EXPERIMENTAL ALLERGIC ORCHITIS : IMMUNOMODULATORY EFFECTS OF GLUCOCORTICOIDS.

By

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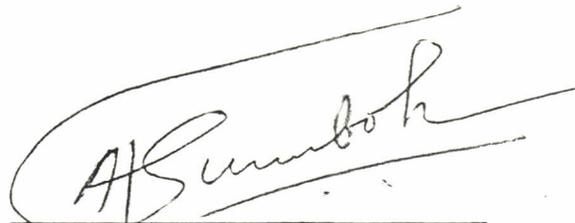
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D E C L A R A T I O N

I, Munuhe, S.J. Wachira, hereby declare that this thesis is my original work and has not been presented for award of degree in any other University.
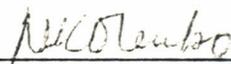
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DEDICATION

To my parents :

Vincenza Ngima and Simon Wachira.

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A B B R E V I A T I O N S

APC	–	Antigen presenting cell
BBB	–	Blood brain barrier
BMP	–	Basic myelin protein
BSA	–	Bovine serum albumin
BTB	–	Blood testis barrier
CFA	–	Complete Freunds adjuvant
CMI	–	Cell mediated immunity
CNS	–	Central nervous system
DDSA	–	Dodecenyl succinic acid
DTH	–	Delayed type hypersensitivity
EAE	–	Experimental allergic encephalomyelitis
EAO	–	Experimental allergic orchitis
EAT	–	Experimental allergic thyroiditis
EM	–	Electron microscopy
H & E	–	Haematoxylin and eosin
HT	–	Hashimoto's thyroiditis
IFA	–	Incomplete Freunds adjuvant
LNCs	–	Lymph node cells
MHC	–	Major histocompatibility complex
MIF	–	Migration inhibition factor
MNA	–	Methyl nadic anhydride
MS	–	Multiple sclerosis
PECs	–	Peritoneal exudate cells
SDID	–	Sequential disease inducing determinant
SLE	–	Systemic lupus erythematosus
TSDA	–	Testis cell-sperm differentiation antigens

UNIT ABBREVIATIONS

μ	–	Micron
μg	–	Microgram
mg	–	Milligram
ml	–	Millilitre

S U M M A R Y

Injection of testicular material in complete Freunds
Adjuvant (CFA) leads to immunologically mediated testicular
injury in the guinea pig. A few testicular proteins are
responsible for the histopathology. Partial purification of
testicular material gave an orchitogenic extract. The
partially purified extract was used to study immunomodulatory
effects of glucocorticoids on the pathogenesis of Experimental
Allergic Orchitis (EAO).

A natural glucocorticoid (cortisol) and a synthetic one
(prednisone) were used in the study. Daily administration of
cortisol starting on day one of immunization with orchitogen
to day 14 lead to a significant reduction in the number of
animals that developed orchitis. The severity of
orchitis in cortisol treated animals was less than the saline
treated control group. Similar treatment with prednisone led
to a similar trend albeit to a lesser extent. Thus, 82 %
of immunized and cortisol treated animals did not develop
orchitis as opposed to only 7 % of the untreated animals.
Of the immunized and prednisone treated animals, 37% did
not develop orchitis whereas in untreated group 29% did not
develop orchitis.

Note, the EAO lesion developed from day 12 post-
immunization to day 14. The appearance of orchitis and
aspermatogenesis were to a large extent concurrent whereas
orchitis and epididymitis were not. Also, epididymitis and

aspermatogenesis did not necessarily occur together. This
means that orchitis occurred almost invariably with aspermato-
genesis with or without epididymitis.

On electron microscopy, the cell types identified to be
involved in the pathology were lymphocytes and macrophages.
Classification of the lymphocytes was not done. The study
therefore showed that glucocorticoids- cortisol and prednisone
modulate EAO by suppressing progression of the lesion in
susceptible guinea pigs. Cortisol, a natural glucocorticoid
was found to be more effective than prednisone, a synthetic
one.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 EXPERIMENTAL ALLERGIC ORCHITIS (EAO) AS AN AUTOIMMUNE DISEASE OF THE TESTIS

1.1.1 Introduction

Autoimmune disease results from immunological reactions against self constituents. This may be due to: changes in the particular constituents, alterations in the antigen seques- stration barrier or defects at the level of effector or regulatory arms of the immune response. The mechanisms may involve antibodies of various subclasses as well as Delayed-Type- Hypersensitivity (DTH) reactions.

The state of non-reactivity to self constituents develops during the perinatal period when the animal has not achieved immunocompetence. Thus foreign materials administered at this stage induce tolerance. Self tolerance is not genetically pre-determined rather it depends on the presence of the constituent (antigen) during this period (Weigle 1980).

1.1.2 Types of Immunological Unresponsiveness (Tolerance)

Two types of immunological unresponsivenees have been described (Weigle 1980). These are peripheral inhibition and central unresponsiveness.

Peripheral inhibition is characterised by the presence of immunocompetent cells in circulation towards the constituent/ antigen and antibodies towards the specific antigen may appear transiently. Thus peripheral inhibition may be due to: the

regulatory activity of suppressor cells, antigen blockade or antibody feedback mechanisms.

The suppresor cells are a class of thymus dependent lymphocytes (T lymphocytes) which dampen immunological responses and can be distinguished using specific markers. In mice, they can be distinguished by antisera against Lyt 2,3+. Suppressor cell activity has been associated with tolerance towards some thymus-dependent and thymus independent antigens (Bejamin 1975). The former are mostly proteins and other antigens with diverse antigenic determinants. Thymus-independent antigens comprise mostly of repeating polymers that present several identical determinants.

B lymphocytes tolerance to both foreign and self antigens is thought to be mediated by antigen blockade (Fernandez, et al. 1979). The antigen in this case persists and neutralizes the specific antibodies. The phenomenon is most likely with thymus independent antigens. The animals thus tolerized however, have antigen reactive cells in peripheral circulation which are potentially capable of differentiating into plasma cells upon transfer into suitable host. The plasma cells can then produce specific antibodies to react with the antigen in question.

Hoffmann and Kappler (1978) postulated that antibody feedback mechanisms operate via two distinct routes. One meachanism operates at low antibody concentration and depends on the Fc portion of the antibody molecule. The other

requires high titres of the antibody and does not depend on the Fc fragment. In the former mechanisms, T or B lymphocytes are not affected directly rather antibody inhibits the immune response by interfering with T, B lymphocyte interactions.

Central unresponsiveness differs with peripheral inhibition in that the host is totally incapable of reacting with the tolerogen. Whereas specific suppressor cells may be concomittant, they are not responsible and lymphocytes transferred to a neutral host remain unresponsive. This is best explained by deletion of antigen specific B-cells. A good example is that a neonate mouse of a particular strain when injected with spleen cells from a different strain, mouse embryo is later able to tolerate grafts from the donor strain (Burnet, 1961).

1.1.3   Breaking of Tolerance

Immunological unresponsiveness to self antigens may exist in different immune states of B and T lymphocytes. Thus, both B and T lymphocytes may be tolerized. A high degree of tolerance may exist in T lymphocytes while B lymphocytes are competent and vice-versa. Finally, both T and B lymphocytes may be competent.

When both T and B lymphocytes are immunocompetent, the state of tolerance is usually maintained by anatomical seclusion of the particular constituent. Examples are the testis and the brain which are secluded by the Blood-Testis-Barrier (BTB) (Dym and Fawcett, 1970) and the Blood-Brain-Barrier (BBB) (Daniel et al 1981) respectively. When the constituents of these tissues are exposed to peripheral circulation, they provoke specific immune

responses provided they are presented in association with Major Histocompatibility Complex (MHC) class II antigens on the surface of the antigen presenting cell (APC).

Where there is proven exposure of antigen/constituent to the immune system, autoreactivity or breakdown of tolerance may result from several factors as argued by Roitt (1988). They may occur at the level of T inducer or regulatory mechanisms.

T-inducer bypass mechanisms may be through;
a) Arising of new determinants to which tolerance does not exist. This can occur due to abnormal synthesis or breakdown. A closely related alien antigen may trigger immunological reaction against a self antigen. A good example for this is post rabies vaccine encephalitis which is thought to occur because of the presence of heterogenous brain material in the vaccine. Viral infection sometimes does cause synthesis of membrane proteins specific for the virus. Some of these proteins when in association with self compotents may lead to recognition of the self-constituent as foreign.
b) Idiotype bypass mechanisms are thought to lead to autoimmunity in the following way : The regulatable idiotype that is believed to appear early in life works by T helpers that have specificity for idiotypes on lymphocyte receptors and are able to stimulate them on interaction. Therefore, a foreign antigen that stimulates the production of an antibody that shares a public idiotype with an autoreactive T or

B lymphocyte can lead to stimulation of these cells and lead to autoimmunity.

c) The third way involves polyclonal activation by some environmental substances. This resembles the adjuvant effect and stimulates production of antibodies of a wide specificity from one cell without the requirement of T helper lymphocytes.

Roitt (1988) has further argued that autoimmunity may result from bypass of regulatory mechanisms. Thus when the population of T suppressor cells is reduced, development of auto-antibodies usually ensues. This is exemplified by neonatal thymectomy which greatly reduces their population. In thyroiditis susceptible animals like obese strain chicken and buffalo rats, neonatal thymectomy leads to spontaneous development of the disease.

Thymectomy in susceptible strain mice 2-4 days after birth causes spontaneous orchitis with consistent appearance of autoantibodies to sperm (Taguchi and Nishizuka 1981). This has been explained as being due to the presence of autoreactive T lymphocytes against sperm during these days in the absence of specific suppressor T lymphocytes. T suppressor cells are believed to appear 7 days after birth in the species.

In brief therefore, tolerance maybe broken by interfering with anatomical barriers where antigens are secluded. It may also be broken by bypass of T inducer or regulatory mechanisms. In the former, the antigen is presented directly to the effector arm of the immune response.

1.1.4   Types of Autoimmune Diseases

Autoimmune diseases may affect one or several tissues. They can therefore be classified as organ-specific like Hashimotos Thyroiditis (HT) and multiorgan related like Systemic Lupus Erythematosus (SLE) (Cooke et al 1983). EAO affects the testis and epididymis only and can therefore be classified as organ-specific autoimmune disease.

Both natural and experimentally induced autoimmune diseases have been described (Roitt 1988). Experimentally induced autoimmune diseases provide invaluable models of natural conditions since single well characterised antigens can be isolated in relatively pure forms and the immunological events studied. This can aid in management of natural autoimmunity and provide insight into the functioning of the immune system. Good examples of such experimental models include Experimental Autoimmune Thyroiditis (EAT) which relates well with HT and Experimental Allergic Encephalomyelitis (EAE) which is a good model for Multiple Sclerosis (MS) (Suckling et al 1983). EAO is gaining ground as a model for both experimentally induced as well as spontaneous male infertility.

The immune status of both B and T lymphocytes during autoimmunity may vary from disease to disease. This offers a possible classification for autoimmune diseases into antibody mediated and cell mediated ones. This often poses a difficulty since it is not always possible to follow the sequence of events and to assign a particular role to either humoral or DTH

reactions.

Some salient features do however arise in EAE and EAT
(Weigle 1980). In one type of EAT that resembles HT, the
humoral immune response appears to be the main player. The
main lines of evidence leading to this conclusion are as follows:
1) attempts to stimulate proliferation of separated T lymphocytes
from rabbits immunized with bovine or rabbit thyroglobulin in
CFA in vitro have been unsuccessful and 2) serum levels of
precipitating antibody to thyroglobulin have been found to
correlate well with the severity of the lesion.

EAE provides a model of autoimmune disease that is mediated by
Cell-Mediated Immunity (CMI)((Weigle 1980). It has close resemblance
EAO in this aspect and therefore merits some further considera-
tion.

EAE is induced by injection of homologous, heterologous,
or syngeneic Central Nervous System (CNS) tissue or Basic
Myelin Protein (BMP) in CFA Bernard and carnegic (1975). Correlatio
between EAE and DTH has been demonstrated by skin testing
and migration inhibition activity generation assays. T-cells and
macrophages are implicated in the induction of the histological
lesions of EAE and appear to be directly involved in demyelina-
tion (Weigle 1980). These cells also play a role in EAO lesion as
will be described later.

Further evidence of CMI in EAE has been obtained from
adoptive transfer experiments. This has been achieved with
lymph node cells (Stone 1961). More specifically, T lymphocytes

have been shown to be important in the induction and pathogenesis of EAE (Ortiz-Ortiz et al 1976) by removal and reconstitution experiments.

In vitro induction whereby thymocytes taken from rats are sensitized with brain antigen and then injected into syngeneic recipients results in lesions typical of EAE (Reviewed by Weigle 1980). Similar findings have been reported for EAO where Lymph Node Cells (LNCs) were used instead of thymocytes (Werkele and Begemann 1976).

All these pieces of data confirm the role of CMI in EAE.

1.1.5   Genetic Basis of Autoimmune Diseases

It has been established that there is a genetic basis for autoimmune diseases (Glasser and Silvers 1974). The importance of H-2 in determining susceptibility of mice to EAT was shown by studies using inbred and congenic strains of mice. Animals of a particular H-2 type showed good correlation between antibody titres and pathology after immunization with thyroglobulin and CFA (Weigle 1980). The genes at the D-end of the H-2 have been shown to have a modifying effect on EAT in mice of both good and poor responder strains (Kong et al 1979).

Also, strain variability has been reported in the development of EAE (Reviewed by Weigle 1980). An autosomal dominant gene (Ir) linked to the histocompatibility locus determines the susceptibility to EAE. A susceptibility pattern has also been found in patients with multiple sclerosis which is related to histocompatibility determinants.

1.2   HISTORY   OF EAO

1.2.1   Introduction

Injury of the testes resulting from subcutaneous injection of testicular material and adjuvants in the guinea pig was first reported in the 1950's (Voisin et al 1951). However, the authors failed to demonstrate specific circulating antibodies. They also demonstrated similar damage when tissue homogenates of kidney and liver were used hence their conclusion that the damage was as a result of stress.

Other workers induced injury of the testis characterised by aspermatogenesis and testis specific autoantibodies by immunization with testicular material purified in several ways or homologous spermatozoa. They however, did not notice inflammatory cells in the testis or epididymis (Freund et al 1953; Waksman 1959).

Subsequent studies did show that not all the testis proteins were responsible for the damage (Brown et al 1963). By autoclaving and phenolic extraction followed by testing, the active fraction appeared in the phenolic phase. The authors also managed to show the presence of an immediate skin reaction by day 9 after immunization and circulating antibody and EAO lesion after day 10. The lesion was concomitant with immediate and delayed type skin reactions. Furthermore, fluorescent antibody staining revealed that in the epididymis, the antibodies were directed at the sperm acrosome. In the testis, staining was confined to spermatocytes, spermatids and spermatozoa. These

authors demonstrated that immunization with organ preparations

other than testicular material in CFA did not produce lesions

in the testis. They conferred protection to induction of

orchitis by prior immunization with testicular autoclavate in

incomplete Freunds Adjuvant (IFA) and also by perinatal

immunization.

EAO, has since been induced in several other mammalian

species. In the rabbit homologous and heterologous guinea pig

testicular material in CFA has been used to induce EAO. When

homologous testis was used, the histopathology resembled that

in the guinea pig testis. The aspermatogenesis and orchitis were

present in the absence of immune complexes in the testis (Tung and

Woodroffe 1978).

In the mouse, this has been achieved by injecting homologous

testicular material in CFA of Bordetella Pertussis (Kohno et al

1983).

EAO has also been successfully induced in non-human

primates (Andrada et al 1969), in humans (Mancini et al 1965)

by active immunization with testicular homogenate.

In addition to active immunization, it has also been possible

to induce EAO in vitro. This has been achieved by culturing

normal LNCs with autologus dissociated testis cells whereby

they form rosette-like aggregates and later undergo blast

transformation and proliferation. When these lymphocytes are

injected into syngeneic recipients, they cause lesions in the

testis characteristic of EAO (Wekerle and Begemann 1976). This

suggests that testicular self antigens are recognized by clonally performed autologus lymphocytes.

Adoptive transfer of EAO with LNCs, peritoneal exudate cells (PECs) and purified T lymphocytes from peritoneal exudate has been achieved (Tung et al 1971a, Kantor and Dixon 1972, Tung et al 1977). The lesions thus obtained are indistiguishable from those in EAO induced by active immunization. The adoptive transfer also required that the recipients be sexually mature indicating that they are antigen specific.

1.2.2. Isolation of Orchitogenic Antigens

Several attempts have been made to isolate and characterise guinea pig testicular orchitogenic autoantigens. A highly soluble aspermatogenic protein named API has been isolated from guinea pig testis and shown to have acrosomal localization. The protein has been shown to induce orchitis at very low concentrations (0.5 to 1 μg). The lesion that is obtained resembles one when "whole testis" is used (Jackson et al 1975).

Other autoantigens that have been isolated and shown to have acrosomal localization are GP1, GP4, S and P. GP1 and GP4 are glycoproteins that have a high orchitogenic activity (Hagopian et al 1975). S and P autoantigens are water soluble and no evidence has been offered to indicate whether they differ from those described by other authors (Lefroit-Joliy et al 1979).

More recently (Teuscher et al 1983) an aspermatogenic polypeptide named AP3 has been isolated and shown to have a high

orchitogenic activity. The activity was unaffected by various denaturing techniques like reduction and alkylation. This suggests that the activity is dependent on the primary structure and was therefore referred to as a Sequential-Disease Inducing Determinant (SDID). The antigen is therefore different from API which was reported to lose activity upon reduction or alkylation and from GP1 and GP4 by its lack of carbohydrate moiety.

Apart from acrosomal autoantigens, another autoantigen named T (Lefroit-Jolly et al 1979) with spermatozoal and testicular cells plasma membranes localization has been isolated. The T autoantigen is very sensitive to denaturation and this rules out its possibility of being AP3.

1.3   IMMUNOLOGY AND IMMUNOGENETICS OF EAO

1.3.1   Immunology of EAO

The role of cell mediated and humoral immune responses in autommunity are obscure and often confusing. Early studies on the immunology of EAO indicated that both antibody and CMI responses are required for full expressin of EAO (Brown et al 1963). Waksman (1959) interpreted the nessity for CFA in the induction of EAO to mean requirement of DTH.

Further data has been obtained from experiments involving prior protection against EAO induction by pretreatment with antigen in Incomplete Freunds Adjuvant (IFA). Brown and coworkers (1967) used testicular autoclavate as the antigen and managed to confer protecti

They suggested that the protection was due to suppression of DTH reactions. They however observed that the appearance of the lesion was concomitant with both circulating antibody and DTH. This led to their conclusion that both DTH and circulating antibody are necessary for full expression of EAO.

Hojo and Hiramine (1982) conferred protection by pretreatment of animals with testicular antigen in IFA two weeks prior to orchitogenic challenge. They showed that CMI responses in vitro in the protected animals were suppressed and that DTH as shown by Skin testing was also depressed. An important observation was that protected animals had higher antitesticular antibody titres than the ones given only orchitogenic challenge. They did not demonstrate any protection when the antibody fraction was administered into other guinea pigs. The authors therefore, confirmed that pretreatment with antigen in IFA confers protection by suppressing CMI responses rather than humoral ones. The role of CMI is further buttressed by the evidence that the animals thus protected can develop orchitis when injected with lymphocytes from actively immunized animals. Furthermore, lymphocytes from the actively immunized unprotected animals do produce Macrophage Inhibitory Factor (MIF) in vitro .

Adoptive transfer experiments have mainly pointed at CMI (Reviewed by Tung et al 1981a). Thus, LNCs and Peritoneal Exudate Cells (PECs) have bbeen shown to have high efficiency in transfering EAO. Also notable is that the rate of success of the transfer correlates directly with the number of T lymphocytes.

Direct evidence for thymus dependence in the pathology of EAO was obtained from reconstitution experiments (Bernard et al 1978). BALB/C nu/nu hypothymic mice have been found not to develop orchitis despite appropriate immunization schedules. However, reconstitution with thymus cells from litter mates BALB/C nu/+ mice resulted in complete restoration of this capability.

Immunoglobulins and complement component C3 appear in the rete testis of animals given orchitogenic challenge prior to development of orchitis and high titres of complement fixing antibodies are found in the sera of the animals (Tung et al 1971b). Also immune sera has been shown to transfer EAO when the animals are primed with CFA before transfer (Tung et al 1981a). The need for priming is unknown, however it can be concluded that antibodies do play a role in the development of EAO.

In vitro studies indicate that T lymphocytes alone do not respond to testicular antigens but the addition of mitomycin C treated macrophages or B lymphocytes enhances the response. Tritiated thymidine incorporation studies show that B lympho-cytes are more effective than macrophages (Hojo et al 1980). The authors therefore suggested that B lymphocytes act as antigen processing and/or presenting cells to the T lymphocytes.

The picture that emerges from the evidence is that although CMI is responsible for EAO lesion, the initial stages involve B lymphocytes, macrophages and perhaps antibodies from B lymphocytes.

resistance to induction of EAO is also inherited as a dominant autosomal trait.

1.4   HISTOPATHOLOGY OF EAO IN GUINEA PIG

The EAO lesion appears between days 9 – 14 after immunization with testicular extract or spermatozoa (Brown et al 1963). Three well-defined histological lesions have been described (Tung and Alexander 1977) i.e.

a)   Aspermatogenesis in the Seminiferous Tubules (ST) which consists of a focal desquamation of spermatids in which deformed acrosomes of spermatids are visualized ultrastructurally. This is followed by entire loss of the germinal epithelium except the Sertoli cells, leydig cells and spermatogonia. The desquamated cells are found in the lumen of the ST, the rete and the epididymis. Peritubular infiltration of mononuclear cells does occur sometimes.

b)   In the initial stages of EAO, focal clusters of lymphocytes and monocytes appear in the interstitial spaces of STs and rete testis.

c)   Later, clusters of macrophages and lymphocytes appear in the lumen of the ST, rete and epididymis. The later stages are characterised by heavy infiltration of monocytes, lymphocytes, eosinophils in the interstitial spaces and of polymorphonuclear neutrophils (PMNs) in the lumen of blood vessels and ST.

Prominent polymorphs are seen between cells along the basement membrane and inside the lumen of the efferent ducts, the caput and caudal epididymis as well as the vas deferens.

Occassionally, giant multinucleate cells appear in the lumen of some seminiferous tubules whose identity is yet to be established (Brown et al 1963).

1.5 NATURAL AUTOIMMUNE ORCHITIS

1.5.1 Spontaneous Autoimmune Orchitis

Tung and coworkers (1981b) have adequately defined an undesirable phenotype that has resulted in the breeding of mink of fine black fur. This phenotype has two types of male infertility. Primary infertility was defined as infertility before the first mating which occurs at the age of 10 months. The second type being secondary infertility defined as infertility after a period of proven successful matings.

Secondary infertility appears to have an immunological basis in that a) the males with this type of infertility had significantly higher antisperm antibody titres than males with primary infertility, b) aspermatogenesis without signs of orchitis or testicular immune complexes appeared only in mink with primary infertility but not those with secondary infertility and c) only males with secondary infertility but not primary showed severe orchitis and testicular immune complexes.

The immunological basis of this secondary infertility gains further credence when the following lines of evidence are considered 1) Aleutian mink disease is an immunological disease that is related to fur colour and 2) After vasectomy of guinea pigs, autoantibody response to sperm surface antigens

is determined by a single autosomal or x-linked gene (Tung et al 1981c).

Spontaenous autoimmune orchitis has been reported from several other species (Alexander and Anderson 1984). A certain strain of beagle dogs has a testicular disease that closely resembles EAO. In mice, a certain strain that is heterozygous for a recessive lethal gene has as high as 40% incidence of EAO. In a normal population of rhesus macaques, a third of all testes have been shown to have monocytic orchitis.

1.5.2   Vasectomy-induced Autoimmune Orchitis

Vasectomy is a surgical procedure of contraception in which the vas deferens is either sectioned or ligated. This in effect obstructs the vas deferens confining the spermatozoa in it and the epididymis.

Autoimmunity following vasectomy of various mammalian species has been reported. In the guinea pig, both bilateral and unilateral vasoligation causes histopathological lesions in both testes indistinguishable from EAO (Tung and Alexander 1977). Further, the lesions obtained can be adoptively transfered to syngeneic recipients (Tung 1978). This shows that the lesions are mediated by specifically sensitized cells of the immune system.

In rabbits, vasectomy leads to orchitis whose histopatho-logical picture is indistinguishable from EAO. In vitro detected CMI responses correlates with the histopathology thus

obtained (Tumbo-Oeri and Roberts 1979). The authors suggested that this maybe due to degeneration and subsequent leakage of spermatozoal antigens into circulation thus provoking specific immune response.

Monocytic orchitis and aspermatogenesis develop in rhesus monkeys after vasectomy. Although it occurs spontaneously in this species, the incidence is much higher and epididymitis occur exclusively in the vasectomized animals (Tung and Alexander 1980).

In addition to orchitis per se, other immunological responses that may or may not be associated with orchitis have been reported after vasectomy. Bigazzi and coworkers (1976) have reported a high incidence of circulating antibodies (46%) to sperm acrosomal antigens after vasectomy of rabbits and 36% of them had both acrosomal and testis antigens antibodies.

Vasectomy in the guinea pig (Tung et al 1981c) causes autoantibodies to Testis Cell-Sperm Differentiation Antigens (TSDA) whose quantity has been found to have a genetic basis. The anti-TSDA antibody response is governed by a single autosomal or x-linked dominant gene.

Evidence of orchitis after vasectomy in humans has met with controversy (Alexander and Anderson 1984). However, high levels of sperm specific autoantibodies in the sera of vasectomized men has been reported (Samuel et al 1975, Tung 1975) CMI responses to sperm after vasectomy in man have also been reported (Nagarkatti and Rao 1976).

All the data indicate that orchitis in natural conditions is similar to that in vasectomy, although the relationship is poorly understood and needs further investigation.

1.5.3 Infection-induced Orchitis

Infection has been shown to cause orchitis. Trypanosomal infection induces testicular degenerative changes leading to suppression of spermatogenesis in goats and laboratory animals. The mechanism of pathology is not known but some authors suggest that it may partly be due to depressed levels of plasma testosterone (Waindi et al 1986). The histological picture that arises shows degenerative changes with testis showing desquamation of germinal epithelia of the seminiferous tubules. This however is not the complete picture of EAO described earlier.

Mumps viral infection in man is known to cause orchitis of unknown origin (Hopps and Parkman 1979). The incidence is as high as 20% of infected adult males. This also needs further investigation as it may lead to the elucidation of EAO pathogenesis.

1.6 IMMUNOMODULATION

1.6.1 Definition

Immunomodulation refers to manipulations of the immune system so as to dampen or enhance its activities to achieve a particular response. It may be broadly subdivided into immunopotentiation, tolerance and immunosuppression.

A comprehensive description of the three has been adequately presented by Waksal (1978). Immunopotentiation is

the augmentation of the hosts non-specific or specific immune responses through a variety of biologically active substances, whereas immunosuppression is the dampening of immune response and can be achieved by various methods among them being radiation, corticosteroids, cytotoxic chemicals which include alkylating agents, antimetabolites, folic acid antagonists and antilymphocyte sera, etc.

1.6.2 Immunomodulation by Glucocorticoids

Glucocorticoids are a group of adrenal corticosteroids that regulate metabolism at the gene level. The effect is mainly at gluconeogenesis and they do so by permitting responses to other treatments such as starvation, diabetes and glucagon rather than a direct effect on the flux. They, together with their synthetic analogues have gained importance in immunotherapy.

The mode of immunomodulation by glucocorticoids varies between corticosteroid sensitive species like the mouse and the rat and the resistant species like man and the guinea pig (Fauci 1979). It involves mainly lysis of lymphoid cells in sensitive species. This although a factor in resistant species, it takes a different form as it is selective. Thus, hydrocortisone in vitro has been shown to lyse T lymphocytes activated in a mixed leucocyte reaction but not resting peripheral leucocytes or mitogen activated T-cells (Cupps and Fauci 1982).

Fauci (1979) has suggested also that, although a marked lymphocytopenia occurs on glucocorticoid administration in pharmacological doses, this is as a result of redistribution of

of circulating lymphocytes into other compartments of the body lymphocyte pool. This may be as a result of changes in the membrane configuration induced by binding glucocorticoids. The lymphocytopenia is characterised by a more marked reduction of the T lymphocytes as compared to B lymphocytes.

Cupps and Fauci (1982) have also suggested that glucocorticoids inhibit most T cell functions by interfering with interleukin-1 stimulation of interleukin-2 production by the T cells. This factor which induces proliferation of T cells after production is reduced.

A difference however, does occur between chronic glucocorticoid treatment and acute treatment (Balow et al 1975). Acute treatment only causes lymphocytopenia without any marked changes in in vitro parameters like MIF and lymphocyte proliferation assays. In contrast, chronic treatment in addition to causing lymphocytopenia, markedly diminishes antigen stimulated MIF and proliferation responses without significant reduction in mitogen stimulated proliferation.

The glucocorticoids also cause a monocytopenia which is similar in kinetics to lymphocytopenia (Cupps and Fauci 1982). This could be due to decreased release of monocytes from bone marrow. The effect on macrophages - monocytes may have a profound effect on the whole immune system due to the central role of these cells in T and B cell responses.

The monocytes, on exposure to various concentrations of glucocorticoids affect immunoglobulin production by B-cells by providing small quantities of interleukin-1 which is required by T cells for production of the cytokine T-cell Replacing Factor dependent on Steroids (TRF-S) which acts on B-cells to increase antibody production (Orson and Auzenne 1988). The net effect of chronic corticosteroid administration is therefore to suppress CMI reactions.

1.6.3   Previous Studies Involving Immunomodulation of EAO
         in the Guinea Pig

Hojo and Hiramine (1982) did show that when guinea pigs were pretreated with testicular antigen in incomplete Freunds adjuvant 2 weeks prior to orchitogenic challenge they developed transient resistance to EAO. This did confirm previous results (Brown et al 1963, Brown et al 1967). In addition, they established that this protection was accompanied by suppresssion of delayed skin response to testicular antigen and abrogation of proliferative response as well as blastogenic factor production by either purified T lymphocytes or unseparated LNCs. These responses, which mmeasure CMI are restored at appoximately the same time that the apparent tolerance breaks.

No correlation between antisperm antibody titres and development of orchitis in the protected animals was observed. The authors were therefore able to conclude that failure of expression of T-cell function confers protection against EAO

induction. This occurs in the presence of competent B-cells as shown by antibody titres.

Similar results have been obtained using cyclosporin A a fungal antimetabolite which blocks T-cell mediated responses by inhibiting the early stages of T-cell activation (Hojo and Hiramine 1985). Specifically, T-cell growth factor (also designated interleukin-2) production by activated T-cells is blocked at the gene expression level (Kronke et al 1984).

Cyclosporin A has been shown to abrogate the induction of EAO in the guinea pig when administered starting on the day of immunization. The unresponsive state is however transitory and is antigen specific (Hojo and Hiramine, 1985).

1.7   AIMS AND OBJECTIVES

Given that EAO is an autoimmune condition modulated by pretreatment with testicular antigen in incomplete Freunds adjuvant (Brown et al 1963; Brown et al 1967, Hojo and Hiramine 1982) and also by treatment with cyclosporin A (Hojo and Hiramine 1985) and that glucocorticoids have been used over the years as therapeutic agents in conditions that are immune mediated   investigations of the effect of glucocorticoids in pathogenesis of EAO are indicated.

Thus, this study was undertaken to assess the effects of glucocorticoids on the pathogenesis of EAO.

More specifically:-

1. To investigate the characteristics of the histopathologic lesion of EAO when cortisol has been administered.

Structure of Cortisol

$$
\begin{array}{c}
H \\
| \\
H-C-OH \\
| \\
C=O \\
\end{array}
$$

HO ⋯ OH

O
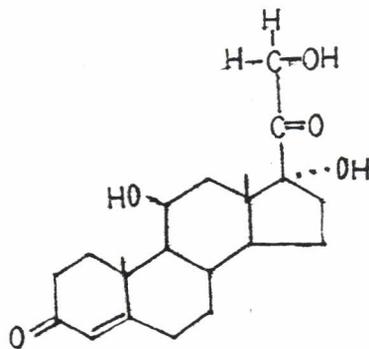
2. To investigate the pathogenesis of EAO when prednisolone a synthetic glucocorticoid and whose antiinflammatory effects compare with those of cortisol, is used.

Structure of Prednisolone

$$
\begin{array}{c}
H \\
| \\
H-C-OH \\
| \\
C=O \\
\end{array}
$$

HO ⋯ OH

O
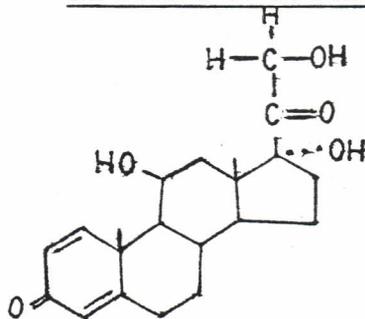
3. To compare the results with those reported in literature on immunomodulation of EAO.

CHAPTER 2

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Animals

Outbred male guinea pigs were obtained from the Department of Biochemistry, University of Nairobi animal house and also from National Public Health Laboratories of the Ministry of Health, Nairobi. Only sexually mature animals weighing between 550 and 700 g were used.

2.1.2 Glucocorticoids

2.1.2.1. Cortisol

Hydrocortison "Ritcher" microcrystalline injectable was purchased from "E.T. Monks" Nairobi and was suspended at a concentration of 25 mg/ml.

2.1.2.2 . Prednisone

Prednisone "Nutritional Biochemical Corporation, Cleveland Ohio" was obtained from "Sigma Chemists", Nairobi and made to a concentration of 2.5 mg/ml.

2.1.3 Adjuvant

Complete Freuds Adjuvant (CFA) (DIFCO, Detroit, Mich.) containing 1 mg killed Mycobacterium Tuberculosis per ml was purchased from Kobian Chemicals" Nairobi.

2.1.4 Light Microscopy Materials

Bouins fixative was prepared as follows (Grimstone and Skaer 1972).

Picric acid (saturated aqueous solution)	75 ml.
Formalin	25 ml.
Acetic acid (glacial)	5 ml.

Haematoxylin stain was prepared as follows (Grimstone and Skaer 1972). To ferric ammonium sulphate (3% in water) 1 volume of haematoxylin (5% in 96% ethanol) was added and the solution allowed to ripen for 2 weeks. Nine volumes of water were then added to the stock solution.

2.1.5   Electron Microscopy Materials

The fixative was prepared as follows:

Glutaraldehyde (25%) 12 ml

0.1 M phosphate buffer, pH 7.4 88 ml.

Epon Resin Mixture

Epon resin mixture was prepared as follows:

Solution A :

Epon 812 - 60 mls

Dodecenyl succinic acid (DDSA) - 100 mls.

Solution B :

Epon 812 - 100 mls

Methyl nadic anhydride (MNA) - 89 mls.

40 mls of solution A was mixed with 60 mls of solution B and mixed thoroughly. 1.8 mls of the catalyst was then added for every 100 mls of the mixture.

2.1.6 Buffers

Phosphate buffered saline contained 57 mM Na_2HPO_4, 3.36 mM NaH_2PO_4 and 43.7 mM NaCl pH 7.4

Physiological saline was NaCl (0.85%) in distilled water.

2.2 METHODS

2.2.1 Antigen Preparation

Guinea pig testis antigenic preparation was prepared according to the fractionation procedure described by Hagopian et al (1975). The testes were obtained from sexually mature male guinea pigs and treated as follows. (All the steps were carried out at 4°C).

i) Fatpads and most of the extraneous muscle were removed. The epididymides were left intact.

ii) The materials were chopped up extensively and the resulting mass homogenized in prechilled chloroform : methanol mixture (2:1) for 5 minutes.

iii) The homogenate was allowed to stand in a separating funnel to obtain 2 layers. The organic layer was discarded and the upper aqueous layer used in the following steps.

iv) The aqueous layer was filtered through 2 sheets of Whatman No. 41 filter papers under suction and the residue washed with liberal amounts of prechilled acetone to remove traces of lipid and water. The filtrate was discarded and the

residue allowed to dry in air.

v)   The dry powder was suspended in 30 volumes of water (w/v) acidified to pH 3 by dropwise addition of 12N HCl. It was stirred overnight with occassional readjustment of pH whenever necessary.

vi)   The mixture was then centrifuged at 10,000 g for 30 minutes.   The precipitate was discarded and the supernatant neutralized to pH 6.0 by dropwise addition of concentrated ammonia solution (NH_4OH).

vii)   The neutralized extract was lyophillized and stored at $-20°C$ until required.

For induction of EAO, the protein concentration was adjusted to 10 mg protein/ml of physiological saline.

2.2.2   Protein Estimation

Protein estimation was carried out by the method of Lowry et al (1951).   The partially purified antigenic preparation was serially diluted after making a stock solution from the lyophillized   powder with physiological saline (0.85% NaCl in distilled water).

A standard protein curve was constructed using bovine serum albumin (BSA) fraction v obtained from Sigma Chemicals Company.   The optical density of the colour developed was read at 750 nm using a Pye Unicam SP 1800 spectrophotometer.

2.2.3   Induction of EAO

EAO was induced by injecting 2 mg of partially purified protein emulsified in 1:1 ratio in CFA.   The emulsion in a total volume of 0.4 mls was injected into the rear footpads and two other areas around the scapular region under asceptic conditions.   Approximately 0.1 mls of the emulsion was injected via the subcutaneous route at every injection site.

2.2.4   Glucocorticoid Administration

A dose of 5mg/100g body weight of cortisol was administered daily for a set of experiments and one of 0.5mg/100g body weight of prednisone was administered for the second set, daily (Fauci 1979).   The injections for both glucocorticoids were given 2 hours prior to orchitogenic challenge intraperitoneally for the initial doses.   This was followed by daily subcutaneous injections for 14 days.

2.2.5   Experimental Design

For evaluation of the glucocorticoids effect on induction of EAO, the animals were in each set of experiments were put into 4 groups.

Group A animals were given orchitogenic challenge and treated with the particular glucocorticoid starting on the day of challenge.

Group B animals were immunized with testicular antigen in CFA and injected with physiological saline in place of the

glucocorticoid.

Group C animals were "immunized" with physiological saline emulsified in CFA and received glucocorticoid treatment to control for stress and the metabolic changes that maybe caused by glucocorticoid administration. Group D animals received no treatment at all.

The animals were bled and killed after 14 days and the testes. removed for histological examination. The blood was tested for precipitating antibodies and some of the testes were prepared for electron microscopy.

2.2.6 Light Microscopy

After removal, the testes and the other tissues were cut up with a sharp knife into thin slices and fixed in bouins solution for a period of 48 hours.

They were then transfered to 50% ethanol for 2 hours, 60% ethanol for 2 hours and then held at 70% ethanol until processed further.

The dehydration process involved 1 change lasting 2 hours in 80% ethanol, 1 change of 2 hours in 90% ethanol, 1 hour in 95% ethanol, then 3 changes of 1 hour each in absolute ethanol.

Clearance was achieved by 2 changes of 1 hour each in xylene and a final one of 2 hours in xylene.

Impregnation was done by transfering the tissues to molten paraffin wax for 3 changes of 2 hours each.

The tissues were then embedded in paraffin wax and sectioned.

Sections of 5µ to 6µ were mounted on glass slides layered with a thin layer of albumin, dewaxed with xylene and rehydrated with decreasing concentrations of ethanol. They were then put in water before staining with hematoxylin and eosin (H & E) and examined by "Leitz Laborlux 12" microscope.

2.2.7   Electron Microscopy

Small blocks of the testis were fixed in 2.3% phosphate buffered glutaraldehyde for a period of 6 - 12 hours at 4°C. The tissues were then washed with phosphate buffer three times with each change lasting 5 minutes. The tissues were then postfixed in 10% Osmium tetroxide (OsO_4) in distilled water for a period of 6 - 12 hours at 4°C. The tissues were then washed with physiological saline for three changes each lasting 5 mins.

Dehydration was done by transfering the tissues to 70% ethanol for 3 changes, each of 5 minutes then 2 changes lasting 10 minutes in 90% ethanol and finally 2 changes of 20 minutes each in absolute alcohol.

The tissues were then cleared in propylene oxide for 2 changes each lasting 30 minutes.

They were then infiltrated with propylene oxide: Epon resin mixture (3:1) for 1 hour. This was followed by infiltration with propylene oxide : Epon resin (1:3) mixture

for another 1 hour. Finally, the tissues were infiltrated with epon resin mixture alone for $1\frac{1}{2}$ hours at 37°C.

Embedding was done in epon resin mixture with catalyst (DMP30) in plastic capsules and at room temperature and then left in the oven at 60°C-70°C for a period of 18 - 24 hours.

Sections of 80 - 150 nm thickness were cut using glass knives and then picked on 300 mesh copper grids which were previously cleaned in formic acid, distilled water and acetone.

The tissues were stained in uranyl acetate for 10 minutes in the dark and rinsed with 4 changes of distilled water. They were then blotted on filter papers before staining with lead citrate for 5 - 7 minutes followed by four rinses in distilled water and blotting on filter paper.

The tissues were scanned using a model EM9 S2 (CARL ZEISS) electron microscope.

2.2.8   Rating of Orchitis, Epididymitis and Aspermatogenesis

The rating of these three features of orchitis was done on arbitrary scale as defined by Hojo and Hiramine (1982). Where there was no evidence of cellular infiltrate, orchitis or epididymitis was rated as zero. 1+ represented one or just a few foci of cellular infiltrate. 2+ represented several foci of cellular infiltrate or moderate, diffuse infiltration or both. 3+ was general involvement of the testis or epididymis with severe infiltration.

Aspermatogenesis was rated from 0 to 3+ with 0 representing no evidence of impaired spermatogenesis. 1+ represented slight aspermatogenesis with very few spermatozoa and spermatids. Aspermia and diminution of spermatogenic cells was rated as 2+. Complete aspermatogenesis was rated as 3+.

C H A P T E R   3

RESULTS

3.1 EFFECT OF CORTISOL ON THE DEVELOPMENT OF EAO

The intensity and incidence of EAO was investigated by light and electron microscopy after chronic cortisol treatment. These experiments were carried out to find out whether, cortisol which is known to inferfere with T-cell activity leading to CMI and is a general antiinflammatory agent (Fauci 1979) could confer protection to animals given orchitogenic challenge.

3.1.1 Light Microscopy

Sections from several parts of the testis were made and examined at low (x250) and high power (x400). The overall picture was what was used in the arbitrary scale described earlier.

The results are summarized in Table 1. They illustrate that of the 28 group A animals (animals immunized with TA-CFA and given chronic cortisol treatment). 23 of them representing 82% developed no orchitis whereas 3 (11%) had only a few foci of cellular infiltrate. Two (7%) developed severe orchitis and had giant cells in the seminiferous tubules lumen. Two animals with no evidence of orchitis had slight epididymitis.

The same table shows the results of aspermatogenesis in the animals. The distribution was 22 (79%) with no evidence of aspermatogenesis, 3 (11%) with slight aspermatogenesis, 1 (4%) with moderate and 2 (7%) with severe aspermatogenesis. The two animals with severe orchitis also had severe aspermatogenesis with no evidence of spermatogenic cells in the STs. The testis of these animals had severe interstitial

inflammation (Plate 2).

Of the 28 animals in group B (animals immunized with TA-CTA and treated with physiological saline), 20 (71%) had severe orchitis presenting as heavy interstitial and ST infiltration by inflammatory cells. The rete testis in all the cases was the most affected. Five animals (18%) exhibited moderate, 1 (4%) slight and 2 (7%) no orchitis. Twenty of the animals developed severe aspermatogenesis, 4 (14%) had moderate and 2 (7%) slight aspermatogenesis. Two of the animals had normal spermatogenesis. In the same group, 6 (22%) had severe epididymitis, 16 (59%) had moderate epididymitis and 5 (18%) had normal epididymides.

In group C animals (24 animals "immunized" with physiological saline and given chronic cortisol treatment) none of them developed orchitis, epididymitis or aspermatogenesis. The same was observed with the 10 group D animals (animals that were not injected at all).

TABLE 1   :   SUPPRESSION OF EAO BY CORTISOL

GROUP	IMMUNIZATION	ADMINISTRATION OF CORTISOL	ORCHITIS O	1+	2+	3+	Total	ASPERMATOGENESIS O	1+	2+	3+	Total	EPIDIDYMITIS O	1+	2+	3+	Total
A	TA–CFA	YES	23	3	0	2	28	22	3	1	2	28	24	2	2	0	28
B	TA–CFA	NO	2	1	5	20	28	2	2	4	20	28	5	0	16	6	27
C	PHYSIOLOGICAL SALINE	YES	24	0	0	0	24	24	0	0	0	24	24	0	0	0	24
D	NONE	NO	10	0	0	0	10	10	0	0	0	10	10	0	0	0	10

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FIGURE 1

Bar graphs showing the incidence of epididymitis,
orchitis and aspermatogenesis in animals immunized with
TA-CFA and treated with cortisol (Group A) alongside
the control group B. The vertical axis is in number of
animals.   The horizontal axis shows the rates of the
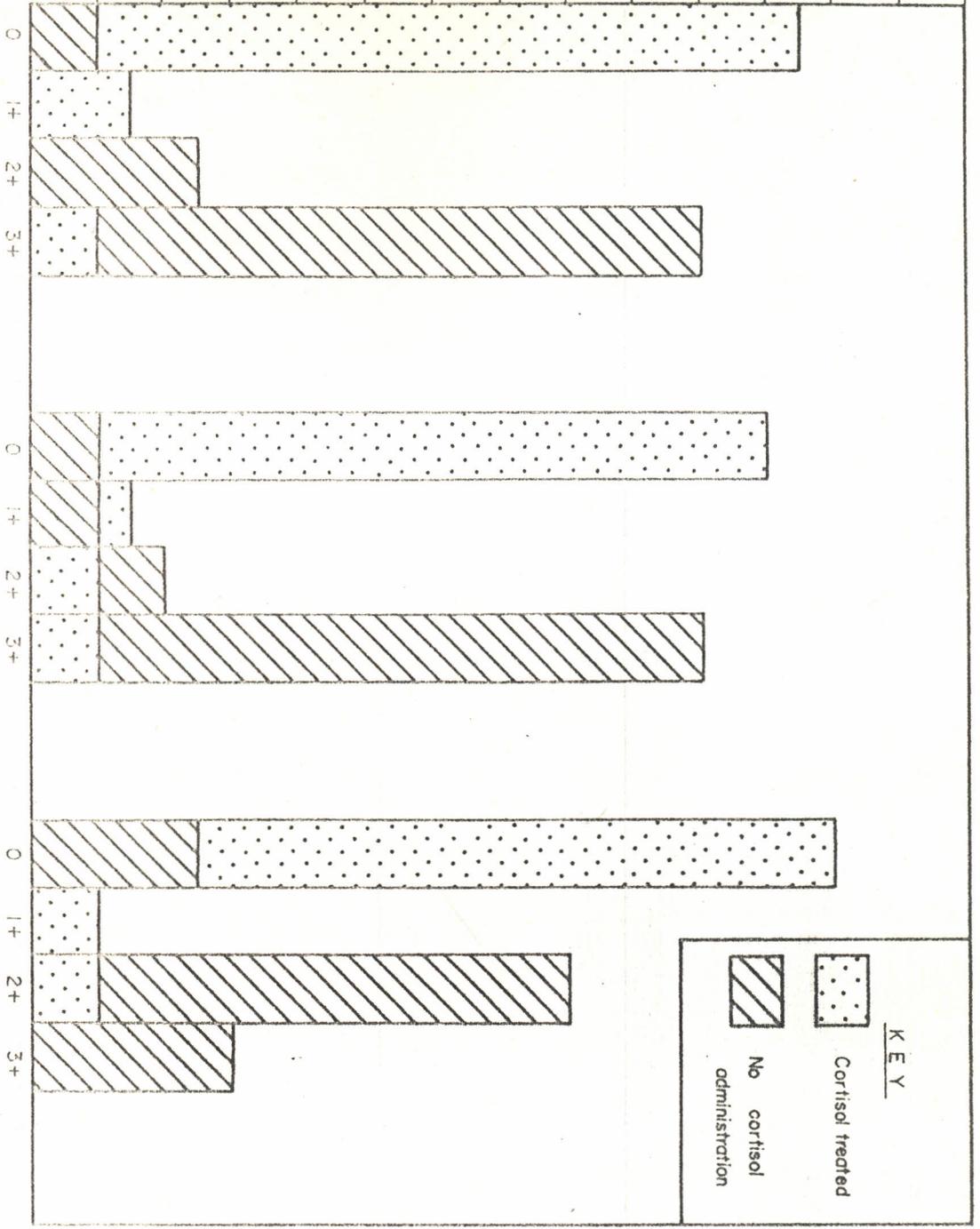three parameters as described in the arbitrary scale
earlier.

NUMBER OF ANIMALS

28 26 24 22 20 18 16 14 12 10 8 6 4 2

ORCHITIS

0 1+ 2+ 3+

ASPERMATOGENESIS

0 1+ 2+ 3+

EPIDIDYMITIS

0 1+ 2+ 3+

K E Y

Cortisol treated

No cortisol administration

3.1.2   Electron Microscopy

Light microscopy (above) revealed definite pathology in the animals given orchitogenic challenge and not treated with cortisol. At the same time, animals given orchitogenic challenge and given chronic cortisol treatment were mostly devoid of pathology. This therefore necessitated the use of EM in order to obtain a more detailed picture on the ultrastructural state of the testis from the two groups.

Animals from group A which demonstrated protection from EAO had normal germ cells seen on electron micrographs between adjacent Sertoli cells (Plate 7).

On the other hand, testis with orchitis had several macrophages and lymphocytes. Although the Sertoli cells did not appear affected, the tight junctions were less well defined (Plate 8).

3.2   PREDNISONE EXPERIMENTS

Prednisone is a synthetic glucocorticoid which in vivo interconverts with prednilone according to the following equation:-

Prednisone   Prednisolone
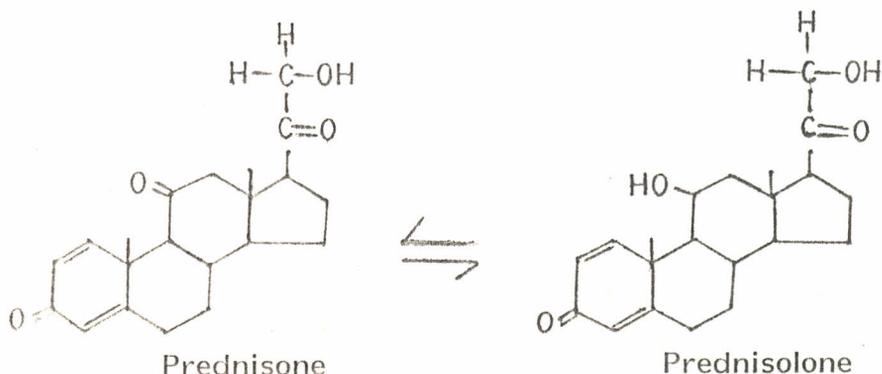
It was used to confirm the results obtained with cortisol given that synthetic steroids have been found to be several times more effective in humans (Wolff 1979).

3.2.1   Histological Examination

Table II summarizes the histological findings in the groups of animals on this set of experiments.   The protected (group A) animals demonstrated lower incidence and lesser severity of orchitis, epididymitis and aspermatogenesis.

Thus 5 animals out of the thirteen in this group had no orchitis on the arbitrary scale described previously.   This represents 39% of the total.   Four of them i.e. 31% total had 1+ orchitis, 2 (15%)   had 2+ and only 2 (15%) had severe orchitis.

More or less, the same trend was observed with aspermatogenesis in the same group. Four animals had no aspermatogenesis i.e.   31% and six (46%)   had slight, 1 animals (8%   had moderate and 2 (15%)   had severe asperma-togenesis.

Majority of animals in this group had no signs of epididymitis.   Seven of them (54%)   had 0 epididymitis, 2 (15%) had slight, 3 (23%)   moderate and only 1 (8%)   had severe epididymitis.

This trend bears sharp contrast to group B animals. Of the seven animals in this group challenged with TA-CFA, 4

of them   (57%)   had severe orchitis, 1   (14%)   had moderate

and only 2(29%)   had no evidence of orchitis.

The trend of aspermatogenesis was similar with 4

animals   (54%)   severes 1(14%)   with moderate, 1 with slight

and only 2 (29%)   had no evidence of aspermatogenesis.

Although the trend of epididymitis in this group was not

as dramatic, it is quite clear that a higher percentage of

animals in this group had more severe epididymitis than for

group A.   Three animals representing 43%   had moderate

epididymitis, 1 (14%)   severe and 1 slight epididymitis.

Two (29%)   animals had no evidence of epididymitis.

Animals in group C and D had no signs of EAO according

to the three parameters described earlier.

TABLE II

SUPPRESSION OF EAO BY PREDNISONE

The table summarizes the results for animals
immunized with testicular antigenic preparation in
complete Freunds adjuvant (TA-CFA) and given
prednisone treatment (Group A). Those given
orchitogenic challenge (TA-CFA) followed by physio-
logical saline treatment (Group B). Those "immunized"
with physiological saline and treated with prednisone
(Group C) and a fourth group that did not receive any
of the above treatment (Group D).

TABLE II   :   SUPPRESSION OF EAO BY PREDNISONE

GROUP	IMMUNIZATION	ADMINISTRATION OF PREDNISONE	ORCHITIS					ASPERMATOGENESIS					EPIDIDYMITIS				
			O	1+	2+	3+	Total	O	1+	2+	3+	Total	O	1+	2+	3+	Total
A	TA–CFA	YES	5	4	2	2	13	4	6	1	2	13	7	2	3	1	13
B	TA–CFA	NO	2	0	1	4	7	2	1	1	4	7	2	1	3	1	7
C	PHYSIOLOGICAL SALINE	YES	5	0	0	0	5	5	0	0	0	5	5	0	0	0	5
D	NONE	NO	5	0	0	0	5	5	0	0	0	5	5	0	0	0	5

FIGURE 2

Bar graphs showing the incidence of orchitis, aspermatogenesis and epididymitis in animals immunized with TA-CFA and given chronic prednisone treatment (Group A) alongside the control group B.   The vertical axis is in percentage per group.   The horizontal axis shows the rates of the three parameters as described in the arbitrary scale earlier.
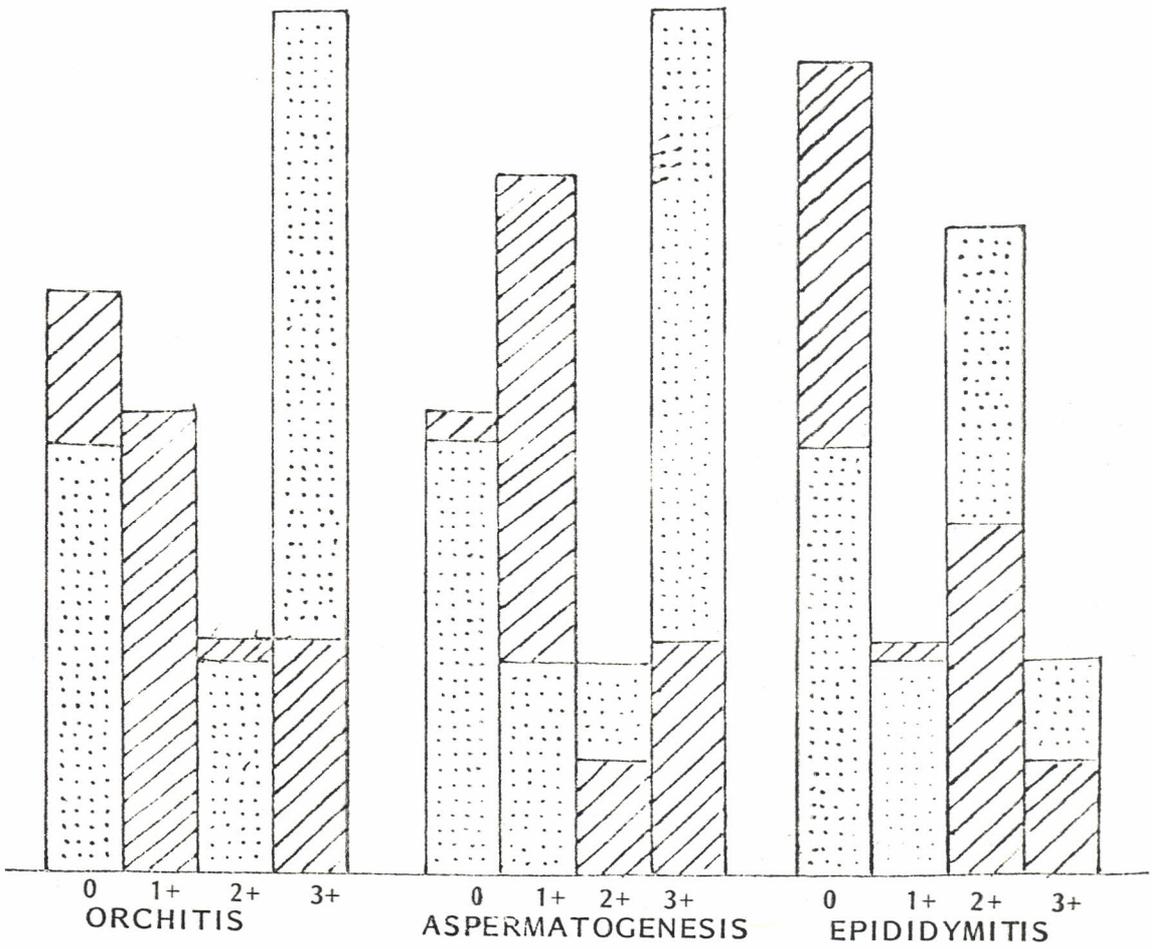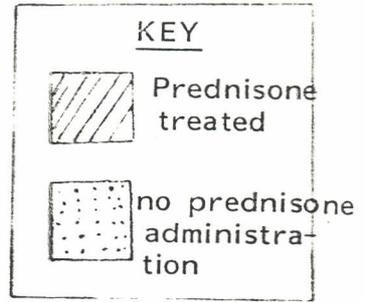
43

TABLE 3

The table gives the percentages of the EAO intensity in each group of animals for both cortisol and prednisone experiments.

TABLE 3 :

		CORTISOL					PREDNISONE			
		A	B	C	D		A	B	C	D
ORCHITIS	0	82.14	7.14	100	100		38.46	28.57	100	100
	1+	10.71	3.57	0	0		30.77	0	0	0
	2+	0	17.85	0	0		15.38	14.29	0	0
	3+	7.14	71.43	0	0		15.38	57.14	0	0
EPIDIDYMITIS	0	85.7	17.85	100	100		53.85	28.57	100	100
	1+	7.14	0	0	0		15.38	14.29	0	0
	2+	7.14	59.25	0	0		23.08	42.86	0	0
	3+	0	22.22	0	0		7.69	14.29	0	0
ASPERMATOGENESIS	0	78.57	7.14	100	100		30.77	28.57	100	100
	1+	10.71	7.14	0	0		46.15	14.29	0	0
	2+	3.57	14.29	0	0		7.69	14.29	0	0
	3+	7.14	71.43	0	0		15.38	57.14	0	0

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PLATE 2

Cross-section of testis of a guinea pig immunized
with TA-CFA and given daily injections of physiological
saline.   Seminiferous tubules showing cellular infiltration
and is devoid of spermatozoa.   Interstitial spaces show
heavy cellular infiltration.   x 250

Arrows show interstitial space with heavy infiltration
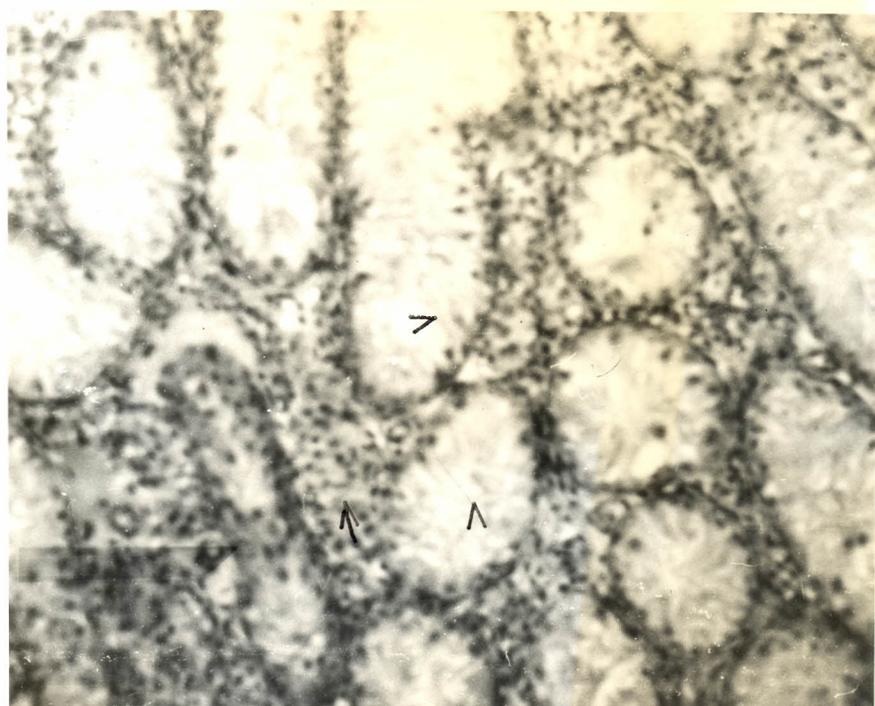Arrow heads show lumen of STs with no spermatozoa

Plate 2

PLATE 3

Cross-section of the rete testis of a guinea that
though it was protected with cortisol, it developed
orchitis after orchitogenic challenge. No prominent
foci are visible. x 250.

Giant cells are visible in the lumen. G
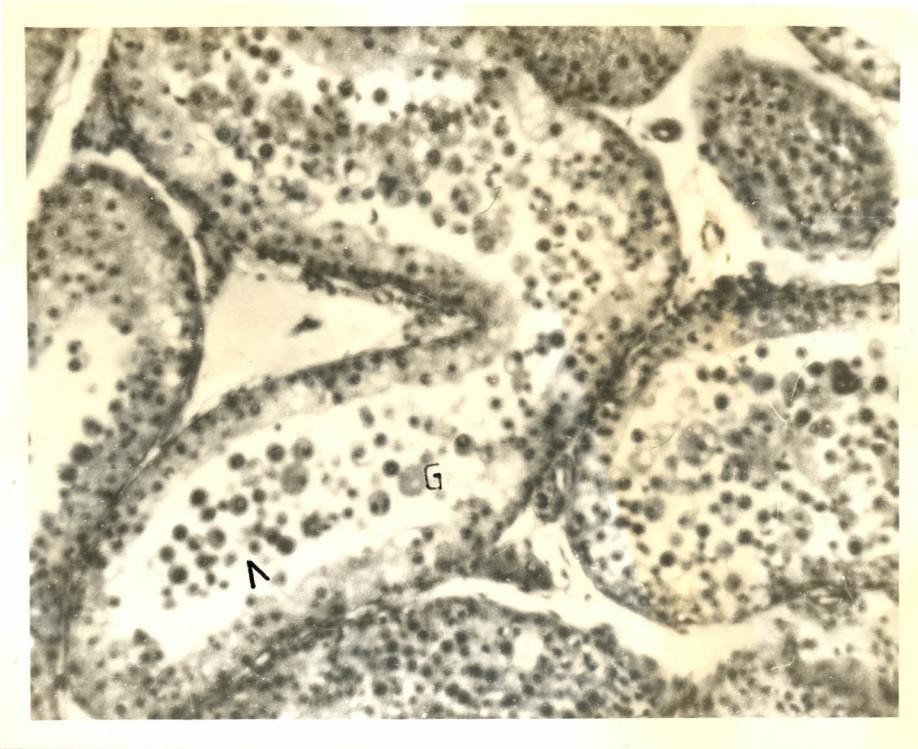Arrow heads show lumen devoid of spermatozoa.

Plate 3

PLATE 4

Cross-section of the epididymis of a normal guinea pig immunized with TA-CFA and given chronic gluco-corticoid treatment. The lumen has mature spermatozoa and no evidence of infiltration in the interstitial spaces. x 250.

Arrow heads show lumen with mature spermatozoa.

Arrows show interstitial space devoid of inflammatory cells.

PLATE 5

Close up of plate 4.

The interstitial space has no inflammatory cells shown by arrows.

Plate 4

Plate 5
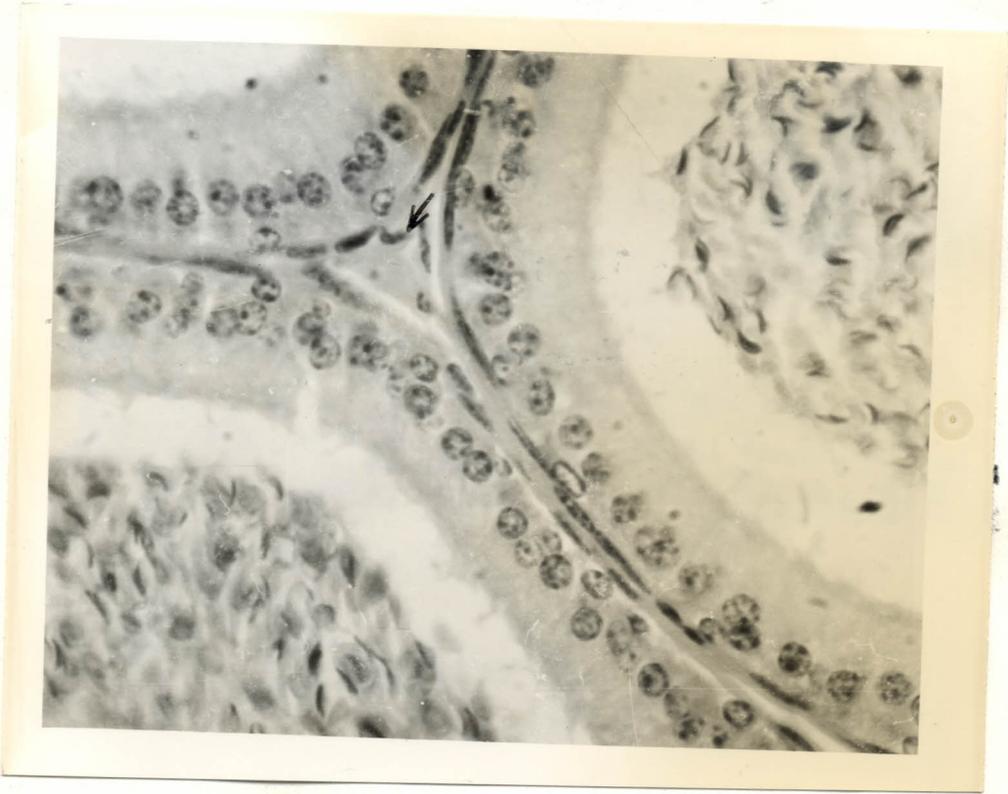
PLATE 6

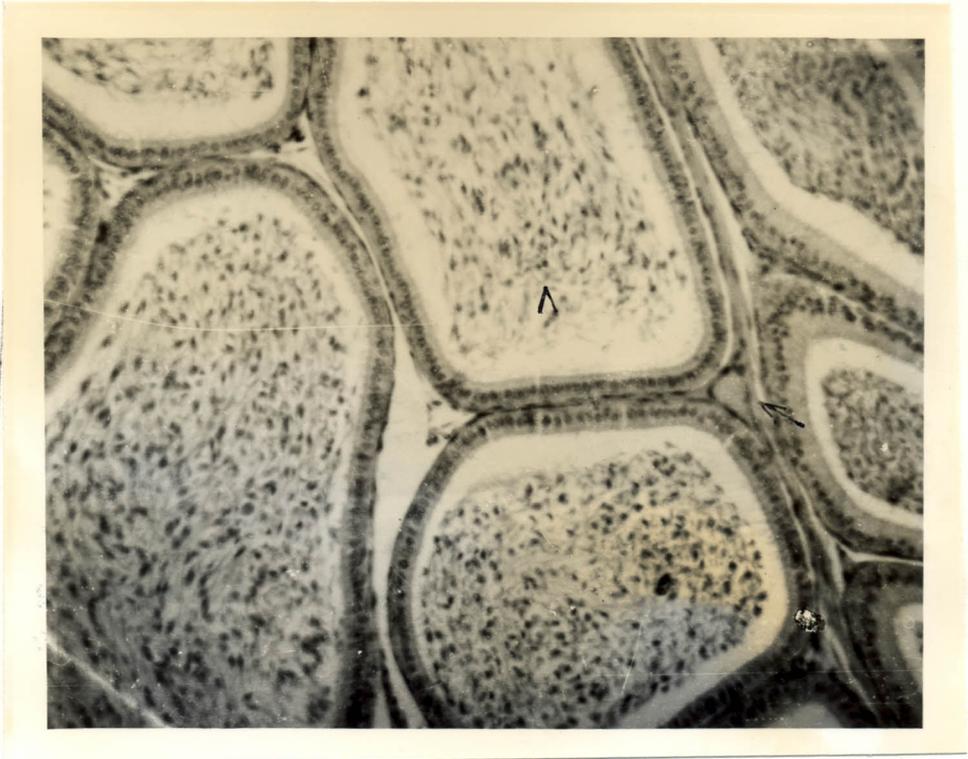Cross-section of a normal GP testis. The STs
have all the stages of spermatogenesis. The lumen
has mature spermatozoa and the integrity of the
interstitium is evident. ×400

Arrows - Interstitial space

Arrow heads - Spermatozoa

Plate 6

PLATE 7

Electron micrographs of ST of testis from an animal treated with cortisol after orchitogenic challenge.   x 8000.

S-   Sertoli cell

P-   Spermatogonia

Plate 7

PLATE 8

a) Electron micrograph of ST of testis with orchitis

showing macrophage (M). x8000
(Arrow shows disrupted tight junctions).

b) A different area of the same section showing a lymphocyte

(in the STs. x8000.

Plate 8 (a)

(b)

CHAPTER 4

DISCUSSION AND CONCLUSIONS

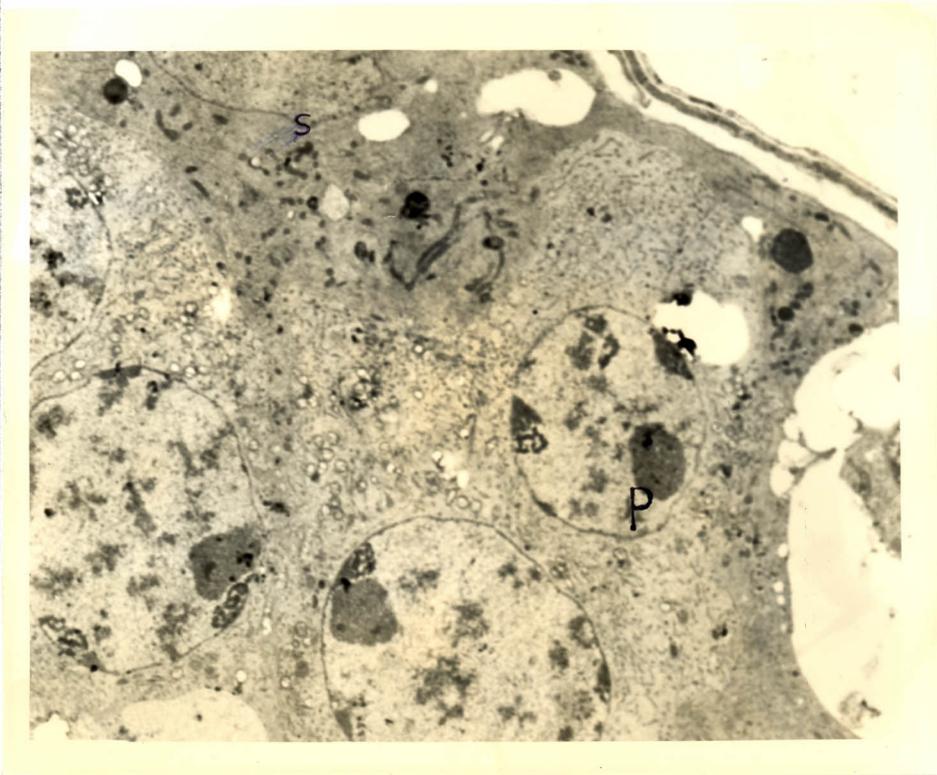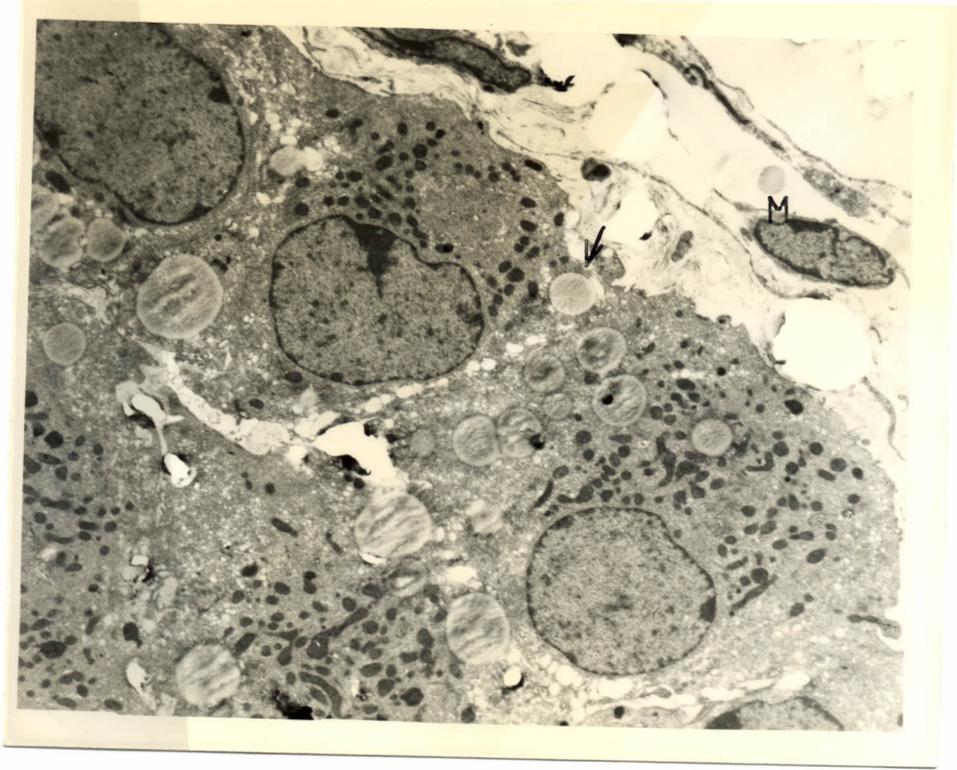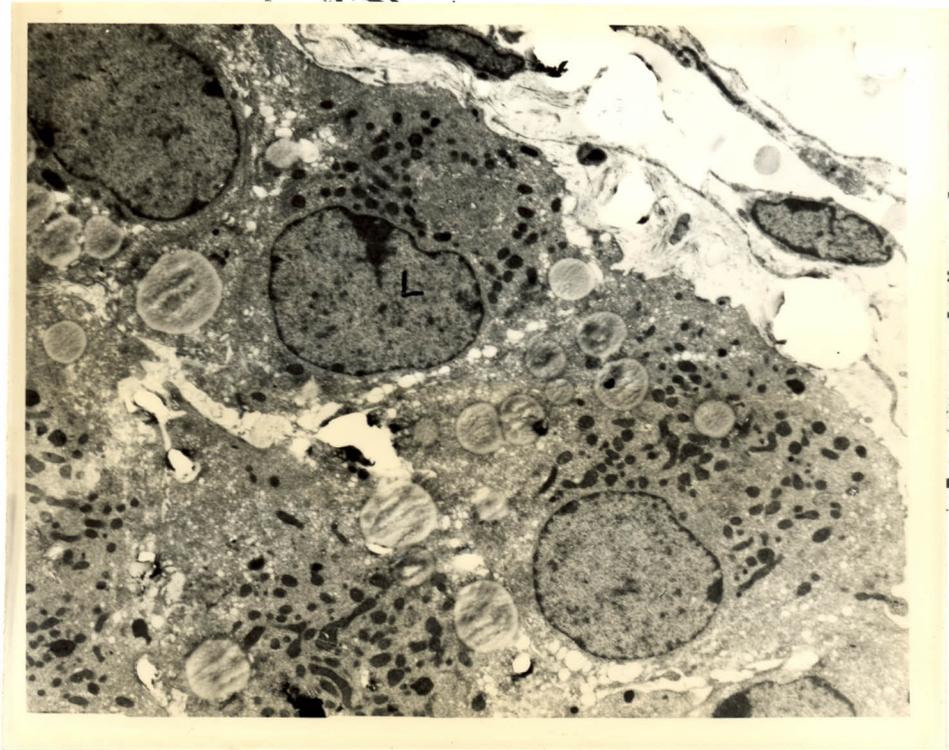The study revealed that glucocorticoids do suppress EAO. The results (Fig 3) revealed that cortisol was more effective than prednisone. This is at variance with the fact that synthetic steroids are usually more effective than natural ones (Wolff 1979). However, this is in humans and species variability both in the rate of metabolism, products of metabolism and effectiveness may appear (Fotherby and James 1972). It is therefore, worthwhile to speculate that prednisone does not persist as long as cortisol to effect its immunosuppression adequately and to predict major differences between the humans and guinea pig in the clearance of synthetic and natural corticosteroids. The dose used was however obtained from human dosages as reported by Fauci (1979).

Glucocorticoids have been reported to have profound effects on T-cell dependent immune responses. Chronic administration which leads to lymphocytopenia causes a reduced MIF and antigen-induced proliferative responses to the antigen and not to mitogens led to a remarkable suppression of EAO induction. The combined effect led to total suppression in some cases and when induction was not suppressed, lymphocytopenia ensured few immune cells at the site of inflammation. This of course would also be as a result of genetic variability in the same species. This may be due to metabolizing rates of the drug as it has been reported in the rabbits that as much as 20-fold variation in liver drug metabolizing microsomal systems do exist between strains (Fotherby and James, 1972).

These findings augur well with those of Hojo and Hiramine (1982) in which protection against EAO induction was achieved by pretreatment with antigen in incomplete Freunds Adjuvant (TA-IFA). The authors reported 94% of animals challenged free of orchitis, aspermatogenesis and epididymitis two weeks post-protection. This tolerance gradually broke to 0% at six weeks. Their results were similar to those of Brown et al (1967) but these authors did not report a kinetic study rather they only did the two weeks post protection case. Chronic cortisol treatment achieved 82% of total animals given orchitogenic challenge free of orchitis, 86% free of epididymitis and 79% free of aspermatogenesis. Cyclosporin A has also been shown to abrogate the induction of EAO (Hojo and Hiramine 1985). The pattern of protection which was achieved by treatment with this immuno-modulatory agent starting on the day of immunization to the day of killing (day 14) was similar to that of cortisol and TA-IFA.

The results suggest that the mode of immunomodulation is similar for the TA-IFA pretreatment, cyclosporin A and gluco-corticoids. Pretreatment with TA-IFA has been shown to make T-cells or unseparated lymph node cells (LNCs) incapable of reacting to testicular antigen (TA) after two weeks (Hojo and Hiramine 1982). This capability is gradually restored with time. The pretreatment therefore suppresses cell-mediated immune responses. Cyclosporin A is fungal metabolite which inhibits transcription of interleukin-2 mRNA therefore acting on T-cells and spares immunocompetent B-cells and macrophages (Kronke et al 1984).

It in effect affects CMI responses alone. Glucocorticoids are also known to effect immunomodulation by interfering with the production of T-cell Growth Factor (TCGF) also known as inter-leukin-2 (Cupps and Fauci 1982).

It is therefore possible that immunomodulation of EAO by glucocorticoids is effected by affecting the T-helper/inducer function of the T-cells. This would gain credibility since it has been reported that during passive transfer of EAO, the subset T cells required bear the CD4 surface marker (Mahi-Brown et al 1988). This corresponds to the human T4 and murine L3T4 whi are for T-helper or delayed-hypersensitivity functions (Roitt 19

The conclusion that immunomodulatory effects of gluco-corticoids on EAO are mainly at the T-cell level are supported the studies of Bernard et al (1978). The authors did show tha hypothymic male BALB/c nu/nu mice were resistant to inductior of EAO. Reconstitution with thymocytes from litter mate nu/+ mice completely restores this capability. This indicates that T-cells are obligatory in the induction phase of EAO.

Although there does not appear to be an apparent rule governing the manifestation of orchitis, aspermatogenesis and epididymitis, they usually occur concurrently. Thus, the thre appear to be suppressed by glucocorticoid treatment. There is however, an obvious relationship between the intensity or orch and extent of aspermatogenesis which is not so for epididymitis The reason for this is easy to speculate since the inflammatory cells clear the antigen which in this case happens to be sperm

togenic cells. Orchitis as defined in this thesis suggests the number of the immunocompetent cells in the testis. The epididymis naturally has a way of breaking down unejaculated spermatozoa and hence may take a longer time before they are actually attacked by the immune response.

The appearance of desquamated spermatozoa in the epididymal lumen suggests that the inflammatory cells in the epididymis may partly appear from the STs by the normal sperm transport processes. This can vary from animal to animal depending on the extent of damage of the structures responsible. This is further supported by the fact that inflammatory cells appeared mostly in the caput rather than caudal epididymis at the time of killing the animals (i.e. on the 14th to 15th day). It may be worthwhile to investigate further whether as the disease progress, there is a relationship in the time that orchitis appears in the rete testis, the caput and the caudal epididymis.

The study established that both natural and synthetic glucocorticoids do suppress the induction and progression of EAO. Th underlines the importance of interleukin-2 activation of T lymphocytes in the yet to be established mechanism of the disease. Immunomodulation of EAO by cyclosporin A an agent that also acts at interleukin-2 level confirms this.

Results of the progression of the disease after successful induction by, manipulations of T-effector cells or in vitro studies are imperative in the elucidation of the mechanism of CMI mediated autoimmunity and EAO specifically.

REFERENCES

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Alexander, N.J. and Anderson, D.J. : Immunological factors in reproductive fitness. In Reproduction in mammals : Reproductive fitness (Austin, C.R. and Short, R.V. eds) Cambridge Univ. Press. 1984. 4 : 176 - 209.

Andrada, J.A., Andrada, E.J. and Witebsky, E. : Experimental autoallergic orchitis in rhesus monkeys. Proc. Soc. Exp. Biol. Med. 1969. 130 : 1106 - 1113.

Balow, J.E., Hurley, D.L. and Fauci, A.S. : Immunosuppressive effects of glucocorticosteroids : Differential effects of Acute vs Chronic administration on cell mediated immunity. J. Immunol. 1975. 114 : 1072 - 1076.

Benjamin, D.C. : Evidence for specific suppression in the maintenance of immunologic tolerance. J. Exp. Med. 1975. 141 : 635 - 646.

Bernard, C.C.A. and Carnegic, P.R. : Experimental autoimmune encephalomyelitis in mice : Immunologic response to mouse spinal cord and myelin basic proteins : J. Immuno. 1975. 1537 - 1540.

Bernard, C.C.A., Mitchel, G.F., Leydon, J. and Bargerbos, A. : Experimental autoimmune orchitis in T-cell deficient mice. Int. Archs. Allergy. Appl. Immun. 1978. 56 : 256 - 263.

Bigazzi, P.E., Kosuda, L.L., Hsu, K.C. and Andres, G.A. : Immune complex orchitis in vasectomized rabbits. J. Exp. Med. 1976. 143 : 382 - 404.

Brown, P.C., Glynn, L.E. and Holborow, E.J. : The
pathogenesis of Experimental Allergic Orchitis in the
guinea pig. J. of Pathology and Bacteriology,
1963. 86 : 505 - 520.

Brown, P.C., Glynn, L.E. and Holborow, E.J. The dual
necessity for a delayed hypersensitivity and circulating
antibody in the pathogenesis of Experimental Allergic
Orchitis in guinea pigs. Immunology, 1967. 13 :
307 - 314.

Burnet, M. : Tolerance and unresponsiveness. In Immunology.
Scientific American. W.H. Freeman and Company 1976.
114 - 118.

Cooke, A., Lydyard, P.M. and Roitt, I.M. : Mechanisms of
autoimmunity : a role for cross-reactive idiotypes.
Immunology Today. 1983. 4 : 170 - 175.

Cupps, T.R. and Fauci, A.S. : Corticosteroid-mediated
immunoregulation in man. Immunol. Rev. 1982 :
65 : 133 - 153.

Daniel, P.M., Lam, D.K.C. and Pratt, O.E. : Changes in
the effectiveness of the blood-brain and blood-spinal
cord barriers in experimental allergic encephalomyelitis-
possible relevance to multiple sclerosis. J. Neurol.
Sci. 1981. 52 : 211 - 219.

Dym, M. and Fawcett, D.W. : The blood-testis-barrier in the
rat and physiological compartmentation of the seminiferous
epithelium. Biol. Reprod. 1970. 3 : 308 - 326

Fauci, A.S. : Immunosuppressive and antiinflammatory
    effects of glucorticoids. In Glucocorticoid Hormone
    Action. Monographs of endocrinology (Barter, J.D. and
    Rousseau, G.G. eds.). Springer-Verlag. 1979. 12 :
    449 - 465.

Fernandez, C. , Hammarstsrom, L., Moller, G., Primi, D. and
    Smith, C.J.E. : Immunological tolerance affects only
    a subpopulation of the antigen-specific B lymphocytes :
    Evidence against clonal deletion as the mechanism of
    tolerance induction. Immunol. Rev. 1979. 43 : 3 - 41.

Fotherby, K. and James, F. : Synthetic steroids metabolism of
    : In Advances in steroid biochemistry and pharmacology.
    (M.H. Briggs & G.A. Christie eds). 1972. 3 : 67 - 167.

Freund, J., Lipton, M.M. and Thompson, G.E. : Aspermatogenesis
    in the guinea pig induced by testicular tissue and
    adjuvants. J. Exp. Med. 1953. 97 : 711 - 725.

Glasser, D.L. and Silvers, W.K. : Genetic determinants of
    immunological responsiveness. Adv. Immunol. 1974.
    18 : 1 - 66.

Grimstone, A.V. and Skaer, R.J. : In a guide book to
    microscopial methods. Cambridge Univ. Press. 1972.
    20 - 27.

Hagopian, A., Jackson, J.J., Carlo. D.J., Limjuco, G.A. and
    Eylar, E.H. : Experimental Allergic Aspermatogenic

Orchitis. III. Isolation of spermatozoal glycoproteins
and their role in allergic aspermatogenic orchitis.
J. Immunol. 1975. 115 : 1731 - 1743.

Hoffmann, M.K. and Kappler, J.W. : Two distinct mechanisms
of immune suppression by antibody. Nature (London).
1978. 272 : 64 - 65.

Hojo, K., Hiramine, C. and Ishitaki, M. : Lymphocyte
proliferative response in vitro and its cellular dependency
in guinea pigs with Experimental Allergic Orchitis.
J. Reprod. Fert. 1980. 59 : 113 - 123.

Hojo, K. and Hiramine, C. : Suppression of Experimental
Allergic Orchitis and Cellular Immune response in the
guinea pig by pretreatment with testis antigen in incomplete
Freunds adjuvant. Int. Archs. Allergy. Appl. Immunol.
1982. 69 : 40 - 49.

Hojo, K. and Hiramine, C. : In vivo effects of cyclosporin A :
Abrogation of the induction of Experimental Allergic Orchitis
and sparing of the generation of suppressor cells. Int.
Archs. Allergy Appl. Immun. 1985. 78 : 63 - 70.

Hopps, E.H.J. and Parkman, P.P. : Mumps virus. In Diagnostic
procedure for viral, Rickettsial and chlamydial infections.
5th edition (E.H. Lennette and N.J. Schimdt eds). Am
Public Health Assoc. Washington, D.C. 1979. 633 - 653.

Jackson, J.J., Hagopian, A., Carlo, D.J. and Limjuco, G.A. :
Experimental Allergic Aspermatogenic Orchitis : Isolation

of a spermatozoal protein (API) which induces Allergic
Orchitis. J. Biol. Chem. 1975. 250 : 6141 - 6150

Kantor, G.L. and Dixon, F.J. : Transfer of Experimental
Allergic Orchitis with peritoneal exudate cells.
J. Immunol. 108 : 329 - 338.

Kohno, S., Munoz, J.A., Williams, T.M., Teuscher, C., Bernard,
C.C.A. and Tung, K.S.K. : Immunopathology of Murine
Experimental Allergic Orchitis. J. Immunol. 1983.
130 : 2675 - 2682.

Kong, Y.M., David, S. Giraldo, A.A., Elrehew, Y.M. and
Rose, N.R. : Regulation of autoimmune response to
mouse thyroglobulin : Influence of H-2D end genes.
J. Immunol. 1979. 123 : 15 - 18.

Kronke, M., Leonard, W.J., Depper, J.M., Arya, S.K.,
Wongstaal, F., Gallo, R.C., Waldmann, T.A. and Green,
W.C. : Cyclosporin A inhibits T-cell growth factor gene
expression at the level of mRNA transcription. Proc.
Natl. Acad. Sci. USA. 1984. 81 : 5214 - 5218.

Lefroit-Joliy, M., Lebar, R. and Voisin, G.A. : Guinea pig
spermatozoal plasma membrane T-autoantigen. Attempts
at solubilization, purification and characterization.
Molecular Immunol. 1978. 16 : 327 - 333.

Lowry, O.H., Rosebrough, N., Farr, A.L. and Randall, R.J.:
Protein measurement with the folin phenol reagent. J.
Biol. Chem. 1951. 193 : 265 - 275.

Mahi-Brown, C.A., Yule, T.D. and Tung, K.S.K. : Evidence
for active immunological regulation in prevention of
testicular autoimmune disease independent of the
blood-testis-barrier. Am. J. Reprod. Immunol. and Microb.
1988. 16 : 165 - 170.

Mancini, R.E., Andrada, J.A., Saraceni, D., Bachmann, A.E.
Levieri, J.C. and Nemirovsky, M. : Immunological and
testicular response in man sensitized with human testicular
homogenate. The Journal of Clin. Endocrinol. and
metabolism. 1965. 25 : 859 - 875.

Nagarkatti, P.S. and Rao, S.S. : Cell-mediated Immunity to
homologous spermatozoa following vasectomy in the human
male. Clin. Exp. Immunol. 1976. 26 : 239 - 242

Orson, F.M. and Auzenne, C.A. : Glucocorticosteroid-induced
immunoglobulin production requires intimate contact between
B cells and monocytes. Cellular Immunology. 1988.
112 : 147 - 155.

Ortiz-Ortiz, L., Nakamura, R.M. and Weigle, W.O. : T-cell
requirement for experimental allergic encelphalomyelitis
induction in the rat. J. Immunol. 1976. 117 : 576 -
579.

Roitt, I.M. : Autoimmune diseases. In Essential Immunology.
(Ivan Roitt Author) Blackwell Sci. Publication. 1988.
176 - 253.

Samuel, T., Kolk, A.H.J., Rumke, P. and Van Lis, J.N.J. :
Autoimmunity to sperm antigens in vasectomized man.
Clin. Exp. Immunol. 1975. 21 : 65 - 74.

Stone, S.H. : Transfer of allergic encephalomyelitis by lymph
node cells in inbred guinea-pigs. Science. 1961.
34 : 619 - 620.

Suckling, A.J., Reiber, H., Kirby, J.A. and Rumsby, M.G. :
Chronic relapsing experimental allergic encephalomyelitis.
Immunological and blood-cerebrospinal fluid. J. of
Neuroimmunology. 1983. 4 : 35 - 45.

Taguchi, O., and Nishizuka, Y. : Experimental autoimmune orchitis
after neonatal thymectomy in the mouse. Clin. Exp.
Immunol. 1981. 46 : 425 - 343

Teuscher, C., Wild, G.C. and Tung, K.S.K. : Experimental allergic
orchitis. The isolation and partial characterisation of an
aspermatogenic polypeptide (AP3) with an apparent
sequential disease inducing determinant(s). 1983.
130 : 2683 - 2688.

Teuscher, C., Smith, S.M., Goldberg, E.H., Sheaver, G.M. and
Tung, K.S.K. : Experimental allergic orchitis in mice 1.
Genetic control of susceptibility and resistance to
induction of autoimmune orchitis. Immunogenetics.
1985. 323 - 333.

Tumbo-Oeri, A.G. and Roberts, T.K. : Immunological and
morphological consequences of vasectomy in the rabbit.
Experimentia. 1979. 35 : 576 - 676.

Tung, K.S.K., Unanue, E.R. and Dixon, F.J. : Pathogenesis
of experimental allergic orchitis. I. Transfer with
immune lymph node cells. J. Immunol. 1971a. 106 :
1453 - 1462.

Tung, K.S.K., Unanue, E.R. and Dixon, F.J. : Pathogenesis
of experimental allergic orchitis. II. The role of
antibody. J. Immunol. 1971b. 106 : 1463 - 1472.

Tung, K.S.K. : Human sperm antigens and antisperm antibodies:
I. Studies on vasectomy patients. Clin. Exp. Immunol.
1975. 20 : 93 - 104.

Tung, K.S.K., Leong, C. and McCarty, T.A. : Pathogenesis of
experimental allergic orchitis. III. T lyphocyte
requirement in local adoptive transfer by peritoneal
exudate cells. J. Immunol. 1977. 118 : 1774 - 1779.

Tung , K.S.K. and Alexander, N.J. : Autoimmune reactions in
the testis. The testis Vol. IV (A.D. Johnson and W.R.
Gomes eds). N.Y. Academic Press. 1977. pp 491- 516.

Tung, K.S.K. and Woodroffe, A.J. : Immunopathology of
experimental allergic orchitis in the rabbit. J.
Immunol. 1978. 120 : 320 - 328.

Tung, K.S.K.: Allergic orchitis lesions are adoptively transferred from vasoligated guinea pigs to syngeneic recipients. Science. 1978. 201 : 833 - 835.

Tung, K.S.K. and Alexander, N.J. : Monocytic orchitis and aspermatogenesis in normal and vasectomized Rhesus macaques (Macaca mulatta). Am. J. Pathol. 1980. 101 : 17 - 27.

Tung, K.S.K. : Teuscher, C. and Meng, A.L. : Autoimmunity to spermatozoa and the testis. Immunol. Rev. 1981a. 55 : 217 - 256.

Tung, K.S.K., Teuscher, C.E., Meng, A.L., Blaustein, J.C., Kohno, S., and Howell, R. : The black mink. A natural model of immunologic male infertility. J. Exp. Med. 1981b. 154 : 1016 - 1032

Tung, K.S.K., Teuscher, C., Goldberg, E.H. and Wild, G.: Genetic control of antisperm autoantibody response in vasectomized guinea pigs. J. Immunol. 1981c. 127 : 835 - 839.

Voisin, G.A., Delaunay, A. and Barber, M. : Sur des lesions testiculaires provoquees Chez Le Cobaye par Iso-te autosensibilisation. Ann. Inst. Pasteur. 1951. 81 : 48 - 63.

Waindi, E.N., Gombe, S. and Oduor-Okelo, D. : Plasma

testosterone in Trypanosoma congolense infected

toggenburg goats. Arch. Andrology. 1986. 17 :

9 - 17.

Waksal, S. : Immunomodulation : Immunopotentiation, tolerance

and suppression. In Immunology II. (Bellanti, J.A. ed)

W.B. Saunders Company. 1978. pp. 243 -265.

Waksman, B.H. : A histologic study of auto-allergic testis

lesion in the guinea pig. J. Exp. Med. 1959. 109 :

311 - 324.

Weigle, W.O. : Analysis of autoimmunity through experimental

models of thyroiditis and allergic encephalomyelitis.

Adv. Immunol. (Dixon, F.J. and Kunkel, G.A. eds).

1980. 30 : 159 - 273.

Wekerle, H. and Begemann, M. : Experimental autoimmune

orchitis : In vitro induction of an autoimmune disease.

J. Immunol. 1976. 116 : 159 - 161.

Wolff, M.E. : Structure-activity relationships in glucocorticoids.

In glucocorticoid Hormone Action. Monographs of

endocrinology (Barter, J.D. and Rousseau, G.G. eds).

Springer-Verlag. 1979. 12 : 97 - 107.