

THE EFFECTS OF ANTITHYMOCYTIC SERUM
ON THE RETICULO-ENDOTHELIAL SYSTEM IN MICE

by

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CHAPTER I

INTRODUCTION

In the mid 19th century, von Recklinghausen (1863) and Ponfick (1869) discovered the existence of cells capable of taking up and storing vital dyes. Metchnikoff called these cells phagocytes, and studied their role in the inflammatory response of the organism (1883; 1905). These, and subsequent studies ultimately led to the formulation of the concept of the reticulo-endothelial system by Aschoff (1924).

The work of Metchnikoff also focussed the attention of scientists to the importance of phagocytosis in immunity. Studies on the spleen (Pfeiffer and Marx, 1898) and lymph nodes (McMaster and Kidd, 1937) implicated the reticulo-endothelial cells in the immunological response of the organism. The fate of a "marker-antigen" was studied in phagocytes by Sabin (1939). The correlation between the appearance of antibodies in the serum and the disappearance of dye-proteins in the phagocytic cells suggested that actively phagocytosing cells were also responsible for the synthesis of antibodies. Although more recent evidence does not support this conclusion,

these early studies proved instrumental in drawing attention to the phagocytic cells of the body as sites of antigen deposition.

Fifty years after Metchnikoff's initial studies on phagocytosis, it was demonstrated that cells engaged in the phagocytosis of antigen do not themselves produce antibody (Harris and Ehrich, 1946). A correlation between the location of the antigen, and subsequent sites of plasma cell development and antibody production was found following the intravenous injection of Salmonella typhii into rabbits (Fagraeus, 1948; 1948). Evidence concerning the cellular sites of γ -globulin synthesis was provided by the development of a two stage fluorescent antibody method (Coons et al, 1950; Coons et al, 1955; Leduc et al, 1955; White et al, 1955). Other experiments using this technique showed the presence of γ -globulin in the germinal centres of the lymphatic nodules, and in the cytoplasm of both mature and immature plasma cells (Ortega and Mellors, 1957). In lymphoid organs, plasma cells were often found clustered about macrophages, forming a so-called "morphologic unit" (Thiery, 1960). A close association between lymphoid cells and macrophages was found in explants of lymphoid tissue from rabbits subjected to antigenic stimulation in vivo. These latter findings

led to the suggestion that such an association might effect a transfer of the antigen-complex from the macrophage to the lymphoid cells, and thereby stimulate antibody production (Sharp and Burwell, 1960).

Elegant in vitro experiments were devised which demonstrated that antigens did not stimulate the antibody producing cells directly, but first had to be processed by macrophages (Fishman, 1961; Fishman and Adler, 1963). A primary antibody response could be induced in lymph node cells by macrophages containing ingested antigen, or by RNA extracts of such cells. Moreover, the direct application of antigen to lymph node cells did not produce antibody, nor was there any antibody produced by the macrophages themselves after antigen ingestion (Fishman, 1961; Cohen, 1967). Enzymatic degradation of the antigen (Levine et al, 1963; Levine and Benacerraf, 1965) and its subsequent coding on RNA (Campbell and Garvey, 1963) were considered essential to the induction of antibody formation.

Macrophage-induction of antibody formation to Shigella antigen in irradiated recipients added credence to such a mechanism (Gallily and Feldman, 1967). The interaction of antigen and lymphocyte-free macrophage suspensions stimulated the production of agglutinating

antibody, whereas *Shigella* antigen alone, or in the presence of lymphoid cells did not promote antibody formation. Furthermore, lymphoid cells from adult donors did not confer immunity on newborn rabbits (Dixon and Weigle, 1957), but peritoneal macrophages, when injected into newborn rabbits, rendered immunologically incompetent animals immunologically reactive (Martin, 1966). It was concluded that a "macrophage signal" or macrophage-processed antigen led to antibody induction, thus involving a bicellular mechanism of macrophage-lymphocyte interaction in the immune reaction (Feldman, 1968).

Although the works of Fishman, Gallilly and Feldman demonstrate the importance of antigen processing by macrophages in the induction of antibody formation, the exact nature of the mechanism responsible for this phenomenon remains unknown. One theory proposes that the major portion of the antigen is degraded by the phagocyte and eliminated. A small portion of the antigen is taken up by precursors of the antibody producing cells, and serves as a stimulus for antibody formation. Apparently plasma cells are capable of ingesting small particles such as ferritin; however, no evidence of the specific antigen in an antibody producing cell has as yet been found (Wellensiek and Coons, 1964; Nossal et al, 1965).

Another theory proposes the passage of informational RNA to an antibody-producing cell. When lymphocytes of a rabbit of one particular γ -globulin allotype are exposed to RNA from an immunized rabbit of a different allotype, some of the antibody produced is of the donor allotype, suggesting that true information transfer has taken place (Fishman, 1967). Similarly, antibody formation could be induced in rabbits by the transfer of macrophages exposed to antigen in vitro, but not by a lysate of these cells, suggesting that the response was not due to a transfer of the antigen with the macrophages (Pribnow and Silverman, 1967).

The third theory postulates an antigen-RNA complex. Phenol extracts from immune cells were found to be mixtures of RNA and antigen (Friedman et al, 1965). RNA extracted from macrophages previously exposed to haemocyanin showed detectable quantities of this compound (Askonas and Rhodes, 1965). The addition of antigen to macrophage preparations resulted in the formation of RNA-protein complex with immunogenic activity (Gottlieb et al, 1967). Treatment of this complex both with RNAase and pronase destroyed its immunogenicity. Apparently, this RNA had very little specificity, which led to the

speculation as to whether its function was merely to protect the antigen, or to facilitate its uptake by immunocompetent cells (Cohn, 1968).

Although the intracellular mechanism by which antigen is processed remains to be solved, the importance of phagocytosis as an initial step of the immune response seems to be established.

If the production of an immune response involves processing of an antigen by macrophages, and the subsequent passage of information as an immunogenic molecule (or antigen) to the antibody producing cells, it should be possible to suppress the immune response by interfering with either the afferent or efferent limb of the immune reflex arc. The search for immunosuppressants was stimulated by the discovery (Medawar, 1944) that homograft rejection was due to an immunological reaction.

Among the agents presently employed as immunosuppressants are 6-mercaptopurine and azothioprine which interfere with protein (eg. antibody) synthesis, cortico-steroids which act on lymphoid cells (i.e. the efferent limb of the immune arc) and amethopterin which inhibits transcription of information to RNA. Unfortunately, these agents are not selective as they suppress reaction to the whole spectrum of antigens, including

the immune response to pathogens. A new approach to immunosuppressive therapy would be to find an agent capable of acting either on the afferent limb of the immune arc or suppressing antibody response to specific antigens. Recent reports that anti-lymphocyte serum (ALS) has a depressing effect on the phagocytic activity of the reticulo-endothelial cells (Sheagren et al, 1969; Pisano et al, 1969) and that it exercises selective cell-mediated immune suppression (Levey and Medawar, 1966; Jooste, 1970) enhance the possibility that this agent may fulfil the above requirements.

Metchnikoff prepared the first ALS in 1899 by injecting guinea pigs with suspensions prepared from rabbit lymph nodes and rat spleens. Injection of the resultant serum into the donor species not only caused destruction of white blood cells, but also agglutination and death of polymorphonuclear leukocytes. Christian and Leen (1905) measured the toxicity of the antiserum by the cessation of the ameboid movement of leukocytes in vitro. In 1917, Papenheimer (a; b) prepared a serum which was effective against lymphocytes and thymocytes when tested in vitro, using the diffusion of trypan blue as an indicator of cell damage.

Equivocal results were reported following in vivo administration of anti-lymphocyte serum. After the injection of heterologous ALS, Flexner (1902) and Bunting (1903) reported an initial cellular depletion in lymphoid organs, followed by an increased cell count whereas Pappenheimer (1917 a; b) demonstrated cellular depletion and subsequent collapse of tissue architecture. Although Cruickshank (1941) was unable to find histological evidence of lymphocyte damage in the lymphoid organs of rats following in vivo administration of heterologous anti-lymphocyte serum, such changes have been reported in mice (Gray et al, 1966; Lance 1968).

In the two decades following the discovery of ALS by Metchnikoff, several investigators (Besredka, 1900; Bunting, 1903; Pappenheimer, 1917 a; b) reported leukocytosis in antiserum treated animals. The first serum to produce a fall in the blood lymphocyte count was developed in 1937 (Chew and Lawrence). These results have now been confirmed for rats (Cruickshank, 1941) and mice (Gray et al, 1966). However, the latter two experiments yielded contradictory results concerning polymorphonuclear leukocyte counts. After antiserum treatment, Cruickshank reported an increase in the number of polymorphonuclear

leukocytes, while Gray and his co-workers reported a decreased white blood cell count, in addition to the lymphopenic effect.

The first serum which could produce profound lymphopenia in vivo without demonstrable agglutination in vitro was developed in 1951 (Woodruff and Forman). The application of antiserum to immunological problems was pioneered by Humphrey (1955) who showed that antisera to neutrophils effectively suppresses the Arthus-type reaction in guinea pigs. This work inspired Inderbitzen (1956) who demonstrated a suppression of sensitivity in guinea pigs to tuberculo-protein by ALS administration. ALS was also found to depress the delayed hypersensitivity reaction to dinitrochlorobenzene (Wilhelm et al, 1958), diphtheria toxin and experimental allergic encephalomyelitis (Waksman et al, 1961). Concurrent with the depression of delayed hypersensitivity, Waksman found a transient depression of the level of blood lymphocytes, with no effect on other circulating cellular elements. He concluded that the small lymphocyte is the primary reactant in the various types of delayed hypersensitivity reactions.

In addition to its importance in depressing the delayed hypersensitivity reaction, the use of anti-lymphocyte serum seems to have great promise in the field of tissue and organ transplantation. The first successful allograft survival in the presence of a major histo-compatibility difference between the donor and the recipient was obtained in rats using rabbit anti-rat lymphocyte serum (Woodruff and Anderson, 1963). Draining of the thoracic duct prior to grafting was shown to prolong allograft survival for a short period of time, indicating that the action of ALS is lymphocyte directed (Woodruff and Anderson, 1963; McGregor and Gowans, 1963). Treatment with rabbit anti-mouse lymphocyte serum resulted in a decreased ability to reject first and second skin allografts and rat skin xenografts (Monaco et al, 1966) in mice.

The precise method by which ALS causes immunosuppression has not yet been established. A number of theories have been proposed in an attempt to explain in the "modus operandi" of ALS - all of which explain some of the results obtained, while leaving others unexplained.

The well documented ability of ALS to cause lysis of lymphocytes is the basis for the cytotoxic theory. This theory suggests that ALS acts primarily through the depletion of a certain "group" or "class" of lymphocytes; the affected groups may be functionally or antigenically different or distributed according to age or degree of immune competence (Chew and Lawrence, 1937; James, 1967; Martin and Miller, 1967; van der Werf et al, 1968).

The observation that lymphoid cells are coated by ALS in vitro led Levey and Medawar (1966) to put forward the blindfolding theory. Coating of the lymphocytes would have a "blindfolding effect" thus preventing recognition of antigens, either on the afferent or efferent limb of the immune reflex arc (Greaves et al, 1967; Field and Gibbs, 1968; Eijssvoogel et al, 1969).

The competitive antigen theory states that heterologous ALS in itself acts as an antigen in the recipient with a preferential affinity for lymphocytes. It is conceivable that ALS would therefore have an advantage over other antigens and thereby cause pre-occupation of the immunological competent cells (Halpern et al, 1963; Guttman et al, 1967).

Other theories are based on the superiority of thymocytes as antigens for ALS production. Although the precise role of the thymus in immunological phenomena is unknown, it has been suggested that ALS inhibits the humoral factor of the thymus (Nagaya and Sieker, 1966) or selectively inactivates or destroys thymic-dependent cells.

Recent studies indicated that ALS has an effect on the phagocytic activity of the reticulo-endothelial cells. Investigators (Sheagren et al, 1969; Pisano et al, 1969) have reported a decrease in the in vivo phagocytosis of particulate matter following ALS treatment, and suggested the blockade of the reticulo-endothelial system as the underlying mechanism. The results also imply that ALS acts primarily on the afferent limb of the immune arc, namely the macrophages.

The aims of the present work were twofold; first, to study the effects of ALS on the morphology of both developing and mature lymphoid tissue; and secondly, to study the in vivo effects of ALS on the phagocytic activity of the reticulo-endothelial cells.

CHAPTER II

MATERIALS AND METHODS

1. Animals

Male C57Bl/J mice (Jackson Memorial Laboratories, Bar Harbor, Maine) and male random-bred Swiss mice (Biological Breeders Laboratories, Ottawa) in the weight range of 20 - 30 grams were used. For studies on the morphology of developing lymphoid tissue, C57Bl/J and Swiss mice of both sexes ranging in age from 1 day to 2 weeks were used. Studies on phagocytic activity were conducted on males of the Swiss strain, weighing between 25 - 30 grams.

2. Sera Used

Three types of sera were employed:

- (a) Active horse anti-mouse thymocyte serum (ATS), Lot No. 2, August 4, 1968;
- (b) Inactive horse anti-mouse thymocyte serum (iATS), Lot No. 1, August 4, 1968; and
- (c) Normal horse serum (NHS) as control sera.

All sera were supplied by the Institute of Microbiology and Hygiene of the University of Montreal. ATS and iATS, which were prepared against C57Bl/J thymocytes, were used unabsorbed.

SECTION I: THE EFFECTS OF ATS ON LYMPHOID TISSUE IN
YOUNG AND ADULT MICE

1. Experimental Protocol for Adult and Young C57Bl/J
and Swiss Mice

A) Adult Mice

(i) C57Bl/J mice were divided into seven groups of four animals each. Groups 1 to 3 received ATS, groups 4 to 6 received iATS and group seven served as an untreated control (Table I). All injections were via the intraperitoneal (i.p.) route. Group 1 received one injection of 0.25 ml. ATS; group 2 received 0.25 ml. ATS each day for two consecutive days, and group 3 received 0.25 ml. each day for four consecutive days. Similarly, group 4 received 0.25 ml. iATS once, group 5 twice, and group 6 four times. In all treated groups, sacrifice was by ether one day after the final injection.

(ii) Swiss random-bred mice were divided into nine groups of six for injection via the i.p. route, nine groups of six for injection via the subcutaneous route (s.c.), and four groups of four for injection via the intravenous (i.v.) route. One group of six mice served as an untreated control (Table II).

(a) Intraperitoneal Route: Group 1 received daily injections of 0.25 ml. ATS for four days. Groups 2 and 3 received daily 0.25 ml. injections of iATS and NHS respectively, for a like number of days. The animals were sacrificed one day after the final injection.

TABLE I
EXPERIMENTAL PROTOCOL FOR ADULT C57Bl/J MICE

Group Number	Sera Used	Number of Injections	Sacrifice (in days) After Final Injection
1	ATS	1	1
2	ATS	2	1
3	ATS	4	1
4	iATS	1	1
5	iATS	2	1
6	iATS	4	1
7	Untreated Control		

Group 4 mice received the same treatment as reported for group 1. Similarly, groups 5 and 6 received the respective treatments of groups 2 and 3. The animals in groups 4 to 6 were sacrificed three days after the final injections.

Group 7 received 0.5 ml. of ATS every third day, a total of three times. Groups 8 and 9 received 0.5 ml. of iATS and NHS respectively. These mice were sacrificed three days after the final injection.

(b) Subcutaneous Route: The three injection schedules described under (a) for the i.p. route of administration were used. Groups 10 to 12 received daily 0.25 ml. doses of ATS, iATS and NHS respectively for four consecutive days, and were sacrificed one day after the final injection. Groups 13 to 15 received daily 0.25 ml. doses of the three sera respectively for four consecutive days and were sacrificed by ether three days after the final injection. Groups 16 to 18 received 0.5 ml. of ATS, iATS and NHS respectively every third day, a total of three times, and were sacrificed three days after the final injection.

(c) Intravenous Route: Group 19 mice received one 0.25 ml. injection of ATS, group 20 one injection each day for two days, while groups 21 and 22 received daily 0.25 ml. injections of ATS for three and four days respectively. Introduction of the serum was by the lateral tail vein. The animals were sacrificed by ether one day after the final injection in each group.

TABLE II

EXPERIMENTAL PROTOCOL FOR ADULT SWISS MICE

Group Number	Route	Sera Used	Number of Injections	Sacrifice (in days) After Final Injection
1	i.p.	ATS	4	1
2	i.p.	iATS	4	1
3	i.p.	NHS	4	1
4	i.p.	ATS	4	3
5	i.p.	iATS	4	3
6	i.p.	NHS	4	3
7	i.p.	ATS	3	3
8	i.p.	iATS	3	3
9	i.p.	NHS	3	3

TABLE II (Cont'd)

Group Number	Route	Sera Used	Number of Injections	Sacrifice (in days) After Final Injection
10	s.c.	ATS	4	1
11	s.c.	iATS	4	1
12	s.c.	NHS	4	1
13	s.c.	ATS	4	3
14	s.c.	iATS	4	3
15 °	s.c.	NHS	4	3
16	s.c.	ATS	3	3
17	s.c.	iATS	3	3
18	s.c.	NHS	3	3
19	i.v.	ATS	1	1
20	i.v.	ATS	2	1
21	i.v.	ATS	3	1
22	i.v.	ATS	4	1
23	UNTREATED CONTROL			

B) Young Mice

C57Bl/J and Swiss mice were divided into fifteen age groups for study (Table III). Groups 1 to 12 received 0.1 ml. of 10% ATS in physiological saline, while groups 13 to 15 received 0.1 ml. undiluted ATS per injection. All sera were injected intraperitoneally. Animals on a multiple injection schedule received one injection each day up to the number of injections designated for the respective groups. In each group, an untreated control animal from the same litter was included. Sacrifice was by decapitation one day after the final injection in each group.

2. Weighing of Animals and Organs

The body weight of each mouse in each group was recorded prior to the first injection, before each subsequent injection, and just before sacrifice. In this way, a percentage weight change for each mouse during the experimental period was calculated. An average weight change for the group was then reported by averaging the weight changes of all the mice in that group.

The wet weights of the kidney, spleen and thymus of C57Bl/J mice, of liver, spleen, thymus, and mesenteric lymph node of Swiss mice, and of spleen and thymus of young C57Bl/J and Swiss mice were expressed as percent of the final total body weight.

TABLE III

EXPERIMENTAL PROTOCOL FOR YOUNG C57Bl/J AND SWISS MICE

Group Number	Number of Injections	Age (in days) at Start of Injection Schedule
1	1	0 - 0.5
2	2	0 - 0.5
3	3	0 - 0.5
4	4	0 - 0.5
5	1	1
6	2	1
7	1	2
8	2	2
9	1	3
10	4	3
11	2	5
12	1	6
13	4	10
14	2	12
15	1	13

3. Histology

Immediately after being weighed, all organs were fixed, dehydrated, cleared and embedded in paraffin. Fixation was either in buffered 10% neutral formalin or in Carnoy's solution. Sections were cut at 5 μ ; formalin-fixed sections were stained with either Meyer's Haematoxylin and eosin or Jenner-Giemsa (Pearse, 1961), while sections fixed in Carnoy's were stained with either methyl-green pyronin (Kurnick, 1955) or 1% toluidine blue buffered to pH 4.4 by sodium phthalate (Bélanger and Hartnett, 1960). In addition to the above staining procedures, the spleen, thymus and mesenteric lymph node from mice treated intravenously with ATS were fixed in buffered 10% neutral formalin, sectioned at 5 μ , stained according to the Feulgen procedure, and counterstained with 1% aqueous fast green.

In addition to the aforementioned organs, tibiae and part of the ileum were also examined. Tibiae were fixed in AFA (a 3:4:1 ratio of 95% alcohol: formalin: acetic acid) followed by demineralization for two weeks in 10% EDTA, using the constant replacement technique (Bélanger et al, 1965). After rinsing in water, the bones were split lengthwise to expose the marrow. They were then dehydrated, cleared and embedded in paraffin.

Sections were cut at 6μ and stained with dilute Wright's stain (Bélanger et al, 1957). Formalin-fixed ileum was sectioned at 5μ and stained with haematoxylin and eosin.

4. Mast Cell Count

After fixation and sectioning at 5μ , spleen sections of young mice were stained with 1% toluidine blue (Bélanger and Hartnett, 1960). Counts were made in twenty adjacent fields at 400X magnification. The Wild #5683 measuring eye piece was used. This grid is divided into 100 squares the area of which represents one field. The area (A) of one field was calculated to be 0.0729mm^2 according to the equation

$$100 \frac{(a \times c)^2}{(b)} = A$$

where a = number of intervals in the eyepiece scale;

b = number of scale intervals on the stage micrometer;

c = width of one stage micrometer interval.

Twenty fields, therefore, represented a total area of 1.46mm^2 .

5. Statistical Methods

The organ weights represent the means $\pm$ standard error of the mean of individual weighings in each group. Student's t-test was used to determine the significance of differences between the mean values obtained for serum-treated mice and untreated controls.

SECTION II: THE EFFECTS OF ATS ON THE PHAGOCYTIC ACTIVITY
OF RETICULO-ENDOTHELIAL CELLS

1. Particulate Matter Used to Study Phagocytosis

Two different types were used:

(a) Polystyrene latex particles (Dow Chemical Company, Midland, Michigan) 1.099μ diameter in a 10% suspension. The stock suspension was diluted 60-fold in physiological saline.

(b) Imferon (Fisons Canada Limited, Toronto) an iron-dextran complex containing 50 milligrams iron per ml.

2. Experimental Protocol for in vivo Administration of Serum and Particulate Matter

Adult male Swiss mice were divided into two sets of thirteen groups, each group containing four mice. In set A, groups 1 to 6 received ATS, groups 6 to 12 received NHS while group 13 received no serum treatment and served as untreated control. All sera were injected intraperitoneally (Table IV).

Group 1 received 0.25 ml. of ATS once, group 2 received 0.25 ml. each day for two days, and group 3 received 0.25 ml. ATS each day for four days. Group 4 received one 0.5 ml. injection of ATS once, group 5 received 0.5 ml. ATS twice with an interval of 2 days and group 6 received four injections at two day intervals. Groups 7 to 9 were on an identical regimen as groups 1 to 3 respectively,

but NHS was given in place of ATS. Groups 10 to 12 received NHS according to the schedule of groups 4 to 6.

Following the prescribed serum treatment for each group, 0.3 ml. of Imferon was administered subcutaneously one day after the final injection in groups 1 to 3 and 7 to 9, and two days after the final sera injections in groups 4 to 6 and 10 to 12. Group 13, the untreated control, also received 0.3 ml. of Imferon subcutaneously. One day after this injection, the animals were sacrificed by ether.

The animals in set B received the identical regimen of serum injections as those in set A groups. However, latex was administered to this set of animals in place of Imferon. Mice received 0.4 ml. latex solution intravenously by way of the lateral tail vein, and were sacrificed by ether four hours after this single injection.

3. Histology

Phagocytic uptake of Imferon was studied in the liver and spleen, and that of latex in the liver.

A. Imferon

The tissues were fixed in buffered 10% neutral formalin, and after dehydration, clearing, and embedding in paraffin, 5 μ sections were cut. Perls' Prussian Blue technique (Pearse, 1961) was used to visualize the sites of iron deposition. Aqueous 1% Safronin O was used as a counterstain.

TABLE IV
IN VIVO ADMINISTRATION OF SERA AND PARTICULATE MATTER

Group Number	Set A (Imferon)	Set B (Latex)	Sera Used	Injection Total Number	Schedule Regimen	Sacrifice (in days) After Final Injection
1		1	ATS	1		1
2		2	ATS	2	Daily	1
3		3	ATS	4		1
4		4	ATS	1		2
5		5	ATS	2	Two-day Intervals	2
6		6	ATS	4		2
7		7	NHS	1		1
8		8	NHS	2	Daily	1
9		9	NHS	4		1
10		10	NHS	1		2
11		11	NHS	2	Two-day Intervals	2
12		12	NHS	4		2
13		13	Untreated			

B. Latex

One mm.³ pieces of liver were fixed for two hours in 2% glutaraldehyde-cacodylate buffer adjusted to pH 7.4. The tissues were post-fixed for two hours in 2% osmium tetroxide and adjusted to pH 7.2 with Millonig's buffer (1962). After fixation and alcohol dehydration at 2°C, the specimens were embedded in Araldite 502 according to the method of Luft (1961). Sections were cut at 1μ on an LKB Pyramitome and stained with 0.5% toluidine blue in a 1% borax solution. The thin sections were used to study the phagocytosis of latex particles.

4. Estimation of Phagocytic Activity

Phagocytic activity in the liver and spleen after Imferon injection was studied by cell counts. Kupffer cells were counted in twenty fields using the 40X objective and 10X ocular lenses. The twenty fields had an area of 1.46mm.² Splenic macrophages were counted in five fields using the 20X objective and 10X ocular lenses. The total area of these five fields was 1.45 mm.². The areas of hepatic and splenic tissue were calculated according to the method described in Section I, Part 3 (Mast Cell Count). Kupffer cells were scored according to the presence or absence of phagocytosed particulate matter, whereas in the spleen, only Imferon-containing cells were counted. In addition to

the studies on phagocytic activity, the effect of ATS on the total number of Kupffer cells in the twenty fields was also studied.

5. Statistical Methods

The number of cells phagocytosing Imferon was calculated as a percentage of the total phagocytic cell count. This percentage was expressed as the mean $\pm$ standard error of the mean for each group. Total Kupffer cell counts were similarly evaluated. Student's t-test was used to determine the significance of differences between the mean values obtained for serum-treated and untreated control mice.

CHAPTER III

RESULTS

I. THE EFFECTS OF ATS ON C57B1/J MICE

a. Survival

Mice receiving one ATS injection daily for four consecutive days (group 3; Table V) exhibited weight loss, symptoms of jaundice, hematuria, diarrhea, hunching of the back and loss of body hair. Fifty percent of the mice in group 3 died during the experimental period. No such changes occurred in the other five groups.

b. Effects on Total Body Weight

All groups, with the exception of group 3, showed weight increases over the experimental periods. In group 3, there was a marked weight decrease.

Group 4 and 5 mice injected with iATS had weight increases comparable to that of the untreated controls. In group 6, the weight increase almost doubled that of the untreated control group (Table V).

c. Effects on Organ Weights

Organ weights expressed as percent of total body weight are summarized in Table V. A progressive increase in splenic weight is noted in groups 1 to 3,

TABLE V

TOTAL BODY AND ORGAN WEIGHTS OF C57 B1/J MICE GIVEN ACTIVE OR INACTIVE
ANTITHYMOCYTIC SERUM
i.p. (MEANS $\pm$ S.E.M.)

Group Number	Sera Used	Number of Doses	% Change in T.B.W.	Spleen Weight % T.B.W.	Thymus Weight % T.B.W.	Kidney Weight % T.B.W.
1	ATS	1	+1.9	.352 $\pm$.021 [•]	.207 $\pm$.016	.636 $\pm$.018
2	ATS	2	+0.2	.568 $\pm$.050 [•]	.190 $\pm$.028	.621 $\pm$.022
3	ATS	4	-5.9	.815 $\pm$.024 [•]	.147 $\pm$.010 [•]	.663 $\pm$.022
4	iATS	1	+2.6	.277 $\pm$.009	.182 $\pm$.003	.660 $\pm$.017
5	iATS	2	+3.6	.276 $\pm$.006	.197 $\pm$.012	.662 $\pm$.019
6	iATS	4	+5.2	.286 $\pm$.009	.199 $\pm$.005	.658 $\pm$.015
7	Untreated Control	-	+2.8	.292 $\pm$.012	.209 $\pm$.007	.612 $\pm$.026

T.B.W. Total Body Weight • P .05 - .02 • P .01 - .001 • P < .001
S.E.M. Standard Error of the Mean

TABLE V

TOTAL BODY AND ORGAN WEIGHTS OF C57 BL/J MICE GIVEN ACTIVE OR INACTIVE
ANTITHYMOCYTIC SERUM
i.p. (MEANS $\pm$ S.E.M.)

Group Number	Sera Used	Number of Doses	% Change in T.B.W.	Spleen Weight % T.B.W.	Thymus Weight % T.B.W.	Kidney Weight % T.B.W.
1	ATS	1	+1.9	.352 $\pm$.021*	.207 $\pm$.016	.636 $\pm$.018
2	ATS	2	+0.2	.568 $\pm$.050*	.190 $\pm$.028	.621 $\pm$.022
3	ATS	4	-5.9	.815 $\pm$.024*	.147 $\pm$.010*	.663 $\pm$.022
4	iATS	1	+2.6	.277 $\pm$.009	.182 $\pm$.003	.660 $\pm$.017
5	iATS	2	+3.6	.276 $\pm$.006	.197 $\pm$.012	.662 $\pm$.019
6	iATS	4	+5.2	.286 $\pm$.009	.199 $\pm$.005	.658 $\pm$.015
7	Untreated Control	-	+2.8	.292 $\pm$.012	.209 $\pm$.007	.612 $\pm$.026

T.B.W. Total Body Weight * P .05 - .02 * P .01 - .001 * P < .001
S.E.M. Standard Error of the Mean

reaching significant levels in groups 2 and 3. The weight of the spleen in groups 4 to 6 did not differ significantly from those of the untreated controls

Thymic weights generally remained constant.

The only significant change was detected in group 3 where a marked weight decrease occurred after ATS treatment. No significant changes were noted in kidney weight in any of the six experimental groups.

d. Histological Changes

i. Spleen

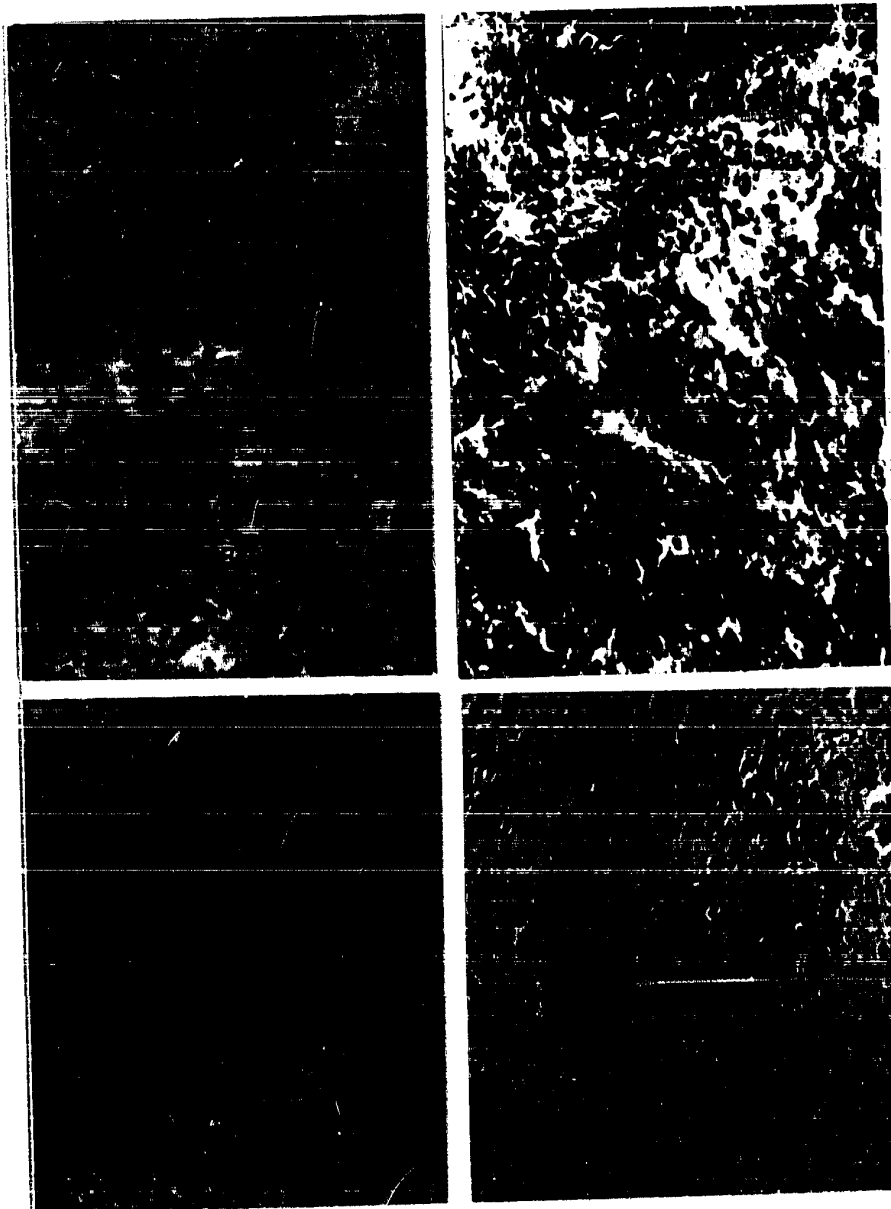
Group 1 spleens had slight increases in the volume of red pulp. The lymph nodules contained germinal centres with some cellular debris. In group 2, some periarteriolar depletion of small lymphocytes was evident. Large lymphocytes and cellular debris were noticeable in the depleted areas. Red pulp volume was more pronounced and the lymph nodules were smaller than in group 1 spleens. Germinal centres were not observed in group 2 spleens. Mice receiving four ATS injections (group 3) had more pronounced periarteriolar depletion than group 2 (figure 1), and an extensive red pulp hyperplasia which effaced the normal red pulp architecture (figure 2). Megakaryocytes and myeloid blast cells increased in number in the red pulp (figure 2). The lymph nodules were greatly

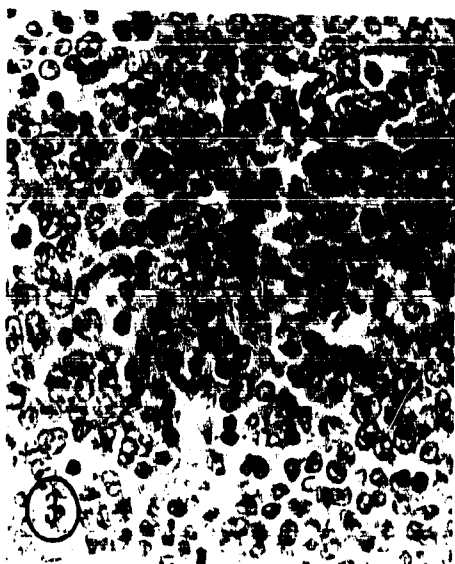
Figure 1 Periarteriolar (A) and perinodular (B) lymphocyte depletion in the splenic nodules after four ATS injections intraperitoneally. Note the large lymphocytes and mitotic figures in the region of periarteriolar depletion. 500 x. H and E stain.

Figure 2 Splenic red pulp from C57Bl/J mouse treated four times with ATS intraperitoneally. Erythroid hyperplasia; increases in the number of myeloid cells and megakaryocytes. 250 x. Toluidine blue.

Figure 3 Denuded splenic lymph nodule after four intraperitoneal injections of ATS. Majority of cells large, lymphocytes and blast cells; mitotic figures evident. 500 x. H and E stain.

Figure 4 Depletion of small lymphocytes in the diffuse lymphatic tissue of the spleen. Note relatively small degree of periarteriolar depletion. 250 x. H and E stain.





reduced in size and were clearly demarcated by the hyperplastic red pulp. In addition to the extensive periarteriolar depletion which in some cases almost completely denuded the splenic nodule of small lymphocytes (figure 3), small lymphocyte depletion was also observed in the diffuse lymphatic tissue of the white pulp (figure 4). In the regions of the periarteriolar and perinodular depletion, many large lymphocytes and reticular cells were seen. The nodules contained no recognizable germinal centres, but did contain cellular debris and some mitotic figures. Generally, group 3 spleens were composed of smaller but more numerous nodules.

Except for minimal changes in group 6 spleens, the splenic architecture of mice receiving iATS was comparable to that of the controls. Group 6 spleens had limited periarteriolar depletion and some large lymphocytes.

ii. Thymus

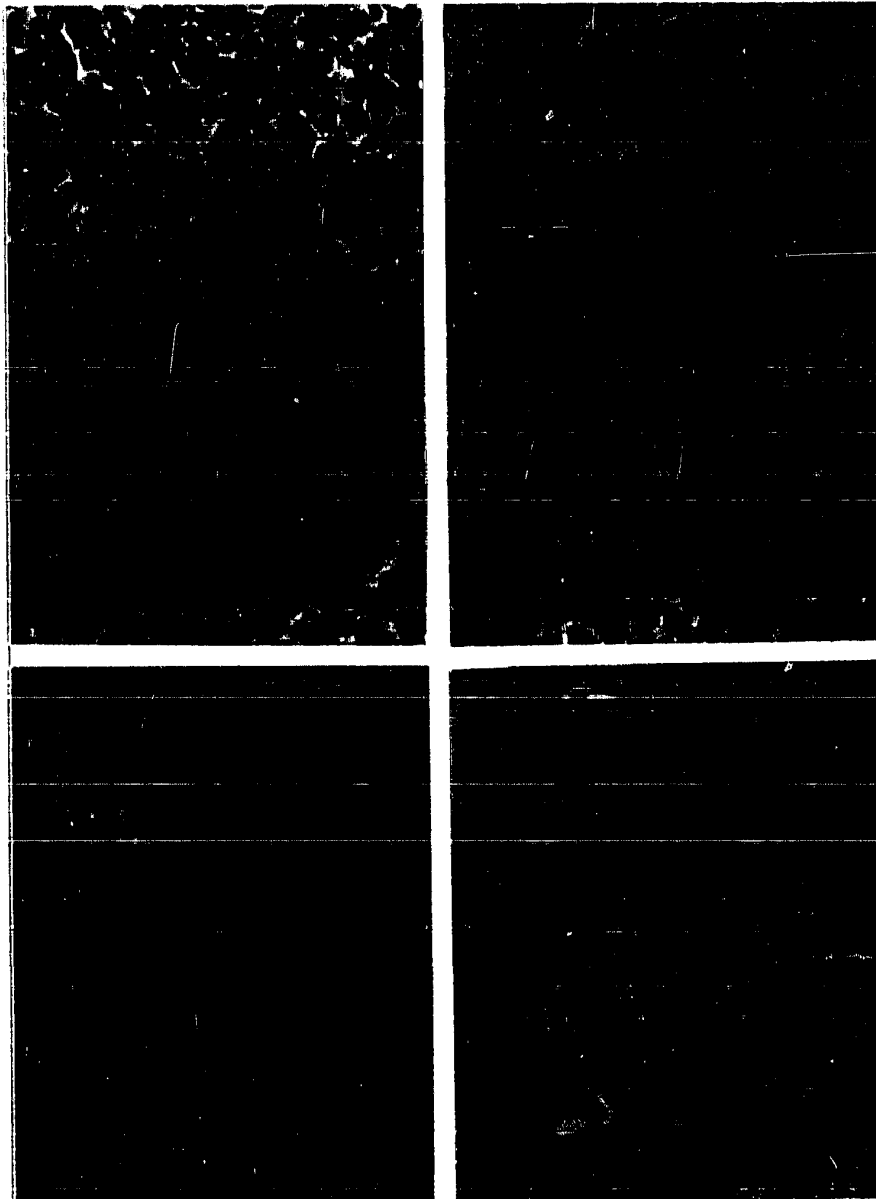
Thymic architecture was unremarkable, except for group 3. In this group, thymocyte depletion occurred at the cortico-medullary junction and as a result, the reticular cells in this region became visible (figure 5).

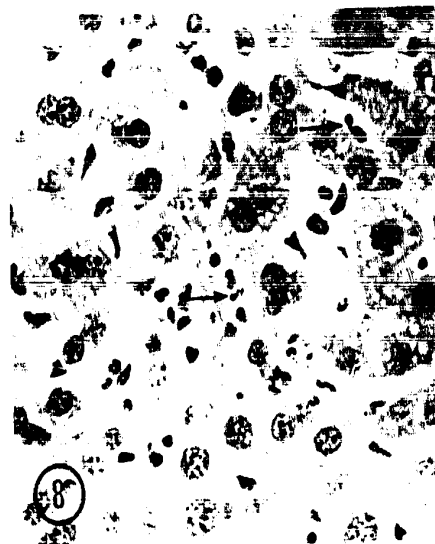
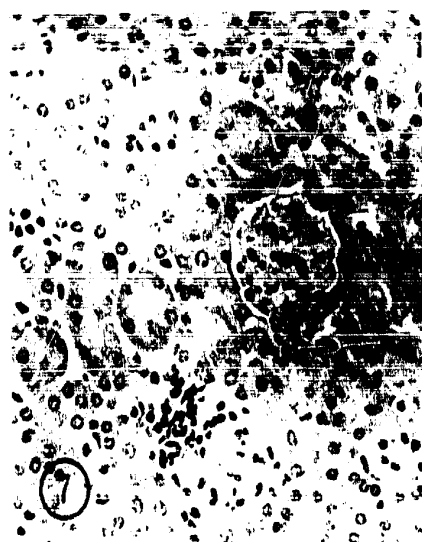
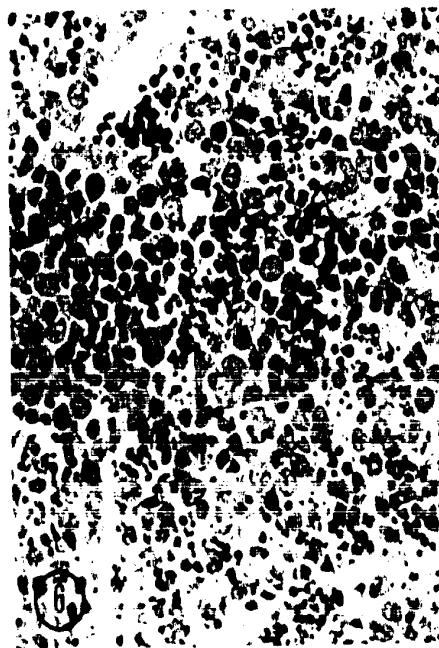
Figure 5 Small lymphocyte depletion at the cortico-medullary junction in the thymus (C) following four intraperitoneal ATS injections. Reticular cells visible. 500 x. H and E stain.

Figure 6 Polymorphonuclear leukocyte (arrows) invasion in the thymus of C57Bl/J mice receiving four intraperitoneal injections of ATS. Cellular debris is also evident. 500 x. H and E stain.

Figure 7 Glomerular swelling after four ATS injections intraperitoneally. Renal tubules are unaffected. 500 x. H and E stain.

Figure 8 Phagocytosis of small lymphocytes and cellular debris (arrows) by Kupffer cells. 500 x. H and E stain.





In the cortex of some group 2 and 3 mice, there were large amounts of cellular debris as well as some polymorphonuclear leukocytes (figure 6). No such observations were made in group 1 and in groups 4 to 6.

iii. Kidney

The structure of the kidney, like that of the thymus, was generally unaffected by ATS treatment. Minor changes did occur in group 3 where some glomerular swelling was found (figure 7). In some cases, the swollen capillary tufts appeared to completely fill Bowman's capsule; however, these changes were not consistent. The tubules were histologically unremarkable.

II. THE EFFECTS OF ATS ON SWISS MICE

a. Survival

Swiss mice receiving ATS, iATS or NHS at daily or at three day intervals, either intraperitoneally or subcutaneously, showed no evidence of distress (Tables VI, VII, VIII and IX). Similar findings were obtained for groups 19 and 20 on the intravenous schedule (Table X). Group 21 mice, however, exhibited all the symptoms described for group 3 mice of the C57Bl/J strain, namely weight loss, hematuria, symptoms of jaundice, diarrhea, hunching of the back and loss of body hair.

Thirty percent of the mice in this group died before sacrifice. No mice in group 22 survived the prescribed intravenous regimen.

b. Effects on Total Body Weight

i. Intraperitoneal treatment

Total body weight changes in these groups are summarized in Tables VI and VII. Increased body weight was least pronounced in group 1 mice; however, no consistent pattern was evident among the different groups except that animals sacrificed three days after the final injection gained considerably more weight than in any other group, including the controls.

Weight increases for the iATS and NHS groups on the three day interval schedule were comparable to those of the daily schedules.

ii. Subcutaneous treatment

A summary of weight changes for these groups of animals is presented in Tables VIII and IX. ATS treated mice sacrificed three days after the final injection (groups 13 and 16) gained considerably more weight than group 10 mice which were sacrificed one day after the final injection of a daily schedule.

Mice receiving iATS at three day intervals (group 17) showed the largest weight increases of all the

subcutaneously treated mice. Groups 11 and 14; 12, 15 and 18 which received iATS and NHS respectively had comparable weight increases (Tables VIII and IX).

No individual weight loss occurred among any of the animals receiving the three sera either intraperitoneally or subcutaneously.

iii. Intravenous treatment

A summary of body weight changes in intravenously treated mice is presented in Table X. Percentage weight losses were recorded for groups 20 and 21. The average weight loss more than doubled when three intravenous injections of ATS were given instead of two. All group 22 mice which received four intravenous injections of ATS died before examination and no weight changes could be determined.

c. Organ Weights

i. Intraperitoneal treatment

The variations in serum administration resulted in varying degrees of hepatic weight increase (Tables VI and VII). ATS produced significant increases in hepatic weight with all three schedules (groups 1, 4 and 7). The two other sera also produced significant increases in liver weight, but only when a three day interval was interposed between the final injection and

sacrifice (groups 5 and 6, Table VI; groups 8 and 9, Table VII).

The greatest percentage weight changes occurred in the spleen. The average weight of the spleen increased four-fold in group 1 mice after ATS treatment compared to a less than two-fold increase when a three day interval was introduced between the final injection and sacrifice (groups 1 and 4, Table VI). Injections of ATS at three day intervals also resulted in marked splenomegaly as did injections of iATS, but to a lesser extent (groups 7 and 8, Table VII).

Thymic weight changes did not reach significant levels with any of the three sera used. A significant decrease in the mesenteric lymph node weights of mice on the daily schedules (groups 1 and 4, Table VI) was contrasted by the significant increase in the weights of this organ following ATS treatment on the three day interval schedule (group 7, Table VII). Lymph node weights of mice receiving iATS and NHS remained at levels comparable to the untreated controls.

ii. Subcutaneous treatment

Administration of all three sera resulted in increased hepatic weight. The greatest increases were observed following the three day interval schedule (Tables VIII and IX).

ATS administration produced significant increases in splenic weight; both daily schedules resulted in a more than two-fold weight increase (groups 10 and 13, Table VIII). Similar significant increases occurred in the spleen of group 16 animals (Table IX). Splenic weight increases were within a narrow range following the three different subcutaneous injection schedules. The two other sera did not significantly increase splenic weight.

Thymic weight changes were comparable to those of the untreated controls, as was the case following intraperitoneal treatment. In the mesenteric lymph nodes however, a significant weight decrease occurred in mice receiving ATS daily (groups 10 and 13, Table VIII). The weight of this organ remained unchanged in mice receiving the active serum at three day intervals (group 16, Table IX). An unexplained weight loss was recorded for the mesenteric lymph nodes of group 17 mice. Aside from this one discrepancy, iATS and NHS had no effect on the weight of this organ.

iii. Intravenous treatment

One intravenous injection (group 19) did not result in significant splenomegaly or hepatomegaly. Increases in liver and spleen weights became significant in groups 20 and 21 (Table X). This was contrasted by

TABLE VI

TOTAL BODY AND ORGAN WEIGHTS OF SWISS MICE GIVEN ACTIVE ATS, INACTIVE ATS,
OR NHS i.p. (MEANS $\pm$ S.E.M.)

Group Number	Sera Used	Number of Doses	% Change in T.B.W.	Liver Weight % T.B.W.	Spleen Weight % T.B.W.	Thymus Weight % T.B.W.	Lymph Node Weight % T.B.W.
1	A) ATS	4	+5.4	6.28 $\pm$.20 [●]	1.82 $\pm$.18 [⊙]	.29 $\pm$.02	.059 $\pm$.007 [⊙]
2	iATS	4	+18.4	6.09 $\pm$.34	.51 $\pm$.06	.32 $\pm$.02	.086 $\pm$.005
3	NHS	4	+16.7	5.88 $\pm$.11	.47 $\pm$.04	.34 $\pm$.02	.097 $\pm$.003
4	B) ATS	4	+26.6	6.72 $\pm$.23 [⊙]	.83 $\pm$.07 [⊙]	.27 $\pm$.01	.064 $\pm$.007 [●]
5	iATS	4	+24.2	6.51 $\pm$.08 [●]	.41 $\pm$.03	.33 $\pm$.01	.080 $\pm$.006
6	NHS	4	+19.5	6.29 $\pm$.11 [●]	.45 $\pm$.03	.34 $\pm$.02	.092 $\pm$.008
23	Untreated Control	-	+12.5	5.49 $\pm$.11	.47 $\pm$.02	.30 $\pm$.03	.093 $\pm$.002

T.B.W. Total Body Weight -

S.E.M. Standard Error of the Mean -

A) Given Sera for 4 consecutive days. Sacrifice 1 day after final injection

B) Given sera for 4 consecutive days. Sacrifice 3 days after final injection

● P .02 - .01

⊙ P .01 - .001

⊙ P < .001

TABLE VII

TOTAL BODY AND ORGAN WEIGHTS OF SWISS MICE GIVEN ACTIVE ATS, INACTIVE ATS,
OR NHS AT 3 DAY INTERVALS i.p.
(MEANS $\pm$ S.E.M.)

Group Number	Sera Used	Number of Doses	% Change in T.B.W.	Liver Weight % T.B.W.	Spleen Weight % T.B.W.	Thymus Weight % T.B.W.	Lymph Node Weight % T.B.W.
7	ATS	3	+12.8	7.53 $\pm$.45 [⊙]	1.42 $\pm$.10 [⊙]	.24 $\pm$.03	.135 $\pm$.007 [⊙]
8	iATS	3	+24.8	6.85 $\pm$.23 [⊙]	.70 $\pm$.07 [●]	.25 $\pm$.02	.108 $\pm$.010
9	NHS	3	+19.6	6.99 $\pm$.14 [⊙]	.65 $\pm$.05	.28 $\pm$.02	.081 $\pm$.006
23	Untreated Control	-	+12.5	5.49 $\pm$.11	.47 $\pm$.02	.30 $\pm$.03	.093 $\pm$.002

T.B.W. - Total Body Weight ● P .01 - .001 ⊙ P < .001

S.E.M. - Standard Error of the Mean

TABLE VIII

TOTAL BODY AND ORGAN WEIGHTS OF SWISS MICE GIVEN ACTIVE ATS, INACTIVE ATS, OR NHS S.C.
(MEANS $\pm$ S.E.M.)

Group Number	Sera Used	Number of Doses	% Change in T.B.W.	Liver Weight % T.B.W.	Spleen Weight % T.B.W.	Thymus Weight % T.B.W.	Lymph Node Weight % T.B.W.
10	A) ATS	4	+10.1	6.78 $\pm$.15 [●]	1.16 $\pm$.12 [●]	.29 $\pm$.01	.059 $\pm$.004 [●]
11	iATS	4	+16.7	6.48 $\pm$.21 [●]	.55 $\pm$.03	.34 $\pm$.03	.081 $\pm$.005
12	NHS	4	+20.2	6.04 $\pm$.13 [●]	.54 $\pm$.02	.32 $\pm$.02	.095 $\pm$.006
13	B) ATS	4	+21.3	6.92 $\pm$.40 [●]	.98 $\pm$.13 [●]	.30 $\pm$.02	.055 $\pm$.004 [●]
14	iATS	4	+19.8	6.26 $\pm$.29 [●]	.42 $\pm$.09	.31 $\pm$.01	.087 $\pm$.007
15	NHS	4	+16.2	6.40 $\pm$.21 [●]	.52 $\pm$.06	.28 $\pm$.03	.096 $\pm$.008
23	Untreated Control	-	+12.5	5.49 $\pm$.11	.47 $\pm$.02	.30 $\pm$.03	.093 $\pm$.002

T.B.W. - Total Body Weight S.E.M. - Standard Error of the Mean

A) Given sera for 4 consecutive days. Sacrifice 1 day after final injection
B) Given sera for 4 consecutive days. Sacrifice 3 days after final injection

● P .02 - .01

● P .01 - .001

TABLE IX

TOTAL BODY AND ORGAN WEIGHTS OF SWISS MICE GIVEN s.c. INJECTIONS OF ACTIVE ATS,
INACTIVE ATS, OR NHS AT 3 DAY INTERVALS
(MEANS $\pm$ S.E.M.)

Group Number	Sera Used	Number of Doses	% Change in T.B.W.	Liver Weight % T.B.W.	Spleen Weight % T.B.W.	Thymus Weight % T.B.W.	Lymph Node Weight % T.B.W.
16	ATS	3	+18.5	7.89 $\pm$.54	1.30 $\pm$.14	.23 $\pm$.02	.096 $\pm$.010
17	iATS	3	+24.0	7.17 $\pm$.25	.61 $\pm$.08	.25 $\pm$.02	.062 $\pm$.004
18	NHS	3	+19.5	7.37 $\pm$.18	.64 $\pm$.06	.25 $\pm$.02	.119 $\pm$.012
23	Untreated Control	-	+12.5	5.49 $\pm$.11	.47 $\pm$.02	.30 $\pm$.03	.093 $\pm$.002

T.B.W. - Total Body Weight • P .01 - .001 ⊙ P < .001

S.E.M. - Standard Error of the Mean

TABLE X

TOTAL BODY AND ORGAN WEIGHTS OF SWISS MICE GIVEN i.v. INJECTIONS OF ACTIVE ATS
AT DAILY INTERVALS (MEANS $\pm$ S.E.M.)

Group Number	Sera Used	Number of Doses	% Change in T.B.W.	Liver Weight % T.B.W.	Spleen Weight % T.B.W.	Thymus Weight % T.B.W.	Lymph Node Weight % T.B.W.
19	ATS	1	+ .04	6.02 $\pm$.27	.64 $\pm$.06	.21 $\pm$.02	.056 $\pm$.007 [•]
20	ATS	2	-5.20	6.98 $\pm$.27 [•]	.70 $\pm$.02	.26 $\pm$.02	.066 $\pm$.006
21	ATS	3	-11.50	6.95 $\pm$.29 [•]	1.14 $\pm$.09 [•]	.14 $\pm$.01 [•]	.058 $\pm$.002 [•]
22	ATS	4	All mice died during experimental period				
23	Untreated Control	-	+12.50	5.49 $\pm$.11	.47 $\pm$.02	.30 $\pm$.03	.093 $\pm$.002

T.B.W. Total Body Weight - [•] P .02-01, [•] P .01-.001, [•] P < .001

S.E.M. Standard Error of the Mean

the consistent decrease in the mesenteric lymph node weight in the three groups examined. The thymic weight remained constant in groups 19 and 20, but decreased significantly in group 21.

d. Histological Changes

i. Intraperitoneal treatment

A. Liver

The hepatic architecture was not markedly affected by the three schedules previously described. A few parenchymal cells were in various stages of mitosis, and there appeared to be increased numbers of Kupffer cells. These changes were accompanied by dilations of the hepatic sinusoids. Small lymphocytes and cellular debris were found in these spaces; some of the latter was also found in the phagocytic cells bordering the sinusoids (figure 8). The amount of debris was most pronounced in group 1 mice and least evident in the livers of group 7 mice. Similar histological findings resulted with iATS and NHS on both the daily and three day interval schedules.

B. Spleen

The spleen, as in the case of C57Bl/J mice, showed marked histological changes. Group 1 mice had

excessive red pulp hyperplasia with concomitant increases in the number of megakaryocytes and myeloid blast cells, a decrease in the size of the lymph nodules, periarteriolar and perinodular small lymphocyte depletion, and an increase in the number of pyroninophilic cells in the red pulp. In the nodules, large lymphocytes and cellular debris were evident in the region of periarteriolar depletion.

Group 4 spleens had less extensive areas of red pulp hyperplasia, nor were the splenic nodules as depleted. Large numbers of megakaryocytes and pyroninophilic cells were evident in the red pulp. The lymph nodules were evident in the red pulp. The lymph nodules contained some large lymphocytes, cellular debris, and germinal centres with mitotic figures (figures 9a and 9b). The ratio of the area of the germinal centre to the lymph nodule was greater in ATS treated animals than in mice receiving either iATS or NHS. Except for very slight periarteriolar depletion and the appearance of some large lymphocytes, iATS and NHS (groups 2 and 5; 3 and 6) produced no marked histological alterations (figure 10).

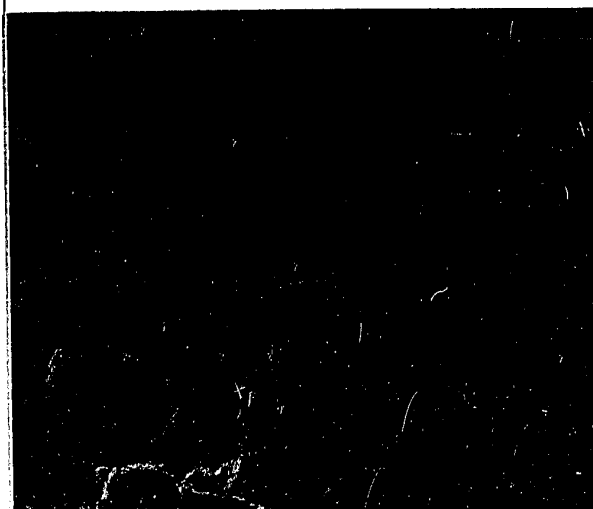
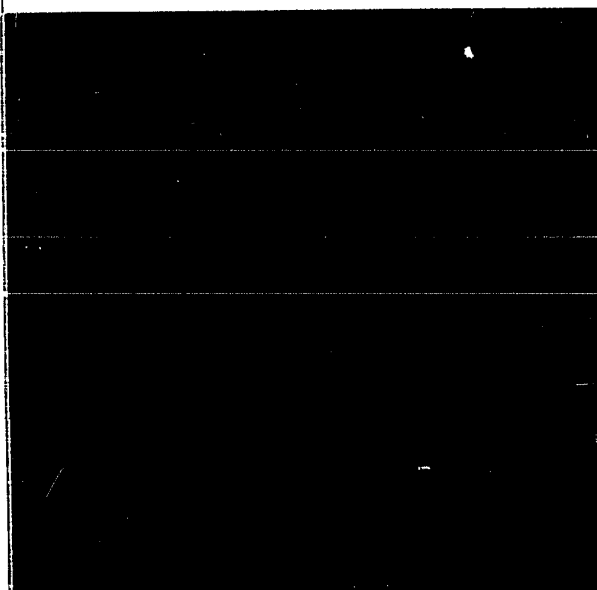
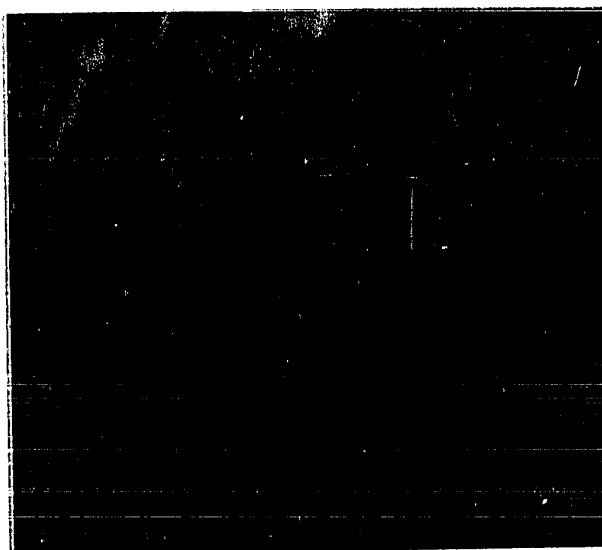
C. Thymus

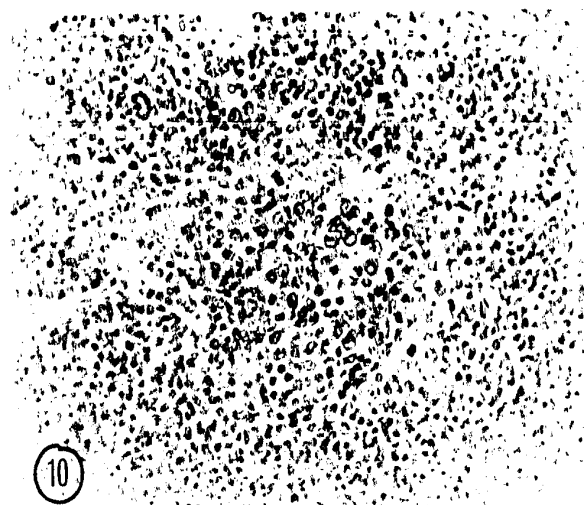
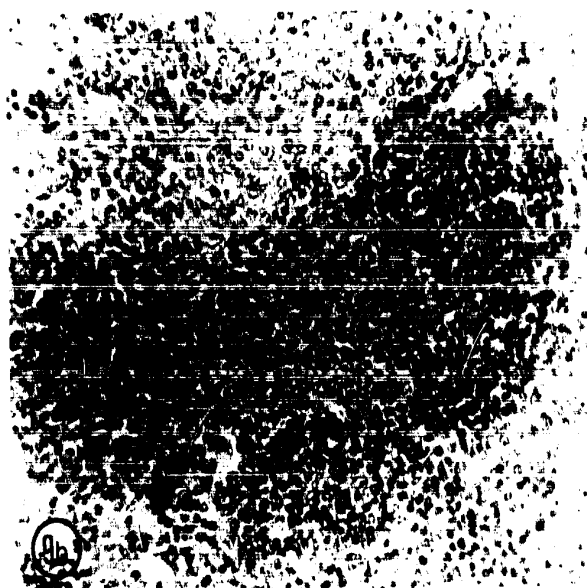
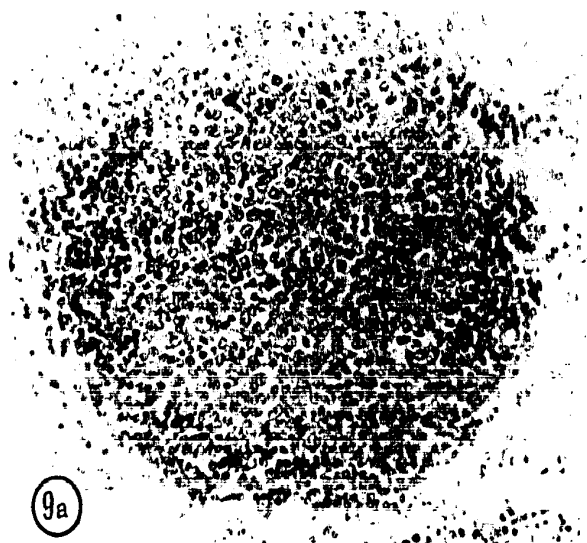
No changes in thymic architecture were seen following treatment with iATS and NHS. ATS, on the other hand, produced a slight degree of small lymphocyte depletion

Figure 9a Germinal centre in splenic white pulp.
Swiss mouse treated four times with ATS
intraperitoneally. Note propensity of large
lymphocytes, blast cells and mitotic figures.
500 x. Methyl green - pyronin stain.

Figure 9b Small germinal centre in the spleen of
the same mouse (9a). Note depletion in
diffuse lymphatic tissue and pyroninophilic
cells in red pulp. 500 x. Methyl green -
pyronin stain.

Figure 10 Splenic germinal centre of a mouse treated
with NHS. Note size of the germinal centre
relative to the nodule. 500 x. Methyl green -
pyronin stain.





at the cortico-medullary junction with the resultant visualization of the reticular cells.

D. Mesenteric Lymph Node

In addition to the spleen, the mesenteric lymph node showed significant histological changes after ATS treatment. In group 1 mice, deep cortical depletion of small lymphocytes occurred (figure 11); the architecture of the subcapsular region was unchanged, however. Isolated clusters of small lymphocytes appeared in the depleted regions (figure 12), and the post-capillary venules, which normally are obscured by small lymphocytes, were clearly demarcated by these cells (figure 13). The lymph nodules of the subcapsular region contained germinal centres and some large lymphocytes, while the depleted deep cortical region contained pyroninophilic cells and cellular debris (figure 14). Group 4 mice had a lesser degree of deep cortical depletion. The lymph nodules contained more large lymphocytes than in group 1, and some pyroninophilic cells within distinctive germinal centres. Most of the pyroninophilic cells were confined to the medullary region.

In group 7 mice, ATS treatment produced cellular hyperplasia in the medullary region of the lymph node. Reticular cells and pyroninophilic cells were much

Figure 11 Deep cortical depletion in the mesenteric lymph node following four ATS injections. The subcapsular region is intact. 250 x. Toluidine blue.

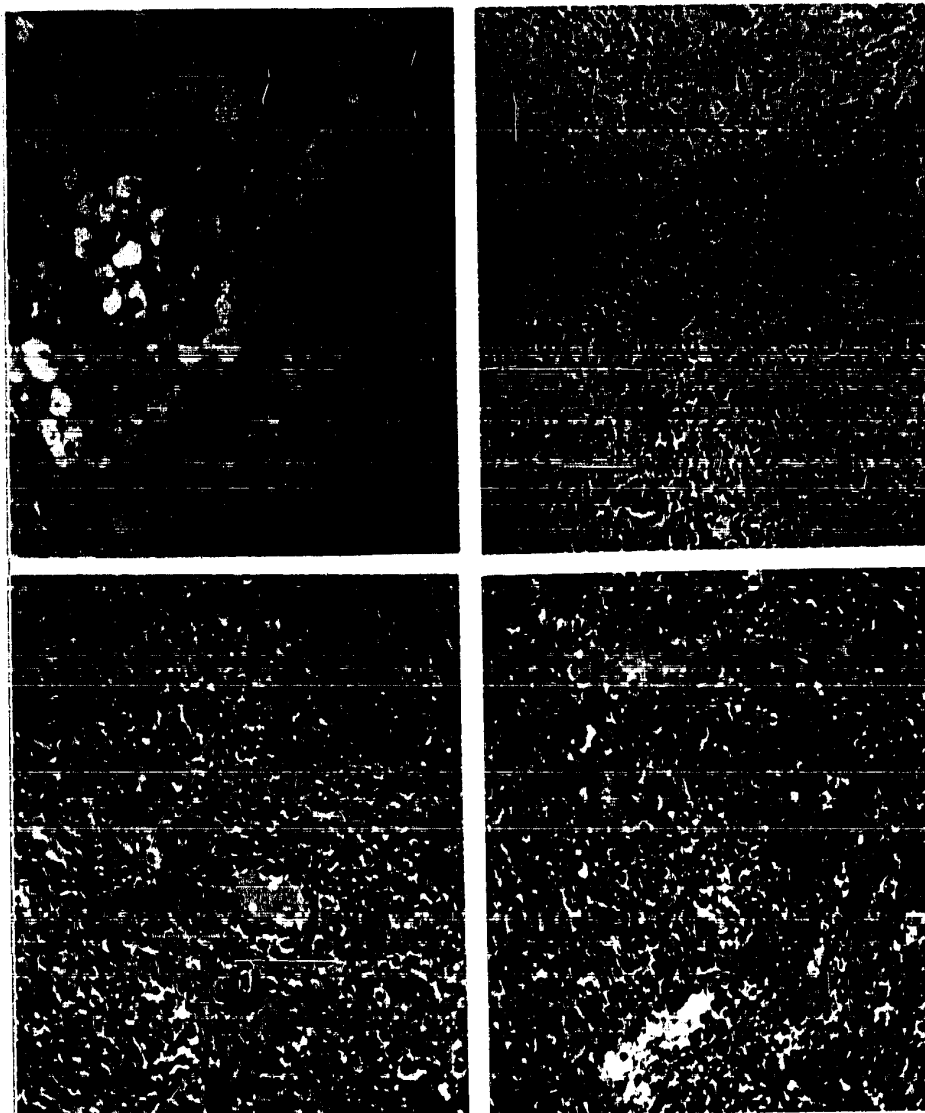
Figure 12 Lymphocyte cluster in the small lymphocyte depleted deep cortical region. Such cellular groupings are not uncommon and are usually seen in association with capillaries and venules (arrows). 250 x. H and E stain.

Figure 13 Post - capillary venules clearly demarcated by deep cortical depletion following four ATS injections. 250 x. H and E stain.

Figure 14 Letters mark three distinct regions in mesenteric lymph node following ATS treatment:

- D - intact subcapsular cortex
- E - slightly enlarged germinal centre with blast cells, large lymphocytes and mitotic figures
- F - depleted deep cortical region

250 x. H and E stain.



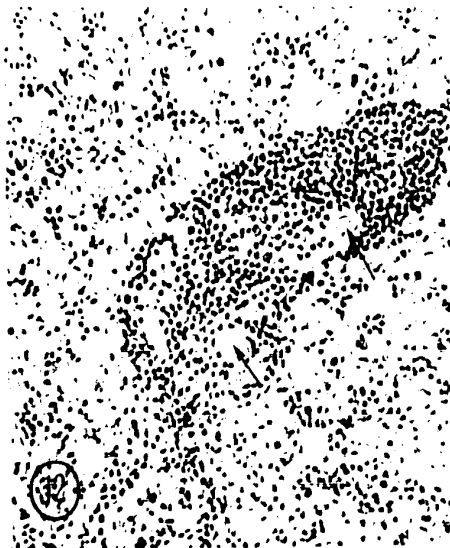
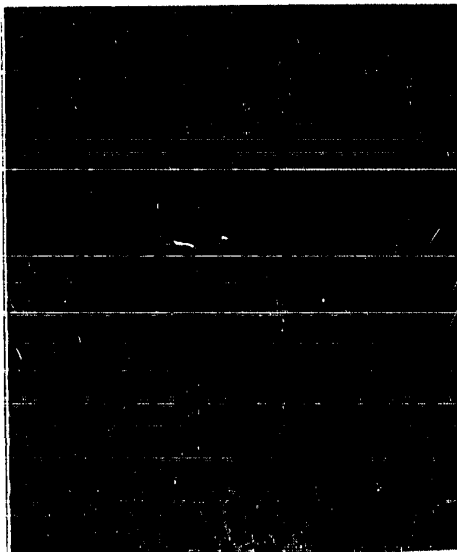
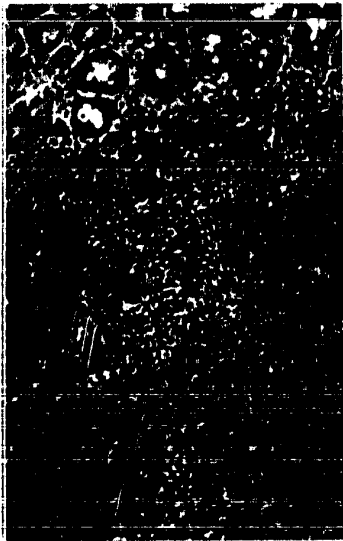


Figure 15 Peyer's patch following intraperitoneal ATS treatment. Few large lymphocytes, pyknotic nuclei and cellular debris in the perifollicular region. 125 x. H and E stain.

Figure 16 Bone marrow of a Swiss mouse after four intraperitoneal ATS injections. Note decreased number of small lymphocytes. Myeloid and erythroid populations appear to be normal. 500 x. Dilute Wright stain.

Figure 17 Bone marrow of a Swiss mouse after four intraperitoneal NHS injections. More small lymphocytes are evident than in figure 16. 500 x. Dilute Wright stain.





more numerous in this region, resulting in a cellular density approximating that of the cortex. Cortical nodules and germinal centres were partially obscured by this proliferation, but did appear to be larger than in the controls. The two other sera did not produce significant changes in lymph node architecture.

E. Other Tissue

The Peyer's patches of the ileum and the bone marrow of the tibiae were also examined. Small lymphocyte depletion in Peyer's patches was minimal with all three injection schedules used (figure 15), thus differing markedly from the patterns observed in the mesenteric lymph node. Bone marrows appeared to have more myeloid blast cells after ATS treatment (figure 16), while the number of small lymphocytes declined slightly in comparison to the control group (figure 17). Treatment with iATS and NHS produced no significant changes.

ii. Subcutaneous treatment

A. Liver and Thymus

Changes in the liver and thymus were comparable to those previously described in the intraperitoneally treated mice.

B. Spleen

Spleens of group 10 mice had depleted lymph nodules which contained a few large lymphocytes and some cellular debris, while in the red pulp, magakaryocytes, pyroninophilic cells and myeloid blast cells were present. Red pulp hyperplasia was not as extensive as in mice of group 1. Spleens of mice sacrificed three days after the final injection of either the daily or three day interval schedules (groups 13 and 16) differed only slightly. Lymphocyte depletion was not as pronounced; however, the number of large lymphocytes remained constant as did the volume of the red pulp. Pyroninophilic cells were present in the nodules and the red pulp. Some mitotic figures were also evident in the nodules of group 13 and 16 mice spleens.

C. Mesenteric Lymph Node

In the mesenteric lymph nodes, deep cortical depletion was pronounced in group 10 and 13 animals. Large lymphocyte numbers in the above two groups were comparable. Germinal centres and cellular debris were readily noticeable in the nodules. Pyroninophilic cells were found in the germinal centres and deep cortical regions, while in group 13, these cells were in the

germinal centres and medulla. In some group 16 lymph nodes, ATS treatment produced a cellular hyperplasia in the medulla. Lymph nodules and germinal centres appeared to be larger than in the controls. Pyroninophilic cells were found in the same regions as reported for group 13 mice.

Except for the occasional large lymphocyte observed in the nodules of the spleen and lymph node, iATS and NHS produced no significant histological alterations.

iii. Intravenous treatment

The architecture of all organs examined was most severely altered in mice receiving ATS intravenously. After two injections, there was an accumulation of cellular debris and lymphocytes in the hepatic sinusoids with a resultant increase in the phagocytosis of this material by the Kupffer cells. Group 20 spleens had pronounced periarteriolar and perinodular depletion (figure 18), while in the mesenteric lymph nodes of this group, marked deep cortical depletion was evident. Thymocytes were depleted at the cortico-medullary junction in the thymus which resulted in the visualization of the reticular cells.

After three intravenous injections, pronounced changes occurred in the spleen, mesenteric lymph node and thymus.

A. Spleen and Mesenteric Lymph Node

Group 21 spleens had extensive red pulp hyperplasia; however, many of the erythrocytes in this region were only "ghosts" (figure 19). Myeloid blast cells and megakaryocytes were greatly reduced in the red pulp. Lymph nodules had greatly distorted shapes and sizes. Whereas the two other routes had produced small lymphocyte depletion in the nodules, intravenously treated mice showed nodules composed of small compact cells. These cells were identified by the Feulgen technique as small lymphocytes (figure 20).

In the deep cortical region of the mesenteric lymph node, there was a lesser degree of depletion than in group 1 mice which had received ATS intraperitoneally. This region contained many compact cells similar to those described for the spleen which were identified as small lymphocytes.

Germinal centres and large lymphocytes were largely lacking in the organs of group 21 mice.

B. Thymus

In contrast to the spleen and mesenteric lymph node, three intravenous injections of ATS produced a sharp decrease in the number of lymphocytes in the thymus (figure 21). Reticular cells and a number of large blast cells were easily recognizable in the depleted cortex. Cellular debris was also found in this region. The medulla contained large numbers of lymphocytes. Many were found clustered around the venules and small veins of this region. There also appeared to be an increased number of Hassall's corpuscles (figure 22).

C. Other Tissue

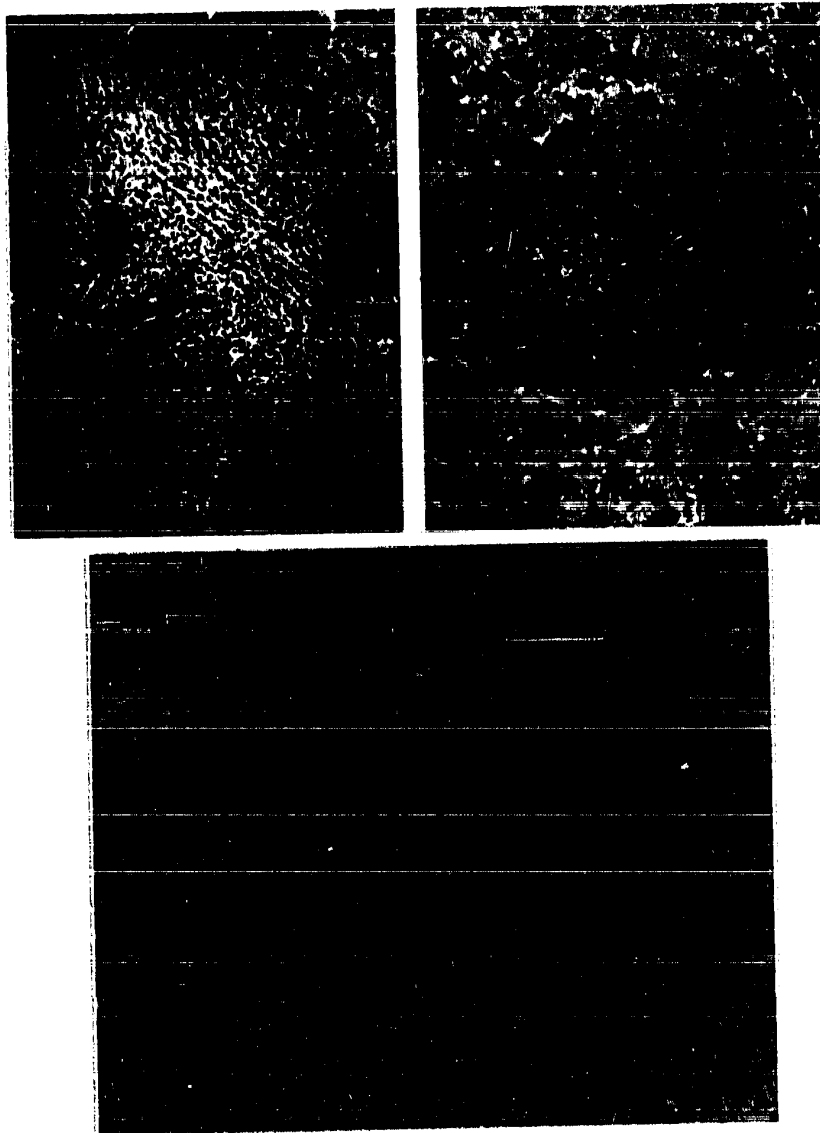
Peyer's patches and bone marrow were also examined from group 21 animals. There appeared to be a slight depletion of small lymphocytes in the Peyer's patches following intravenous ATS treatment (figure 23) and in the bone marrow the small lymphocyte population was greatly reduced (figure 24). Giant cells (figure 25) and abnormal mitotic figures (figure 26) were observed amongst the cells of the bone marrow.

In summary, ATS affects both C57Bl/J and Swiss strains of mice in a similar fashion thereby indicating a species-specific rather than strain-specific effect.

Figure 18 Splenic nodule of a Swiss mouse after two intravenous injections of ATS. Note the pronounced periarteriolar and perinodular depletion. 250 x. H and E stain.

Figure 19 Splenic nodule after three intravenous injections of ATS. Erythroid hyperplasia in the red pulp characterized by red cell "ghosts". 250 x. H and E stain.

Figure 20 Splenic nodule after three intravenous injections of ATS. Nodule composed mostly of small lymphocytes. 250 x. Feulgen - fast green.



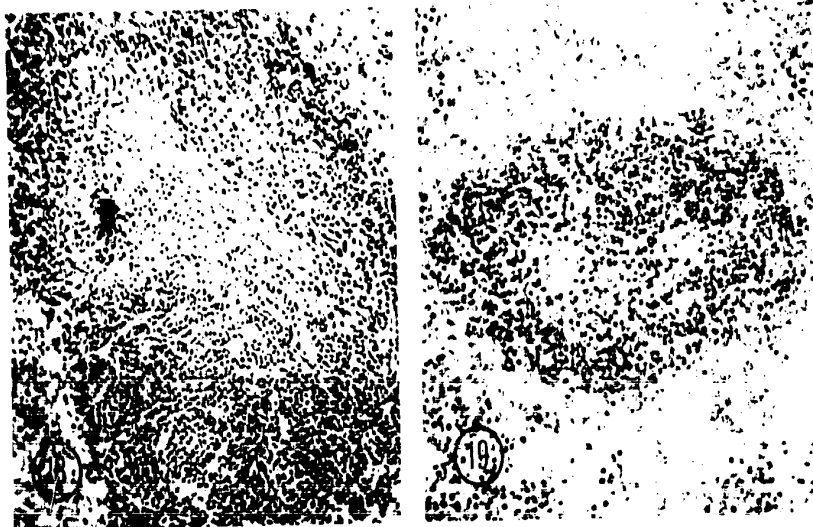
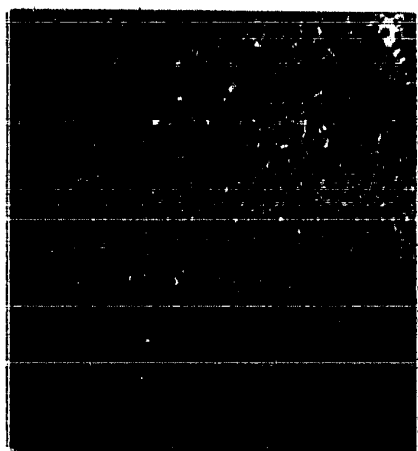


Figure 21 Thymus following three intravenous injections of ATS. The cortex has been severely depopulated while the medulla contains increased numbers of small lymphocytes. Note the cellular debris evident in the cortex (arrow). 125 x. H and E stain.

Figure 22 Medulla of the same thymus (figure 21). The concentration of small lymphocytes in this region has increased. Note increased numbers of Hassall's corpuscles and the lymphocyte accumulation around the vein. 250 x. H and E stain.

Figure 23 Peyer's patch following three intravenous injections of ATS. Small lymphocytes predominant; blast cells, pyknotic nuclei, cellular debris also evident. 125 x. H and E stain.



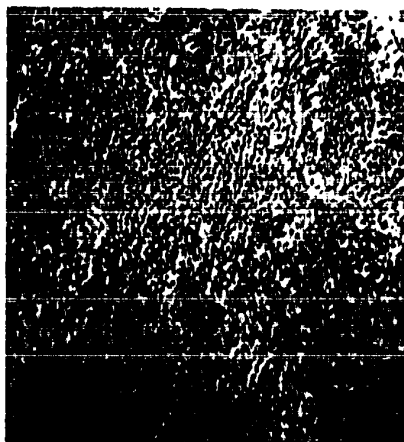


Figure 24 Bone marrow following three intravenous injections of ATS. Small lymphocyte population depleted. Some abnormal myeloid cells (arrow) are present. 500 x. Dilute Wright stain.

Figure 25 Giant cell within the marrow cell population of figure 24. 1250 x. Dilute Wright stain.

Figure 26 Abnormal mitotic figure (arrow) within the marrow cell population of figure 24. 1250 x. Dilute Wright stain.





The route by which ATS is administered is important. Subcutaneous injections produce gradual histological changes which persist over longer periods of time. This is contrasted by the dramatic effects of intravenously injected ATS which results in pronounced histological changes and in many cases, death.

III. THE EFFECTS OF ATS ON YOUNG C57B1/J AND SWISS MICE

A. Survival

All mice in the fifteen groups survived intraperitoneal serum administration (Table XI).

b. Effects on Total Body Weight

There were marked increases in groups 3, 4, 10 and 13. Weight increases in the other groups were less pronounced.

c. Effects on Organ Weight

Thymic weights did not vary significantly from those of the untreated controls in any of the fifteen groups. This was contrasted by the increase in splenic weights following ATS treatment, reaching significant levels for groups 3, 4, 8, 10, 13 and 14. These groups had received at least two injections of ATS (Table XI).

TABLE XI THE EFFECTS OF ATS ON THE ORGAN AND BODY
WEIGHTS OF YOUNG C57B1/J AND SWISS MICE

THE EFFECTS OF ATS ON THE ORGAN AND BODY WEIGHTS OF YOUNG C57 BL/J AND SWISS MICE

Group No.	Sera Used	Number of Injections	Age (in days) at sacrifice	% Change in T.B.W. over Control	Thymus		Spleen	
					Treated % of T.B.W.	Control % of T.B.W.	Treated % of T.B.W.	Control % of T.B.W.
1	Diluted ATS♦	1	1	2.2	.44 ± .03	.46 ± .02	.22 ± .02	.23 ± .01
2	"	2	2	3.8	.45 ± .02	.47 ± .02	.32 ± .03	.25 ± .02
3	"	3	3	7.9	.40 ± .03	.44 ± .03	.41 ± .02⊙	.29 ± .03
4	"	4	4	12.7	.41 ± .02	.43 ± .01	.50 ± .03⊙	.31 ± .01
5	"	1	2	1.9	.42 ± .02	.47 ± .02	.32 ± .03	.25 ± .02
6	"	2	3	4.4	.40 ± .03	.44 ± .03	.36 ± .03	.29 ± .02
7	"	1	3	3.7	.41 ± .02	.44 ± .03	.34 ± .02	.29 ± .02
8	"	2	4	3.9	.38 ± .02	.43 ± .01	.39 ± .02⊙	.31 ± .01
9	"	1	4	4.6	.38 ± .03	.43 ± .01	.34 ± .03	.31 ± .01
10	"	4	7	8.8	.39 ± .02	.38 ± .02	.59 ± .04●	.29 ± .02
11	"	2	7	2.9	.37 ± .03	.38 ± .02	.39 ± .03	.29 ± .02
12	"	1	7	3.1	.35 ± .02	.38 ± .02	.34 ± .02	.29 ± .02
13	ATS	4	14	9.7	.36 ± .03	.36 ± .02	.69 ± .04●	.30 ± .03
14	ATS	2	14	5.2	.34 ± .02	.36 ± .02	.43 ± .02⊙	.30 ± .03
15	ATS	1	14	3.3	.33 ± .01	.36 ± .02	.34 ± .02	.30 ± .03

T.B.W. Total Body Weight

⊙ P .02 - .01

* For each group, untreated littermates were used as controls

⊙ P .01 - .001

♦ 10% ATS in physiological saline

● P < .001

d. Histological Changes

i. Thymus

Thymic architecture in the fifteen groups of mice treated with ATS was comparable to that of the untreated controls.

ii. Spleen

Untreated littermates from each of the fifteen groups of mice were used as control. The first sign of splenic organization in the untreated controls appeared in three day old mice where a cellular alignment was observed around small arterioles. One day later, the periarteriolar population of cells consisting of small lymphocytes started to form lymph nodules. Many nodules were observed in one week old mice where the number of small lymphocytes had increased markedly. In addition to small lymphocytes, two week old splenic nodules also contained large lymphocytes and germinal centres.

After one ATS injection, spleens of group 1 mice already showed early signs of splenic nodule formation (figure 27). This development was easily recognized in two day old spleens in which nodules containing small and large lymphocytes were noticeable (figure 28). Spleens of three day old mice (group 3) were not as well organized

as those of group 2. Lymph nodules contained few small lymphocytes; the majority of the cells in the nodules were large lymphocytes. In group 4 mice, no lymphoid nodules were observed. Most of the cells around the arterioles were large lymphocytes while in the rest of the spleen, many reticular cells and megakaryocytes were seen (figure 29).

Groups receiving two ATS injections (6, 8, 11 and 14) had periarteriolar depletion in the developing lymph nodules. In this depleted region, large lymphocytes and cellular debris were observed. Germinal centres were present within the nodules and were separated from the depleted periarteriolar region by small lymphocytes.

In groups 10 and 13, periarteriolar small lymphocyte depletion resulted following four ATS injections (figure 30). The small lymphocyte depletion was more severe in these two groups as the large lymphocytes of the periarteriolar region and of the germinal centre merged (figure 31). The nodules in these two groups were smaller than in their respective controls while the volume of the red pulp was greatly enlarged.

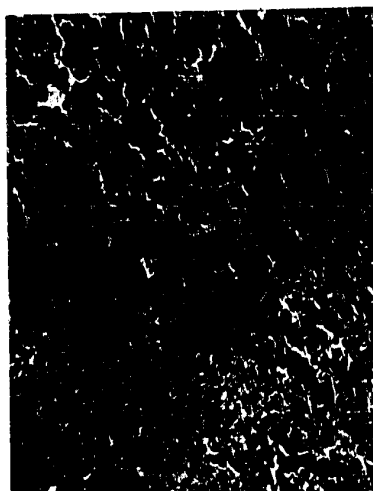
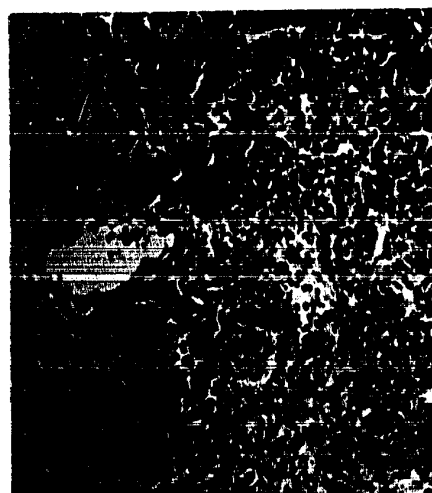
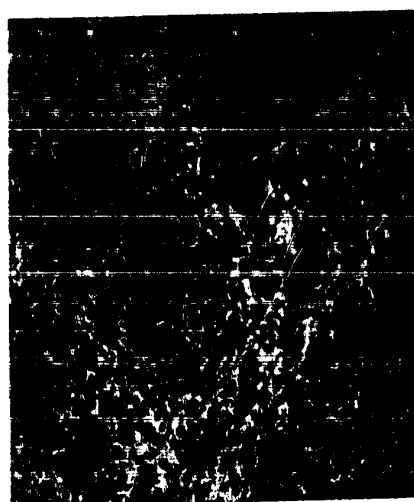
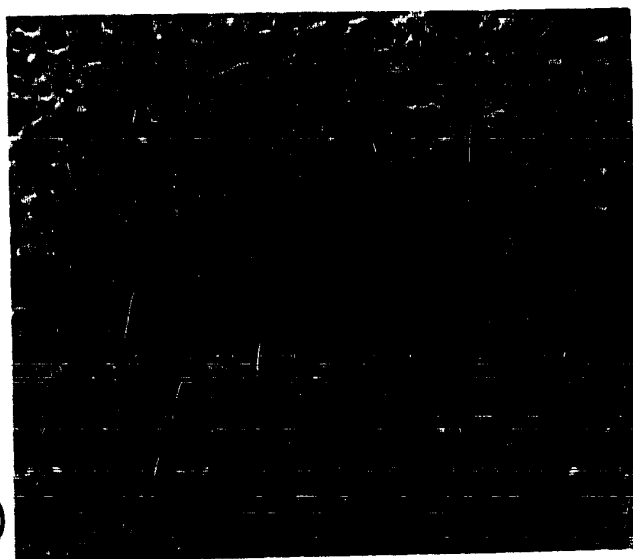
Figure 27 Spleen from a one-day old mouse following one intraperitoneal injection of ATS. Note the developing lymph nodule (arrow) and the abundance of mast cells throughout. 500 x. Toluidine blue.

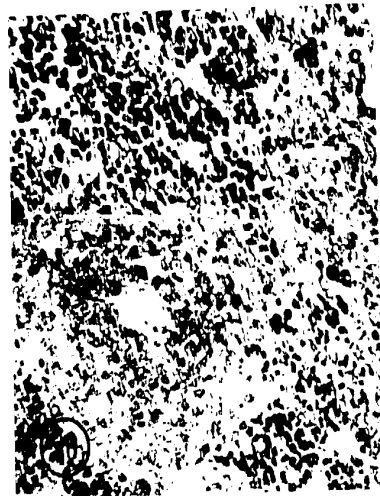
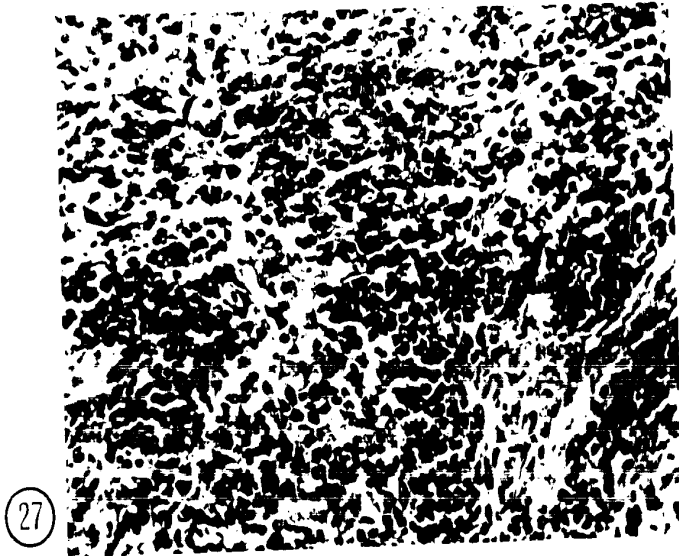
Figure 28 Spleen from a two-day old mouse following two intraperitoneal injections of ATS. Note beginning organization of lymph node. 500 x. H and E stain.

Figure 29 Spleen from a four-day old mouse following four intraperitoneal injections of ATS. No lymph nodules are evident. Splenic architecture is disorganized. Note the large number of blast cells in the white pulp. 500 x. H and E stain.

Figure 30 Splenic lymph nodule from a seven-day old mouse treated four times with ATS. Periarteriolar depletion and blast cell transformation are evident. 500 x. H and E stain.

(27)





e. Effects on Mast Cells

In the controls, mast cell counts were highest at birth (Table XII). Between birth and one week of age, the number of mast cells decreased gradually; thereafter, their number levelled off.

One injection of ATS produced an increase in the number of mast cells in a given splenic area (figure 27). From these levels which were only slightly higher than the controls, the mast cell count in these groups (1, 5, 7, and 9) decreased in a pattern similar to that of their respective controls, reaching comparable levels in two weeks (group 15).

Two ATS injections produced significantly higher numbers of mast cells in groups 2, 6 and 8 (figure 32). From these markedly higher levels, the number of mast cells decreased and reached the control level within two weeks.

Significant increase in the number of splenic mast cells was also observed in groups receiving three and four ATS injections (3, 4, 10 and 13). In mice less than seven days old (3, 4 and 10), the increase in the disorganized spleen was threefold (figure 33). However, in two week old animals, four ATS injections had only a minimal effect on the splenic mast cell count as their number approximated the level of their respective controls (group 13).

TABLE XII
THE EFFECT OF ATS ON THE SPLENIC MAST CELL COUNT

Group Number	Age (in days) at Time of Sacrifice	Mast Cell Count	
		Treated	Control
1	1	160 (1)	149
2	2	217 (2) *	108
3	3	271 (3) *	85
4	4	241 (4) *	57
5	2	130 (1) θ	108
6	3	134 (2) Δ	85
7	3	96 (1)	85
8	4	89 (2) Δ	57
9	4	74 (1) θ	57
10	7	47 (4) *	10
11	7	21 (2) Δ	10
12	7	19 (1) Δ	10
13	14	14 (4) θ	9
14	14	9 (2)	9
15	14	11 (1)	9

θ P .02 - .01

Δ P .01 - .001

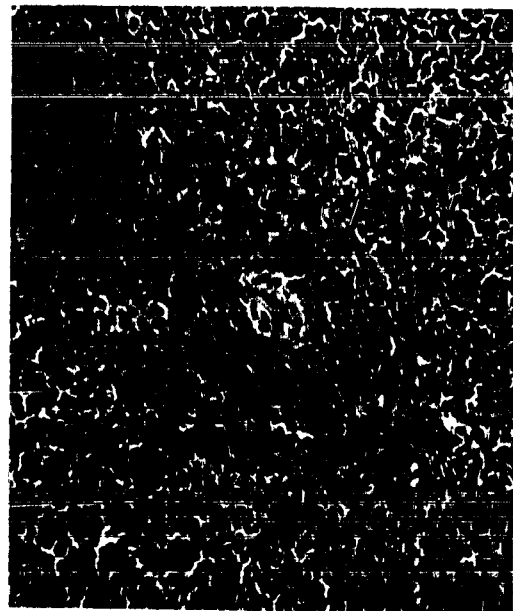
* P < .001

Number of injections in parenthesis.

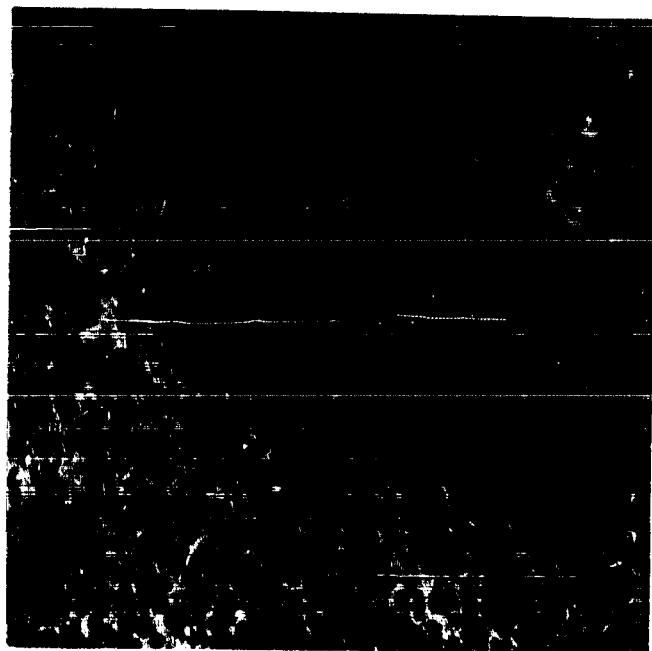
Figure 31 Splenic lymph nodule from a two week old mouse treated four times with ATS. Small degree of periarteriolar depletion in the well formed nodule. 250 x. H and E stain.

Figure 32 Two-day old spleen following two ATS injections. Mast cells situated concentrically around the lymph nodule and in the red pulp. 500 x. Toluidine blue.

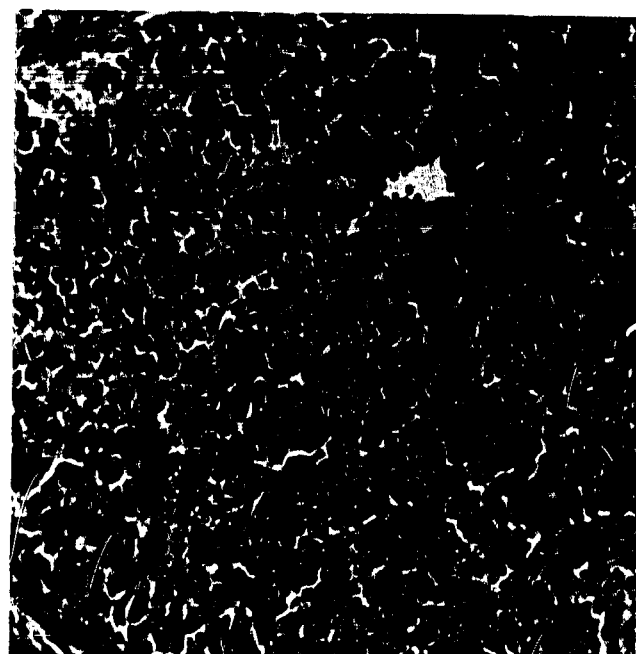
Figure 33 Four-day old spleen following four ATS injections. Splenic architecture disorganized. Blast cells (arrow) and large numbers of mast cells are evident. 500 x. Toluidine blue.



31



32

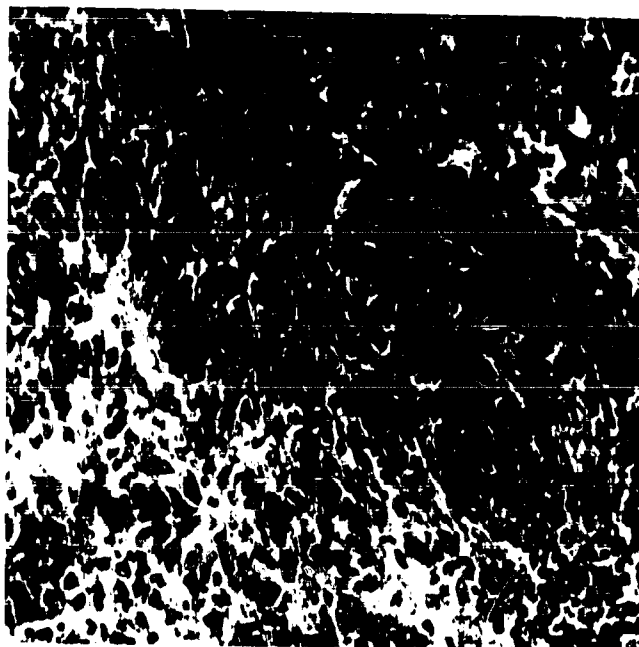


33

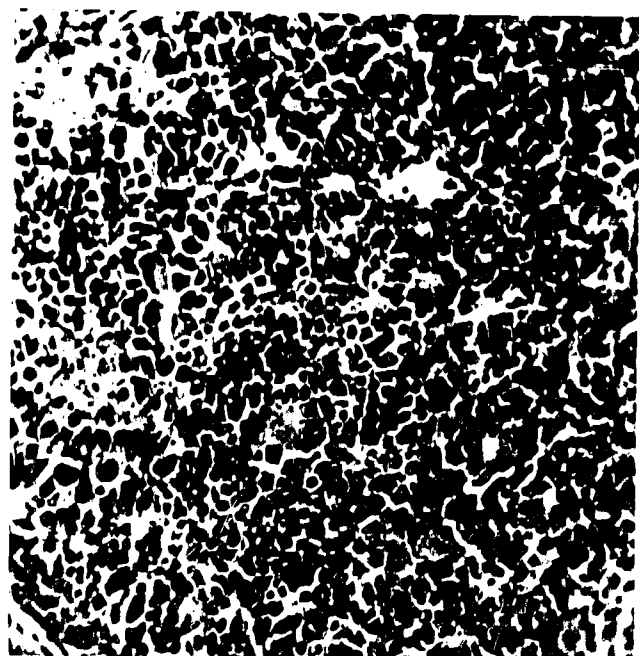
31



32



33



In summary, ATS administration to young mice was accompanied by significantly higher mast cell counts in the spleen. As the animals grew older, the number of splenic mast cells decreased in a pattern similar to that of the untreated controls. At two weeks of age, ATS injections did not produce the significant increases that were observed in the younger groups.

IV. THE EFFECTS OF ATS ON THE PHAGOCYTIC ACTIVITY OF HEPATIC AND SPLENIC MACROPHAGES IN ADULT SWISS MICE

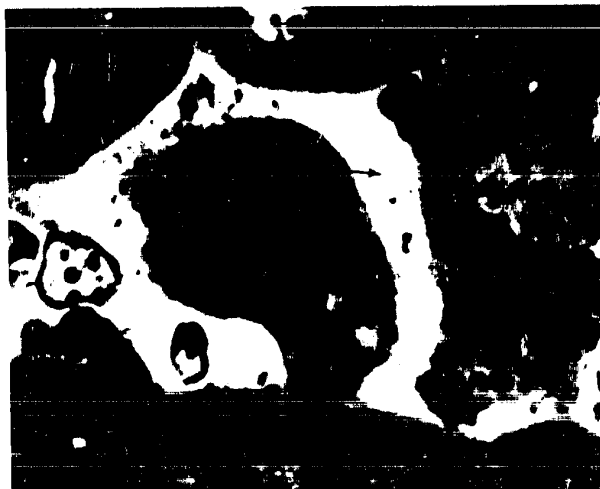
a. Latex Particles (Set A)

After one ATS injection, Kupffer cells were found to contain ingested small lymphocytes, cellular debris and some polystyrene latex particles (Table XIII). The majority of the visible particles was in the hepatic sinusoids (figure 34). After two ATS injections, more phagocytes were observed with ingested cellular debris, while the uptake of latex particles was minimal (figure 35). This pattern reversed itself after four ATS injections (groups 3 and 9, set A). The Kupffer cells of these groups contained ingested latex particles with only minimal amounts of cellular debris noted in either the phagocytes or in the hepatic sinusoids (figure 36).

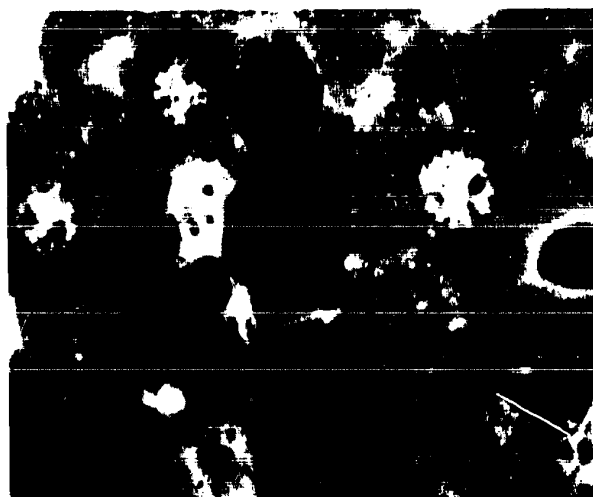
Figure 34 Phagocytosis of latex particles by Kupffer cells in an untreated control animal. Latex particles (arrow) are found within the sinusoids and in the cytoplasm of the Kupffer cells. 1250 x. Toluidine blue.

Figure 35 Aggregated lymphocytes adhere to the Kupffer cells following two ATS injections. Phagocytosis of latex particles is reduced. 1250 x. Toluidine blue.

Figure 36 Phagocytosis of latex particles as well as that of small lymphocytes and cellular debris is observed following four ATS injections. 1250 x. Toluidine blue.



34



35



36

TABLE XIII
PHAGOCYTOSIS OF POLYSTYRENE LATEX PARTICLES BY KUPFFER CELLS

Group Number (Set A)	Sera Used	Injection Schedule	Number of Injections	Kupffer Cells Containing Latex (% of Control)
1	ATS		1	49 ± 2.4 *
2	ATS		2	14 ± 0.2 *
3	ATS	Daily	4	85 ± 5.3
4	NHS		1	52 ± 1.6 *
5	NHS		2	32 ± 0.9 *
6	NHS		4	88 ± 7.2
7	ATS		1	53 ± 2.3 *
8	ATS		2	22 ± 0.3 *
9	ATS	Two-day Intervals	4	81 ± 4.4 *
10	NHS		1	59 ± 1.4 *
11	NHS		2	33 ± 1.1 *
12	NHS		4	87 ± 5.9

* P < .01 * P < .001

Normal horse serum had a similar effect as ATS. An initial decrease in the number of phagocytosed latex particles after two NHS injections (groups 5 and 11, set A) was followed by a return to near control levels in groups 6 and 12 which received four NHS injections. Most of the material taken up by the Kupffer cells after two NHS injections was cellular debris and small lymphocytes; after four injections, the uptake of polystyrene latex particles was predominant.

b. Imferon (Set B)

i. Phagocytic Activity

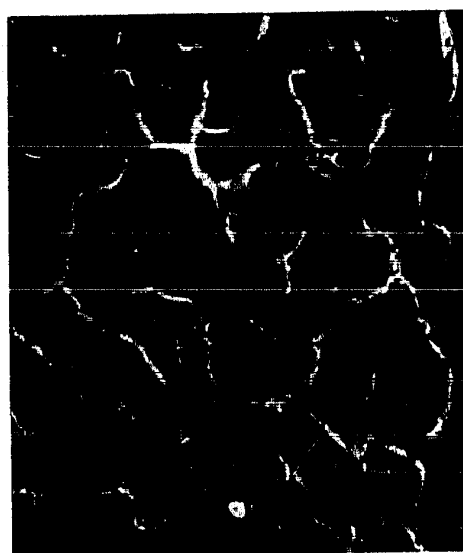
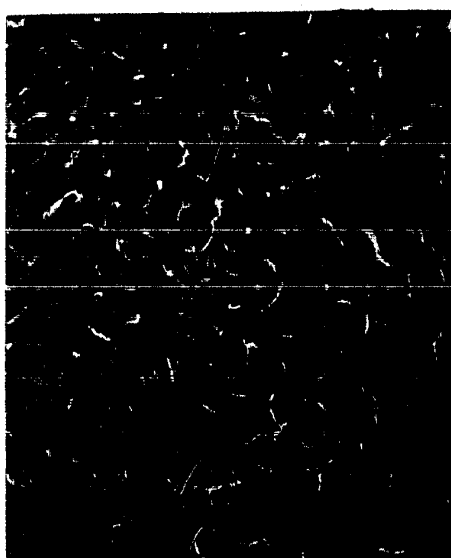
The uptake of Imferon by Kupffer cells was significantly depressed in mice which received one ATS injection (group 1, figure 37). A further decrease was obtained in group 2 mice. The macrophages contained small lymphocytes and cellular debris, while the Imferon was mostly confined to sinusoids (figure 38). After four injections on the daily regimen, an increase in the uptake of Imferon was noted in group 3 surpassing the levels of the untreated controls. Kupffer cells were gorged with the ferric complex; cellular debris was minimal in both the sinusoids and in the hepatic phagocytes (figures 39 and 40).

Figure 37 Phagocytosis of Imferon by Kupffer cells following one injection of ATS. 500 x. Perls' Prussian blue - Safronin O.

Figure 38 Phagocytosis of Imferon by Kupffer cells following two ATS injections is greatly reduced in comparison to figure 37. 250 x. Perls' Prussian blue - Safronin O.

Figure 39 Four ATS injections resulted in greatly enhanced phagocytosis of Imferon by the Kupffer cells. 250 x. Perls' Prussian blue - Safronin O.

Figure 40 Phagocytosis of Imferon following four ATS injections. Note the phagocytosis of some cellular debris as well (arrow). 500 x. Perls' Prussian blue - Safronin O.





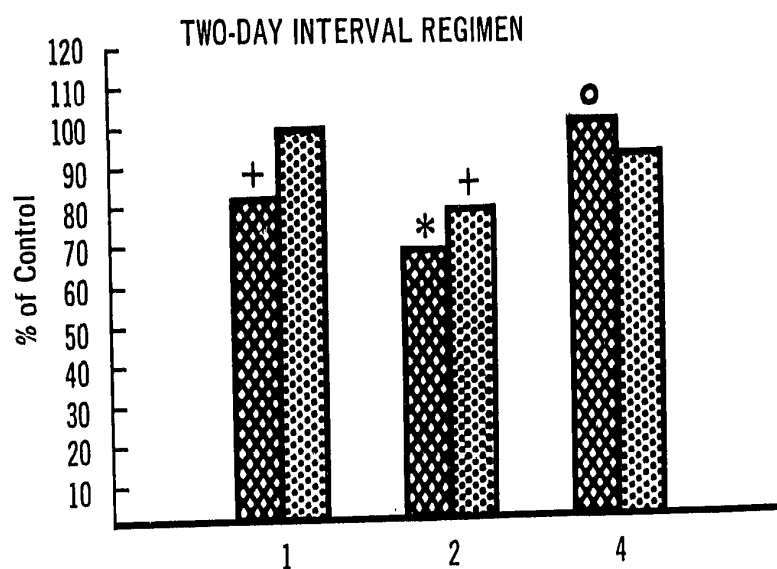
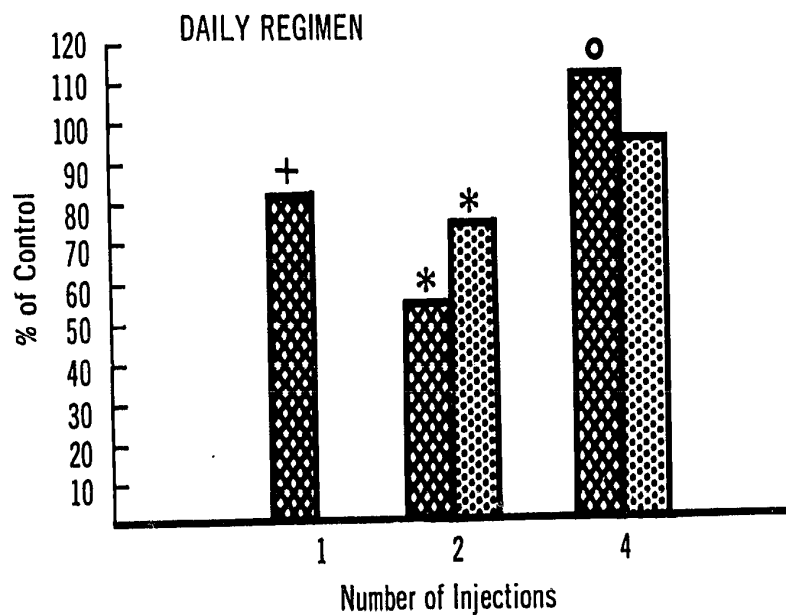
The pattern of initial decline followed by increased phagocytosis surpassing the levels of the controls was also observed in mice on the two day interval schedule (groups 7 to 9). Similar patterns of decline and recovery were obtained using NHS daily (groups 4 to 6) and at two day intervals (groups 10 to 12). This pattern is presented in figure 41.

In the spleen, the number of cells containing Imferon decreased after one ATS injection (group 1; set B). Further decrease occurred after two and four injections (groups 2 and 3). These findings are presented in figure 42. Splenic macrophages containing Imferon clearly demarcated the lymph nodules (figure 43 and 44). This demarcation began to fade with each successive administration of ATS, reaching a low after four injections when only a few Imferon-containing cells were visible. Similar patterns persisted in mice receiving ATS at two-day intervals.

Two NHS injections also produced a decrease in the number of phagocytic cells, but after four injections (group 6), their number increased (figure 45). A similar pattern was obtained in animals receiving NHS at two day intervals (groups 10 to 12). The fading demarcation of lymph nodules which occurred after four ATS injections (groups 3 and 9) was contrasted by a return to more clearly

Figure 41 Phagocytosis of Imferon by hepatic
Kupffer cells.

PHAGOCYTOSIS OF IMFERON BY HEPATIC KUPFFER CELLS
(Area: 1.46 mm²)



ATS NHS (o) P.02-.01 (+) P.01-.001 (*) P < .001

PHAGOCYTOSIS OF IMFERON BY HEPATIC KUPFFER CELLS
(Area: 1.46 mm²)

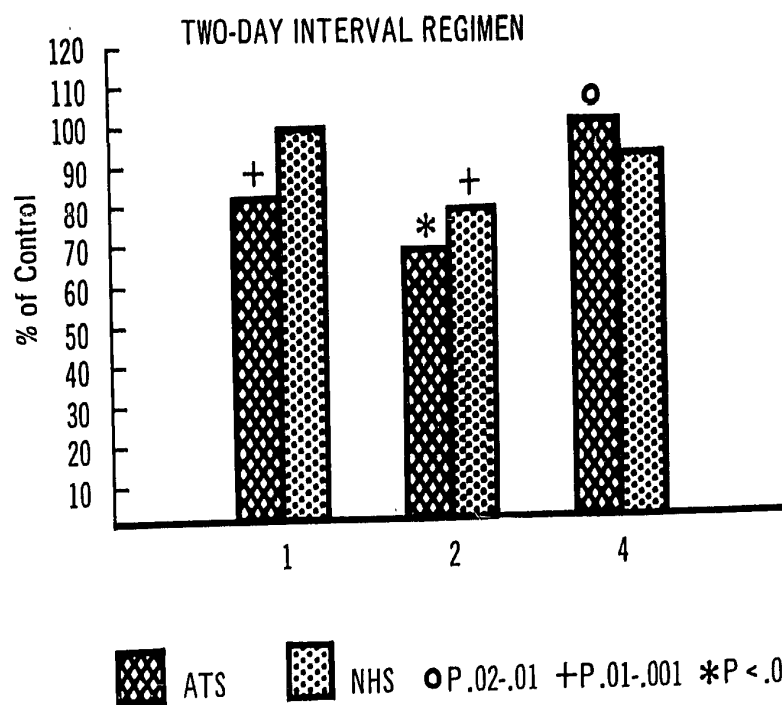
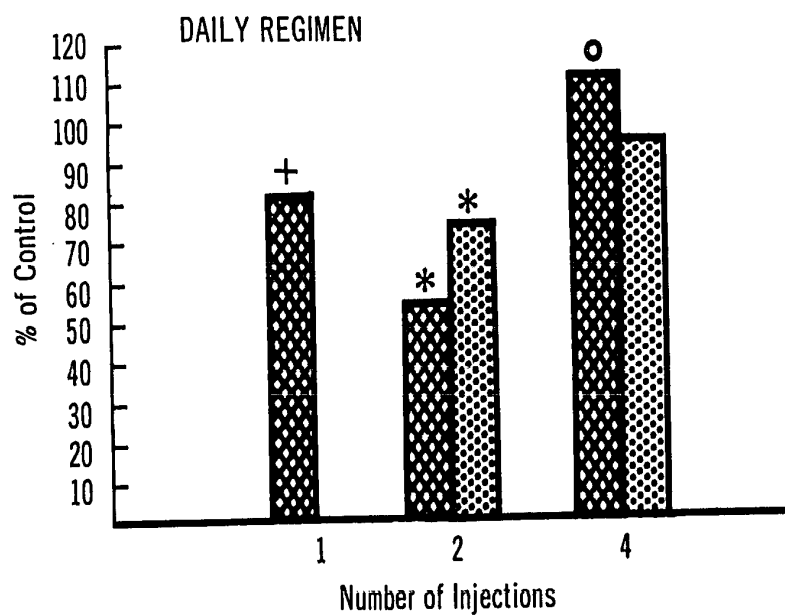
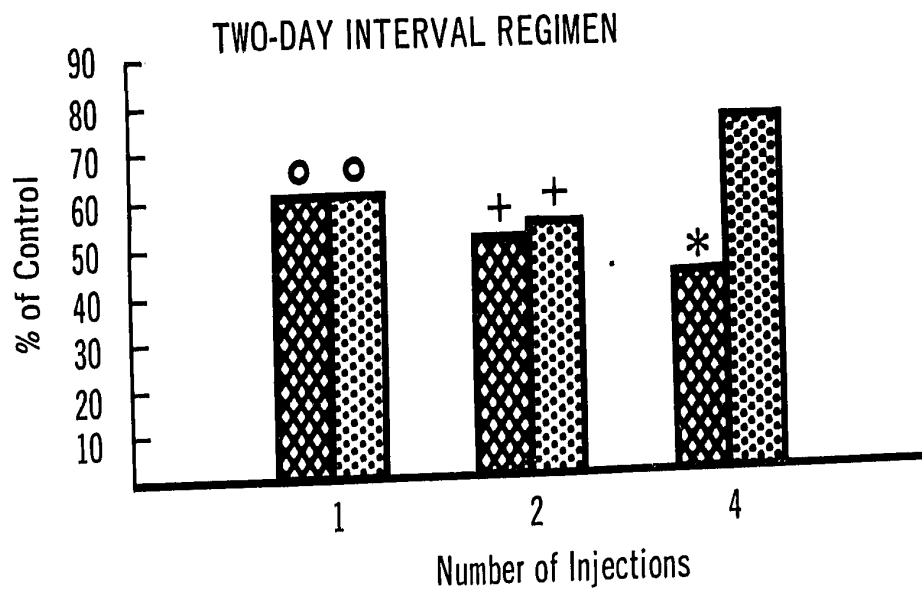
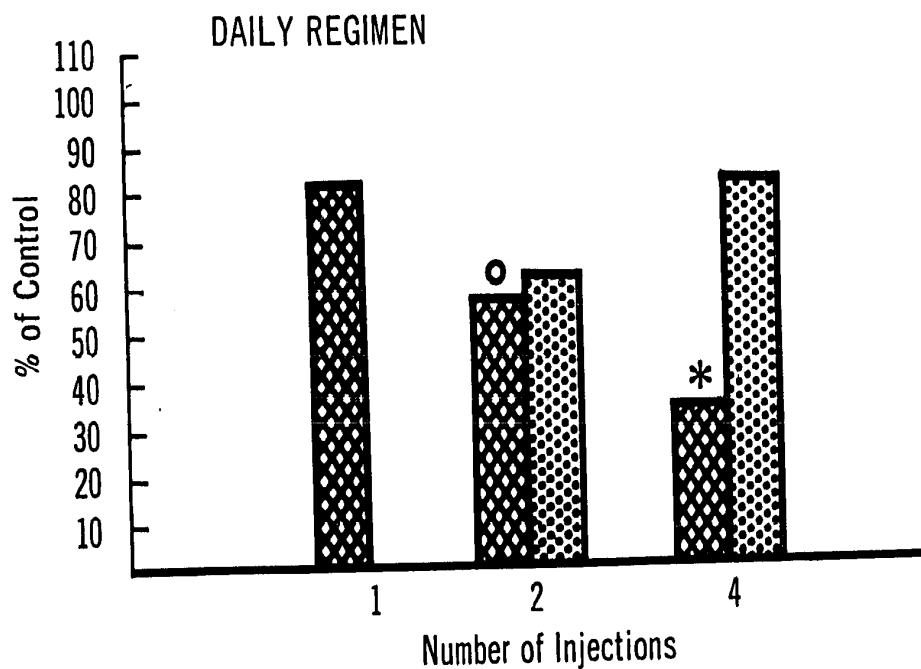


Figure 42 Phagocytosis of Imferon by splenic
macrophages.

PHAGOCYTOSIS OF IMFERON BY SPLENIC MACROPHAGES (Area: 1.45 mm²)



ATS



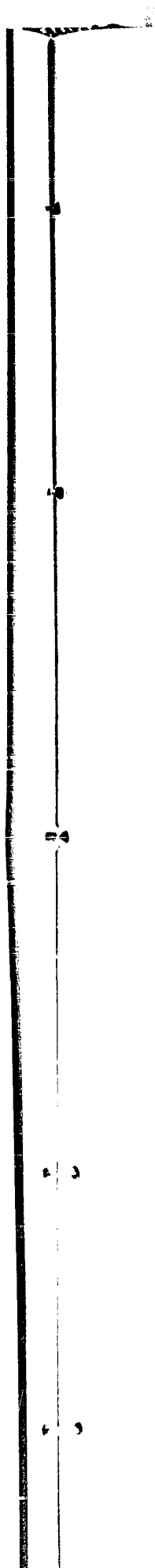
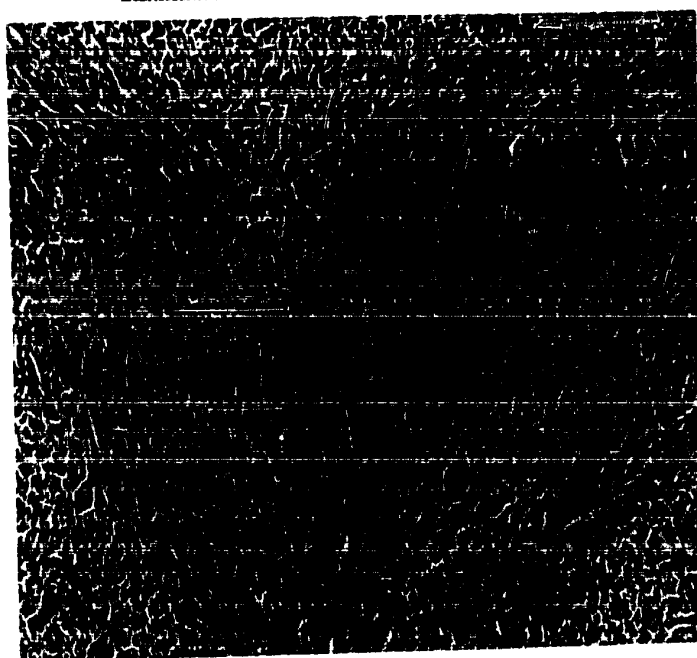
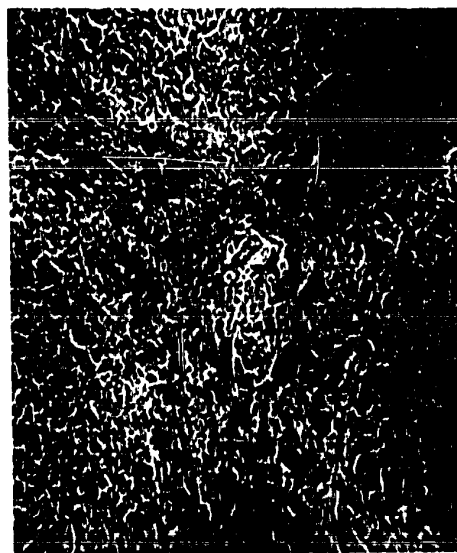
NHS

[○]P.02-.01 ⁺P.01-.001 ^{*}P<.001

Figure 43 Phagocytosis of Imferon by splenic macrophages in the diffuse lymphatic tissue. Untreated control. 250 x. Perls' Prussian blue - Safronin O.

Figure 44 Decreased phagocytosis of Imferon by splenic macrophages following one ATS injection. 250 x. Perls' Prussian blue - Safronin O.

Figure 45 Phagocytosis of Imferon by splenic macrophages following four NHS injections. Number of phagocytosing cells comparable to the number in figure 44. 250 x. Perls' Prussian blue - Safronin O.



demarcated nodules after four NHS injections (groups 6 and 12).

In summary, two injections of ATS produced a decrease in the phagocytosis of particulate matter in both the Kupffer cells and splenic macrophages. After four ATS injections, an increase in phagocytosis by Kupffer cells occurred whereas in the spleen, decreased phagocytic activity persisted. NHS treatment produced an initial decline followed by a recovery in phagocytic activity in both the Kupffer cells and splenic macrophages.

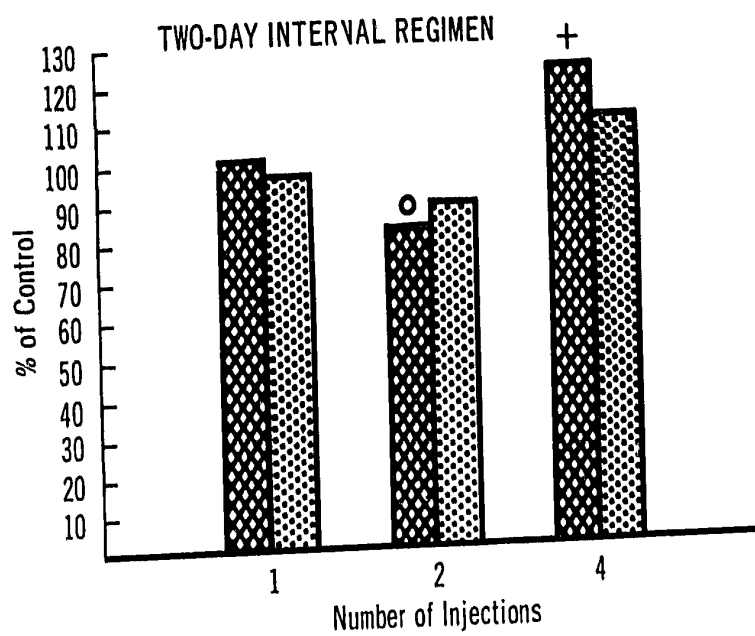
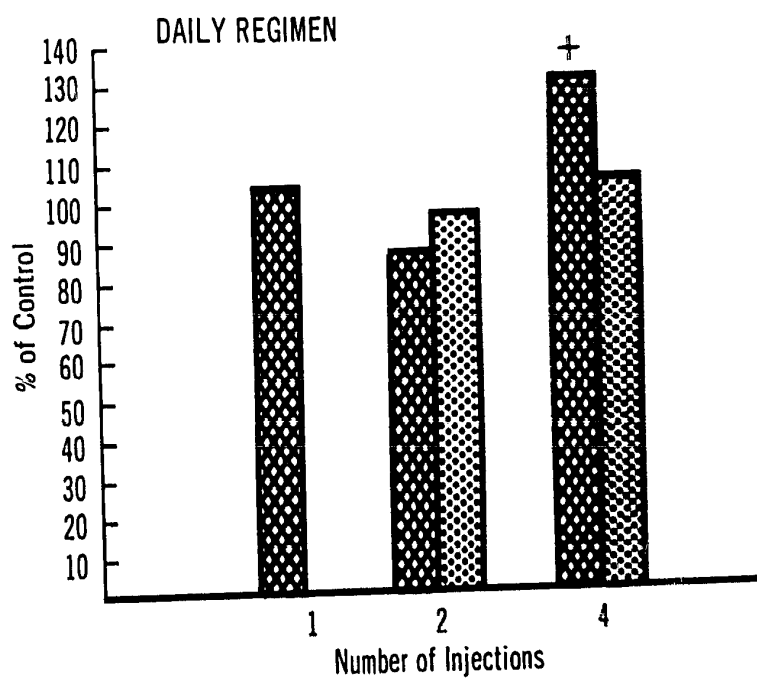
ii. The Effect of ATS on the Number of
Kupffer Cells

The number of Kupffer cells was counted over a total area of 1.46 mm. After two ATS injections, a slight decrease in the total number of littoral cells was noted; four ATS injections resulted in a significant increase in the hepatic phagocyte number (groups 2 and 3, set B). The use of NHS did not affect the total number of phagocytic cells (figure 46).

This pattern was observed in all mice whether on the daily injection schedule or on the two day interval regimen.

Figure 46 Effect of ATS on the number of Kupffer cells.

EFFECT OF ATS ON THE NUMBER OF KUPFFER CELLS
(Area: 1.46 mm^2)



ATS

NHS

o P.02-.01

+ P.01-.001

CHAPTER IV

DISCUSSION

Two populations of small lymphocytes are known to exist - recirculating and non-recirculating (Gowans and McGregor, 1965). The majority of the recirculating lymphocytes are long-lived, thymic derived cells (Everett et al, 1964; Everett et al, 1970) whereas twenty percent of the recirculating lymphocytes in the peripheral blood are short-lived, bone marrow derived cells (Osmond and Everett, 1964).

These two populations of lymphocytes are believed to originate from a common stem cell residing in the bone marrow (Miller, 1962; Craddock et al, 1971). The long-lived lymphocytes are continuously recirculating under normal conditions and are found in the thoracic duct, blood, deep cortical regions of the lymph nodes and the periarteriolar region of the spleen. Thoracic duct cannulation (McGregor and Gowans, 1963) and neonatal thymectomy (Miller, 1962; Osoba, 1965) are known to deplete these cells from the above regions; accordingly, these areas are referred to as "thymic-dependent zones" (Parrott et al, 1966), and the recirculating lymphocytes are, therefore,

thought to be under thymic influence. There are two ways in which thymic programming of the lymphocytes is thought to occur:

- (1) a humoral mechanism wherein an active factor essential for such a cellular population to arise is released from the thymus to affect precursor cells at a distance (Osoba and Miller, 1963; Osoba, 1965; Globerson and Auerbach, 1967).
- (2) cells from the marrow first go to the thymus where they are programmed. They then leave as immunocompetent cells capable of expressing thymic dependent lymphocyte function (Leuchars et al, 1964; Davies et al, 1966).

The majority of the short-lived lymphocytes are non-recirculating. These cells, which have a life-span of a few days to a few weeks, are located in the lymph node medulla, splenic red pulp, and in the germinal centres of the lymph nodes and spleen. These cells also make up a large percentage of the cells of the Peyer's patches and the appendix which are considered by some investigators to be the "bursal equivalent" tissues in mammals (Cooper et al, 1966; Craddock et al, 1971). Thoracic duct cannulation and neonatal thymectomy have no apparent effects on these regions (Ford and Gowans, 1969). Craddock et al (1971) termed the cells directed along this pathway "bursal-directed

lymphocytes". The end point of bursal lymphocyte differentiation is the plasma cell which has a survival period of two to three days (Vasquez, 1964; Parrott et al, 1966).

The long-lived thymic lymphocytes are responsible for cell-mediated immunity which includes graft rejection, delayed hypersensitivity, graft-versus-host reactions and defense against intracellular organisms. The bursal lymphocytes on the other hand, are responsible for the humoral antibody response. Neonatal thymectomy (Miller, 1962), thoracic duct cannulation (McGregor and Gowans, 1963) and treatment with ALS and ATS (Levey and Medawar, 1966; *ibid*, 1967; Röpke, 1970) decreases the number of thymic lymphocytes with a resultant defect in the cellular immune response. Plasma cells are unaffected. Removal of the Peyer's patches and the appendix or pathological disorders such as Bruton-type agammaglobulinemia (Bruton, 1952) results in severely depressed immunoglobulin levels, greatly decreased humoral antibody production and absence of plasma cells in the lymphoid tissue.

The production of at least some antibodies by plasma cells seems to be largely dependent upon thymic lymphocyte integrity (Miller and Mitchell, 1969). The role of the recirculating thymic lymphocytes in binding

antigen processed by the macrophages and its subsequent presentation to the less mobile bursal lymphocytes may be an important factor in the initial recognition of some antigens (Claman and Chaperon, 1969).

Bone marrow stem cells give rise to both populations of lymphocytes. However, not all the precursor cells are directed along the two aforementioned pathways. Many remain uncommitted. The ontogenetic reasons for the retention of this "wasteful" and "ineffective" marrow lymphopoiesis are the continuing need for totally uncommitted cells to meet antigenic challenge.

I. THE EFFECTS OF ATS ON ADULT C57Bl/J AND SWISS MICE

The action of ATS can be divided into three groups:

(1) a specific action involving the active IgG fraction which is responsible for depleting the long-lived lymphocytes and inducing immunosuppression;

(2) a non-specific heterologous protein fraction (horse serum in this case) which induces lymphocyte-blast cell transformation, and may be immunogenic; and

(3) an irrelevant and potentially toxic fraction which is capable of causing physical distress and death.

(a) Intraperitoneal and Subcutaneous Routes

i. Active fraction and immunosuppression

It is assumed that ALS and ATS exert their

immunosuppressive effects by the destruction of recirculating lymphocytes (Levey and Medawar, 1966; *ibid*, 1967) since lymphocytes which had already "homed" to lymphoid organs were relatively resistant to their action (Taub and Lance, 1968 a). This was substantiated by the findings of Denman and Frenkel (1968) that ALG treatment resulted in a shift within the small lymphocyte population from the long-lived to the short-lived variety. Furthermore, Denman and Frenkel (1968) observed that such a depletion could only occur outside the lymphoid tissue as fluorescein-labelled ALG penetrated such tissue to a very minor degree.

The destruction of the recirculating lymphocytes observed in our experiments resulted in depleted deep cortical regions in the lymph nodes and depleted periarteriolar regions in the splenic white pulp, the so-called "thymic-dependent" zones. Our findings in these organs are consistent with previous findings in mice and rats receiving either ATS or ALS (Woodruff and Anderson, 1963; Russe and Crowle, 1965; Gray et al, 1966; Monaco et al, 1966; Turk and Willoughby, 1967; Lance, 1968; Taub and Lance, 1968 b; Barth et al, 1969 a; Gotjamanos and Gill, 1970; Turk, 1970). In some of our mice, extensive depletion of the cortical as well as the deep cortical region of the lymph node and of the splenic nodules occurred after intensive ATS treatment. Similar observations were reported by Gray et al (1966).

Taub and Lance (1968 b) suggest that such extensive depletions are unnecessary for immunosuppression and that small lymphocyte depletion from the "thymic-dependent" zones is sufficient to bring about such an effect.

Cellular depletion in the diffuse lymphoid tissue at the periphery of the splenic nodules which has been noted by a number of investigators (Pichlmayr et al, 1968; Barth et al, 1969 a; Gotjamanos and Gill, 1970) was also a feature of the present study. This region which contains macrophages capable of phagocytosing antigen and particulate matter (Nossal et al, 1966) may also constitute a "thymic-dependent" zone.

Although many investigators claim no alteration in thymic architecture following ATS or ALS treatment (Lance, 1968; Taub and Lance, 1968 b; Röpke, 1970), our findings of small lymphocyte depletion at the cortico-medullary junction support similar observations by Gray et al (1966). Small lymphocytes within the thymus are basically of the short-lived variety; therefore, lymphocyte depletion at the cortico-medullary junction may indicate a zone of long-lived thymic-dependent lymphocytes. The relative ineffectiveness of ATS on the thymus does not

exclude the possibility that some of the immunosuppressive activity of the antiserum is mediated through this organ as has been suggested by some investigators (Russe and Crowle, 1965; Parrott, 1967).

The Peyer's patches which play a role in the humoral antibody response were relatively unaffected by ATS treatment. The few pyknotic nuclei and the minute amounts of cellular debris were the result of ATS acting on the small population of thymic lymphocytes found in the perifollicular region of the Peyer's patches. We found a slight decrease in the small lymphocyte population of the bone marrow whereas others reported that ALS and ATS had no effects on this cell population (Taub and Lance, 1968 b; Jacobsen and Griffiths, 1970). The myeloid and erythroid cell populations appeared to be unaffected.

Lymphocyte destruction by ATS treatment was accompanied by increases in the corticosterone levels (Gray et al, 1966). Repeated cortisone administration has been shown to destroy lymphocytes (Dukor and Dietrich, 1967). Experiments on rats have led to the suggestion that the action of ATS is mediated through the adrenal cortex, and immunosuppression is the result of the secretion of gluco-and mineralocorticoids (Brendel, 1970).

- ii. Heterologous protein fraction, cellular transformation and immunogenicity

These changes included medullary hyperplasia in the lymph node, germinal centre formation and increases in the number of blast cells, large lymphocytes and plasma cells.

Medullary hyperplasia and increases in the number of blast and plasma cells are consistent with the findings of Starzl et al (1966), Rule and Judd (1968) and Bach et al (1970). These findings are interesting since they demonstrate the unique capacity of ATS to discriminate between humoral and cell mediated immune responses by suppressing the latter only (Lance and Batchelor, 1968). Mice given immunosuppressive doses of ALS were still capable of producing antibodies to Salmonella-H antigens and to specific H-2 antigens of the donor (Balner and Dersjant, 1967; Lance, 1968). However, discrimination is not absolute since very high dosages of ALS and ATS are capable of suppressing both immune responses (Waksman et al, 1961).

The occurrence of blast cells and large lymphocytes in the "thymic-dependent" zones of the spleen and lymph node, in the germinal centres of the spleen and lymph node and in the medulla of the lymph node observed in our

experiments is consistent with the findings of Levey and Medawar (1966) and Tyler et al (1969). Röpke (1970) was unable to demonstrate their presence after one ATS injection. Lymphocyte transformation is not a unique sequel of ATS administration as it was also demonstrated to occur with some non-specific mitogens such as phytohemagglutinin (Nowell, 1960) and with specific antigens such as tuberculin (Nowell, 1965).

Lymphocyte transformation by ALS and ATS in vitro has been demonstrated by a number of investigators (Holt et al, 1966; Gray et al, 1966; Woodruff et al, 1967; Martin and Miller, 1967; Anderson et al, 1968; Antoine et al, 1970). In vivo transformation, on the other hand, appears to be the result of non-specific antigenic stimulation by heterologous serum protein. This view is supported by our findings that both inactive ATS and NHS gave rise to large lymphocytes and blast cells in the spleen and lymph node, and is in agreement with similar in vivo results obtained by Taub and Lance (1968 b) who used rabbit anti-mouse lymphocyte serum (RAMLS) and normal rabbit serum (NRS).

ATS produces in vitro lymphocyte transformation in the absence of complement (Holt et al, 1966). However, in the presence of complement, a resultant lysis of these cells occurs (Sacks et al, 1964). This latter system could

account for the presence of cellular debris in the "thymic-dependent" zones of the spleen and lymph node following ATS treatment.

iii. Irrelevant and potentially toxic fraction

A side effect of ATS treatment accompanying periarteriolar depletion in the spleen was red pulp erythroid hyperplasia. A striking feature of this condition was a proliferation of bone marrow derived cells in the splenic red pulp. ATS eliminates a sub-population of lymphocytes (Martin and Miller, 1967) with increase in the number of bone-marrow derived cells. These cells which serve as stem cells can differentiate into lymphocytes as was demonstrated with the chromosome marker cell technique developed by Harris and Ford (1964). The proliferation of bone-marrow derived cells in the splenic red pulp may be a source of lymphocytes for the depleted lymphoid nodules.

Hepatomegaly, which occurred following ATS, iATS and NHS administration was probably not caused by the immunosuppressive or immunogenic fractions but rather by the irrelevant fraction of the sera. Gotjamanos and Gill (1970) reported increased RNA synthesis in the enlarged livers following ALS and NRS treatment, as well as increased

concentration of purines. Purines are important constituents of RNA needed for the development of normal bone marrow and peripheral tissue cells (Lajtha and Vane, 1958). Hepatic hypertrophy appears to be a compensatory mechanism; it may possibly provide the increased amounts of purines required for the maintenance of cellular proliferation in the regenerating depleted lymphoid tissue.

(b) Intravenous Route

The effects of ATS on mice injected either intraperitoneally or subcutaneously were consistent with observations documented by a number of investigators. Multiple intravenous injections have produced additional interesting changes.

Prolonged intravenous treatment resulted in marked histopathological changes in the thymus. A shift in the small lymphocyte population from the cortex to the vascular medulla was noted in our experiments. A similar movement was observed in the experiments of Sainte-Marie and Leblond (1964) but to a lesser extent. The amount of cellular debris and the number of Hassall's corpuscles increased. Blau (1970) suggested that these organelles are phagocytic, and their increase may be causally related to the increased amount of cellular debris.

A synergism was found to exist between the thymic cells and the antibody forming precursor cells of the bone marrow (Claman et al, 1966; Mitchell and Miller, 1968). The thymus and marrow cells of ALS-treated mice could repair immunological defects present in the spleen (Jeejeebhoy, 1970). Following multiple intravenous injections we observed a marked effect on bone marrow cells. Bone marrow small lymphocytes were noticeably depleted and the myeloid cell population was found to contain many abnormal cells. Erythroid hyperplasia still persisted and was most evident in the spleen. Hemolysis, which may or may not have been due to an irrelevant factor introduced with ATS occurred concurrently and red cell "ghosts" were prominent in the hyperplastic tissue. It is possible that as a result of multiple intravenous ATS injections, the thymic cell-bone marrow precursor cell balance was disrupted. The depletion of the bone marrow cells appeared to increase the significance of the thymus in such a system. In order that the thymus meet the added requirements, two criteria would have to be met:

- (1) the production of large numbers of small lymphocytes;
- and
- (2) the ability of these cells to move into the circulation and to peripheralize.

The thymus is described as lymphoid organ "par excellence" (Miller, 1964) as it has a high mitotic rate and is capable of producing small lymphocytes independent of antigenic stimulation. Under normal conditions, the majority of these cells are short-lived and are retained within the organ. Those cells which are exported are long-lived however. Such export was demonstrated by Sainte-Marie and Leblond (1964) in rats as more lymphocytes were found in the venous than in the arterial circulation. These observations have been supported by the studies of Nossal (1964) on guinea pigs, and by Weissman (1967) who used the tagged marker cell technique. This cellular movement from the thymus is probably not the result of a direct action of ATS on this organ; the poor penetration of fluorescein-labelled ALG into thymic tissue (Denman and Frenkel, 1968) suggests some indirect mechanism.

Small lymphocytes are depleted from the recirculating pool and the bone marrow. It is conceivable that when blood depleted of small lymphocytes passes through the thymus, it triggers some mechanism which results in the release of thymic lymphocytes into the blood. From the circulation they pass into the spleen and lymph nodes and as a result, the "thymic-dependent" zones are repopulated.

It is conceivable that the cells newly released from the thymus are not yet fully differentiated or programmed and consequently do not take part in complement-mediated lysis by ATS. Their sole function may be to serve as a transitional cell population until the bone marrow can recover and restore the immunocompetent cell population.

(c) Survival

ATS caused death of intraperitoneally treated C57Bl/J mice and of intravenously treated Swiss mice. Similar findings were reported by Nagaya and Sieker (1965) in rats and by Monaco et al (1966) in mice following intraperitoneal administration of ALS, and in mice following one large intravenous dose of antiserum (Taub and Lance, 1968 b).

Based on our experiments, three possible pathomechanisms can be considered:

- (1) A prominent symptom noted prior to death was hematuria. Examination of mice revealed signs of jaundice. Since this condition greatly decreases the absorption of the fat soluble vitamins, the developing K-vitamin deficiency results in reduced prothrombin synthesis and subsequent hemorrhagic incidents. This possibility is substantiated by the frequent occurrence of gastro-intestinal hemorrhage.

(2) ALS administration is known to enhance the susceptibility of mice to certain mycobacterial infections (Gaugas and Rees, 1968) which may lead to death.

(3) Sera produced against lymphocytes or thymocytes have produced glomerulonephritis in a variety of animals (Katz et al, 1967; Guttman et al, 1967; Lance, 1968; Orr et al, 1970; Denman et al, 1970; Taylor, 1970; Taylor, 1971). These studies have revealed the presence of an anti-kidney antibody in both the anti-lymphocytic serum and in the globulin fraction which reacted with the glomerular basement membranes in a fashion similar to the pathomechanism of Masugi nephritis. In mice, such a condition resulted if ATS was administered weekly for more than eight weeks (Lance, 1968) while in monkeys, three weekly injections over a three week period produced glomerulonephritic symptoms (Taylor, 1971). Therefore it is highly unlikely that death observed in our studies was the result of glomerulonephritis since the injection schedules were not prolonged sufficiently to elicit such a response. The only histological sign related to renal lesions was slight glomerular swelling, but none of the animals showed acute glomerulonephritic symptoms. The glomerular edema, however, could have been the indicator of the incipient stage of glomerulonephritis.

(d) Route

Various investigators use different routes of ATS administration and this also influences the effects of the antiserum on the recipients. Some toxic substances are present in our ATS which resulted in the death of intraperitoneally treated C57Bl/J and intravenously treated Swiss mice. These toxic substances are absorbed onto tissue receptor sites when the antiserum is injected subcutaneously. Because of the relative safety of this latter route, it is widely used (Sacks et al, 1964; Pichlmayr et al, 1968). These findings are confirmed by the observation that the longest allograft survival occurs after subcutaneous treatment, followed by intraperitoneal and intravenous administration (Dalton, 1970; Shorter and Elveback, 1970).

II. THE EFFECTS OF ATS ON YOUNG C57Bl/J AND SWISS MICE

(a) General Effects

Mice thymectomized at birth and sacrificed up to sixty days later were found to have hyperplasia of the reticulo-endothelial system, proliferation of reticular cells and histiocytes replacing the cortex of lymph nodal tissue, splenomegaly associated with extensive extra-medullary hemopoiesis, accumulation of pyroninophilic

cells in the splenic red pulp, increased size and number of hepatic Kupffer cells, with increased phagocytic activity, diminished immunological competence, terminal wasting and death (Miller and Howard, 1964). These changes were somewhat similar to those found in adult mice following ATS treatment, and support the concept that ATS administration is capable of conferring "immuno-thymectomy" when administered to adult animals (Russe and Crowle, 1965).

The splenomegalic condition was common to both young and adult mice following ATS treatment; Miller and Howard (1964) attribute this condition to extramedullary hemopoiesis. Red pulp hyperplasia was however less evident in the young animals. Wasting and death which followed some injection schedules in adult mice did not occur in young mice. This was consistent with the findings of Jooste (1968) who found no toxic effects when administering ALS daily during the first ten days after birth. Furthermore, Jooste (1968; 1970) reported a doubling of body weight between days 10 to 21 following antiserum administration for the first ten days. Although such significant weight increases were not obtained in the present study, marked weight increases were, nevertheless, evident.

Histologically thymic architecture appeared to be unaltered. The function of the thymus is much more pronounced in the young than in older animals. This organ has been found to export large numbers of lymphocytes whereas in the adult, peripheralization is significantly diminished (Nossal, 1964). Furthermore, in mice up to three weeks of age, the thymus alone is capable of restoring immune reactivity. Thymic cells from newborn or three week old donors given to thymectomized radiation chimeras restored immune reactivity whereas thymocytes from prenatal and older animals afforded virtually no protection or restoration (MacGillivray et al, 1970). In animals older than three weeks, bone marrow as well as thymus cells are required for such restoration (Jeejeebhoy, 1970).

(b) Mast Cells

Following ATS treatment, an increase in the mast cell number occurred in the spleens of young mice. Mast cells contain heparin, histamine, serotonin or 5-hydroxytryptamine, and other active substances (Selye, 1965). These metachromatic cells are believed by some investigators to be of thymic origin and have been found to arise in rat fetuses between the fifteenth and eighteenth day of gestation (Csaba et al, 1965; Törö et al, 1969).

Mast cells in early splenic organization are localized almost exclusively in the red pulp. In developing lymph nodules, these cells are also found concentrically situated around the developing nodules of the splenic white pulp. The appearance of these spindle and spherical shaped cells in both regions of the spleen in young mice (Metcalf, 1965) as compared to their exclusive localization in the red pulp of adult animals (Vicklický, 1967) might indicate a possible biosynthetic difference between the mast cells of younger and older animals. Such a difference was indeed found to exist between mast cells cultured from young and old animals as S^{35} -labelled sodium sulphate was incorporated faster into the mast cells of younger animals (Guidotti and Spinelli-Rossi, 1964).

Early investigations of mast cells suggested that they could arise from cultured chicken lymphocytes (Dantschakoff, 1909). However, this occurrence could have been the result of contamination of the lymphocyte cultures by basophils, macrophages and reticular cells. Murray (1948) has postulated that reticular cells can transform into mast cells. Combs et al (1965), using histochemical and radioactive methods, described a four stage development

of mast cells. Cells of the first stage were described as lymphocyte-like cells with a weakly staining reaction for sulphated mucopolysaccharides. Burnet (1965) has also postulated a lymphocyte-mast cell transformation.

If mast cells indeed originate in the thymus, they must leave this organ as lymphocytes or lymphocyte-like cells as no mast cells are observed in the circulation; these cells would have to have the potential to transform into mast cells in the peripheral tissue. The movement of large numbers of lymphocytes from the thymus of young animals could possibly initiate the occurrence of an increased number of mast cells observed in the spleen. In adult animals, the thymus sends out decreasing numbers of lymphocytes; this could account for the paucity of mast cells in the spleen of adult animals as has been demonstrated by a number of investigators (Katzberg, 1954; Arvy and Quivy, 1955) and is consistent with the results of the present study.

An important constituent of the mast cells is histamine, a potent vasodilator. One could speculate that the large number of mast cells in the young spleen are exerting a vasodilatory effect which could result in the more rapid passage of programmed thymocytes and bone

marrow derived precursor cells to the spleen. As the development of splenic architecture progresses and the "seeding" of the spleen stabilizes at a lower level, there is less need for histamine and the number of mast cells decreases.

Upon administration of ATS, lymphoid cells are depleted. The increase in the number of mast cells following such treatment is similar to a mast cell increase following irradiation (Murray, 1948) which is a known lymphoid tissue depleting agent. The increase in the mast cell number could be either by migration of the thymic lymphocytes to the spleen followed by differentiation into mast cells or by mitosis of the existing mast cells.

Although our study demonstrated an apparent increase in the number of mast cells following ATS treatment which is consistent with the earlier findings of Lance (1968), this conclusion must be viewed with certain limitations;

- (1) Mast cell counts were not correlated with the total cell count and an increase in the number of mast cells may be the result of an overall cell hyperplasia; and
- (2) The increase may have been the result of a tissue condensation (Murray, 1948).

III. THE EFFECTS OF ATS ON PHAGOCYTTIC ACTIVITY

The experiments on phagocytic activity were conducted in order to determine the effects of ATS on the hepatic and splenic macrophages and to resolve some of the equivocal results obtained using the antiserum. Findings of in vivo (Sheagren et al, 1969; Pisano et al, 1969; Patterson et al, 1970) and in vitro (Woodruff et al, 1967; Loewi et al, 1969) depression of phagocytic activity are contradicted by findings of in vivo (Gill and Gotjamanos, 1969; Grogan, 1969; Kinnaert et al, 1969) and in vitro (Hughes, 1970; Argyris and Plotkin, 1970) enhancement of phagocytosis.

Earlier in vitro studies showed that phagocytosis of starch particles was dependent on antibody and complement (Nelson and Lebrun, 1956). Some investigators (Jenkin and Rowley, 1961; Normann and Benditt, 1965) have found that phagocytosis in some instances occurs only in the presence of certain specific antibodies (opsonins). Stuart (1967) claimed that mouse anti-lymphocyte serum has opsonizing properties as demonstrated by the adherence of mouse lymphocytes to ALS treated mouse macrophages and their subsequent phagocytosis. This observation was substantiated by the work of Berken and Benacerraf (1968)

who demonstrated that anti-sheep erythrocyte opsonins were a part of the IgG fraction. The IgG fraction is considered to be the "active" fraction of ALS (James, 1967).

(a) Liver

Pisano et al (1969) and Sheagren et al (1969) have described depressed phagocytosis by Kupffer cells following an initial injection of ATS. Patterson et al (1970) further demonstrated that the injection of hepatic macrophages would reconstitute normal phagocytic activity.

A similar depression in the clearance of colloidal carbon from the peripheral blood was noted by Chare and Boak (1970) following a single injection of ALS. However, they obtained a similar depression with NHS treatment and concluded that the initial depression was not a specific property of ALS. Grogan (1969) noted enhanced phagocytic activity following ALS treatment in rats. Gill and Gotjamanos (1969) proposed that the initial phagocytic depression was due to non-specific changes in the serum while Vreeken et al (1969) concluded that the depression was the result of accompanying platelet destruction.

In our experiments, an initial decrease in phagocytosis by the Kupffer cells after one and two ATS or NHS injections was followed by enhanced phagocytosis after four injections. This pattern is consistent with similar findings of Gotjamanos and Gill (1970) and is compatible with the concept of an indirect action of ATS on macrophages. One or two administrations of ATS seems likely to result in macrophage and lymphocyte adherence and aggregation, with consequent fall in circulating immunocytes. Such adherence could be the result of the cytophilic antibody content of the serum. Greaves et al (1969) have suggested that the cytophilic effect of the antiserum may be more important than the cytotoxic effect on the lymphocytes. Electron microscopy of ALS treated peritoneal cell monolayers has confirmed the speculation of a marked increase in close cytoplasmic contacts between macrophages and between macrophages and lymphocytes (MacLaurin and Humm, 1970). The adherence of lymphocytes to the macrophages and their subsequent phagocytosis resulted in impaired phagocytosis of unrelated antigen. As a result, antigen processing is decreased and the primary immune response is impaired. In our

experiments, antigen is represented by latex and Imferon particles; initial ATS treatment results in the depressed uptake of these particles.

The biphasic curve which results after ATS treatment can be explained as follows:

(1) The adherence of lymphocytes to macrophages and their destruction by "allogeneic" inhibition (Möller et al, 1966) or by cytotoxic activity (Levey and Medawar, 1966) initially requires cytophilic antibody and/or opsonins. As a result, phagocytosis of unrelated antigen is impaired.

(2) As ATS injections are repeated and the number of recirculating lymphocytes and the amount of cellular debris (as a result of complement-mediated lysis) decreases, less cytophilic antibody and/or opsonin is required for phagocytosing lymphocytes and cellular debris. More becomes available for the uptake of unrelated antigen and their phagocytosis is enhanced.

The increase in hepatic mass and the significant increase in the number of Kupffer cells may account for the increased phagocytic activity following ATS treatment for four to eight days. Our findings therefore differed from those of Grogan (1969) who reported enhanced phagocytosis

with no increase in the hepatic weight. Benacerraf et al (1955) demonstrated that the rate of carbon uptake was a function of the size of the particles and the amount of blood flowing through the liver. Particle size could account for the marked differences observable between latex and Imferon uptake. An increase in the liver blood flow would increase the phagocytic rate through an increase in the number of latex and Imferon particles coming into contact with the Kupffer cells per unit of time. The increased blood flow also serves to supply nutrients to a hypertrophied liver which results after multiple injections of ATS and NHS with a concomitant increase in phagocytic activity.

Our experiments tend to support the proposal that phagocytic depression of the Kupffer cells is not a direct consequence of ATS administration. Furthermore, such a depression is not unique to this situation. Intravenous injections of carbon, iron oxide or killed typhoid bacilli also produced an early depression of the phagocytic activity (Biozzi et al, 1953).

b. Spleen

Significant decreases in splenic phagocytic activity resulted following ATS treatment. NHS administration, on the other hand, produced an initial decrease followed by a return to near normal levels, somewhat similar to the

biphasic pattern obtained with both sera in the liver.

Some investigators claim that two populations of "macrophages" exist within the spleen - the classical macrophages and the dendritic reticular cells (Mitchell and Abbot, 1965). The splenic macrophages are mostly found within the red pulp and in the diffuse lymphatic tissue of the white pulp (Nossal et al, 1966); these cells are capable of taking up any foreign material, be it antigenic or not. The dendritic reticular cells are located in the lymphoid follicles (Mitchell and Abbot, 1965; Nossal et al, 1966; Nossal et al, 1968); their primary function is believed to be the localization of antigen. Trapping of antigen is not thought to occur indiscriminately, but rather as a result of an antibody dependent process (Nossal et al, 1965; McDevitt et al, 1966). Apparently, the antigen retaining reticulum cells and the true macrophages are functionally different cells.

The study of splenic phagocytosis following ATS treatment yielded varied results. These variations can best be explained by the two "macrophage" populations concept.

Barth et al (1969 a; b) reported that following the injection of I^{125} -labelled flagellin of Salmonella typhii, ALS treated mice retained far less antigen in the

lymphoid follicles than did the normal sera treated mice and the untreated controls. Similar results were obtained in vitro in which depressed antigen uptake and retention were observed in macrophages incubated with ALS (Woodruff et al, 1967; Loewi et al, 1969). The mechanism of antigen trapping was found to be sensitive to X-irradiation, a powerful immunosuppressive agent (Jaroslow and Nossal, 1966). Similar results were obtained using Actinomycin D, cyclophosphamide and cortisone acetate (Nettesheim and Hammons, 1970). This latter investigating team noted no defect in the uptake of antigen and its retention by the liver, whereas splenic tissue was significantly impaired in this respect. They concluded that the mechanism of antigen uptake and/or retention is not the same in these two organs. These studies conducted on the splenic follicles would indicate that the dendritic reticular cells responsible for antigen trapping within this region are directly affected by immunosuppressive agents, among them ATS. One can only speculate as to how this depression is accomplished; it is possible that the antiserum acts on the antibody which is necessary for antigen trapping and in some way, inactivates it.

Findings in our experiments of depressed phagocytosis of particulate matter by the splenic macrophages in the diffuse lymphatic tissue following multiple injections of ATS indicated that ATS also had an effect on this phagocytic population. Some investigators believe that the depression of phagocytic activity by splenic macrophages is the result of an indirect action of ATS (Martin, 1969; Chare and Boak, 1970). They concluded that since localization of antigen, cellular debris or particulate matter in organs other than the liver and spleen was relatively negligible and unaffected by the antiserum, increased liver phagocytosis was at the expense of material destined to localize in the spleen.

The findings in our experiments that after four NHS injections phagocytosis of Imferon returned to near normal levels whereas four ATS injections further depressed phagocytosis suggested that ATS had some direct effect on the splenic macrophages. These differences could not have been due to the differences in the phagocytosis by the Kupffer cells as both ATS and NHS proved to enhance their phagocytic activity. The following two factors led us to this conclusion:

(1) The depletion of the diffuse lymphatic tissue at the periphery of the lymphoid nodules after multiple ATS injections could have affected the splenic macrophages in this region and reduced their ability to phagocytose Imferon.

(2) Multiple injections of ATS and NHS produced patterns of decline and recovery of the phagocytic activity of the Kupffer cells. A similar biphasic pattern was obtained with NHS in the spleen whereas multiple ATS injections resulted in a further depression of phagocytosis by the splenic macrophages.

The role of macrophages in the initiation of some immune responses has been demonstrated (Fishman, 1961; Fishman and Adler, 1963). ATS probably does not influence the initial uptake of antigen in the liver; this, however, is apparently not the case in the spleen. The dendritic reticular cells of this organ have a reduced ability to trap and retain antigen following ATS treatment. Furthermore, these cells may not process antigen but rather serve as a point of aggregation to which the lymphocytes are attracted to be programmed. The splenic macrophages do phagocytose and process antigen; whether

the depressive action of ATS on the phagocytic activity of this macrophage population is similar to that on the dendritic reticular cells is unknown. Finally, even though ATS may have no apparent effects on the uptake of antigen by the hepatic macrophages, one can not exclude the possibility that ATS acts intracellularly on the lysosomes, rendering the enzymes of these organelles unavailable for the processing of antigen.

IV. CONCLUDING REMARKS

Much variation exists in the reports on the effects of ATS on the histology and phagocytic activity of the reticulo-endothelial organs. This undoubtedly is due to differences in the immunization schedules, variability in the times of examination after injection or differences in the specificity of the sera used - whether ATS or ALS raised by lymph node or thoracic duct lymphocytes or the purified IgG fraction have been used.

The use of ATS as an immunosuppressant in transplantation and delayed hypersensitivity reactions is gaining acceptance. However, before full clinical use can be made of its beneficial properties, the "modus operandi" of ATS will have to be elucidated and the undesirable side-effects associated with immunosuppression controlled.

SUMMARY

1. The action of ATS on the morphology and phagocytic activity of the reticulo-endothelial organs was investigated.
2. The effects of ATS are species rather than strain-specific in that it affects C57B1/J and Swiss mice similarly.
3. ATS depletes the periarteriolar and deep cortical regions of the spleen and lymph node respectively, the so-called "thymus-dependent" zones.
4. The heterologous protein fraction (horse serum) induces lymphocyte-blast cell transformation in vivo; it is not a specific reaction to ATS.
5. Erythroid hyperplasia is evident in the spleen following ATS treatment. This is thought to be the result of some irrelevant fraction in the antiserum.
6. Hepatomegaly is not a specific reaction to ATS, as both iATS and NHS are capable of producing this condition.
7. Intraperitoneal and subcutaneous ATS injections produce some corticomedullary depletion in the thymus. After intravenous treatment, thymocytes shift from the cortex to the medulla which may indicate a cellular migration from this organ. Furthermore, the "thymus-dependent" zones of the spleen and lymph node are repopulated.
8. Peyer's patches are virtually unaffected by ATS treatment. Intravenous ATS treatment depresses the small lymphocyte population of the bone marrow and produces some abnormal myeloid cells.
9. Young mice treated from birth with multiple injections of ATS were found to lack splenic organization. Blast cells were noticeable, but

lymph nodules and germinal centres were lacking.

10. Spleens of mice younger than one-week had significantly increased numbers of mast cells following ATS treatment; in older animals, ATS proved to be largely ineffective in increasing the mast cell count.

11. A biphasic pattern of decline and recovery in the phagocytosis of particulate matter and antigen following ATS treatment indicates that the antiserum does not have a direct effect on the Kupffer cells.

12. Multiple injections of ATS do not produce a biphasic phagocytic pattern in the spleen, but rather causes a further depression in the phagocytic activity of the splenic macrophages. This indicates a direct influence of ATS on these macrophages.

13. It is conceivable that the immunosuppressive action of ATS may involve two mechanisms, either singly or in combination:

a) The number of recirculating long-lived lymphocytes is reduced (either by the cytophilic or cytotoxic action of ATS); consequently, fewer cells are available for programming by antigens.

b) ATS exerts a direct effect on the splenic macrophages and reduces their ability to take up and retain antigen; as a result, fewer cells are programmed.

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APPENDIX

Total body weights from which percentage organ weights
were calculated
(organ weight changes calculated against sacrifice weights)

TABLE VC57B1/J MICE

Group	Total Body Weight (gms)	
	Beginning of Schedule	Sacrifice
1	23.1	23.3
2	22.9	23.0
3	24.7	23.4
4	24.5	25.1
5	25.3	25.8
6	23.6	24.8
7	23.6	24.2

TABLE VISWISS MICE

1	21.5	22.6
2	22.4	26.3
3	20.7	24.1
4	19.5	24.7
5	21.3	26.4
6	21.0	25.1
23	25.4	28.5

TABLE VII

7	23.2	26.2
8	22.5	28.1
9	23.0	27.5
23	25.4	28.5

TABLE VIII

10	21.3	23.4
11	20.2	23.6
12	22.0	26.5
13	21.5	26.0
14	22.5	27.0
15	24.2	28.1
23	25.4	28.5

TABLE IX

16	23.5	27.8
17	23.2	28.8
18	22.5	26.9
23	25.4	28.5

TABLE X

19	21.4	21.5
20	20.9	19.8
21	20.7	18.4
22	NO DATA AVAILABLE	
23	25.4	28.5

TABLE XI

YOUNG C57B1/J and SWISS MICE

Group	Weight of Control (gms)
1	1.46
2	1.31
3	1.42
4	1.27
5	1.67
6	1.55
7	2.05
8	2.34
9	2.74
10	2.50
11	3.15
12	3.37
13	5.05
14	5.24
15	5.75