INFLUENCE OF ACETYLSALICYLIC ACID, AN INHIBITOR OF PROSTAGLANDIN BIOSYNTHESIS, ON THE MALE REPRODUCTIVE TRACT OF THE RAT

A Thesis

Presented to the Faculty of Graduate Studies,

University of Manitoba, in Partial Fulfillment

of the Requirements for the Degree of

Master of Sciences

by

James Elliott Scott

December 1976

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For my parents and

For Kate

ACKNOWLEDGEMENTS

I would like to express my thanks to Dr. T.V.N. Persaud, Professor of Anatomy, for allowing me to work in his laboratory and for his time and guidance during this study.

My sincere thanks also to Ms. Brenda Bell for her excellent preparation of the photographs, and to Ms. Susan Pylypas, Mrs. Rodica Schurath and Mr. Paul Perumal for their kindness and help in the preparation of histological material.

Thanks are also due to Mrs. Roberta Van Aertselaer for typing the manuscript.

ABSTRACT

Although the occurrence of prostaglandins in the male reproductive organs is consistent, their physiological role in fertility and reproduction is not known.

The influence of acetylsalicylic acid, an inhibitor of prostaglandin synthesis, on the male reproductive tract was investigated in Sprague-Dawley rats of proven fertility. Acetylsalicylic acid dissolved in phosphate buffer was administered once a day at different dose levels (300mg/kg body weight and 150mg/kg body weight) for periods of 12 and six days. The animals were killed twenty-four hours after the final treatment and specimens of the testes, epididymides, ductus deferens, seminal vesicles, kidneys and adrenal glands were prepared for light microscopic and histochemical study.

Organ weights were not significantly altered in the animals that were treated with acetylsalicylic acid. However,cell counts indicated that the drug caused an increased rate of spermatogenesis when it was administered at the lowest treatment level (150mg/kg body weight for six days). Higher dosages (150mg/kg body weight and 300mg/kg body weight for periods of 12 and six days respectively) inhibited spermatogenesis, caused shrinkage of the seminiferous tubules and produced morphological changes in the germinal epithelium. The latter included intra-epithelial spaces, cells with pyknotic nuclei,and greater numbers of necrotic cells in the

peripheral layers of the germinal epithelium of stage IX seminiferous tubules from which spermatozoa are released. The highest treatment level (300mg/kg body weight for 12 days) did not inhibit spermatogenesis or produce morphological changes in the germinal epithelium, but caused a significant increase in the mean diameter of the seminiferous tubules.

Enzyme histochemical study revealed that treatment with acetylsalicylic acid at different dosages (150mg/kg body weight and 300mg/kg body weight) for an extended period (12 days) produced an increase in free acid phosphatase activity, but a decrease in the activity of alkaline phosphatase. This could be related to the observed morphological changes. The activity of cytochrome oxidase was also altered in animals showing morphological changes in the testes. The drug may therefore affect the respiratory chain which involves cytochrome oxidase. How these morphological and enzyme-histochemical changes are related to the specific action of acetylsalicylic acid still remains uncertain.

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STATEMENT OF THE PROBLEM

1. STATEMENT OF THE PROBLEM

The prostaglandins are a unique group of naturally occurring chemical regulators with a wide range of physiological and pharmacological activities. These substances are ubiquitous and are widely distributed in animal tissues. There is now evidence that the prostaglandins are physiologically important for several reproductive processes, including male fertility, ovulation, embryonic development, and parturition. The effects of certain prostaglandins and inhibitors of prostaglandin synthesis on the female reproductive system and the developing conceptus have been investigated in several species of laboratory animals. Little is known,however,of the role of prostaglandins in these processes, including male fertility.

Acetylsalicylic acid, a non-steroidal antiinflammatory substance, is a potent inhibitor of prostaglandin synthesis. The objectives of this investigation were to determine:

a) the morphological changes (histological and histochemical) in the testes, epididymides, ductus deferens, seminal vesicles, adrenal glands and kidneys following treatment with acetylsalicylic acid,

b) the effects of this treatment on different stages of spermatogenesis,

c) and to relate these findings to problems of male infertility.

SECTIONS 1 - 3

REVIEW OF RELATED LITERATURE

2. REVIEW OF RELATED LITERATURE

2.1 THE MALE REPRODUCTIVE TRACT OF THE RAT

2.1.1 Development

2.1.1.1 The Testis

Testicular development in the rat begins with the appearance of thickenings, the genital ridges, medial to the mesonephros. The basal lamina beneath the ridges disappears and cell cords grow from the epithelium into the underlying mesenchyme. Subsequently, primordial germ cells from the yolk sac migrate along the dorsal mesentery and become concentrated in the gonadal ridges (Martinovitch, 1938; Chiquoine, 1954). The seminiferous cords were formerly thought to form by downgrowth from the surface epithelium; recently,it has been shown that in rodents Sertoli cells within the gonad enlarge, make contact with each other, and engulf the germ cells to form the seminiferous cords (Jost et al, 1973). The primordial germ cells were also thought to exert an inductive effect on testicular development. However, new evidence suggests a passive role for the germ cells (Boczkowski, 1973).

A dense fibrous layer, the tunica albuginea, separates the surface germinal epithelium from the testicular cords which extend into the medulla and a basal lamina forms about the cords. The cords are lined by sustentacular Sertoli cells and primordial germ cells. Mesenchymal cells around the cords develop into the interstitial cells of Leydig.

2.1.1.2 The Genital Ducts

The male genital ducts develop from the ducts of the mesonephric kidneys. As the mesonephroi degenerate, some of the tubules become associated with the gonad which lies medial to the mesonephros; these form the ductuli efferentes. Connections are established through the rete testis to the seminiferous tubules. The organization of the Wolffian ducts and regression of the Müllerian ducts are dependent, respectively, on androgen production by the Leydig cells and a factor or hormone produced by the Sertoli cells (Peters, 1976). Caudal to the union of the ductuli efferentes and the mesonephric duct, the latter becomes greatly elongated and convoluted to form the ductus epididymidis. The remaining portion of the mesonephric duct acquires a thick muscular coat and becomes the ductus deferens.

2.1.1.3 The Seminal Vesicles and Ejaculatory Ducts

Close to the caudal origin of the urogenital sinus, each ductus deferens dilates to form an ampulla from which a glandular diverticulum gives rise to the seminal vesicle. Each ejaculatory duct is derived from the mesonephric duct between the developing seminal vesicle and the urethra.

2.1.2 Microscopic Structure

2.1.2.1 The Testis

The testis is a compound tubular gland lying in the scrotal sac. A thickened fibrous sac, the tunica albuginea, encloses the testis. Towards the superior pole, at a point along the periphery, a thickening of the tunica forms the mediastinum testis. Fibrous projections from the

latter into the body of the testis divides it into lobuli testis in which one or more seminiferous tubules lie. Germinal epithelium lines the seminiferous tubules. The structure of the germinal epithelium will be discussed later (see Spermatogenesis, 2.2).

Each seminiferous tubule is surrounded by a basal lamina. Around the basal lamina a layer of epitheloid cells of varying thickness encloses the tubules. Ultrastructural studies indicate that these cells have the character of smooth muscles fibers (Clermont, 1958). Loose connective tissue fills much of the interstitial space between the seminiferous tubules. Blood vessels pass through the connective tissue while macrophages, fibroblasts, mast cells and Leydig cells are embedded in it. The latter constitute the endocrine portion of the testis.

At the mediastinum testis the seminiferous tubules empty into the rete testis through the tubuli recti. Simple cuboidal epithelium lines both the tubuli recti and the rete testis.

2.1.2.2 The Genital Ducts

From the rete testis, twelve to twenty tubules, the ductuli efferentes pass to the epididymidis. Alternating areas of high and low columnar cells line the ductuli efferentes. Two cell types are recognized in the epithelium. One type bears cilia whereas the other has sparse microvilli. The cilia beat in the direction of the epididymidis.

The ductuli efferentes fuse to form the single ductus epididymidis. Three regions of the epididymidis are recognized. The caput epididymidis lies at the superior pole of the testis. The corpus epididymidis lies along the side, whereas the cauda epididimidis is located at the inferior pole. Psuedostratified columnar epithelium lines the ductus epididymidis in which two cell types are distinguished. The principal epithelial cells are tall columnar in the caput, becoming low columnar to cuboidal in the cauda. Stereocilia are present on the luminal border of the principal cells. Intraepithelial lymphocytes with heterochromatic nuclei are sporadically present in the epithelium.

A basal lamina lies beneath the epithelium. External to it is a layer of smooth muscle cells showing a proximo-distal increase in thickness.

The ductus epididymidis is continuous with the ductus deferens. The latter is lined by pseudostratified columnar epithelium which with the lamina propria is thrown into longitudinal folds. Stereocilia are present at the luminal border of the epithelium. The thick muscular wall of the ductus deferens is composed of inner and outer longitudinal layers and a thick circular middle layer. An outer adventitial connective tissue layer surrounds the ductus deferens.

2.1.2.3 The Seminal Vesicles and Ejaculatory Ducts

The seminal vesicles are irregular saccular organs. The epithelium consists of tall columnar cells with small basal cells irregularly placed at their bases. Extensive folds increase the secretory area. The basal lamina and lamina propria separate the mucous membrane from the peripheral muscle coat. Inner circular and outer longitudinal muscle layers surround the seminal vesicle. Fibrous connective tissue encloses the entire structure.

The secretory cells of the mucous membrane elaborate a thick yellow fluid which provides a vehicle, as well as nutritional requirements, for the sperm.

The ejaculatory ducts are lined by simple or pseudostratified columnar epithelium. Some glandular function has been ascribed to the mucosa. Near the openings of the ducts the epithelium often appears transitional in structure.

2.2 SPERMATOGENESIS

2.2.1 The Germinal Epithelium

2.2.1.1 Spermatogonia

Two types of spermatogonia may be distinguished. Type A spermatogonia were first described as "dust" cells based on the appearance of the nuclear chromatin; type B spermatogonia were described as "crusty" because of the coarse chromatin granules in the nucleus (Regaud, 1901). Subsequent investigations showed that A spermatogonia divide mitotically five or six times before differentiating into the B type (Roosen-Runge and Giesel, 1950; Roosen-Runge, 1952); the latter, after two more mitotic divisions, give rise to preleptotene spermatocytes (Ortavant, 1959). Clermont and Leblond (1953) identified three kinds of spermatogonia (type A, intermediate, and type B) and also confirmed five peaks of mitotic activity. Correlation between cell associations, which these authors had previously described (Leblond and Clermont, 1952), and spermatogonial counts indicated that at the beginning of each cycle a single A spermatogonia gives rise to two and these in turn to four type A cells. One of these four cells becomes "dormant" and will not divide before the next cycle. Mitotic divisions of the other type A cells produce intermediate and B spermatogonia and preleptotene spermatocytes (Clermont and Leblond, 1953). Additional investigations

have indicated that 8-10% of the A spermatogonia degenerate at stage XII, before entering prophase (Clermont, 1962).

Subsequent cytological studies have tended to modify the concept of spermatogonial renewal. Five classes of type A spermatogonia (A_0, A_1, A_2, A_3, A_4) were identified in the germinal epithelium; types A_1-A_4 spermatogonia are the proliferating and differentiating stem cells, whereas the type A_0 spermatogonia are reserve stem cells and are not actively involved in the production of spermatogonia and spermatocytes (Clermont and Bustos-Obregon, 1968). Four mitotic peaks in the germinal epithelium occurring at stages IX, XII, XIV and I correspond to division of spermatogonial types A_1-A_4. The population of type A_1 spermatogonia is believed to be renewed by cells derived from A_4 spermatogonia. However, Huckins and Kopriwa (1969) found the origin of A_1 spermatogonia is not associated with the mitotic peak of the A_4 cells. Further investigations of stem cell populations in the seminiferous tubules of rodents indicated that A_s (corresponding to A_0 cells of Clermont and Bustos-Obregon, 1968) spermatogonia are the stem cells (Huckins, 1971a and b; Oakberg, 1971; DeRooij, 1973). They divide throughout the cycle particularly from stages IX to I; when the population attains its maximum, mitotic activity ceases among the stem cells and most are transformed to A_1 spermatogonia. Subsequent divisions give rise to A_2 spermatogonia (at stage IX), A_3 spermatogonia (at stage XI), and A_4 spermatogonia (at stage I) (Huckins, 1971c). However, reevaluation of the problem of identifying the stem cells of the germinal epithelium led Clermont and Hermo (1975) to confirm the model originally proposed by Clermont and Bustos-Obregon (1968) that the A_0 spermatogonia were

largely non-proliferative. The latter did not divide more at stage I than at other stages of the cycle, and since no other source was present to account for the appearance of A_1 spermatogonia at stage II, type A_4 cells were considered to be the parent cells.

A reparative function has been ascribed to the population of reserve A_0 spermatogonia (Dym and Clermont, 1967). Irradiation of the testis destroyed most type A_1-A_4 spermatogonia; the A_0 cells were resistant to irradiation. Subsequently, A_1 spermatogonia reappeared along the walls of the seminiferous tubules presumably from proliferation of the undamaged A_0 cells. In normal testicular tissue, the presence of a chalone which inhibits mitotic activity of A_0 spermatogonia has been demonstrated (Clermont and Mauger, 1974).

Intermediate spermatogonia develop from mitotic activity of A_4 spermatogonia dividing at stages XIV - I. Type B spermatogonia appear from division of the intermediate cells; the former cells give rise to preleptotene spermatocytes.

2.2.1.2 Spermatocytes

Spermatocytes are present in the germinal epithelium for a relatively long period. Primary spermatocytes are conspicuous because of the appearance of their nuclei in the long prophase of the first maturation division. With the exception of chromosomal changes (reviewed by Bishop and Walton, 1960) the development of these cells has not been thoroughly investigated.

Measurements, using Chalkley's technique (1943), of the volume of primary spermatocytes indicates an increase beginning at the zygotene stage (Roosen-Runge, 1955). Merckle (1957) found a fourfold increase in the size of the nuclei of primary spermatocytes during prophase.

Primary spermatocytes are found in the germinal epithelium at all stages (Leblond and Clermont, 1952). During the first maturation division the cells spend most of the time in the preleptotene and pachytene stages. In this period a degeneration rate of 12% has been demonstrated among primary spermatocytes (Clermont, 1962). As a result of the first stage of meiotic division, secondary spermatocytes are formed. These cells are smaller than the primary spermatocytes, lie closer to the lumina of the seminiferous tubules,and are restricted to tubules at stage XIV of the cycle. They soon form a new layer of spermatids in stage I (Leblond and Clermont, 1952). Clermont (1962) demonstrated a 15% degeneration rate among the secondary spermatocytes.

2.2.1.3 Spermatids

Spermatids are formed as a result of the second maturation division of the spermatocytes. The spermatids show no tendency to grow (Roosen-Runge 1955) and it is generally accepted that spermatids do not divide (Roosen-Runge, 1952). Spermatids are present in all stages of the germinal epithelium; their development is characterized by four phases (Leblond and Clermont, 1952). The first or Golgi phase leads to the

formation of the acrosomic granule. The cap phase results in the acrosomic granule forming the head cap which enlarges to cover a third of the nucleus. Subsequently, during the acrosome phase, the head cap is oriented towards the basal lamina, the cytoplasm is displaced to the side away from the basal lamina, and the nucleus of the spermatid elongates. In the fourth or maturation phase, the nucleus continues to flatten and elongate; by the end of this phase the spermatid has a typical curved appearance and is released into the lumen (spermiation). Approximately 10% of spermatids degenerate before spermiation occurs (Clermont, 1962).

2.3 PROSTAGLANDINS

2.3.1 Nature, Action and Synthesis

Prostaglandins are a group of pharmacologically active, modified fatty acids. Structurally, the prostaglandins are unsaturated hydroxy acids with a five member ring within a twenty carbon skeleton. Four series of natural prostaglandins, designated as E, F, A, and B, corresponding to differences in the cyclopentyl group of the basic 20-carbon prostanoic acid, have been described. Compounds of the E type typically exhibit a hydroxyl group at carbon eleven and fifteen and an oxygen at carbon 9. Type F prostaglandins have a hydroxyl group replacing the oxygen at the alpha position in the cyclopentyl ring. Of the other groups of prostaglandins, the A prostaglandins have been most widely studied. Prostaglandins of the A series have an oxygen located at carbon 9, while the hydroxyl at position eleven has been replaced by a double bond between carbons 10 and eleven. The individual prostaglandins are designated with a subscript 1, 2 or 3 corresponding to the degree of saturation of the compound i.e., the number of double bonds.

The prostaglandins show important biological properties, and possess a broad spectrum of physiological activities. In a biological system, the compounds of a particular group generally have the same biological action, although it may differ quantitatively. A particular prostaglandin may have different effects on different tissues; likewise, different prostaglandins may have opposite effects on any particular tissue. Ninety percent of the prostaglandins of the E and F series are

metabolized in passing through the lungs and liver (Dawson et al, 1970; Samuelsson et al, 1971); only minute quantities of prostaglandins are required to stimulate certain organs (Karim and Hillier, 1974). Certain breakdown products of the prostaglandins are more resistant to metabolism and may be biologically active (Bygdeman et al, 1974). Prostaglandins also affect the production of cyclic AMP. Presumably, hormonal interaction with specific sites on the plasma membrane results in the enzymatic degradation of adenosine triphosphate by adenyl cyclase, increasing the levels of cyclic AMP (Sutherland et al,1965; Sutherland et al, 1968). Prostaglandins of the E series have been shown to increase the formation of cyclic AMP in various tissues (Horton, 1969), including the anterior pituitary (Zor et al, 1 70), the corpus luteum of rabbits (Jonsson, 1973), and the ovaries of the mouse (Kuehl et al, 1970). Thus, the action of prostaglandins at the cellular level may be to modify the production of cyclic AMP.

Prostaglandins are distributed in almost all tissues and body fluids of higher mammals. Human semen is a rich source of these compounds (Von Euler and Eliasson, 1967), and it was in the semen that they were first identified (Von Euler, 1935; Goldblatt, 1935). The prostaglandins are not stored to any great extent in the tissues (Ramwell and Shaw, 1970, 1971); their release may be ellicted (Piper and Vane 1971) by a wide variety of stimuli (neural, chemical or mechanical). For this reason the prostaglandins are probably synthesized by the tissues prior to their release.

Prostaglandin synthesis involves oxidative cyclization of polyunsaturated fatty acids of twenty-carbons in length (Samuelsson, 1972), catalyzed by the enzyme prostaglandin synthetase. Prostaglandins E_2 and F_2 are formed from archidonic acid. The acid is released from its phospholipid and enzymatically converted to prostaglandin. Activity of the prostaglandin synthetase enzyme complex has been demonstrated in different organs, but the activity was found to be greatest in the seminal vesicles (Nugteren et al , 1965).

2.3.2 Pharmacological Effects of Prostaglandins

2.3.2.1 Platelet Aggregation

Platelet aggregation influences cutaneous circulation and is related to thrombus formation and stasis of the blood. The E prostaglandins in particular have been found to affect the platelets. PGE_1 inhibits ADP-induced platelet aggregation, whereas PGE_2 stimulates aggregation (Kloeze,1966). The former compound was also found to suppress platelet thrombus formation (Berman et al, 1969). Similarly, Chandrasekhar (1967) found that PGE_1 given at a dose of 2 mg/kg I.V. inhibited platelet aggregation in rats. Platelet aggregation was found to be inhibited close to the site of intravenous infusion of PGE_1, but it was normal at a more distant site (French and Hillier, Cited in Karim and Hillier, 1974).

That prostaglandins are involved in homeostasis is evident from several studies. Smith and Willis (1970) demonstrated that unstimulated

platelets did not contain prostaglandins, but they were subsequently synthesized and released after the platelets were exposed to thrombin. Similarly, investigations in the rat have indicated that prostaglandins are produced by platelets during the process of clotting, production coinciding with second phase aggregation and release in the platelets (Glenn et al, 1972; Orczyk et al, 1972).

2.3.2.2 Inflammation and Vascular Permeability

Bergstrom et al (1959) demonstrated in man that infusion of PGE produced a dull red erythema in the skin; more recently, prostaglandins have been shown to increase vascular permeability (Crunkhorn and Willis, 1969; Williams and Morley, 1973). Prostaglandins have also been extracted from inflammatory exudates in animals (Willis, 1969) and man (Greaves et al, 1971), and in vitro have been found to cause emigration of leucocytes (Kaley and Weiner, 1971).

Prostaglandins have been implicated in phagocytic activity in the inflammatory infiltrate. These compounds are produced by polymorphonuclear leucocytes during phagocytosis in vitro (Higgs and Youlten, 1972). Similarly, a release of prostaglandins during phagocytosis has been reported (Macmorine et al ,1968).

Sondergaard and Wolf-Jürgensen (1972) and Sondergaard et al (1973) attributed a chemotactic factor to prostaglandins E_1 and F_2 ; intradermal injection of the two prostaglandins was followed initially by infiltration of the area with polymorphonuclear cells which were subsequently replaced by mononuclear cells.

2.3.3 Prostaglandins and the Male Reproductive System

Investigations into the role of prostaglandins in the male reproductive process have been few (Labhsetwar, 1974). At least 13 prostaglandins are present in seminal plasma (Samuelsson, 1963; Hamburg and Samuelsson, 1966). Prostaglandins of the E type occur in the highest concentration; in particular, PGE_1 and PGE_2 predominate (Bygdeman and Samuelsson, 1966). Eliasson (1959) demonstrated by the "split-ejaculate" technique that the prostaglandins in semen of normal individuals occur in the same fraction as fructose and therefore originate in the seminal vesicles. The addition of prostaglandin E_1 to the semen had no effect on the motility and metabolism of the spermatozoa. Bygdeman et al (1970) found that in a group of infertile men, the level of the E prostaglandins was significantly lower in 40% compared to the semen from fertile males; infertility in these patients could not be attributed to any specific cause.

Prostaglandins may also influence the release of interstitial cell stimulating hormone (ICSH or LH). Prostaglandins have been shown to increase the concentration of cyclic AMP in the anterior pituitary (Zor et al, 1970); Ratner et al (1974) found greater LH release following the administration of PGE_1 in a single dose of 20 µg per rat. In the testis, Ericsson (1972, cited by Tso et al ,1975), Bartke et al (1973) and Tso et al (1975) found degenerative changes, including atrophy, inhibition of spermatogenesis, and immature germinal cells in the seminiferous tubules of testes from animals treated with prostaglandins. Lowered levels of plasma testosterone were also detected following prostaglandin treatment in the mouse (Bartke et al, 1973).

However, the rate of spermatogenesis, determined by appearance of radioactive tracer in the semen, was increased following prolonged administration of PGE_2 or PGF_2 (Hunt and Nicholson, 1972); testicular biopsies or semen samples were not examined. Abbatiello et al (1975), in a quantitative study of the cells of the germinal epithelium, found a depression of spermatogenesis during the meiotic phase following subcutaneous administration of prostaglandin E_1 and E_2.

Little is known about the physiological role of prostaglandins in male reproduction, although there is some indication that they might be involved in release of gonadotrophin and the transport of spermatozoa. Further investigations are clearly required in this area.

2.3.4 Inhibitors of Prostaglandin Synthesis

2.3.4.1 Introduction

Aspirin and other non-steroidal anti-inflammatory agents are among the most widely distributed and most frequently used drugs available today. They alter most biological systems in which they have been tested. For example, these substances inhibited oxidative phosphorylation (Brody, 1955; Skidmore and Whitehouse, 1966), although the doses involved were much greater than the therapeutic range (Vane, 1974); affected platelet function (Zucker and Peterson, 1968; Gordon, 1974); and blocked leucocyte migration (Phelps, 1969).

Vane (1971) showed that acetylsalicylic acid inhibits the enzyme prostaglandin synthetase and thereby reduces the biosynthesis of prostaglandins. Simultaneously, Smith and Willis (1971) demonstrated that thrombin-induced liberation of prostaglandin E_2 and F_2 by human platelets could be reduced by aspirin or indomethacin. Ferreira et al (1971) also found that aspirin and indomethacin inhibit the release of prostaglandins in the perfused spleen of the dog. Blackwell et al (1975) has recently confirmed by radiometric assay in kidney microsomes of the rabbit that aspirin and indomethacin inhibit prostaglandin biosynthesis; indomethacin proved more effective than aspirin.

The effects of inhibition of prostaglandin synthetase activity by non-steroidal anti-inflammatory agents have been investigated in different species of animals and different tissues (Robinson and Vane, 1973).

Lau et al (1973) have found in the rat that administration of indomethacin for a period of four days post-conception completely inhibited implantation. Parturition in the rat was delayed 16-25 hours following oral administration of various prostaglandin synthetase inhibitors (Chester et al,1972; Csapo et al,1973). Persaud (1974) has also shown that indomethacin markedly prolonged pregnancy and caused an increased number of fetal deaths, resorptions and congenital defects in rats. Similar observations were made in mice (Persaud and Moore 1974, 1975). Prostaglandin synthetase inhibitors have also been found to block compensatory hypertrophy of the ovary following unilateral ovariectomy (Castracane et al,1975) and hypertrophy of the uterus induced by an IUD (Chaudhuri 1975).

The biological role of prostaglandins has also been investigated in other tissues by using antagonists of prostaglandin synthetase. Ojeda et al (1975) demonstrated in the rat that inhibitors of prostaglandin synthesis can suppress the release of LH from the pituitary; but the release of FSH was not altered. Chobsieng et al (1975) found that intravenous administration of PGE_2 increased the levels of serum LH; this response could be abolished by prior treatment with an antiserum to the hypothalamic gonadotrophin releasing hormone, thus indicating a hypothalamic site of action for the prostaglandins.

Inhibitors of prostaglandin synthesis also cause ulceration of the mucosal lining of the stomach (Rainsford 1975; Jorgenson 1976). However, administration of prostaglandins has been shown to reduce secretion of gastric acid and to either increase or decrease mucosal

blood flow, depending on the dosage and the secretory state of the stomach (Carter et al ,1974; Carmichael et al ,1975).

2.3.4.2 The Male Reproductive System

There have been few published studies relating to the effects of inhibition of prostaglandin synthesis on the male reproductive system. Collier and Flower (1971) found that acetylsalicylic acid, taken orally over a period of seven days, did not affect the volume of the semen; however, the concentrations of E and F prostaglandins in the semen were significantly decreased compared to the control samples. Horton et al (1973) confirmed that prostaglandin levels in human seminal fluid are significantly reduced after aspirin treatment; but prostaglandin synthesis was not abolished by high doses of the drug.

Inhibitors of prostaglandin synthesis have demonstrated a marked influence on male fertility. Cenedella and Crouthamel (1973) found that acetylsalicylic acid administered by gavage resulted in a significant increase in the fertility of male mice that were initially considered to be infertile on the basis of the number of implantation sites after mating; no change was found in the fertility of normal mice after treatment with acetylsalicylic acid. Abbatiello et al (1975) demonstrated that inhibition of prostaglandin synthesis, induced by oral administration of acetylsalicylic acid (100 mg/kg twice daily; 200mg/kg twice daily) and indomethacin (1.0mg/kg) for a period of 15 days, enhanced spermatogenesis. This was evident from the significant increases in the numbers of spermatocytes and spermatids.

The morphological and histochemical aspects of the male reproductive tract under the influence of prostaglandin synthetase inhibitors have not been further investigated.

SECTIONS 1 - 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 ANIMALS

Albino Sprague-Dawley rats of the Holtzman strain weighing 250-300g were obtained from Biolab (St. Paul, Minn.). Animals were housed in wire-mesh cages under controlled environmental conditions (temperature 68°-72°F; 12 hour light-dark cycle) and maintained on Teklab Mouse and Rat diet and water ad libitum.

Male rats were placed with females overnight and the following morning vaginal smears were taken and examined for spermatozoa. The presence of spermatozoa indicated a mature and fertile male rat.

3.2 EXPERIMENTAL DESIGN

Experiments were designed to determine the effects of inhibition of prostaglandin synthesis on the male reproductive tissues of the rat, induced by acetylsalicylic acid (ASA) (Vane, 1971; Abbatiello et al., 1975). The kidneys and adrenal glands were also studied.

The animals, all of proven fertility, were randomly assigned to eight groups according to the following treatment schedule:

Table 1: Treatment of Male Rats With ASA

Experimental Group	Number of Animals	Treatment	Duration
I	8	ASA 300mg/kg	12 DAYS
II	6	CONTROL	12 DAYS
III	6	ASA 150mg/kg	12 DAYS
IV	4	CONTROL	12 DAYS
V	4	ASA 300mg/kg	6 DAYS
VI	2	CONTROL	6 DAYS
VII	4	ASA 150mg/kg	6 DAYS
VIII	4	CONTROL	6 DAYS

The amount of acetylsalicylic acid to be injected was weighed out and dissolved in Sorensen's phosphate buffer; the pH of the solution was found to be slightly acidic (pH=6.0). Animals of Groups I, III, V, and VII were treated with the ASA solution subcutaneously each morning for six or twelve days. The injection site was between the scapulae.

An equal volume of the solvent (phosphate buffer) adjusted to pH 6.0 was administered to the animals of Groups II, IV, VI, and VIII. These served as controls. All animals were weighed at intervals of two days.

3.2.1. Histological and Histochemical Studies

Animals were killed by ether administration twenty-four hours after the final treatment. One control and one experimental animal were killed simultaneously. Blood samples were obtained by cardiac puncture for testerone assay.

The testis, epididymidis, ductus deferens, seminal vesicle, kidney and adrenal from one side of each animal were removed and placed in Bouin's solution. The other testis, epididymidis, ductus deferens, seminal vesicle, kidney and adrenal were processed for enzyme-histological studies. Control and experimental tissue specimens were placed on a cryostat chuck and covered with embedding medium (Ames O.C.T. Compound, Fisher Scientific Co. Ltd.).

A styrofoam container was used as a freezing chamber. Dry ice was packed around a beaker approximately half filled with 2-methylbutane (practical grade). A cooling period of forty-five minutes was necessary to lower the temperature of the liquid sufficiently for snap freezing. The specimens were quickly immersed in the solution for one to one and a

half minutes and immediately placed in the cryostat which was maintained at a temperature of -20C. A minimum of 30 minutes was required for specimens to reach the temperature of the cryostat. Sections were cut at 4μ and these were placed on a slide. Each slide contained one to four sections and four slides were prepared for each tissue. The tissue sections were then left standing at room temperature for 45-75 minutes. Histochemical tests (reagents were obtained from Sigma Chemical Co., St. Louis, Ms.) were carried out for the following enzymes: acid phosphatase, (Burstone, 1958; Kaplow and Burstone, 1964) alkaline phosphatase (Burstone, 1958, Kaplow, 1963) and cytochrome oxidase (Burstone, 1960, 1961). The fourth slide was fixed in buffered formalin for additional studies if required.

Tissues fixed in Bouin's solution were cleaned and weighed, samples were embedded in paraffin and processed for histological examination by the hematoxylin and eosin, Mallory's trichrome, and periodic acid-Schiff's staining techniques.

3.3 QUANTITATIVE EVALUATION OF SPERMATOGENESIS

3.3.1 Organ Weights

Organs were weighed and expressed as grams per 100 grams body weight of the animal at autopsy. The weight of the ductus deferens was expressed as milligrams per millimeter length per 100 grams body weight. The Gonadosomatic index (Kalla and Bansal, 1975) was also determined.

A mixed analysis of variance and Duncan's multiple range test of significance was applied to the data.

3.3.2 Tubular Diameters

Sixty tubular diameters were measured for each animal using an eye piece scale where one division was equal to 5μ. One hundred measurements were randomly chosen for each group. A mixed analysis of variance and Duncan's multiple range test of significance was applied to the data. The independent factor was the treatment schedule with eight levels.

3.3.3 Spatial Dimensions and Relationships

Testicular cross sections were analyzed by the method of Chalkley (1943). Four pointers were placed in the ocular lens of the microscope and an oil immersion objective was used. The stage was moved randomly and the number of hits on the following areas recorded:

a) interstitial material (connective tissue, blood vessels and Leydig cells)

b) intertubular space (excluding interstitial material)

c) luminal space (space lying above the germinal epithelium)

d) germinal epithelium (the lining of the seminiferous tubules including cytoplasm being shed by spermatids during spermiogenesis, and the basement membrane about the seminiferous tubules)

A mixed analysis of variance and Duncan's multiple range test of significance was applied to each measurement. The independent factor was treatment schedule.

3.3.4 Quantitative Analysis of the Germinal Epithelium

Germinal epithelium was analyzed by the method of Oakberg (1956). Stage VII tubules (Clermont and LeBlond, 1955) were used (Abbatiello et al., 1975; Croft and Bartke, 1976). Tubules were selected randomly according to the following criteria:

a) the tubular cross section must be round to oval in shape

b) the cross section should pass through the lumen

Sertoli cells, Type A spermatogonia, preleptotene and pachytene spermatocytes and spermatids were counted in six tubular cross sections for each animal. Crude counts were corrected to account for differences in nuclear diameter (Abercrombie, 1946) and expressed per ten Sertoli cells (Croft and Bartke, 1976).

A mixed analysis of variance and Duncan's multiple range test of significance was applied to each cell type. The independent factor was the treatment schedule.

3.3.5 Testosterone Assay

Blood samples obtained by cardiac puncture were allowed to clot and centrifuged. Serum was removed and testosterone levels were determined by radioimmunoassay (Reyes et al., 1974; Stearns et al., 1974).

A mixed analysis of variance and Duncan's multiple range test of significance was applied to the data.

SECTIONS 1 AND 2

RESULTS

4. RESULTS

4.1 QUANTITATIVE AND MORPHOLOGICAL DATA

4.1.1 Organ Measurements

4.1.1.1 Organ Weights

The mean organ weights expressed as grams per 100 grams body weight* are presented in Tables 2a & b and Figs. 1-7.

The mean weights of the testes were greater in all groups of animals that were treated with ASA than in the corresponding controls (Fig. 1). However,the differences were not statistically significant.

Comparison of the mean organ weights of the animals that were treated with ASA for different periods and at different doses are presented in Tables 3a & b; 4a & b. At a dosage of 300mg/kg body weight ASA, the mean testicular weight was significantly greater ($p < 0.05$) after treatment over a period of 12 days than after treatment for six days (Table 3a). Comparison of the results of treatment at different dosages (Table 4) revealed a significant increase ($p < 0.05$) in the mean testicular weight after the animals were treated with ASA at a dose level of 300mg/kg body weight for a period of 12 days (Table 4a). The corresponding control values did not differ significantly.

The mean weights of the epididymides were not significantly affected as a result of treatment with ASA (Table 2a & b). The mean

*The weights of the ductus deferens are expressed as milligrams per millimeter per 100 grams body weight.

weights tended to approximate each other in groups receiving ASA at a dose of 150mg/kg body weight for either six or 12 days and 300mg/kg body weight for six days (Fig. 2). No significant differences were evident when comparing the mean weights of the epididymides of the animals after different treatment periods when ASA was administered at a dose of 150mg/kg body weight (Table 3b); similarly no significant difference was found in the mean weights when ASA was administered to the animals for a period of six days at different dosages (Table 4b). However, a significant increase ($p < 0.01$) in the mean weight of the epididymides resulted after treatment with ASA at a dose of 300mg/kg body weight for 12 days, compared to the animals that received ASA at a dose of 150mg/kg body weight for 12 days (Table 4a). When the animals were treated for six days at a dose level of 300mg/kg body weight, the mean weights of the epididymides were significantly greater ($p < 0.05$) in the group receiving ASA for 12 days at a rate of 300mg/kg body weight (Table 3a).

Treatment with ASA produced no significant difference in the mean weights of the seminal vesicles compared to the control values (Table 2a & b). The mean weights of the seminal vesicles of animals receiving ASA at a dosage of 150mg/kg body weight for either six or 12 days, and 300mg/kg body weight for six days tended to approximate each other (Fig. 3) and were not significantly different (Table 3b & 4b). Comparison of the mean weights of the seminal vesicles between the groups receiving ASA at a dose of 300mg/kg body weight for six or 12 days revealed no significant difference (Table 3a); the

mean weights also did not differ significantly between the animals that were administered ASA for 12 days at the two dose levels (Table 4a).

A significant difference ($p < 0.01$) in the mean weights of the ductus deferens was present between the animals treated with ASA for 12 days at a dose of 300mg/kg body weight and the controls (Table 2a). The differences in the mean weights of the ductus deferens were not statistically significant when the animals that were treated with ASA at a dose of 150mg/kg body weight for six or 12 days and 300mg/kg body weight for six days were compared with the controls. The mean weights of the ductus deferens of these latter animals treated with ASA approximated each other (Fig. 4); the differences were not statistically significant (Tables 3b &4b). In the groups of animals treated with ASA at a dose level of 300mg/kg body weight, the mean weight of the ductus deferens was significantly increased ($p < 0.01$) after the treatment period of 12 days (Table 3a). The mean weight of the ductus deferens also showed a significant increase ($p < 0.01$) in the group receiving 300mg/kg body weight ASA compared to the animals treated at a dose level of 150mg/kg body weight for a period of 12 days (Table 4a). However, the mean weight of the ductus deferens in the two corresponding control groups also differed significantly.

No statistically significant differences were found in the mean weights of the kidneys between the animals treated with ASA or the solvent (Tables 2a & b). The mean weights of the kidneys from animals treated with ASA at a dose level of 150mg/kg body weight for either six or 12 days, and with 300mg/kg body weight for six days tended to approximate each other (Fig. 5); the differences were not statistically

significant (Tables 3b & 4b). The mean weight of the kidneys was significantly greater ($p < 0.01$) when ASA was administered to the animals at a dose of 300mg/kg body weight than at a dose of 150mg/kg body weight over a 12 day period (Table 4a).

When the animals were treated with ASA, the mean weights of the adrenal glands were greater than those of the corresponding control animals (Fig. 6); the differences were not statistically significant (Table 2a & b). Comparing animals treated with 150mg/kg body weight ASA for six days and for 12 days revealed a significant increase ($p < 0.05$) in the mean weights of the adrenal glands after treatment for a period of six days (Table 3b). Treatment for a period of six days at different dose levels produced a significant increase ($p < 0.05$) in the mean weight of the adrenal glands of animals that received ASA at a dose of 150mg/kg body weight (Table 4b).

The mean GSI was increased in the groups of animals treated with ASA for periods of 12 days (300mg/kg body weight) and six days (300mg/kg body weight and 150mg/kg body weight) compared to the control animals (Table 2a & b and Fig. 7); the differences were not statistically significant. Treatment of animals with ASA for a period of 12 days at a dose level of 150mg/kg body weight caused a significant decrease ($p < 0.05$) in the mean GSI (Table 2). Comparison of the mean GSI (gonadosomatic indices) of animals that received ASA for different periods (Tables 3a & b) and at different dose levels (Tables 4a & b) revealed no significant differences.

Table 2a: Mean organ weights (grams per 100 grams body weight[+]) after treatment of animals with ASA

	Treatment with ASA			
	I ASA 300 mg/kg body weight for 12 days	Control	III ASA 150 mg/kg body weight for 12 days	Control
Testes	0.514 ± .062	0.420 ± .019	0.374 ± .031	0.365 ± .019
Epididymides	0.158 ± .006	0.152 ± .006	0.120 ± .013	0.130 ± .005
Seminal Vesicles	0.091 ± .007	0.117 ± .015	0.114 ± .009	0.097 ± .011
Ductus Deferens	0.781 ± .019*	0.682 ± .024	0.491 ± .021	0.482 ± .025
Kidneys	0.399 ± .016	0.346 ± .014	0.333 ± .014	0.321 ± .022
Adrenal Glands	0.0066 ± .0066	0.0057 ± .003	0.0054 ± .0005	0.0046 ± .0005
GSI	5.77 ± .58	5.48 ± .30	6.54 ± .59**	8.57 ± .23

*Significantly Greater ($p < 0.01$) than control values.
**Significantly Less ($p < 0.05$) than control values.

[+]The weight of the ductus deferens is expressed as milligrams per millimeter per 100 grams body weight.

Table 2b: Mean organ weights (grams per 100 grams body weight[+]) after treatment of animals with ASA

	Treatment with ASA			
	V ASA 300 mg/kg body weight for 6 days	Control	VII ASA 150 mg/kg body weight for 6 days	Control
Testes	0.370 ± .014	0.350 ± .005	0.470 ± .011	0.441 ± .021
Epididymides	0.131 ± .006	0.134 ± .001	0.125 ± .003	0.121 ± .011
Seminal Vesicles	0.128 ± .007	0.089 ± .009	0.111 ± .011	0.122 ± .021
Ductus Deferens	0.554 ± .25	0.555 ± .048	0.540 ± .022	0.566 ± .028
Kidneys	0.354 ± .025	0.329 ± .033	0.332 ± .010	0.335 ± .007
Adrenal Glands	0.0052 ± .0001	0.0042 ± .0006	0.0070 ± .0006	0.0062 ± .0005
GSI	6.86 ± .30	7.83 ± .33	5.65 ± .37	6.24 ± .29

[+]The weight of the ductus deferens is expressed as milligrams per millimeter per 100 grams body weight.

Table 3a: Mean organ weights (grams per 100 grams body weight) in rats treated with ASA for different periods

	Treatment with ASA		
	I 300 mg/kg body weight for 12 days	V 300 mg/kg body weight for 6 days	Significance of difference between the corresponding control groups
Testes	0.514 ± .062**	0.370 ± .014	No Significant Difference
Epididymides	0.158 ± .006**	0.131 ± .006	No Significant Difference
Seminal Vesicles	0.091 ± .007	0.128 ± .007	No Significant Difference
Ductus Deferens	0.781 ± 0.19*	0.554 ± .025	Significantly Different ($p < 0.01$)
Kidneys	0.399 ± .016	0.354 ± 0.25	No Significant Difference
Adrenal Glands	0.0066 ± .0006	0.0052 ± .0001	No Significant Difference
GSI	5.77 ± .58	6.86 ± .30	Significantly Different ($p < 0.05$)

*Significantly Greater ($p < 0.01$)
**Significantly Greater ($p < 0.05$)

3b: Mean organ weights (grams per 100 grams body weight) in rats treated with ASA for different periods

	Treatment with ASA		
	III 150 mg/kg body weight for 12 days	VII 150 mg/kg body weight for 6 days	Significance of difference between the corresponding control groups
Testes	0.374 ± .031	0.470 ± .011	No Significant Difference
Epididymides	0.120 ± .013	0.125 ± .003	No Significant Difference
Seminal Vesicles	0.114 ± .009	0.111 ± .011	No Significant Difference
Ductus Deferens	0.491 ± .021	0.540 ± .022	No Significant Difference
Kidneys	0.333 ± .014	0.332 ± .101	No Significant Difference
Adrenal Glands	0.0054 ± .0005	0.0070 ± .0006*	No Significant Difference
GSI	6.54 ± .59	5.65 ± .37	Significantly Different ($p < 0.01$)

*Significantly Greater ($p < 0.05$)

Table 4a: Mean organ weights (grams per 100 grams body weight) in rats treated with ASA at different doses

	Treatment with ASA		
	I 300 mg/kg body weight for 12 days	III 150 mg/kg body weight for 12 days	Significance of difference between the corresponding control groups
Testes	0.514 ± .062**	0.374 ± .031	No Significant Difference
Epididymides	0.158 ± .006*	0.120 ± .013	No Significant Difference
Seminal Vesicles	0.091 ± .007	0.114 ± .009	No Significant Difference
Ductus Deferens	0.781 ± .019*	0.491 ± .021	Significantly Different ($p < 0.01$)
Kidneys	0.399 ± .016*	0.333 ± .014	No Significant Difference
Adrenal Glands	0.0066 ± .0006	0.0054 ± .0005	No Significant Difference
GSI	5.77 ± .58	6.54 ± .59	Significantly Different ($p < 0.01$)

*Significantly Greater ($p < 0.01$)
**Significantly Greater ($p < 0.05$)

Table 4b: Mean organ weights (grams per 100 grams body weight) in rats treated with ASA at different doses

	Treatment with ASA		
	V 300 mg/kg body weight for 6 days	VII 150 mg/kg body weight for 6 days	Significance of difference between the corresponding control groups
Testes	0.370 ± 0.14	0.470 ± .011	No Significant Difference
Epididymides	0.131 ± .006	0.125 ± .033	No Significant Difference
Seminal Vesicles	0.128 ± .007	0.111 ± .011	No Significant Difference
Ductus Deferens	0.554 ± .025	0.540 ± .022	No Significant Difference
Kidneys	0.354 ± .025	0.332 ± .010	No Significant Difference
Adrenal Glands	0.0052 ± .0001	0.0070 ± .0006*	No Significant Difference
GSI	6.86 ± .30	5.65 ± .37	No Significant Difference

*Significantly Greater ($p < 0.05$)

Table 5: Sources of Variation Among Treatment Schedules and Organ Weights

Source of Variation	Level of Significance	Required F Value
1. Testis Weights		
Experimental F = 2.258	Not Significant	F > 3.07 @ 0.05
Control F = 3.680	5%	F > 3.29 @ 0.05
2. Epididymidis Weights		
Experimental F = 4.717	5%	F > 3.07 @ 0.05
Control F = 3.815	5%	F > 3.29 @ 0.05
3. Seminal Vesicle Weights		
Experimental F = 3.521	5%	F > 3.13 @ 0.05
Control F = 0.707	Not Significant	F > 3.29 @ 0.05
4. Ductus Deferens Weights		
Experimental F = 46.842	1%	F > 4.87 @ 0.01
Control F = 10.434	1%	F > 5.42 @ 0.01
5. Kidney Weights		
Experimental F = 4.081	5%	F > 3.07 @ 0.05
Control F = 0.452	Not Significant	F > 3.29 @ 0.05
6. Adrenal Weights		
Experimental F = 2.635	Not Significant	F > 3.07 @ 0.05
Control F = 3.801	5%	F > 3.29 @ 0.05
7. GSI		
Experimental F = 0.933	Not Significant	F > 3.07 @ 0.05
Control F = 23.052	1%	F > 5.42 @ 0.0o

Fig. 1 (Upper): Mean testicular weight (grams per 100 grams body weight) following treatment of the animals with acetylsalicylic acid at different dose levels for 12 or six days.

Fig. 2 (Lower): Mean weight of the epididymides (grams per 100 grams body weight) following treatment of the animals with acetylsalicylic acid at different dose levels for 12 or six days.

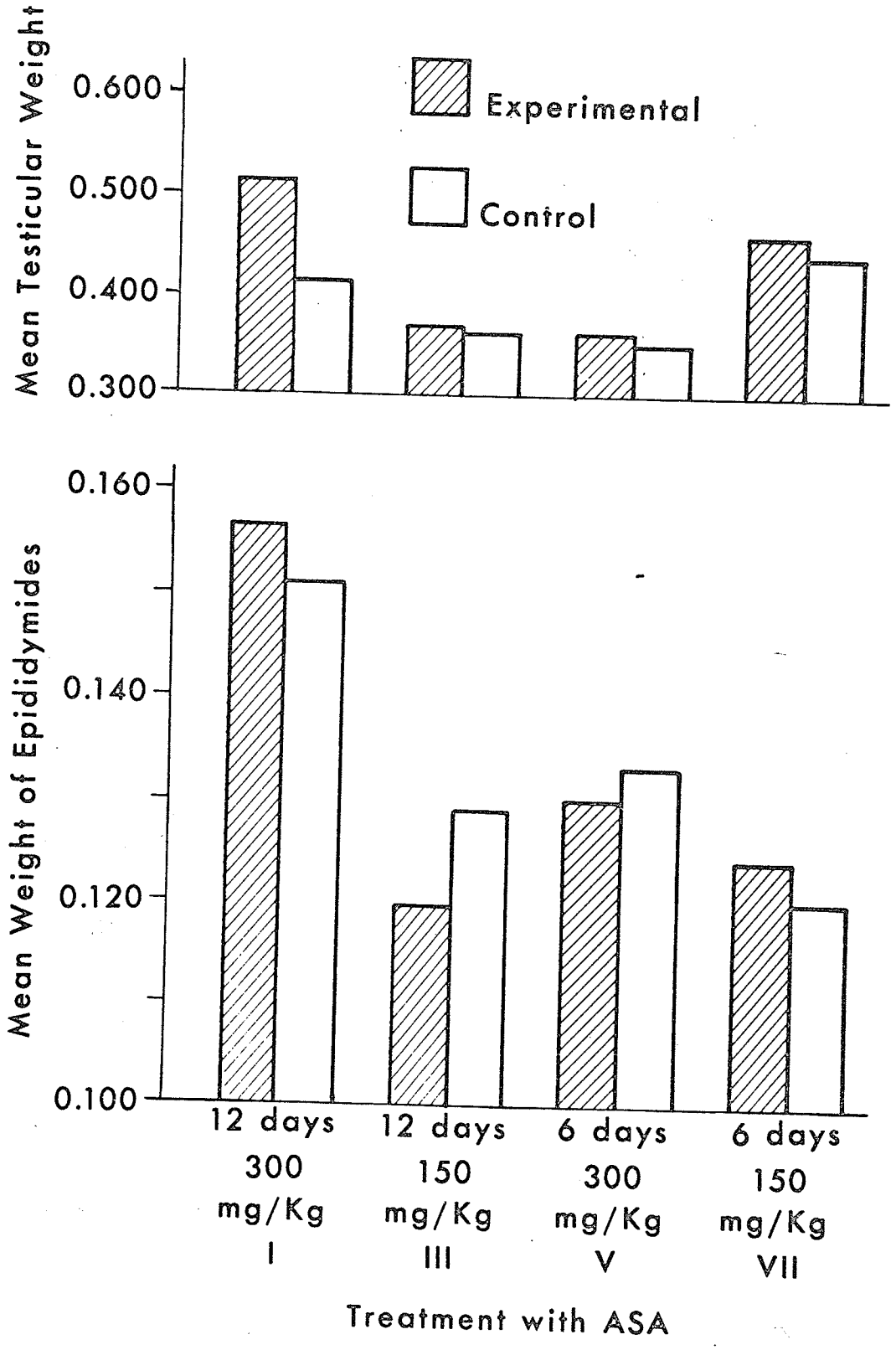

Mean Testicular Weight
0.600
0.500
0.400
0.300
Experimental
Control
Mean Weight of Epididymides
0.160
0.140
0.120
0.100
12 days 300 mg/Kg I
12 days 150 mg/Kg III
6 days 300 mg/Kg V
6 days 150 mg/Kg VII
Treatment with ASA

Fig. 3 (Upper): Mean weight of the seminal vesicles (grams per 100 grams body weight) following treatment of the animals with acetylsalicylic acid at different dose levels for 12 or six days.

Fig. 4 (Lower): Mean weight of the ductus deferens (milligrams per millimeter per 100 grams body weight) following treatment of the animals with acetylsalicylic acid at different dose levels for 12 or six days.

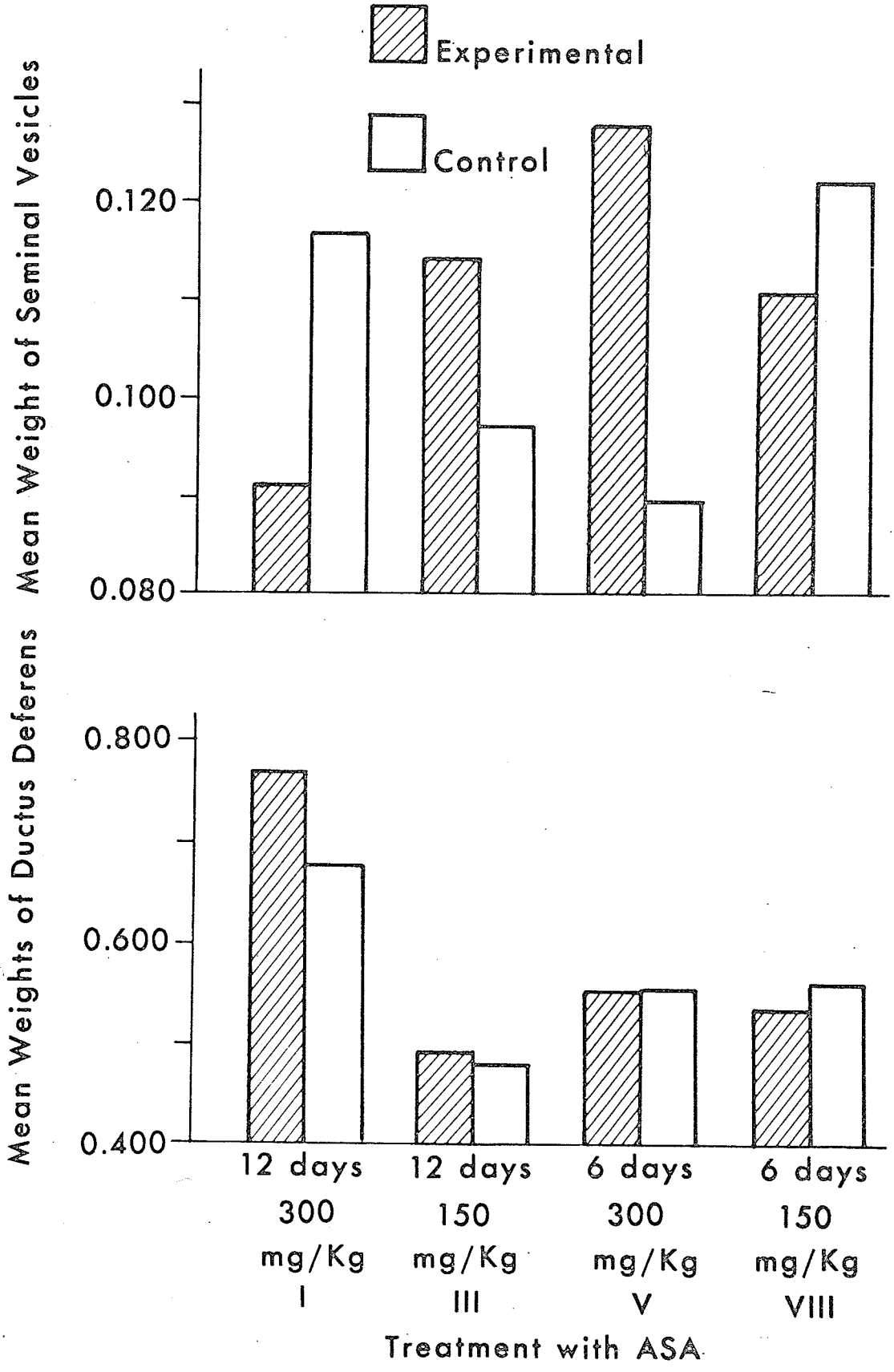

Experimental
Control
Mean Weight of Seminal Vesicles
0.120
0.100
0.080
Mean Weights of Ductus Deferens
0.800
0.600
0.400
12 days 300 mg/Kg I
12 days 150 mg/Kg III
6 days 300 mg/Kg V
6 days 150 mg/Kg VIII
Treatment with ASA

Fig. 5 (Upper): Mean weight of the kidneys (grams per 100 grams body weight) following treatment of the animals with acetylsalicylic acid at different dose levels for 12 or six days.

Fig. 6 (Lower): Mean weight of the adrenal glands (grams per 100 grams body weight) following treatment of the animals with acetylsalicylic acid at different dose levels for 12 or six days.

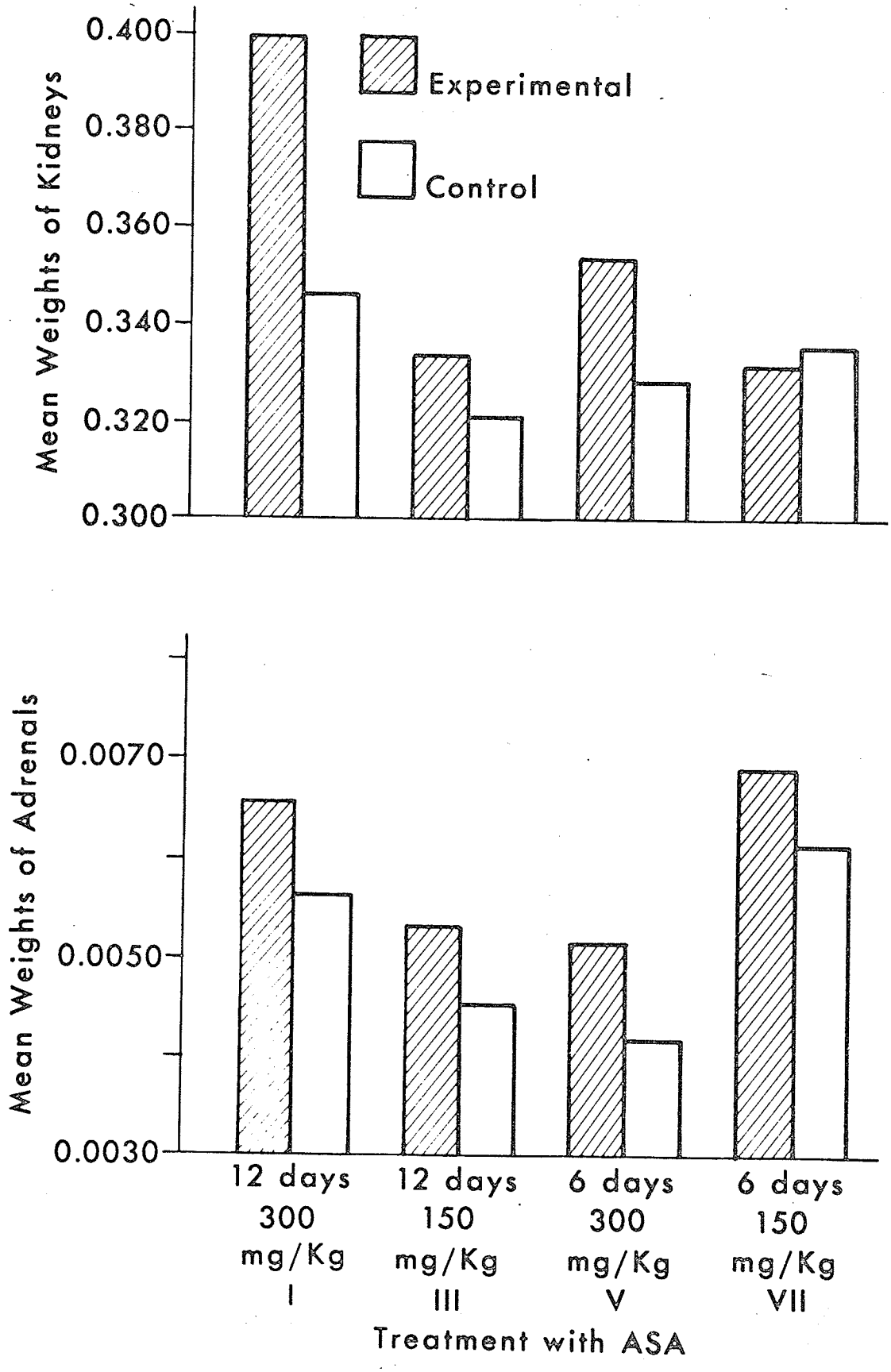
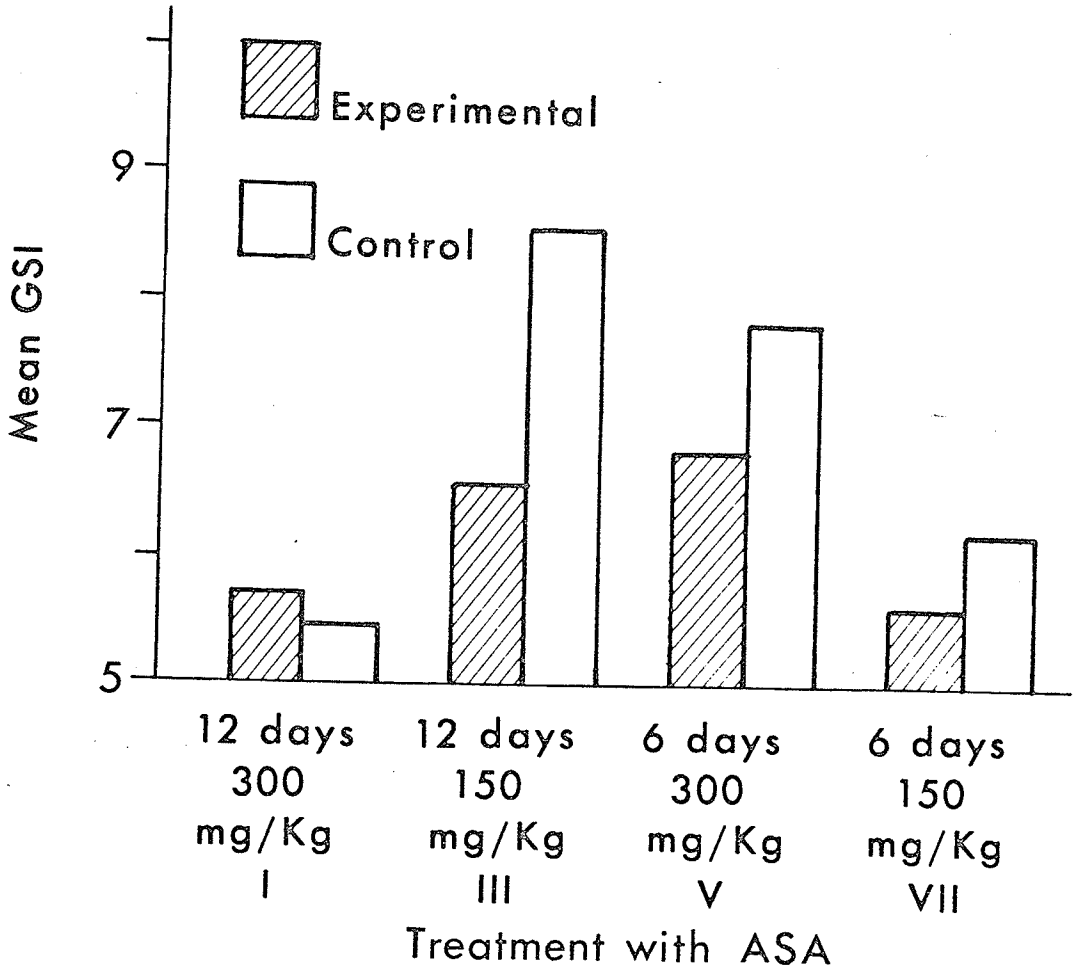

Mean Weights of Kidneys
0.400
0.380
0.360
0.340
0.320
0.300
Experimental
Control
Mean Weights of Adrenals
0.0070
0.0050
0.0030
12 days 300 mg/Kg I
12 days 150 mg/Kg III
6 days 300 mg/Kg V
6 days 150 mg/Kg VII
Treatment with ASA

Fig. 7: Mean gonadosomatic index $\left(\frac{\text{Testicular weight x body weight}}{100}\right)$ following treatment of the animals with acetylsalicylic acid at different dose levels for 12 or six days.


Experimental
Control
Mean GSI
9
7
5
12 days 300 mg/Kg I
12 days 150 mg/Kg III
6 days 300 mg/Kg V
6 days 150 mg/Kg VII
Treatment with ASA

4.1.1.2 Tubular Diameters

The mean diameters of the seminiferous tubules are presented in Table 6. Treatment of animals with ASA for a period of 12 days (300mg/kg body weight) produced a significant increase ($p < 0.05$) in the mean tubular diameter compared to the controls. In contrast, treatment with ASA for a period of 12 days at a dose of 150mg/kg body weight resulted in a significant decrease ($p < 0.01$) of the mean tubular diameter. The mean tubular diameters of the control animals and the animals that were treated with ASA for a period of six days did not differ significantly.

Comparison of the animals that were treated with ASA at a dose level of 300mg/kg body weight for different periods (Table 7a) revealed a significant increase ($p < 0.01$) in the mean tubular diameter over a treatment period of 12 days; however, the mean tubular diameters of corresponding control groups also differed significantly.

Animals receiving ASA at a dose level of 150mg/kg body weight showed a significant increase ($p < 0.01$) in the mean diameter of the seminiferous tubules when treated for six days (Table 7b); the mean tubular diameters of the corresponding control groups also showed significant differences. When the animals were treated for a period of 12 days the mean diameter of the seminiferous tubules was significantly greater in the group receiving ASA at a dose of 300mg/kg body weight than the group treated with ASA at a dose of 150mg/kg body weight (Table 8a). The mean diameters of the seminiferous tubules did not differ significantly in animals treated at the two

different dose levels for a period of six days (Table 8b).

The sources of variation of the mean tubular diameters with respect to the treatment schedule are presented in Table 9. The mean diameters of the seminiferous tubules of animals that were treated with ASA and those that were treated with solvent tended to differ ($p < 0.01$)

Table 6a: The mean diameters of the seminiferous tubules of animals after treatment with ASA

	Treatment Dosage			
	I ASA 300 mg/kg for 12 days	Control	III ASA 150 mg/kg for 12 days	Control
Mean tubular diameters (μ)	252.14 ± 3.29	240.37 ± 3.11	206.20 ± 3.30	242.15 ± 2.97
	Significantly greater than Controls ($p < 0.05$)		Significantly less than controls ($p < 0.01$)	

Table 6b: The mean diameters of the seminiferous tubules of animals after treatment with ASA

	V ASA 300 mg/kg for 6 days	Control	VII ASA 150 mg/kg for 6 days	Control
Mean tubular diameters (μ)	223.15 ± 2.71	224.80 ± 3.26	215.55 ± 2.77	222.70 ± 3.20
	Not Significant		Not Significant	

Table 7a: The mean diameters of the seminiferous tubules after treatment with ASA for different periods

	Treatment with ASA	Mean tubular diameter (μ)
I	300 mg/kg body weight for 12 days	252.14 ± 3.29*
V	300 mg/kg body weight for 6 days	223.15 ± 2.17
	Significance of difference between corresponsing control groups	Significantly different ($p < 0.01$)

*Significantly greater ($p < 0.01$)

Table 7b: The mean diameters of the seminiferous tubules after treatment with ASA for different periods

	Treatment with ASA	Mean tubular diameter (μ)
III	150 mg/kg body weight for 12 days	206.20 ± 3.30
VII	150 mg/kg body weight for 6 days	215.55 ± 2.77*
	Significance of difference between the corresponding control groups	Significantly different ($p < 0.01$)

*Significantly greater ($p < 0.05$)

Table 8a: Mean diameters of the seminiferous tubules after treatment with ASA at different dosages

	Treatment with ASA	Mean tubular diameters (μ)
I	300 mg/kg body weight for 12 days	252.14 ± 3.29*
II	150 mg/kg body weight for 12 days	206.20 ± 3.30
	Significance of difference between the corresponding control groups	No significant difference

*Significantly greater ($p < 0.01$)

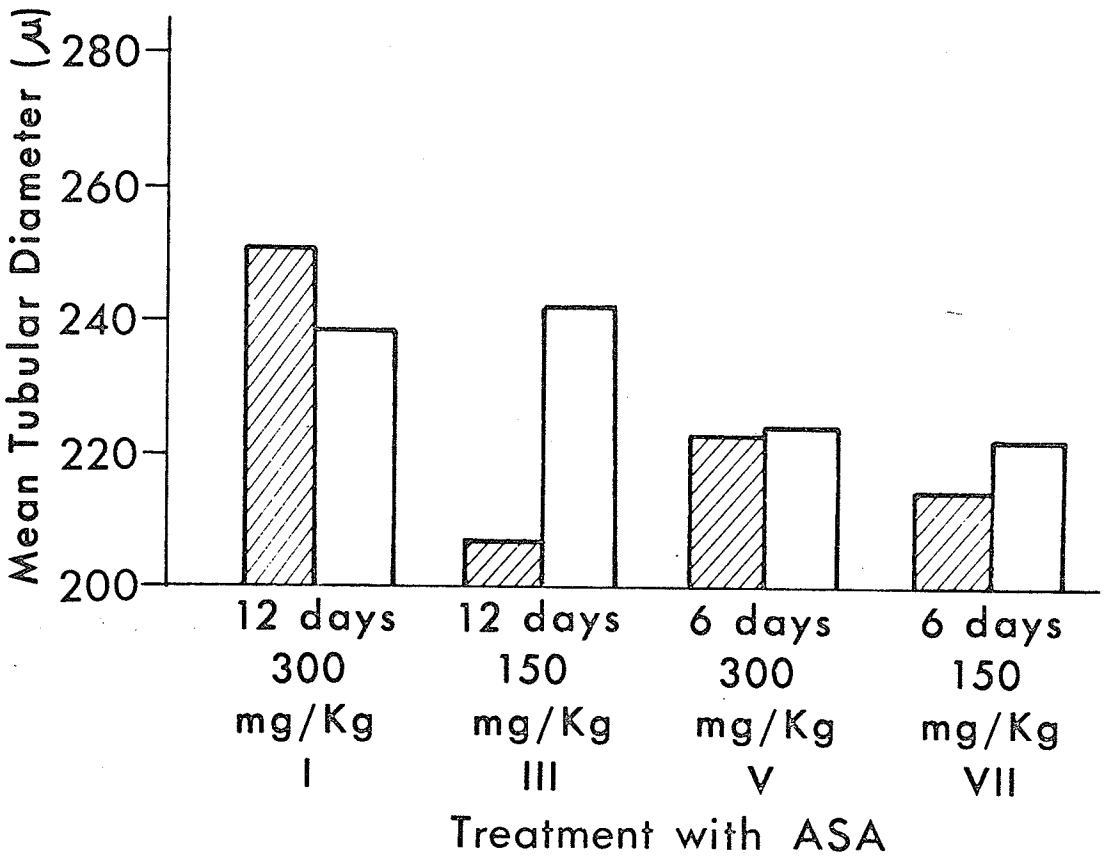
Table 8b: Mean diameters of the seminiferous tubules after treatment with ASA at different dosages

	Treatment with ASA	Mean tubular diameters (μ)
V	300 mg/kg body weight for 6 days	223.15 ± 2.71
VII	150 mg/kg body weight for 6 days	215.55 ± 2.77
	Significance of difference between the corresponding control groups	No significant difference

Table 9: Sources of variation relating to treatment schedules and mean tubular diameters

Source of variation	Level of significance	Required F Value
Tubular diameter		
Experimental F = 42.452	1%	F > 3.78 @ 0.01
Control F = 10.356	1%	F > 3.78 @ 0.01

Fig. 8: Mean tubular diameter (μ) following treatment of the animals with acetylsalicylic acid at different dose levels for 12 or six days.


Mean Tubular Diameter (μ)
280
260
240
220
200
12 days 300 mg/Kg I
12 days 150 mg/Kg III
6 days 300 mg/Kg V
6 days 150 mg/Kg VII
Treatment with ASA

4.1.1.3 Spatial Dimensions and Relationships

The mean number of random "hits"* in different areas of testicular cross-sections is presented in Table 10.

The mean number of "hits" on the interstitial tissue of the testes of animals treated with ASA were decreased compared to the control groups (Fig. 9), but the differences were not significant in any group (Table 10). The mean area occupied by the interstitial tissue was similar in animals that were treated with ASA for periods of 12 days (150 mg/kg body weight) and six days (300mg/kg body weight and 150m/kg body weight). No significant differences were present when the results of the different treatment periods (Table 11) and of the different dosages (Table 12) were compared.

No significant changes were detected in the mean area occupied by the spaces between the seminiferous tubules (intertubular spaces) after treatment with ASA (Table 10). A slight decrease in intertubular space was found after the animals were treated with ASA for 12 days at a dose of 150mg/kg body weight and for six days at doses of 300mg/kg body weight and 150mg/kg body weight (Fig. 10). The mean areas occupied by these intertubular spaces did not differ significantly at different treatment periods (Table 11) or following different treatment dosages (Table 12).

After treating the animals with ASA for a period of 12 days, the mean area occupied by the lumina of the seminiferous tubules

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*See Section 3.3.1.3 (in Materials and Methods). "Hits" are defined as "the contact of the image of the point with the clearly observed image of the specimen" (Chalkley, 1943).

was increased (Fig. 11); the increase was significant ($p < 0.05$) at a dose level of 300mg/kg body weight (Table 10). In animals that were treated with the substance for a period of six days, the mean area occupied by the lumina was less than that of the control groups (Fig. 11). At a dose level of 300mg/kg body weight, administered over different periods, the lumina of the seminiferous tubules occupied an area that was significantly increased after treatment for 12 days (Table 11a). The mean area occupied by the lumina was also significantly greater ($p < 0.05$) after the animals were treated for a period of 12 days at a dose of 300mg/kg body weight than at a dose of 150mg/kg body weight (Table 12a).

The space occupied by the germinal epithelium was increased in animals treated with ASA for periods of 12 days (150mg/kg body weight) and for six days (300mg/kg body weight and 150mg/kg body weight), when compared with the control animals (Fig. 12); the differences, however, were not significant (Tables 11b and 12a). The space occupied by the germinal epithelium was significantly decreased ($p < 0.05$) in animals that were treated with ASA at a dose of 300mg/kg body weight for 12 days (Table 10). The space occupied by the germinal epithelium did not differ significantly when compared at different treatment periods (Table 11) or dosages (Table 12).

The sources of variation among groups of animals that were treated with ASA (experimental) or with the solvent (control), and spatial relationships are presented in Table 13. Treatment with ASA did not result in a significant variation of the mean area of the interstitial tissue between the groups. Similarly, the mean areas of the intertubular space in the groups that were treated with ASA

did not differ significantly; control values, however, showed significant differences ($p < 0.01$). The areas occupied by the luminal space and the germinal epithelium were significantly different among groups of animals that received ASA ($p < 0.01$ and $p < 0.05$ respectively).

Table 10a: Mean number of random "hits" in different areas in a testicular cross-section after treatment with ASA

	Treatment with ASA			
	I 300mg/kg body weight for 12 days	Control	III 150 mg/kg weight for 12 days	Control
Interstitial Tissue	6.84 ± .55	7.05 ± .67	5.45 ± .61	6.90 ± .73
Intertubular Space	18.15 ± 1.73	17.65 ± .42	20.05 ± 1.29	23.95 ± 1.49
Luminal Space	22.70 ± 1.70*	16.90 ± 1.59	18.05 ± 1.02	16.80 ± 1.64
Germinal Epithelium	53.15 ± 1.34**	58.70 ± 1.28*	56.45 ± 1.49	52.45 ± 1.98

*Significantly greater ($p < 0.05$) than control values.
**Significantly less ($p < 0.05$) than control values.

Table 10b: Mean number of random "hits" on different areas in a testicular cross-section after treatment with ASA*

	Treatment with ASA			
	V 300mg/kg body weight for 6 days	Control	VII 150mg/kg body weight for 6 days	Control
Interstitial Tissue	6.05 ± .81	7.00 ± .63	5.70 ± .68	7.00 ± .57
Intertubular Space	21.35 ± .88	22.15 ± 1.54	18.50 ± 1.53	18.85 ± 1.06
Luminal Space	14.90 ± 1.57	15.70 ± 1.58	17.25 ± 1.87	18.55 ± 1.20
Germinal Epithelium	57.95 ± 1.23	55.15 ± 1.63	58.50 ± 1.44	55.60 ± 1.94

*Differences between experimental and control values were not statistically significant in any case.

Table IIa: The mean number of random "hits" on different areas in a testicular cross-section after treatment with ASA for different periods

	Treatment with ASA		
	I ASA 300mg/kg body weight for 12 days	V ASA 300mg/kg body weight for 6 days	Significance of difference between the corresponding control groups
Interstitial Material	6.84 ± .55	6.05 ± .81	No significant difference
Intertubular Space	18.15 ± 1.73	21.35 ± .88	Significantly different ($p < 0.05$)
Luminal Space	22.70 ± 1.70*	14.90 ± 1.57	No significant difference
Germinal Epithelium	53.15 ± 1.34	57.45 ± 1.23	No significant difference

*Significantly greater ($p < 0.01$)

Table IIb: The mean number of random "hits" on different areas in a testicular cross-section after treatment with ASA for different periods*

	Treatment with ASA		
	III ASA 150mg/kg body weight for 12 days	VII ASA 150mg/kg body weight for 6 days	Significance of difference between the corresponding control groups
Interstitial Material	5.45 ± .61	5.70 ± .68	No significant difference
Intertubular Space	20.05 ± 1.29	18.50 ± 1.53	Significantly different ($p < 0.05$)
Luminal Space	18.05 ± 1.02	17.25 ± 1.87	No significant difference
Germinal Epithelium	56.45 ± 1.49	58.50 ± 1.44	No significant difference

*Differences between groups treated with ASA for different periods were not significant.

Table 12a: The mean number of random "hits" on different areas in a testicular cross-section after treatment with ASA for different periods

	Treatment with ASA		
	I ASA 300mg/kg body weight for 12 days	III ASA 150mg/kg body weight for 12 days	Significance of difference between the corresponding control groups
Interstitial Material	6.84 ± .55	5.45 ± .61	No significant difference
Intertubular Space	18.15 ± 1.73	20.05 ± 1.29	Significantly different ($p < 0.05$)
Luminal Space	22.70 ± 1.70*	18.05 ± 1.02	No significant difference
Germinal Epithelium	53.15 ± 1.32	56.45 ± 1.49	Significantly different ($p < 0.05$)

*Significantly greater ($p < 0.05$)

Table 12b: The mean number of random "hits" on different areas in a testicular cross-section after treatment with ASA for different periods*

	Treatment with ASA		
	V ASA 300mg/kg body weight for 6 days	VII ASA 150mg/kg body weight for 6 days	Significance of difference between the corresponding control groups
Interstitial Material	6.05 ± .81	5.70 ± .78	No significant difference
Intertubular Space	21.35 ± .88	18.50 ± 1.53	No significant difference
Luminal Space	14.90 ± 1.57	17.25 ± 1.87	No significant difference
Germinal Epithelium	57.95 ± 1.23	58.50 ± 1.44	No significant difference

*Differences between groups treated with ASA at different dosages were not significant.

Table 13: Sources of variation among treatment schedules and spatial relationships

Sources of variation	Level of significance	Required F Value
1. Interstitial Material		
Experimental F = 0.792	Not significant	F > 2.76 @ 0.05
Control F = 0.010	Not significant	F > 2.76 @ 0.05
2. Intertubular Space		
Experimental F = 0.933	Not significant	F > 2.76 @ 0.05
Control F = 5.155	1%	F > 4.13 @ 0.01
3. Luminal Space		
Experimental F = 4.327	1%	F > 4.13 @ 0.01
Control F = 0.604	Not significant	F > 2.76 @ 0.05
4. Germinal Epithelium		
Experimental F = 3.055	5%	F > 2.76 @ 0.05
Control F = 2.197	Not significant	F > 2.76 @ 0.05

Fig. 9 (Upper): Mean number of random "hits" on the interstitial tissue of testes from animals treated with acetylsalicylic acid at different dose levels for 12 or six days.

Fig. 10 (Lower): Mean number of random "hits" on the intertubular space of testes from animals treated with acetylsalicylic acid at different dose levels for 12 or six days.

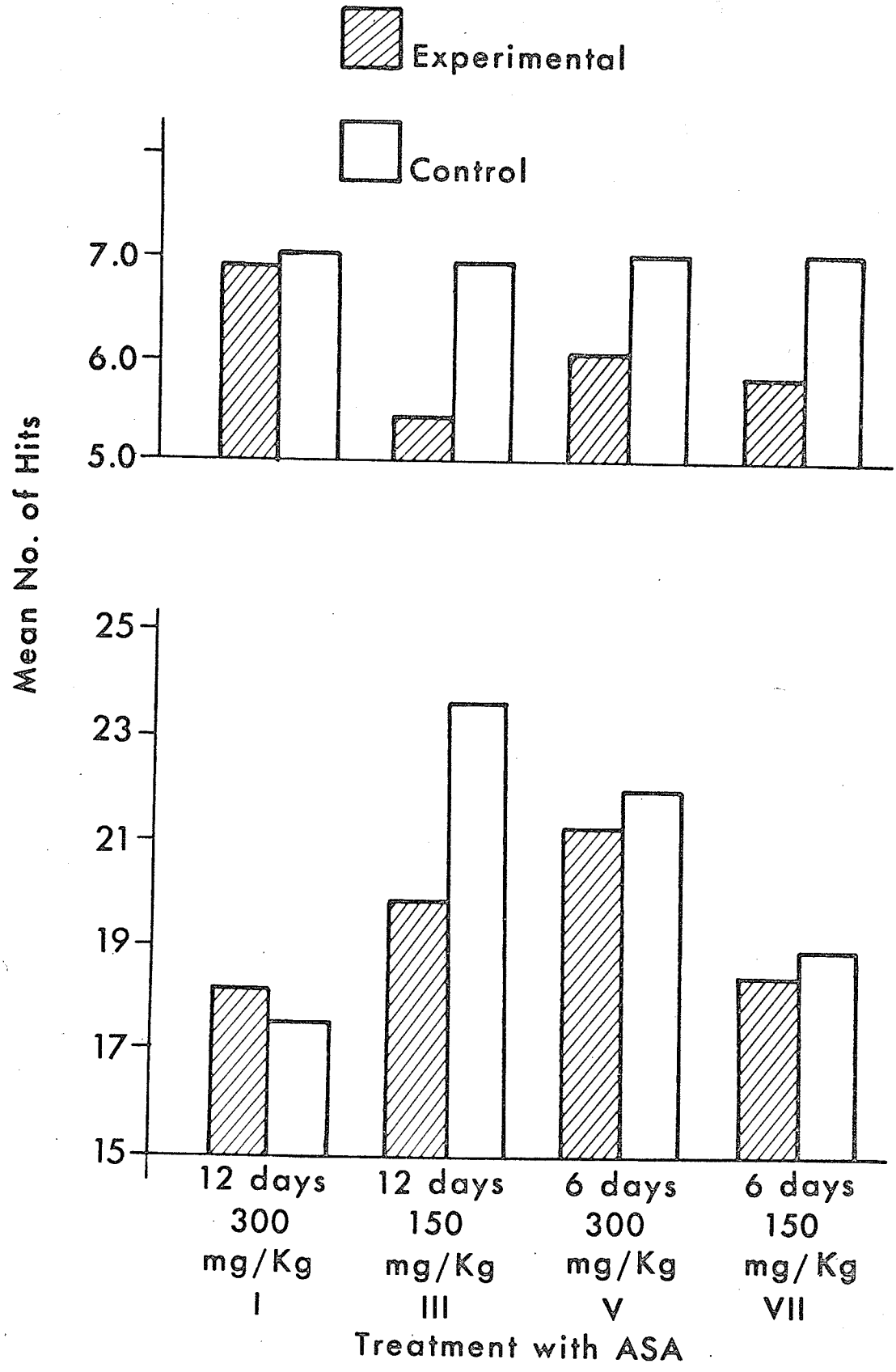
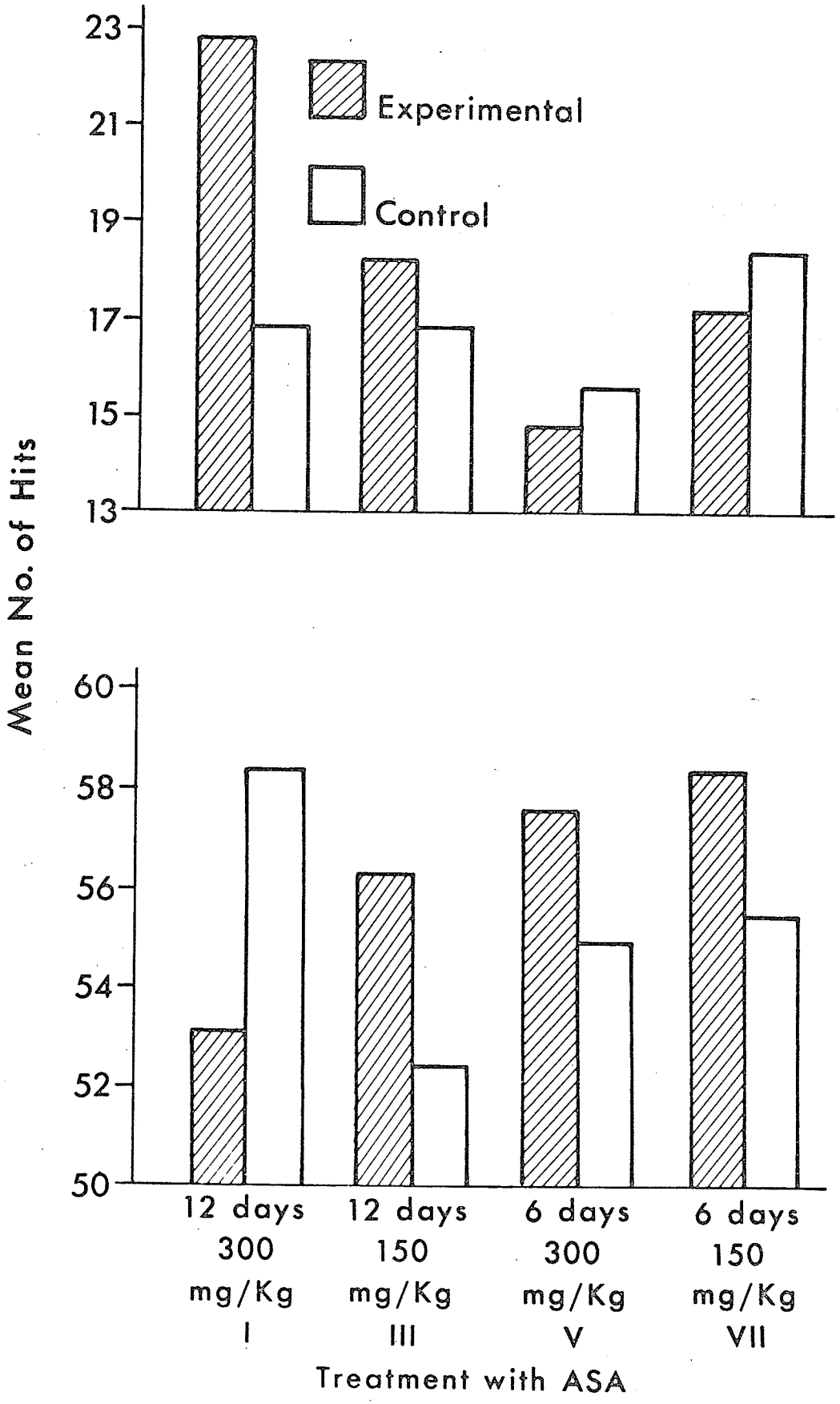

Experimental
Control
Mean No. of Hits
7.0
6.0
5.0
25
23
21
19
17
15
12 days 300 mg/Kg I
12 days 150 mg/Kg III
6 days 300 mg/Kg V
6 days 150 mg/Kg VII
Treatment with ASA

Fig. 11 (Upper): Mean number of random "hits" on the luminal space of testes from animals treated with acetylsalicylic acid at different dose levels for 12 or six days.

Fig. 12 (Lower): Mean number of random "hits" on the germinal epithelium of testes from animals treated with acetylsalicylic acid at different dose levels for 12 or six days.


23
21
19
17
15
13
Experimental
Control
60
58
56
54
52
50
Mean No. of Hits
12 days 300 mg/Kg I
12 days 150 mg/Kg III
6 days 300 mg/Kg V
6 days 150 mg/Kg VII
Treatment with ASA

4.1.1.4 Quantitative Analysis of the Germinal Epithelium

The mean numbers of type A spermatogonia, preleptotene spermatocytes, pachytene spermatocytes and spermatids per cross-section of stage VII seminiferous tubules are presented in Tables 14a and b and Figs. 13-16.

The mean numbers of A spermatogonia per tubular cross-section did not differ significantly between the animals treated with ASA and the animals treated with the solvent (Tables 14a and b). The mean numbers of A spermatogonia per cross-section of seminiferous tubules decreased in animals that were treated with ASA at a dose level of 300mg/kg body weight compared to the control values (Fig. 10); however, the mean numbers of type A spermatogonia were greater in animals receiving ASA at a dose level of 150mg/kg body weight (Fig. 10).

The mean numbers of A spermatogonia per tubular cross-section approximated each other in animals that were treated with ASA for a period of 12 days at dose levels of 300mg/kg body weight and 150mg/kg body weight and a period of six days at a dose of 150mg/kg body weight (Fig. 10); however, the differences were not statistically significant (Tables 15b and 16a). When the animals were treated for a period of six days, the mean number of A spermatogonia per tubular cross-section was significantly greater ($p < 0.05$) when ASA was given at a dose of 300mg/kg body weight than at a dose of 150mg/kg (Table 16b); however, the mean numbers of A spermatogonia of the control groups differed significantly ($p < 0.01$).

Treatment of animals with ASA for a period of 12 days produced a decrease in the mean numbers of preleptotene spermatocytes per cross-section of seminiferous tubules at stage VII, compared to the controls (Table 14a and Fig. 11); the difference was only significant ($p < 0.01$) in those animals treated with ASA at a dose level of 150mg/kg body weight. In contrast, animals treated with ASA for a period of six days showed an increased in the mean numbers of preleptotene spermatocytes per cross-section when compared to the control animals (Table 14b and Fig. 11); the difference was significant ($p < 0.01$) in animals treated at a dose level of 150mg/kg body weight.

The mean numbers of preleptotene spermatocytes per tubular cross-section approximated each other when the animals were treated with ASA for periods of 12 days (300mg/kg body weight) and six days (300mg/kg body weight and 150mg/kg body weight) (Fig. 11). The results of these treatments did not differ significantly (Tables 15a and 16b). Treatment of animals with ASA at a dose level of 150mg/kg body weight produced a significant increase ($p < 0.01$) in the mean number of preleptotene spermatocytes over a period of six days compared to animals treated for 12 days (Table 15b). The mean number of preleptotene spermatocytes per tubular cross-section in corresponding control groups were significantly decreased ($p < 0.01$) when the control animals were treated for a period of six days (Table 15b and Fig. 11). A significant increase in the mean number of preleptotene spermatocytes was found in animals that were treated with ASA over a period of 12 days at a dose level of 300mg/kg body weight (Table 16a).

The mean numbers of pachytene spermatocytes per tubular cross-section were decreased in animals that were treated with ASA for periods of 12 days (300mg/kg body weight and 150mg/kg body weight) and six days at a dose of 300mg/kg body weight compared to control values (Fig. 12); the differences were statistically significant ($p < 0.01$) only in the group treated for a period of 12 days at a dose of 150mg/kg body weight (Table 14). When the animals were treated with ASA for a period of six days at a dose of 150mg/kg body weight, the mean number of pachytene spermatocytes per tubule was increased (Fig. 12).

Comparing animals treated with ASA for different periods at dose levels of 300mg/kg body weight (Table 15a) and 150mg/kg body weight (Table 15b) revealed a significant increase ($p < 0.01$) in the mean number of pachytene spermatocytes per tubule after treatment for six days compared to the 12 day treatment period; however, the mean numbers of pachytene spermatocytes per tubule differed significantly ($p < 0.01$) between the control groups corresponding to the animals treated with ASA at a dose level of 300mg/kg body weight (Table 15a). In the groups of animals treated with ASA at different dose levels for periods of 12 days (Table 16a) or for six days (Table 16b), the mean number of pachytene spermatocytes per tubule was significantly increased ($p < 0.01$) only after treatment for a 12 day period at a dose of 300mg/kg body weight.

Treatment of the animals with ASA at a dose of 300mg/kg body weight for either six or 12 days did not significantly affect the mean

numbers of spermatids per tubule compared to control animals. When the animals were treated with ASA at a dose of 150mg/kg body weight the mean numbers of spermatids per tubule was significantly increased ($p < 0.01$) after treatment for six days; treatment at the same dose level for a period of 12 days produced a significant decrease ($p < 0.01$) in the mean number of spermatids per tubule (Table 14 and Fig. 13).

The sources of variation between the mean numbers of the different types of cells and treatment schedule are summarized in Table 17. The mean numbers of preleptotene and pachytene spermatocytes and spermatids tended to differ between the groups at a significant level ($p < 0.01$) after treatment with ASA (experimental) or the solvent. The mean numbers of A spermatogonia did not differ significantly between the groups that received ASA for different periods and dosages; groups that received the solvent, however, differed significantly ($p < 0.01$).

Table 14a: Mean numbers of spermatogonia preleptotene and pachytene spermatocytes and spermatids after treatment with ASA*

	I ASA 300 mg/kg body weight for 12 days	Control	III ASA 150 mg/kg body weight for 12 days	Control
A Spermatogonia	0.822 ± .096	0.956 ± .118	0.667 ± .137	0.635 ± .154
Preleptotene Spermatocytes	32.15 ± .96	34.85 ± 1.11	25.19 ± .83**	36.29 ± 1.78
Pachytene Spermatocytes	29.10 ± .82	30.56 ± .67	24.56 ± .81**	33.20 ± 1.35
Spermatids	109.11 ± 2.61	114.54 ± 3.63	95.92 ± 3.92**	130.1 ± 5.11

*Mean numbers of cells corrected according to Abercrombie's formula (1946) and expressed per 10 Sertoli cells
**Significantly less ($p < 0.01$) than control values.

Table 14b: Mean numbers of spermatogonia preleptotene and pachytene spermatocytes and spermatids after treatment with ASA*

	V ASA 300mg/kg body weight for 6 days	Control	VII ASA 150 mg/kg body weight for 6 days	Control
A Spermatogonia	1.233 ± .151	1.255 ± .261	0.717 ± .234	0.370 ± .103
Preleptotene Spermatocytes	34.03 ± 1.28	33.87 ± 1.48	32.64 ± 1.36**	24.88 ± 1.26
Pachytene Spermatocytes	34.34 ± 1.51	35.64 ± 1.09	31.75 ± .96	30.14 ± 1.27
Spermatids	108.15 ± 4.08	115.83 ± 2.65	131.26 ± 5.37**	97.80 ± 2.51

*Mean numbers of cells corrected according to Abercrombie's formula (1946) and expressed per 10 Sertoli cells.
**Significantly greater ($p < 0.01$) than control values.

Table 15 : Mean numbers of spermatogonia preleptotene and pachytene spermatocytes and spermatids in rats treated with ASA for different periods*

	Treatment with ASA		
	I mg/kg body weight for 12 days	V mg/kg body weight for 6 days	Significance of difference between the corresponding control groups
A Spermatogonia	0.822 ± .096	1.233 ± .151***	No significant difference
Preleptotene Spermatocytes	32.15 ± .96	34.03 ± 1.28	No significant difference
Pachytene Spermatocytes	29.10 ± .82	34.34 ± 1.51**	Significantly different ($p < 0.01$)
Spermatids	109.11 ± 2.61	108.15 ± 4.08	No significant difference

*Mean numbers of cells corrected according to Abercrombie's formula (1946) and expressed per 10 sertoli cells.
**Significantly greater ($p < 0.01$).
***Significantly greater ($p < 0.05$).

Table 15b: Mean numbers of spermatogonia preleptotene and pachytene spermatocytes and spermatids in rats treated with ASA for different periods*

	Treatment with ASA		
	III 150 mg/kg body weight for 12 days	VII 150 mg/kg body weight for 6 days	Significance of difference between the corresponding control groups
A Spermatogonia	0.667 ± .137	0.717 ± .234	No significant difference
Preleptotene Spermatocytes	25.19 ± .83	32.64 ± 1.36**	Significantly different ($p < 0.01$)
Pachytene Spermatocytes	24.56 ± .81	31.75 ± .96**	No significant difference
Spermatids	95.92 ± 3.92	131.26 ± 5.37**	Significantly different ($p < 0.01$)

*Mean numbers of cells corrected according to Abercrombie's formula (1946) and expressed per 10 sertoli cells.
**Significantly greater ($p < 0.01$).

Table 16a: Mean numbers of spermatogonia preleptotene and pachytene spermatocytes and spermatids in rats treated with ASA at different doses*

	Treatment with ASA		
	I 300 mg/kg body weight for 12 days	III 150 mg/kg body weight for 12 days	Significance of difference between the corresponding control groups
A Spermatogonia	0.822 ± .096	0.667 ± .137	No significant difference
Preleptotene Spermatocytes	32.15 ± .96**	25.19 ± .83	No significant difference
Pachytene Spermatocytes	29.10 ± .82**	24.56 ± .81	No significant difference
Spermatids	109.11 ± 2.61***	95.92 ± 3.92	Significantly different ($p < 0.01$)

*Mean numbers of cells corrected according to Abercrombie's formula (1946) and expressed per 10 Sertoli cells.
**Significantly greater ($p < 0.01$).
***Significantly greater ($p < 0.05$).

Table 16b: Mean numbers of spermatogonia preleptotene and pachytene spermatocytes and spermatids in rats treated with ASA at different doses*

	Treatment with ASA		
	V 300 mg/kg body weight for 6 days	VII 150 mg/kg body weight for 6 days	Significance of difference between the corresponding control groups
A Spermatogonia	1.233 ± .151***	0.717 ± .234	Significantly different ($p < 0.01$)
Preleptotene Spermatocytes	34.03 ± 1.28	32.64 ± 1.36	Significantly different ($p < 0.01$)
Pachytene Spermatocytes	34.34 ± 1.51	31.75 ± .96	Significantly different ($p < 0.01$)
Spermatids	108.15 ± 4.08	131.26 ± 5.37**	Significantly different ($p < 0.05$)

*Mean numbers of cells corrected according to Abercrombie's formula (1946) and expressed per 10 Sertoli cells.
**Significantly greater ($p < 0.01$).
***Significantly greater ($p < 0.05$).

Table 17: Sources of variation among treatment schedules and cell counts

Source of variation	Level of significance	Required F Value
1. Type A Spermatogonia		
Experimental F = 2.571	Not significant	F > 2.68 @ 0.05
Control F = 5.495	1%	F > 4.13 @ 0.01
2. Preleptotene Spermatocytes		
Experimental F = 13.157	1%	F > 4.13 @ 0.01
Control F = 12.797	1%	F > 4.13 @ 0.01
3. Pachytene Spermatocytes		
Experimental F = 16.113	1%	F > 4.13 @ 0.01
Control F = 6.503	1%	F > 4.13 @ 0.01
4. Spermatids		
Experimental F = 13.089	1%	F > 4.13 @ 0.01
Control F = 10.419	1%	F > 4.13 @ 0.01

Fig. 13 (Upper): Mean number of type A spermatogonia* in stage VII seminiferous tubules in the testes of animals treated with acetylsalicylic acid at different dose levels for 12 or six days.

Fig. 14 (Lower): Mean number of preleptotene spermatocytes* in stage VII seminiferous tubules in the testes of animals treated with acetylsalicylic acid at different dose levels for 12 or six days.

*Numbers corrected according to Abercrombie's formula (1946) and expressed per 10 Sertoli cells.

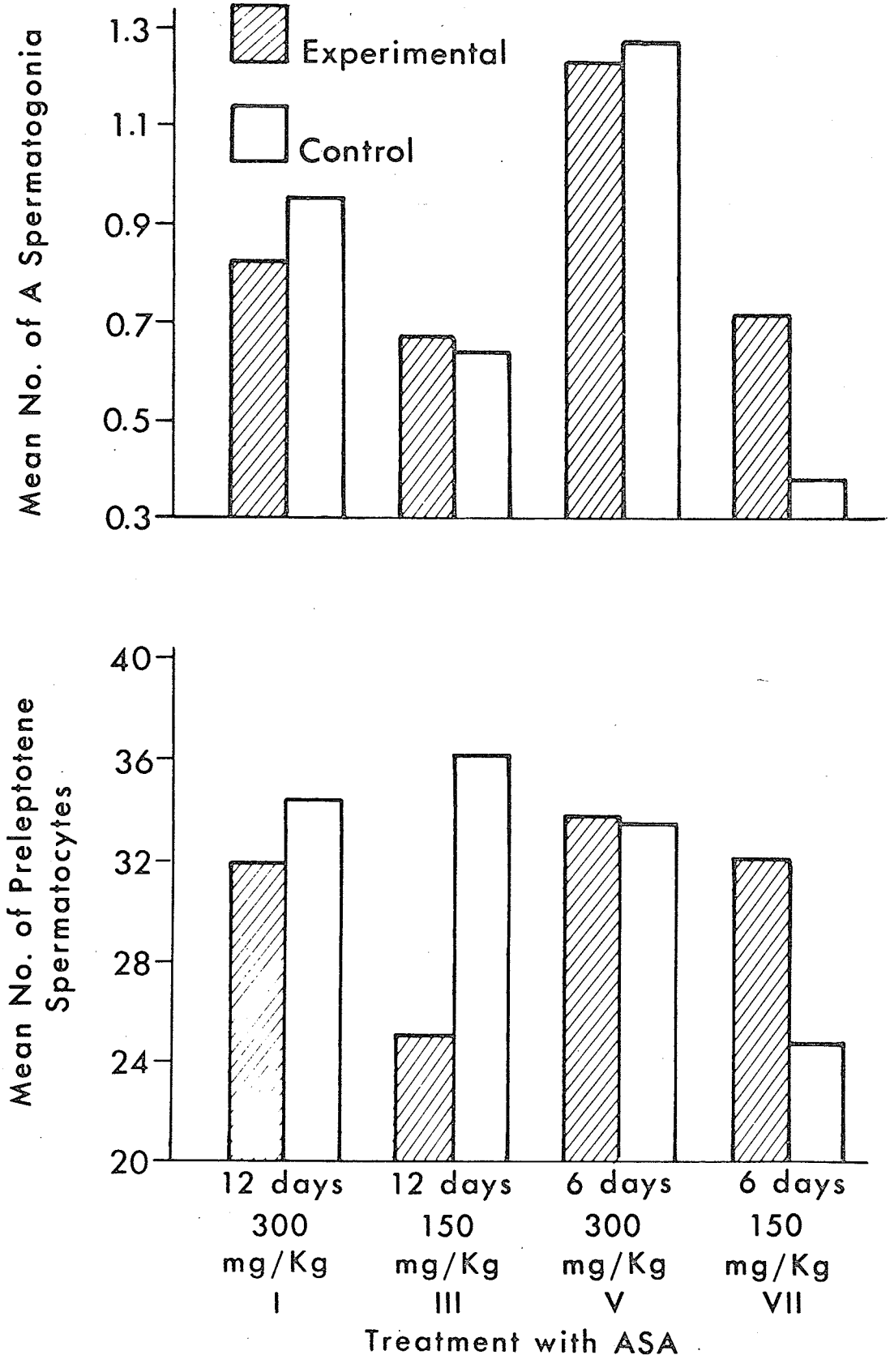
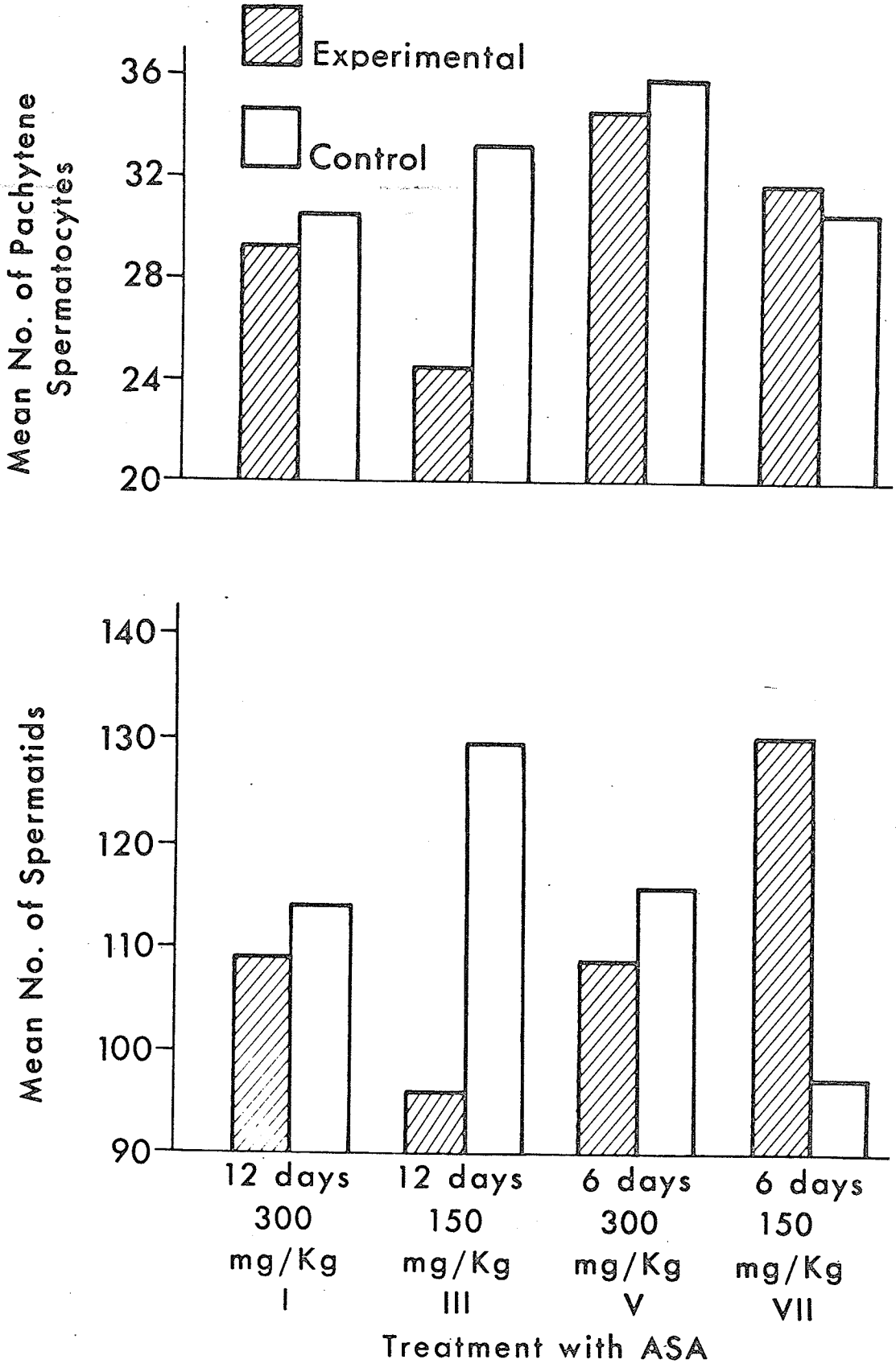

Mean No. of A Spermatogonia
1.3
1.1
0.9
0.7
0.5
0.3
Experimental
Control
Mean No. of Preleptotene Spermatocytes
40
36
32
28
24
20
12 days 300 mg/Kg I
12 days 150 mg/Kg III
6 days 300 mg/Kg V
6 days 150 mg/Kg VII
Treatment with ASA

Fig. 15 (Upper): Mean number of pachytene spermatocytes* in stage VII seminiferous tubules in the testes of animals treated with acetylsalicylic acid at different dose levels for 12 or six days.

Fig. 16 (Lower): Mean number of spermatids* in stage VII seminiferous tubules in the testes of animals treated with acetylsalicylic acid at different dose levels for 12 or six days.

*Numbers corrected according to Abercrombie's Formula (1946) and expressed per 10 Sertoli cells.


Experimental
Control
Mean No. of Pachytene Spermatocytes
36
32
28
24
20
Mean No. of Spermatids
140
130
120
110
100
90
12 days 300 mg/Kg I
12 days 150 mg/Kg III
6 days 300 mg/Kg V
6 days 150 mg/Kg VII
Treatment with ASA

4.1.1.5 Testosterone Assay

The mean testosterone levels in the serum of animals treated with either ASA or the solvent are given in Table 18. The mean levels of testosterone did not differ significantly between the ASA treated and the control groups of animals (Table 18). After treatment with ASA for a period of 12 days at a dose level of 300mg/kg body weight, the mean testosterone level was slightly decreased compared to either the controls or the normal testosterone level in the serum (Fig. 17).

A mixed analysis of variance revealed that the mean serum levels of testosterone did not differ in groups of animals that were treated with ASA or solvent (Table 19).

Table 18: Mean testosterone levels (ng %) in serum of rats after treatment with ASA*

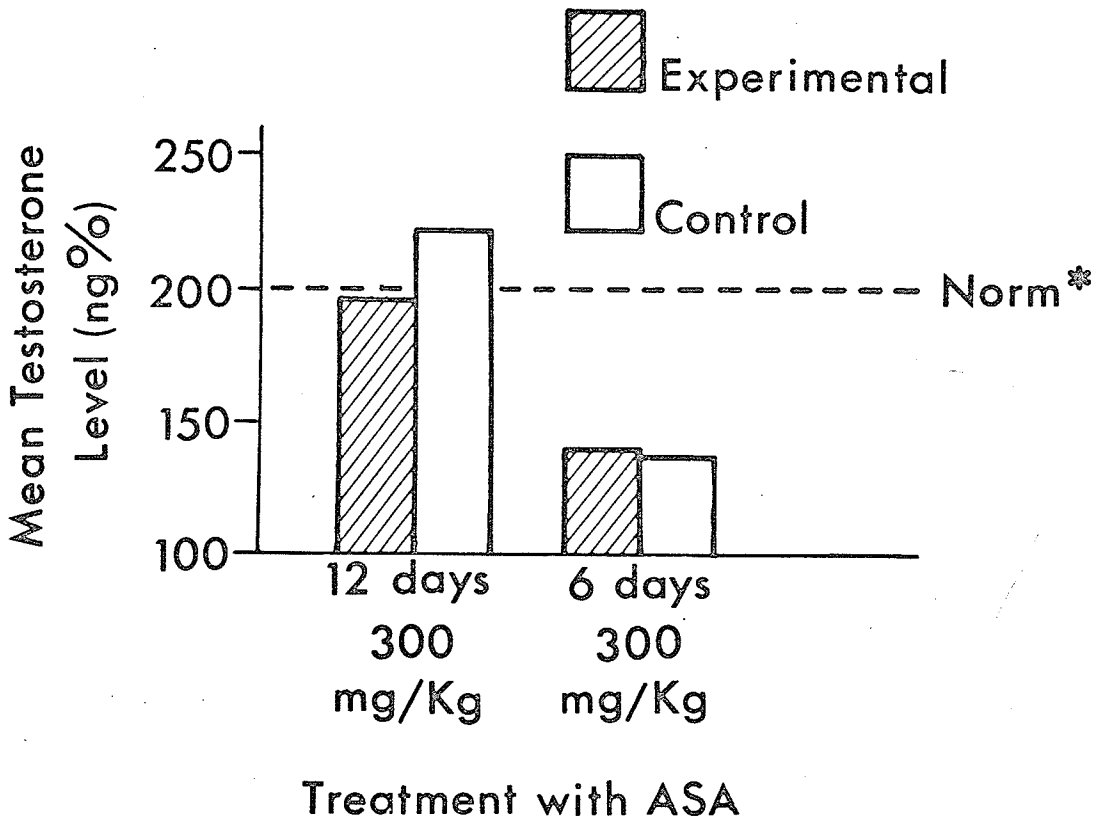
Treatment with ASA	No. of animals	Mean testosterone level (ng %)
300mg/kg body weight for 12 days	4	195.00 ± 34.10*
Control	4	223.75 ± 76.25
300mg/kg body weight for 6 days	4	137.50 ± 37.67*
Control	2	135.00 ± 25.00

*Differences between experimental and control values were not statistically significant.

Table 19: Sources of variation among treatment schedules and mean testosterone levels in the serum of rats

Source of variation	Level of significance	Required F Value
Serum		
Testosterone levels		
Experimental F = 1.281	Not significant	F > 5.59 @ 0.05
Control F = 0.592	Not significant	F > 6.61 @ 0.05

Fig. 17: Mean level of testosterone (ng %) in the serum of animals treated with acetylsalicylic acid at a dose level of 300mg/kg body weight for 12 or six days.



*DeJong et al (1973)

4.1.2 Morphological Observations

4.1.2.1 Testes

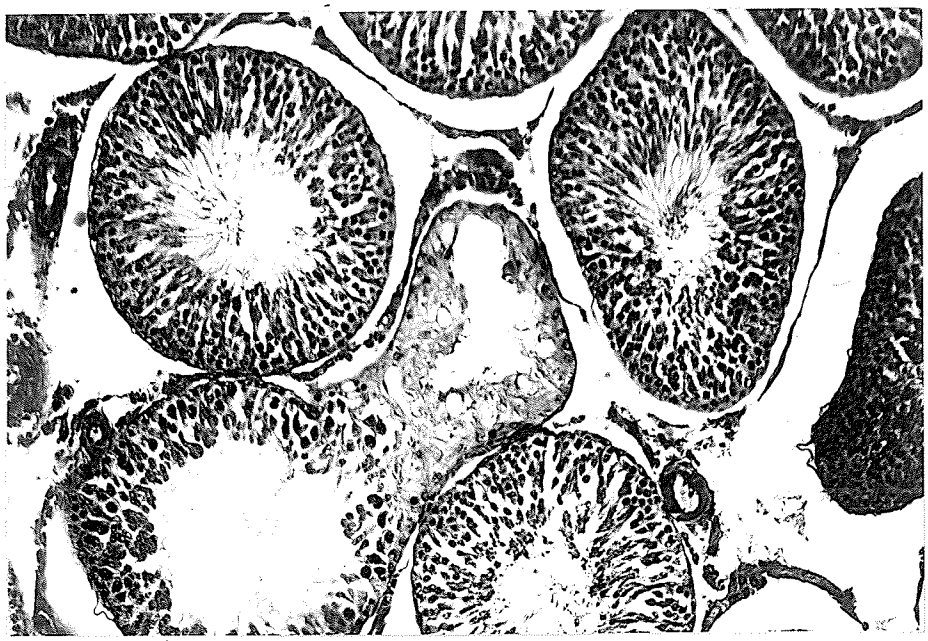
In animals that were treated with ASA at a dose of 300mg/kg body for a period of 12 days, no extensive lesions were observed in cross-sections of the testes compared to those of control animals. However, the testes of animals treated with ASA or the solvent occasionally exhibited focal degenerative areas in the seminiferous tubules (Fig. 18). The basal lamina around the tubules was separated from the basal cells of the germinal epithelium in many areas (Fig. 19), particularly in the center of the testes. In this area, the seminiferous tubules were separated from each other and were of a smaller diameter than those located in the periphery (Fig. 20). Deep to the tunica albuginea, the seminiferous tubules were packed closely together and separation of the basal lamina was minimal (Fig. 21). Neither the testes of the animals treated with ASA nor those treated with solvent showed any inflammatory infiltration, congestion of blood vessels or edema.

Treatment with ASA did not produce any visible alterations in the amount or distribution of interstitial tissues. The cells of Leydig were largely restricted to the triangular spaces formed by adjacent tubules (Fig. 22). Loose connective tissue surrounded the Leydig cells and adjacent blood vessels in the interstitium.

Examination of the germinal epithelium revealed no obvious changes after treatment with ASA for a period of 12 days at a dose of 300mg/kg body weight. Intercellular spaces were occasionally present between the primary spermatocytes and within the upper

Fig. 18 (Upper): A focal area of degeneration of the germinal epithelium in a seminiferous tubule of an animal treated with the solvent.
(130X)

Fig. 19 (Lower): Seminiferous tubules near the center of the testis of an animal treated with solvent. The basal lamina is separated from the tubules in several places (arrows).
(254X)



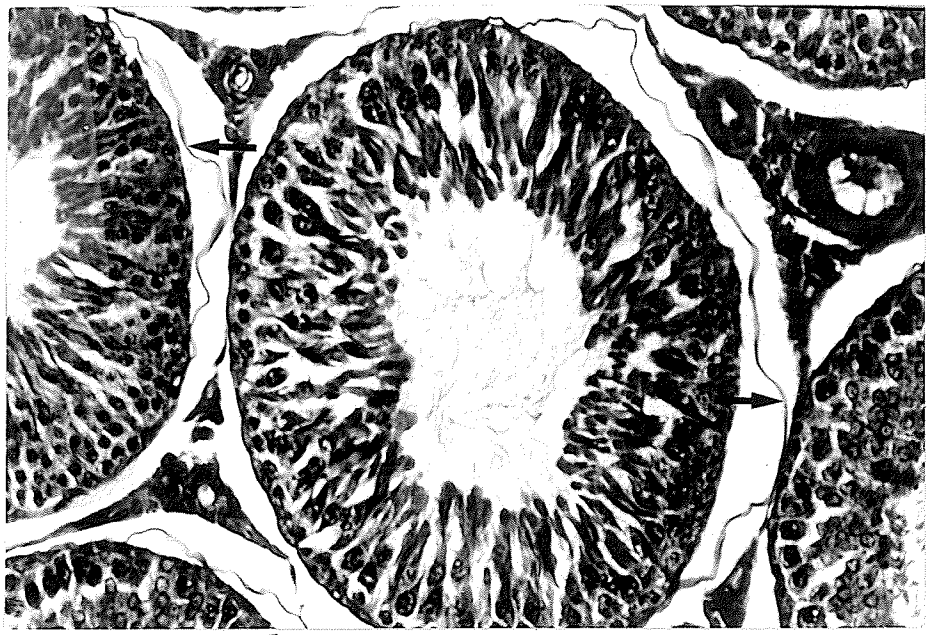
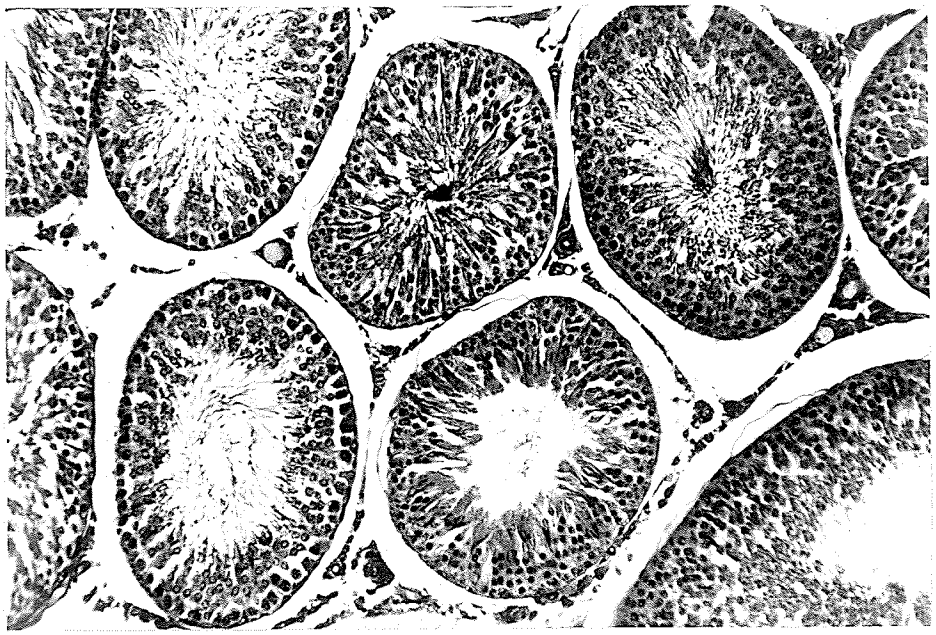


Fig. 20 (Upper): Seminiferous tubules near the center of the testis from an animal treated with the solvent. The tubules are separated from each other and from the interstitial tissue.
(130X)

Fig. 21 (Lower): Seminiferous tubules beneath the tunica albuginea (arrow) are closely packed; the basal laminae are not separated from the tubules.
(32.5X)



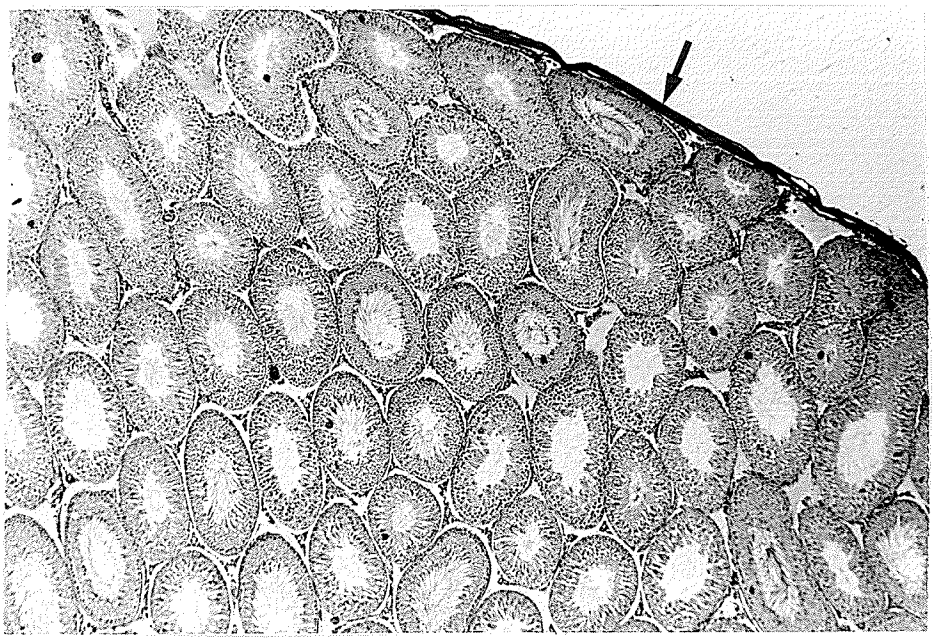
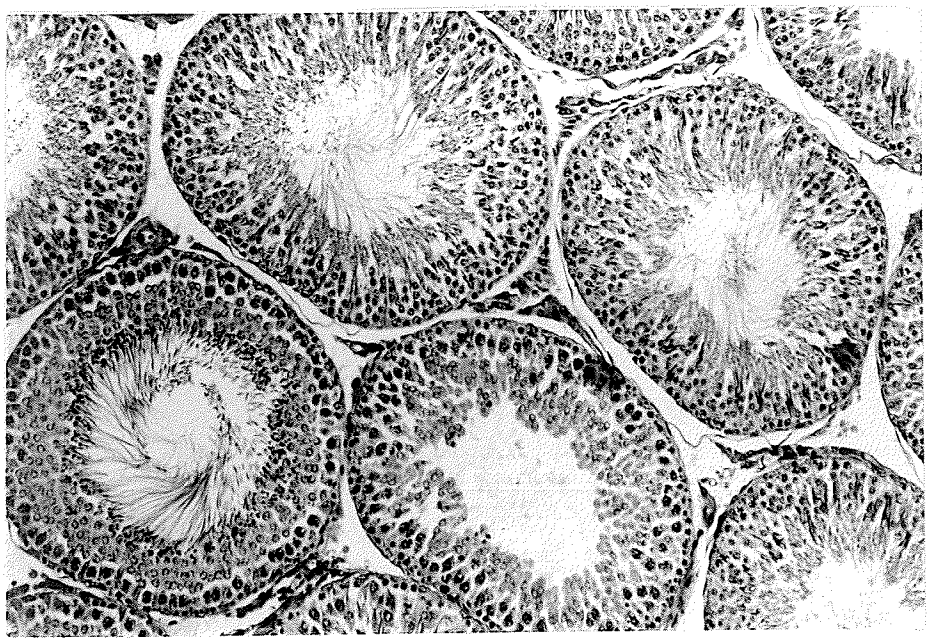
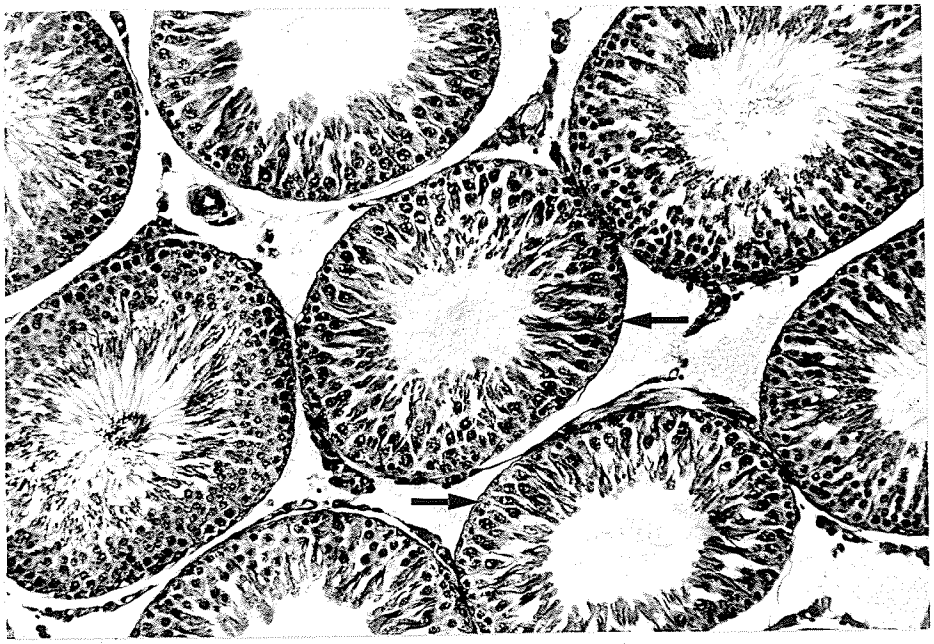


Fig. 22 (Upper): Testicular cross - section from an animal treated with acetylsalicylic acid for 12 days at a dose of 300mg/kg body weight. The distribution of interstitial tissue was not altered by the drug.
(130X)

Fig. 23 (Lower): Seminiferous tubules in stages XII - XIII (arrows) from an animal treated with acetylsalicylic acid for 12 days at a dose of 300mg/kg body weight. Some intra - epithelial spaces are present in the germinal lining.
(130X)





layer of spermatids in the germinal epithelium of stage XII - XIII tubules (Fig. 23). Necrotic nuclei were infrequently associated with the larger spaces. Similarly, some intercellular spaces were observed in the germinal epithelium of stage VII tubules of animals after treatment with the solvent or ASA for a period of 12 days at a dose of 300mg/kg body weight (Fig. 24). Karyolytic nuclei, probably of type A spermatogonia (Clermont, 1962), were frequently present along the luminal border of stage IX tubules. Immature cells were seldom present in the lumina of the seminiferous tubules.

Treatment of the animals with ASA for a period of 12 days at a dose of 150mg/kg body weight produced observable changes in the epithelium of the seminiferous tubules. Intercellular spaces were prominent in the germinal epithelium of most tubular cross-sections causing the affected areas to stain less intensely (Fig.25); smaller similar areas were infrequently detected in control cross-sections (Fig.26). In the tubules of the animals treated with ASA, necrotic cells probably of type A spermatogonia, zygotene or diplotene spermatocytes were associated with the intra-epithelial spaces present in the tubules of stages XII and XIII(Fig. 27). Necrotic cells were also frequently present along the basal lamina of stage IX tubules (Fig. 28); fewer in number were concentrated along the luminal margin of tubules from animals treated with the solvent (Fig. 29). The distribution of the interstitial tissue was similar in the animals treated with ASA or the solvent; there was no evidence of inflammation, congestion, or edema.

Fig. 24: A stage VII tubule (arrow) from the testis of an animal treated with acetylsalicylic acid for 12 days at a dose of 300mg/kg body weight. Some intercellular spaces are present in the germinal epithelium. (130X)

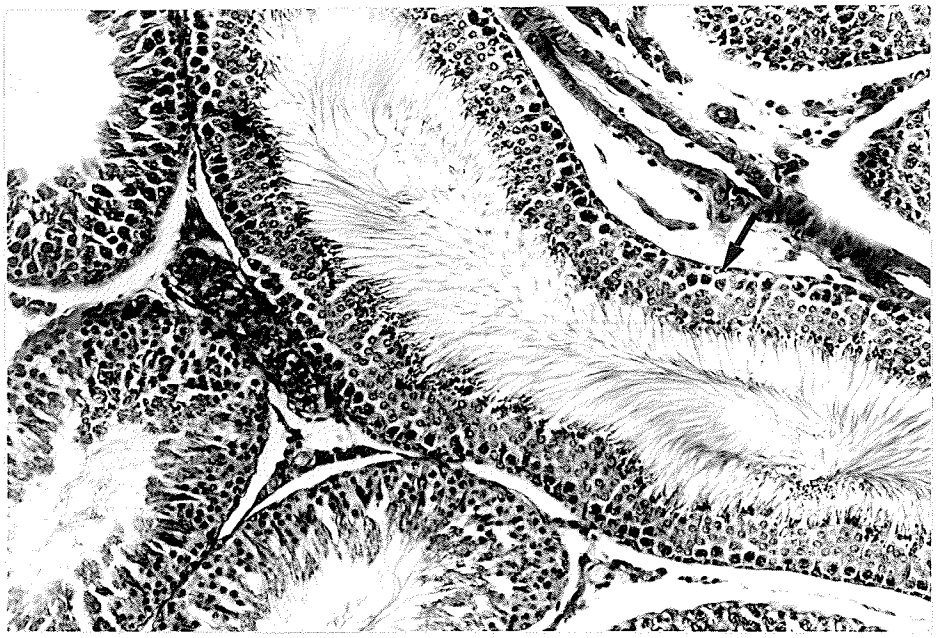
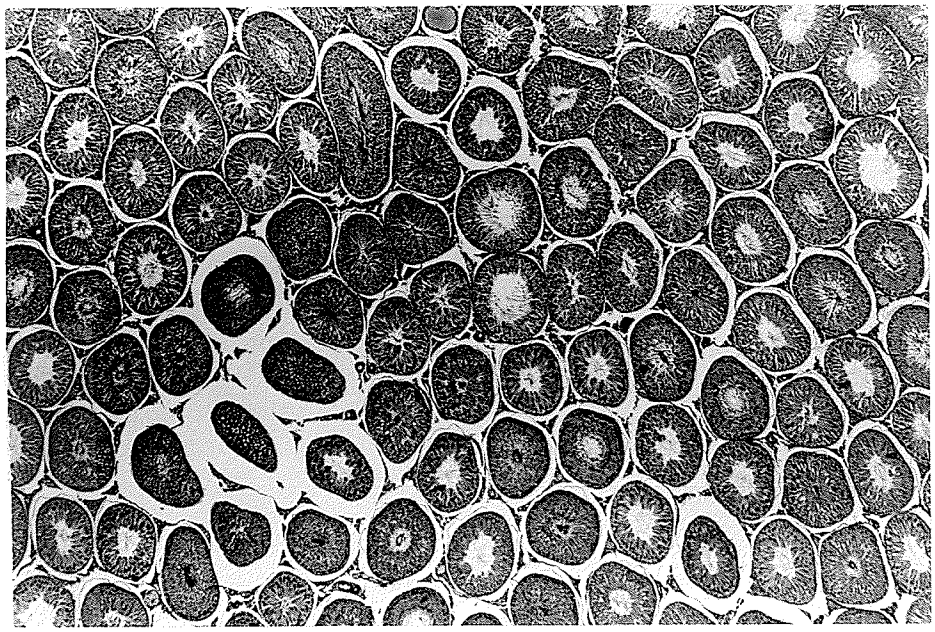


Fig. 25a (Upper): Typical testicular cross - section from an animal treated with the solvent. Intra - epithelial spaces are minimal.
(32.5X)

Fig. 25b (Lower): Testicular cross - section from an animal treated with acetylsalicylic acid at a dose of 150mg/kg body weight for 12 days. Extensive intercellular spaces caused the tissue to stain less intensely.
(32.5X)



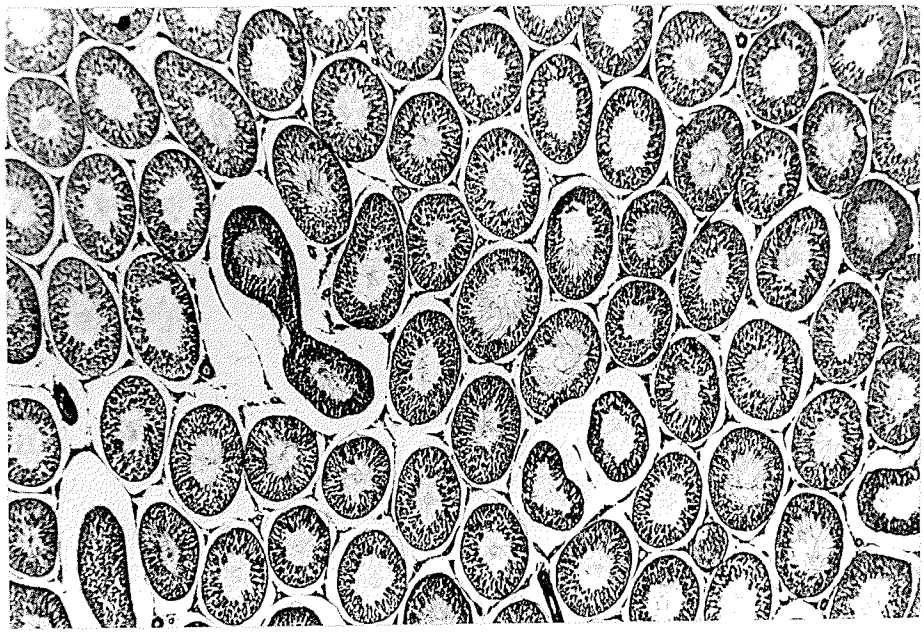
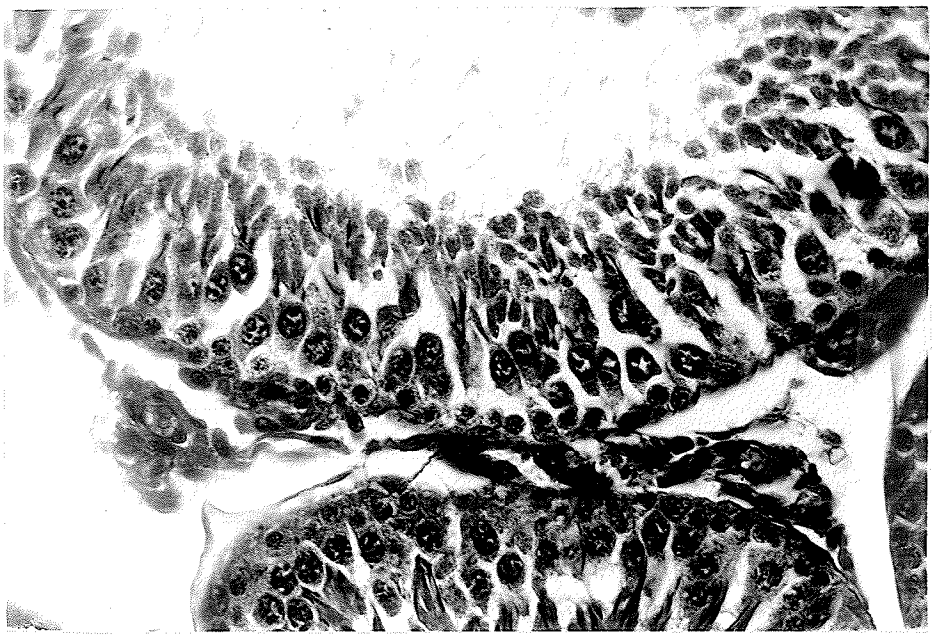


Fig. 26 (Upper): Testicular cross - section from an animal treated with solvent. Intercellular spaces are minimal.
(32.5X)

Fig. 27 (Lower): Seminiferous tubules at stages XII - XIII of an animal treated with acetylsalicylic acid at a dose of 150mg/kg body weight for 12 days. Some necrotic cells are present in the germinal epithelium.
(325X)



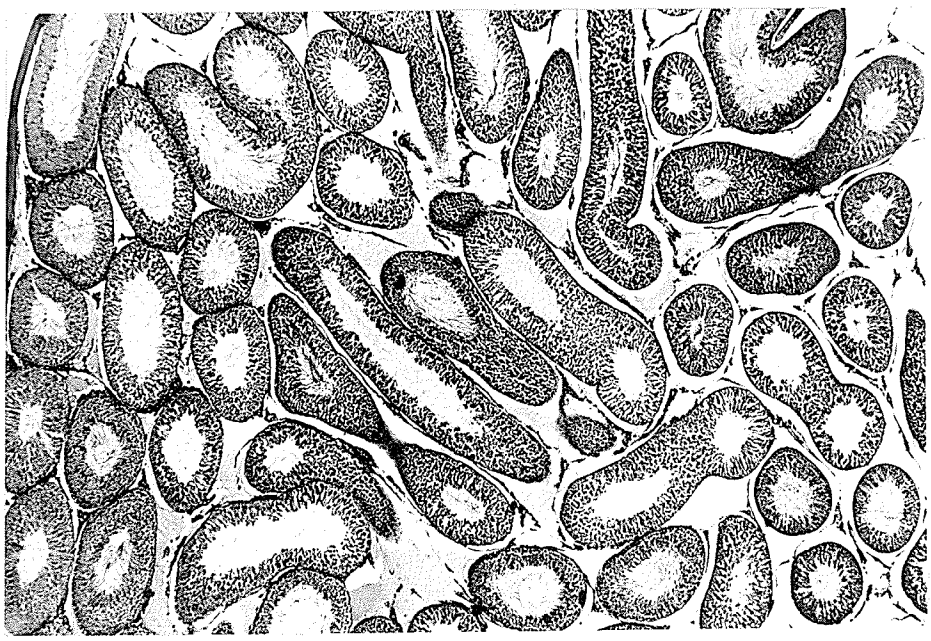
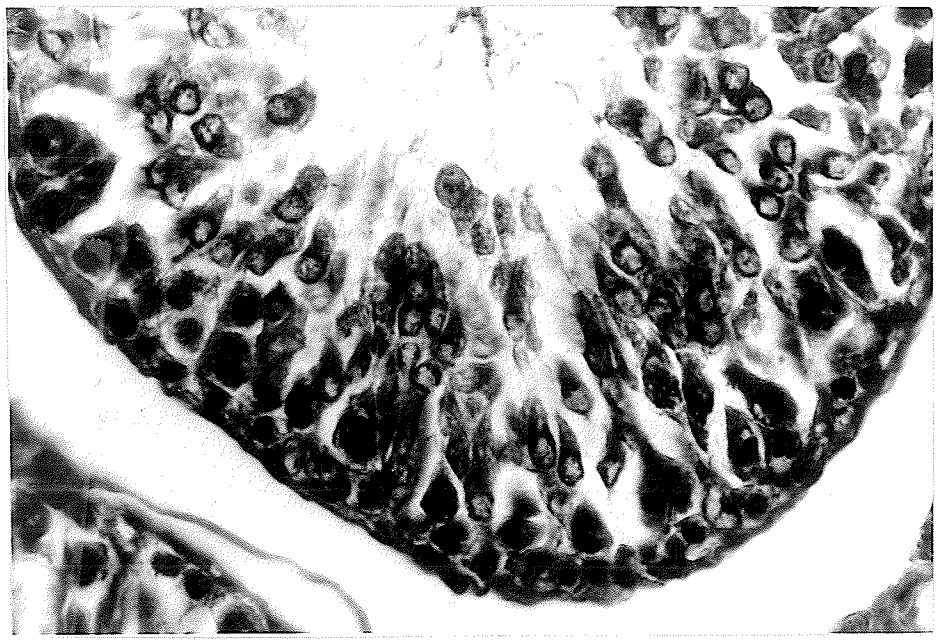


Fig. 28 (Upper): Seminiferous tubule in stage IX from an animal treated with acetylsalicylic acid at a dose of 150mg/kg body weight for 12 days. Necrotic cells with pyknotic nuclei are present near or along the basal lamina. Intercellular spaces are also present. (520X)

Fig. 29 (Lower): Stage IX seminiferous tubule from an animal treated with the solvent. Necrotic cells are concentrated around the lumen (arrow). (325X)



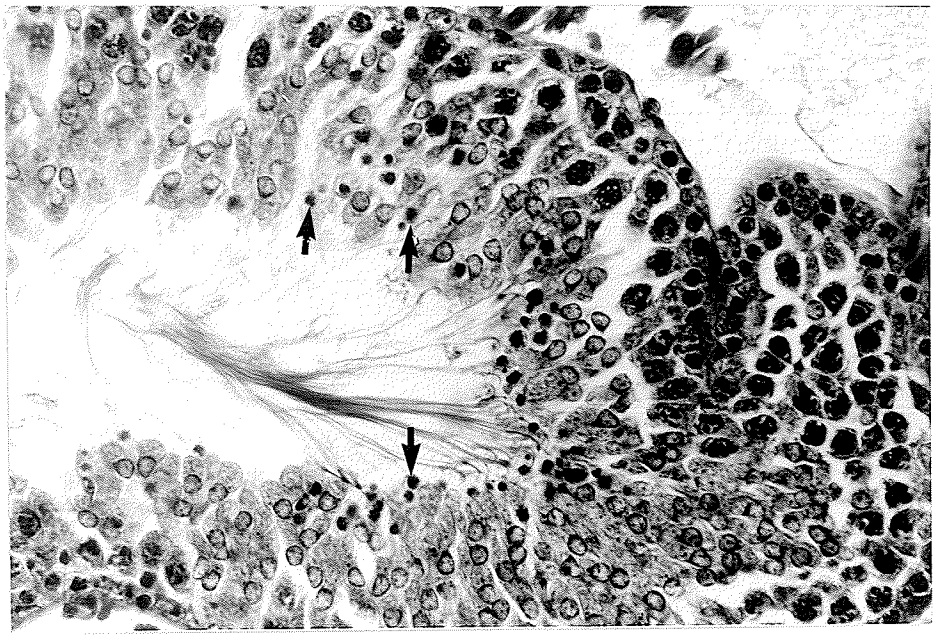
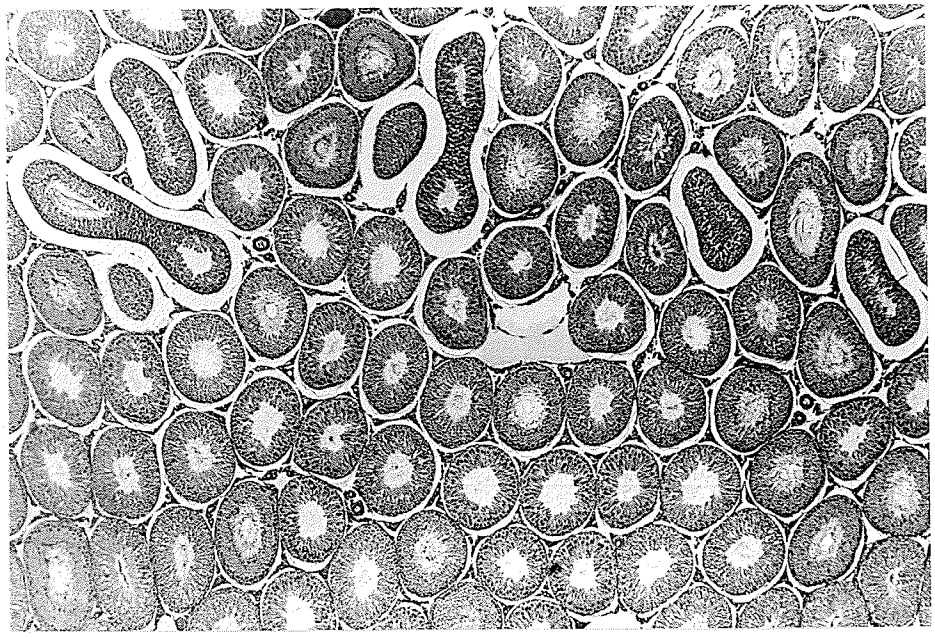


Fig. 30a (Upper): Testicular cross - section from an animal treated with solvent. Intercellular spaces are minimal.
(32.5X)

Fig. 30b (Lower): Testicular cross - section from an animal treated with acetylsalicylic acid at a dose of 300mg/kg body weight for six days. Extensive intra - epithelial spaces in the germinal epithelium caused the affected areas to stain less intensely.
(32.5X)



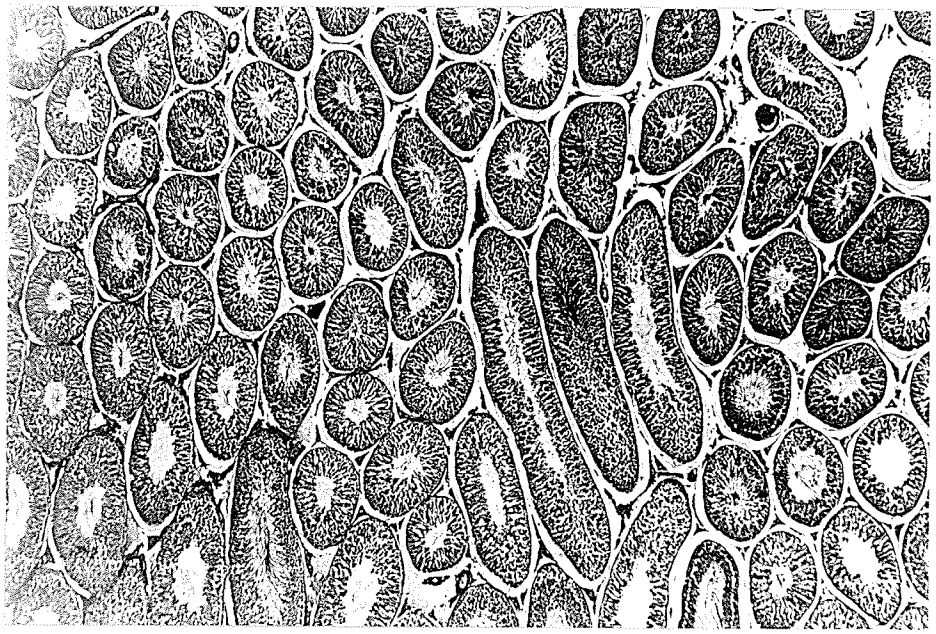
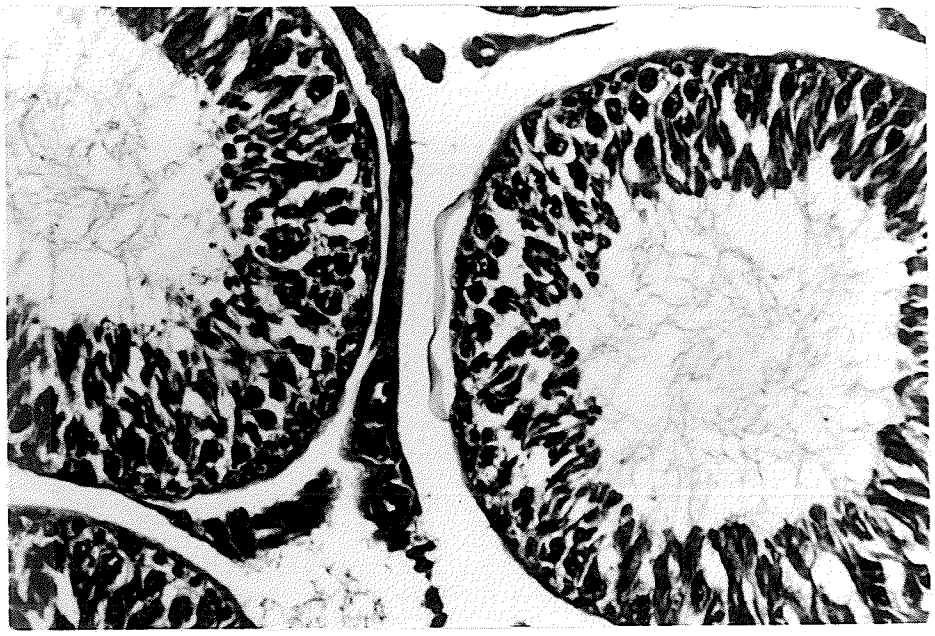


Fig. 31 (Upper): Seminiferous tubules in stages I - IIIof the cycle. The germinal epithelium is disrupted by intercellular spaces and numerous cells with pyknotic nuclei.
(254X)

Fig. 32 (Lower): Two tubular cross - sections at stage XIV (arrows). Intercellular spaces are present and in some areas extend to the basal lamina.
(130X)



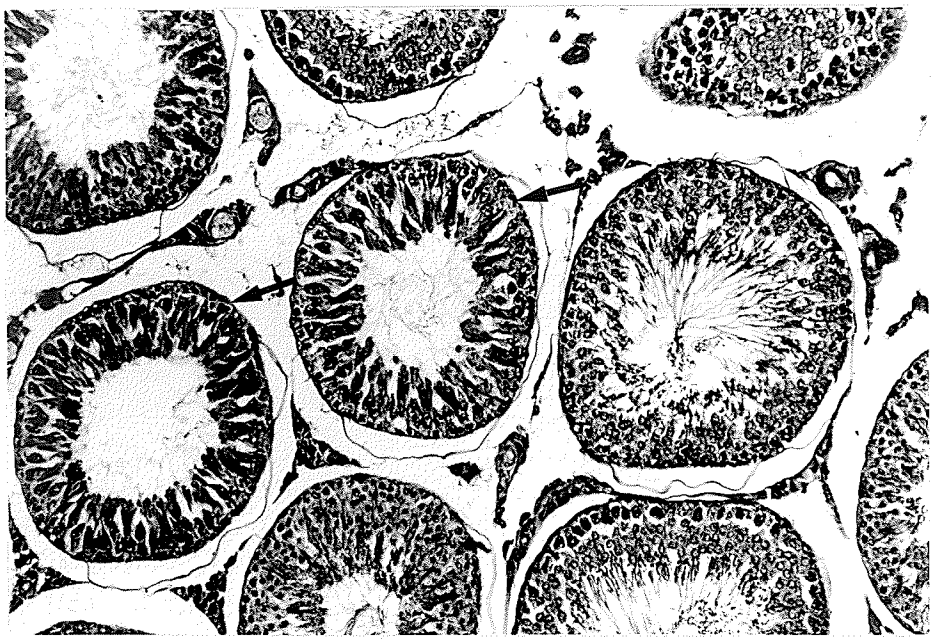
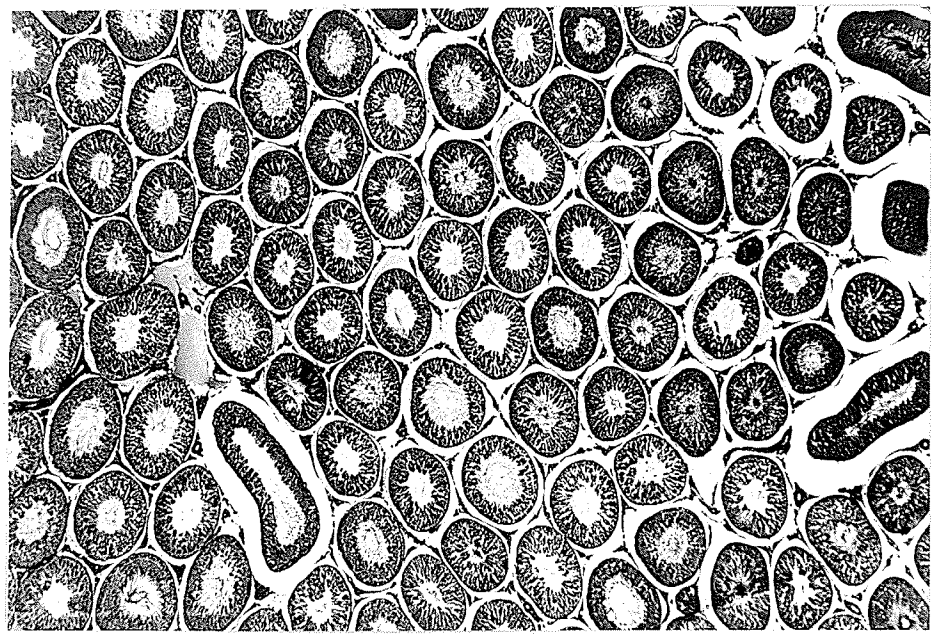


Fig. 33 (Upper): Stage IX seminiferous tubule from an animal treated with acetylsalicylic acid at a dose of 300mg/kg body weight for six days. Cells with pyknotic nuclei are present along the basal lamina (arrows).
(520X)

Fig. 34 (Lower): Testicular cross - section from an animal treated with acetylsalicylic acid for six days at a dose of 150mg/kg body weight. Intercellular spaces in the germinal epithelium are minimal.
(32.5X)



Obvious changes were present in the germinal epithelium of animals treated with ASA at the higher dose of 300mg/kg body weight for a period of six days. Extensive intercellular spaces were detected in the germinal epithelium causing it to stain less intensely than control cross-sections (Fig. 30). The germinal lining of tubules at stages I-III was disrupted and necrotic cells were frequently present (Fig. 31). Similar spaces were also evident in the germinal epithelium of stage XIV tubules and in some cases extended right up to the basal lamina (Fig. 32). Treatment with ASA at a dose of 300mg/kg body weight for six days produced extensive degenerative changes in the germinal lining of stage IX tubules; the necrotic cells were associated with the peripheral layers of the epithelium, and were also frequently present along the basal lamina of the tubules (Fig. 33). Intercellular spaces were minimal in the germinal epithelium of the seminiferous tubules of stage IX. No changes were evident in the amount or distribution of interstitial tissue; inflammation changes, congestion or edema were not detected.

Treatment of the animals with ASA at a dose of 150mg/kg body weight for a period of six days did not produce any extensive lesions. Intra-epithelial spaces in the seminiferous tubules of animals treated with ASA were restricted to small areas, similar to the controls (Fig. 34). The distribution of necrotic lesions did not differ in animals treated with ASA or the solvent.

Treatment of animals with ASA did not modify the staining properties of testicular tissue to periodic acid-Schiff's (PAS) or to Mallory's trichrome reagents. PAS activity was restricted to the interstitial tissues and to the basal lamina of the seminiferous tubules. Some activity was also associated with stage XIV tubules in the displaced cytoplasm of the spermatids. Mallory's trichrome revealed an intense blue reaction restricted to the basal lamina of the seminiferous tubules and the interstitial tissue.

In all groups of animals treated with ASA at different doses and for various periods, minimal degenerative changes were detected in stage VII tubules (where cell counts were done).

4.1.2.2 Epididymides

No changes were observed in the caput epididymidis of animals that were treated with ASA (Fig.35). Most of the tubules were packed with spermatozoa. The epithelial lining was continuous; stereocilia formed an uninterrupted border around the lumina. Intra-epithelial lymphocytes did not appear to be affected by treatment with ASA. PAS staining in the caput epididymidis was similar in animals treated with ASA or the solvent; the luminal margin of the lining cells, the basal lamina and the intervening connective tissue were reactive to PAS. In animals treated with ASA or the solvent, Mallory's trichrome stained the basal lamina, the stereocilia, and the stroma of the caput epididymidis intensely blue.

No evidence of necrotic cells, inflammation, edema or congestion was found in the caput epididymidis of animals that were treated with ASA.

The cauda epididymidis was not affected as a result of treatment of the animals with ASA. Large numbers of spermatozoa were present in the lumina of the epididymidis; there was no apparent difference in the number of spermatozoa present in the cauda epididymidis of animals treated with ASA or the solvent (Fig. 36). The lining epithelium formed a continuous membrane interrupted only by secretory cells. The mucosa appeared unaffected (Fig. 37).

PAS activity in the cauda epididymidis of animals treated with either ASA or the solvent was restricted to the luminal margin of the epithelial cells, the basal lamina, and the connective tissue between the tubules. Mallory's trichrome stained the stereocilia, basal lamina and connective tissue stroma blue in the region of the cauda epididimidis.

4.1.2.3 Ductus Deferens

No changes were present in the pseudostratified columnar epithelium of the ductus deferens in animals treated with ASA or the solvent (Fig. 38); the distal portion of the ductus deferens was packed with spermatozoa (Fig. 39). A degree of hypercellularity was found in the lamina propria of the ductus deferens in animals treated with ASA at a dose of 300mg/kg body weight for a period of 12 days; some control animals exhibited similar features (Fig. 40).

PAS activity was present along the upper border of the pseudostratified epithelium and the stereocilia in the ductus deferens of animals receiving the solvent. In animals treated with ASA for a period of 12 days at a dose of 300mg/kg body weight, PAS activity was minimal along the upper border of the cells; PAS activity in the other groups of animals treated with ASA was similar to that of control animals. The lamina propria of the ductus deferens reacted intensely to PAS in animals receiving ASA or the solvent; similarily, Mallory's trichrome demonstrated the presence of extensive connective tissue in the lamina propria and muscularis of the ductus deferens of animals treated with either ASA or solvent.

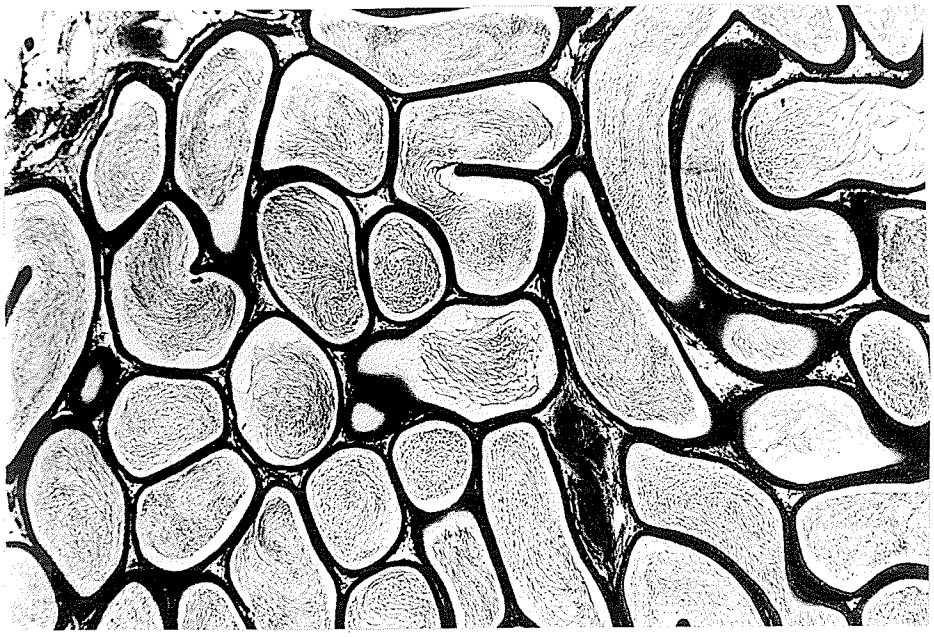
4.1.2.4 Seminal Vesicles

No lesions were detected in the seminal vesicles of animals treated with ASA (Fig. 41). The pseudostratified columnar epithelium was continuous along the inner surface of the glands. Secretory cells were frequent in the epithlium. There was no evidence of inflammation or edema.

The staining reaction of PAS and Mallory's trichrome in the seminal vesicles of animals treated with ASA or solvent was similar; the basal lamina and lamina propria were areas demonstrating maximal activity.

Fig. 35a (Upper): The caput epididymidis of an animal treated with the solvent.
(32.5X)

Fig. 35b (Lower): The caput epididymidis from an animal treated with acetylsalicylic acid. The tubules are packed with spermatozoa.
(32.5X)



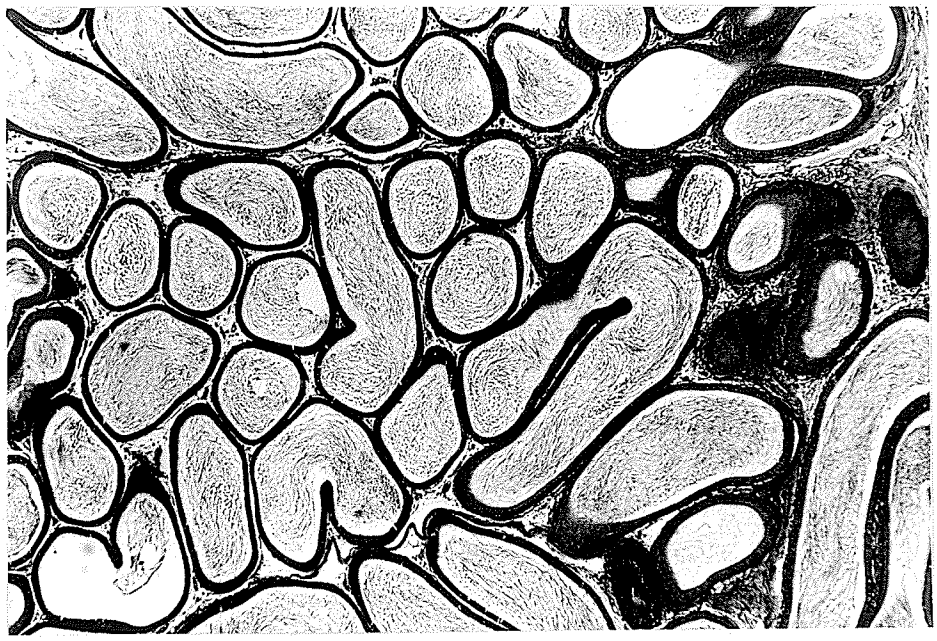
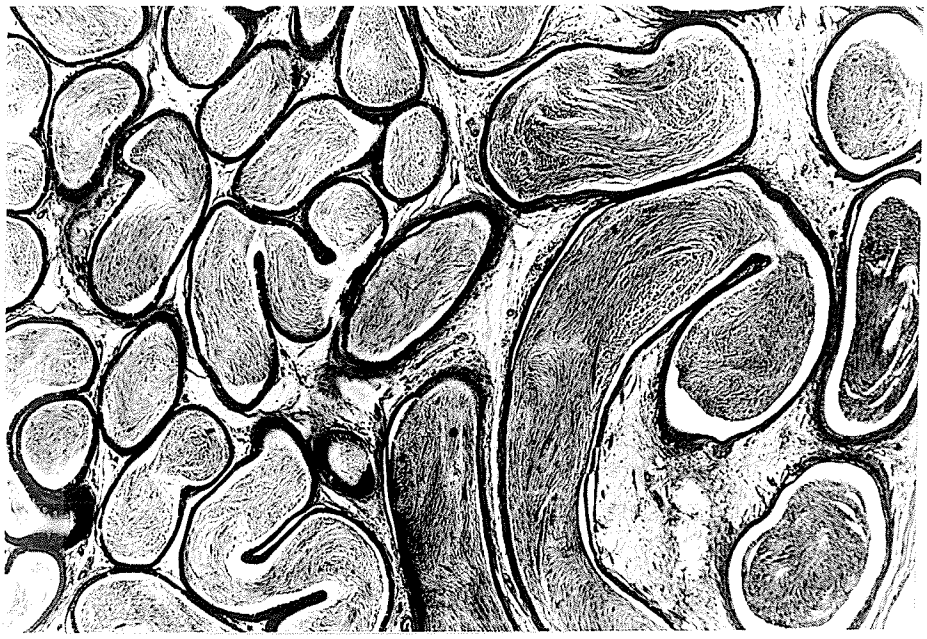


Fig. 36a (Upper): The cauda epididymidis of an animal treated with solvent.
(32.5X)

Fig. 36b (Lower): The cauda epididymidis of an animal treated with acetylsalicylic acid. No morphological changes were detected.
(32.5X)



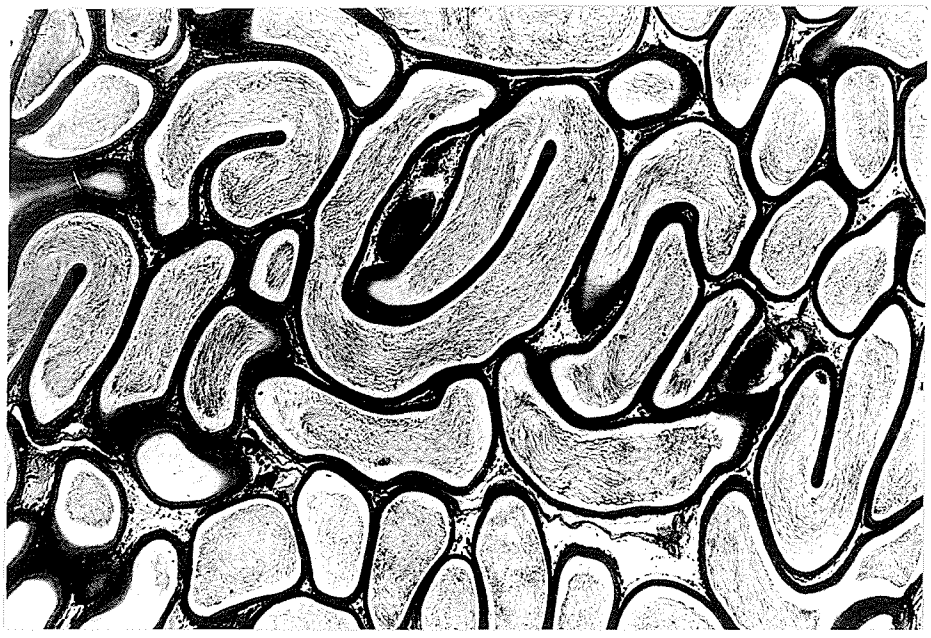


Fig. 37: The lining epithelium of the cauda epididymidis of an animal treated with acetylsalicylic acid. The mucosa was not affected by the drug. Secretory cells are apparent (arrows).
(520X)

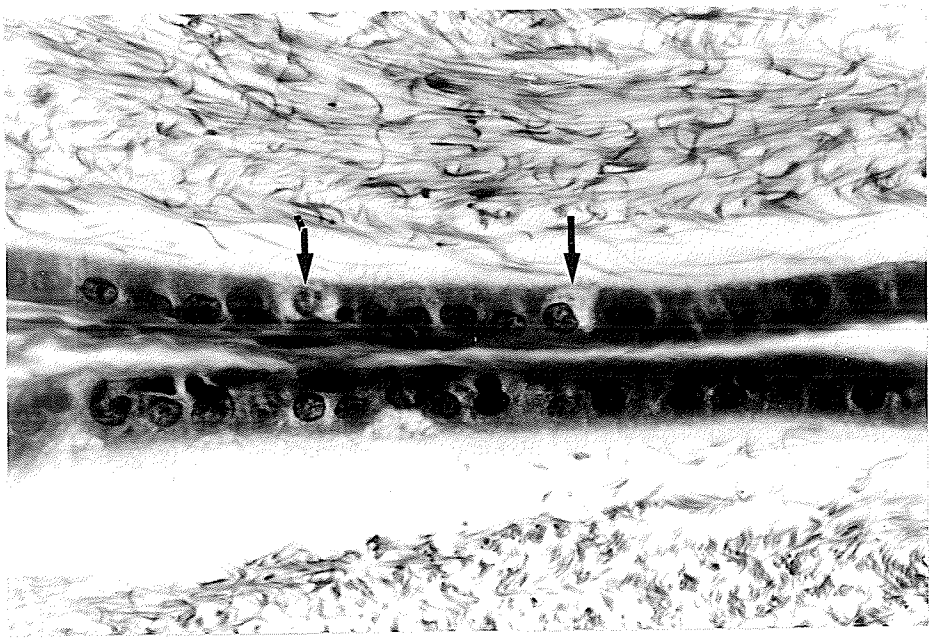
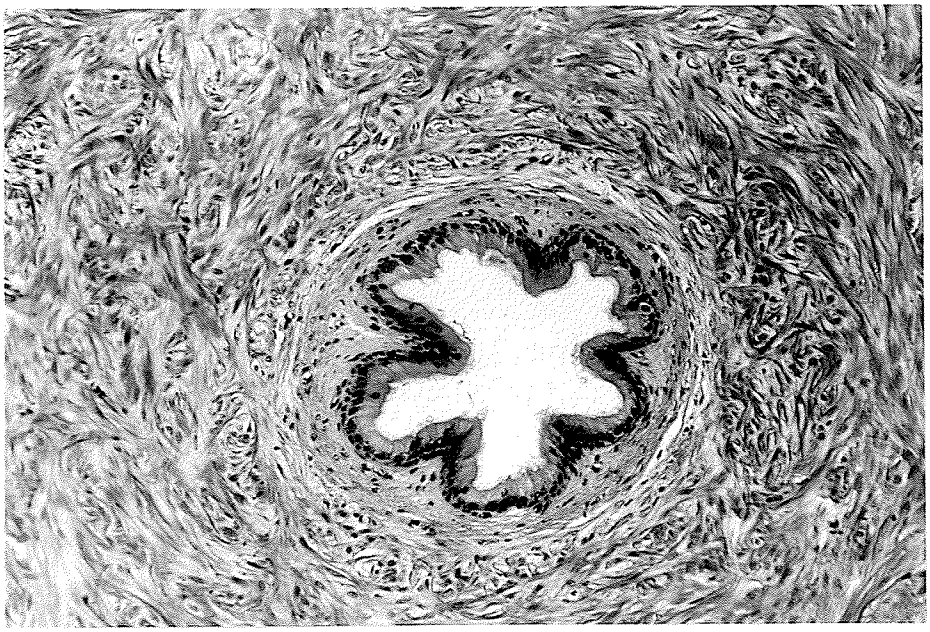


Fig. 38a (Upper): Ductus deferens of an animal treated with the solvent.
(130X)

Fig. 38b (Lower): Ductus deferens of an animal treated with acetylsalicylic acid. No lesions were apparent in the mucosa, lamina propria or muscularis.
(130X)



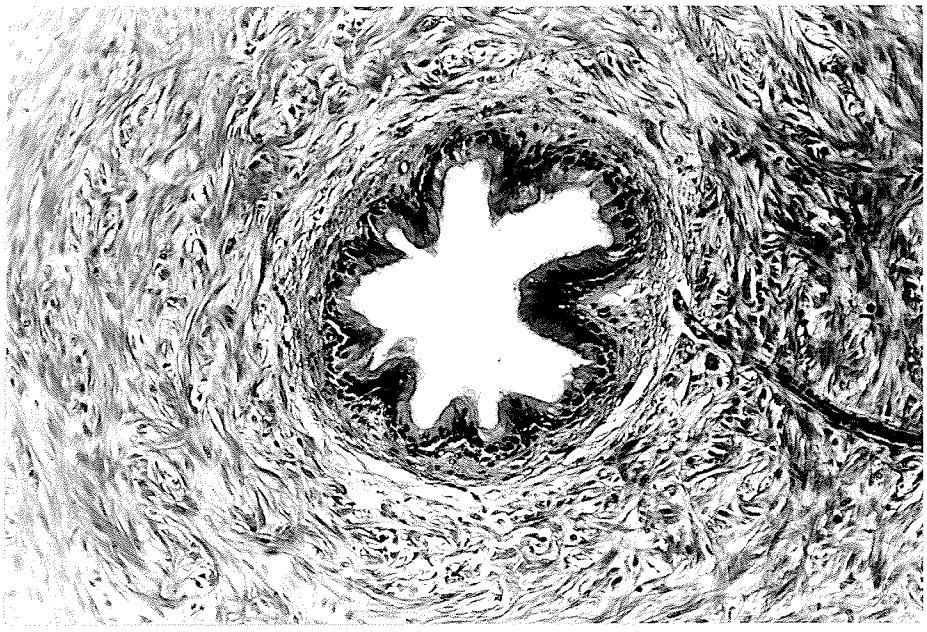
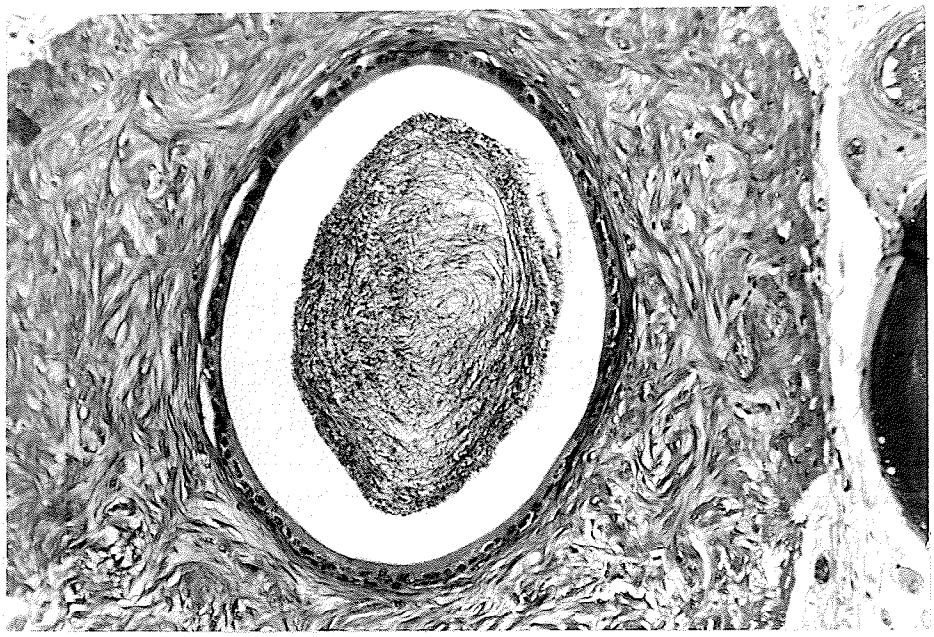


Fig. 39a (Upper): Distal portion of the ductus deferens of an animal treated with solvent.
(130X)

Fig. 39b (Lower): Distal ductus deferens of an animal treated with acetylsalicylic acid. The lumen is filled with spermatozoa.
(130X)



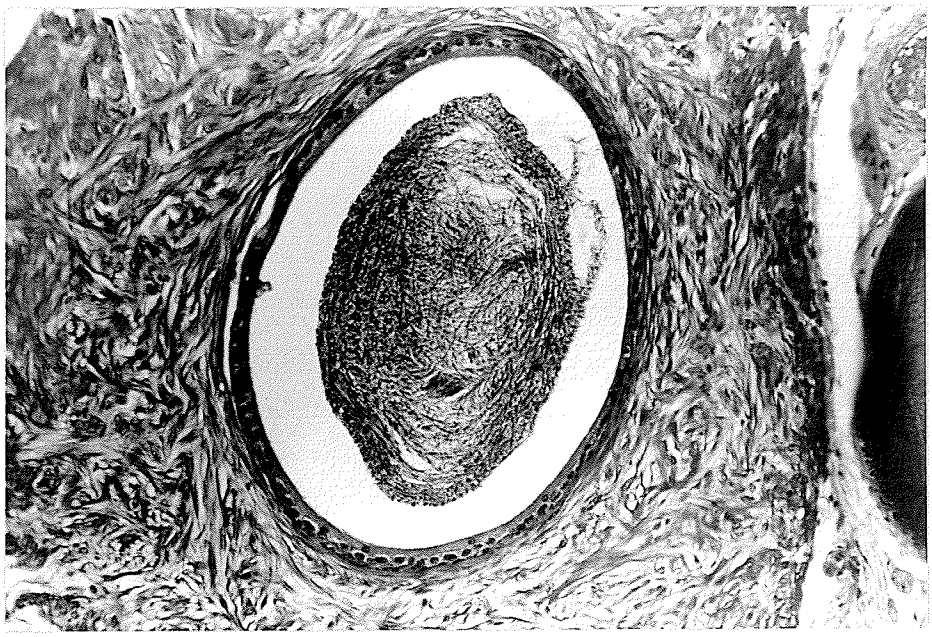


Fig. 40: Ductus deferens of an animal treated with acetylsalicylic acid for 12 days at a dose of 300mg/kg body weight. Some hypercellularity is present in the lamina propria near the top of the picture.
(130X)

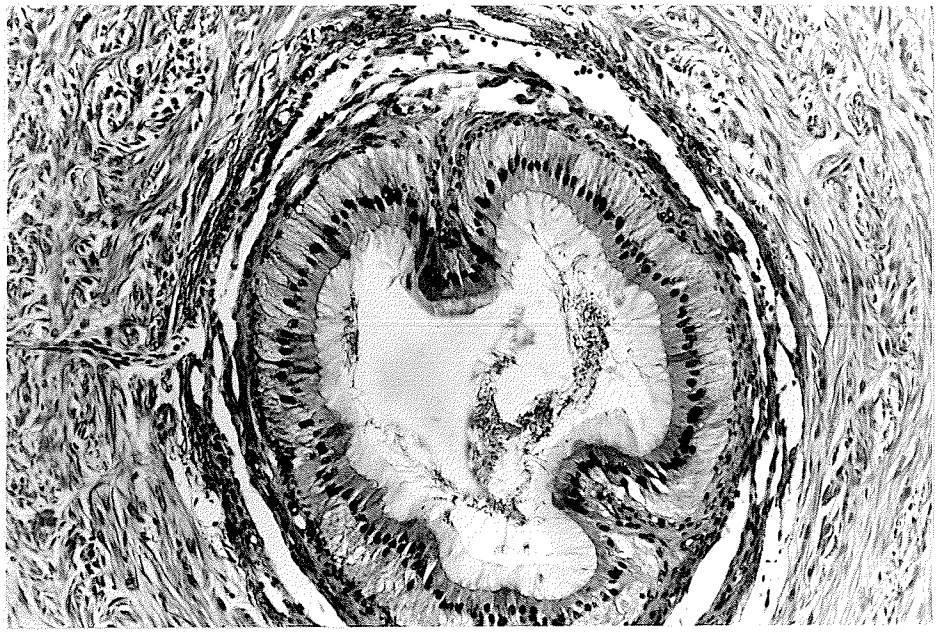
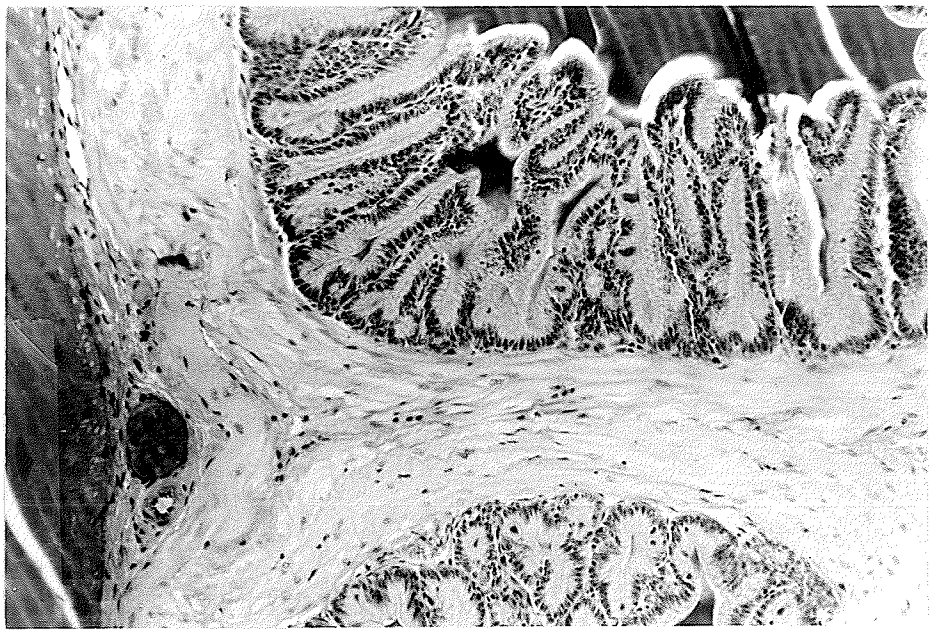
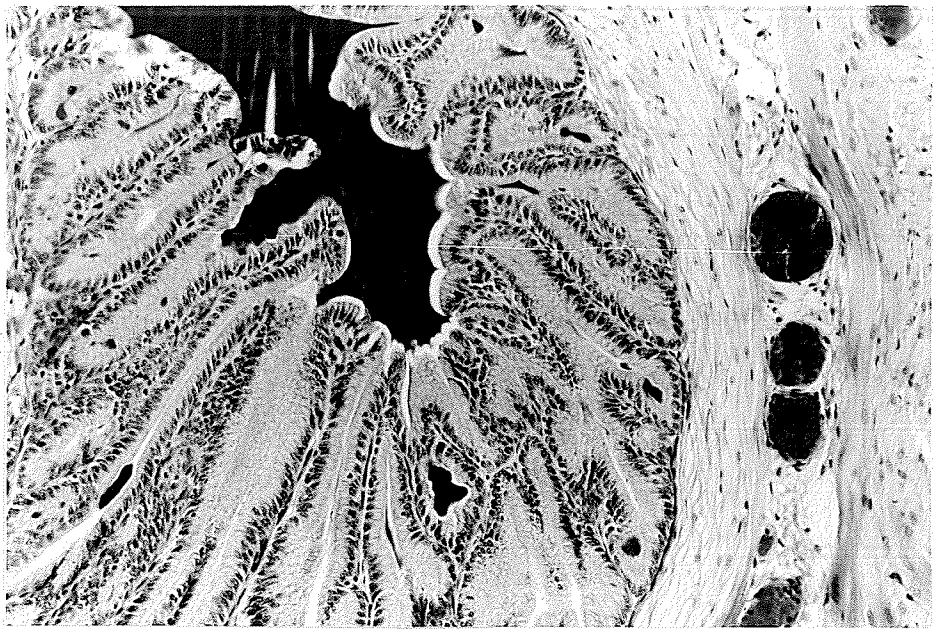


Fig. 41a (Upper): Seminal vesicla from an animal treated with solvent.
(130X)

Fig. 41b (Lower): Seminal vesicle from an animal treated with acetylsalicylic acid. No lesions were apparent in the gland.
(130X)





4.1.2.5 Kidneys

The kidneys of the animals treated with ASA did not reveal any pathological changes. The glomeruli did not exhibit any hypercellularity or crescent forms; no protein or vascular formed elements were present in the renal tubules to a significant degree (Fig. 42).

PAS activity was similar in the kidneys of animals treated with ASA or the solvent. In the glomeruli, a positive PAS reaction was associated with the capsular and glomerular basal laminae. PAS activity was distributed in the cuboidal cells lining the proximal convoluted tubules; it tended to be more concentrated towards the luminal side and brush border of the cells. The high PAS activity along the lumen was more pronounced towards the medulla of the kidney. The cells lining Henle's loop in the medulla of the kidney showed very little PAS activity. The cells of the distal convoluted tubules showed some PAS activity concentrated in the basal region.

4.1.2.6 Adrenal Glands

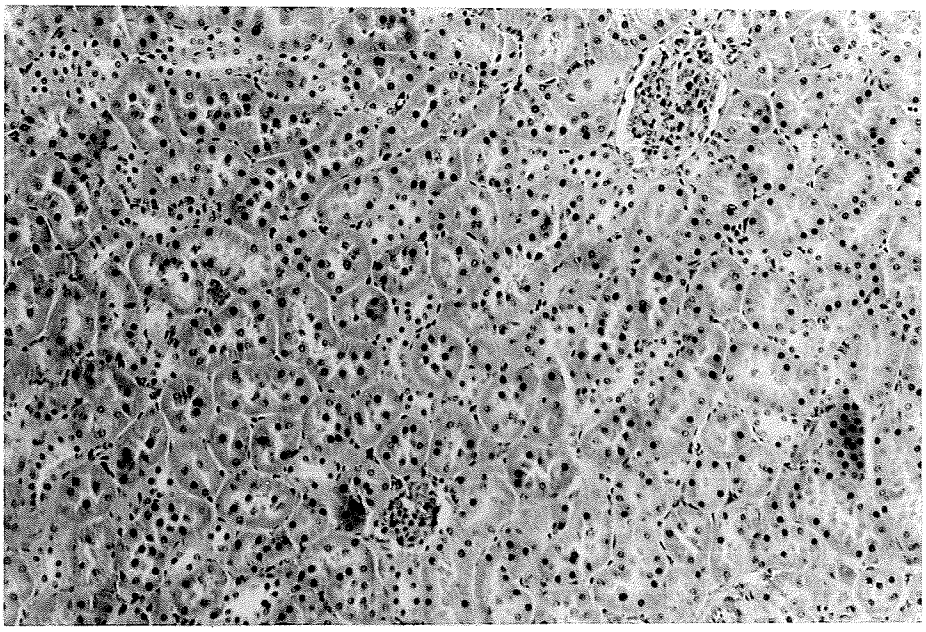
The adrenal glands of animals treated with ASA showed no pathological changes (Fig. 43). Congestion of the blood vessels, inflammation or edema were not detected after treatment with ASA.

PAS activity in the adrenal glands following treatment of the animals with ASA or the solvent was restricted to the capsule and the sinusoids between the parenchymal cell cords; some slight

activity was also present in the zona reticularis. The medulla of the adrenal gland did not show any PAS activity. The Mallory's trichrome staining technique revealed an increasing distribution of connective tissue towards the medulla.

Fig. 42a (Upper): Cortex of the kidney from an animal treated with the solvent.
(130X)

Fig. 42b (Lower): Renal cortex of an animal treated with acetylsalicylic acid. No lesions attributable to treatment with the drug were found in the kidneys.
(130X)



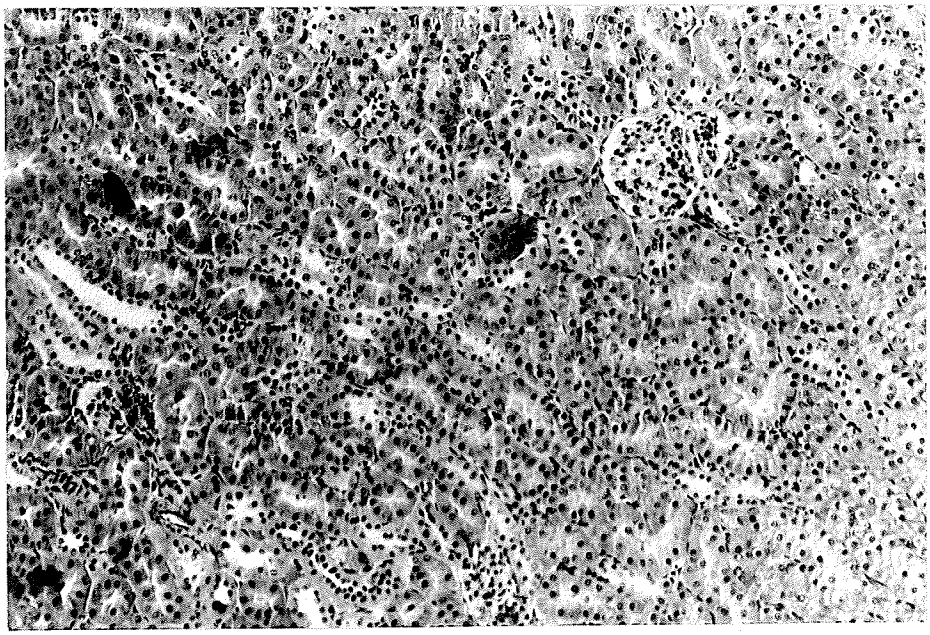
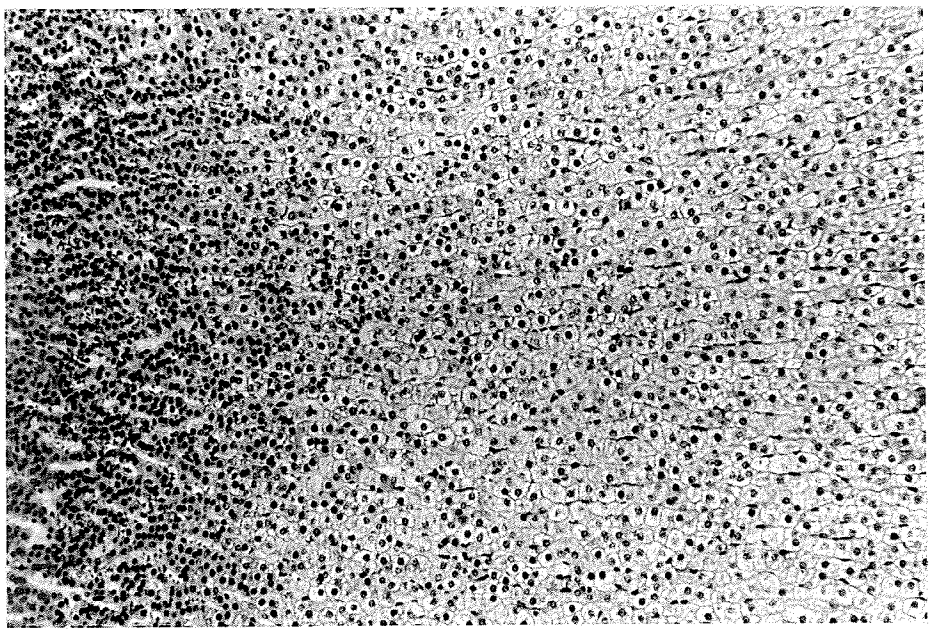
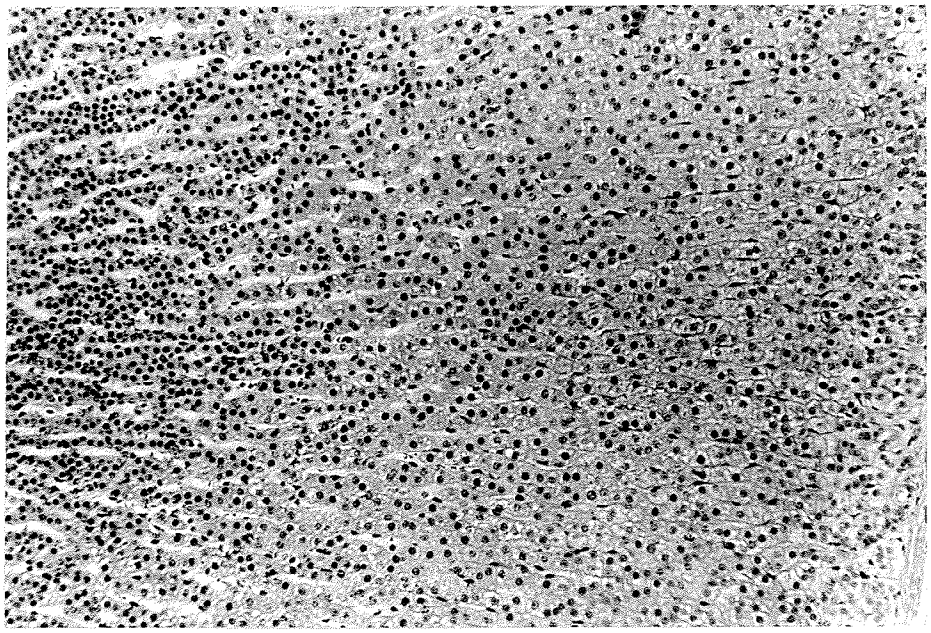


Fig. 43a (Upper): Cortex of the adrenal gland from an animal treated with the solvent.
(130X)

Fig. 43b (Lower): Adrenal cortex following treatment of the animal with acetylsalicylic acid. No morphological changes were detected.
(130X)





4.2 ENZYME HISTOCHEMICAL RESULTS

4.2.1 Acid Phosphatase

The activity of acid phosphatase in different tissues of animals that were treated with ASA is summarized in Table 20.

4.2.1.1 Testes

Acid phosphatase activity in the testes of animals treated with ASA or the solvent was demonstrated by the presence of small blue granules scattered throughout the germinal epithelium and interstitial tissues (Fig. 44). In some of the seminiferous tubules the granules were more concentrated in the luminal layers of the germinal lining. Intracellular activity was generally localized in the peripheral cytoplasm.

After treatment with ASA at all dose levels for a period of 12 days, acid phosphatase activity was slightly greater than that in animals treated only with the solvent (Table 20). However, treatment with ASA at a dose level of 300mg/kg body weight for a period of six days resulted in a slight decrease in activity; no difference was detected at a dose of 150mg/kg body weight.

4.2.1.2 Epididymides

Acid phosphatase activity in the caput epididymidis was concentrated in the pseudostratified columnar epithelium lining the tubules (Fig. 45); in these cells, the area towards the lumen was more reactive. Some activity was also observed in spermatozoa present in the lumen.

The caput epididymidis of all animals treated with ASA showed

a slight decrease in activity of acid phosphatase (Table 20).

In the cauda epididymidis, the activity of the enzyme was also restricted to the epithelial lining of the tubules (Fig. 46).

After treatment with ASA at a dose of 300mg/kg body weight over a period of six days the cauda epididymidis was moderately less reactive (Fig. 46); over a period of 12 days, activity was only slightly less than that of the controls (Table 20).

4.2.1.3 Ductus Deferens

In the ductus deferens, acid phosphatase activity was concentrated in the pseudostratified columnar epithelium lining the lumen (Fig. 47); some activity was also present along the basal lamina.

Following treatment with ASA at a dose level of 300mg/kg body weight for a period of six days, acid phosphatase activity was slightly increased in the ductus deferens (Table 20). The activity of the enzyme in the other ASA treated groups did not differ from that of the corresponding controls.

4.2.1.4 Seminal Vesicles

The activity of acid phosphatase in the seminal vesicles was distributed throughout the columnar epithelium of the mucosa (Fig. 48). A narrow band at the luminal surface of the lining epithelium was more reactive than other areas.

The seminal vesicles revealed moderately increased enzymatic activity in animals that were treated with ASA at a dose of 300mg/kg body weight for 12 days (Fig. 48); the activity in other animals treated with ASA did not differ from that of the controls (Table 20).

4.2.1.5 Kidneys

Acid phosphatase activity in the kidneys was most intense in the cortex (Fig. 49); the apices of the cuboidal cells lining the proximal and distal convoluted tubules showed the greatest reactivity. Some activity was also distributed throughout the cytoplasm of these cells. Enzymatic activity in the glomeruli was minimal. In the medulla, some granules were present in the thicker segments of Henle's loop.

The activity of acid phosphatase was moderately decreased in the kidneys of animals treated with ASA at a dose of 300mg/kg body weight for six or 12 days (Table 20 and Fig. 49). Treatment at a dose of 150mg/kg body weight resulted in a slight decrease in enzymatic activity when administered for a 12 day period.

4.2.1.6 Adrenal Glands

Acid phosphatase activity was uniformly distributed throughout the parenchymal cells of the cortex of the adrenal glands (Fig. 50). Granules of enzyme activity were larger in the zona glomerulosa and reticularis than in the zona fasciculata. The medulla exhibited only minimal activity. The adrenal glands were slightly more reactive only after ASA treatment at a dose of 300mg/kg body weight for a period of 12 days (Table 20). In other ASA treated groups, the activity of the enzyme showed no differences from that of the corresponding controls.

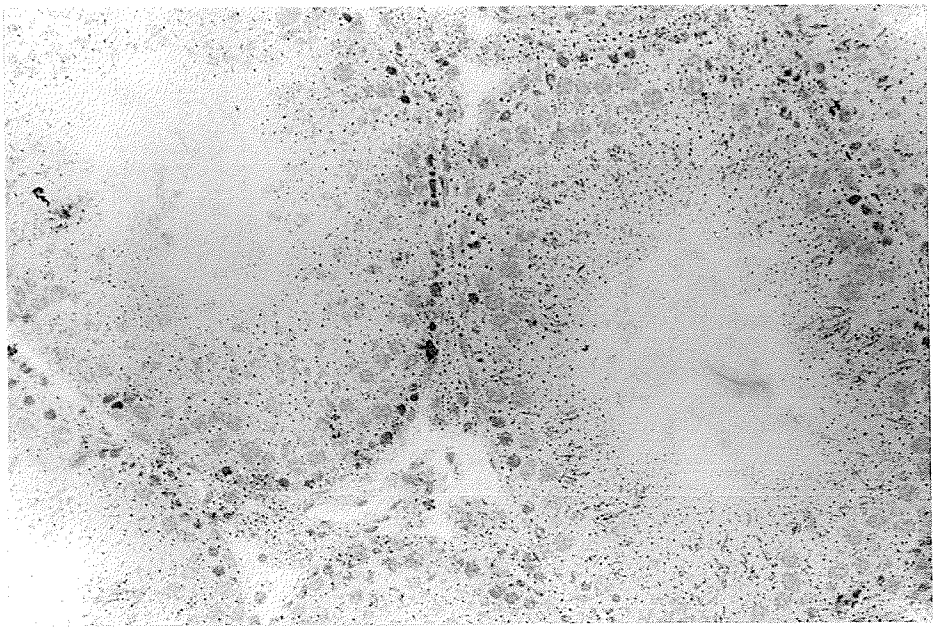
Table 20: Acid phosphatase activity after treatment with ASA

Tissues	Treatment with ASA I 300mg/kg body weight for 12 days	III 150mg/kg body weight for 12 days	V 300mg/kg body weight for six days	VII 150mg/kg body weight for six days
Testes	+	+	-	o
Caput Epididymidis	-	-	-	-
Cauda Epididymidis	-	o	--	o
Ductus Deferens	o	o	+	o
Seminal Vesicles	++	o	o	o
Kidneys	--	-	--	o
Adrenal Glands	+	o	o	o

+++Greatly increased compared to controls.
++Moderately increased compared to controls.
+Slightly increased compared to controls.
oNo difference compared to controls.
-Slightly decreased compared to controls.
--Moderately decreased compared to controls.
---Greatly decreased compared to controls.

Fig. 44 (Upper): Distribution of acid phosphatase activity (granules) in the testis of an animal treated with solvent. The activity is uniformly present throughout the germinal epithelium and interstitial tissue. Enzyme activity was only slightly altered in the testes of animals treated with acetylsalicylic acid.
(166X)

Fig. 45 (Lower): Acid phosphatase activity in the caput epididymidis of an animal treated with the solvent. Activity is concentrated along the luminal border of the epithelial cells. Treatment with acetylsalicylic acid slightly altered the enzymatic activity in the caput epididymidis.
(130X)



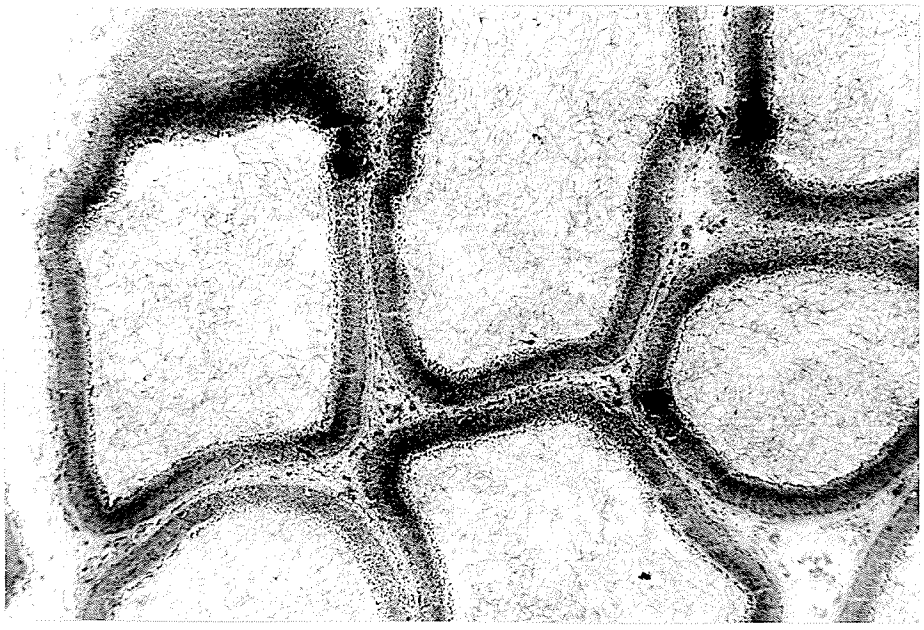
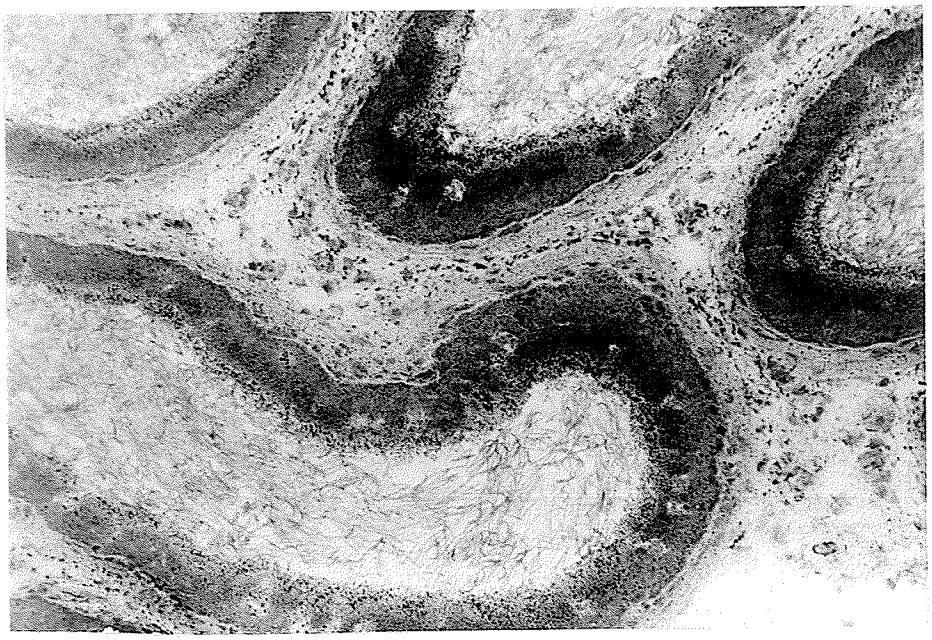


Fig. 46a (Upper): Acid phosphatase activity in the cauda epididymidis of an animal treated with solvent. Enzyme activity is most intense along the luminal margin of the epithelium. (208X)

Fig. 46b (Lower): Acid phosphatase activity in the cauda epididymidis of an animal treated with acetylsalicylic acid for six days at a dose of 300mg/kg body weight. Enzymatic activity was moderately decreased. (208X)



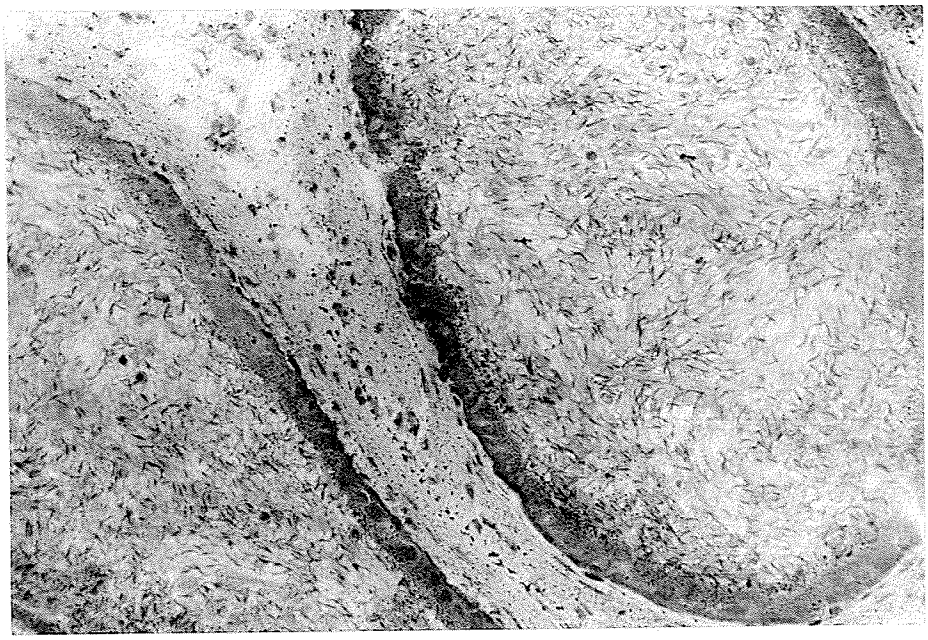


Fig. 47: Distribution of acid phosphatase activity in the ductus deferens of an animal treated with solvent. Enzyme activity is concentrated in the epithelium and the basal lamina. In the animals treated with acetylsalicylic acid, slight alterations of enzyme activity were found.
(130X)

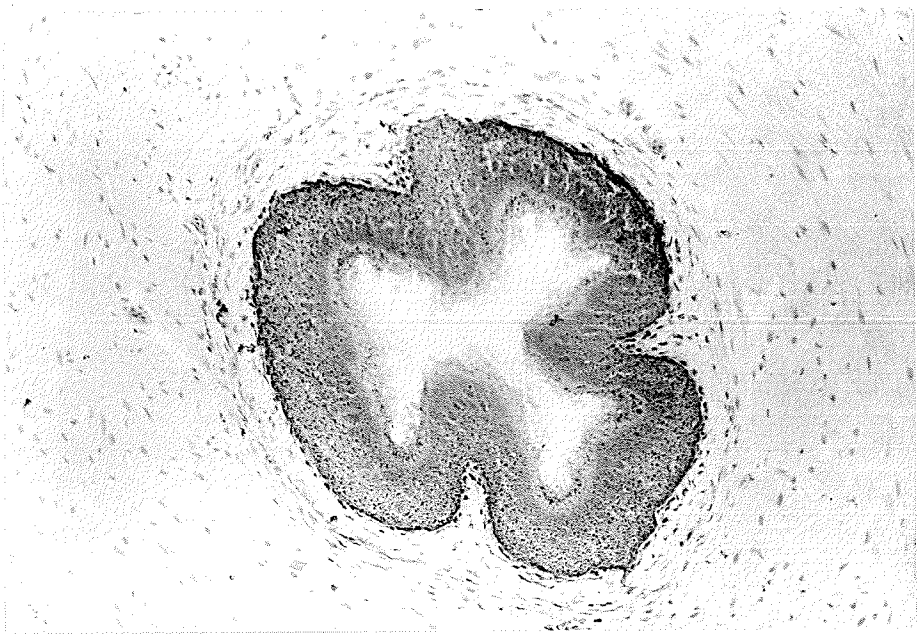
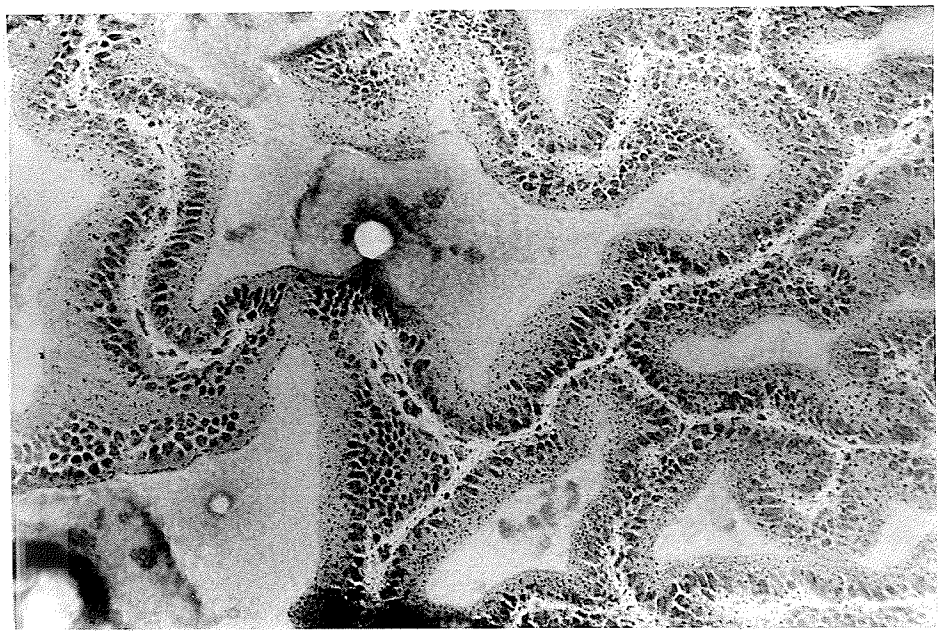


Fig. 48a (Upper): Distribution of acid phosphatase activity (granules) in the seminal vesicle of an animal treated with solvent. The activity is concentrated in the columnar epithelium. The luminal border of the cells is most reactive. (208X)

Fig. 48b (Lower): Seminal vesicle of an animal treated with acetylsalicylic acid for 12 days at a dose of 300mg/kg body weight. Acid phosphatase activity was moderately increased along the luminal side of the lining cells. (208X)



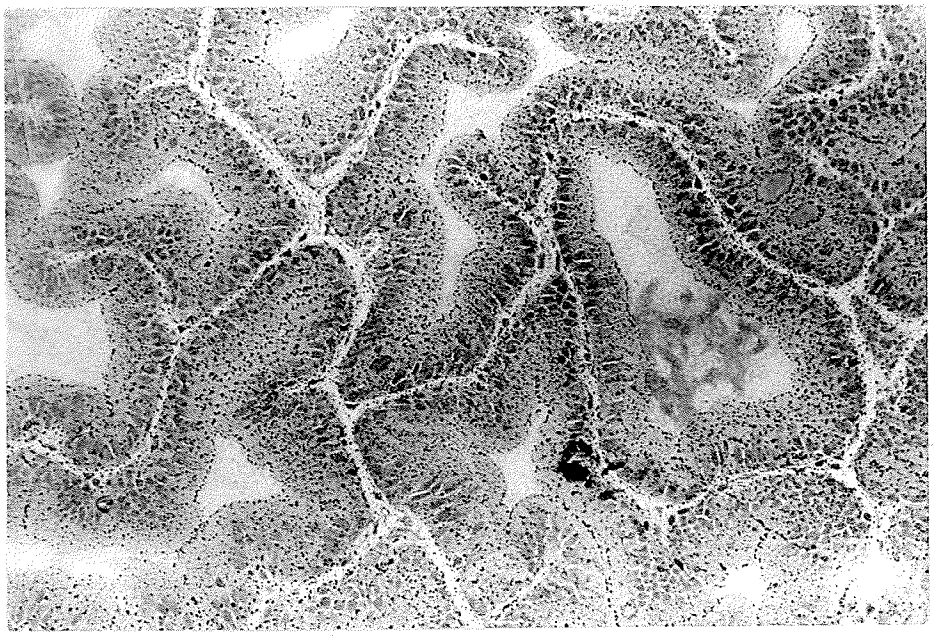
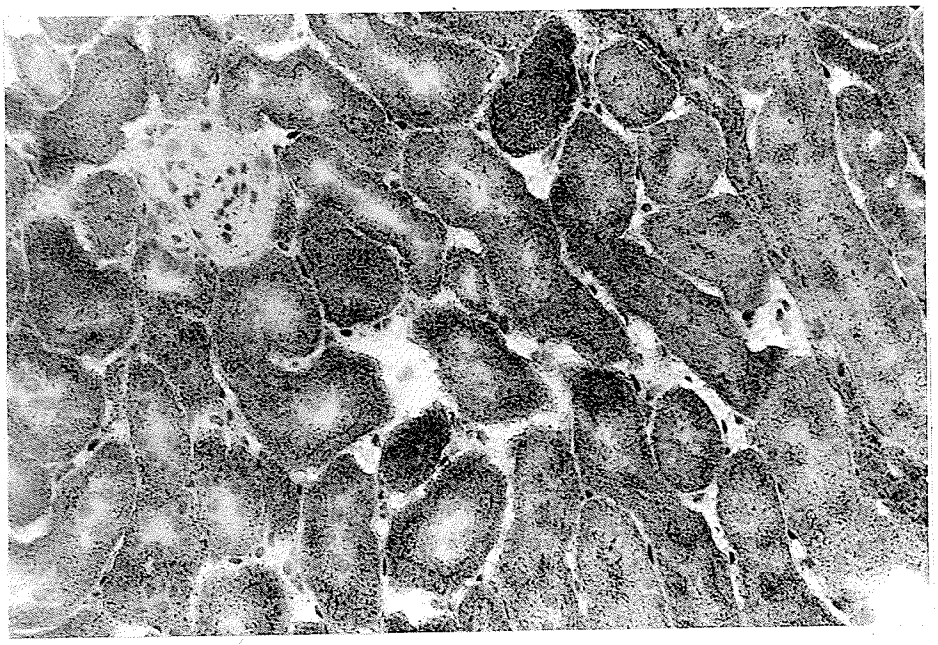
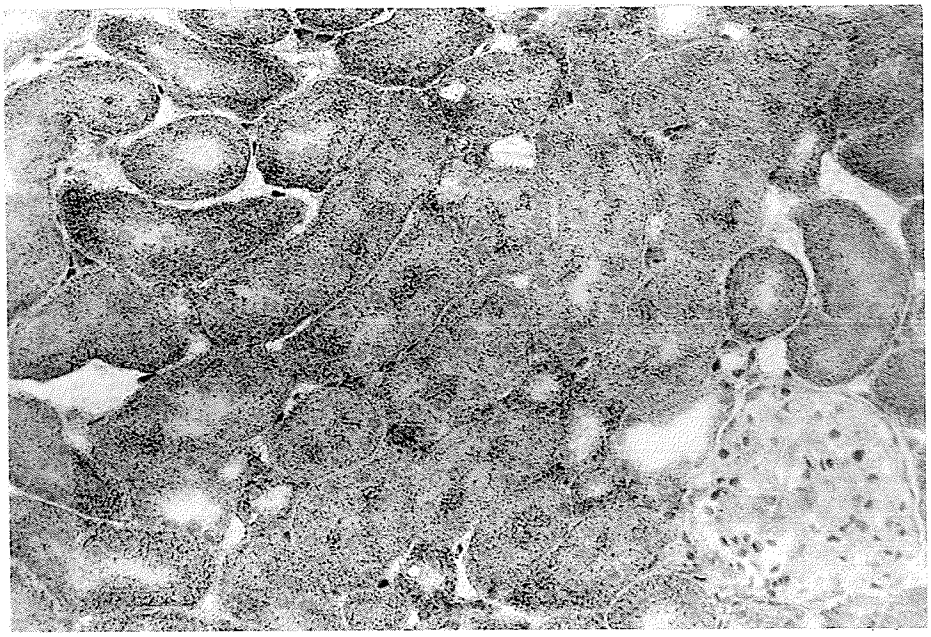


Fig. 49a (Upper): Acid phosphatase activity in the cortex of the kidney of an animal treated with solvent. Enzyme activity is most intense at the apices of the cells lining the tubules.
(208X)

Fig. 49b (Middle): Activity of acid phosphatase in the renal cortex following treatment of the animals with acetylsalicylic acid for six days at a dose of 300mg/kg body weight. Enzyme activity was moderately decreased.
(208X)

Fig. 49c (Lower): Acid phosphatase activity in the renal cortex after the animal was treated with acetylsalicylic acid for 12 days at a dose of 300mg/kg nody weight. The activity was moderately decreased.
(208X)





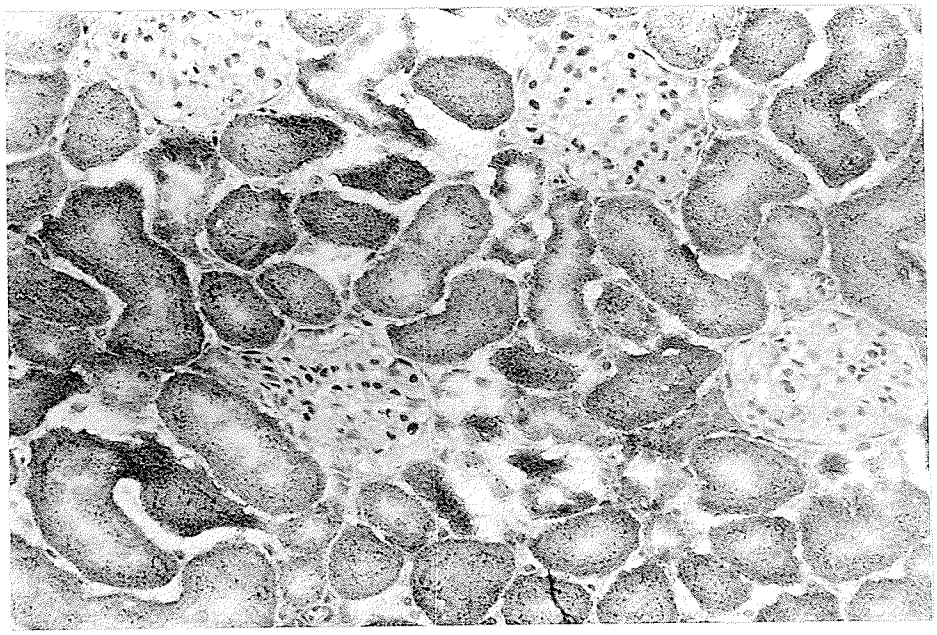
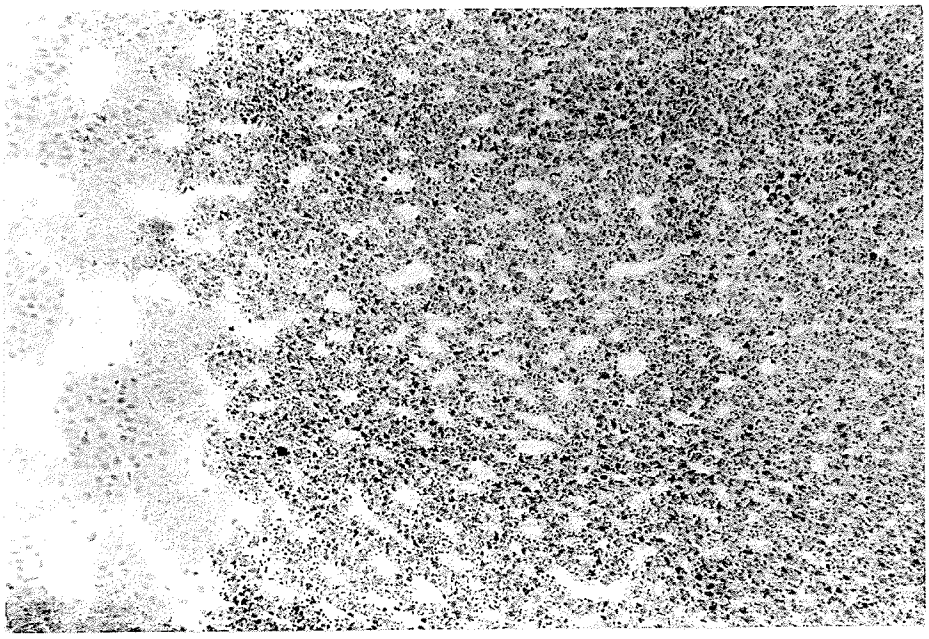


Fig. 50: Acid phosphatase activity in the cortex (right) and the medulla (left) of the adrenal gland of an animal treated with solvent. The activity is evenly distributed in the cortex.
(130X)



4.2.2 Alkaline Phosphatase

The activity of alkaline phosphatase in different tissues of animals that were treated with ASA is presented in Table 21.

4.2.2.1 Testes

Intense alkaline phosphatase activity was demonstrated by the presence of small blue granules around the blood vessels in the interstitium and in the basal lamina of the seminiferous tubules (Fig. 51). The germinal epithelium showed minimal enzymatic activity as did the Leydig cells in the interstitial tissue.

Alkaline phosphatase activity was less intense in the testes of all groups of animals treated with ASA (Table 21); the decrease was slight in all groups except those animals treated for a period of 12 days at a dose of 300mg/kg body weight where enzyme activity was moderately decreased (Fig. 51).

4.2.2.2 Epididymides

In the caput epididymidis, enzyme activity was confined largely to the blood vessels, the basal lamina and the smooth muscle around the tubules (Fig. 52).

The caput epididymidis of animals treated with ASA showed no differences in enzyme activity with the exception of a slight decrease after treatment with ASA for six days at a dose of 300mg/kg body weight (Table 21).

Enzyme activity in the cauda epididymidis was localized in the basal lamina, blood vessels and smooth musculature (Fig. 53).

Treatment of animals with ASA at a dose of 300mg/kg body weight produced a slight increase in alkaline phosphatase activity in the cauda epididymidis; however, treatment at a dose of 150mg/kg body weight over the same period resulted in a slight decrease (Table 21). Animals treated with ASA for a period of six days at a dose of 300mg/kg body weight showed a moderate decrease in staining intensity (Fig. 53); in those receiving 150mg/kg body weight, enzymatic activity in the cauda epididymidis was the same as the controls (Table 21).

4.2.2.3 Ductus Deferens

Alkaline phosphatase activity in the ductus deferens was present as a deep staining blue ring associated with the lamina propria around the lumen. Activity was also present around the blood vessels and in the muscularis (Fig. 54). A slight decrease in enzyme activity was observed in the ductus deferens of animals that were treated with ASA at a dose of 300mg/kg body weight for a period of 12 days (Table 21). The intensity of the staining in the ductus deferens of the other groups of animals treated with ASA did not differ from that of the controls.

4.2.2.4 Seminal Vesicles

In the seminal vesicles, the activity of alkaline phosphatase was largely confined to the lamina propria and to the basal lamina underlying the mucous membrane (Fig. 55).

After the animals were treated with ASA, for a period of 12 days at a dose of 300mg/kg body weight, enzymatic activity was slightly increased (Table 21).

4.2.2.5 Kidneys

Alkaline phosphatase activity was concentreated in the apical region of the cells lining the tubules of the renal cortex (Fig. 56); some scattered granules were also present in the basal region. The glomeruli showed only a slight reaction. In the medulla very minimal enzyme activity was present. Treatment with ASA at a dose of 300mg/kg body weight over a period of 12 days produced a moderate decrease in enzyme activity (Fig. 56); after treatment for a period of six days, the activity of the enzyme was slightly decreased (Table 21).

4.2.2.6 Adrenal Glands

Enzyme activity in the adrenal glands was distributed throughout the parenchymal cells of the cortex (Fig. 57); in the medulla it was confined to the blood vessels. After treatment with ASA, alkaline phosphatase activity was slightly decreased in all treatment groups, with the exception of the animals that were treated for 12 days at a dose of 150mg/kg body weight (Table 21).

Table 21: Alkaline phosphatase activity after treatment with ASA

Tissues	Treatment with ASA I 300mg/kg body weight for 12 days	III 150mg/kg body weight for 12 days	V 300mg/kg body weight for six days	VII 150mg/kg body weight for six days
Testes	--	-	-	-
Caput Epididymidis	o	o	-	o
Cauda Epididymidis	+	-	--	o
Ductus Deferens	-	o	o	o
Seminal Vesicles	+	o	o	o
Kidneys	--	o	-	o
Adrenal Glands	--	o	-	-

+++Greatly increased compared to controls.
++Moderately increased compared to controls.
+Slightly increased compared to controls.
oNo difference compared to controls.
-Slightly decreased compared to controls.
--Moderately decreased compared to controls.
---Greatly decreased compared to controls.

Fig. 51a (Upper): Alkaline phosphatase activity in the testis of an animal treated with the solvent. Intense activity is restricted to the blood vessels and the basal lamina around the tubules.
(130X)

Fig. 51b (Lower): Alkaline phosphatase activity in the testis of an animal treated with acetylsalicylic acid for 12 days at a dose of 300mg/kg body weight. Enzyme activity was moderately decreased along the basal lamina.
(130X)

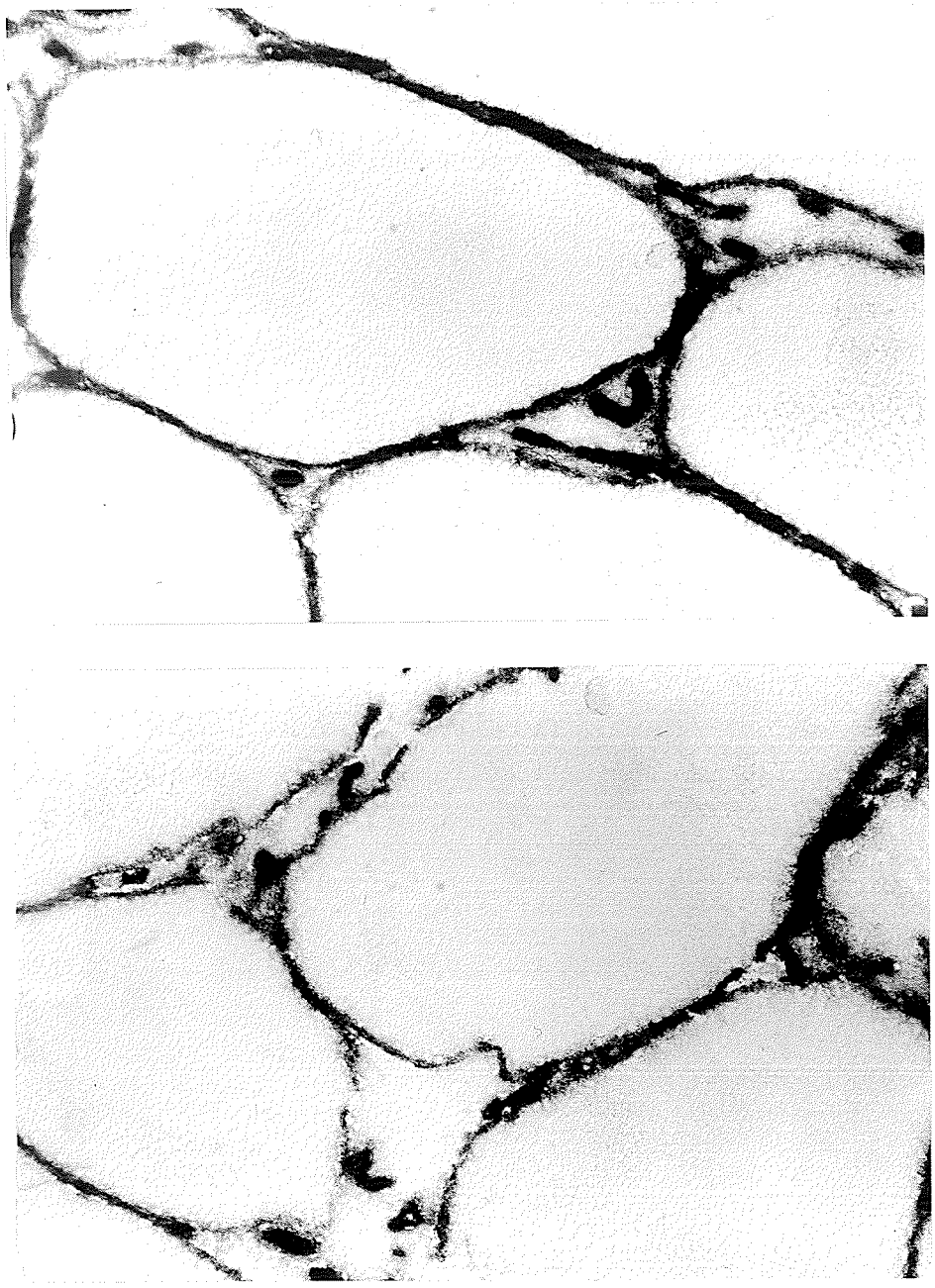


Fig. 52: Alkaline phosphatase activity in the caput epididymidis of an animal treated with the solvent. The basal lamina around the seminiferous tubules, the smooth musculature and the blood vessels show most intense enzymatic activity. Acetylsalicylic acid produced only slight alterations in activity.
(130X)

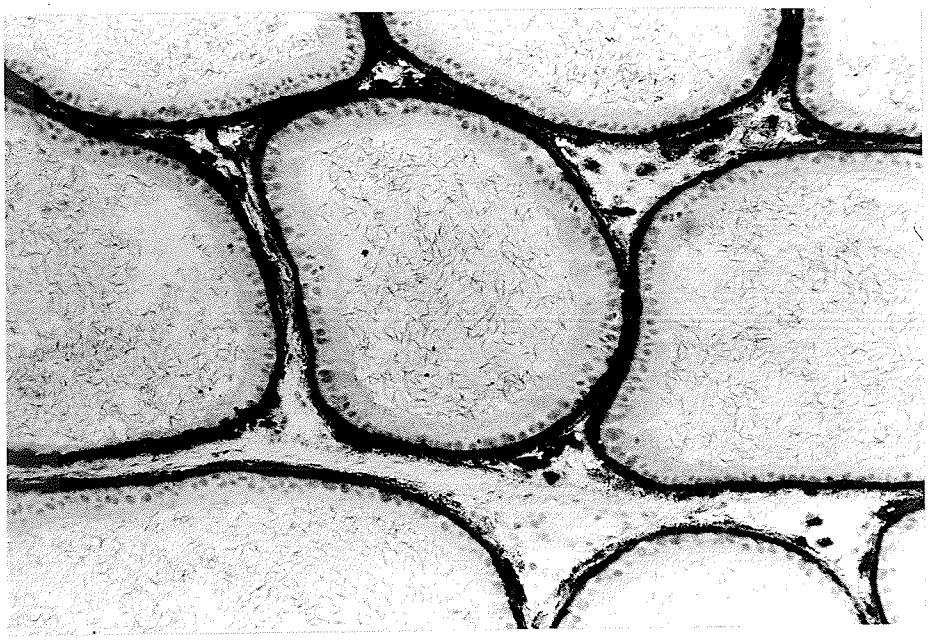
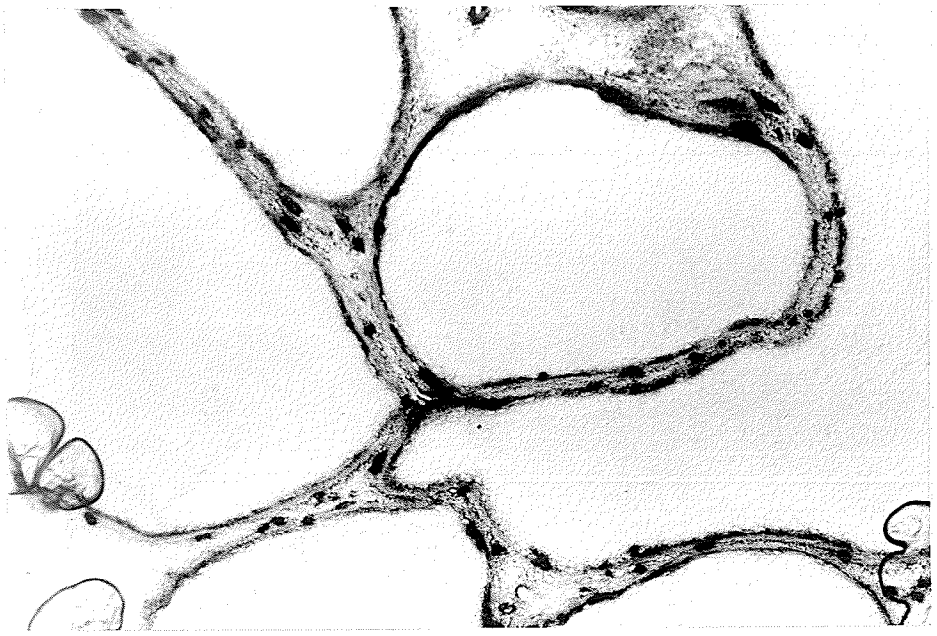


Fig. 53a (Upper): Distribution of alkaline phosphatase in the cauda epididymidis of an animal treated with solvent. Enzymatic activity is present in the basal lamina, blood vessels and to some degree in the smooth musculature around the tubules.
(130X)

Fig. 53b (Lower): Alkaline phosphatase activity in the cauda epididymidis of an animal treated with acetylsalicylic acid for six days at a dose of 300mg/kg body weight. There was a moderate decrease in enzyme activity.
(130X)



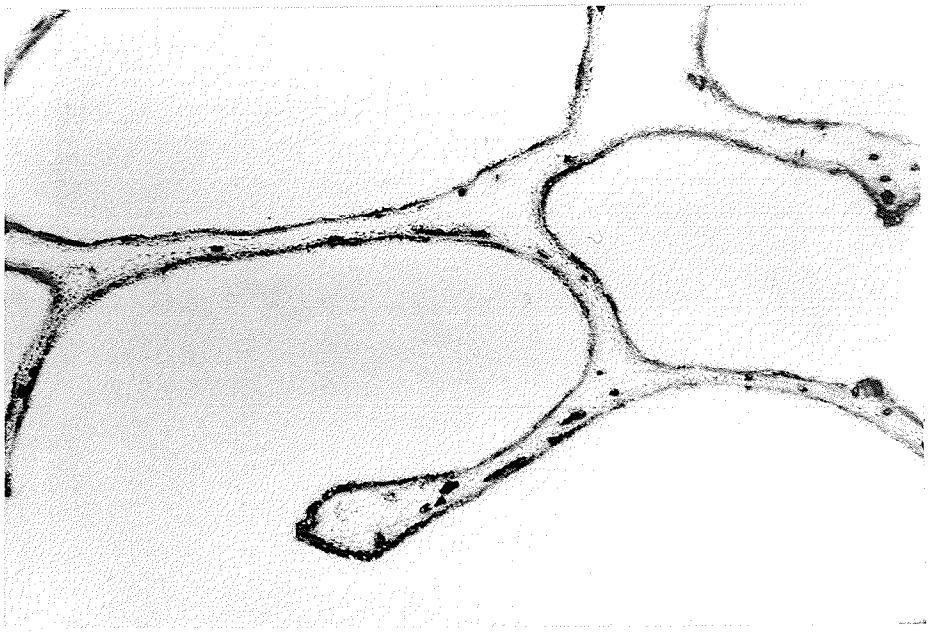


Fig. 54 (Upper): Alkaline phosphatase activity in the ductus deferens of an animal treated with the solvent. Reactivity is present in the lamina propria, blood vessels and muscular coat. Acetylsalicylic acid produced slight changes in enzyme activity.
(130X)

Fig. 55 (Lower): Alkaline phosphatase activity in the seminal vesicle of an animal treated with solvent. The enzyme is present in the basal lamina and the lamina propria. Activity was slightly altered following treatment with acetylsalicylic acid.
(130X)

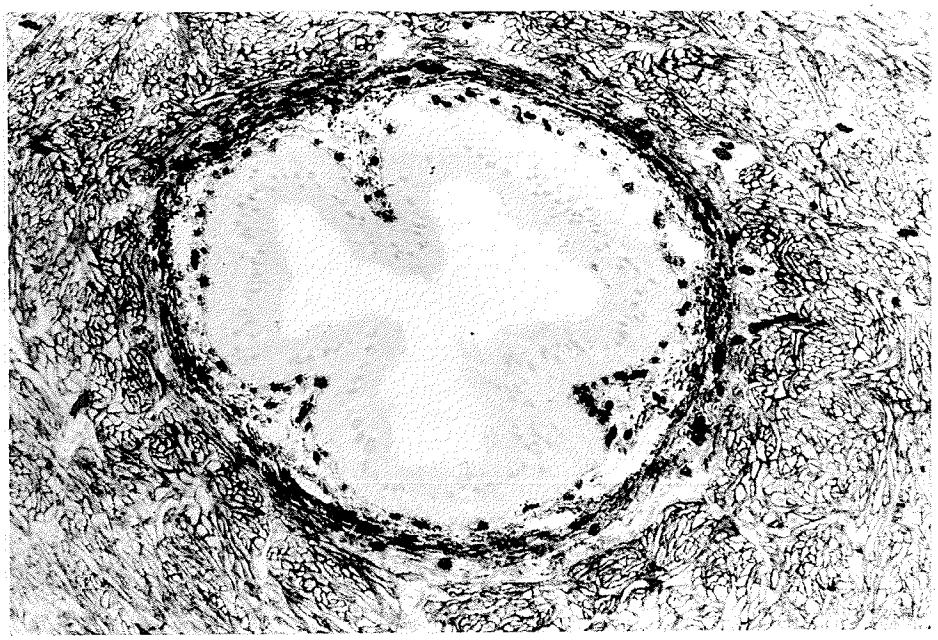
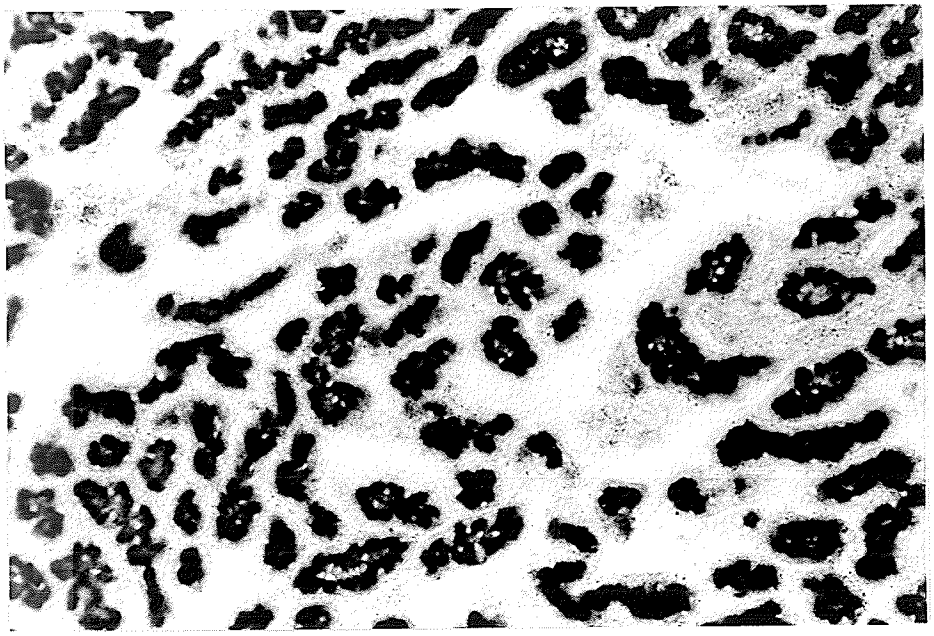


Fig. 56a (Upper): Alkaline phosphatase activity in the renal cortex of an animal treated with solvent. The apices of the cells lining the tubules shows intense activity.
(130X)

Fig. 56b (Lower): Alkaline phosphatase activity in the renal cortex of an animal treated with acetyl-salicylic acid for 12 days at a dose of 300mg/kg body weight. Enzyme activity was moderately decreased.
(130X)



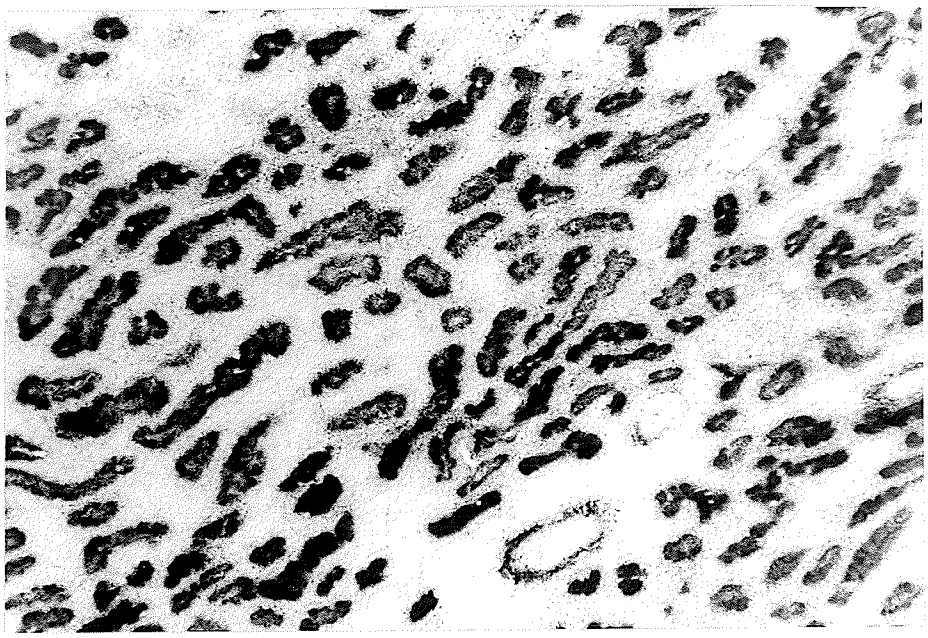
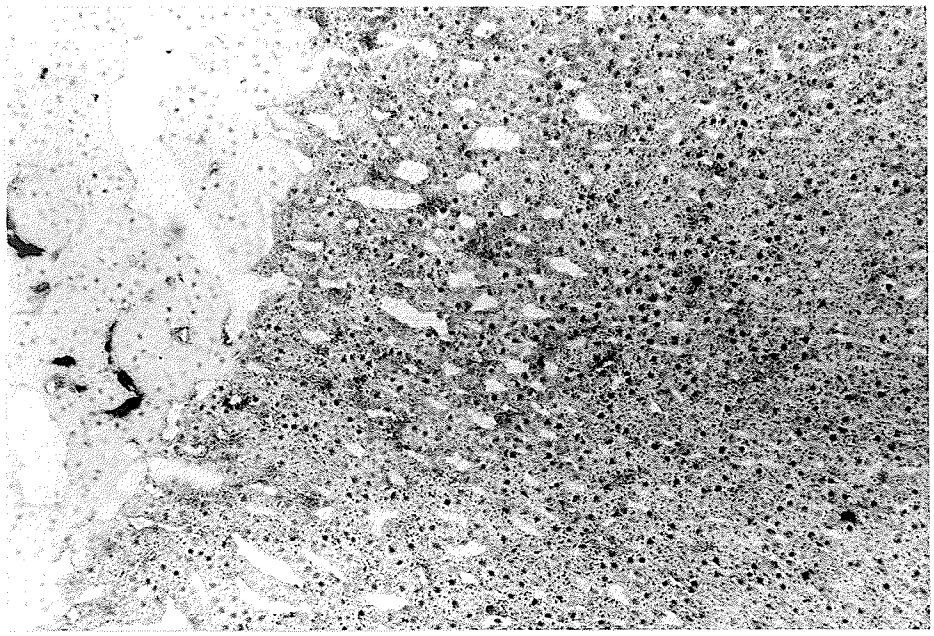


Fig. 57: Alkaline phosphatase activity (granules) in the cortex (right) and medulla (left) of the adrenal gland from an animal treated with the solvent. The activity is distributed throughout the cortex but is restricted to the blood vessels in the medulla. Treatment with acetylsalicylic acid produced slight changes in enzymatic activity.
(130X)



4.2.3 Cytochrome Oxidase

The activity of cytochrome oxidase in different tissues of animals treated with ASA is presented in Table 22.

4.2.3.1 Testes

Cytochrome oxidase activity was minimal in the testes and was restricted to the germinal epithelium. Animals treated with ASA at a dose of 150mg/kg body weight for 12 days or 300mg/kg body weight for six days showed a slight increase in cytochrome oxidase activity (Table 22).

4.2.3.2 Epididymides

In the caput epididiymidis, enzyme activity was confined to the luminal side of the pseudostratified columnar epithelium lining the tubules. Treatment with ASA for 12 days at a dose of 300mg/kg body weight resulted in a slight decrease in cytochrome oxidase activity; the activity in the caput epididymidis of the other groups treated with ASA did not differ from that of the corresponding controls (Table 22).

In the cauda epididymidis, enzyme activity was distributed in the upper parts of the cells of the lining epithelium similar to that in the caput epididymidis. Enzymatic activity was slightly decreased in the cauda epididymidis of animals treated with ASA for six days at a dose of 300mg/kg body weight (Table 22).

4.2.3.3 Ductus deferens

The ductus deferens exhibited an even distribution of cytochrome oxidase activity in the pseudostratified columnar epithelium. Slight activity was also detected in the muscular wall. Enzyme activity was only slightly decreased in the ductus deferens of animals treated with ASA for a period of six days at a dose of 300mg/kg body weight (Table 22); the intensity of the reaction did not differ in other groups of animals treated with ASA when compared with the controls.

4.2.3.4 Seminal Vesicles

Cytochrome oxidase activity was not evident in the seminal vesicles of animals treated with ASA or solvent.

4.2.3.5 Kidneys

A positive enzyme reaction was present in the basal region of cells lining the proximal and distal convoluted tubules in the renal cortex (Fig. 58); enzyme activity decreased towards the medulla of the kidney. The tissues of the glomeruli appeared unreactive.

The kidneys of animals treated with ASA at a dose 300mg/kg body weight displayed a moderate increase in reaction at both treatment periods (Table 22 and Fig. 58). Enzyme activity in the kidneys was not affected by treatment with ASA at a dose of 150mg/kg body weight.

4.2.3.6 Adrenal Glands

Cytochrome oxidase activity was not detected in the medulla of the adrenal gland. However, a slight reaction of the enzyme was evenly distributed in the parenchymal cells of the cortex. Enzyme activity was unaffected by ASA treatment (Table 22).

Table 22. Cytochrome oxidase activity

after treatment with ASA

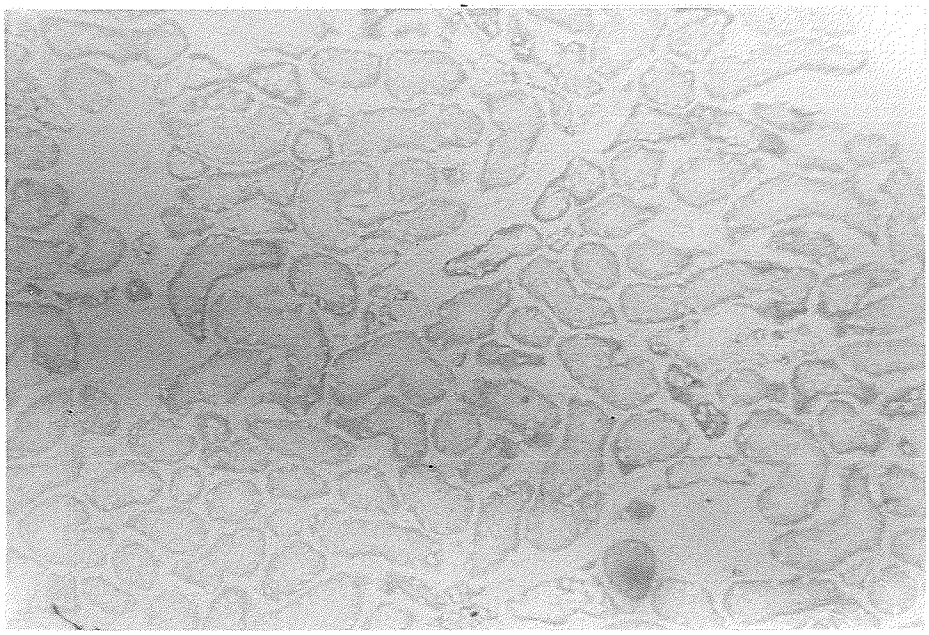
Tissues	Treatment with ASA			
	I 300mg/kg body weight for 12 days	III 150mg/kg body weight for 12 days	V 300mg/kg body weight for six days	VII 150mg/kg body weight for six days
Testes	o	+	+	o
Caput Epididymidis	-	o	o	o
Cauda Epididymidis	o	o	-	o
Ductus Deferens	Not available	o	-	o
Seminal Vesicles	o	o	o	o
Kidneys	++	o	++	o
Adrenal Glands	o	o	o	o

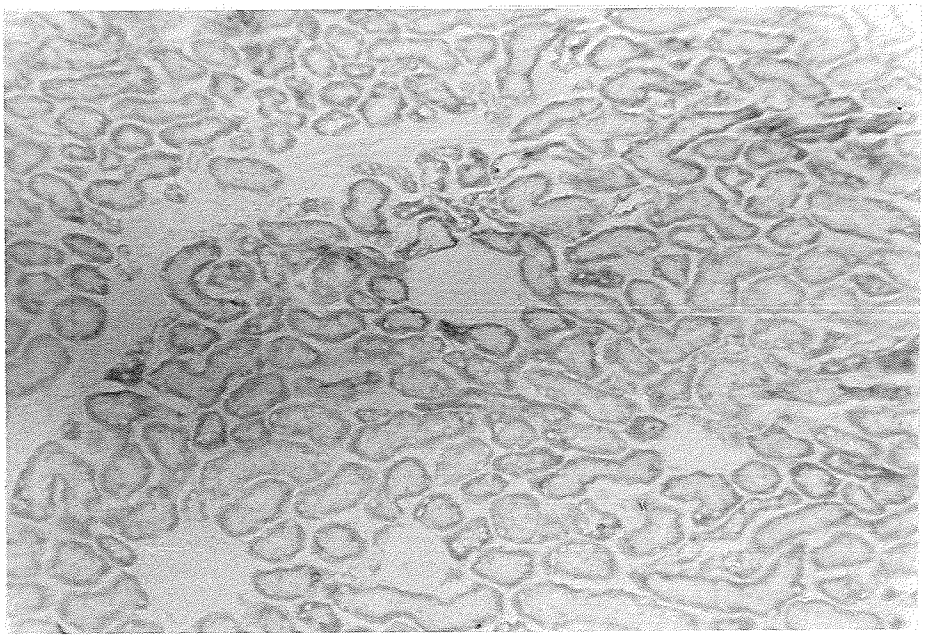
+++Greatly increased compared to controls.
++Moderately increased compared to controls.
+Slightly increased compared to controls.
oNo difference compared to controls.
-Slightly decreased compared to controls.
--Moderately decreased compared to controls.
---Greatly decreased compared to controls.

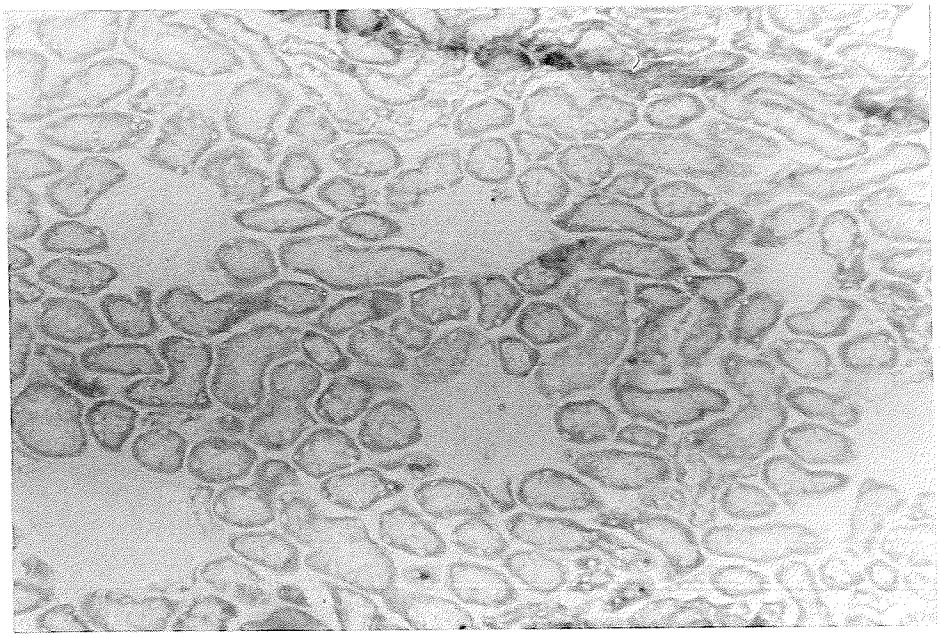
Fig. 58a (Upper): Cytochrome oxidase activity in the renal cortex of an animal treated with the solvent. The basal regions of the cells lining the tubules are most reactive.

Fig. 58b (Middle): Activity of cytochrome oxidase in the renal cortex of an animal treated with acetylsalicylic acid for six days at a dose of 300mg/kg body weight. Cytochrome oxidase activity was moderately increased.
(130X)

Fig. 58c (Lower): Cytochrome oxidase activity in the renal cortex following treatment of the animal with acetylsalicylic acid for 12 days at a dose of 300mg/kg body weight. Enzymatic activity was moderately increased.
(130X)







SECTIONS 1 AND 2

DISCUSSION

5. DISCUSSION

Acetylsalicylic acid, a non-steroidal anti-inflammatory drug, inhibits prostaglandin synthesis (Vane, 1971; Blackwell et al, 1975). Recently, its effects on reproductive processes in the female have been reported (Aiken, 1974; Lindner et al, 1974). On the other hand, the influence of this substance on male reproduction has received little attention. The present investigation attempted to determine the morphological and enzyme-histochemical changes in the reproductive tract of the male rat, following treatment with acetylsalicylic acid (ASA).

5.1 QUANTITATIVE AND MORPHOLOGICAL STUDY

Administration of acetylsalicylic acid produced an increase in mean testicular weight. Analysis of variance revealed that the mean testicular weights did not differ significantly after the animals were treated with ASA, whereas those of the control groups differed at a significant level ($p < 0.05$). In contrast, Duncan's multiple range test of significance showed that the highest treatment level significantly altered the mean testicular weight when compared to those of animals receiving ASA for a shorter period or at a lower dose. The results of the two statistical procedures may be an indication of their sensitivity.

Determination of the mean diameter of the seminiferous tubules, the random "hits" on different testicular areas, and the cell counts of pre-meiotic cells, cells in meiosis, and post-meiotic cells indicated that

spermatogenesis was enhanced in those animals receiving acetylsalicylic acid at the lowest treatment level. In contrast, the drug had an adverse effect on spermatogenesis at higher treatment levels.

The lowest treatment level (150mg/kg body weight for six days) of acetylsalicylic acid caused a significant increase ($p < 0.01$) in the mean numbers of pre-meiotic and post-meiotic cells. This suggests that either the rate of maturation and division of these cells was increased or that the number undergoing necrosis was decreased. Similar observations were made by Abbatiello et al (1975) after treatment of mice with aspirin or indomethacin.

The treatment period was of sufficient duration to significantly increase ($p < 0.01$) the production of preleptotene spermatocytes and spermatids. Preleptotene spermatocytes develop from spermatogonia over a period of 111 hours (Leblond and Clermont, 1952). An increased rate of maturation and subsequent mitotic division of the precursor spermatogonial cell types during the six day treatment period would therefore account for the number of pre-meiotic cells. Similarly, the significant increase in the numbers of spermatids may reflect an increase in the rate of: a) maturation of the primary spermatocytes from pachytene stage to diakinesis, and/or b) division of the primary and secondary spermatocytes. This increased number of cells could not be attributed to a decrease in the population of necrotic cells or to changes in their distribution. That the number of pachytene spermatocytes were not significantly increased indicated that the maturation rate of primary spermatocytes from the preleptotene stage to the pachytene stage was not increased after treatment with the drug.

Histological study of testicular cross-sections did not reveal any alterations which would parallel the quantitative increase in certain cell types, i.e., crowding of cells or enlargement of the seminiferous

tubules. It is likely that there was not a sufficient increase in cell numbers to produce prominent morphological changes.

Acetylsalicylic acid had an adverse effect on the cells of the germinal epithelium when the drug was administered either for extended periods or at higher dosages. In most cases, the mean number of each cell type was decreased compared to the corresponding control value. In those animals that were treated with ASA for 12 days at a dose of 150mg/kg body weight, the numbers of pre-meiotic cells, cells in meiosis, and post-meiotic cells were significantly decreased ($p < 0.01$) compared to the controls. This is indicative of an overall depression of spermatogonial mitotic division as reflected in the decreased numbers of preleptotene spermatocytes. A depression in the formation and maturation rate of the primary spermatocytes resulted in fewer spermatids being formed. Furthermore, histological study indicated that the damage was more severe at stages other than that in which cell counts were done, particularly stages XII-XIV and I-II. These stages are also associated with mitotic peaks of the type A spermatogonia and spermatocytes (Clermont and Hermo, 1975). The necrotic nuclei consistently seen along the luminal border of the germinal epithelium in stage IX tubules may reflect an increased rate of necrosis of the spermatocytes and spermatids in animals that were treated with the drug.

One could anticipate that because of cell loss, the thickness of the germinal epithelium would be reduced. However, the random "hits" on the germinal epithelium showed that it was thickened. Taking into consideration the mean tubular diameters, the observed increase in thickness of the germinal epithelium might have been caused by shrinkage of the seminiferous tubules with concurrent loss of cells; in fact, the tubular diameters were significantly decreased ($p < 0.01$)

in those animals that showed significant loss of germinal cells.

Administration of acetylsalicylic seemed to affect the testes in a dose-dependent manner. It enhanced spermatogenesis when it was administered at a lowest treatment level, and at the higher treatment levels spermatogenesis was generally inhibited. Bartke et al (1973) found an increased rate of spermatogenesis in mice that were treated with prostaglandin; this is partially in agreement with the present findings.

Within the groups of animals receiving the higher treatment levels, significant alterations were found in the germinal epithelium of the testes of animals treated with ASA for a period of 12 days at doses of 150mg/kg body weight and at doses of 300mg/kg body weight for six days; such extensive lesions were not present in the testes of those animals that received the drug at the highest treatment level (300mg/kg body weight for 12 days). Furthermore, in only this group of animals was the mean diameter of the seminiferous tubules significantly increased ($p < 0.05$), compared with the control value. The thickness of the germinal epithelium and the size of the lumina were also significantly altered ($p < 0.05$) as would be expected if the tubules were enlarged. The results of administration of the drug at the highest treatment level to the animals indicate that at higher dosages (similar to those used by Abbatiello et al, 1975) inhibitors of prostaglandin synthesis may indeed enhance spermatogenesis.

In most cases, the mean weights of other organs (epididymides, seminal vesicles, kidneys, and adrenal glands) derived from animals that were treated with acetylsalicylic acid were not significantly altered compared to the corresponding control values. Furthermore, histological examination of these organs did not reveal any changes that could be directly attributed to treatment with the drug.

5.2 HISTOCHEMICAL STUDY

Acid and alkaline phosphatases are hydrolytic enzymes that split phosphate esters; the former enzymes are optimally effective under acidic conditions, the latter are active under basic conditions.

Acid phosphatase enzymes are concentrated in the lysosomes of various tissues in the rat (Straus, 1967). The lysosomes originate from the Golgi apparatus or the endoplasmic reticulum and are involved in atrophy, necrosis and in the disposal of insoluble material (Pearse, 1968).

In the testis, the presence of acid phosphatase activity suggests that lysosomes may be involved in mitosis and meiosis (Allison, 1969) as well as changes in spermatid morphology (Blackshaw, 1973). Niemi and Kormano (1965) found that variations in the activity of the enzyme in the germinal epithelium of the testis reflected different stages of the cycle. Enzyme activity was distributed throughout the seminiferous lining, probably associated with the Golgi zone in the spermatogenic cells; in stage VII tubules, the activity was concentrated along the luminal border where spermatozoa were being released.

Acetylsalicylic acid, an inhibitor of prostaglandin synthesis, did not alter the activity of acid phosphatase in most of the tissues derived from animals that were treated at the lowest level (150mg/kg body weight for six days), although the testes of these animals revealed an increased rate of spermatogenesis. The activity of acid phosphatase was slightly increased in the testes of those animals that

were treated for a period of 12 days with different dose-levels of the drug. These animals also demonstrated a loss of spermatogenic cells, but this was not associated with any detectable increase in local enzyme activity. In contrast, Blackshaw and Hamilton (1970) found a great increase in acid phosphatase activity in the spermatocytes following application of heat to the testes. The effect of acetylsalicylic acid on the germinal epithelium might have been minimal, without directly leading to necrotic changes.

In the other tissues, the activity of acid phosphatase was generally decreased or absent at the lower treatment levels, whereas treatment with the drug at the highest dose level caused a slight to moderate increase in activity in the adrenal glands and the seminal vesicles. The latter revealed a moderate increase in activity of the enzyme, particularly along the luminal border of the epithelium. This gland was found to be a rich source of prostaglandins (Hamberg and Samuelsson, 1966). The increased activity of the enzyme may therefore reflect alterations in the secretory activity of the gland and in prostaglandin release after treatment of the animals with acetylsalicylic acid.

Alkaline phosphatase, a hydrolytic enzyme which is effective under basic conditions is localized in the Golgi apparatus and lysosomes in cells of different tissues (Cohn and Hirsch, 1960; Binkley, 1961; Persijn, 1961). In addition to its hydrolytic functions, alkaline phosphatase activity has been associated with uninhibited protein synthesis in the liver (Persaud et al, 1970).

In those animals that received acetylsalicylic acid at the lowest treatment level, alkaline phosphatase activity in most tissues was not altered; the testis, however, showed a slight decrease in activity. In the groups of animals treated with the drug at higher dose levels, enzyme activity was generally slightly to moderately decreased. The activities of alkaline phosphatase and acid phosphatase showed opposite trends in most tissues studied. It would therefore appear that the loss of spermatogenic cells and other morphological changes observed in the testes of animals treated at the higher dose levels are associated with increased levels of acid phosphatase, but a decrease in the activity of alkaline phosphatase. The implications of these findings are not clear at the present time.

Cytochrome oxidase, the terminal cytochrome of the respiratory chain, is a protein capable of transferring electrons to molecular oxygen. This enzyme and others involved in electron transport and oxidative phosphorylation are located in the inner membrane of the mitochondria.

In the testis, cytochrome oxidase activity was not affected in those animals that received acetylsalicylic acid at the lowest treatment level. However, there was a slight increase in enzyme activity in animals that were treated with the drug at higher levels (150mg/kg body weight for 12 days and 300mg/kg body weight for six days) and showed significant changes in the germinal epithelium, including loss of spermatogenic cells. Also, the activity of cytochrome oxidase in the kidneys was moderately increased following treatment with acetylsalicylic acid at a dose of 300mg/kg body weight for 12 or six days. This increase in activity of cytochrome oxidase induced by acetylsalicylic acid indicates that the rate of reduction of molecular oxygen may be altered.

CONCLUSIONS

CONCLUSIONS

Histological and histochemical study of the male reproductive tract of rats treated with acetylsalicylic acid (ASA), an inhibitor of prostaglandin synthesis, revealed the following:

a) Low daily doses of ASA (150mg/kg body weight), administered for six days, caused an increase in the rate of spermatogenesis. This treatment did not affect the activity of acid phosphatase, alkaline phosphatase or cytochrome oxidase in the testis. In the other tissues studied (epididymides, ductus deferens, seminal vesicles, adrenal glands and kidneys) there was no significant morphological or enzyme histochemical changes.

b) Treatment of the animals with acetylsalicylic acid at a higher dose level (300mg/kg body weight for six days) or for a longer period (150mg/kg body weight for 12 days) impaired spermatogenesis, as revealed by decreased numbers of spermatogenic cells and tubular shrinkage. Enzymatic activity was slightly altered as a result of the treatment, although the change could not be related to cell necrosis occurring in the germinal epithelium.

c) The results of treatment at different dose levels indicated that the spermatogonia, primary spermatocytes and secondary spermatocytes were primarily affected by the drug. Low dose levels (150mg/kg body weight) administered over a period of six days increased the rate of differentiation and division of these cells. At higher dose levels

(150mg/kg body weight for 12 days; 300mg/kg body weight for six days), the drug produced cellular necrosis.

d) Administration of acetylsalicylic acid caused shrinkage of the seminiferous tubules (except in those animals treated at the highest treatment level). This was particularly marked in those animals which exhibited a significant loss of spermatogenic cells (group III, 150mg/kg body weight for 12 days). It is not clear whether tubular shrinkage or loss of spermatogenic cells represents the primary effect of the drug.

e) At the highest treatment level (300mg/kg body weight for 12 days) acetylsalicylic acid did not significantly alter the number of spermatogenic cells; the tubular diameters were significantly increased, but morphological changes were not evident. It would therefore appear that over the extended treatment period (12 days), reparation of tissue damage had occurred.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abbatiello, E.R., Kaminsky, M., and Weisbroth, S. (1975). The effect of prostaglandins and prostaglandin inhibitors on spermatogenesis. Int. J. Fert. 20 (3), 177-182.

Abercrombie, M. (1946). Estimation of nuclear population from microtome sections. Anat. Rec. 94, 239-247.

Aiken, J.W. (1974). Prostaglandins and prostaglandin synthetase inhibitors: Studies on uterine motility and function. In, Prostaglandin Synthetase Inhibitors (eds., Robinson, H.J. and Vane, J.R.). Raven Press. pp. 289-301.

Allison, A.C. (1969). Lysosomes in cancer. In, Lysosomes in Biology and Pathology, Vol. 2 (eds. Neuberger, A. and Tatum, E.L.). North Holland Publishing Co.

Bartke, A., Musto, N., Caldwell, V. and Behrman, H.R. (1973). Effects of a cholesterol esterase inhibitor and of prostaglandin $F_{2\alpha}$ on testis cholesterol and on plasma testosterone in mice. Prostaglandins 3, 97-104.

Bergström, S., Duner, H., von Euler, U.S., Pernow, B. and Sjövall, J. (1959). Observations on the effects of infusion of prostaglandin E in man. Acta physiol. scand. 45, 145-151.

Berman, H.J., Tangen, O., Ausprunk, D. and Collins, H. (1969). Prostaglandin E_1 inhibition of aggregation of hamster platelets. Bibl. anat. 10; 507-515.

Binkley, F. (1961). Purification and properties of renal glutathionase. J. biol. Chem. 236, 1075-1082.

Bishop, M.W.H. and Walton, A. (1960). Spermatogenesis and the structure of the mammalian spermatozoa. In, Marshall's Physiology of Reproduction Vol. 1, pt. 2 (ed. Parkes A.S.).

Blackwell, G.J., Flower, R.J. and Vane, J.R. (1975). Some characteristics of the prostaglandin synthesizing system in rabbit kidney microsomes. Biochim. biophys. Acta 398 (1), 178-190.

Blackshaw, A.W. (1973). Testicular enzymes and spermatogenesis. J. Reprod. Fertil. (suppl.) 18, 55-64.

Blackshaw, A.W. and Hamilton, D. (1970). The effect of heat on lysosomal enzymes and spermatogenesis in the rat testis. J. Reprod. Fertil. 22, 569-571.

Boczkowski, K. (1973). Male and female differentiation of the human gonad. Clin. Genet. 4, 213-219.

Brody, T.M. (1955). Uncoupling of oxidative phosphorylation as mechanism of drug action. Physiol. Rev. 7, 335-363.

Burstone, M.S. (1958). Histochemical demonstration of acid phosphatase with napthol AS-MX phosphates. J. nat. Cancer Inst. 21, 523-539.

Burstone, M.S. (1960). Histochemical demonstration of cytochrome oxidase with new amine reagents. J. Histochem. Cytochem. 8, 63-70.

Burstone, M.S. (1961). Modifications of histochemical techniques for the demonstration of cytochrome oxidase. J. Histochem. Cytochem. 9, 59-65.

Bygdeman, M., Fredricsson, B., Svanborg, K. and Samuelsson, B. (1970). The relation between fertility and prostaglandin content of seminal fluid in man. Fert. Steril. 21, 622-630.

Bygdeman, M., Green, K., Toppazada, M., Wiqvist, N. and Bergström, S. (1974). The influence of prostaglandin metabolites on the uterine response of $PGF_{2\alpha}$. A clinical and pharmacokinetic study. Life Sci. 14, 521-531.

Bygdeman, M. and Samuelsson, B. (1966). Analyses of prostaglandins in human semen. Clin. chim. Acta 13, 465-474.

Carmichael, H.A., Nelson, L., Chandra, V., Lyon, A., Cochran, K.M. and Russell, R.I. (1975). Proceedings: Inhibition of aspirin and taurocholic acid- induced gastric mucosal bleeding by prostaglandin 15 (R) 15 methyl-E_2 methyl ester. Gut 16 (10), 822.

Carter, D.C., Ganesan, P.A., Bhana, D. and Karim, S.M.M. (1974). The effect of locally administered prostaglandin 15 (R) 15 methyl E_2 methyl ester on gastric ulcer formation in the Shay rat preparation. Prostaglandins 5, 455-465.

Castracane, V.D., Tankenow, L.F. and Shaikh, A.A. (1975). The effect of inhibition of prostaglandin synthesis on ovarian hypertrophy in the rat. J. Reprod. Fertil. 44 (3), 473-479.

Cenedella, R.J. and Crouthamel, W.G. (1973). Effect of aspirin upon male mouse fertility. Prostaglandins 4, 285-290.

Chalkley, H.W. (1943). Method for the quantitative morphological analysis of tissue. J. nat. Cancer Inst. 4, 47-53.

Chandrasekhar, N. (1967). Inhibition of platelet aggregation by prostaglandins. Blood 30, 554.

Chaudhuri, G. (1975). Inhibition by aspirin and indomethacin of uterine hypertrophy induced by an IUD. J. Reprod. Fertil. 43, 77-81.

Chester, R., Dukes, M., Slater, S.R. and Walpole, A.L. (1972). Delay of parturition in the rat by anti-inflammatory agents which inhibit the biosynthesis of prostaglandins. Nature 20, 37-38.

Chiquoine, A.D. (1954). The identification, origin and migration of primordial germ cells in the mouse embryo. Anat. Rec. 118, 135-146.

Chobsieng, P., Naor, Z., Koch, Y., Zor, U. and Lindner, H.R. (1975). Stimulatory effect of PGE_2 on LH release in the rat: evidence for hypothalamic site of action. Neuroendocrinology 17 (1), 12-17.

Clermont, Y. (1958). Contractile elements in the limiting membrane of the seminiferous tubules of the rat. Exp. Cell. Res. 15, 438-440.

Clermont, Y. (1962). Quantitative analysis of spermatogenesis of the rat: a revised model for the renewal of spermatogonia. Am. J. Anat. 111, 111-129.

Clermont, Y. and Bustos-Obregon, E. (1968). Re-examination of spermatogonial renewal in the rat by means of seminiferous tubules mounted "in toto". Am. J. Anat. 122, 237-248.

Clermont, Y. and Hermo, L. (1975). Spermatogonial stem cells in the albino rat. Am. J. Anat. 142 (2), 159-175.

Clermont, Y. and Leblond, C.P. (1953). Renewal of spermatogonia in the rat. Am. J. Anat. 93, 475-501.

Clermont, Y. and Leblond, C.P. (1955). Spermiogenesis of man, monkey, ram and other mammals as shown by the "periodic acid- Schiff technique. Am. J. Anat. 96, 229-254.

Clermont, Y. and Mauger, A. (1974). Existence of a spermatogonial chalone in the rat testis. Cell Tissue Kinet. 7, 165-172.

Cohn, Z.A. and Hirsch, J.G. (1960). The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leucocytes. J. Exp. Med. 112, 983-1004.

Collier, J.G. and Flower, R.J. (1971). Effect of aspirin on human seminal prostaglandins. Lancet ii, 852-853.

Croft, B.T. and Bartke, A. (1976). Quantitative study of spermatogenesis in vasectomized mice. Int. J. Fertil. 21, 61-64.

Crunkhorn, P. and Willis, A.L. (1969). Actions and interactions of prostaglandins administered intradermally in the rat and in man.

Csapo. A.I., Csapo, E.F., Fay, E., Henzl, M.R. and Salau, G. (1973). The delay of spontaneous labour by naproxen in the rat model. Prostaglandins 3, 827-837.

Dawson, W., Jessup, S.J., McDonald-Gibson, W., Ramwell, P.W. and Shaw, J.E. (1970). Prostaglandin uptake and metabolism by the perfused rat liver. Brit. J. Pharmacol. 39, 585-598.

DeJong, F.H., Hey, A.H. and Van Der Molen, H.J. (1973). Effect of gonadotrophins on the oestradiol-17β and testosterone by the rat testis. J. Endocr. 57, 277-284.

DeRooij, D.G. (1973). Spermatogonial stem cell renewal in the mouse. I. Normal situation. Cell Tissue Kinet 6, 281-287.

Dym, M. and Clermont, Y. (1967). Effects of x-rays on type A spermatogonia in the rat. Anat. Rec. 157, 238.

Eliasson, R. (1959). Studies on prostaglandin occurence, formation, and biological action. Acta physiol. scand. (suppl.) 158, 1-73.

Ferreira, S.H., Moncada, S. and Vane, J.R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature New Biology 231 (25), 237-239.

Glenn, E.M., Wilks, J. and Bowman, B. (1972). Platelets, prostaglandins, red cells, sedimentation rates, serum and tissue proteins and non-steroidal anti-inflammatory drugs. Proc. Soc. Exp. Biol. 141, 879-886.

Goldblatt, M.W. (1935). Properties of human seminal plasma. J. Physiol. (Lond.) 84, 208-218.

Gordon, J.L. and Macintyre, D.E. (1974). Proceedings: Inhibition of human platelet aggregation by aspirin in vitro and ex vivo. Brit. J. Pharmacol. 52 (3), 451P.

Greaves, M.W., Sondergaard, J. and McDonald-Gibson, W. (1971). Recovery of prostaglandins in human cutaneous inflammation. Brit. med. J. 2, 258-260.

Hamberg, M. and Samuelsson, B. (1966). Prostaglandins in human seminal plasma. J. biol. Chem. 241, 257-263.

Higgs, G.A. and Youlten, L.J.F. (1972). Prostaglandin production by rabbit peritoneal polymorphonuclear leukocytes in vitro. Brit. J. Pharmacol. 44, 330P.

Horton, E.W. (1969). Hypotheses on physiological roles of prostaglandins. Physiol. Rev. 49, 122-161.

Horton, E.W., Jones, R.L. and Marr, C.G. (1973). Effects of aspirin on prostaglandin and fructose levels in human semen. J. Reprod. Fertil. 33, 385-392.

Huckins, C. (1971a). Spermatogonial stem cell population in the rat. I. Their morphology, proliferation and maturation. Anat. Rec. 169, 533-558.

Huckins, C. (1971b). The spermatogonial stem cell population in adult rats. II. A radiographic analysis of their cell cycle properties. Cell Tissue Kinet. 4, 313-334.

Huckins, C. (1971c). Cell cycle properties of differentiating spermatogonia in adult Sprague-Dawley rats. Cell Tissue Kinet. 4, 139-154.

Huckins, C. and Kopriwa, B.M. (1969). A technique for the radioautography of germ cells in whole mounts of seminiferous tubules. J. Histochem. Cytochem. 17, 848-851.

Hunt, W.L. and Nicholson, N. (1972). Studies on semen from rabbits injected with ^{3}H-thymidine and treated with prostaglandin E_2 and $F_{2\alpha}$. Fert. Steril. 23. 763-768.

Jonsson, H.T. (1973). Stimulation of adenyl cyclase by prostaglandins in the rabbit corpus luteum. In, Prostaglandins and Cyclic AMP. Biological Actions and Clinical Applications (eds. Kahn, R.H. and Lands, W.E.M.). Academic Press Inc. pp. 183-186.

Jörgenson, T.G. (1976). Ulcer formation and histochemical changes in rat-stomach mucosa induced by acetylsalicylic acid. Acta path. microbiol. scand. 84 (1), 64-72.

Jost, A., Vigier, B., Prepin, J. and Perchellet, J.P. (1973). Studies on sex differentiation in mammals. Recent Prog. Horm. Res. 29, 1-41.

Kaley, G. and Weiner, R. (1971). Effect of prostaglandin E_1 on leukocyte migration. Nature New Biology 234, 114-115.

Kalla, N.R. and Bansal, M.P. (1975). Effects of carbon tetrachloride on gonadal physiology in male rats. Acta Anat. 91, 380-385.

Kaplow, L.S. (1963). Cytochemistry of alkaline phosphatase. Am. J. Clin. Path. 39 (5), 439-449.

Kaplow, L.S. and Burstone, M.S. (1964). Cytochemical demonstration of acid phosphatase in hematopoietic cells in health and in various hematological disorders using azo dye techniques. J. Histochem. Cytochem. 12, 805-811.

Karim, S.M.M. and Hillier, K. (1974). Prostaglandins: Pharmacology and clinical application. Drugs 8, 176-207.

Karnovsky, M.J. (1963). The fine structure of mitochondria in the frog nephron correlated with cytochrome oxidase activity. Expl. molec. Path. 2, 347-366.

Kloeze, J. (1966). Influence of prostaglandins on platelet adhesiveness and platelet aggregation. In, Prostaglandins, proceedings of Nobel Symposium 2. (eds. Bergström, S. and Samuelsson, B.). Almqvist and Wilsell, pp. 241.

Kuehl, J.A., Humes, J.L., Tarnoff, J., Cirillo, V.J. and Ham, E.A. (1970). Prostaglandin receptor site: evidence for an essential role in the action of luteinizing hormone. Science 169, 883-886.

Labhsetwar, A.P. (1974). Prostaglandins and the reproductive cycle. Fedn. Proc. 33 (1), 61-77.

Lau, I.F., Saksena, S,K. and Chang, M.C. (1973). Pregnancy blockade by indomethacin, an inhibitor of prostaglandin synthesis: its reversal by prostaglandins and progesterone in mice. Prostaglandins 4 (6), 795-803.

Leblond, C.P. and Clermont, Y. (1952). Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulfurous acid" technique. Am. J. Anat. 90 (2), 167-215.

Lindner, H.R., Zor, U., Bauminger, S., Tsafriri, A., Lamprecht, S.A., Koch, Y., Antebi, S. and Schwartz, A. (1974). Use of prostaglandin synthetase inhibitors in analysing the role of prostaglandins in reproductive physiology. In, Prostaglandin Synthetase Inhibitors (eds. Ribonson, H.J. and Vane, J.R.). Raven Press, pp. 271-288.

Macmorine, D.R.L., Movat, H.Z., Neta, R. and Takeuchi, Y. (1968). *In vitro* release of permeability factors and a slow reacting substance from phagocytosing PMN-leukocytes. Fedn. Proc. 27, 249.

Martinovitch, P.N. (1938). The development *in vitro* of the mammalian gonad, ovary and ovogenesis. Proc. R. Soc. Biol. 125, 232.

Merckle, U. (1957). Volumetrische befunde an den samenzellen und den Sertoli-zellen der ratte. Z. mikr.-anat. Forsch 63, 252-273.

Niemi, M. and Kormano, M. (1965). Cyclic changes in and significance of lipids and acid phosphatase activity in the seminiferous tubules of the rat testis. Anat. Rec. 151, 159-170.

Nugteren, D.H., Beerthuis, R.K. and Van Dorp, D.A. (1965). The participation of molecular oxygen in the biosynthesis of prostaglandins. Biochim. biophys. Acta 98, 654-656.

Oakberg, E.F. (1956). A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. Am. J. Anat. 99, 391-413.

Oakberg, E.F. (1971). Spermatogonial stem cell renewal in the mouse. Anat. Rec. 169, 515-532.

Ojeda, S.R., Harms, P.G. and McCann, S.M. (1975). Effect of inhibitors of prostaglandin synthesis on gonadotropin release in the rat. Endocrinology 97 (4), 834-854.

Orczyk, G.P. and Behrman, H.R. (1972). Ovulation blockade by aspirin or indomethacin - in vivo evidence for a role of prostaglandin in gonadotrophin secretion. Prostaglandins 1, 3-20.

Ortavant, R. (1959). Spermatogenesis and morphology of the spermatozoon. In: Reproduction in Domestic Animals, Vol. 2, Chap. 1 (eds. Cole, H.H. and Cupps, P.T.).

Pearse, A.G.E. (1968). Acid phosphatases. In: Histochemistry, Theoretical and Applied, Vol. 1. J. and A. Churchill Ltd. pp. 547-575.

Persaud, T.V.N. (1974). Inhibitors of prostaglandin synthesis during pregnancy. 2. The effects of indomethacin in pregnant rats. Anat. Anz. Bd. 136, 354-358.

Persaud, T.V.N. and Moore, K.L. (1974). Inhibitors of prostaglandin synthesis during pregnancy. 1. Embryopathic activity of indomethacin in mice. Anat. Anz. Bd. 136, 349-353.

Persaud, T.V.N. and Moore, K.L. (1975). Teratogenicity of indomethacin in mice. Tenth International Congress of Anatomy, Tokyo. pp. 128.

Persaud, T.V.N., Putzke, H.-P., Tessmann, D. and Bienengräber, A. (1970). Microscopic and enzyme histochemical studies on the liver and kidney of rats treated with fulvine, the toxic alkaloidal constituent of Crotolaria fulva. Acta Histochem. Bd. 37, 369-378.

Persijn, J.-P., Daems, W., de Man, J.C.H. and Meijer, A.E.F.H. (1961). Demonstration of adenosine triphosphatase activity with the electron microscope. Histochemie 2, 372-382.

Peters, H. (1976). Intrauterine gonadal development. Fert. Steril. 27 (5), 493-500.

Phelps, P. (1969). PMN leucocyte motility in vitro. II. Stimulatory effects of monosodium urate crystals and urate in solution. Partial inhibition by colchicine and indomethacin. Arthitis Rheum. 12, 189-196.

Piper, P.J. and Vane, J. (1971). The release of prostaglandins from lung and other tissues. Ann. N.Y. Acad. Sci. 180, 363-385.

Rainsford, K.D. (1975). The biochemical pathology of aspirin - induced gastric damage. Agents and Actions 5 (4), 326-344.

Ramwell, P. and Shaw, J. (1970). Biological significance of the prostaglandins. Recent Prog. Horm. Res. 26, 139-187.

Ramwell, P. and Shaw, J. (1971). The biological significance of the prostaglandins. Ann. N.Y. Acad. Sci. 180, 10-13.

Ratner, A., Wilson, M.C., Strivastava, L. and Peake, G.R. (1974). Stimulatory effects of PGE_1 on rat anterior pituitary cyclic AMP and luteinizing hormone release. Prostaglandins 5, 165-171.

Regaud, C. (1901). Etudes sur la structure des tubes seminiferes et sur la spermatogenese chez les mammiferes. Arch d'Anat. Micr. 4, 101-156.

Reyes, F.I., Boroditsky, R.S., Winter, J.S.D. and Faiman, C. (1974). Studies on human sexual development. II. Fetal and maternal serum gonadotropin and sex steroid concentrations. J. clin. Encodr. Metab. 38, 612-617.

Robinson, H.J. and Vane, J.R. (1973). Prostaglandin Synthetase Inhibitors. Their effects on physiological functions and pathological states. Raven Press.

Roosen-Runge, E.C. (1952a). Kinetics of spermatogenesis in mammals. Ann. N.Y. Acad. Sci. 55, 574-584.

Roosen-Runge, E.C. (1952b). The third maturation division in mammalian spermatogenesis. Anat. Rec. 112, 463.

Roosen-Runge, E.C. (1955). Quantitative studies on spermatogenesis in the albino rat. III. Volume changes in the cells of the seminiferous tubules. Anat. Rec. 123, 385-398.

Roosen-Runge, E.C. and Giesel, L.O. (1950). Quantitative studies on spermatogenesis in the albino rat. Am. J. Anat. 87, 1-30.

Samuelsson, B. (1963). Isolation and identification of prostaglandins from human seminal plasma. J. biol. Chem. 238, 3229-3234.

Samuelsson, B. (1972). Biosynthesis of prostaglandins. Fedn. Proc. 31, 1442-1450.

Samuelsson, B., Granström, E., Green, K. and Hamberg, M. (1971). Metabolism of prostaglandins. Ann. N.Y. Acad. Sci. 180, 138-163.

Skidmore, I.F. and Whitehouse, M.W. (1966). Concerning the regulation of some diverse biochemical reactions underlying the inflammatory response by salicylic acid, phenylbutazone and other rheumatic drugs. J. Pharm. Pharmac. 18, 558-560.

Smith, J.B. and Willis, A.L. (1970). Formation and release of prostaglandins by platelets in response to thrombin. Br. J. Pharmac. 40, 545P.

Smith, J.B. and Willis, A.L. (1971). Aspirin selectively inhibits prostaglandin production in human platelets. Nature New Biology 231 (25), 235-237.

Sondergaard, J., Helin, P. and Jorgenson, H.P. (1973). Human cutaneous inflammation induced by prostaglandin E_1. J. Path. 109, 239-243.

Sondergaard, J. and Wolf-Jürgenson, P. (1972). The cellular exudate of human cutaneous inflammation induced by prostaglandins E_1 and $F_{1\alpha}$. Acta Derm-Vener Stockh. 52, 361-364.

Stearns, E.L., MacDonnell, J.A., Kaufman, B.J., Padua, R., Lucman, T.S., Winter, J.S.D. and Faiman, C. (1974). Declining testicular function with age. Am. J. Med. 57, 761-766.

Straus, W. (1967). Lysosomes, phagosomes and related particles. In: Enzyme Cytology (ed. Roodyn, D.B.). Academic Press, pp. 239-306.

Sutherland, E.W., Oye, I. and Butcher, R.W. (1965). The action of epinephrine and the role of the adenyl cyclase system in hormone action. Recent Prog. Horm. Res. 21, 623-646.

Sutherland, E.W., Robinson, G.A. and Butcher, R.W. (1968). Some aspects of the biological role of adenosine 3', 5'-monophosphate (cyclic AMP). Circulation 37, 279-306.

Tso, C.F. and Lacy, D. (1975). Effects of $PGF_{2\alpha}$ on the reproductive system of the male rat. J. Reprod. Fertil. 44, 545-550.

Vane, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biology 231 (25), 232-235.

Vane, J.R. (1974). Mode of action of aspirin and similar compounds. In: Prostaglandin Synthetase Inhibitors (eds. Robinson, H.J. and Vane, J.R.) Raven Press, pp. 155-164.

von Euler, U.S. (1935). A depressor substance in the vesicular gland. J. Physiol. (Lond.) 84, 21P-22P.

von Euler, U.S. and Eliasson, R. (1967). Prostaglandins. Medicinal Chemistry, A series of monographs, Academic Press.

Williams, T.J. and Morley, J. (1973). Prostaglandins as potentiators of increased vascular permeability in inflammation. Nature (Lond.) 246, 215-217.

Willis, A.L. (1969). Release of Histamine , kinin and prostaglandins during carrageenin induced inflammation in the rat. In, Prostaglandins, Peptides and Amines (eds. Mantegazza, and Horton, E.W.). Academic Press, pp. 31.

Zor, A., Kaneko, T., Schneider, H.P.G., McCann, S.M. and Field, J.B. (1970). Further studies on stimulation of anterior pituitary cyclic adenosine 3', 5'- monophosphate formation by hypothalamic extract and prostaglandins. J. biol. chem. 245, 2883-2888.

Zucker, M.B. and Petersen, J. (1968). Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet functions by acetylsalicylic acid ingestion. Proc. Soc. Exp. Biol. Med. 127, 547-551.