others, the concept of hypersensitivity was advanced and von Pirquet coined the word "allergy" to denote an altered reactivity or changed response in an individual, due to the presence of specific antibody. Hypersensitivity and allergic states are definitely disadvantageous to the host, and can be fatal.

About sixty years ago, Landsteiner discovered the human blood groups, and since then numerous isoantigens and antibodies have been
discovered. All these systems have confirmed the basic concept of "horror autotoxicus" as advanced by Ehrlich. Briefly stated, it proposes that an organism will never produce antibodies to any substance normally present in its tissues, i.e., the organism will not form auto-antibodies. The concept, however, has been questioned from its inception, and today, the known exceptions in humans take the form of severe afflictions ranging from hematologic dyscrasias to specific multi-organ pathology.

Investigators of the autoimmune disorders are immediately concerned with two very real problems which are mutually exclusive, but are interrelated. There is the diligent search for the antigen responsible for the initiation of the production of specific pathologic change. Failure to demonstrate tissue specific antigens makes it difficult to pinpoint an immunologic mechanism at work. There are, however, several organ systems in which tissue-specific antigens have been demonstrated, namely, lens, brain, spermatozoa and thyroid. Secondly, there is the search for circulating antibody, either precipitating or incomplete. However, certain experimental and clinical autoimmune diseases may progress without the presence of detectable serum antibody. In others, notably, experimental allergic encephalomyelitis (EAE), experimental thyroiditis or Hashimoto's disease of man, circulating antibody have not been ascertained to be related to the process. The affected organs demonstrate severe degenerative changes characterized by round cell infiltrates and perivascular cuffing. These observations have led some observers to speculate on the possibility that the lesions are the
result of cell-bound antibody or as it is commonly called, delayed hypersensitivity. Proponents of this concept (68) maintain that pathologic changes are mediated by sensitized lymphocytes and/or macrophages which invade the antigen-containing tissues causing the destruction of these tissues; and, that in most autoimmune disorders, the disease cannot be initiated in a normal recipient animal by the passive transfer of circulating serum antibodies, but only by sensitized lymphoid cells. The recent successful passive transfer of experimental autoallergic diseases including EAE, nephritis and thyroiditis by sensitized lymphoid cells supports this concept (68). All attempts to produce lesions comparable to those observed in the donor animal by means of passive serum transfer have been uniformly unsuccessful except in the case of antibodies to the formed elements of the blood (68), and in another instance in which antithyroid antibodies were involved (62).

Recently, a state of apparently true autoimmunization, associated with demonstrable serum autoantibodies and multi-organ pathology has been described in rabbits injected with rat and rabbit liver ribosomes (20). The production of comparable pathology in animals which received serum containing antibodies to these cytoplasmic constituents constitutes an exception to the usual negative experimental results involving passive transfer of autoimmune disease by circulating antibodies, and definitely establishes the causal role of the antibodies in this disease. Furthermore, the specificity of antiribosome serum has been associated with the ribose nucleic acid fraction (RNA) of the ribosomes, and many aspects of the disease have been repeated by the injection of
RNA in Freund's adjuvant. The results reported here constitute an effort to depict the specific histopathology produced in rabbits injected with ribosomes and antiribosome sera. For comparison, the tissues of animals receiving RNA, ribosomal protein or nuclei, as well antisera to these substances, are included.
HISTORICAL REVIEW

Within the past ten or fifteen years a biologic concept has re-emerged, which, because of the enormity of its implications, has challenged the talents of numerous investigators. This concept states simply that the tissues of the body of a host may act as an antigenic stimulus for the production of antibodies directed against these same tissue antigens, to produce, in effect, anti-tissue antibodies, the mechanism being known as autoimmunization. The problem has not been nearly resolved nor is it expected to be for some time to come as there are as many conflicting points of view and theories as there are diseases which have been classified as "autoimmune" for want of a better pathological or clinical niche within which to place them.

The possibility that disease may be initiated by an autoimmune mechanism is not new. Donath and Landsteiner(21) were the first to investigate and correctly characterize paroxysmal cold hemoglobinuria as an autoimmune disease as early as 1900. But it has only been within fifteen years or so with the advent of newer serologic and biometric techniques, and sufficient clinical studies correlated with definitive experimental applications, that the concept has emerged as a definite biologic and medical entity.

Any review of autoimmunity requires a discussion of experimentally produced states as well as the natural occurrences in man. Most of the
experimental diseases discussed, with the exception of renal organs, but which involve the thyroid, central, and peripheral nervous systems, testes, uves, articular tissues, adrenal and skin can be reproduced in animals by immunizing them with autologous, homologous or heterologous tissue homogenates or extracts, preferably with Freund's adjuvant. Regardless of whether auto-, homo-, or heteroimmunization is employed, it is likely that in the pathogenesis of the resulting disease, the host's own tissues serve as target antigens and are injured as a result of the immune response. Only rarely do comparable diseases develop in humans as a result of immunization with tissue antigens, as in the case of paralysis developing after the Pasteur treatment for rabies. These experimental diseases, usually produced by immunologic means, serve as models, which is justified by the fact that they resemble clinically and pathologically to greater or lesser degree certain human diseases of unknown, but long-suspected immunologic origin. On the basis of this resemblance, the autoimmune pathogenesis which appears likely to exist in animals has been postulated to operate in such human diseases as thyroiditis, demyelinating diseases, and sympathetic opthalmitis.

The evidence supporting an autoimmune pathogenesis of these human diseases cannot be as definitive as that available for the autoimmune experimental diseases for two reasons. First, it is necessary to assume an identity between the experimental entity in the animal and the human disease on the basis of clinical and pathological comparison which can never be entirely satisfactory. Second, while an autoimmune pathogenesis for these experimental diseases seems a virtual certainty
on the basis of the method by which they are produced, final demonstration of the responsible immunologic agent or mechanism is still lacking. Attempts to transfer these diseases passively by serum of the affected animal have not been successful, but transfer with lymphoid elements have been accomplished in experimental allergic encephalomyelitis and thyroiditis, but whether these sensitized cells are the direct cause of the observed lesions is still an open question. This failure, however, does not rule out a possible role for circulating antibodies since even if circulating antibodies were mediating these diseases, their concentration in the serum might not be sufficient to allow passive transfer because the antibodies might be removed from the blood by the target tissue as rapidly as they were formed. While detectable circulating antibodies or cellular or delayed sensitivity, or both, may develop during the course of experimental auto- or homoinmunication to tissue antigens, there has not, until recently (20), been produced conclusive evidence that either of these immunologic responses is the cause of the associated disease.

Historically, the recognition that certain diseases were probably allergic or might even represent the consequences of autosensitization has frequently antedated the demonstration of disease of the same type in an experimental animal. Cases in point are sympathetic ophthalmia, phakoanaphylactic endophthalmitis, the demyelinating diseases, and aspermatogenesis. Uhlenhuth (66), in 1903, was the first to demonstrate that the lens of the eye contained an organ-specific auto-antigen and that antibody which reacted with autologous lens material was present in
patients' sera. In 1899, Landsteiner(41) and Metchinkoff(50) demonstrated antibodies against heterologous sperm. Metalnikoff(49) working independently verified their work and was able to produce antibody against autologous sperm.

The introduction of the Pasteur vaccine for rabies prophylaxis in 1885 was soon followed by reports of an encephalomyelitis associated with the injections of dried rabbit spinal cord containing "fixed" rabies virus(7). Beginning as early as 1899, many investigators(3) attempted to induce paralysis and neurologic lesions in animals following the injections of normal nervous tissue. Although some animals did develop paralysis, these early studies were inconclusive owing to the irregular appearance of neurologic signs and an absence of definite pathologic changes.

Unequivocal evidence for the paralytogenic nature of rabbit brain in monkeys was provided by Rivers and his associates(60, 61) during the 1930's. The method, however, required many injections, was time-consuming and too unwieldy for general use. However, Morgan(48) and Kabat(38) independently found that acute disseminated encephalomyelitis could regularly be produced with but a single injection of brain tissue incorporated in the adjuvants of Freund and Mc Dermott(26). This disease has now been studied in a number of animals(27,32,34,37,42,44,51,56), and the evidence accumulated by these studies indicates that experimental allergic encephalomyelitis (EAE) is an immunologic phenomenon involving organ-specific antigens(Maksman 68).
In 1900, Lindemann noted the injurious action of anti-renal serum (43), but it wasn't until 1934, when Masugi(45), showed that anti-rat kidney rabbit serum would induce nephritis and chronic renal damage when injected into rats, that interest in the subject was aroused, and the question seriously debated, whether nephritis as observed in man, could be in some cases, the result of the action of anti-kidney antibodies produced by autoimmunization. Antikidney antibodies, detected by Coons' fluorescent antibody technique(14), have been found to localize in the glomerular basement membranes and in some instances in the tubular epithelium of kidneys of rabbits injected with heterologous anti-kidney antiserum(46). The fluorescent tracer technique when applied to kidney sections from cases of human glomerulonephritis revealed localization of gamma globulin in the damaged glomeruli(47).

The high degree of organ and even intra-organ specificity of nephrotoxic antibodies is indicated now by the fact that the apparent antigens concerned are shared by other organs such as the placenta and lung of the same species and by other species(64). May, however, has suggested that such renal damage resulted from a reaction between antibodies produced by the host to this injected foreign nephrotoxic globulin and portions of this foreign protein specifically bound to the kidney(40). This hypothesis has been supported by the work of Seegal (63), but more recently has been challenged by Erdmann(23).

In spite of much experimental work, no convincing demonstration of an autoimmunologic pathogenesis has been obtained. One exception to the series of failures was the observations by the Calvettis(9) that in
rats, injection of mixtures of homologous or autologous kidney and killed beta hemolytic streptococci produced morphological and functional renal changes suggestive of glomerulonephritis. Circulating antibodies to the injected antigens were also demonstrable. Numerous attempts to repeat this observation plus variations on this experimental theme, such as the use of Freund's adjuvant admixed with streptococcal or staphylococcal toxins, or allowing kidneys to sustain ischemic necrosis, have been unsuccessful and the results still await confirmation. While heteroimmune nephrotoxic serum nephritis is understood in considerable detail, the method of producing the disease, i.e., the injection of heterologous anti-tissue antibodies, does not greatly help in the understanding of the immunologic process, if any, involved in pathogenesis of human glomerulonephritis. The fact that the morphologic changes in nephrotoxic serum nephritis resemble those in human glomerulonephritis may attest more to the limited variety of histologic response of the kidney than to a similarity of pathogenesis in the two conditions. Almost all attempts to induce an autoimmune response to kidney in various laboratory animals utilizing either heterologous, homologous, or autologous tissue have been unsuccessful and Grabar(30) and Cruickshank(16) both categorically deny the possibility that autoimmunization may be induced by injection of autologous kidney into experimental animals. Even the use of Freund's adjuvant, which has brought out the best antigenicity of most tissues has not enabled anyone to demonstrate an autoimmune response to kidney antigens.
It was suggested many years ago by von Pirquet and Schick that glomerulonephritis might be a disease of hypersensitivity on the basis of the interval between overt infection by hemolytic streptococci and the appearance of nephritis. The recent demonstration (39) that streptococcal M protein is localized in the glomeruli for at least eight days after injection lends some support to the hypersensitivity theory.

Another interesting development which has recently received quite considerable attention has been the production of renal glomerular lesions and vascular lesions simulating those of human glomerulonephritis and periarteritis nodosa respectively by the injection of soluble, preformed antigen-antibody complexes in slight antigen excess into experimental animals (18). Morphologic lesions of serum sickness were also repeatedly demonstrated in rabbits by injections of soluble complexes of bovine serum albumin and antibovine serum albumin. Dixon (19) has recently extended these observations to an experimental model in which in vivo soluble antigen-antibody complexes are formed with much the same results. Immunologic hepatic injury characterized by hepatocellular necrosis and fibrosis has also been produced by the injections of soluble pre-formed antigen-antibody complexes. The disease, though, was self-limited and not chronic as were the glomerular and vascular lesions produced by Dixon. However, both fluorescent tracing and electron microscopy employing ferritin-conjugated globulin (2) have detected host gamma globulin, which was presumed to be antibody, within the lesions caused by antigen-antibody complexes. Beyond these observations there is little agreement concerning the mechanisms
by which antigen–antibody reaction produces the characteristic lesions
of the disease, although the role of hypersensitivity of the immediate
type with the production of Arthus-type injury certainly cannot be
ruled out.

To the diseases just described, for which an autoimmunologic
etiology may be attributed, may be added a growing list of other dis-
orders either afflicting specific organs, or causing widespread tis-
sular injury and associated with a spectrum of reactive serum globulin
components. EAE has been amply described in experimental animals both
as to histologic manifestations and serologic demonstrations, but the
relationship to human demyelinating diseases cannot be determined with
certainty. It is based largely on morphologic evidence except in the
case of the infrequent post-Pasteur treatment paralysis in which the
genesis of the human disease is comparable to EAE, although complement-
fixing antibrain antibodies such as are found in EAE, are not evident
in the sera of humans suffering from post-rabies treatment with killed
virus and tissue antigens. However, on the basis of morphologic study
most workers feel there is reasonable correspondence between EAE and
acute disseminated encephalomyelitis, seen as an occasional sequela of
measles, smallpox, chickenpox, mumps, influenza, and immunization
against rabies or smallpox. The relationship of EAE to multiple scle-
rosis is not so evident on morphologic grounds. What has been dis-
cussed concerning EAE may be applied to experimental results obtained
from studies regarding injection of homologous or heterologous peri-
pheral nerve plus Freund's adjuvant into various laboratory animals.
Lesions, located in the spinal ganglions, dorsal roots, and peripheral nerves themselves, are formed within 2 to 2½ weeks, and the course and progress of morphologic alterations bear a certain resemblance to acute infectious polyneuritis, Landry's paralysis, and infectious polyradiculitis (Guillain-Barre syndrome). Whether the experimental and natural processes are similar and whether an autoimmune pathogenesis operates in the human diseases are questions that remain to be answered.

The injection of guinea pigs and monkeys with homologous uvea in Freund's adjuvant produces an inflammatory reaction in the uvea, (13), characterized by focal infiltrates of lymphocytes and macrophages. In immunized animals, trauma to the iris precipitated a reaction in the traumatized eye. No information concerning antibody response or development of cutaneous sensitivity to the uveal antigen is available, but the experimental lesion closely resembles that of sympathetic ophthalmia of man, long thought to be caused by an autoimmune response to the antigens of the injured eye.

Pearson (52) has reported the production of an extensive polyarthritis in rats given repeated injections of macerated homologous muscle in Freund's adjuvant, and recent observations reported by this worker (53), revealed that the disease was produced just as efficiently without the use of muscle homogenates but by Freund's adjuvant only. The presence of circulating antibodies and cutaneous hypersensitivity was not investigated. If this disorder can be established with some degree of certainty as an autoimmune reaction, it may be an important development in our understanding of rheumatoid diseases.
An interesting group of human maladies for most of which there are no experimental counterparts, and which display a curious pathologic feature, that of fibrinoid degeneration, have for this reason been categorized as connective tissue disorders and are known as the so-called "collagen diseases". The diseases included under this nomenclature are: rheumatic fever, rheumatoid arthritis, dermatomyositis, scleroderma, periarteritis nodosum, lupus erythematosus, and some of the nephritides. Since the early 1930's, at which time they were first grouped in this manner by Klinge, pathologists have suggested a hypersensitive state as the basic cause of these disorders. Some want to regard the etiology as autoimmunization. The evidence for the role of hypersensitivity is well founded, as fibrinoid degeneration, the classic pathologic lesion found in collagen disease, is found in experimental anaphylaxis and in the Arthus phenomenon(11) and (58). Periarteritis nodosa-like lesions have been produced in animals sensitized to egg albumin and by the massive injections of horse serum into rabbits(57). The injections of egg white into mice yielded pathologic changes of rheumatic carditis(10). Antihistaminics will prevent development of a horse serum induced myocarditis, an effect similar to that exerted by antihistaminics in protecting animals against anaphylactic shock. Furthermore, total body irradiation 24-72 hours before sensitization with horse serum brings about a marked reduction in the number and intensity of the vascular lesions and carditis(15). Perhaps the most convincing evidence has been the production
of vascular lesions induced in normal recipient rabbits by passive sensitization (12).

A great amount of time and effort have been devoted to the study of two of the above listed collagen diseases; lupus erythematosus or LE as it is commonly designated, and rheumatoid arthritis. Of the two, LE is the most spectacular in its spectra of antibodies, mostly directed against cell nuclei, which may be demonstrated in sera from patients suffering from the disease. The evidence that LE may arise on an immunologic basis is substantial (55). Studies of the so-called LE factor in the serum suggest that it reacts in a rather specific manner with nuclear material from diverse sources. Other observations have demonstrated the absorption of LE factor by isolated nuclei, with nucleoprotein, and specifically by DNA (36), the source of which was not important since serum from lupus patients react with DNA from human leucocytes, beef thymus, pneumococcus, and bacteriophage. Immunohistochemical study of LE inclusions showed them to be coated with gamma globulin (29), confirming the above observations. Other antibodies are directed toward the patients own red cells, white cells and platelets.

Rheumatoid arthritis shares many of the features of LE except the antigen appears to be a gamma globulin of the usual molecular size. The antibody, or rheumatoid factor (RF), shares many of the characteristics of the various reactive globulins of LE in that it may also be found in other of the collagen diseases as, scleroderma, dermatomyositis
and periarteritis nodosum. LE cells may also be observed in patients suffering from rheumatoid arthritis. An immunologic basis for these diseases has much to recommend it due to the amazing array of tissue-reactive antibodies present and their occurrence in high frequency in subjects already known to be producers of anti-tissue antibodies. However, opposed to this view is the fact that these diseases may occur in individuals with agammaglobulinemia, who are incapable of producing the antibodies discussed, and also the fact that the lesions of LE nor rheumatoid arthritis have never been experimentally produced.

In the course of studies on the possible role of antibodies in homograft rejection, Voisin and Maurer(67) noticed that rabbits injected with homologous skin in Freund's adjuvant developed an atrophy of the skin and alopecia. Dermatologic disorders such as eczemas and neurodermatitis(35) have long been associated with autoimmunization.

A possible experimental model which may provide information regarding certain non-tuberculous cases of Addison's disease was described by Steiner(65). An inflammatory disease in guinea pigs was produced by injection of whole guinea pig adrenal in Freund's adjuvant. The lesions appeared much like those seen in the human counterpart.

A recent addition to the list of diseases in which an autoimmune mechanism may play a possible role is that of ulcerative colitis. The data, briefly summarized, show that antibodies against human colon are directed towards an antigen extracted by phenol with water from embryonic, juvenile and adult colon mucosa. Immunohistochemical studies
located specific fluorescence on the epithelial cells of the mucosa, preferentially in the crypts. These preliminary unconfirmed experimental observations are mentioned primarily to indicate the variety of diseases that may prove to be capable of eliciting destructive autoimmune responses.

Only one experimental autoimmune disease has almost fulfilled the criteria needed to prove the role of an autoimmune mechanism in the pathogenesis of the disease. The diseases just described certainly point toward such a mechanism, but only in experimental thyroiditis does the evidence justify calling it a disease caused essentially by autoimmunization. The most significant evidence is that the antigenic stimulus has been isolated, characterized and identified. These antigens, thyroglobulin and a microsomal fraction of the acinar epithelial cells, stimulate the formation of specific antibody which has been detected by immunofluorescence techniques both in the thyroids of animals immunized with thyroglobulin and in thyroids of humans suffering from chronic thyroiditis or Hashimoto's disease (22). The circulating antibodies in both experimental animals and in humans are easily detected, but do not always reflect the severity of the disease. The disease has been transferred by sensitized lymphoid elements into normal recipients and lesions which appeared were identical to those observed in the donor animals and in human cases of thyroiditis (24). The lesions which are formed exhibit morphologic evidence as being mediated by delayed hypersensitivity accompanied by cellular infiltrate, but all
these compelling facts still do not give enough information to establish this as a disease of autoimmunization. Passive transfer of the disease by serum anti-organ antibodies has not been accomplished even when high levels of circulating antibodies are maintained.

It is obvious that the proof required to establish any of these so-called immunologic diseases as autoimmune, rests mainly on the nature of the mechanism responsible for the initiation of organ-specific lesions. There are two schools of thought regarding this important subject, and both have their adherents. It is one of the main contentions of adherents of the delayed hypersensitivity school that all lesions in all experimental autoallergies are fundamentally the same. In every case the earliest and mildest lesion is described as a perivenous collection of inflammatory cells, mainly of the lymphocytic or histiocytic type, originating from the blood stream or vascular adventitia. In more extensive lesions, there is invasion of parenchyma by these cells, usually with destruction of specific parenchymal elements, such as myelin, testicular germinal epithelium, thyroid colloid, and in homografts, the newly placed skin. Vascular necrosis and scarring occur if the lesion is severe, and if mild, no trace of scarring is left behind. Passive transfer of sensitized lymphocytes from immunized donor animals to normal recipients have produced the lesions in such experimental diseases as EAE, thyroiditis, and peripheral nerve disorders as polyneuritis(70). On the other hand, proponents of the serum antibody mechanism must admit that circulating antibody, when demonstrable, has never produced lesions of any of the diseases by
passive transfer of such serum into normal recipients. Neither has the concentration of circulating antibody always paralleled the severity of the disease. Only in disorders as acquired hemolytic anemia(71), agranulocytosis(17), and thrombocytopenic purpura(33) have passively transferred antibodies produced disease similar to that observed in the donor animals. Roitt(62), however, succeeded in establishing thyroiditis by passive transfer of heterologous circulating antibodies in normal rats pre-treated with one injection of Freund's adjuvant.

It is interesting at this point, to speculate on the mechanism by which Freund's adjuvant influences the development of lesions in experimental animals injected with tissue antigens. It is obvious that any and all the experimental diseases of autoimmune origin have been produced by a mixture of tissue antigen and Freund's adjuvant. Most, if not all these diseases would be very difficult, if not impossible to produce without the use of adjuvant. No one knows the answer, but Freund(28) believes that the killed tubercle bacilli produce a cellular reaction, composed of mononuclear cells, some with the morphologic character of epitheloid cells, lymphocytes, and plasma cells, that brings about an allergic injury to certain organs if appropriate tissue antigens are injected with complete adjuvant. The paraffin oil establishes a persistent depot of antigen at the site of injection and promotes the transport of antigen to other sites of antibody formation. The synergistic effect of these components then boosts antibody production to high levels and, at the same time, promotes a delayed type hypersensitivity. This being the case, then, organ-specific injury could be the
result of both circulating antibody and immigration of sensitized lymphoid cells.

In both humans and animals, the mechanism of autoimmunization, the antigens involved, and the role of the autoantibodies are either unknown or disputed(68). In 1953, Billingham, Brent and Medawar(6) set out deliberately to establish the condition known as immunologic tolerance, which they succeeded in establishing in embryo mice. The tolerant state, established during embryonic existence, or during the first few days of extra-uterine life, operates during adult life so as to prevent autoimmunization normally(8). This situation further complicates the concept of disease initiated by autoimmune process. In order to explain the autoimmune diseases, which involve autoantigens to which tolerance should have been established, various proposals have been made. One of these, proposed by Burnet, suggests that certain "forbidden clones" of autoantibody producing cells somehow "escape" eradication in perinatal life or that similar clones arise later in life by somatic mutation. Another theory suggests that certain tissues such as brain and thyroid are not available to the antibody forming mechanism normally, so that no tolerance to these tissues has been established. When injury, pre-existing disease or injection of these tissues occur, these tissue antigens are foreign to the antibody-forming mechanism and produce corresponding autoantibodies.

In the face of such formidable, and seemingly factual conclusions, it is interesting that the findings, from which the present work arose, showed that circulating autoantibodies to tissues constantly available,
to the antibody-forming mechanism, were produced in normal rabbits by
the injection of rat or rabbit liver ribosomes in Freund's adjuvant
(20). It will be shown here that this process was accompanied by the
development of pathology in various tissues. Furthermore, the much
debated question of the mechanism of autoimmune disease is answered
here by the proof of the initiation of an identical pathologic process
in normal animals injected with antiribosome serum from the above
animals, which is the sine qua non of the establishment of true auto-
immunization.
MATERIALS AND METHODS

Preparation of Cytoplasmic and Nuclear Materials

The cytoplasmic and nuclear materials employed in this investigation were prepared by Dr. George Webster and Mr. Richard Sutter formerly of the Department of Agricultural Biochemistry. For a complete description of the extraction procedures, see (5).

Immunization of Rabbits

Albino female rabbits were injected with rat liver nuclei, the cytoplasmic ribosomes from rat and rabbit livers, with soluble ribosomal protein extracted from rat liver ribosomes. These materials were incorporated in Freund's adjuvant and injected intramuscularly into the thigh muscles of each rear limb. One animal received intravenous injections of the ribosomal protein solution. Five rabbits were injected with quantities of nuclear materials ranging from 16.8 mg to 67.5 mg protein. Two of these animals receiving lower dosages died during immunization. Five rabbits received rat liver ribosomes ranging in concentration from 77 to 140 mg protein. One of these animals died. One rabbit received a total of 77 mg protein concentration of rabbit liver ribosomes. Ribosome antigen was measured as the protein represented in ribosomes and not the total amount of material injected. The assumption was made that protein represents 50 per cent of the ribosomal material. Three rabbits were injected sRNA (10 mg to 98 mg) and one of
these animals died. Two animals were injected with ribosomal protein. One received 25 mg of ribosomal protein in Freund's adjuvant intramuscularly and 16 days later was given 10 mg intravenously in solution. The other animal received a total of 32 mg of ribosomal protein solutions intravenously over a 25 day period.

The time of sacrifice for these animals was predicated on the fact that animals which received ribosome material showed signs of obvious distress at 70-78 days after initial injection, and, if allowed to live, would invariably succumb shortly afterward. Animals which received materials other than ribosomes were electively sacrificed at times approximating those of ribosome-injected animals. Thus, two of the three animals injected with nuclear material were sacrificed at 85 days and the remaining animal at 55 days; sRNA-injected animals were sacrificed at 77 and 81 days. Two of the five rabbits injected with rat liver ribosomes were used in this study; one was sacrificed at 77 and the other at 78 days. The one rabbit which received rabbit liver ribosomes was sacrificed at 69 days. Both animals which received ribosomal protein were sacrificed at 60 days.

Passive Serum Transfer.

Rabbits were injected intravenously with one to ten ml of serum from animals actively immunized with the various antigenic preparations described above. The days after injections of the donor animals on which the serum samples were obtained are in parentheses. Sixteen rabbits received antiserum to rat or rabbit liver ribosomes (70-78 days). Six were chosen for this study and were electively sacrificed at 5, 23,
and 44 days after injection. Two animals received antiserum to mRNA (88 days) and one was sacrificed at 2 and the other at 7 days; one rabbit received antiserum to rat liver nuclei (55 days) and sacrificed 10 days later; three animals received antiserum to ribosomal protein (60 days) and two were sacrificed at 15 and the remaining animal at 22 days after injection. One animal received antiserum to rabbit ribosomes which was absorbed twice with rabbit liver ribosomes coupled to normal human Type O Rh positive erythrocytes and sacrificed 36 days later.

Sacrifice was accomplished by exsanguination by cardiac puncture and neither intravenous nor gaseous anesthetics were administered.

**Histologic Techniques.**

Animals were autopsied immediately after sacrifice and their tissues placed in 10 per cent neutral formalin. Sections were prepared on an AO rotary microtome at an indicated thickness of 6 to 8 microns. In most instances, except where an alcian blue-PAS stain for mucopolysaccharides of epithelial and connective tissue origin was indicated, Harris' hematoxylin-eosin stain was utilized after fixation and drying.

The preparation of histologic sections was made possible through the cooperation of Dr. Francis W. McCoy, Dept. of Medicine, Ohio State University, and his technical staff.

**Photomicroscopy.**

A Reichert "Biozet" binocular microscope employing a Reichert 35mm camera attachment with built-in light intensity meter was utilized for
photomicrographic work. The light source was a built-in 6 volt coil filament tungsten lamp capable of producing a color temperature of 3200 degrees Kelvin. Kodak High Speed Ektachrome Type B was found to be satisfactory without the use of light balancing filters for most work. High magnifications required the use of either Kodak Kodachrome II (Professional) or Kodak Kodachrome X in combination with an 82 and/or 82B Kodak light balancing filter(s) for the purpose of raising the color temperature to the required levels demanded by these films. All photomicrographs, however, were taken with the aid of a 0.1 per cent light transmitting neutral density filter fitted over the substage light exit. All exposed film was developed by a franchised Kodak processing plant.
RESULTS

As previously noted (20) rabbits injected with rat or rabbit liver ribosomes in Freund’s adjuvant become ill 70-78 days later, exhibiting signs of physical distress, such as lethargy and extreme muscular weakness. This was accompanied by a marked, severe autoimmune hemolytic anemia and leucopenia. Autoantibodies for erythrocytes, leucocytes and ribosomes were demonstrable in their sera as well as antibodies to yeast αRNA, which were inhibited by various nucleotides, nucleosides and bases associated with RNA(5). These previous reports also noted pathologic changes in the heart, liver, kidney, lung, spleen and peripheral blood. Identical hemolytic anemia, leucopenia and tissue pathology were described in normal rabbits injected with antiribosomal rabbit serum from donor animals receiving ribosomes.

In the present work, an attempt has been made to describe certain specific lesions occurring in both animals injected with ribosomes and with antiribosomal serum which appear to be the result of, or associated with an autoimmune process. Animals injected with yeast αRNA and anti-αRNA sera developed very similar lesions. Animals injected with ribosomal protein and rat liver nuclei did not show similar tissue changes. Certain other changes occurred in all animals and were considered non-specific, such as interstitial nephritis, hepatocellular vacuolization, and interstitial fibrosis associated with other non-
specific pulmonary findings, as these lesions have been demonstrated frequently in normal stock animals.

Comparative histological study of the heart, liver, lung, and spleen of these animals is presented. Observations on the peripheral blood were limited to animals receiving ribosomes and antiribosome serum.

Heart—Rabbits Injected with Ribosomes

Gross inspection revealed a slightly enlarged, pale, flabby myocardium, the most notable characteristic of which was the complete lack of muscular tone, and when held by the apex, collapsed upon itself. Cut sections did not reveal any gross irregularities of the valves, internal structures or endocardium.

Microscopic examination of the myocardium revealed swollen, pale myofibers separated by large spaces, some filled with pale-staining fluid. The edematous process extended to the connective tissue spaces surrounding small arteries and veins. The sarcoplasm exhibited a granular appearance and myofiber striations were smudged and indistinct. Other areas demonstrated myofiber fragmentation which resulted in nuclear dislodgment accompanied by the presence of free nuclei (Figure 1, Plate I), although the cell nuclei appeared relatively unaffected. The histologic change just described affected the entire myocardium and was especially prominent near areas of foci of necrosis, which were randomly distributed throughout the myocardium and usually exhibited two forms conditioned by the presence or absence of cellular infiltrate. Their appearance was usually round or oval, never large or extensive (one to two mm in diameter), and associated with a sharply
circumscribed perimeter which endowed them with a punched-out appearance. About one-third of these foci were observed to be quite acellular, except for a very few macrophages situated near the borders of the lesions (Figure 2, Plate I). The remaining foci, however, contained a cellular infiltrate comprised of macrophages, lymphocytes, fibroblasts, and a few plasma cells (Figure 3, Plate I). Other pinpoint foci, considered to be early lesions, appeared to surround small venules and capillaries, accompanied by a few lymphocytes, fibroblasts, and abundant plasma cells, Figure 4, Plate I. Lesions, somewhat chronologically older, demonstrated fewer inflammatory cells and increased fibrogenesis, (Figure 1, Plate II).

The pericardium was not remarkable but examination of the endocardium revealed a minimal endothelial proliferation which included portions of the base of the leaflets of the tricuspid valve. The structure of the remaining valve was not remarkable. (Figure 2, Plate II) exhibits a subendothelial fibrosis and thickening, a condition never observed in normal, healthy rabbits. The fibrotic process was quite extensive and extended along the wall of the endocardium of the left ventricle from the tricuspid valve to the apex. The lesion ranged from a few hundredths of a millimeter in depth to about one millimeter in thickness, (Figure 3, Plate II). The valves were slightly involved by minimal thickening.

Study of the cardiac vasculature revealed no irregularities in the coronary arteries and veins and their larger branches. However, medial hyalinization and intimal activation, (Figure 4, Plate II), associated
with a fibrinoid change in the adventitia of small arteries and arterioles, (Figure 1, Plate III), were observed. The lymph vessels were enlarged and filled with pale-staining material. Similar vascular changes were observed in the auricles but were not as extensive as noted in the ventricular musculature. The histologic changes just described were noted in all animals injected with rat and rabbit liver ribosomes.

Heart—Rabbits Injected With Antiribosome Serum

Gross inspection revealed histologic features essentially identical to those observed in ribosome-injected animals. The pericardium was grey in color, rough, and thickened.

Microscopic examination of the myocardium of animals sacrificed five days after intravenous injection of antiribosome rabbit serum revealed enlarged, pale-staining myofibers widely separated by edema and exhibited smudged, indistinct striations. Most fibers demonstrated ruptured sarcolemma, thin strands of which bridged the wide edematous spaces between myofibers. The sarcoplasm had disappeared along the length of a few fibers leaving broken, fragmented ends lying free. Both the auricular and ventricular myocardium were involved. A striking feature was the constant presence of a round cell infiltrate, which was predominately histiocytic, and which bulged apart the partially separated fibers, (Figure 2, Plate III). Note the infiltrate and also a small area of focal necrosis and fibroblastic activity at the top of the figure. Focal necrosis also occurred around small, thin-walled veins, (Figure 3, Plate III), and was never observed around larger arteries and veins. The most commonly observed form of necrosis was a
PLATE I

Figure 1.—Section of myocardium from rabbit injected with ribosomes demonstrating edematous, hypertrophied myofibers. x100.

Figure 2.—An area of focal necrosis in myocardium of rabbit injected with ribosomes. Note the almost complete lack of cellular infiltrate. x100.

Figure 3.—Area of focal necrosis in myocardium of rabbit injected with ribosomes. This lesion originated somewhat later than the lesion as shown in Figure 2, Plate I, as demonstrated by the presence of an infiltrate. x100.

Figure 4.—Small focal necrotic area situated around thin-walled capillaries. Note the presence of plasma cells. Myocardium of rabbit injected with ribosomes. x312.
linear lesion about four to five mm. in length and about one mm in width, infiltrated and surrounded with macrophages, plasma cells, and histiocytes. (Figure 4, Plate III). These foci possessed no predilection for vasculature or other distinguishing structures. Compare with (Figures 2 and 3, Plate I).

An outstanding feature observed in hearts of animals sacrificed 23 days after injection of ten ml of antiribosome rabbit serum was the severe damage sustained by the myocardium. Lesions ranged from swollen, edematous myofibers separated by edema to a complete disruption of the sarcolemma; the sarcoplasm appeared as a precipitated, eosinophilic mass resembling coagulation necrosis which left naked nuclei surrounded by a clear peri-nuclear halo. Striations were smudged and indistinct, and in some, fibers were entirely lost to view. Overall, there was an almost complete loss of normal architecture and an increased interstitial fibroblastic activity was evidenced, (Figure 1, Plate IV). Compare with Figure 1, Plate I. A few areas of hyaline necrosis were observed near small, thin-walled veins. Also prominent was an organizing pericarditis composed of newly formed connective tissue and associated with large areas of fresh hemorrhage.

One outstanding histologic finding was a severe arteriolar medial hypertrophy characterized by a greatly thickened media which almost occludes the lumen, (Figure 2, Plate IV).

Examination of the remaining vasculature revealed only a slight arterioloaerclerosis and intimal activation of arterioles.
PLATE II

Figure 1.—Border of focal necrotic area much earlier in origin than that shown in Figure 4, Plate I. Note increased fibrosis and the presence of fibroblasts and scavenging macrophages. Rabbit injected with ribosomes. x312.

Figure 2.—Section of endocardium of rabbit injected with ribosomes, demonstrating subendothelial fibrosis. The adjacent endocardium presents hypertrophied, edematous myofibers. x100.

Figure 3.—Rabbit injected with ribosomes. Subendothelial fibrosis of greater severity than that presented in Figure 2, Plate II. x100.

Figure 4.—Rabbit injected with ribosomes. Small myocardial artery demonstrating medial hyalinization and intimal activation. x430.
PLATE III

Figure 1.—Rabbit injected with ribosomes. Small myocardial artery demonstrating a fibrinoid change in the adventitial sheath. Lesions such as this were present in animals injected with antiserum to ribosomes also. x100.

Figure 2.—Rabbit injected with antiribosome serum and sacrificed five days after injection. A histiocytic infiltrate is seen bulging apart the myocardial fibers. Note the small area of focal necrosis. x100.

Figure 3.—Rabbit injected with antiribosome serum and sacrificed five days after injection. A focal necrotic area borders a small, thin-walled vein in the myocardium. x100.

Figure 4.—Typical linear focal necrosis in myocardium of rabbit injected with antiribosome serum and sacrificed five days after injection. x100.
Heart—Rabbits Injected With aRNA

Gross inspection revealed a pale, soft, flabby myocardium. The injected coronary vessels were patent throughout, and the pericardial surface was smooth and glistening.

Microscopic examination revealed hypertrophied, edematous myofibers separated by an edematous process; cell nuclei appeared relatively unaffected, however. Pinpoint lesions of focal necrosis associated with disruption of individual myofibers, (Figure 3, Plate IV), were observed scattered throughout the myocardium. Compare with (Figure 2, Plate III). There was apparently no predilection for the vasculature. Patchy areas of hyaline necrosis bounded by normal myocardium was observed in both auricular and ventricular musculature. More severe lesions were demonstrated by foci of necrosis associated with heavy fibrosis and scar formation. These areas were usually bounded by small hemorrhages and edematous processes which separated the muscle bundles. Cellular infiltrate was absent, (Figure 4, Plate IV). Compare with (Figures 2 and 3, Plate I). In other areas of the ventricular musculature, necrotic foci, fewer in number than those previously described, were observed in the outer margins of the ventricular musculature. They were represented by fibrotic, shredded, eosinophilic masses surrounded by proliferating fibroblasts and an occasional small, dark round cell. Moderate fibrogenesis was seen around the periphery of the injury, (Figure 1, Plate V). Compare with (Figure 3, Plate III).

The pericardium, endocardium and valvular structures were not remarkable. The vasculature was not remarkable except for slight arteriolar intimal activation.
Figure 1.—Focal coagulation necrosis in myocardium of rabbit injected with antiribosome serum and sacrificed 23 days after injection. Note absence of cellular infiltrate. x100.

Figure 2.—A portion of an organized pericarditis in an animal sacrificed 23 days after injection of antiribosome serum. Note the severe arteriolar medial hypertrophy. The lumen is almost occluded. x125.

Figure 3.—Pinpoint focal necrotic areas surrounded by an edematous myocardium from rabbit injected with sRNA. x100.

Figure 4.—Focal myocardial necrosis characterized by heavy fibrosis and scar formation. Animal injected with sRNA. x100.
Heart—Rabbit Injected With Anti-sRNA

Gross inspection revealed a slightly enlarged, flabby, pale myocardium. The coronary vessels and their branches were injected but patent throughout. Cut section revealed no remarkable findings, as valves, endocardium and internal structures appeared normal. A small mural thrombus, presumably post mortem, was noted on the right ventricular wall.

Microscopic examination revealed swollen hypertrophied myofibers separated by edema. Localized areas of hyaline necrosis were observed near large thin-walled veins, (Figure 2, Plate V). Overall, the myocardium did not appear to be as severely affected as those just previously presented. Compare with (Figure 1, Plate I and Figure 3, Plate IV). Scattered throughout the muscle bundles were patches of focal necrosis associated with moderate fibrosis and cellular infiltrate which consisted of mononuclear histiocytes, (Figure 3, Plate V). Figure 4 Plate V depicts these cells under higher magnification.

Lesions similar to those just described were observed in close proximity to small veins; Figure 1 Plate VI shows one such lesion near the root of the mitral valve, which in itself, showed no remarkable changes. The small vein can be seen lying just to the right of the lesion. The same cellular components were observed in these foci as for the foci found in the myocardium. Some portions of the myocardium presented a picture of necrosis associated with histiocytic infiltrate and surrounding hyaline change in the muscle fibers, plus an increased
Figure 1.—Focal myocardial necrosis of later origin than that as shown in Figure 4, Plate IV. Rabbit injected with sRNA. x100.

Figure 2.—Myocardium of an animal injected with anti-sRNA serum. Note the dense, spreading band of hyaline necrosis around the small vein. The remaining myocardium is edematous. x50.

Figure 3.—Focal myocardial necrosis characterized by a cellular infiltrate shown here comprised mainly of macrophages which are invading the interstitial spaces. Animal injected with antiserum to sRNA. x100.

Figure 4.—Enlarged view of the cellular infiltrate shown in Figure 3, Plate V. Note the necrotic myofibers. x450.
interstitial fibrosis accompanied by a few fibroblasts and an occasional macrophage. Fresh hemorrhage was seen in the immediate vicinity.

Prominent was an endothelial proliferation which extended up to and included the valve leaflets. Coexistent with proliferation was a prominent subendocardial fibrosis, (Figure 2 Plate VI), comprised of dense bands of collagen and proliferation fibroblasts which extended from the apex to the origin of the valve roots. Compare with (Figures 2 and 3, Plate II). The auricular endocardium and endothelium were not affected in this manner. Examination of the coronary arteries and veins revealed a normal architecture. Minimal arteriolar intimal activation was noted.

**Heart—Rabbits Injected With Riboprotein**

Gross inspection revealed a somewhat enlarged, pale heart with less than normal muscular resilience. The coronary vessels appeared within normal limits. The pericardium was dull-gray and roughened over the entire muscle.

Microscopic examination revealed swollen, edematous fibers separated by edema; the nuclei were unaffected. Small patches of hyaline necrosis were evident throughout and focal necrosis and hemorrhage were not observed.

An organizing pericarditis characterized by thin, elongated bundles of collagenous fibers, capillaries, fibroblasts, histiocytes and a few polymorphonuclears was observed overlying the myocardium,
(Figure 3 Plate VI). The mesothelium overlying the pericarditis presented large, proliferating, oval cells containing compact, basophilic nuclei surrounded by a large eosinophilic cytoplasm. Invasion of the myocardium by a fibrotic process was observed at the base of the pericarditis, and, in some areas was replaced by hyalinized muscle fibers. Compare with (Figure 2, Plate IV and Figure 3, Plate VI). For the majority of the circumference of the pericardial inflammation, the base consisted of a densely staining eosinophilic layer of collagenous material, but in a few places, this base gave way to an infiltrating fibrosis which adversely affected the fibers situated in close proximity and produced inroads of fibrosis with a consequent cellular reaction consisting mainly of fibroblasts and a few histiocytes. The fibers of papillary muscles appeared edematous and their surfaces were covered with a moderate endothelial proliferation with a suggestion of interpapillary adhesions.

Medium-sized arteries and veins were within normal limits, although there was a slight hyalinization of arteriolar and capillary walls. The coronary vessels were normal in all respects.

Heart—Rabbits Injected with Antiserum to Riboprotein

Gross inspection revealed a pale, flabby musculature otherwise not remarkable. The coronary vessels appeared within normal limits.

Microscopic examination revealed an extensive edematous process which separated most of the swollen, hypertrophied fibers, (Figure 4 Plate VI), which encompassed most of the ventricular and auricular
PLATE VI

Figure 1.—A focal necrotic area near a small thin-walled vein at the root of the mitral valve. Animal injected with antiserum to sRNA. The valve itself was not remarkable. x125.

Figure 2.—Subendocardial fibrosis in the heart of a rabbit which received antiserum to sRNA. x125.

Figure 3.—A portion of an organizing pericarditis which completely enveloped the myocardium of an animal injected with riboprotein. x100.

Figure 4.—A myocarditis characterized by the presence of swollen, edematous fibers among which is interspersed a round cell infiltrate. Animal injected with antiserum to riboprotein. x250.
musculature. Compare with (Figure 1, Plate I and Figure 3, Plate IV). Focal necrosis, interstitial fibrosis, perivascular infiltrate or hemorrhage were not evident. A few portions of the myocardium exhibited hyaline necrosis in close proximity to thin-walled veins. Examination of the pericardium, endocardium, endothelium, valves and associated structures were negative. All vascular elements were essentially negative.

Heart-Rabbits Injected With Rat Liver Nuclei

Gross inspection revealed a heart which possessed normal muscular tone and resilience, and its size was within normal limits. A dull-gray roughened pericardium enveloped the entire organ.

Microscopic examination revealed normal myocardial architecture. Close study of the myofibers and their nuclei demonstrated no remarkable histologic findings, except in one or two areas where old hemorrhage characterized by hemosiderin in crystals lying free and contained within a few macrophages were observed coincidentally with old scar formation. (Figure 1, Plate VII). Scattered areas of hyalinization were present near small thin-walled veins. Focal necrosis, perivascular infiltrate, and interstitial fibrosis were not demonstrable. Examination of the valves, endothelium and endocardium were not remarkable. Study of the vasculature was not remarkable except for very slight hyaline changes in the media of small arteries and associated with slight intimal activation. Small, medium, and large veins appeared normal. A very extensive pericarditis composed of organized connective
tissue in which fresh hemorrhages were present was noted. Fatty changes were evident in some portions of this inflammatory reaction. (Figure 2, Plate VIII). Compare with (Figure 2, Plate IV and Figure 3, Plate VI).

Heart—Rabbits Injected With Antiserum To Nuclei

Gross and microscopic examination revealed essentially negative histologic findings.

Kidney—Rabbits Injected With Ribosomes

Gross examination revealed slightly enlarged, pale, clay-colored organs which were considered to be less turgid than normal. Hemorrhagic petechiae and a few pitted scars which averaged about one-half mm in diameter, were scattered over the surface of the cortex. The capsules stripped with ease, and cut section revealed a slight thinning of the cortex, the color of which was dull and clay-colored. The medullary rays were not remarkable and plainly visible. The pyramids and pelvis were within normal limits.

Microscopic examination revealed relatively normal proximal and distal convoluted tubules, although beginning cloudy swelling, and just a hint of hyaline droplet formation in a few of the tubular epithelial cells was noted. Approximately 90 percent of the glomeruli observed appeared normal. The remainder exhibited a beginning glomerulitis characterized by an increased size of the glomerulus due to epithelial and endothelial cell proliferation, and possibly, proliferation of the
PLATE VII

Figure 1.—Scar formation surrounded by fresh hemorrhage in the myocardium of rabbit injected with rat liver nuclei. x125.

Figure 2.—A pericarditis composed of an organized connective tissue and associated with fatty changes from a rabbit injected with nuclei. x125.

Figure 3.—A picture of beginning proliferative glomerulitis characterized by swollen, proliferating endothelial cells. The capillary and capsule basement membranes are not thickened. Note the normal tubular structures. Animal injected with ribosomes. x125.

Figure 4.—Subacute glomerulohephritis in an animal injected with ribosomes. Especially prominent is the formation of an epithelial crescent opposite the glomerular tuft. Note the round cell infiltrate in the edematous interstitial tissue. x250.
mesangial cells. (Figure 3, Plate VII). The basement membranes of
Bowman's capsule and the glomerular capillaries were not thickened, nor
was there any exudate or cells present in Bowman's space. Hemorrhage
or casts were never observed in any of the tubules. Of the total
glomerular population, about 5 percent exhibited more severe glomerular
changes as depicted in (Figure 4, Plate VII). Pictured here is a
typical example of beginning subacute glomerulonephritis characterized
by swelling of the capillary endothelial cells containing dark,
pyknotic nuclei. The epithelial cells lining Bowman's capsule were
activated and a partly formed epithelial crescent is present opposite
the contracted glomerulus. Contrast this picture with (Figure 3,
Plate VII), a picture of acute glomerulitis. Note also (Figure 4,
Plate VII) the adhesion between the capillary loops and Bowman's cap-
sule. Also present is an interstitial nephritis consisting of round
cells and edema, and concomitant tubular changes consisting of cloudy
swelling and epithelial cell degeneration. About one per cent of the
total glomeruli examined demonstrated complete scarring and fusion of
Bowman's capsule and glomerulus into a fibrotic, non-functional,
esinophilic mass, (Figure 1, Plate VIII). These combined changes
were evident in at least two of the three animals examined.

A curious feature regarding one rabbit of this series was the
presence of narrow bands of cellular infiltrate and fibrosis situated
in the medullary rays associated with necrosis and destruction of the
renal tubular epithelium, (Figure 2, Plate VIII). Figures 3 and 4,
Plate VIII depict the histologic features characteristic of these
PLATE VIII

Figure 1.—Chronic sclerosing glomerulonephritis in an animal injected with ribosomes. The glomerular structure is obliterated by dense fibrosis. The surrounding tubular structures present necrotic changes in their epithelium. x250.

Figure 2.—A slight medullary, interstitial, round cell infiltrate in an animal injected with ribosomes. Note partial desquamation of the tubular epithelium also. x50.

Figure 3.—A portion of the severe interstitial nephritis near a small vein, in an animal injected with ribosomes. Note the heavy cellular infiltrate with an associated fibrosis. Bowman's capsule is greatly dilated, its basement membrane thickened and contains pale-staining fluid. The glomerulus is relatively ischemic. x125.

Figure 4.—An enlarged view of the cellular infiltrate as seen in Figure 3, Plate VIII. Note the presence of plasma cells, fibroblasts, lymphocytes, macrophages, and degenerate tubular epithelial cells. x397.
lesions. The cellular infiltrate consisted of plasma cells and small, dark round cells with little or no discernible cytoplasm which contained a dark, basophilic nucleus, the chromatin pattern of which was in dense, block-like masses. A few histiocytic elements and pseudo-eosinophiles were present admixed with fibroblasts, (Figure 1, Plate II). The infiltrate swept around and into the interstitial spaces separating the tubules into compact masses, the epithelial cells of which have ballooned, lost their nuclei, and appeared as large empty globules. See (Figure 4, Plate VIII). Glomeruli which were caught up in this lesion demonstrated greatly expanded Bowman's capsules filled with edema fluid or albuminous material. The capsule is thickened and the glomerulus is essentially normal, but is relatively ischemic.

Evident also were perivascular infiltrates usually situated near small, thin-walled veins. Note in (Figure 1, Plate IX) the various cell types just described plus the presence of a number of pseudo-eosinophiles. These latter cells were always seen in perivascular infiltrates but never observed far removed from these vascular structures. Note also the elongated ring of tubular epithelium surrounded by infiltrate and fibrosis. Small isolated foci comprised of the same cellular and histologic findings were observed scattered throughout the cortex, and were not associated with vascular structures. The collecting tubules appeared within normal limits. No histologic changes were observed in the large and medium-sized arteries and veins, although arteriolar intimal activation was observed. Afferent glomerular arterioles were not remarkable.
Kidney—Rabbits Injected With Antiribosome Serum

Gross inspection revealed enlarged, pale, edematous organs. Cortical petechiae and scarring were not evident. The capsules stripped with ease and cut section revealed that the cortex, pyramids and pelvis were within normal limits.

Microscopic examination of renal tissue from an animal sacrificed five days after passive transfer of antiribosome rabbit serum revealed intense hyperemia of the entire kidney, as the arcuate arteries and veins and arteriae spuriae were filled with blood. The tubules were not remarkable and appeared normal except for slight cloudy swelling of the tubular epithelium. Tubular basement membranes were not thickened.

Most of the glomeruli examined demonstrated a proliferative glomerulitis; the increased glomerular cellularity resulting from endothelial cell proliferation. (Figure 2, Plate IX). Compare with (Figure 3, Plate VII). Exudation of proteinaceous material into Bowman's space was noted and the basement membrane of Bowman's capsule was slightly thickened. There was a moderate degree of cortical interstitial cellular infiltrate observed. (Figure 3, Plate IX), but the medulla was the site of severe lymphocytic interstitial nephritis and attendant hemorrhage. These infiltrates were either scattered small foci, (Figure 4, Plate IX), or presented narrow linear bands extending from the medulla to the cortex. (Figure 3, Plate IX). Compare with (Figure 2, Plate VIII). The vasculature was not remarkable.
PLATE IX

Figure 1.—An enlarged view of the renal interstitial infiltrate showing predominance of pseudoeosinophiles among fibroblasts, macrophages and lymphocytes. Animal injected with ribosomes. x397.

Figure 2.—A beginning proliferative glomerulitis in an animal which received antiribosome serum and sacrificed five days after injection. Note the extensive endothelial and epithelial cell proliferation. The basement membrane of Bowman's capsule is slightly thickened. Note also the round cell infiltrate in the interstitial tissue. x125.

Figure 3.—Cortical lymphocytic infiltrate. Note the beginning glomerulitis characterized by glomerular proliferation. The tubules are separated by slight edema. x50.

Figure 4.—Focal lymphocytic infiltrate with attendant hemorrhage in medulla of rabbit injected with antiribosome serum. Sacrificed five days after injection. x125.
Gross inspection of kidneys from animals sacrificed 23 days after passive transfer revealed small, hard, pale organs and the capsules stripped with ease. Cut section revealed thinning of the cortex, but the pyramids and pelvis appeared normal.

Microscopically, a severe cortical and medullary interstitial nephritis and fibrosis was apparent by a massive overall tubular degeneration characterized by hyalinization and homogenation of the epithelial cells comprising the tubules, into amorphous eosinophilic masses which occluded the tubular lumens. (Figure 1, Plate X). In other instances, the epithelial cells were compressed against thickened basement membranes and their dark, pyknotic nuclei displaced from their cytoplasmas. Both tubular atrophy and hypertrophy were present amid severe fibrosis accompanied by an infiltrate of small dark round cells which surrounded and invaded the interstitial spaces and tubules leaving behind a jumbled mass of tubules and scarred glomeruli. Observed also was hyalinization and scarring of glomeruli by a process of periglomerular fibrosis. (Figure 2, Plate X). Compare with (Figure 3, Plate VIII). These non-functional glomeruli were seen in abundance and were characterized by thickened, fibrosed Bowman's capsules containing avascular, atrophic glomeruli. Tubular, interstitial, or capsular hemorrhages and exudation was not observed. Tubular casts were not evident in proximal, distal, or collecting tubules.

Examination of the vasculature revealed lesions limited to the small arteries and arterioles of the cortex and were most likely branches of the arcuate arteries and possibly the arteriae spuriae.
PLATE X

Figure 1.—Severe interstitial nephritis with associated tubular degeneration and pericapsular thickening and fibrosis. Note the scarred, contracted glomerulus. A round cell infiltrate is present. Animal injected with antiserum to ribosomes and sacrificed 23 days after injection. x125.

Figure 2.—Severe pericapsular fibrosis and hyalinization. A degenerate, contracted, non-functional glomerulus is contained with the dilated capsular space. Animal injected with antiserum to ribosomes and sacrificed 23 days after injection. x312.

Figure 3.—A small cortical artery demonstrating a fibrinoid change in the media associated with a severe adventitial edema. Note the pronounced intimal proliferation. Animal injected with antiserum to ribosomes and sacrificed 23 days after injection. x312.

Figure 4.—A severe cortical interstitial nephritis. Note the marked hypertrophy of the tubules and dense interstitial fibrosis and infiltrate, from an animal injected with antiserum to ribosomes and sacrificed 44 days after injection. x125.
The lesions were characterized by medial edema, which separated the smooth muscle fibers, and by a fibrinoid change in the media. Intimal proliferation was present, characterized by the swollen hyperbasophilic nuclei of the endothelial cells, (Figure 3, Plate X). The adventitia was relatively unaffected. Intense perivascular edema, characterized by wide, clear zones in the interstitial connective tissue surrounding the vessels was observed in both cortex and medulla, (Figure 3, Plate X). The above vascular pathology was not noted in animals which received ribosomes.

Animals sacrificed 44 days after passive transfer presented small, hard kidneys, which upon cut section, revealed a thin cortical layer and normal pyramids and pelvis. The capsules stripped with ease.

Microscopically, severe interstitial nephritis and fibrosis which resulted in gradual tubular atrophy and/or hypertrophy was present. Very broad bands of tubular degeneration and fibrosis which arose in the medulla and ascended by way of the medullary rays, reached to the capsule, (Figure 4, Plate X). Normal glomeruli were the exception and not the rule, as most of them presented a picture of hyalinization and scarring by a process of periglomerular fibrosis and capsular thickening, (Figure 1, Plate XI). The majority of the glomeruli appeared as shown in (Figure 2, Plate XI), in which pericapsular thickening and fibrosis were prominent and the basement membrane of Bowman's capsule was thickened. Compare with (Figures 1 and 2, Plate X). The glomerulus was contracted and avascular and the capillaries presented slightly thickened basement membranes. Glomerular adhesions have attached
PLATE XI

Figure 1.—Periglomerular fibrosis and hyalinization with an associated tubular hypertrophy and degeneration in kidney of a rabbit injected with antiserum to ribosomes and sacrificed 44 days after injection. x125.

Figure 2.—An ischemic, degenerate glomerulus which demonstrates focal adhesions to the thickened capsule wall. The upper portion of the glomerular tuft shows evidence of degenerating epithelial cells. The tubules are hypertrophied, their basement membranes are thickened and the epithelium has desquamated. From a kidney of an animal injected with antiserum to ribosomes. x312.

Figure 3.—Broad bands of an interstitial infiltrate and fibrosis in the kidney from an animal injected with sRNA. Note the greatly dilated tubules and the thickened basement membranes of Bowman's capsules. x50.

Figure 4.—A beginning proliferative glomerulitis associated with slight exudation into Bowman's space in a kidney from an animal injected with sRNA. The tubules are within normal limits. x312.
themselves to a portion of Bowman's capsule and dense eosinophilic masses, considered to be degenerate epithelial cells, were noted within the glomerular capillary loops. This description fits the picture of focal membranous nephritis coexistent with interstitial nephritis. No abnormalities were noted concerning the collecting and ascending and descending loops of Henle. Examination of the vasculature revealed no remarkable findings.

Kidney—Rabbits Injected With eRNA

Microscopic examination revealed broad bands of interstitial nephritis and fibrosis which swept from the medullary portion toward the cortex by way of the medullary rays. (Figure 3, Plate XI). Compare with (Figure 2, Plate VIII and Figure 3, Plate IX). There was the usual tubular degeneration accompanied by atrophied and hypertrophied tubules which contained pale-staining material; a cellular infiltrate which consisted of plasma cells, fibroblasts, histiocytes and a large cell with little demonstrable cytoplasm but which contained a large vesicular nucleus. These latter cells may represent a degenerate cell resulting from anoxia.

Examination of the glomeruli revealed a spectrum of pathology which ranged from proliferative glomerulitis, (Figures 4, Plate XII, I, Plate XII). Compare with Figure 3, Plate VII, and Figure 3, Plate VIII). However, many normal glomeruli were observed throughout the cortex. Most proximal and distal convoluted tubules appeared within normal limits except in those areas where interstitial nephritis had
wrought its damage. Upon closer inspection, however, many tubular epithelial cells were observed to contain hyaline droplets which stained intensely with eosin, (Figure 3, Plate XII). A partial fibrinoid change of the media of small arteries accompanied by slight intimal activation and perivascular edema, were the only demonstrable histologic changes of the vasculature. Engorged lymphatics filled with pale-staining material were observed over most of the cortex and medulla, (Figure 1, Plate XII). Compare with (Figure 3, Plate X).

Kidney—Rabbits Injected With Antiserum To eRNA

Microscopic examination revealed an interstitial nephritis accompanied by fibrosis equal in severity as to the kidneys just described. The usual characteristics of interstitial nephritis were noted---tubular degeneration and atrophy, interstitial fibrosis, round cell infiltrate, periglomerular hyalinization and fibrosis, and thickened Bowman's capsules which contained avascular, non-functional glomeruli, (Figure 4, Plate XII). Compare with (Figure 3, Plate XI). Glomerular morphology ranged from those that appeared normal in structure to those which revealed a proliferative glomerulitis, accompanied by exudation into Bowman's space, (Figure 1, Plate XIII). Compare with (Figure 3, Plate VII; Figure 2, Plate IX; Figure 4, Plate XI). The glomerulus depicted in (Figure 2, Plate XIII), appears quite bloodless and the endothelial and epithelial cells are smudged and indistinct, although the capillary basement membranes are within normal limits. An exudate is apparent at the top of the glomerulus and a focal adhesion to the
PLATE XII

**Figure 1.**—A proliferative glomerulitis in kidney of rabbit injected with sRNA. The tubular structures are within normal limits, but the small artery demonstrates a slight edematous process in the adventitia. x125.

**Figure 2.**—Thickened, dilated Bowman's capsules, each containing an ischemic glomerulus and pale-staining exudate. Note the beginning tubular epithelial cell desquamation and degeneration. Rabbit injected with sRNA. x500.

**Figure 3.**—Hyaline droplet degeneration in tubular epithelium of rabbit injected with sRNA. x500.

**Figure 4.**—A severe cortical inflammatory infiltrate and fibrosis in kidney of rabbit injected with antiserum to sRNA. Note the degenerate tubules surrounding the inflammatory process. x50.
basement membrane of Bowman's capsule may be seen at this point. This is a picture of subacute glomerulonephritis superimposed upon a diffuse membranous nephritis of early origin. Note the relatively unaffected proximal and distal convoluted tubules.

Figure 3, Plate XIII demonstrates the identical vascular pathology as noted in sRNA-injected animals, namely, beginning fibrinoid change and edema in the medial coat. Compare with (Figure 3, Plate X, and Figure 1, Plate XII). Also shown is a severe intimal edema accompanied by an activation and proliferation as evidenced by the presence of large block-like cells lining the lumen. There appears to be considerable edema within the adventitia also. The internal elastic lamina appears to be somewhat thickened, although it is difficult to determine whether it has sustained a hyaline change. Examination of large arteries and veins disclosed no remarkable findings. Capillary hyalination and endothelial activation were observed throughout the cortex and medullary zones.

Occasional portions of either distal or proximal convoluted were observed undergoing partial calcification.

Kidney—Rabbits Injected With Ribosomal Protein

The most outstanding lesion observed in sections of these kidneys was a severe interstitial nephritis located predominately in the cortex, but in certain areas it dipped into the medulla, (Figure 4, Plate XIII). The tubules were severely affected as they were either atrophied or hypertrophied and presented necrotic, desquamated, epithelial
Figure 1.—A proliferative glomerulitis with associated thickening of the basement membrane of Bowman's capsule. A slight exudation into Bowman's space is noted. The tubules present necrotic epithelium. Rabbit injected with antiserum to sRNA. x312.

Figure 2.—A subacute glomerulonephritis super-imposed upon an membranous nephritis. Note the smudged appearance of the necrotic capillary endothelial and epithelial cells. Exudate appears in the capsular space. Slight cloudy swelling swelling of the tubular epithelium is evident. Rabbit injected with antiserum to sRNA. x312.

Figure 3.—A small cortical artery in kidney of animal which received antiserum to sRNA, demonstrating severe edematous changes in the media and intima. Note the presence of activated endothelial cells lining the intima. x500.

Figure 4.—A view of the cortex from the kidney of an animal which received riboprotein. Note the hypertrophied tubules, many of which have lost their epithelium. Note also the dilated glomerular capsules. x50.
cells. Glomerular histologic changes ranged from those that appeared
to be within normal limits to those that demonstrated focal adhesions,
and in which proliferative changes were not observed, (Figure 1,
Plate XIV). Compare with (Figure 2, Plate IX). Hemorrhage and/or
exudate were not observed in either the capsular spaces or tubules.
The vasculature was not remarkable. A few lesions, characterized by
broad, eosinophilic bands of coagulation necrosis and which lacked an
accompanying cellular infiltrate, were observed at the cortico-
medullary junction. These probably represented old, focal infarcts,
(Figure 2, Plate XIV).

Kidney—Rabbits Injected With Antiserum To Ribosomal Protein

Microscopic examination revealed an advanced degree of severe
interstitial nephritis with concomitant glomerular histologic change.
Dense bands of cellular infiltrate composed of small, dark, round cells,
plasma cells, and fibroblasts were observed admixed with dense colla-
genous bands of fibrosis. The tubules were either atrophic or hyper-
trophied and exhibited necrotic, flattened epithelial cells many of
which had desquamated. A suggestion of fluid within the tubules was
demonstrated by the presence of a pale-staining material within their
lumens, (Figure 3, Plate XIV). Figure 4, Plate XIV, demonstrates a
higher magnification of the fibrotic process and cellular infiltrate.
Note the increased interstitial connective tissue and thickening of
the tubular basement membranes. None of the tubules exhibit normal
tubular epithelium.
PLATE XIV

**Figure 1.**—A relatively normal, though ischemic glomerulus which demonstrates focal adhesions between it and the capsule basement membrane. The surrounding tubules are relatively normal. From a rabbit injected with riboprotein. x125.

**Figure 2.**—In the center of this figure is an area of coagulation necrosis which presumably resulted from an old infarction. Note the accompanying inflammatory infiltrate and tubular hypertrophy. From kidney of a rabbit injected with riboprotein. x50.

**Figure 3.**—A severe cortical inflammatory infiltrate and fibrosis associated with atrophic and hypertrophied tubules. Pericapsular thickening and fibrosis is evident also. Rabbit injected with antiserum to riboprotein. x50.

**Figure 4.**—A portion of severe cortical interstitial nephritis from kidney of rabbit injected with antiserum to riboprotein. Thickened tubular basement membranes which demonstrate necrotic, desquamated epithelium are completely surrounded by increased interstitial fibrosis and accompanying cellular infiltrate. x125.
Although normal glomeruli were observed, much of the cortex was filled with degenerate, non-functional, ischemic glomerular structures as demonstrated in (Figure 1, Plate XV). Note the pale-staining material within Bowman's space and the degenerate condition of the surrounding tubules. Figure 2, Plate XV, demonstrates much the same pathology, upon which an additional glomerular insult in the form of adhesions and degenerating epithelial cells is superimposed.

Study of the vasculature produced no remarkable findings. A slight degree of calcification in a proximal convoluted tubule and a low grade, round cell medullary infiltrate associated with interstitial fibrosis were the only other noteworthy histologic changes.

**Kidney—Rabbits Injected With Nuclei**

Microscopic examination revealed a paucity of histologic change. The tubules appeared within normal limits and interstitial fibrosis and infiltrate were absent. The vasculature appeared normal in all respects. The majority of the glomeruli were normal in all respects but on occasion a few exhibit changes which resembled acute exudative glomerulonephritis characterized by exudation of albuminous material into the capsular space and tubular lumens in association with the presence of leucocytes within the glomerular capillaries. (Figure 3, Plate XV).
PLATE XV

Figure 1.—A glomerulus from a kidney of a rabbit injected with antiserum to riboprotein, which demonstrates a complete degenerative change of the endothelial and epithelial cells, together with severe tubular necrosis. Note the presence of fluid within the contracted capsular space. x312.

Figure 2.—A contracted, ischemic glomerulus showing two early synechiae which are forming between the tuft and Bowman’s capsule. Note the complete loss of tubular epithelium and thickened tubular basement membranes. Rabbit injected with antiserum to riboprotein. x312.

Figure 3.—A typical glomerulus in an area of interstitial nephritis in the kidney of a rabbit injected with nuclei. The glomerulus is ischemic and presents degenerate endothelium and epithelium. Small globular droplets of possible albuminous origin lie with the capsular space. The tubules present an atrophic necrotic epithelium, some of which has desquamated and fills the tubular lumens. x312.

Figure 4.—A slightly degenerate glomerulus from a kidney of a rabbit which received antiserum to riboprotein. Most of the glomeruli in this series of animals appeared as shown. x312.
Kidney—Rabbits Injected With Antiserum To Nuclei

These kidneys were totally without lesions except for a slight calcification of one or two proximal convoluted tubules, and what might be considered a very slight proliferative change noted in a very few glomeruli, (Figure 4, Plate XV). Compare with (Figure 3, Plate VII).

Liver—Rabbits Injected With Ribosomes

Grossly, the livers appeared pale and exhibited a light-brown color with a faint reddish cast, and they did not appear to be enlarged. The lobule edges were sharp and slightly scalloped. The capsule was not thickened and the surface was smooth and glistening. Cut section revealed a firm homogenous matrix which was not easily scraped off with a knife.

Microscopic examination revealed varying degrees of parenchymal injury which ranged from small pinpoint areas of focal hepatic cell necrosis, (Figure 1, Plate XVI), in which a few cells had experienced complete cytoplasmic degeneration, to complete hepatocellular vacuolization accompanied by total precipitation of the cell cytoplasm and dissolution of cell membranes, (Figure 2, Plate XVI). The focal necrotic lesions were not related to the portal triads or vasculature but were scattered over the entire parenchyma in a random fashion. Each lobule was affected equally both qualitatively and quantitatively over its entirety as regards the hepatocellular vacuolization, as preferential centrilobular, midzonal or peripheral distribution was not observed.
PLATE XVI

Figure 1.—Pinpoint areas of focal hepatocellular necrosis in the liver of an animal injected with ribosomes, characterized by cell necrosis and loss of the cytoplasmic membranes. The nuclei appear relatively unaffected. x312.

Figure 2.—Hepatocellular vacuolization characterized by precipitation and/or loss of cell cytoplasm. A few nuclei appear pyknotic. From the liver of an animal injected with ribosomes. x312.

Figure 3.—An interlobular hepatocellular infiltrate composed of plasma cells, lymphocytes and fibroblasts. Note how the infiltrate extends between the biliary cords and which completely destroys the hepatic parenchyma. Animal injected with ribosomes. x125.

Figure 4.—A perivascular inflammatory infiltrate comprised of plasma cells, lymphocytes, and macrophages situated around an interlobular vein. Animal injected with ribosomes. x100.
A lesion common to all animals of this group was a perilobular hepatitis plus an interlobular hepatocellular degeneration accompanied by an inflammatory process. Figure 3, Plate XVI demonstrates this interlobular hepatocellular degeneration characterized by an inflammatory process which consisted of small dark round cells invading the sinusoids and surrounding the hepatic cells, causing them to degenerate and finally disappear in the fibrotic process accompanying the infiltrate. Figure 4, Plate XVI, shows the type of inflammatory reaction and infiltrate observed around thin-walled veins, the periportal and perilobular connective tissue. It consists of plasma cells, fibroblasts, lymphocytes, and a few cells present with dense chromatin patterns and dark, homogeneous, non-granular eosinophilic cytoplasm, the latter cell resembling those observed in the renal interstitial inflammation. Examination of the vasculature and biliary tree was not remarkable.

Liver—Rabbits Injected With Antiserum To Ribosomes

Microscopic examination of the livers of animals sacrificed five days after receiving antiribosome serum, revealed moderate passive hyperemia and hepatic cell lesions which appeared not unlike post mortem changes, (Figure 1, Plate XVII), and characterized by cloudy swelling and wide edematous sinusoids which separated the hepatic cords. The hepatic cell nuclei appeared relatively unaffected. Compare with hepatic parenchyma of rabbits injected with ribosomes, (Figure 2, Plate XVI). A few nests of small dark round cells were observed scattered here and
there throughout the lobule and with no apparent preference for vascular of secretory structures. (Figure 1, Plate XVII).

An inflammatory infiltrate comprised of a few plasma cells, lymphocytes, macrophages, and fibroblasts and associated with early fibrogenesis, which led to the formation of newly formed connective tissue, (Figure 2, Plate XVII), was observed around the periportal areas, and which infiltrated into the interlobular connective tissue spaces. Compare with (Figure 3, Plate XVI). A perivenous inflammatory response, which consisted of plasma cells, fibroblasts, lymphocytes, and a few macrophages, was observed infiltrating the surrounding hepatic parenchyma, (Figure 3, Plate XVII).

As sacrifice times increased to 23 and 44 days after injection of serum, so did hepatic cell vacuolization increase in severity. Figure 4, Plate XVII, depicts this process at 23 days and (Figure 1, Plate XVIII) at 44 days. An additional lesion observed in the latter group (44 day), was the presence of nests of small dark round cells, which seemed to arise from the scant sinusoidal connective tissue, (Figure 1, Plate XVIII). Compare with (Figures 1, 3 Plate XVII). The hepatic parenchyma in the immediate vicinity appeared to be degenerate. Perilobular and interlobular fibrosis, together with an inflammatory response, were increased in severity in animals sacrificed 23 and 44 days after the injection of serum, which by this time, consisted almost entirely of proliferating fibroblasts and dense bundles of collagen packed closely together. Examination of the portal triads and vasculature revealed no remarkable findings.
Figure 1.—Parenchymal changes in the liver of an animal injected with antiserum to ribosomes and sacrificed five days after injection. Cloudy swelling of the hepatic cells and interstitial edema are prominent features. Note the presence of a nest of small, dark, round cells. x125.

Figure 2.—Perilobular inflammatory infiltrate comprised of plasma cells and lymphocytes. Early fibrogenesis associated with newly formed connective tissue is evident. Hepatocellular necrosis bordering the infiltrate is also displayed. Animal injected with antiserum to ribosomes and sacrificed five days after injection. x125.

Figure 3.—A perivenous inflammatory infiltrate composed of plasma cells, lymphocytes, and macrophages. Note destruction of hepatic parenchyma bordering this lesion. Rabbit injected with antiserum to ribosomes and sacrificed five days after injection. x312.

Figure 4.—A severe hepatocellular vacuolization in an animal which received antiserum to ribosomes and sacrificed 23 days after injection. x125.
Liver—Rabbits Injected With eRNA

Microscopic examination revealed a slight to moderate periportal and interlobular fibrosis and associated inflammatory process with heavy extension into the parenchyma. There was a concentrated band of fibrosis and giant cell formation around some, but not all, of the central veins, (Figure 2, Plate XVIII). Parenchymal lesions ranged from a wide-spread hepatic cell necrosis characterized by cloudy swelling, hyaline droplet formation, pyknosis, and karyorrhexis of cell nuclei, (Figure 3, Plate XVIII), to an associated total hepato-cellular vacuolization, (Figure 4, Plate XVIII). A moderate degree of perivascular inflammatory reaction was observed which infiltrated the surrounding parenchyma and destroyed the hepatic cells with which the infiltrate came in contact. A peculiarity of these inflammatory foci was the formation of an "adenoma-like" circlet of large oval-shaped cells containing vesicular nuclei and no visible cytoplasm, (Figure 1, Plate XIX). Compare this lesion with those in (Figures 3, Plate XVII, and 1, Plate XVIII). Speculations as to what these cells are will be given in the discussion. Nothing remarkable was demonstrated by the vasculature and portal triads.

Liver—Rabbits Injected With Antiserum To eRNA

Microscopic examination revealed considerable parenchymal injury in the form of hepatic cell vacuolization as described for all animals thus far examined, although vacuolization was not as extensive as that
exhibited by the animals previously described, (Figure 2, Plate XIX). Periportal, perilobular, and interlobular fibrosis was minimal or non-existent, and when present, was accompanied by a low grade inflammatory process which consisted of the usual cellular components. Especially noticeable were islands of foci of inflammatory cells which seemed to arise from the sinusoidal connective tissue, and which were scattered randomly over the entire parenchyma with no association or preference for either vasculature or biliary tree. The cellular infiltrate was composed of small dark round cells characterized by dense chromatin and scanty cytoplasm, a few lymphocytes, and large cells associated with dense nuclear chromatin patterns and non-granular, eosinophilic cytoplasm. These latter cells were identical to those observed in the liver and kidney of animals injected with ribosomes and antiribosome rabbit serum. Hepatic cells caught up in this inflammatory process were either degenerate and necrotic or were entirely missing from the centers of the cellular mass, (Figure 3, Plate XIX). Compare with (Figures 1, 3, Plate XVII, and Figure 1, Plate XVIII). In the liver of one animal, widespread activation and phagocytosis by Kupffer cells lining the sinusoids was observed, (Figure 4, Plate XIX). The material being phagocytized can only be surmised, but probably represented hemosiderin crystals from hemolyzed erythrocytes. Examination of the vasculature and biliary tree was not remarkable.
Figure 1.—Hepatocellular vacuolization. A nest of small, dark, round cells is shown apparently arising from the sinusoidal connective tissue. A structure which appears to be a giant cell can be seen within the center of the lesion. Rabbit injected with antiserum to ribosomes and sacrificed 44 days after injection. x125.

Figure 2.—An inflammatory infiltrate and fibrosis associated with giant cell formation around a central vein from liver of an animal injected with sRNA. x50.

Figure 3.—Severe hepatocellular degeneration and necrosis characterized by cloudy swelling and necrotic changes in the hepatic parenchyma. Note also karyolysis and pyknosis of the hepatic cell nuclei. A dilated lymphatic, containing pale-staining fluid is seen on the left. Rabbit injected with sRNA. x125.

Figure 4.—Severe hepatocellular vacuolization in the liver from a rabbit injected with sRNA. x125.
PLATE XIX

Figure 1.—A perivenous inflammatory infiltrate. Its center contains a circlet of oval-shaped cells which might possibly represent bile ductal proliferation. Rabbit injected with sRNA. x125.

Figure 2.—Hepatocellular vacuolization from liver of an animal injected with antiserum to sRNA. x125.

Figure 3.—An island of round cell infiltrate arising from the sinusoidal connective tissue. The cells comprising the infiltrate appear to be lymphocytic. Note the invasion and destruction of the surrounding hepatic cells. Animal injected with antiserum to sRNA. x312.

Figure 4.—An activated Kupffer cell containing phagocytized material from the liver of an animal which received antiserum to sRNA. Phagocytosis by these littoral cells was quite common in the livers of animals injected with ribosomes, sRNA or the antisera to these antigens. x312.
Liver—Rabbits Injected With Riboprotein

Microscopic examination revealed no evidence of fibrosis or inflammatory infiltrate. The portal triads and vasculature were within normal limits and presented no remarkable findings, (Figure 1, Plate XX). The normal picture was, however, disturbed by the presence of a severe hepatocellular vacuolization, (Figure 2, Plate XX).

Liver—Rabbits Injected With Antiserum To Riboprotein

There was no evidence of periportal or perilobular inflammatory infiltrate, (Figure 3, Plate XX). The lobular architecture was within normal limits except for hepatocellular vacuolization, (Figure 4, Plate XX). An edematous process separated the hepatic cords and dilated the lymphatics. The portal triads, vasculature, and biliary tree presented no remarkable findings.

Liver—Rabbits Injected With Nuclei

Microscopic examination presented severe hepatocellular vacuolization; the nuclei appeared unaffected, (Figure 1, Plate XXI). No zonal pattern of this cellular injury was observed as the entire lobule was equally affected, as were its neighbors. Severe periportal, interlobular, and perilobular fibrosis were observed, and was especially heavy perivascularly, (Figure 2, Plate XXI), in which masses of small dark round cells, plasma cells and fibroblasts invaded and engulfed the surrounding hepatic cells, many of which were observed to have been totally destroyed by the advancing infiltrate. Compare with
Figure 1.—An overall view of the hepatic parenchyma from an animal injected with riboprotein. Note the lack of an infiltrate or fibrosis. x50.

Figure 2.—A higher magnification of Figure 1, Plate XX, demonstrating severe hepatocellular vacuolization. x312.

Figure 3.—An overall view of the hepatic parenchyma from an animal injected with antiserum to riboprotein. An inflammatory infiltrate and fibrosis are absent. x50.

Figure 4.—A higher magnification of Figure 3, Plate XX, which demonstrates hepatocellular vacuolization. x312.
(Figure 4, Plate XVI). One other animal in this series presented only severe hepatocellular vacuolization and no other noteworthy histologic changes were observed. Except for a moderate periportal fibrosis, the triads presented no other change. The liver was not congested nor edematous and many pseudoeosinophiles were observed lying in the sinusoids.

Liver—Rabbits Injected With Antiserum To Nuclei

Histologic changes in the livers of animals in this group were almost non-existent. Figure 3, Plate XXI depicts a low power view which demonstrates an almost normal hepatic architecture as fibrosis and infiltrate were absent. The biliary tree and vasculature presented no remarkable changes. Only hepatocellular vacuolization of moderate severity remained to present the only noteworthy hepatic lesion. (Figure 4, Plate XXI). The nuclei remained unaffected.

Lungs—Rabbits Injected With Ribosomes

Gross inspection revealed two normal-appearing lungs lying free in their respective pleural cavities and from which about ten ml of clear, straw-colored fluid was obtained. The color was grayish-pink and the lung substance resilient to the touch. Cut sections, however, yielded an abundance of clear, frothy fluid which exuded from the cut surfaces whenever the lungs were compressed. Consolidation was not evident and the pleura was not remarkable.
Figure 1.—Hepatocellular vacuolization in the liver from a rabbit injected with nuclei. The hepatic cell nuclei appear relatively unaffected. x312.

Figure 2.—An inflammatory infiltrate surrounding an interlobular vein. The infiltrate and fibrosis have separated small islands of apparently normal hepatic cells from those which have degenerated. Animal injected with nuclei. x125.

Figure 3.—An overall view of the hepatic parenchyma from an animal which received antiserum to nuclei. Note the absence of perilobular and periportal infiltrate and fibrosis. x50.

Figure 4.—Severe hepatocellular vacuolization in the liver from an animal which received antiserum to a nuclei. x125.
Upon microscopic examination it was noted that almost all sections of lung were identical in appearance histologically. The most striking lesions were confined to the parenchyma, i.e., the alveolar septa, in which was revealed a severe interstitial inflammatory response characterized by greatly thickened septa, which gave the lung an atelectatic representation, (Figure 1, Plate XXII). The inflammatory exudate consisted of wandering macrophages, polymorphs, and septal cells separated by fibrosis and an abundance of edema fluid, (Figure 2, Plate XXII). The entire parenchyma was affected by this process and, in addition, was associated with a severe granulomatous reaction characterized by the presence of multiple, discrete, randomly distributed epithelioid granulomata, (Figure 3, Plate XXII), which were non-caseating nodules, the main cellular component of which was a macrophage, demonstrating epithelioid cell characteristics and an occasional giant cell of the Langerhans type. A peribronchiolar lymphocytic infiltrate, comprised of small dark round cells, which surrounded and gradually infiltrated the surrounding parenchyma or, as is shown in (Figure 4, Plate XXII), infiltrated the smooth muscle wall of the bronchiole. Prominent also was an increased activation of the germinal centers of the lymphoid nodules which accompanied the bronchioles, (Figure 1, Plate XXII). Although septal thickening and the inflammatory response were severe, no discernable septal cell desquamation was observed.

Examination of the vasculature revealed a severe edematous process associated with complete disruption of the media of some, but not all, of the peribronchiolar arteries, (Figure 1, Plate XXIII), in which the
Figure 1.—An interstitial inflammatory infiltrate characterized by thickened alveolar septa, giving the lung an atelectatic picture. The bronchiole contains blood within its lumen. The accompanying bronchiolar artery is normal in all respects. Rabbit injected with ribosomes. xl25.

Figure 2.—An enlarged view of the inflammatory cells within a thickened alveolar septa. A fibrinous exudate and edema separates the cellular elements, most of which are macrophages. Rabbit injected with ribosomes. x500.

Figure 3.—Non-caseating epithelioid granulomata in the lung from an animal injected with ribosomes. Note the central core filled with macrophages demonstrating epithelioid characteristics. A ring of small lymphocytes surrounds each granuloma. xl25.

Figure 4.—A peribronchiolar lymphocytic infiltrate is shown partially invading the smooth muscle wall of the bronchiolus, the epithelium of which is denuded. Rabbit injected with ribosomes. xl25.
necrotic wall was almost completely breached by a degenerative process accompanied by macrophages, histiocytes, and polymorphonuclears. Intimal proliferation and fibrinoid change in the medial and adventitial coats were prominent also. The large branches of the pulmonary artery and vein were not remarkable except for the finding of a nest of polymorphonuclears located subintimally in one of the arteries, (Figure 2, Plate XXIII). Alveolar capillary hyalinization was observed in an occasional thickened alveolar wall.

The pleura presented neither thickening nor fibrosis and its accompanying lymphatics were not remarkable. Aside from peribronchial lymphoid hyperplasia and infiltrate by inflammatory cells into the walls of the bronchioles, and aspirated blood within their lumens, no serious histologic change was noted in the respiratory tree.

Lungs—Rabbits Injected With Antiserum To Ribosomes

Gross inspection revealed identical findings as described for the lungs of animals injected with ribosomes.

Microscopic examination of animals sacrificed five days after passive transfer revealed a severe interstitial inflammatory process characterized by thickened, collapsed, alveolar septa and septal wall infiltrate comprised of wandering macrophages, lymphocytes, septal cells, polymorphs and an occasional plasma cell, (Figure 3, Plate XXIII). Compare with (Figure 1, Plate XXII). The alveolar capillaries and bronchiolar arteries were filled with fresh hemorrhage and presented a passive hyperemia. Mucus plugs filled the lumens of bronchioles and
PLATE XXIII

Figure 1.—A peribronchiolar artery from the lung of an animal injected with ribosomes. A portion of the media is completely necrotic; the degenerative process almost breaching the wall and reaching the lumen. The remainder of the media is extremely edematous. Note the activated endothelium lining the intima. x500.

Figure 2.—A nest of pseudoeosonophiles located just beneath the intima of a large branch of the pulmonary artery. This situation was not seen in animals which received antiserum to ribosomes. x500.

Figure 3.—A severe interstitial pneumonia in the lung from a rabbit injected with antiserum to ribosomes and sacrificed five days after injection. The bronchioles contain blood or mucous plugs and are surrounded by a lymphocytic infiltrate. x125.

Figure 4.—A lymphoid hyperplasia surrounding a respiratory bronchiole. Note the desquamated epithelial debris within the lumen. Rabbit injected with antiserum to ribosomes and sacrificed five days after injection. x312.
a slight lymphocytic infiltrate was observed perivascularly, (Figure 3, Plate XXIII).

Associated with the alveolar wall inflammatory process was a pronounced peribronchiolar lymphoid hyperplasia (Figures 4 and 1, Plates XXIII and XXIV). Note how, in (Figure 1, Plate XXIV), the lymphocytic infiltrate has invaded and breached the muscular wall of this bronchiole. Note also the thick, cord-like structure of the alveolar septa. The walls of most small peribronchiolar arteries were extremely edematous, the tissue and cellular components widely separated by fluid-filled spaces. Moderate intimal activation was present. (Figure 2, Plate XXIV). Compare with animal injected with ribosomes, (Figure 1, Plate XXIII).

Lungs of animals sacrificed 23 days after passive transfer showed identical histologic findings with the exception of less severe vascular changes. However, old widespread hemorrhage was present, which completely filled the alveolar spaces with destroyed erythrocytes and edema. Septal cells filled with pigment were observed everywhere. Only a hint of the presence of erst-while septal walls was provided by a few dark basophilic cells which lay about in random disorder, (Figure 3, Plate XXIV).

An overall increase in the amount of fibrosis, interstitial inflammation, capillary congestion, septal thickening, and peribronchiolar lymphoid hyperplasia was observed in animals sacrificed 44 days after passive transfer, (Figures 4, Plate XXIV and 1, Plate XXV). Compare with (Figures 2, 3, Plate XXII), respectively. Note the massive
PLATE XXIV

Figure 1.—A severe peribronchiolar lymphoid hyperplasia and infiltrate shown invading the muscular wall. A few cells appear within the lumen, accompanied by mucous plugs. Rabbit injected with antiserum to ribosomes and sacrificed five days after injection. x125.

Figure 2.—The muscular wall of this peribronchiolar artery presents a severe edematous change associated with an area of fibrinoid necrosis. The internal elastic lamina is thickened and tortuous. Nothing remains of the intima save a few endothelial cells lining the lumen. Rabbit injected with antiserum to ribosomes and sacrificed five days after injection. x500.

Figure 3.—A view of a lung from an animal injected with antiserum to ribosomes and sacrificed 23 days after injection. The presence of old fresh intra-alveolar hemorrhage associated with phagocytizing macrophages, demonstrates what might be considered a stage of pneumonic consolidation. Edema fluid fills a few of the alveolar spaces. x125.

Figure 4.—A portion of the septal wall inflammatory infiltrate associated with a dense fibrinous exudate. This is the cause of septal thickening. Rabbit injected with antiserum to ribosomes and sacrificed 44 days after injection. x500.
PLATE XXV

Figure 1.—A massive peribronchiolar lymphocytic infiltrate which has completely breached the bronchiolar wall, and cells appear within the lumen. Rabbit injected with antiribosome serum. Sacrificed 44 days after injection. x125.

Figure 2.—Fibrinoid change accompanied by edema in the wall of a respiratory bronchiole. Note blood within the lumen. A septal wall inflammatory infiltrate surrounds the bronchiole. Animal injected with antiserum to ribosomes and sacrificed 44 days after injection. x500.

Figure 3.—Moderate intra-alveolar hemorrhage in the lung of an animal injected with sRNA. Septal wall thickening and inflammatory infiltrate are minimal. x50.

Figure 4.—Severe septal thickening associated with an interstitial inflammatory infiltrate. The clear spaces represent what is left of the alveolar structures. Rabbit injected with sRNA. x50.
lymphocytic invasion of the wall of a bronchiolus at the left of the figure. Accompanying the histologic changes just described was a fibrinoid change noted in a few of the respiratory bronchioles, (Figure 2, Plate XXV), a finding not observed in lungs of animals sacrificed at five and 23 days. Bronchiolar changes other than those just noted were not evident except for the presence of mucous plugs and aspirated blood within their lumens.

Lungs—Rabbits Injected With sRNA

Parenchymatous changes ranged from areas of lung which appeared relatively normal both structurally and functionally to those in which the alveolar spaces were filled with both old and fresh hemorrhage, (Figure 3, Plate XXV), as well as extremely thickened, cord-like septa associated with an abundant inflammatory reaction and fibrosis, (Figure 4, Plate XXV). Figure 1, Plate XXVI demonstrates a view of the cell population within a septal wall. Note the presence of fibroblasts, macrophages, polymorphs, and a debris-laden macrophage; all cellular elements are well separated by profuse edema. Compare with (Figure 2, Plate XXII). These changes were noted in sections of lung taken from random sources and point to the fact that various regions of lung tissue were affected by varying degrees of injury. Most of the bronchioles did not exhibit any remarkable abnormalities, but an occasional few presented thickened laminae propriae associated with a proliferative epithelial cell response, (Figure 2, Plate XXVI). Epithelial cell necrosis and desquamation were not observed though aspirated blood was noted within the lumens. Peribronchiolar lymphoid
hyperplasia was present but was not nearly so severe as in animals injected with ribosomes and animals injected with antiribosome rabbit serum. (Figure 3, Plate XXVI). Compare with (Figure 4, Plate XXIII). Small bronchiolar arteries revealed a measurable degree of medial thickening and intimal activation, (Figure 2, Plate XXVI). Examination of the large branches of the pulmonary artery and veins and alveolar wall capillaries was not remarkable.

The terminal bronchioles revealed severe edematous changes in their muscular walls and mucous plugs in their lumens, (Figure 4, Plate XXVI). Compare with (Figure 2, Plate XXV). Figure 1, Plate XXVII, demonstrates what appeared to be a nematode cut in cross-section and lying within the lumen of a small artery which appeared normal in all respects. The internal structure of the object and its disassociation with the intimal walls made it unlikely that it was an organized thrombus.

No evidence of the severe granulomatous response as observed in animals injected with ribosomes was seen in the animals just described.

Lung—Rabbits Injected With Antiserum To eRNA

Alveolar wall thickening and fibrosis with associated inflammatory response was not nearly so severe as in the lungs of animals which received ribosomes and eRNA and those which were injected with antiribosome serum, but focal septal thickening randomly scattered over the parenchyma, was the usual finding, (Figure 2, Plate XXVII). Other areas presented normal parenchymal architecture and vasculature,
Figure 1.—An enlarged view of septal inflammatory infiltrate comprised of macrophages, some of which demonstrate phagocytic activity. An abundant fibrinous exudate separates the cellular elements. Animal injected with sRNA. x500.

Figure 2.—A portion of an interstitial inflammatory infiltrate and septal wall thickening. The bronchiolus demonstrates a thickened lamina propria and necrotic material within the lumen. The accompanying artery is within normal limits. Rabbit injected with sRNA. x50.

Figure 3.—A peribronchiolar lymphoid hyperplasia. Note the presence of fluid within their lumens. Rabbit injected with sRNA. x312.

Figure 4.—An edematous change and fibrinoid necrosis in the wall of a respiratory bronchiole. Desquamated epithelial cells and fluid are shown within the lumen. Rabbit injected with sRNA. x500.
(Figure 3, Plate XXVII), although the artery shown in the figure does exhibit slight intimal activation and edema in the adventitial coat. A few larger peribronchiolar arteries revealed an extensive medial hypertrophy as evidenced by the thick muscular wall and pronounced intimal proliferation, the intima itself being thickened also, (Figure 4, Plate XXVII). Compare with (Figure 1, Plate XXIII, and Figure 2, Plate XXIV). The alveolar capillaries were not remarkable.

The bronchioles exhibited edematous walls and degenerate, desquamating epithelial cells which were observed lying free within their lumens, and associated with what appeared to be a purulent exudate, (Figure 1, Plate XXVIII). Also evident was a severe peribronchiolar lymphoid hyperplasia, which gradually thinned as it spread circumferentially from the bronchiolar wall, (Figure 1, Plate XXVIII). Evidence of pleural thickening and/or fibrosis was not observed; the pleural lymphatics were not remarkable.

The histologic changes observed in the lungs of rabbits injected with riboprotein and rat liver nuclei and rabbits injected with the antisera to these antigens will be described together, as the pathology presented in these organs was quite similar.

Figure 2, Plate XVIII demonstrates the changes as observed in lungs of animals actively immunized with either riboprotein or rat liver nuclei. A granulomatous response, characterized by non-caseating granulomas, and, which consisted of a core of epithelioid cells surrounded by a dense ring of lymphocytes, was observed scattered throughout the parenchyma. A peribronchiolar lymphoid hyperplasia was
Figure 1.—A cross section of what appears to be a nematode contained within the lumen of a small artery. From the lung of a rabbit injected with sRNA. x125.

Figure 2.—Parenchyma of lung from an animal injected with anti-serum to sRNA demonstrating focal septal thickening. x50.

Figure 3.—Moderate edematous change in the adventititia surrounding a small artery, associated with a slight intimal endothelial cell activation. The alveolar walls are relatively free of inflammatory infiltrate. Rabbit injected with antiserum to sRNA. x125.

Figure 4.—A large peribronchiolar artery demonstrating severe hypertrophy of the media. Intimal hypertrophy and proliferation is also pronounced. Rabbit injected with antiserum to sRNA. x125.
present, accompanied by a severe bronchiolitis characterized by lymphocytic infiltration of the bronchiolar smooth muscle and epithelium, edematous bronchiolar walls, and desquamation of necrotic epithelium into the lumens, (Figure 3, Plate XXVIII). Septal thickening, associated with an inflammatory infiltrate which consisted of mononuclear cells, polymorphonuclear cells, and edema was characteristic, (Figure 4, Plate XXVIII and Figure 1, Plate XXIX). Compare the character of this infiltrate with that observed in animals receiving ribosomes and antiribosome serum, (Figure 2, Plate XXII and Figure 4, Plate XXIV). Note the mitotic figure in (Figure 4, Plate XXVIII). Hemorrhage was absent and the pleura was not involved in any fibrosis or thickening.

Animals which had received antisera exhibited only minor histologic changes such as old intra-alveolar hemorrhage associated with the presence of large numbers of phagocytizing macrophages, (Figure 2, Plate XXIX). Septal thickening, associated with an inflammatory reaction was very slight or non-existent as much of the parenchyma presented normal alveolar architecture. Only in one animal which received rat liver nuclei antiserum, was a pleuritis, characterized by an organized connective tissue, demonstrable, (Figure 3, Plate XXIX). Note also in (Figure 3, Plate XXIX), the large emphysematous bullae lying just beneath the newly formed connective tissue.

Spleen—Rabbits Injected With Ribosomes and Antiribosome Serum

Because of certain common histologic features, rabbits receiving ribosomes and antiserum to them are discussed together.
Figure 1.—Peribronchiolar lymphoid hyperplasia. Note the necrotic, desquamated, bronchiolar epithelium lying within the lumen of the bronchus near the top of the figure. Purulent exudate fills the lumen of an accompanying bronchiole at left of figure. Rabbit injected with antiserum to sRNA. x125.

Figure 2.—This figure demonstrates the minimal septal thickening and infiltrate associated with granuloma formation as demonstrated in lungs of animals injected with either riboprotein or nuclei. x50.

Figure 3.—A severe bronchiolitis characterized by a chronic lymphocytic infiltrate which has invaded the muscularis and lining epithelium of a small bronchiolus. Note the desquamated, necrotic material within the lumen. This was a common finding in animals injected with riboprotein or nuclei. x50.

Figure 4.—An enlarged view of the septal infiltrate common to animals injected with riboprotein or nuclei. The majority of the cells present are either wandering macrophages or septal cells, separated by a fibrinous exudate. Note the mitotic figure. x500.
An extensive chronic inflammatory process associated with a moderate plasma cell response and lymphoid hyperplasia was present in animals injected with ribosomes, (Figure 4, Plate XXIX), and also present in animals sacrificed 44 days after passive transfer of antiribosomal serum, (Figure 1, Plate XXX). A reticulum-cell hyperplasia was present in both groups of animals, but was more prominent in animals sacrificed five days after serum injection, (Figure 2, Plate XXX). Also prominent in both groups of animals was severe medial edema, intimal proliferation and fibrinoid change in the adventitia of the central arteries, (Figure 3, Plate XXX). Other vascular injury consisted of arteriolosclerosis of small arterioles traversing the red pulp in the spleens of rabbits sacrificed five days after the injection of antiribosome rabbit serum. A peculiar perifollicular deposition of an amorphous, hyaline-like substance tentatively identified as a glycoprotein by PAS staining, was demonstrable in rabbits injected with ribosomes and sacrificed at 70-78 days, (Figure 4, Plate XXX), and in animals five days after injection of serum, (Figure 1, Plate XXI). It increased both circumferentially and in amount by the 23rd day after injection of serum, (Figure 2, Plate XXXI), and was substantially diminished in animals given serum 44 days before, (Figure 3, Plate XXXI). Another peculiar finding was the presence of what might be considered nuclear debris of some description interspersed among reticulum cells, plasma cells and the macrophages of the red pulp, (Figure 4, Plate XXXXI). This phenomenon was restricted to animals injected with ribosomes and those sacrificed 44 days after the injection of antiribosomal serum.
PLATE XXIX

Figure 1.—Alveolar wall inflammatory infiltrate comprised predominately of pseudoeosinophiles and exudate. Common to animals injected with riboprotein or nuclei. x500.

Figure 2.—The changes presented in this figure were common to animals injected with antiserum to riboprotein or nuclei. Note the old intralveolar hemorrhage characterized by the presence of macrophages, presumably containing hemosiderin crystals. The alveolar walls are relatively free from infiltrate and only slightly thickened. Septal cell desquamation is considerable, however. x125.

Figure 3.—A pleuritis composed of organized connective tissue. Especially prominent is the presence of two emphysematous bullae lying just beneath the organized layer. Rabbit injected with nuclei. x125.

Figure 4.—Spleen from an animal injected with ribosomes demonstrating follicular hypertrophy associated with an activated germinal center. The surrounding splenic cords are thickened and show evidence of a chronic inflammatory response. x50.
PLATE XXX

Figure 1.—Spleen from an animal injected with antiserum to ribosomes and sacrificed 44 days after injection. Chronic inflammatory response. x50.

Figure 2.—A reticulum cell hyperplasia from the spleen of an animal injected with antiserum to ribosomes and sacrificed five days after injection. x500.

Figure 3.—Small branch of the splenic artery which demonstrate severe medial edema and intimal proliferation. A fibrinoid change is noted in the adventitial sheath. Common to animals injected with ribosomes and antiserum to ribosomes. x125.

Figure 4.—A perifollicular deposition of an amorphous hyaline-like substance presumably a glycoprotein, and which possesses characteristics of amyloid. PAS positive. Rabbit injected with ribosomes. x125.
Many megakaryocytes were observed randomly distributed throughout the red pulp in both actively immunized animals and animals sacrificed 23 days after passive transfer, (Figure 1, Plate XXXII). An extensive capillary proliferation was present and an homogenous appearing exudative material filled the intercellular and intercapillary spaces. This material when stained with alcian-blue PAS exhibited a fibrinous network which may represent a condensation of the exudate, and, which apparently came from the numerous capillaries in these areas and not the central arteries which were absent in many of the follicles. Evidence of increased red cell destruction was demonstrated by the presence of an abundance of hemosiderin lying free within the sinusoids and wandering macrophages. Present also in most animals of both series was the presence of myeloid metaplasia, characterized by small foci of cells which resembled young, juvenile forms of the granulocytic series, distributed throughout the red pulp.

Spleen—Rabbits Injected With sRNA and Antiserum To sRNA

Animals injected with sRNA and those that were injected with anti-sRNA rabbit serum will be discussed together.

Microscopic examination revealed a moderate inflammatory response characterized by slight reticulum-cell hyperplasia and lymphoid follicular hyperplasia, (Figure 2, Plate XXXII), which was slightly more prominent in sRNA-injected animals. Moderate passive congestion was observed in both groups and was associated with widely dilated sinusoids. Signs of increased red cell destruction were not evident. A
PLATE XXXI

Figure 1.—Perifollicular deposition in spleen from a rabbit injected with antiserum to ribosomes. Sacrificed five days after injection. x125.

Figure 2.—Perifollicular deposition in spleen from a rabbit injected with antiserum to ribosomes. Sacrificed 23 days after injection. x125.

Figure 3.—Perifollicular deposition in spleen from an animal injected with antiserum to ribosomes. Sacrificed 44 days after injection. x125.

Figure 4.—Unidentified debris with a pronounced affinity for basophilic dye, and which possibly represents nuclear remnants. x500.
slight plasma cell response was demonstrable in the red pulp of animals of both series. Most central and red pulp arteries exhibited a pronounced intimal activation and hyaline sclerosis of the media and elastic lamina. (Figure 3, Plate XXXII). Compare with (Figure 3, Plate XXX). Megakaryocytes were observed in spleens of animals which received anti-sRNA rabbit serum. (Figure 4, Plate XXXII). The peculiar perifollicular deposition and capillary proliferation with associated exudate observed in ribosome-injected animals were not observed in the animals just described.

The histologic findings recorded in spleens of animals injected with riboprotein and rat liver nuclei, and animals injected with the antisera to these antigens, were so distinctly alike and yet so few that separate descriptions are not necessary. Those lesions which were present corresponded quantitatively and qualitatively to those present in rabbits injected with sRNA and anti-sRNA. One exception, though, was the absence of megakaryocytes in animals of this last group.

Studies of the Peripheral Blood

A striking feature observed in animals injected with ribosomes was the production of a severe autoimmune hemolytic anemia on or about 77-78 days post-injection. A comparable hemolytic anemia was observed in animals within six days after one intravenous injection of anti-ribosome rabbit serum. The anemia was characterized by hemoglobin values of 6.0 grams percent or lower, very low red cell counts, a leucopenia, hematocrit measurements of around 16 percent, a
PLATE XXXII

Figure 1.—A megakaryocyte situated among the cells comprising the splenic cords; a finding common to animals injected with ribosomes or antiserum to ribosomes. The presence of this cell is indicative of extramedullary erythropoiesis. x312.

Figure 2.—A follicular hyperplasia associated with a chronic inflammatory response; commonly observed in all animals examined. x50.

Figure 3.—A small splenic artery situated in the red pulp which demonstrates hyaline sclerosis and intimal activation. This finding was commonly observed in all animals examined. x312.

Figure 4.—A megakaryocyte in the spleen of an animal injected with antiserum to sRNA. x312.
reticulocytosis, and hemoglobinemia. Examination of blood smears revealed a picture of severe anemia characterized by red cell anisocytosis, poikilocytosis, polychromatophilia, target cells, and severe central pallor, (Figure 1, Plate XXXIII). Note the dividing basophilic erythroblast, a sure sign of increased erythropoietic activity on the part of the bone marrow. Another morphologic evidence of anemia is illustrated in (Figure 2, Plate XXXIII), in which an erythrocyte is depicted containing three variably-sized basophilic dots, the so-called Howell-Jolly bodies. Large histiocytes, displaying foamy, vacuolated cytoplasms which contained large azurophilic nuclei and which were associated with a finely-skeined chromatin pattern, were observed on or about 77 days post-injection in animals receiving antiribosome rabbit serum, of which time these cells gradually diminished and disappeared by the 10th day post-injection. Many of these histiocytes were engaged in erythrophagocytosis, (Figure 3, Plate XXXIII), or leucophagocytosis, (Figure 4, Plate XXXIII). Demonstrable also was a chronologic development of polymorphonuclear leucocyte degeneration characterized by a rounding-up and contraction of the nuclei into small, compact, pyknotic masses surrounded by lightly-staining, indistinct, eosinophilic granules. The phenomenon initiated in animals which received antiribosome rabbit serum on or about the 2nd day post-injection and progressively worsened until it culminated in leucocytic cytolysis on the 4th or 5th day with the dispersion of cytoplasmic granules and extrusion of nuclei, (Figure 1, Plate XXXIV), and eventual nucleophagocytosis, (Figure 2, Plate XXXIV).
FIGURE 1.—Peripheral blood from rabbit injected with antiserum to ribosomes and sacrificed five days after injection. Red cell anisocytosis, poikilocytosis, and central pallor are evident. Prominent also is a dividing basophilic erythroblast. These changes were also evident in animals injected with ribosomes and sacrificed 70 to 78 days later. x630.

FIGURE 2.—Peripheral blood from a rabbit injected with antiserum to ribosomes and sacrificed five days after injection. The erythrocyte at the left of the figure contains Howell-Jolly bodies. Note the presence of two large histiocytes, one of which demonstrates erythrophagocytosis. x312.

FIGURE 3.—A large primitive histiocyte containing three ingested erythrocytes. From the peripheral blood of an animal injected with antiserum to ribosomes and sacrificed five days after injection. These cells were also observed in animals injected with ribosomes. x450.

FIGURE 4.—A large histiocyte containing a polymorphonuclear leucocyte. Note the smudged, amorphous nuclei and cytoplasmic granules of the ingested cell. x450.
CONTROLS

Rabbits given one intramuscular injection of Freund's adjuvant (10 ml) and sacrificed 77-78 days later were utilized as controls. At no time during the interim between injection and sacrifice did these animals demonstrate the behavior or hematologic patterns described for those injected with ribosomes and antiribosome rabbit serum. Histologic examination of organ systems failed to demonstrate lesions comparable to the test animals with the lone exception of liver and lung. Only these two organs will be described; the remainder will be displayed as the normal histology for the particular organ system, so that comparisons may be made.

Liver

Extensive, but not severe, hepatocellular vacuolization and cytoplasmic precipitation were observed over most of the parenchyma examined. (Figure 3, Plate XXXIV). The process demonstrated identical histologic similarities with those of test animals. Infiltrates and fibrosis were not observed. The vasculature and biliary tree were within normal limits.

Lung

A moderate interstitial inflammatory reaction and septal wall thickening associated with a moderate granulomatous response which consisted of scattered, discrete, small, non-caseating granulomas comprised of a central core of epithelioid cells and surrounded by a narrow band
Figure 1.—Leucocyte cytolysis as demonstrated in the peripheral blood of an animal injected with antiserum to ribosomes and sacrificed five days after injection. Note the dark, pyknotic nuclei and the dispersion of cytoplasmic granules. A nucleus, lying free, can be seen in the lower portion of the figure. x630.

Figure 2.—Nucleophagocytosis as demonstrated by a large histiocytocyte. Note the presence of a small, pyknotic, globular object within the cell cytoplasm. From the peripheral blood of an animal injected with antiserum to ribosomes and sacrificed five days after injection. x630.

Figure 3.—Control rabbit injected with Freund's adjuvant alone. The livers were free of inflammatory infiltrate and fibrosis, but hepatocellular vacuolization is evident. x125.

Figure 4.—Control rabbit injected with Freund's adjuvant alone. A moderate alveolar wall thickening and associated inflammatory infiltrate is evident in the lungs. Present also are epithelioid granulomas. x50.
of small lymphocytes was observed, (Figure 4, Plate XXXIV). A mild bronchiolitis associated with a slight peribronchiolar lymphoid cell activation was demonstrated over portions of the parenchyma. The vasculature, lymphatics, and pleura were not remarkable.

Figure 1, Plate XXXV demonstrates a section of normal myocardium from an animal which had experienced weekly cardiac punctures for a total 12 weeks.

Figure 2, Plate XXXV exhibits a low-power view of a section of kidney, the histology of which is within normal limits.

Figure 3, Plate XXXV demonstrates a higher magnification of normal glomeruli.

Figure 4, Plate XXXV demonstrates an overall view of normal splenic red pulp and follicles.
Figure 1.—Control. Section of myocardium from a rabbit injected with Freund's adjuvant alone. The myocardium is normal in all respects. The animals had experienced weekly cardiac punctures for a period of 12 consecutive weeks. x125.

Figure 2.—Control. Section of renal cortex from a rabbit injected with Freund's adjuvant alone. The tubules and glomeruli are within normal limits. x50.

Figure 3.—Control. Section of renal cortex from an animal injected with Freund's adjuvant alone. The glomerular structures are not remarkable. x125.

Figure 4.—Control. Splenic section from an animal injected with Freund's adjuvant alone. A chronic inflammatory reaction is shown. Present also was a moderate follicular hyperplasia. x50.
DISCUSSION

Obviously, the results indicate that all animals injected with ribosomes, sRNA, ribosomal protein and nuclei, as well as those receiving antisera to these substances, displayed a diverse range of tissue changes and in certain aspects, an overall similarity was observed.

The serologic demonstration of autoantibodies to ribosomes and sRNA, together with the marked autoimmune hemolytic anemia and leukopenia previously reported in animals injected with ribosomes and anti-ribosome serum, indicated, at the outset, that at least some of the pathology noted by a general examination of the tissues of these animals, was due to an immunologic mechanism involving autoantibodies to RNA. Since some of these phenomena occurred in rabbits injected with sRNA from yeast, it was expected that these too were involved with a similar process and would show corresponding pathology.

However, as noted above, some of the lesions described in these animals were also present in rabbits receiving ribosomal protein and nuclei, although these animals did not develop the hematologic manifestations noted above even though autoantibody for nuclei was demonstrable in rabbits injected with nuclei and antibody to ribosome protein was present in animals given the protein. Furthermore, many of the lesions in these latter two groups were found in practically all
animals, even controls receiving Freund's adjuvant alone. These le-
sions are considered as non-specific, that is, unrelated to the
immunologic mechanism proposed.

The essential problem here, was the separation and characteri-
ization for those lesions considered to be the result of an immune gene-
sis, as distinct from those that were generally thought of as being
indigent to and quite commonly seen in normal, caged, domestic rabbits.
To this end, then, all of the changes encountered in the various organ
systems will be reviewed, and an attempt made to resolve this distinc-
tion.

Enlarged, pale, flabby hearts associated microscopically with
hypertrophied myocardial fibers separated by edema, areas of focal
necrosis with or without cellular infiltrate, subendocardial fibrosis,
pericarditis, and moderate vascular changes were noted. Examination of
kidneys revealed both normal and degenerate tubules, progressive
glomerular injury culminating in complete glomerular insufficiency.
Associated histologic changes as interstitial nephritis and pyelo-
nephritis were noted. Vascular changes were not prominent.

The lungs revealed moderate to severe interstitial inflammation,
alveolar septal thickening, and fibrosis associated with a peribron-
chiolar lymphoid hyperplasia and bronchiolitis. Associated vascular
changes ranged from minimal to severe. An organizing pleuritis was
evident in one animal. Associated exclusively with animals receiving
antigen incorporated in Freund's adjuvant was a moderate to severe
granulomatous response.

All livers examined revealed hepato-cellular vacuolization of
varying intensity. A moderate to severe periportal and perilloobular fibrosis accompanied by an inflammatory infiltrate was consistently observed in some, but not all animals. Nests of round cells which originated from sinusoidal connective tissue and associated possibly with hepato-cellular regeneration were observed only in animals which received antiribosome or anti-sRNA rabbit serum. The biliary and vascular trees were not remarkable. Infrequently observed were pinpoint areas of hepato-cellular necrosis without a coexistent inflammatory response.

The spleens were enlarged and congested and presented evidence of increased red cell destruction. The lymphoid follicles were moderately enlarged and activated and were especially characterized by a perifollicular deposition of an amorphous, hyaline-like substance. An associated reticulum cell hyperplasia, combined with a moderate plasma cell activation was noted. The vasculature presented extreme medial hyalinization and edematous changes. Focal areas of myeloid metaplasia associated with megakaryocytes were observed.

Peripheral blood examination revealed an attempt on the part of the bone marrow to compensate for the extreme anemia. Large histiocytes demonstrating erythro-, leuco-, and nucleophagocytosis, together with progressive leucocytic cytolyis was observed only in animals injected with ribosomes and those injected with antiribosome rabbit serum.

A critical study of the histologic changes observed in all the animals immunized with the various preparations, revealed a certain
similarity which involved certain lesions. These lesions appeared to be non-specific since they were present in animals having no other evidence of autoimmune disease such as those receiving ribosomes and sRNA. The latter statement is made with certain reservations, as these changes cannot be divorced easily from those to which an autoimmune etiology has been attached.

All animals except those which had received antiribosome rabbit serum and sacrificed five days later, revealed an hepatocellular vacuolization which ranged from moderate to severe in its extent. This lesion was not related to any specific zonal distribution, but occurred evenly over the entire lobule, each lobule appearing quite like its neighbor. Since vacuolization was revealed in control rabbits also, it appears as though all livers thus affected had been in a high state of metabolic activity, which biochemically, indicated that glycogen was apparently being stored in quite considerable quantities. Vacuolization then was due to glycogen depletion which resulted primarily from the washing-out of glycogen by the fixing process. If a specific glycogen stain such as Best's carmine were used, the cells would have appeared quite filled with red-stained glycogen granules. Vacuolization then, seems to be a result of a quite normal nutritional function and not the result of an intracellular antigen-antibody reaction.

Rabbits which had received antiribosome rabbit serum and were killed five days later did not reveal signs of vacuolization, but early indications of hepatic injury were manifested by cloudy swelling edematous changes. Cloudy swelling is a very mild form of degeneration,
easily reversible and best observed in the liver and kidneys. It is characterized by opaque, swollen cells which exhibit a granular cytoplasm, the granules being of a protein nature and the swelling due mainly to an increased water content of the cytoplasm. Cloudy swelling could possibly have progressed and terminated in vacuolization as was exhibited by rabbits which received antiribosome rabbit serum, but which were sacrificed 23 to 44 days later. The observed differences between these two groups of animals could be explained on the basis of the time intervening between injection and sacrifice. In an animal sacrificed a few days after injection, hepatic injury would manifest itself by early degenerative changes. and perhaps due to the toxic effect of antiserum, a temporary state of anoxemia caused by the severe anemia would ensue. As the animal sickened, its food intake would necessarily be curtailed, thus leading to decreased glycogen storage. Animals sacrificed at a later date would have survived the initial toxic effects, regained their well-being and fed well. Changes in the liver would reflect this series of events by revealing cloudy swelling and then later, vacuolization.

In addition to vacuolization, a moderate to severe periportal and perilobular fibrosis accompanied by an inflammatory infiltrate, was observed in those groups of animals suspected of harboring lesions mediated by an autoimmune mechanism, i.e., animals immunized with ribosomes and sRNA and those which were passively sensitized by the injection of the corresponding antisera. Only one other group, those injected with rat liver nuclei, demonstrated these
lesions. Control animals injected with Freund's adjuvant only, were not affected. The question as to whether these lesions represent the end result of autoimmunization or the normal consequence of a non-specific irritant commonly observed in normal stock rabbits (59) is difficult to resolve. The appearance of these lesions can be explained on a time basis regardless of whether they are the result of an immune process or a sequence of events observed in normal, non-immunized animals. Both etiologies could apply.

It is observed in animals which received antiribosome rabbit serum and sacrificed five days after injection that the perportal and perilobular interstitial spaces consisted of newly formed, somewhat edematous, connective tissue associated with very little fibrosis. This acute lesion was complemented by the early degenerative changes evident in the hepatic parenchyma. When compared to the observed changes in rabbits sacrificed 23 and 44 days after receiving antiribosome rabbit serum, it was evident that a process of long standing chronicity characterized by abundant, dense bands of collagenous tissue, the presence of lymphocytes and abundant fibroblasts, had ensued. From this description, it may be inferred that either an immune process initiated the early lesion, and then progressive autoimmunization directed the course toward chronicity; or it may be inferred that in all these rabbits, a non-specific degenerative process was just beginning which was first observed in animals sacrificed at five days and progressed toward chronicity in direct proportion to the animals longevity.
Chronic periportal fibrosis and infiltrate was especially pronounced in rabbits injected with ribosomes and sacrificed 70-78 days later. A factor which helps substantiate an immunologic basis as the cause of these lesions, is the fact, that whereas vacuolization was observed in control animals injected with Freund's adjuvant only, periportal and perilobular fibrosis were not. From this it may also be inferred that histologic change was not usually produced in the liver of animals immunized with Freund's adjuvant. An exception of this condition was noted, however, in one animal which demonstrated giant cell formation around a central vein. (Figure 2, Plate XVIII).

The histologic changes observed in the lungs of all animals examined were quite similar in that a particular pattern was established which varied only in degree of severity from animal to animal. Alveolar septal thickening associated with a massive inflammatory response, fibrosis and edema together with peribronchiolar lymphoid hyperplasia and bronchiolitis were consistently demonstrated in experimental and normal stock animals. Most rabbits when caged together within a small area of confinement exhibit communicable upper respiratory diseases due to bacterial and protozoan agents.

The condition seen in sections from the lungs of all animals examined was related to both an acute and chronic interstitial pneumonia associated with passive congestion together with old and fresh intra-alveolar hemorrhage resulting from capillary hyperemia and extravasation. Passive congestion was noted only in the lungs of animals which received
anti-εRNA sera. Actual thrombosis with resultant infarction was never observed. Blood within the lumens of small bronchioles and/or respiratory bronchioles was most likely due to aspiration from punctured lungs which occurred during exsanguination. The presence of mucous plugs and degenerate bronchiolar epithelium, together with invasion of bronchiolar walls by lymphoid elements were indicative of a chronic bronchiolitis, probably of infectious origin. The epithelioid granulomata, commonly observed in animals receiving antigen incorporated in Freund's adjuvant, or Freund's adjuvant only were in all probability initiated by the mycobacteria present in such adjuvant, whereas animals injected with antisera only were negative in this respect. Furthermore, similar observations have been made in rats made arthritic by injections of Freund's adjuvant only (53). The pleuritis, associated with emphysematous changes observed in an animal injected with rat liver nuclei, was the consequence of the extension of a severe pulmonary inflammation. One other point regarding the thickened alveolar septa and diminished volume of the alveolar spaces themselves should be expanded upon. Whether this observation is real and due to the existing inflammatory exudate, or is the product of an artifact, is a moot question, as the manner in which the lungs were prepared after removal from the animal determines the final histologic picture. Compression effects due to sectioning are a distinct possibility, especially if sections were cut too thick, as some of the sections examined appeared to be. Only by intratracheal instillation of fixative prior to removal
and sectioning, can suitable sections of lung, free from artificial
devices be obtained.

The severe changes as noted in the vasculature of animals injected
with ribosomes and those injected with antiribosome rabbit serum, con­
trasted vividly with the slight to moderate change observed in all
other animals, including controls. The presence of edematous muscular
walls, medial hypertrophy, intimal activation and a fibrinoid change
in the adventitial sheaths does not justify considering all these
changes were due to an immune response. Medial hypertrophy is espe­
cially noticeable in pulmonary arteries and their branches in normal
stock rabbits(31). Also the fact that the most severe vascular ab­
normalities occurred in organs which were affected by non-specific in­
flammatory conditions, such as interstitial nephritis and pyelonephritis
in kidneys and interstitial inflammation in lung tissue, lends consider­
able weight to the theory that the surrounding parenchymal inflammation
indirectly caused these vascular changes.

An immune genesis initiating these pulmonary vascular changes,
though, has much to recommend it. First, they were present only in
animals which received ribosomes and sRNA and those which received the
respective antisera by passive transfer. Secondly, the presence of
nests of polymorphonuclears located sub-intimally in the large branches
of the pulmonary artery, (Figure 2, Plate XXIII), is indicative of an
immune reaction. The presence of an edematous media also substantiates
this finding. Thirdly, lesions somewhat similar to those just described
have been produced by the injection of soluble, pre-formed antigen-
antibody complexes of bovine albumin and anti-bovine albumin or by an
in vivo complexing utilizing the same antigen and antibody(19). The
injury was described by the authors as being similar to an Arthus re-
action. Vascular injury in the present instances could then be the
result of in vivo ribosome-antiribosome complexing.

The kidneys of all animals examined demonstrated changes so
similar in their morphologic and cellular constituents, that only a
non-specific etiology can be assigned to their causation. A moderate
to severe interstitial nephritis, characterized by an inflammatory in-
filtrate comprised predominately of round cells, and which resemble
lymphocytes, was associated with severe pericapsular fibrosis and
thickening. Glomeruli and tubules, caught up in the advancing wave of
fibrosis were severely damaged and presented changes which reflected
the severity of the interstitial inflammatory process. Both atrophic
and hypertrophied proximal and distal convoluted tubules were observed,
some filled with fluid, but most exhibited a change in the tubular epi-
thelium, which reflected increased intra-tubular pressure, most likely
caused by urinary stasis. These changes just described were most
likely the result of an acute pyelonephritis superimposed upon an acute
interstitial nephritis of non-specific etiology. Both conditions have
been verified many times by a number of investigators in normal stock
rabbits, which somehow seem prone to the development of such lesions.
Pyelonephritis of bacterial origin is common to most laboratory animals
and is easily acquired but difficult to eradicate.
While one or the other of these non-specific conditions could produce the observed glomerular and tubular lesions, kidneys of control animals injected with Freund's adjuvant only, rat liver nuclei or antinuclear sera were not so affected. These facts could be explained as individual differences in the animals concerned, whether or not these non-specific lesions would be present at the time of sacrifice. Some believe interstitial nephritis to be caused by an allergic factor, usually bacterial in origin (25). An associated pyelonephritis could supply the needed bacteria for the production of an allergic lesion, but blood cultures and examination of tissue sections were negative for the presence of organisms. This is not to say that a slight transient bacteremia was not present, or that bacteria were not contained in the tissues, as blood cultures were not obtained at close intervals, and, hematoxylin and eosin staining procedures leave much to be desired when examinations for bacteria are to be performed. Furthermore, a time sequence relating the severity of glomerular and tubular lesions to the length of time an animal survived before sacrifice, as postulated in the discussion concerning the hepatic lesions, was demonstrated also. For instance, animals, which were sacrificed 70-78 days after initial injection sustained lesions of comparable severity, and in contrast, animals which received antiribosome rabbit serum and sacrificed five days later exhibited changes resembling acute interstitial nephritis only in the medullary portion of the kidney. This area is usually the first to become affected by such change, which in time, advances peripherally toward the cortex to involve the structures contained therein.
As the time of sacrifice was extended, from 23 to 44 to 78 days, so did the advancing fibrosis and infiltrate, until a picture of chronicity was established associated with the attendant degenerative consequences. To add weight to this argument, early degenerative changes as cloudy swelling and hyaline droplet formation, were not observed in animals sacrificed at later dates, but only in animals sacrificed shortly after injection, such as those which received antiribosome rabbit serum. These generalized early changes were probably the result of the influence of an acute interstitial process situated in the medulla, but could also conceivably be the result of a disturbance of cell metabolism initiated by an intracellular antigen–antibody reaction if complexes can be taken up by parenchymatous cells as advocated by (Benacerraf 4).

The wide range of injury exhibited by glomeruli, that is, from beginning glomerulitis together with normal tubular structures, to severe pericapsular thickening, fibrosis, and hyalinization with its associated atrophic, ischemic, scarred glomerular counterparts, was attributed to the presence of either mild or severe interstitial inflammatory changes, respectively. Glomeruli which lay outside the influence of either the pyelonephritis or interstitial changes were usually within normal limits both morphologically and functionally. Certain reservations must be made, however, in animals which were immunized with ribosomes, as a spectrum of glomerular changes which ranged from early glomerulitis, through acute glomerulohephtritis and ending in complete glomerular fibrosis and scarring, were observed in the absence
of any demonstrable interstitial nephritis or pyelonephritis. These lesions could conceivably have been the result of an immune process initiated by the localization of precipitated immune complexes, injuring the endothelium of capillaries comprising the glomerular tuft. An experimental counterpart has been provided by the work of Dixon(19). Even though glomerular changes resembled those often observed in human cases of acute and subacute glomerulonephritis and focal membranous nephritis, it is difficult to relate these changes as being purely a glomerulonephritis per se, simple because certain attendant and coexistent exudative phenomena, which must be demonstrated, were never observed. Specifically, the presence of hemorrhage into Bowman's space or tubules, the presence of tubular casts associated with an albuminuria, or the presence of polymorphonuclears associated with a fibrinous exudate, either within glomerular capillary loops or Bowman's space would help establish a true picture of glomerulonephritis. Urine samples were not obtained in any of the animals examined, but in the course of later investigations, urine samples were obtained at weekly intervals from animals injected with ribosomes in Freund's adjuvant and from others which were injected with antiribosome rabbit serum. Neither albuminuria, nor the presence of white or red cells, nor tubular casts were demonstrated. From these observations, it would seem that glomerular and tubular lesions were the direct result of an interplay between an acute to chronic interstitial nephritis and an associated pyelonephritis.

Other non-specific lesions such as tubular calcification and focal
coagulation necrosis were the result, presumably, of local ischemic process which involved a small artery which supplied these areas, leading to infarction with subsequent necrosis and final deposition of calcium into the infarcted area, whether it be a tubule or interstitial tissue. These lesions were limited to one or two animals injected with riboprotein or rat liver nuclei.

A rather constant yet peculiar finding, was the presence of a cell which demonstrated a rather small, dark basophilic nucleus, contained within an eosinophilic, granular cytoplasm. These cells were observed only in animals which had sustained severe anemias and were therefore most numerous in animals injected with ribosomes and those injected with antiribosome serum. Their usual habitat was limited primarily to liver and kidney, but on occasion, were observed within the abundant cellular infiltrate which comprised the thickened walls of alveolar septa. In renal sections, they were usually found lying at random throughout the medullary portion and on occasion could be observed scattered among plasma cells, lymphocytes and macrophages which comprised the interstitial infiltrate. In the livers, they usually were demonstrated in close proximity to areas of perilobular and periportal fibrosis. From their peculiar appearance and association only with rabbits which had sustained a gross reduction of circulating erythrocytes, they were considered to be a degenerating cell type which is often observed in parenchymatous tissues in which anoxic conditions are prevalent.
The discussion has so far been limited to an appraisal of lesions considered to have been engendered by non-specific agents unrelated to an immune mechanism. Certainly there can be no definite lines drawn between a lesion which is of immune origin and that which is not. Lesions observed in the heart, spleen and changes in peripheral blood of animals injected with ribosomes were considered to be the result of an autoimmune process on the basis of the fact that they were accompanied by an autoimmune hemolytic anemia and leucopenia, associated with circulating antibodies for ribosomes and sRNA, within 70 to 78 days after injection. All aspects of the disease were reproduced in animals within five days after injection of antiribosome serum. It was first thought that animals injected with yeast sRNA and those injected with anti-sRNA serum duplicated the autoimmune lesions observed in ribosome injected animals, but only somewhat similar lesions occurred in their cardiac musculature.

The most prominent and consistent cardiac lesions were areas of focal necrosis, either situated in close proximity to small thin-walled veins in animals which received antiribosome or anti-sRNA rabbit serum, or scattered randomly over the myocardium in animals immunized with ribosomes or sRNA. The lesions were similar in morphology, but quite different in the supporting cellular elements between animals immunized with ribosomes and those which were injected with antiribosome rabbit serum. The former contained necrotic foci which were larger and displayed an increase in fibrosis associated with fibroblasts and a few scattered macrophages. The lesions observed in passively sensitized
animals tended to be smaller, contained less fibrosis, but demonstrated a more complete complement of plasma cells, macrophages, histiocytes and a few fibroblasts. Compare (Figure 2, Plate I) with (Figure 4, Plate III). A few necrotic foci were, however, evident as long, narrow, linear lesions which resembled those observed in ribosome injected animals. Areas of focal necrosis observed in animals injected with sRNA and rabbits injected with antiserum to sRNA were almost identical in their predilection for small thin-walled veins, but necrotic areas of actively immunized animals were more chronic in appearance. Compare (Figure 1, Plate V) with (Figure 2, Plate V). Infiltration by debris-laden macrophages into an area which appeared to contain fragmented, degenerate myofibers with no association of fibrosis or cellular infiltrate other than macrophages characterized the lesions observed in animals injected with anti-sRNA rabbit serum. The overall differences noted above indicate that the focal necrotic lesions produced in animals actively immunized with ribosomes or sRNA were definitely more chronic in nature as attested by the presence of fibrosis and a scattering of a few lymphocytes, whereas, the same type of lesion produced by injection of antiserum indicates an acute necrotizing process in which fibrosis and its attendant chronic cellular element have not had sufficient time to make their appearance. However, as the time between injection and sacrifice increased, focal necrotic areas were not observed in animals injected with antiribosome rabbit serum and sacrificed at 23 and 44 days after injection. Either these lesions appeared shortly after passive transfer, became fibrotic and finally disappeared without leaving a trace of the former injury, or they were not produced at all.
The question of an immune mechanism or non-specific force in the development of a subendothelial fibrosis associated with endothelial proliferation and fibrinoid changes in the adventitia of small myocardial arteries, coexistent with the presence of a generalized myocarditis characterized by spongy hypertrophied myofibers and separated by edema, is equivocal. Non-specific conditions, such as malnutrition associated with lowered blood volume and resultant anoxia; or an extension of an inflammatory process from inflamed lungs to the heart musculature by a process of contiguity across the opposing visceral surfaces of the lungs and pericardium, could easily initiate pericardial inflammation and thence involvement of the entire myocardium, as interstitial pneumonia was observed in animals injected with ribosomes and antiribosome rabbit serum. The myocardium of these animals presents a picture of chronicity as observed in debilitating diseases or an avitaminosis. It is difficult to relate this picture to those animals injected with antiribosome serum and sacrificed five days after injection with an immune process unless the serum antibody concentration was of such high titer and avidity, that immune reactions occurred within the cells comprising the myocardium very soon after the injection. The appearance of the acute nature of the focal necrotic lesions tend to bear out this observation. The possibility of an immune mechanism is indicated by the fact that these changes were not observed in any other group of animals immunized except those actively immunized with riboprotein and rat liver nuclei, in which animals, only a pericarditis was initiated, and which was most likely the result of pulmonary
inflammation. Furthermore, similar histologic changes have been observed in animals injected with soluble antigen–antibody complexes, and which were especially pronounced in small arteries as a fibrinoid change in the adventitial and medial costs, combined with a mild form of subendocardial fibrosis (19).

The histologic changes observed in the spleens served as a sensitive barometer regarding the physiologic status of the internal milieu of animals injected with the various substances. Only in ribosome-injected animals and those injected with antiribosome rabbit serum was there manifested a peculiar perifollicular deposition of hyaline-like substance which stained positively be PAS techniques, and which appeared to be composed primarily of glycoprotein. This substance was demonstrated in actively immunized animals but it appeared also in spleens of passively transferred animals as early as 5 days after injection of antiribosome rabbit serum. The deposit increased both in amount and circumferentially in animals sacrificed 23 days after passive transfer of serum and thence gradually diminished in those animals sacrificed 44 days after injection of serum. The material was deposited in intercellular spaces around cells rather than in cells. Material which gave positive PAS staining and which appeared to be in more dispersed form was also demonstrated between red pulp capillaries in areas of capillary proliferation. The substance in question could be a form of amyloid, as this peculiar substance stains with PAS by virtue of its mucopolysaccharide and glycoprotein content. Amyloid deposition is associated with long-continued, infected tissue destructive processes
such as tuberculosis, leprosy, etc. Some consider the reticulo-endothelial system to be active in formation of amyloid; others look on amyloid as a kind of antigen-antibody precipitate, prevalent wherever conditions of hyperglobulinemia exist. The concept of amyloid deposition would certainly pertain to those animals injected with ribosomes, because of the picture of a long-continued chronic, wasting disease presented by these animals. However, it is more difficult to fit this rationale into the puzzle presented by passively transferred animals in which perifollicular deposits were first encountered within five days after injection of serum, and perhaps, earlier. Perhaps, in these animals the initial lesions were the direct result of injected ribosome antibody reacting directly with ribosome fractions within diverse tissue cells. In the process, degraded protein products were released in such large quantities as to form within five days time, a sufficient pool which was recognized as amyloid. As sacrifice times increased to 23 and 44 days, reimmunization to autologous ribosomes occurred, releasing more protein degradation products culminating in perifollicular deposits. As an alternative, it may be construed that soluble antigen-antibody complexes were precipitated, if a state of equivalence was attained, and lodgement of this precipitate occurred within the sieve-like interstices of the splenic cords near the follicles.

The remaining splenic histologic changes were regarded as secondary expressions, induced primarily by either the influence of Freund's adjuvant or the severe anemia produced in ribosome-injected animals and those injected with antiribosome rabbit serum. Lymphoid cell
hyperplasia associated with activated germinal centers and plasma cell response was observed in all animals including controls. It is a well known fact that any antigenic stimulus with or without Freund's adjuvant, will stimulate organs comprising the reticulo-endothelial system in this manner, and Freund's adjuvant when administered alone, possesses this stimulatory property (28). Arteriolosclerosis of the central arteries and those of the red pulp, together with capillary hyalinization are normal degenerative, vascular processes consistent with ageing, and are most pronounced in the spleen (31). The severe medial edema of ribosome-injected animals most likely reflected the terminal toxic effects of a long, chronic illness. Fragile, spheroid erythrocytes, coated with autoantibody, and trapped within the meshes of the splenic cords, were the cause of signs of increased red cell destruction, characterized by the presence of free and phagocytized crystals of hemosiderin, far in excess of what would normally be observed. An attempt to compensate for the leucopenia, was demonstrated by small, focal areas of myeloid metaplasia, characterized by the presence of meta-myelocytes and myelocytes; juvenile forms of the granulocytic series. The presence of megakaryocytes almost guarantees the possibility that compensatory extramedullary erythropoiesis was initiated in the splenic red pulp, although basophilic or polychromatophilic erythroblasts were not observed.

The presence of a severe anemia, leucopenia, and reticulosis in rabbits injected with ribosomes in Freund's adjuvant, was the first laboratory confirmation that a severe, self-sustaining disease process
had been initiated in these animals, which was self-limiting only in the sense that death was the eventual outcome. Autoantibodies against autologous, homologous and heterologous red cells, associated with anti-leucocyte antibodies, were demonstrated in the sera of these animals within 14 days post-injection, and were present in increased amounts until sacrifice. Sera from these animals, when transferred passively to normal rabbits, produced within five days, identical hematologic findings. The anemia was established to be an acquired hemolytic type on the basis of the presence of erythrocyte autoantibody and examination of blood smears revealed gross erythrocytic abnormalities.

Associated with these various cellular elements was the presence of large histiocytes, characterized by vacuolated, foamy cytoplasms in which were contained large azurophilic nuclei. These cells were usually demonstrated within 70-78 days after injection with ribosomes and within five days after injection in animals which received antiribosome rabbit serum. The amount of serum injected was very roughly correlated with the time of appearance and in what numbers these cells would be demonstrated. The most striking feature regarding these histiocytes was their ability to phagocytize erythrocytes and leucocytes presumably sensitized with autoantibody. One cell, in particular, was in the process of engulfing six erythrocytes at one time. In all probability, the origin of these histiocytes was the bone marrow, as this organ is primarily the seat of primitive stem cells. However, the entire reticulo-endothelial system may have supplied these forms, as the fixed reticulum cells possess phagocytic properties, and when
the occasion demands, can be converted to wandering macrophages. Histioctytosis has been described in a number of disease states, and it is especially prominent in allergic states(1). If these cells can be related to an immune mechanism operating in animals injected with ribosomes, and those injected with antiribosome rabbit serum, this factor would further substantiate the facts already given in support of this theory.

Anti-leucocyte antibody, in addition to its sensitizing capacity was also demonstrated directly by its cytolitic activity, presumably in conjunction with complement. Progressive leucocytic cytolysis, ending in the dispersion of cytoplasmic granules, extrusion of nuclei and eventual nucleophagocytosis, could be followed chronologically from about the 2nd day after injection of antiribosome rabbit serum. At this time, slight degenerative changes within the intact leucocyte were first noticed. By the 4th day, extrusion of granules and nuclei were predominant, and by the 5th day, nucleophagocytosis was the eventual outcome. These changes have never been observed in any other group of animals immunized. Animals injected with Freund's adjuvant displayed normal hematologic findings, although on occasion, a slight lymphocytosis was encountered.

Considerable evidence is available concerning the occurrence of autoimmune diseases in humans, and the production of experimental auto-allergic diseases in animals(68). In both humans and animals, the mechanism of autoimmunization is as yet unknown or disputed. Target organs are injured by one of two means, or perhaps both; either by
circulating anti-organ antibody or delayed hypersensitivity. Waksman (69) states categorically that all human and experimental autoallergies are mediated by delayed hypersensitivity.

It is a fact that all human and experimental autoallergies are characterized by a "perivascular island" reaction which is characterized by focal collections of lymphocytes, histiocytes and plasma cells in and around the adventitial sheaths of small veins, and associated with slight proliferative changes in the intimal cells themselves. Invasion of the parenchyma by these wandering cells takes place. Parenchymal elements are destroyed, and eventually phagocytized elements of the injured parenchyma are observed within macrophages. A situation whereby the parenchymal cells of the thyroid and synovia have actually been "invaded" by "lymphocytes" and destroyed, has been described by Waksman, to which he gives the name "emperipolesis"(70). Tissue injury is supposedly heightened by the release of "transfer factor" from sensitized lymphocytes. Non-sensitized lymphocytes take up this "transfer factor", presumably cell-bound antibody, and distribute it over larger and larger antigen-containing areas, so that the entire organ is eventually infiltrated with macrophages and lymphocytes.

A few of the experimental autoimmune diseases have been passively transferred by means of sensitized cells. Although circulating anti-organ antibody can be demonstrated serologically, there is no definite proof that it can directly affect target organs, as the diseases have never been produced in normal animals by passive transfer of circulating antibodies, except in experimental blood disorders, no matter what
quantity of serum was used. Again, there is a rough correlation between the degree of delayed hypersensitivity as measured by skin testing and the severity of organ injury, but no such correlation exists for circulating antibody, which, for that matter, may be entirely lacking even in the face of severe parenchymatous lesions. Lesions, if mediated by circulating antibody, are usually termed vasculonecrotic or Arthus-type which is characterized by edema, the presence of polymorphonuclears and plasma cells, associated with thrombosis, resultant infarction and eventual necrosis.

It is not known definitely how the lesions described here, and thought to be of immune origin, were caused. Evidence for an immediate or delayed type mechanism, or possible complexing in the case of those animals injected with antisera, is equivocal. Scattered areas of focal myocardial necrosis were abundant in ribosome and sRNA-injected animals. A very few were observed near small, thin-walled veins. Yet these lesions appeared to be the result of a long, chronic illness as attested by their fibrotic nature, and evidence of the typical perivascular cuffing so characteristic of delayed type hypersensitivity were not present. It is possible, however, that in passively sensitized animals, an Arthus reaction could have been the causation of foci of necrosis around thin-walled veins. Here an abundant supply of antigen (ribozymes) and antiribosome antibody, separated only by the thin vein wall, could have reacted to cause these lesions. This reasoning may also hold for the massive tubular degeneration was observed in the cortex of the kidneys, where there were large areas of destruction surrounding
thin-walled veins accompanied by a cellular infiltrate. However, precipitating type antibody has never been demonstrated in these sera. It must be remembered also, that any chronic non-specific inflammatory lesion will consist of these cell types, and for this reason, it is felt that the renal lesions were all due to non-specific causes.

In the liver, nests of round cells which seemed to arise from the sinusoidal connective tissue, and comprised predominately of lymphocytes, surrounded and infiltrated the hepatic parenchyma with resultant cellular necrosis. The presence of these cells, coupled with the nature of the lesion, point toward delayed hypersensitivity. However, similar lesions have been produced by the intravenous injection of soluble antigen-antibody complexes in the livers of rats, an organ in which chronic, progressive autoimmunization has been postulated. Coexistent with hepatocellular necrosis mediated by these complexes, was the production of bile ductal proliferation, a phenomenon which was seemingly evident in both ribosome and rRNA injected rabbits and in animals passively sensitized with antisera. It is difficult to visualize how these lesions were produced in passively sensitized animals, as only serum antibodies were transferred and so another possible mechanism can be postulated.

Evidence has been presented that the autoantigen concerned in the present experiments are nucleic acids, presumably RNA characterized by finer determinant groups such as nucleotides and nucleosides. The nucleic acid is contained within ribosomes and ribosomes are the seat of protein synthesis, and thus antibody production. Once inside a
competent antibody synthesizing cell, it is only speculative whether substances act as template or modify cellular metabolism to produce complimentary globulin. The mechanism would be unusual in that homologous ribosomes were used and should be already present within these cells. But ribosome antibody was synthesized, and released into the blood. Since globulins, and specifically, antibody have been shown to be taken up by macrophages and parenchymatous cellular elements, and have been shown to penetrate the cytoplasmic and nuclear membranes (14), probably by a process of pinocytosis, it is not difficult to visualize entrance of ribosome antibody into these same cells. It is known that RNA is always associated with protein, and once separated from this protein, intracellular nucleases, inactive only when in the presence of intact RNA-protein combination, become activated and destroy RNA. Perhaps this delicate balance between normalcy and self-destruction is upset by the continual uptake of antiribosome globulin. A state of saturation is reached and exceeded, ribonuclease is activated and the cell is autolysed. The same mechanism would be true for antigen-antibody complexes. Determinant groups on ribosomes complimentary to the specific antiribosome globulin are neutralized by the presence of intact RNA-protein moiety, but with sufficient increments of ingressing antisera, protein-RNA bondings are ruptured, ribonuclease is activated and the result is self-destruction. It is also conceivable that anti-sRNA antibody as demonstrated in antiribosome serum could interfere with protein synthesizing capacities of cells especially during transfer of key amino acids from the nuclear DNA to the RNA
ribosome template to produce, in effect, "nonsense" proteins which would not aid in the economy of the cell. These antibodies could also neutralize the free flow of cellular eRNA and thence bring the total cellular enzyme synthesizing capabilities to a complete standstill. These are the events which could possibly be operative in ribosome-injected animals as this process would take time to manifest itself. This is borne out by the fact that these animals exhibited no clinical or hematologic sign of illness until about 70-78 days post-injection.

The mechanism postulated for animals passively sensitized with antiribosome rabbit serum is quite similar except for the short time interval between transfer and the expression of symptoms. Responsibility for the prompt appearance of lesions in animals sacrificed within five days post-passive transfer, was due to the large volume (10ml) of antiserum injected, and which was complemented by the production of a high ratio of antibody content to blood volume. Most of the antibody was quickly absorbed into the tissues and sufficient amounts were present to saturate the cells so that tissueal damage was in evidence early in the course of the disease. As sacrifice times increased to 23 and 44 days, the original antibody injected was depleted by degradation and excretion. However, reimmunization could have proceeded from autologous ribosomes released from necrotic tissues and autoimmunization would then become self-sustaining.

The results of these experiments indicate that some other mechanism of "self-recognition" other than that proposed by Burnet(8) may be operating to guarantee the suppression of an autoimmune state.
Certainly ribosomes and the mesenchymal elements of the blood, such as erythrocytes, leucocytes and platelets are present before, during and after immunologic tolerance of embryonic existence or the first few days post-natally. This tolerant state functions during adult life and is presumably the reason autoimmunization does not occur normally, and yet breaks down when threatened by the injection of homologous cytoplasmic organelles to affect elements such as the erythrocytes leucocytes and cells of organs to which tolerance should have been acquired. Presumably, intracellular antibody in the form of RNA-associated protein acts as the deterrent against autoimmunization, and only when this relationship is disturbed, then the stage is set for an autoimmune mechanism to play out its role.
SUMMARY

1. Rabbits injected with heterologous and homologous liver ribosomes developed an autoimmune disease characterized by an hemolytic anemia, a leucopenia and lesions in the heart, kidney, liver, lung and spleen.

2. Practically identical results were obtained from rabbits injected intravenously with antiribosome rabbit serum.

3. An autoimmunologic role was thought to be operative in cardiac, splenic and hematologic tissues of those animals injected with ribosomes, but only in cardiac tissue of animals injected with yeast sRNA. This was emphasized because identical lesions occurred in animals receiving antisera, containing antibody to ribosome and yeast sRNA.

4. Lesions in lung, liver, and kidney were considered as non-specific, due possibly to other causes, since they were found in all animals, including controls.

5. Heterologous rat ribosomes were equally as effective in producing lesions described as homologous ribosomes, and demonstrated neither species nor organ specificity. Yeast sRNA also demonstrated a lack of species specificity.

6. The degree of tissular injury, both specific and non-specific, evoked in animals passively sensitized, was not influenced by variation in the volume of antisera injected.
7. It was postulated that the autoimmune mechanism involved in the production of specific lesions was the result of the autoantibody to RNA or complexes of this antibody and RNA.
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