CHAPTER IV

PHARMACOLOGICAL INVESTIGATIONS
INTRODUCTION
INTRODUCTION

The testis has a dual function of producing large numbers of spermatozoa in sexually mature males as well as secretion of androgens for the maintenance of male reproductive system, libido and potency. In a microscopic section of an adult testis, it can be seen that the seminiferous tubules, arranged in lobules contain many cells, the vast majority of which are in various stages of division; these are spermatogenic cells. In the rat, the long coiled tubules form palisades which open into an intra-testicular rete. The rete testis leads through a variable number of different ducts into the epididymis. The architecture of the seminiferous tubules provides the epithelium a large surface area and allows the long tubules to be packed compactly. In the connective tissue between the seminiferous tubules lie the endocrine-secreting interstitial cells (Leydig cells), fibroblasts and blood vessels. Each seminiferous tubule is surrounded by a basement membrane; these are undifferentiated germ cells called spermatogonia, which provide continuous source of new cells by mitotic division. Some spermatogonia move away from the basement membrane and increase markedly in size.
Each of these large cells, now termed a primary spermatoocyte, divides to form two secondary spermatoocytes, each of which divides into two spermatids. The latter ultimately transform into mature spermatozoa. This process is known as spermatogenesis.

The changes in the spermatids leading to the formation of spermatozoa constitute the process of spermiogenesis. The series of changes by which the spermatids are transformed into spermatozoa are divisible into a number of steps (numbered by Arabic numerals) on the basis of morphological criteria. These phases signify these steps - Golgi phase, cap phase, acrosome phase, and maturation phase.

**Hormonal Control of Spermatogenesis:**

Pituitary gonadotropins, FSH and LH, and testosterone secreted by the testes regulate spermatogenesis in mammals. The division of spermatogonia leading to the formation of spermatoocytes in the rat are apparently independent of action of gonadotropins while in the human, gonadotropins appear to be essential for stimulating synthesis and secretion of androgens by the Leydig cells. The androgens in turn stimulate spermatogenesis. In oligospermic, or hypophysectomised men, administration of human menopausal gonadotropin
Figure 1:
Summary of hormonal control of testicular function.
The negative signs indicate that testosterone inhibits LH secretion via both the hypothalamus and the anterior pituitary. Testosterone reaches the seminiferous tubules to stimulate spermatogenesis both by local diffusion and by release into the blood and recirculation to the testes. FSH influences spermatogenesis via an action on the Sertoli cells and is, in turn, inhibited by a substance, inhibin, released by the Sertoli cells.
(HMG), or human chorionic gonadotropin (HCG), or a combination of both results in the completion of spermatogenesis, indicating the role of FSH in stimulation of spermatogonial divisions and of HCG or LH in completion of spermatogenesis. Low doses of testosterone suppress spermatogenesis in rats by inhibition of pituitary gonadotropins while high dose of testosterone stimulates and maintains spermatogenesis in spite of inhibition of LH and FSH. See figure 1.

High concentrations of androgens in the testis are needed for the maintenance of spermatogenesis in the human male. While the action of LH on the Leydig cells has been established, the site of action of FSH is still not clear. Data from patients with oligozoospermia or non-obstructive azoospermia, or other conditions in which seminiferous epithelium is depleted of its cellular elements, show elevated levels of FSH, suggesting correlation between FSH and spermatogenic activity. FSH stimulates protein synthesis in the testis which may be a sequel to binding of FSH to plasma membranes of Sertoli cells resulting in the elevation of cyclic AMP and protein kinase. FSH also stimulates the production of an androgen-binding protein which is formed in the Sertoli cells.
and is secreted into the lumen of the seminiferous tubule. The stimulation of secretion of secretion of androgen-binding protein by FSH may increase the transport of androgen from the intertubular, testicular lymph and make it available for action on the seminiferous epithelium and also for transport into the epididymis. 1-3

The accessory reproductive organs that offer a passage as well as a site for transient storage of spermatozoa released from the testis are the epididymis and the ductus deferens. A number of glands are associated with the reproductive tract and secrete the seminal plasma, a fluid medium for transporting spermatozoa into the female tract during ejaculation. These glands are of two types. One, those arising embryologically from the Wolffian duct (ductus deferens), i.e. the ampullary glands and seminal vesicles; two, those arising from urinogenital sinus or urethra, i.e. prostate and Cowper’s glands. Usually the embryological and anatomical association is retained in the adult. The seminal vesicles open with the ductus deferens to form an ejaculatory duct or the two may independently open into the pelvic urethra. The prostate empties through multiple ducts into the prostatic urethra. Cowper’s
glands open into the urethral bulb. In addition, the mucus secreting glands of lillre open into the urethra along its length while the preputial glands secrete into the prepuce.

Mammalian spermatozoa, released from the testis, undergo maturation or a series of morphological and physiological changes during their transit through the epididymis and acquire the characteristic pattern of motility and ability to fertilize ova. The epididymis is a long coiled duct which opens proximately into the efferent ducts and continues distally as the ductus deferens. The epididymis is divisible into caput, corpus and cauda epididymides or the initial, middle and terminal segments. The different segments have specialised group of cells which perform secretory and absorptive functions of this organ. The major biochemical constituents synthesized and/or secreted by the epididymis include glycercy1phosphorylcholine (GPC), lipids, carnitine, sialic acids, proteins and steroids. GPC is believed to maintain the osmotic balance in the lumen of the epididymis of the epididymis. Sialic acids, free or bound to proteins, are secreted by epididymis of a number of mammals. Sialic acid may be involved in the maint-
ence of ionic balance in the epididymis, in antigenic interactions between the epididymis and spermatozoa, and in the maturation of spermatozoa and maintenance of structural integrity of the membranes of spermatozoa.

The concentration of phospholipids is high in the caput epididymis and decreases to a considerable extent as the spermatozoa migrate through the epididymal canal. The phospholipids may either serve as a substrate for spermatozoa during their epididymal maturation or as a source of epididymal GPC.

Carnitine occurs in the epididymis; its concentration is highest in the cauda epididymides. The epididymis does not synthesize carnitine but accumulates and concentrates in the epididymal plasma from the blood. Sperm from the testis contain very little amounts of carnitine but accumulate it to reach a maximum in the cauda epididymides and this may be related to the maturation and consequent development of fertilizing ability by the spermatozoa. Carnitine may be involved in energy metabolism of sperms by oxidation of fatty acids.

The epididymis provides a favourable milieu for storage
of sperms in a motile and fertilizable state. The structural and functional integrity of the epididymis is dependent on androgens. However, the epididymis has a higher threshold requirement of androgens than the other accessory organs for the maintenance of its functional integrity.

The epididymis receives androgen both from the peripheral circulation and also directly from the testicular fluid entering the epididymis through the efferent ducts. Testicular androgens may be transported into epididymis either as free testosterone or bound to an androgen-binding protein of testicular origin which transports androgen to the epididymis. Testosterone is metabolised to 5-dihydrotestosterone or 5-androstanediol in exerting its action on the epididymis.4

The accessory glands secrete a variety of chemical substances and contribute to the formation of seminal plasma. The seminal plasma provides an ionically balanced nutritive milieu for the survival of sperm in the female reproductive tract. The chemical composition of the glandular secretions has been studied extensively by Mann (1964).5

The testes, in addition to their function of providing
spermatozoa, manufacture steroid hormones which control secondary sexual characteristics, the reproductive cycle and the growth and development of the accessory reproductive organs, excluding the testes. The sex organs also exert potent protein anabolic effects.

Of the number of differently naturally occurring androgens, testosterone, the principal male hormone, is the most potent, followed by dihydrotestosterone, androstenediol, androstenedione, dihydroepiandrosterone and androsterone in order of their potency.

Testosterone is synthesized by the interstitial (Leydig) cells of the testes from cholesterol through pregnenolone, progesterone, and hydroxyprogesterone, which is then converted to the C-19 ketosteroid, androstenedione, the immediate precursor of testosterone. Pregnenolone is found to be a common precursor of the testosterone, adrenocortical hormones and progesterone, as suggested by reactions in the biosynthetic pathway in the adrenal that is responsible for the formation of androgenic (C-19) steroids.

About 99% of the testosterone circulating in the plasma is bound to specific plasma protein (testosterone-binding globulin, TBG). In the normal male, 4-12mg of testosterone are secreted per day. Direct measurement of testosterone in plasma by isotope dilution or radioreplacement assay
indicates that about 0.6μg/dL is present in the normal male. Androgens circulate in blood, in bound as well as unbound forms; the unbound form is physiologically active. Although testosterone is bound to albumin, this binding is weak and non-specific compared to binding with β-globulin which is pronounced in the female.

The increase in size of the penis, scrotum and testes at the same time of puberty is promoted by androgens. Accompanying the testicular growth the sack of the scrotum becomes elongated and pendulous, and rugal folds appear in the scrotal skin. The penis and scrotum become pigmented.

Androgen stimulates the growth and secretory activities of the secondary sex organs such as epididymis, vas deferens, prostate and seminal vesicles. The maturation of spermatозoa during the passage through the epididymis, where they acquire motility and fertilizing ability is also androgen-dependent.

Androgen produces a more aggressive attitude and stimulates libido and sexual potency. Psychosexual development is a very gradual process. Although it becomes accelerated at puberty it begins well before sexual maturity.⁵,⁷
Deficient Androgen Secretion:

Chemically, androgen deficiency is encountered more often than the excess. Deficiency may arise from primary defect in the testis and Leydig cells, secondary defect in the testis or as a result of more generalised endocrine disorder. Primary defects are encountered following castration and in cases of anorchia and bilateral cryptorchidism, in some cases of Clinefelter's syndrome, oligo and azoospermia, and leprosy. Secondary defects may be associated with hypogonadotrophic hypogonadism, pan-hypopituitarism, cirrhosis of the liver, and haemochromatosis.

Clinical manifestations of androgen deficiency varies depending upon degree of failure and the time of its onset. Following pre-puberal castration in man the secondary sex organs including penis, scrotum, prostate, seminal vesicles and epididymis fail to develop. Muscular strength diminishes significantly, and the body growth may be eunuchoid. Varying degrees of these manifestations are observed in other forms of hypogonadism. The testicular deficiency could be selectively gametogenic or both gameto- and androgenic. In the former the individual is sterile, but has normal secondary sex characters; in the second case both the functions are affected.

Failure of testes before puberty causes infantile penis,
feminine distribution of fat (more on buttocks, breast and hips) and arrest of laryngeal and hip growth. The muscles become soft, skin becomes smooth and there is a lack of sexual drive.

In a patient with post-puberal hypogonadism, the general body proportion and size of the penis are not affected. But the body hair become sparse and silky, voice becomes high pitched and libido is generally reduced. Skeletal muscles become flabby and weak and there is a tendency to develop obesity. The volume of semen is markedly decreased and there is a change in the emotional get up, anxiety, fear and inferiority complex replacing the original confidence and vigour.

Administration of testosterone in prepuberal castrated individuals reverses most of the changes seen in hypogonadism. The secondary sex characteristics develop, muscle strength is increased and sexual desire and erections occur normally. Androgen is shown to maintain spermatogenesis in hypophysecotomised rats.

The androgenic activity of new compound is assayed by observing growth of a capon comb of a newly hatched chick. The other method involves the measurement of growth of the seminal vesicles, or the prostate in castrated rats.
Androgen Therapy:

1. **Hypogonadism**: In conditions of androgen deficiency arising from either primary or secondary defects in the testis, androgen therapy is beneficial. As mentioned earlier, such conditions are encountered following castration, and in anorchia, hypogonadotropic hypogonadism, eunuchoidism and panhypopituitarism.

2. **Impotence**: Both sex drive and erectile function of man are sustained by a combination of hormonal and psychic forces. Most often it is difficult to ascertain whether a hormonal deficiency exists in cases of impotency. A course of trial therapy with androgen is perhaps the best procedure in questionable cases. Failure to respond to an adequate dose of androgen given for two or three weeks rules out fairly well, androgen deficiency as the cause of the disorder. A positive response to such therapy however, does not establish the diagnosis unless one excludes the effect of suggestion by appropriate placebo therapy.

3. **Sterility**: Although it has been demonstrated in animals that androgen has a direct stimulatory action on spermatogenesis, administration of androgen in therapeutic doses causes inhibition of FSH secretion, thus causing further suppression of sperm production. Administration
of relatively high doses of androgen for a rebound phenomenon has been found beneficial in certain oligospermic individuals. Another form of effective therapy is administration of androgen along with gonadotropin.

If administered immediately after hypophysectomy, the hormone will prevent tubular atrophy and will maintain spermatogenesis. The evidence is therefore valid that at least in this animal androgen plays a part in the sustenance of the testicular tubules. In the normal human male, it has been established that the ordinary therapeutic doses actually depress the sperm count. It is probable that, although a normal amount of circulating androgen is beneficial and certainly not harmful to the tubular epithelium, the excess amount which accrues from injected hormone added to the normal endogenous supply may suppress the secretion of pituitary gonadotropic hormone (FSH) which normally serves to support tubular integrity.

If occasional cases of sperm inadequacy exist which are due to an inadequate supply of androgen it would be anticipated that male hormone would be beneficial. One would expect this type of case to be characterized clinically by the usual eunuchoid features which are associated with androgen deficiency. There have, in fact, been several
cases of hypogonadotropic eunuchoidism reported in which androgen therapy has been attended by the appearance of sperm in the ejaculate. In some of these patients spermatogenesis was known to have been absent before therapy. Whether in these cases the androgen exerts a direct effect on tubular maturation or simply acts as a trigger to initiate pituitary activity cannot be said with certainty. In other instances, where spermatogenesis has persisted after therapy has been withdrawn, a trigger mechanism is suggested. There are several cases on record in which low sperm counts were seen to rise during therapy in individuals who were not described as frankly eunuchoid. Such an effect, however, has not been commonly observed.

Heller, Heckel, and co-workers have demonstrated a remarkable rebound of spermatogenesis when androgen was given to men who showed oligospermia but whose endocrine status had appeared normal. During the period while testosterone propionate (LXXXIX) was being given in doses of approximately 50 mg. three times a week for 1 to 3 months the sperm count was further reduced to levels of azoospermia, and biopsies revealed a more severe derangement of germinal elements than before. There was tubular necrosis with hyalinization, and disappearance of Leydig cells. Gonadotropin excretion declined. Within 5 to 6 months after discontinuance of androgen the sperm counts rebounded
far above pretreatment levels in approximately half the cases, whereas testicular biopsies showed not only recovery from the depression which attended androgen administration but also an appearance often much improved over that of the control specimens. The improvement was still apparent two years later, in some instances, but in others recession occurred several months after the peak of the rebound.

**Fertility Affecting Factors:**

Several possible changes in the male sex organs and reproductive physiology as a whole can discount on or severely impair fertility, many of which can, however, be reversed by restorative methods. Such changes as follows also form the basis for artificially preventing fertility in man:

I. **Interference with sperm survival**
   a. Prevention of maturation process in epididymis
   b. Prevention of function of accessory glands
   1. Prevention of androgen action on accessory glands
   2. Prevention of formation of accessory-gland secretion
   3. Prevention of accessory-gland secretion from entering urethra

II. **Interference with testicular function**
   a. Prevention of androgen action on seminiferous tubules
   b. Prevention of action of FSH on seminiferous tubules
   c. Prevention of sperm division
III. Interference with hypothalamic or pituitary function

a. Prevention of FSH secretion

b. Prevention on action of Gn-releasing hormone on pituitary

c. Prevention of secretion of Gn-releasing hormone

d. Use of inhibin to inhibit FSH secretion 8-10
REFERENCES:


CHROMOSOMAL STUDIES
AND
ACD 50 DETERMINATION
Chromosomes are discrete basophilic bodies observed in the dividing cell nuclei. During the final stages of division -- metaphase and anaphase -- they undergo contraction, thus facilitating visualisation by conventional staining techniques. Chromosomes appear as intensely basophilic bodies of a characteristic shape as reflected by kinetochore position (De Robertis, 1960). It is now unequivocally established that the chromosome number is constant for a given species and the primary units of inheritance (gene, cistron, mucon, recon) are composed of DNA arranged in a linear array along the length of the chromosomes (Benzer, 1957).

Complex chemical and physical analysis of isolated chromosomes and nuclei indicates that DNA is ever bound to a basic protein, histone and thus exists as nucleoprotein (Daly et al., 1957). About 92% portion of the chromosome is constituted of nucleoprotein and the remainder is rather ill-defined nucleoprotein containing a mixture of residual protein (Mitsky and Ris, 1974). Histone is considered to be mainly responsible for aggregation of DNA through histone bridges (Wilkins and Zubay, 1959). Such aggregation paves the way for comprehending the structure of chromosome as a supermacromolecule. Genetic specificity is believed to be determined by purine and pyrimidine base sequence (Watson and Crick, 1954).
The squash technique of Heitz (1936)\(^7\) renders the chromosomes distributed in a single plane of focus without significant overlapping. Reconstruction in three dimensions from observations taken at different plane of focus characterise the entire chromosome complement of a cell.

A chromosome is regarded as a nuclear component, having a special organisation of individuality and function. It maintains its morphological and physiological properties as a result of successive cell divisions and is capable of cell reproduction.

**Mutagenesis:**

Mutagenesis refers to the irregular breakage and rearrangement in the chromosomes. Spontaneous rearrangements include naturally occurring changes while the induced rearrangements include the induction of chromosomal change by certain external agents. The chromosomal change or the aberrations may bring about changes in the phenotype and in the expected genetic rations, which in the present context of study of role of plant compounds in promoting fertility are undesirable.

Recent concepts categorise external agents into two, weak agents and strong agents. Weak mutagenic activity of agents is a major problem area, as the compounds of widespread
use by the human population are generally weakly mutagenic. To demonstrate weak mutagenic activity is difficult and attempts to do so can lead to controversy between scientists. For example, caffeine, a weak mutagen, produces conflicting effects in a variety of individuals. Susceptibility varies from species to species.

Of late, to test mutagenicity Carrano et al. (1978)\(^8\) have provided that sister chromatid exchange (SCE) could be an indicator of mutagenesis. According to them, a number of SCEs seen in cells after treatment with a range of known mutagens may be directly related to genetic damage and mutation rate. Sister chromatid exchange can be seen in chromosome studied at the metaphase stage of cell division. At this stage, each chromosome is made up of two equal chromatids which arise when the chromosomal DNA is replicated at the synthetic(s) phase of the cell cycle, a few hours before cell division starts. Any exchange between the two sister chromatids would mean that DNA breakage and repair must have taken place in between (S) phase and metaphase. Alkylation agents such as ethylmethane sulfonate, mitomycin C or nitrogen mustard which can also induce chromosomal damage, considerably increase the number of SCEs per cell at the concentration which is significant for morphological damage. This evinces that SCE number is a sensitive test for assessing chromosomal damage.
Before describing the implications of mutagenesis it is necessary to perceive the fate of an external agent administered into the body. When an external agent or the drug is administered the relationship between dose and response yielded is generally an exponential one. The response varies with the dose, but as a rule, the response varies directly with the logarithm of the dose. The logarithm of the response can give a linear relationship. If data from an individual upon which drugs act are plotted, an exponential curve is yielded. The curve is readily relevant to well known Freundlich's Isotherm,

$$KC^{1/n} = \frac{X}{M}$$

in which $K$ is constant, $C$ is the concentration of substance absorbed, $n$ also a constant, $X$ the quantity absorbed and $M$ the weight of the absorbing substance. The logarithm form of the above equation becomes:

$$10g K + 1/n \log C = 10g X - 10g M$$

The $10g X/M$ plotted against $10g C$ gives a linear relationship in true absorption. It is important to be acquainted with the relationship existing between drug dosage and response which may decrease with increasing concentration and follow an exponential pattern. Most often, when small uniform increases in drugs are administered, no significant response
is observed until the total amount of drug attains concentration termed the threshold level. Above the threshold level, a quantal response is observed with each additional increment in dose concentration, until a maximal response is observed which remains unaltered with further administration of the drug. A plot of the response results in the sigmoid or S-shaped curve. The potency of drugs may be compared by determining the concentration of drug required to produce 50% of maximal response. The minimal effective dose is that which is sufficient to produce a minimal response; the effective dose (ED$_{50}$) is that amount necessary for a response of fifty per cent maximal. The drug toxicity could be assessed by therapeutic index.

\[
\frac{LD_{50}}{ED_{50}} = \text{Therapeutic index}
\]

The amount of drug that is lethal to 50 per cent of the individuals is defined as the LD$_{50}$ dose. When the therapeutic index is low, then it should be utilised cautiously. No two animals will respond exactly alike to the same drug dose.

**Response Difference and Causes:**

**Mechanism of chemical and drug action** -

Various factors responsible for reactions within a cell
obey certain physico-chemical laws. Most of the cell's life within comparatively narrow limits of their thermodynamic environment. They are thermolabile. They are sensitive to small changes in hydrogen ion concentration. Their membranes exhibit a higher degree of selectivity for ions and potential foodstuffs. The phenomenon of absorption renders its influence over the actual passage of external agent. Before inducing external agent one must also consider the relative magnitude of the cell on which the molecule of the drug acts. The molecular weight of any compound expressed in grams contains $6.02 \times 10^7$ molecules.

The presence of active patches on the surface of cells has now been generally accepted. The absorption of drug molecules on these prosthetic protoplasmic groups is one of the most plausible explanations of drug action. The activity is confined to certain active patches on the cell surface upon which the molecules act. The capacity of an external agent to exert its action when bound to a receptor site has been referred to, by Ariens (1957)\(^9\) as the intrinsic activity of the drug. A drug may antagonize the action of a second external agent by occupying nearly all the cell receptor sites (receptor affinity) and yet exert little action of its own because of its lack of intrinsic activity. Beutner (1933)\(^10\) indicated that the membrane potential
established between the cell and its environmental fluid is responsible for stimulation or depression.

The toxic external agents which are administered into the body may undergo biochemical transformation within the cell and hence may get detoxified eventually. Biotransformation and detoxication of drugs are mostly mediated by the catalytic action of enzymes. Since the synthesis and activity or the possible repression of enzymes are controlled by gene action, the variation in genetic information is the basis of differing sensitivity to drugs. Simultaneously pattern of excretion of the external agent induced, varies from species to species. These are excreted either as unchanged or as products of oxidation, reduction, hydrolysis and as conjugated products. The relative proportions of these forms excreted depend on the nature of the drug, the species of animal.

Cellular response to drugs is a complex study. A few chemicals and drugs can produce a degree of cellular change such as to provoke chromosomal damage leading to marked change in cellular function or behaviour. Many chemicals and drugs cause stimulation at a low tissue concentration but cellular depression at higher dosage. The external agents may stimulate cellular activity at concentration that produce no irreversible alteration in cell function. The protein synthesis and the genetic code have a very close relationship. A slight disruption of the chromosomal body results in the
disruption of genetic code. DNA molecules carry the genetic code of protein synthesis for the 20 common amino acids and direct their sequence in protein structure. Some of the active external agents have the capability of breaking up the genetic code of protein synthesis. The studies of Handschumacher and Welch (1960) have shown that some drugs block the nucleic acid synthesis essential for protein synthesis and cell proliferation. It is notable that during the early period of gestation, when cell proliferation in the foetus is occurring at a rapid rate, the use of thalidomide as a mild hypnotic drug in pregnant women caused the births of thousands of babies with deformities (Phocomelia). In all probability the drug was responsible for the disruption of the normal genetic code.

The studies on the chromosomes and the subsequent production of aberration with external agents have been further enriched (since 1930s) by Painter (1934), Peto (1935), Levan (1939a), Calvin et al., (1940), Derman (1940 & 1941), Kodani (1942), Barber and Callan (1943), Auerbach and Robson (1946), Haggquist (1948), Levan and Tjio (1948), Darlington (1950), Oehlers (1953), Von Rosen (1954), Kihlman (1955), Nambiar (1955), Novick (1956), Ehrenberg and Gustafsson (1957), Andrew (1959), Lerman (1961), Ray-Chaudhuri (1961), Cattanach (1962), Shankel (1962), Manna and Parida (1965), Kihlman (1966), Manna and Mukerjee (1966), Lobbecke (1967),

**METHODOLOGY**

For the rapidity of fixation and rapidity of handling the gonad tissue, squash method has been given priority. It is quite easy to examine single layers of cells in their totality with efficiency. In instantaneous squashes prepared mainly for assessing the drug affected chromosomes, the acetic-orcein stain recommended by La Cour (1947) proved quite good for rat tissue. The edges of the cover slip touched with acetylglycerine let a slide material remain intact for quite a long time.

Apart from the squash preparation, the paraffin sectioning of the embedded tissue was also done, but it has not given good results. However, besides the acetic stains which
act as combined stain fixatives, basic dye has been suitably employed in the experimental studies.

The leuco-basic fuchsine method first developed by Feulgen and Rossenbeck (1924)\textsuperscript{62} as a microchemical test for the deoxyribosenucleic acid (DNA) found in the chromosomes (Gullick, 1940)\textsuperscript{63} depending upon Schiff's aldehyde reaction gives a translucent and permanent stain to the chromosomes. Liberated aldehyde groups of the nucleic acid on mild hydrolysis with normal hydrochloric acid (N, HCl) at 60° reacting with leuco-basic fuchsine give a violet coloration to the chromosomes. Before mounting, the material passed through alcohol series serves for dehydration. Mounting medium for tissue slides has been found to be of better value than Balsam.

A number of stains are being used generally for staining the chromosomes besides acetic-orcein (after La Cour, 1941) and leuco-basic fuchsine (Modified formula after De Tomasi, 1936 and Coleman, 1938).\textsuperscript{64} They are as mentioned below:

2. Aceto-lacmoid (La Cour).
3. Crystal violet (after Newton).
4. Heidenhain's haematoxylin.
6. Orange G-aniline blue (Mallory, after La Cour et al., 1958).

However the rat gonad tissues was best stained with acetic-orcein and leuco-basic fuchsir. The squashes were made permanent after acetic orcein treatment by:

1. Inverting the slide in a covered smearing dish containing acetic acid: alcohol (1:3).
2. Passing through absolute alcohol, 2 changes 1 min. in each and mounting in DPX finally.

Staining procedure has been mentioned in preceding lines. The study of chromosomal rearrangements and changes has been done on uniformly spread tissue in the squashes. Magnification at 15 x 100 could easily trace out good stage in both treated and the untreated ones.
Experimental Studies:

Determination of Approximate Lethal Dose (ACL$_{50}$) of the samples $S_1$ to $S_6$:

Approximate lethal doses 50 (ACL$_{50}$) of the samples $S_1$ to $S_6$ were determined in albino mice by using Horn's formula and table. Albino mice, with an average weight of 25 grams, were divided into six groups of four rats each. These were administered intraperitoneally (i.p.) with graded doses of test samples $S_1$, $S_2$, $S_3$, $S_4$, $S_5$ and $S_6$. Observations were made for mortality (after 24 hours). The ACL$_{50}$ and confidence limits were taken from Horn's table (Tables 5A and 5B below):

Table 5A and 5B: Approximate Lethal Dose 50 (ACL$_{50}$) and Confidence Limit of Mucuna pruriens Baker Seed Compounds ($S_1$ to $S_5$) and Seed Powder ($S_6$).

Table 5A:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Mortality per cent</th>
<th>ACL$_{50}$ mg/kg</th>
<th>Confidence Limit mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10.0 25.0 46.4 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>$S_1$</td>
<td>25 25 100 100</td>
<td>46.4</td>
<td>22.5-95.7</td>
</tr>
<tr>
<td>2</td>
<td>$S_2$</td>
<td>0 0 75 100</td>
<td>38.3</td>
<td>26.1-56.2</td>
</tr>
<tr>
<td>3</td>
<td>$S_3$</td>
<td>0 0 25 100</td>
<td>27.8</td>
<td>10.8-39.1</td>
</tr>
</tbody>
</table>
Table 5B:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Mortality per cent</th>
<th>ALD$_{50}$ mg/kg</th>
<th>Confidence limit mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>$S_2$</td>
<td>0 0 25 50</td>
<td>1000</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>$S_5$</td>
<td>0 0 25 50</td>
<td>1000</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>$S_6$</td>
<td>0 0 0 25</td>
<td>1000</td>
<td>-</td>
</tr>
</tbody>
</table>

One-fifth of the ALD$_{50}$ of the samples $S_1$ to $S_6$ were considered for reproductive pharmacology.

**Sample** | **Dose**
--- | ---
$S_1$ | 9 mg/kg/rat/day
$S_2$ | 200 mg/kg/rat/day
$S_3$ | 8 mg/kg/rat/day
$S_4$ | 6 mg/kg/rat/day
$S_5$ | 200 mg/kg/rat/day
$S_6$ | 250 mg/kg/rat/day
Chromosomal Studies:

Experiment 1:

Method and Methodology:

The squashes of the freshly dissected testes tissue of the rats of group I to IV were prepared using aceto-orcein, and stored temporarily in acetoglycerin (45 : 55). The squashes were examined under microscope. Several stages of male gamete formation from the germinal epithelial cells were visible. Chromosomes in spermatogonia, primary and secondary spermatocytes were observed and photographed under magnification (x 675). Diploid chromosomes of spermatogonia and haploid chromosomes of primary and secondary spermatocytes were photographed to note effects of the test samples S₁ to S₆ (in the doses on fifth of their respective \( \text{LD}_{50} \)), estradiol propionate (10 \( \mu \)g/rat/day) and of blanks of the samples and EP in the Controls (Group I) to check whether they caused any abnormalities in the chromosomes.

Procedure:

Colony-bred 16 adult male albino rats of Springer Dawley strain, acclimatized to the laboratory environment for four days at room temperature prior to start of the experiment, were taken for studying effects of the test samples on chromosomes of testis tissue—germinal epithelium.
The rats were divided into three groups (of which I and II comprised two rats - Group I of Control, Group II of rats treated with estradiol propionate and Group III of rats treated with the test samples S₁ to S₆). For each sample used in Group III, two rats were taken, the total rats for Experiment I were sixteen. Group I of control received blanks of estradiol propionate and samples S₁ to S₆.

Group II rats received estradiol propionate in sterile arachis oil intramuscularly (10 µg/rat/day).

Group rats received samples S₁ to S₆, each given to a set of two rats, in the doses and solvents/medium as discussed under Table 7A and 7B.

On the 30th day of the experiment, the rats of all the Groups I to III were sacrificed by cervical dislocation and their testes were dissected out. One of every pair of testes was kept in 45% acetic acid and their squashes were prepared using aceto-orcein. The squashes observed under microscope showed chromosomes in the transforming germinal cells - spermatogonia, primary and secondary spermatocytes - to note any abnormalities caused in the morphology and arrangement of the chromosomes. The photographs (Plates 1 to 8) were taken by microscope camera attachment using 10 X and 15 X eyepieces and 10 X and 45 X objective lenses of the microscope, MEOPTA.
Plate 1- Chromosomes of a Control rat (675x3).
Haploid chromosomes of a secondary spermatocyte.

Plate 2- Normal chromosomes of a rat treated with estradiol propionate (10 µg/rat/day).
Diploid chromosomes of spermatogonia (675x3).
Plate 3- Normal chromosomes of a rat treated with the sample $S_1$ (9 mg/kg). Haploid chromosomes of a primary spermatocyte (675x3).

Plate 4- Normal chromosomes of a rat treated with the sample $S_2$ (200 mg/kg). Haploid chromosomes of secondary spermatocytes in formation (675x3).
Plate 5- Normal chromosomes of a rat treated with the sample $S_3$ (8 mg/kg). Haploid chromosomes of primary spermatocytes during formation (675x3).

Plate 6- Normal chromosomes of a rat treated with the sample $S_4$ (6 mg/kg). Haploid chromosomes of secondary spermatocytes (675x3).
Plate 7- Normal chromosomes of a rat treated with the sample S_5 (200 mg/kg). Haploid chromosomes of a secondary spermatocyte (675x3).

Plate 8- Normal chromosomes of a rat treated with the sample S_6 (250 mg/kg). Haploid chromosomes of a secondary spermatocyte (675x3).
Summary:

In Experiment 1 the comparison of chromosomes of germinal epithelial cells of Controls of Group I rats (Plate 1) with those in the cells of Group II and Group III subjects (Plates 2 to 7) showed that estradiol propionate (EP) and samples S₁ to S₆ caused no chromosomal abnormalities in their morphology and arrangement. The normal appearance of chromosomes strongly indicated the absence of cytotoxicity relating to mutagenicity in the administered doses of EP and the samples S₁ to S₆.

The samples S₁ to S₆ were considered for male reproductive pharmacology upon confirmation of their non-cytotoxic nature in rats in the tested doses.
REFERENCES


CASTRATION
AND
HYPOPHYSECTOMY
Castration and Hypophysectomy

Castration, or gonadectomy, leads to marked retrogressive changes in the reproductive organs. In the mouse the seminal vesicles respond to castration according to the age at operation. Castration at 5 days of age, when the seminal vesicles are relatively undifferentiated, does not inhibit differentiation or formation of columnar epithelium. The adrenal X-zone hypertrophies at this time and supplies androgens. Castration at 21 days, when the X-zone hypertrophies and the columnar epithelium has differentiated, causes little change for as long as 100 days. Uniform seminal vesicle degeneration in the castrated mouse is found when the operation is performed after sexual maturity and when X-zone has disappeared.

In contrast to the androgens which stimulate the epithelium, estrogens cause hypertrophy of the fibro-muscular components of the seminal vesicles. Involution of prostate after castration may be prevented by androgen. Similar results have been obtained with a variety of androgens in both rats and monkeys. The principal effect of androgens is exerted on the epithelial component of the gland. Estrogens, on the other hand, cause atrophy of the epithelium. Androgens produce increases in prostate weight in the castrated animal. In the intact animal large doses of estrogen inhibit the action of androgens. The mechanism probably consists of direct antagonism plus the indirect action of decreasing androgen secretion through the pituitary suppression. Androgens stimulate prostatic secretion.
Thus in castrated dogs decreases in prostatic secretion have been found during the first few days after castration, and complete cessation within the first three weeks after castration. Administration of effective doses of androgens renews the prostatic secretion within the first few weeks of treatment. In human hypogonadism androgens initiate or increase prostatic secretion. The relationship between the acid phosphatase of prostatic tissue and androgens has been studied in experimental animals as well as in human subjects. The administration of estrogens causes a sharp decrease in the concentration of this enzyme. Other structures which have been shown to be under the influence of androgens in the male, are the vas deferens, the epididymis, Cowper's gland and the preputial gland. Castration has been shown to cause degenerative changes in the vas deferens, such as decreased secretion, decreased size of the epithelium, and degeneration of the cilia, and these changes can be prevented by androgens. Testosterone brings about a significant increase in the weight of seminal vesicle and ventral prostate of castrated-non-hypophysectomised rats. Similarly, testosterone brings significant increase in the acid phosphatase activity in these organs in the absence of hypophysis. The enzyme activity in these organs is controlled by the pituitary gland factor. Cowper's gland and the preputial gland, quite like the prostate and seminal vesicles, undergo the usual atrophy after removal of the testes and may be stimulated by androgens.
Role of Hypophysis

The endocrine influence of the testes on the formation of fructose in accessory organs is integrated closely with the functioning of the anterior pituitary gland. Hypophysectomy, like gonadectomy, invariably results in a rapid decline in the level of fructose in the seminal plasma (Mann and Parsons, 1950). In the rabbit, for instance, a three to four weeks' period after castration or hypophysectomy alike, usually leads to complete disappearance of fructose so that an ejaculate collected by means of an artificial vagina three weeks after the operation contains no more than 20 μg. fructose, as compared with 500 to 100 μg., before the operation. Both castrated as well as hypophysectomized animals promptly respond to the subcutaneous implantation or injection of testosterone with renewed secretion of fructose. The same happens if instead of testosterone pregnant mare serum gonadotrophin is injected into a hypophysectomized animal.

The effect of malnutrition manifests itself in a progressive decline of the citric acid level in semen and accessory gland secretion and is due to a state of so-called pseudo-hypophysectomy. A state of 'pseudo-hypophysectomy' was described by Mulinos and Pomerantz (1941) in rats as the results of a diet which was qualitatively adequate
but halved in quantity; further supporting evidence was later provided by several groups of investigators (Pazos and Huggins, 1945; Goldsmith and Rigrelli, 1950; Grayhack and Scott, 1952).4, 5, 6
Plan of Experiment 2:

Castration or gonadectomy, in rats and other mammals brings about marked retrogressive changes in the secondary sex organs, especially in their weights and secretions, on account of causing abrupt cut-off of supply of testicular testosterone, thus diminishing fertility.

Androgen replacement therapy, executed by administering testosterone to the castrates, causes reversal of these changes, which confirms the effects to be a consequence of response to the androgen supply. For experimental fertility studies, therefore, castration was employed as an important parameter for studying the effects of the test samples of *Mucuna pruriens* Baker extract on gonadectomised rats to confirm whether they were caused by triggering any androgenic response.

In Experiment 2, adult male albino rats and immature castrated rats, both of Wistar strain, were considered for study of the effects of samples $S_1$ to $S_6$ on reproductive system, for a period of 7 days. Immature castrates were 25 days of age.

The study involved four groups of rats. Group I comprised 9 adult males (intact controls). Group II comprised 9 immature castrated rats. Both these groups were administered with the blanks of testosterone propionate (sterile arachis oil, intramuscularly), samples $S_1$ to $S_5$ (water for injection) and sample $S_6$ (pellets of wheat flour) daily, for 7 days.
Group III comprised 9 immature castrated rats, administered daily with 250 µg of testosterone propionate (TP) for androgen replacement therapy, for 7 days. Group IV comprised six sub-groups A to F, each of 9 immature castrates. Samples S₁ to S₆ were administered individually to each of these sub-groups daily for seven days in the doses as mentioned below (calculated by Horn's formula and table) --

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of rats</th>
<th>Sample</th>
<th>Dose</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9</td>
<td>S₁</td>
<td>9mg/kg/rat/day</td>
<td>intramuscular</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>S₂</td>
<td>200mg/kg/rat/day</td>
<td>intramuscular</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>S₃</td>
<td>8mg/kg/rat/day</td>
<td>intramuscular</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>S₄</td>
<td>6mg/kg/rat/day</td>
<td>intramuscular</td>
</tr>
<tr>
<td>E</td>
<td>9</td>
<td>S₅</td>
<td>200mg/kg/rat/day</td>
<td>intramuscular</td>
</tr>
<tr>
<td>F</td>
<td>9</td>
<td>S₆</td>
<td>250mg/kg/rat/day</td>
<td>oral</td>
</tr>
</tbody>
</table>

On the 7th (last) day of the experiment, the rats of Groups I to IV were autopsied and their accessory sex organs were removed, i.e. of epididymis (caput, corpus and cauda, together), seminal vesicles and ventral prostate. The organs were weighed accurately to note any change in weight of the secondary sex organs in Groups III and IV and were compared with Groups I and II, to confirm triggering of androgenic response.
Experiment 2:

Determination of the Effects of Mucuna pruriens Baker Compounds on the Weights of Secondary Sex Organs in Immature Castrated Rats

Four groups of male albino rats (I to IV) were considered for this experiment for a seven-day study. Group I comprised 9 adult males (intact Controls). Group II comprised 9 immature castrates (castrated Controls). Group III comprised 9 immature castrates rats receiving testosterone replacement therapy (testosterone propionate, 250 μg/rat/day, intramuscularly, for 7 days). Group IV comprised 54 animals, 9 for each of the six sub-groups (A to F). Groups I and II were administered the blanks of testosterone propionate (sterile arachis oil, intramuscularly), samples S₁ to S₅ (water for injection) and sample S₆ (pellets of wheat flour). The subgroups IV-A to IV-E were administered the samples S₁ to S₅ in one-fifth of ACD₅₀ as calculated by Horn's formula and table. Sub-group IV-F was administered with S₆ in the dose of 250 mg/kg/rat/day, orally, for 7 days.

Procedure:

Immature albino rats of no more than 25 days of age (of Wistar strain) of groups II, III and IV were castrated as follows:

The preparations were made for aseptic operation. The rats were anaesthetized (one by one) and a ventral, mid-line incision was made through the skin of the scrotum. The rats showed a
tendency to retract the testes into the abdominal cavity. Slight pressure over the pelvis, however, forced them back into the scrotum. They were freely movable within the scrotum, and one testis was drawn through the skin incision. The spermatic chord and the testicular blood vessels attached to the testis were ligated carefully, that is, two ties, close together, were made, and the chord was cut between the ties. Now a slit was made through the tunica and the testis was freed (plates 9 to 12). The other testis was similarly removed. No thread sutures were necessary, the skin incision being merely closed with wound clips. The animal received post-operative antibiotic therapy for 4 days. First day of the experiment (in groups II to IV) was counted after allowing the castrated animals four days of post-operative rest and therapy.

Table-6 includes the observations of the effects of androgen replacement therapy and the samples $S_7$ to $S_6$ on the weights of secondary sex organs of immature castrates.
Plate 9- It shows a ventral, middle-line incision made through the skin of the scrotum of a rat. The testes in the scrotum are in view.

Plate 10- It shows one of the testes drawn through the skin incision. The spermatic chord (to be ligated) attached to the testes is in view.
Plate 11- It shows one of the testes drawn out of the scrotum for cutting as also the spermatic chord being ligated with the help of sterile threads. The other testes in view was similarly removed.

Plate 12- It shows scrotal sac closed with wound clips of steel.
**Experiment 2:**

**Table 6: Effects of Mucuna pruriens Baker Seed Compounds on the Weight of Secondary Sex Organs of Castrated Immature Rats**

*Period of treatment - 7 days*

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Total epididymis (mg)</th>
<th>Seminal vesicles (mg)</th>
<th>Ventral prostate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact</td>
<td>6</td>
<td>56.40 ± 2.23</td>
<td>34.52 ± 2.68</td>
<td>36.2 ± 1.72</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(I.C.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Castrated</td>
<td>6</td>
<td>29.24 ± 1.15</td>
<td>15.88 ± 0.77</td>
<td>15.63 ± 0.68</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(C.C.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Immature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Castrates</td>
<td>6</td>
<td>51.62 ± 2.3</td>
<td>38.34 ± 4.3</td>
<td>37.38 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>(250 µg/rat/day; i.m.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Total epididymis (mg)</th>
<th>Seminal vesicles (mg)</th>
<th>Ventral prostate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>S₁</td>
<td>6</td>
<td>49.72 ± 3.2</td>
<td>28.49 ± 4.8</td>
<td>29.31 ± 2.6</td>
</tr>
<tr>
<td>B</td>
<td>S₂</td>
<td>6</td>
<td>28.24 ± 2.18</td>
<td>14.69 ± 0.66</td>
<td>15.42 ± 1.2</td>
</tr>
<tr>
<td>C</td>
<td>S₃</td>
<td>6</td>
<td>22.36 ± 3.2</td>
<td>11.63 ± 0.98</td>
<td>11.92 ± 1.7</td>
</tr>
<tr>
<td>D</td>
<td>S₄</td>
<td>6</td>
<td>43.42 ± 2.7</td>
<td>28.63 ± 0.87</td>
<td>31.42 ± 1.61</td>
</tr>
<tr>
<td>E</td>
<td>S₅</td>
<td>6</td>
<td>30.39 ± 1.5</td>
<td>14.97 ± 1.1</td>
<td>15.26 ± 0.82</td>
</tr>
<tr>
<td>F</td>
<td>S₆</td>
<td>6</td>
<td>47.72 ± 2.8</td>
<td>34.78 ± 3.4</td>
<td>32.20 ± 4.3</td>
</tr>
</tbody>
</table>

Continued overleaf
Table 6 Contd.

P values:

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Total epididymis</th>
<th>Seminal vesicles</th>
<th>Ventral prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I vs III</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>III vs IIII</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>$A - (S_7)$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>$B - (S_2)$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>$C - (S_3)$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>$D - (S_4)$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>$E - (S_5)$</td>
<td>$P &lt; 0.02$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.02$</td>
</tr>
<tr>
<td>$F - (S_6)$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
</tr>
</tbody>
</table>
Summary

In Experiment 2 (Table 6), the effects of the samples $S_1$ to $S_6$ observed on the weights of secondary sex organs -- epididymis (caput, corpus and cauda, together), seminal vesicles and ventral prostate of immature castrated rats (Group IV) were compared with the weights of reproductive organs in Groups I, II and III. It was found that the samples $S_1$, $S_4$ and $S_6$ produced significant increase in the weights of their accessory sex organs, which indicated their eliciting of androgenic response and, therefore, their profertility activity.

Sample $S_3$ was found to have brought about a significant decrease in weights of the reproductive organs, indicating the contribution of the sample to the postcastrate degenerative changes in the animals. Samples $S_2$ and $S_5$ were found to have caused insignificant changes in weights of the reproductive organs.
Plan of Experiment 3:

In this experiment the role of the test samples S1 to S6 in fertility was studied on adult male albino rats rendered 'functionally sterile' (reversibly sterile) by estradiol propionate administered in microdose.

Estrogen-induced 'functional sterility' in rats is experimentally useful in confirming whether the role of test samples in fertility is via influencing anterior pituitary. In 'functional sterility' the vital functions of the testis—spermatogenesis and the secretion of testosterone from Leydig cells, which is vital for testicular and extratesticular (sex accessory organs) functioning and growth—are not affected irreparably and can be restored, therefore, by drugs and hormones that have influence on anterior pituitary to result in release of LH which stimulates interstitial cells to cause secretion of testosterone from the Leydig cells.

Estrogen exerts antiandrogenic action as well as suppresses pituitary gonadotropin release and thus depresses male fertility; this condition is reversible when estrogen is administered in microdoses (5-10 µg/rat/day, intramuscularly) for 30 days, but administration of higher doses between 50 and 100 µg/rat/day, intramuscularly, result in hundred per cent sterility, a condition of irreversible impairment of male sexual function.
Doses and Routes of Administration of Samples \(S_1\) to \(S_6\):

<table>
<thead>
<tr>
<th>Group</th>
<th>Sub-group</th>
<th>No. of rats</th>
<th>(EP^* + Sample) and Dose</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>(A_1)</td>
<td>14</td>
<td>(EP + S_1(9\text{mg/kg/rat/day}))</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(B_1)</td>
<td>14</td>
<td>(EP + S_2(200\text{mg/kg/rat/day}))</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(C_1)</td>
<td>14</td>
<td>(EP + S_3(8\text{mg/kg/rat/day}))</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(D_1)</td>
<td>14</td>
<td>(EP + S_4(6\text{mg/kg/rat/day}))</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(E_1)</td>
<td>14</td>
<td>(EP + S_5(200\text{mg/kg/rat/day}))</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(F_1)</td>
<td>14</td>
<td>(EP + S_6(250\text{mg/kg/rat/day}))</td>
<td>oral</td>
</tr>
</tbody>
</table>

*\(EP = 10 \text{ug/rat/day}\)*

Group IV comprised 84 rats which were divided into six sub-groups \(A_2\) to \(F_2\), each consisting of 14 rats. Each sub-group received a sample from \(S_1\) to \(S_6\) for a period of 30 days, in the doses as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Sub-group</th>
<th>No. of rats</th>
<th>Sample</th>
<th>Dose</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>(A_2)</td>
<td>14</td>
<td>(S_1)</td>
<td>9\text{mg/kg/rat/day}</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(B_2)</td>
<td>14</td>
<td>(S_2)</td>
<td>200\text{mg/kg/rat/day}</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(C_2)</td>
<td>14</td>
<td>(S_3)</td>
<td>8\text{mg/kg/rat/day}</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(D_2)</td>
<td>14</td>
<td>(S_4)</td>
<td>6\text{mg/kg/rat/day}</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(E_2)</td>
<td>14</td>
<td>(S_5)</td>
<td>200\text{mg/kg/rat/day}</td>
<td>intramuscular</td>
</tr>
<tr>
<td></td>
<td>(F_2)</td>
<td>14</td>
<td>(S_6)</td>
<td>250\text{mg/kg/rat/day}</td>
<td>oral</td>
</tr>
</tbody>
</table>

The doses of the samples \(S_1\) to \(S_6\) were calculated by Horn's formula and table as mentioned in the tables 5A and 5B.
Hypophysectomy (removal of pituitary) results in cessation of growth and functioning of sexual organs on account of a cut-off in supply of the anterior pituitary hormones—LH and gonadotropins—which, in turn, leads to blockage of release of testosterone from the Leydig cells. Estrogen-induced diminished fertility in male rats (in the dose of 10 µg/rat/day) simulates the effects of hypophysectomy and is termed as 'chemical hypophysectomy', a condition close in connotation to the term 'pseudo-hypophysectomy,' which is a state of subdued fertility arising from acute malnutrition.

Experiment 3 was carried out in adult male albino rats of Wistar strain of proven fertility record. The rats were divided into four groups. Group I which comprised 14 Controls, received the blanks of estradiol propionate (sterile arachis oil, intramuscularly), of samples S₁ to S₅ (water for injection, intramuscularly) and of sample S₆ (pellets of wheat flour, orally) for a period of 30 days. Group II comprised 14 estradiol propionate (EP)-treated rats, rendered 'functionally sterile' or 'chemically hypophysectomised' (EP - 10 µg/rat/day; intramuscularly).

Group III comprised 84 rats which were divided into six sub-groups A₁ to F₁, each consisting of 14 rats. Each sub-group received a combination of estradiol propionate (10 µg/rat/day, intramuscularly) and one sample from S₁ to S₆ for a period of 30 days, in the doses as follows overleaf:
Rats of Groups I to IV were autopsied on the last (30th) day of the experiment for observation of effects of the samples $S_1$ to $S_6$ on the parameters of fertility assessment (each forming a part of Experiment 3) as follows:

- **Change in weight of testes and accessory sex organs** - Experiment 3A

- **Histology of testes and epididymis (caput and cauda)** - Experiment 3B

- **Sperm count assessment** - Experiment 3C

- **Sperm motility assessment** - Experiment 3D

- **Fructose estimation** - Experiment 3E

- **Glycerylphosphocholine estimation** - Experiment 3F
Experiment 3A:

Determination of Effects of Mucuna pruriens Baker Seed Compounds on the Weight of Genital Organs of Estradiol Propionate - Treated (Functionally Sterilised or Chemically Hypophysectomised) Rats

Procedure:

Colony-bred adult male albino rats (150 - 180 g) of Wistar strain and with good collective fertility record were used in the study. They were kept caged in a cool environment at 20°C under uniform husbandry conditions throughout the experimental period of 30 days. The experiment comprised 196 rats in total in four groups. Group I comprised 14 controls; group II 14, treated with estradiol propionate (EP) (10 µg/rat/day, intramuscularly); group III, 84 (14 per sub-group from A1 to F1) treated with EP with each of S7 to S6, separately; group IV, 84, treated with the samples S7 to S6, separately i.e. each of the six sub-groups A2 to F2.

Additional four rats, over 10 per group/sub-group (for the samples) were taken to allocate for 2 or 3 deaths per group/sub-group on account of cannibalism, illness, etc. Recordings of only ten animals per group/sub-group were taken to ensure uniformity in statistical computations.

In Group III, estradiol propionate (EP) was injected, intramuscularly, on a separate site from those where the samples S7 to S5 were administered. Group I was injected with the blanks of estradiol propionate (that is sterile arachis oil), and of samples S7 to S5 (water for injection) and of S6 (pellets of wheat flour), administered orally.
The rats were sacrificed on the day (30th day) following the last injection the testes, seminal vesicles, epididymides and ventral prostate, all pressed between folds of filter paper, were carefully and accurately weighed for observation of any changes in weight upon administration of samples S₁ to S₆ (Tables 9 and 10).

For histological studies the tissues of testes and epididymis (caput and cauda) were fixed in Bouin's fluid and their serial paraffin sections were cut and stained with Ehlrich's haematoxylin and eosin (as discussed in Experiment 3B).

For 'sperm count' and 'sperm motility' studies both caput and corpus portions of epididymis were studied (separately, in case of sperm motility). Only one of the epididymides (from the right and the left portions) was taken for both these studies. The other epididymis was kept for histology.

For estimation of fructose the coagulating gland was removed from the rat and frozen immediately (as discussed under 'Fructose Estimation', Experiment 3E, Table 11).

Glycerylphosphocholine (GPC) was estimated in the epididymis which were surgically removed for preparing tissue homogenates of its caput and cauda portions (as discussed under 'Glycerylphosphocholine Estimation', Experiment 3F, Table 12).
Plate 13 -

Testis of a control rat

Active spermatogenesis. (X 175)

Plate 14 -

Testis of a rat treated with estradiol propionate (10 μg/rat)

Spermatogenesis is arrested at spermatid stage. (X 175)
Plate 15 -

Caput epididymis of a control rat

Immature spermatozoa in view. (X 175)

Plate 16 -

Caput epididymis of a rat treated with estradiol propionate (10 μg/rat)

Reduction in tubular diameter

Thick intertubular stroma. (X 200)
Plate 17 -

Cauda epididymis of a control rat

Mature spermatozoa in view. (X 175)

Plate 18 -

Cauda epididymis of a rat treated with estradiol propionate (10 μg/rat)

Reduction in tubular diameter
Thick intertubular stroma and desquamated germ cells in the lumen are evident. (X 175)
Plate 19 -
Testis of a rat treated with
$S_1$ (9 mg/kg) with all stages
of spermatogenesis.
Active spermatogenesis. (X 175)

Plate 20 -
Caput epididymis of a rat treated with
$S_1$ (9 mg/kg).
Normal tubular diameter and
intertubular stroma. (X 175)

Plate 21 -
Cauda epididymis of a rat treated
with $S_1$ (9 mg/kg).
Normal tubular diameter and
intertubular stroma. (X 175)
Plate 22 -

Testis of a rat treated with $S_2$
(200 mg/kg).
Spermatogenesis arrested at the spermatid stage. (X 175)

Plate 23 -

Caput epididymis of a rat treated with $S_2$ (200 mg/kg).
Reduced tubular diameter and edematous stroma. (X 175)

Plate 24 -

Cauda epididymis of a rat treated with $S_2$ (200 mg/kg).
Thick intertubular stroma and columnar epithelial cells show no changes. (X 175)
**Plate 25**

Testis of a rat treated with 
$S_3$ (8 mg/kg).

Spermatogenesis arrested at spermatid 
stage.

Leydig cells are atrophied with 
numerous fibroblast like elements. (X 175)

**Plate 26**

Caput epididymis of a rat treated 
with $S_3$ (8 mg/kg).

Edematous stroma and desquamated 
germ cells are evident. (X 175)

**Plate 27**

Cauda epididymis of a rat treated with 
$S_3$ (8 mg/kg).

Arrow marks indicate desquamated germinal 
cells in view. (X 175)
Plate 28 -
Testis of a rat treated with $S_4$
(6 mg/kg).
Active spermatogenesis with all stages. (X 175)

Plate 29 -
Caput epididymis of a rat treated with
$S_4$ (6 mg/kg).
Normal tubular diameter.
Normal germ cells and non-edematous
stroma. (X 175)

Plate 30 -
Cauda epididymis of a rat treated with $S_4$
(6 mg/kg).
Normal intertubular stroma and
germin cells. (X 300)
Plate 31 -

Testis of a rat treated with $S_5$
(200 mg/kg).

All stages of spermatogenesis. (X 175)

Plate 32 -

Caput epididymis of a rat treated with $S_5$
(200 mg/kg).

Normal germ cells, intertubular stroma and
tubular diameter. (X 175)

Plate 33 -

Cauda epididymis of a rat treated with $S_5$
(200 mg/kg).

Tubules, dense with spermatozoa,
with normal diameter. (X 175)
Plate 34 -

Testis of a rat treated with S₆ (250 mg/kg).

Active spermatogenesis with all stages in view. The lumen contains abundant germ cells in various stages. (X 175)

Plate 35 -

Caput epididymis of a rat treated with S₆ (250 mg/kg).

Normal tubular diameter and intertubular stroma. (X 175)

Plate 36 -

Cauda epididymis of a rat treated with S₆ (250 mg/kg).

Lumen with abundant spermatozoa and normal intertubular stroma. (X 175)
**Experiment 3A:**

**Table 7: Effects of Mucuna pruriens Baker Seed Compounds on the Weight of Genital Organs of Estradiol Propionate-Treated (Functionally Sterilised or Chemically Hypophysectomised) Rats**

Period of treatment - 30 days

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Testis (mg/100g body weight)</th>
<th>Seminal vesicle (mg/100g body weight)</th>
<th>Ventral prostate (mg/100g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Controls</td>
<td>10</td>
<td>648.4 ± 12.4</td>
<td>143.6 ± 4.8</td>
<td>124.0 ± 4.1</td>
</tr>
<tr>
<td>(2)</td>
<td>Estradiol propionate (EP) (10 µg/rat/day, intramuscularly)</td>
<td>10</td>
<td>144.4 ± 6.2</td>
<td>56.3 ± 3.8</td>
<td>20.6 ± 1.9</td>
</tr>
<tr>
<td>(3)</td>
<td>A&lt;sub&gt;1&lt;/sub&gt; (EP + S&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>10</td>
<td>556.3 ± 9.8</td>
<td>119.4 ± 6.8</td>
<td>85.7 ± 8.7</td>
</tr>
<tr>
<td>(4)</td>
<td>B&lt;sub&gt;1&lt;/sub&gt; (EP + S&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>10</td>
<td>145.6 ± 17.3</td>
<td>55.1 ± 5.3</td>
<td>20.3 ± 2.1</td>
</tr>
<tr>
<td>(5)</td>
<td>C&lt;sub&gt;1&lt;/sub&gt; (EP + S&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>10</td>
<td>133.6 ± 6.3</td>
<td>50.3 ± 3.8</td>
<td>16.4 ± 1.7</td>
</tr>
<tr>
<td>(6)</td>
<td>D&lt;sub&gt;1&lt;/sub&gt; (EP + S&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>10</td>
<td>570.3 ± 9.9</td>
<td>123.2 ± 7.0</td>
<td>93.1 ± 8.6</td>
</tr>
<tr>
<td>(7)</td>
<td>E&lt;sub&gt;1&lt;/sub&gt; (EP + S&lt;sub&gt;5&lt;/sub&gt;)</td>
<td>10</td>
<td>145.3 ± 7.6</td>
<td>57.4 ± 4.3</td>
<td>20.2 ± 1.9</td>
</tr>
<tr>
<td>(8)</td>
<td>F&lt;sub&gt;1&lt;/sub&gt; (EP + S&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>10</td>
<td>597.5 ± 10.1</td>
<td>127.1 ± 6.9</td>
<td>102.0 ± 9.1</td>
</tr>
</tbody>
</table>

*Continued overleaf*
### Table 7 Contd.:

<table>
<thead>
<tr>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_2 \ (S_1)$</td>
<td>10</td>
<td>661.3</td>
<td>± 9.6</td>
<td>157.8</td>
<td>± 8.3</td>
</tr>
<tr>
<td>$B_2 \ (S_2)$</td>
<td>10</td>
<td>642.7</td>
<td>± 7.3</td>
<td>144.2</td>
<td>± 8.8</td>
</tr>
<tr>
<td>$C_2 \ (S_3)$</td>
<td>10</td>
<td>533.6</td>
<td>± 6.9</td>
<td>119.4</td>
<td>± 13.2</td>
</tr>
<tr>
<td>$D_2 \ (S_4)$</td>
<td>10</td>
<td>659.7</td>
<td>± 11.6</td>
<td>155.4</td>
<td>± 9.4</td>
</tr>
<tr>
<td>$E_2 \ (S_5)$</td>
<td>10</td>
<td>646.2</td>
<td>± 8.1</td>
<td>145.4</td>
<td>± 7.7</td>
</tr>
<tr>
<td>$F_2 \ (S_6)$</td>
<td>10</td>
<td>657.4</td>
<td>± 8.6</td>
<td>159.6</td>
<td>± 12.6</td>
</tr>
</tbody>
</table>

### P values:

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Testis</th>
<th>Seminal vesicle</th>
<th>Ventral prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J$ vs $JJ$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>$JJ$ vs $III$</td>
<td>$B_1$ $P &lt; 0.2$</td>
<td>$P &lt; 0.2$</td>
<td>$P &lt; 0.1$</td>
</tr>
<tr>
<td></td>
<td>$C_1$ $P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td></td>
<td>$D_1$ $P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td></td>
<td>$E_1$ $P &lt; 0.1$</td>
<td>$P &lt; 0.2$</td>
<td>$P &lt; 0.2$</td>
</tr>
<tr>
<td></td>
<td>$F_1$ $P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
</tr>
</tbody>
</table>

Continued overleaf
Table 7 Contd.:

P values:

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Testis</th>
<th>Seminal vesicle</th>
<th>Ventral prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>B2</td>
<td>$p &lt; 0.1$</td>
<td>$p &lt; 0.1$</td>
<td>$p &lt; 0.1$</td>
</tr>
<tr>
<td>C2</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>D2</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>E2</td>
<td>$p &lt; 0.2$</td>
<td>$p &lt; 0.2$</td>
<td>$p &lt; 0.1$</td>
</tr>
<tr>
<td>F2</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
</tbody>
</table>

I vs JU
**Experiment 3A:**

**Table 8: Effects of the Mucuna pruriens Baker Seed Compounds on the Weight of Epididymides (Caput, Corpus and Cauda) of Estradiol Propionate - Treated (Functionally Sterilised or Chemically Hypophysectomised) Rats**

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Epididymis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caput (mg)</td>
</tr>
<tr>
<td>(1) Controls</td>
<td>10</td>
<td>85.68 ± 11.0</td>
<td>49.32 ± 9.6</td>
</tr>
<tr>
<td>Estradiol propionate (EP) (10µg/rat/day, intramuscularly)</td>
<td>10</td>
<td>24.56 ± 7.8</td>
<td>17.55 ± 4.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Epididymis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caput (mg)</td>
</tr>
<tr>
<td>A₁</td>
<td>EP + S₁</td>
<td>10</td>
<td>64.25 ± 4.9</td>
</tr>
<tr>
<td>B₁</td>
<td>EP + S₂</td>
<td>10</td>
<td>25.72 ± 3.7</td>
</tr>
<tr>
<td>C₁</td>
<td>EP + S₃</td>
<td>10</td>
<td>20.41 ± 6.9</td>
</tr>
<tr>
<td>D₁</td>
<td>EP + S₄</td>
<td>10</td>
<td>62.12 ± 4.3</td>
</tr>
<tr>
<td>E₁</td>
<td>EP + S₅</td>
<td>10</td>
<td>25.22 ± 3.8</td>
</tr>
<tr>
<td>F₁</td>
<td>EP + S₆</td>
<td>10</td>
<td>68.17 ± 8.9</td>
</tr>
</tbody>
</table>

*Continued overleaf*
Table 8 Contd.:

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
<td>(5)</td>
<td>(6)</td>
</tr>
<tr>
<td>$A_2$ (S₁)</td>
<td>10</td>
<td>90.39</td>
<td>± 11.6</td>
<td>54.38</td>
<td>± 9.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$B_2$ (S₂)</td>
<td>10</td>
<td>84.92</td>
<td>± 7.1</td>
<td>50.19</td>
<td>± 6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_2$ (S₃)</td>
<td>10</td>
<td>50.89</td>
<td>± 7.3</td>
<td>32.19</td>
<td>± 9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D_2$ (S₄)</td>
<td>10</td>
<td>91.44</td>
<td>± 11.1</td>
<td>53.79</td>
<td>± 4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_2$ (S₅)</td>
<td>10</td>
<td>86.12</td>
<td>± 7.8</td>
<td>50.34</td>
<td>± 12.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_2$ (S₆)</td>
<td>10</td>
<td>89.66</td>
<td>± 12.1</td>
<td>54.99</td>
<td>± 13.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P Values:

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Caput</th>
<th>Corpus</th>
<th>Cauda</th>
</tr>
</thead>
<tbody>
<tr>
<td>I vs II</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>$A_1$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>$B_1$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.2$</td>
<td>$p &lt; 0.2$</td>
</tr>
<tr>
<td>II vs III</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>$C_1$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>$D_1$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.2$</td>
<td>$p &lt; 0.2$</td>
</tr>
<tr>
<td>$E_1$</td>
<td>$p &lt; 0.1$</td>
<td>$p &lt; 0.2$</td>
<td>$p &lt; 0.2$</td>
</tr>
<tr>
<td>$F_1$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
</tbody>
</table>

Continued overleaf
Table 8 Contd. :

P values:

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Caput</th>
<th>Corpus</th>
<th>Cauda</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₂</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>B₂</td>
<td>$p &lt; 0.2$</td>
<td>$p &lt; 0.2$</td>
<td>$p &lt; 0.2$</td>
</tr>
<tr>
<td>G vs IV</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>D₂</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>E₂</td>
<td>$p &lt; 0.2$</td>
<td>$p &lt; 0.4$</td>
<td>$p &lt; 0.2$</td>
</tr>
<tr>
<td>F₂</td>
<td>$p &lt; 0.02$</td>
<td>$p &lt; 0.02$</td>
<td>$p &lt; 0.02$</td>
</tr>
</tbody>
</table>
Summary:

In Experiment 3A (Tables 7 and 8), the effect of the samples $S_1$ to $S_6$ on the weights of genital organs of the rats treated with estradiol propionate (functionally sterilised) (Group III) as also in the subjects of normal fertility (Group IV) were observed after 30 days of treatment. In Groups III and IV rats the samples $S_1$, $S_4$ and $S_6$ were found to produce significant increase in the weights of testes, seminal vesicles and epididymis (caput, corpus and cauda). Samples $S_2$ and $S_5$ caused insignificant weight changes in the genital organs in Groups III and IV animals. Sample $S_3$ caused reduction in weight of genital organs, simulating the activity exhibited by Group II rats treated with estradiol propionate. The increase in weight effected by the samples $S_1$, $S_4$ and $S_6$ was indicative of their effect on rise in levels of supply of testosterone to the reproductive organs, whereas for $S_3$ the fall in testosterone level was indicated. Their modes of action have been discussed under 'Results and Discussion' in the last chapter.
Experiment 3B:

Histology of Testis and Epididymis:

Method and Methodology:

Colony-bred adult male albino rats (150-180g) of Wistar strain and with good collective fertility record were divided into four groups. Group I of controls and group II of estradiol propionate (EP) - treated rats, comprised 10 rats each. Group III with sub-groups A₁ to F₁ and group IV with sub-groups A₂ to F₂, comprised 10 rats each for sub-groups of III and IV.

Group III rats were treated with S₁ to S₆ in combination with EP for every sample, separately for each sub-group of 10 rats. Group IV rats were treated with samples S₁ to S₆ separately for each sub-group of 10 rats. EP and the samples S₁ to S₆ were administered in the doses mentioned in the tables 7A and 7B.

On the 30th (last) day of the experiment the rats of groups I to IV were sacrificed by cervical dislocation and the testes, and epididymis (caput, corpus and cauda) were removed. Of the right and left portions of each of these genital organs, one portion of each was kept aside for histology, and were subjected to the following treatment:

One portion of each of the genital organs -- testes, and epididymis was cut into three pieces and fixed in Bouin's and aceto-alcohol fixatives for 6 hours. They were then
dehydrated by passing through an ethanol series of varying percentages 30%, 50%, 70%, 90% and absolute, in the ascending order (the ethanol being taken in water), and finally collected in xylene. Small shreds of paraffin wax were then put into the vials containing xylene-dipped pieces (of the portions of each of these genital organs) just sufficient to allow formation of a jelly-like mass of the medium, and the pieces were kept as such for 3 hours. These were then transferred to copper pans containing molten wax, in the oven at 62°C. Traces of xylene present in the pieces were, thus, evaporated over a period of 30 minutes. Fresh paraffin was put into the pans after replacing the previous one and the heating for 30 minutes was repeated. A watch glass, of 12 cm. diameter, was rubbed on its inner surface with a drop of glycerin and molten wax was poured over it. The pieces of genital organs in the copper pans were immediately transferred into the molten wax in the watch glasses by heated forceps and were embedded sufficiently in the wax layer. Gradual cooling at normal temperature was allowed and after the upper wax surface solidified adequately, cold water was poured over it for complete cooling. The wax plate was then cut into fine rectangular blocks, having straight edges. With the help of a knife the wax block was mounted on a wooden block by setting molten wax on the surface of the wooden block holding it and cooled in the air, alternately, to allow its firm fixation on the wooden block. Two sides (upper and lower)
of the wax blocks were coated with a thin film of soft wax
to ensure the continuous ribbon formation during sectioning
by microtome knife. Sections at 6 μ were cut and the ribbons
were carefully picked up on the Mayer's albumen-coated
(thin film) slides. They were floated on distilled water
drops and slowly heated on hot plate for very short intervals,
just sufficient to allow sticking and uniform spreading of
the ribbons on the slides. They were kept for drying at this
stage at room temperature.

For staining in haematoxylin and eosin, the slides with
the sections of testes and caput and cauda epididymis were
passed successively through the solvents in the following
order: xylene and absolute ethanol, absolute ethanol, 90%
ethanol, 70% ethanol, 50% ethanol, 30% ethanol, distilled
water, haematoxylin stain, distilled water, 30% ethanol,
50% ethanol, 70% ethanol, 90% ethanol, eosin stain, 90%
ethanol absolute ethanol, absolute ethanol + xylene, and,
finally, xylene. The slides were passed from one solvent
trough into another successively with a regular interval
of five minutes. They were then taken out and any excess
of xylene was drained off and were permanently mounted in DPX
with the aid of microslides. They were, at this stage,
observed under microscope at varying magnifications
using 10X and 15X eyepieces and 10X and 45X objective lenses,
and photomicrographed.
Summary:

In Expt 3B, the histological examination of the genital organs in group I to IV rats revealed that Group I (Controls) animal exhibited normal features of testes (with active spermatogenesis), caput and cauda epididymis (with normal tubular diameters) (Plates 13, 15 and 17). Group II rats showed testes with spermatogenesis arrested at the spermatid stage, and reduced tubular diameters of caput and cauda epididymis. Cauda portion also showed thick intertubular stroma and desquamated germ cells (Plates 14, 16 and 18). These retrogressive changes in the genital organ tissues were a result of the estradiol propionate (EP) - induced reversible sterility. EP is known to interfere with Leydig cells function and cause antiandrogenic changes - decline in growth of testes and accessory organs and changes in their histological features indicating subnormal or abnormal state of the organ, or induced sterility.

Group III rats receiving a combination of EP and a sample in each subgroup (A1 to F1) showed for samples S1, S4 and S6 active spermatogenesis, in the testes, with its all stages clear (Plates 14, 28 and 34), normal tubular diameter and germ cells and non-edematous stroma of caput and cauda epididymis (Plates 20, 21, 29, 30, 35 and 36).

These observations, identical to those in Group I controls, indicated that samples S1, S4 and S6 caused reversal of the estradiol propionate-induced effects of sterility, to bring about normality. Samples S2, S3, and S5 showed no alteration of estradiol propionate-induced retrogressive changes.
The testes showed spermatogenesis arrested at spermatid stage with atrophic Leydig cells in view (Plates 20, 25, and 31). The caput epididymis showed reduced tubular diameter and edematous stroma (Plates 23, 26 and 32). The cauda epididymis showed thick intertubular stroma and reduced tubular diameter (Plates 24, 27 and 33).
REFERENCES


SPERM COUT

ASSESSMENT
SPERM COUNT

'Whole semen' as ejaculated, generally appears as a viscous, creamy, slightly yellowish or greyish fluid, and consists of spermatozoa or 'sperm', suspended in the fluid medium, called seminal plasma; its composition depends in the first place, on the proportion of sperm and plasma, and is further determined by the size, storage capacity, and secretory output of several different organs which comprise the male reproductive tract. The volume of the ejaculate and the concentration of spermatozoa or the 'sperm density' in ejaculated semen vary widely from one species to another. A single ram ejaculate for instance, amounts to 0.7-2 ml. only, but is distinguished by a very high sperm density, 2-5 million per ml. semen; when subjected to high-speed centrifugation, ram semen separates, on the average, into about two-thirds of seminal plasma and one-third of firmly packed sperm. Boar semen ejaculates on the other hand, may reach a volume of as much as 500 ml.; this is not due to spermatozoa, but to the seminal plasma generated in very capacious accessory organs; a sperm density not exceeding 100,000 cells/ml. is quite usual for boars, and even lower sperm densities would still be regarded as normal. In man, the average volume of a single ejaculate is about 3 ml., but the sperm density is frequently less than 100,000 cells/ml., so that only
a small portion of the ejaculate, much less than 10%, is represented by the sperm and the rest is seminal plasma. The two components of semen, sperm and seminal plasma, differ in their origin, composition and function, and must be considered separately, in much the same sense as for instance, blood corpuscles and blood plasma.

Apart from the test based upon accomplished fertilization, the means available at present for the evaluation of semen quality include the histological and physico-chemical methods. Histochemical examination of semen involves procedures such as the determination of sperm concentration or 'density' (number of spermatozoa per 1 µl. or 1 ml. of semen) with a cytometer (Walton, 1927; Geisman, 1942)\(^1,2\); differential count of abnormal forms of spermatozoa (Lagerlof, 1934; Harvey and Jackson, 1945; Lane Roberts et al., 1948; Almquist, Prince and Reid, 1949; Wu Elliker and Mc Kenzie, 1952-3)\(^3-7\); determination of incidence of dead spermatozoa by means of 'live-dead staining' methods (Lasley, Casley and Mc Kenzie, 1942; Lasley and Bograt, 1943; Madden, Herman and Berousek, 1947; Crooke and Mandl, 1949; Blom, 1950; Mayer, Squiers, Bogart and Olaufa, 1951; Ortavant, Dupont, Pauthe and Roussel, 1952; Campbell, Hancock and Rothschild, 1953).\(^8-15\)
Phadke et al. (1973) studied correlation between seminal fructose levels and the sperm counts. He found that the fructose content in semen varied inversely with the sperm count: the higher the sperm count the lower the value for seminal fructose noted.

The mean seminal fructose concentration in cases of moderate oligospermia, severe oligospermia, and nonobstructive azoospermia were 293, 309 and 326 mg./100 ml. respectively. These cases differed significantly from normospermic patients with sperm counts of more than 40 million/cu.cm. (at the 0.05% confidence level). Evidently, the fructose level was inversely proportional to the sperm count. In the present series the fructose content in semen was found to vary inversely with the sperm count. Davis and McCune and Schirren had noted such an inverse relationship.

It was suggested that the low values for seminal fructose in normospermic men could result from the utilization of fructose by spermatozoa. This tempting suggestion was obviously based on knowledge gained from studies of the metabolic activities of spermatozoa. Several investigators had proved that, under anaerobic conditions, spermatozoa were capable of utilizing fructose for their energy requirements. The progressively higher values for seminal fructose in cases of oligospermia and azoospermia were
explained by the same logic: namely, that in such cases "nonutilization" of fructose by the progressively declining number of spermatozoa was responsible for the higher seminal fructose content. According to Tyler\textsuperscript{18} and MacLeod and Freund\textsuperscript{19} in specimens with high concentrations of spermatozoa, the spermatozoa themselves occupy an appreciable volume of the ejaculate and hence the fructose concentration per cubic centimeter is low.

According to all of the above-mentioned theories, the seminal fructose values in cases of obstructive azoospermia ought to have been the highest, as there were no spermatozoa in semen either to utilize the fructose or to occupy the volume of the ejaculate appreciably. Rationally, therefore, it was concluded that the fructose production itself varied inversely with the sperm count and that the seminal fructose content depended on the state of the germinal epithelium rather than on the number of spermatozoa present in the semen.
**METHOD AND METHODOLOGY**

Determination of spermatozoal counts (by the method of Bishop et al. 1954). The rat sperm cells were counted by using haemocytometer i.e. usual red blood cells counting chamber.

**Reagent(s):**

Phenol-bicarbonate solution: Phenol (1.25 g) and NaHCO₃ (4 g) were dissolved in distilled water (100 ml.)

**Procedure:**

After thorough mixing of the samples, a preliminary microscopic observation of the sperm was made. Depending upon the sperm density, observed on a plain smear, the sample was diluted 1:2 by phenol-bicarbonate solution. A drop of this solution that immobilizes the sperm cells, was placed in the counting chamber and observed under a low magnification (1:10) to confirm that all the sperm cells were immobilized.

The immobilized sperm cells within the RBC field of the counting chamber were examined and counted. All the sperm cells lying within 5 blocks of 16 small squares each, i.e. 80 squares, or 1/5th of the entire RBC field, were counted.
The purpose of consistency, all cells overlying the lines at the left side and top of the squares were included in the count while those overlying the lines at the right side and bottom were excluded. Further, for greater accuracy the counts were performed twice, once on each side of the chamber and the mean value was taken for computation.

Computation:

The sperm cell count in millions/ml was then computed as follows:

\[(10)(5)(20)(1000)x = \text{Sperms per ml.}\]

- 10 - is correction for the fact that the chamber is 1/10 mm deep
- 5 - is the multiplication factor because the area counted is 0.2 sq. mm.
- 20 - is dilution factor of 1:20.
- 1000 - is common factor.
- x - is the number of sperms in 5 blocks.
Experiment 3C

Sperm Count Assessment

Determination of sperm count by Bishop et al method.

The sperm cells were obtained by squashing the pieces of the caput and cauda portions of epididymis (from the rats sacrificed in Experiment 3) in 0.9% saline and were counted on a haemocytometer, i.e. usual red blood cells (RBC) counting chamber.

All the groups I, II, III and IV of rats of Experiment 3 were dissected on the 30th (last) day of the Experiment and their epididymides were subjected to the treatment for sperm counting as in the procedure below:

Procedure:

The squashes of the caput and cauda portions of epididymis were prepared in 0.9% saline with the help of a tweezer. After thorough squashing, a preliminary observation of the sperms was performed on a plain smear. The sample was diluted 20 times by phenol carbonate solution which immobilised the sperm cells.

A drop of this diluted mixture was placed in the counting chamber and observed under a low magnification (1:10) to confirm that all the sperm cells were immobilised for a facile counting. The immobilised sperm cells within the RBC field of the counting chamber, were counted. All the sperm cells lying within 5 blocks of 16 small squares i.e. 80 squares in all, or one-fifth of the entire RBC field, were counted.
For consistency in observations all the cells overlying the lines at the left side and the top of the squares were included in the count while those overlying the lines at the right side and bottom were eliminated.

Further, for the purpose of greater accuracy, the counts were performed twice, once on each side of the chamber and the mean value was considered for computation to have sperm count in millions/ml as follows:

\[(10) \cdot (5) \cdot (20) \cdot (1000) \cdot x = \text{Sperms per ml.}\]
Table 10

Effects of the Plant Compounds on Sperm Count in Estradiol propionate-treated (chemically hypophysectomised or reversibly sterilised) Rats.

Period of treatment = 30 days

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Sperms in millions/ml from the right and left Epididymides</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Controls</td>
<td>10</td>
<td>60.6</td>
</tr>
<tr>
<td>II</td>
<td>Estradiol propionate (EP) (10 µg/rat/day)</td>
<td>10</td>
<td>35.4</td>
</tr>
<tr>
<td>III A_1</td>
<td>EP + S_1</td>
<td>10</td>
<td>53.2</td>
</tr>
<tr>
<td>B_1</td>
<td>EP + S_2</td>
<td>10</td>
<td>36.6</td>
</tr>
<tr>
<td>C_1</td>
<td>EP + S_3</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>D_1</td>
<td>EP + S_4</td>
<td>10</td>
<td>55.4</td>
</tr>
<tr>
<td>E_1</td>
<td>EP + S_5</td>
<td>10</td>
<td>34.6</td>
</tr>
<tr>
<td>F_1</td>
<td>EP + S_6</td>
<td>10</td>
<td>58.5</td>
</tr>
<tr>
<td>IV A_2</td>
<td>S_1</td>
<td>10</td>
<td>63.4</td>
</tr>
<tr>
<td>B_2</td>
<td>S_2</td>
<td>10</td>
<td>60.2</td>
</tr>
<tr>
<td>C_2</td>
<td>S_3</td>
<td>10</td>
<td>40.7</td>
</tr>
<tr>
<td>D_2</td>
<td>S_4</td>
<td>10</td>
<td>60.8</td>
</tr>
<tr>
<td>E_2</td>
<td>S_5</td>
<td>10</td>
<td>60.5</td>
</tr>
<tr>
<td>F_2</td>
<td>S_6</td>
<td>10</td>
<td>62.7</td>
</tr>
</tbody>
</table>

P Values: I vs II (P < 0.05)

II vs III A_1 - (P < 0.05)  I vs IV A_2 - (P < 0.05)
B_1 - (P < 0.60)  B_2 - (P < 0.60)
C_1 - (P < 0.05)  C_2 - (P < 0.05)
D_1 - (P < 0.05)  D_2 - (P < 0.05)
E_1 - (P < 0.10)  E_2 - (P < 0.10)
F_1 - (P < 0.05)  F_2 - (P < 0.05)
Summary

In Experiment 3C 'sperm count' was considered as a parameter of fertility. The influence of the samples S1 to S6 on sperm population was noted in rats rendered experimentally subfertile by estradiol propionate which diminished sperm count (Group II) significantly -- on the 30th (last) day of the treatment.

It was found that the samples S1, S4 and S6 brought about significant increase in sperm count in adult male albino rats, both in normal subjects (Group IV) treated with the samples S1 to S6, individually, and in estradiol propionate-treated ones (Group III). Samples S2 and S5 effected insignificant changes in sperm population in both Group III and Group IV rats. Sample S3 caused significant decrease in sperm count which showed its contributory action to the estrogen-induced diminished sperm count, and hence to subfertility.

The reversal of estradiol propionate-lowered sperm count (Group III) by the samples S1, S4 and S6 was considered as a significant profertility action. In Group IV, samples S1, S4 and S6 were also found to have brought about significant increase in sperm count, showing their individual profertility activity, free of interference of estradiol propionate action. Sample S3 was also found to have exerted a significant sperm-lowering effect independent of estradiol propionate action, thus discounting on possibility of synergism. Samples S2 and S5 exhibited insignificant activity as in Group III.
REFERENCES:


SPERM MOTILITY
ASSESSMENT
SPERM MOTILITY:

The seminal fluid of the male has been associated with procreation since antiquity but the scientific knowledge on fertilization really starts from Leeuwenhoek's early observations when he discovered living motile cells in the seminal fluid of all species examined under the microscope and also in the uterus of the dog after mating.1,2

Morphology of the sperm has been studied quite extensively. The head is the real vital part of the sperm and contains the nucleus. There is an articulation socket in the head in which the articulation plate of the tail fits. The tail gives the sperm its motion and carries its head to the ultimate destination. The head containing the nucleus must be carried and thus without the propulsive power of the tail, it is lost for all practical purposes. The most constant component of the tail is ring of nine doublet figures called the α or primary fibres. There is a central pair of two fibres called the β or secondary fibres, and an outer ring of coarse longitudinal fibres, the Y or tertiary fibres. The fibres are composed of two subunits, one member is a hollow tubular structure, whereas the outer has a compact appearance and it is from this part side arms usually project literally towards adjacent double figures.
Also, spokes extend radially inwards from the α fibres towards the central β fibres. The central pair of β fibres may be the skeletal or supporting units as well as conductile elements. The Y fibres are well developed in higher species. The mammalian spermatozoa is characterised by the presence of mitochondrial sheath. By the end of sperm development the mitochondria adopts a more condensed appearance than the mitochondria of a somatic cell, probably to meet the peculiar metabolic requirement of the mature sperm. 3

Various techniques are developed to study the patterns of sperm motility. Microscopic assessment of the degree of motility performed directly in semen (Harvey and Jackson, 1945; McLeod, 1946a; Emmens, 1947; Farris, 1960) 4, 5, 6, 7 or by the 'cervical mucus penetration test', in which a drop of semen is placed on the microscopic slide next to cervical mucus and the passage of spermatozoa through the mucus is followed by microscopic observation (Barter & Weisner, 1946; Harvey & Jackson, 1948). 8, 9

The determination of concentration of motile spermatozoa in a semen sample is generally held to be the criterion most clearly correlated with the actual fertility rate. Other techniques to study patterns of sperm motility involve the turbidimetric analysis of buffalo bull sperm,
study of light scattering determination of various
characteristic parameters of spermatozoa motility in a
series of sperm, stroboscopic illumination for the
assessment of hyperactivated motility of mouse spermatozoa,
sperm motile efficiency, motility evaluation of spermatozoa
by photo correlation spectroscopy, and computerised
measurements of sperm velocity and percentage of motile sperm.
Several recent studies have revealed the importance of
epididymal factors in the initiation and maintenance of
sperm motility in rodent, bovine and human sperm. The
factors responsible for forward motility occur in the
caudal region of the epididymis. The corpus portion of
epididymis has an essential role in the development
of the motility and fertilizing capacity of ram epididymal
spermatozoa. Glucosidases secreted by the epididymal
epithelium are believed to provide optimal levels
of energy for spermatozoidal maturation and have been
correlated with the percentage of sperm motility in some
studies. While some materials are localised in the cauda
epididymis and are involved in sperm motility, some
extrinsic factors also play a part in the development of
sperm motility such as cyclic AMP. A proteinaceous factor,
of testicular origin, is important in preventing the
initiation of motility while sperms reside in the distal
reproductive tract of the male rat.
Experiment 3D -

Sperm Motility Assessment

Types of sperm motility can be assessed in many ways but they generally fall into two types. First is a subjectively based ranking system(s) using a microscope. In this system, which has proven expedient for motility assessment and has simplicity of description, the percentage of progressively moving cells is determined and expressed as a percentage motility index. The second type of ranking system is a growing and diverse group of quantitative methods. Although they are mainly quantitative, some techniques are in part, a subjective determination. The use of lasers, complex microscopes, cameras, nuclear magnetic resonance and spectrophotometry are examples of some of these techniques. Repeatability of quantitative methods is good, but the results are expressed in abstract units in some of the methods (Brokaw, 1972; Fawcett, 1975; Nelson, 1974). 17,12,13 These modern methods, for assessing sperm motility, have their own limitations and are feasible in labs equipped with them. For example, optical method, involving single cell photography that emphasises the flagellar activity of single cells (Rikmenspoel, 1962; Katz & Dott, 1975), 14,15 is found largely applicable to routine clinical use only. It is of significant value in quantitating special cases or establishing of laboratory standards (Phillips, 1972). 16
The per cent sperm motility was determined by Bishop et al. method (1954) recommending the counting of number of dead spermatozoa which on subtraction from the total sperm count denoted the number of motile spermatozoa.

**Determination of per cent motility of spermatozoa in epididymis of rat**

In rats the motility of spermatozoa was studied after collecting sperm from the epididymides, a site for maturation and storage of spermatozoa. Squashes of caput and cauda epididymides, removed from rats in Experiment 3, were prepared and they were transferred to 0.9% saline, a medium that diluted sperms for convenient counting and a solution to keep sperms motile for about two hours during the period of counting.

Percentage motility was calculated by counting the dead spermatozoa using haemocytometer, i.e. usual red blood cells (RBCs) counting chamber. The dilution and multiplication factor, to get the number of dead spermatozoa, were the same as in the experiment 3C of sperm count—

\[(10) \cdot (5) \cdot (20) \cdot (1000) \cdot x = \text{dead sperms per ml.}\]

\[
\frac{\text{No. of spermatozoa in total}}{\text{No. of sperms dead}} = \text{No. of living sperms.}
\]

\[
\frac{\text{No. of living spermatozoa}}{\text{No. of total spermatozoa}} \times 100 = \text{No. of motile sperms.}
\]
Table 9 shows the per cent motility of sperms observed at various intervals of 15th min., 30th min., 1st hour and 2nd hour. The per cent motility is an equivalent of number of living sperms per total number of sperms as counted by the formula discussed previously.

For statistical calculations, per cent motility figures of 15th min. of caput and cauda portions of all groups I to IV were considered.

Figures A to N and A₁ to N₁ show per cent sperm motility at the intervals of 15th min., 30th min., 1st hr. and of Groups I to IV rats 2nd hr., of caput and cauda epididymides. A to N for caput and A₁ to N₁ for cauda portion.
| Table 9 |

Per-cent Motility of Spermatozoa in Epididymis

Period of treatment - 30 days

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Treatment</th>
<th>Epididymis</th>
<th>Cauda</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Caput</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 Min</td>
<td>30 Min</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>18.87 ± 0.17</td>
<td>17.64 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>Estradiol propionate (EP) ( ug/kg/rat/day)</td>
<td>9.29 ± 0.43</td>
<td>7.24 ± 1.21</td>
</tr>
<tr>
<td>II</td>
<td>A1 (EP+S1)</td>
<td>18.43 ± 1.24</td>
<td>16.39 ± 1.12</td>
</tr>
<tr>
<td></td>
<td>B1 (EP+S2)</td>
<td>9.63 ± 0.83</td>
<td>6.46 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>C1 (EP+S3)</td>
<td>19.03 ± 0.36</td>
<td>7.43 ± 0.84</td>
</tr>
<tr>
<td></td>
<td>D1 (EP+S4)</td>
<td>17.58 ± 0.88</td>
<td>14.63 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>E1 (EP+S5)</td>
<td>13.63 ± 0.47</td>
<td>9.63 ± 1.23</td>
</tr>
<tr>
<td></td>
<td>F1 (EP+S6)</td>
<td>17.64 ± 0.33</td>
<td>14.78 ± 0.79</td>
</tr>
<tr>
<td>III</td>
<td>A2 S1</td>
<td>19.98 ± 1.24</td>
<td>17.78 ± 1.02</td>
</tr>
<tr>
<td></td>
<td>B2 S2</td>
<td>9.87 ± 0.78</td>
<td>6.59 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>C2 S3</td>
<td>9.08 ± 1.16</td>
<td>7.07 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>D2 S4</td>
<td>19.86 ± 0.64</td>
<td>14.78 ± 1.22</td>
</tr>
<tr>
<td></td>
<td>E2 S5</td>
<td>14.24 ± 0.78</td>
<td>9.77 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>F2 S6</td>
<td>19.93 ± 1.17</td>
<td>14.93 ± 0.87</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

Each recording in the table represents a mean of three observations in duplicate.
Table 9 Contd.

P values:

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Epididymis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caput</td>
</tr>
<tr>
<td>I vs II</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(A_1(S_1))</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(B_1(S_2))</td>
<td>P &lt; 0.60</td>
</tr>
<tr>
<td>(C_1(S_3))</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>II vs III</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(D_1(S_4))</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(E_1(S_5))</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>(F_1(S_6))</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>IV vs IV</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(A_2(S_1))</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(B_2(S_2))</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(C_2(S_3))</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(D_2(S_4))</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(E_2(S_5))</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>(F_2(S_6))</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>
**Figure C**

**Caput**

- EP + S₁

**Cauda**

- EP + S₁

- % Motility

- Hours: 1, 2

**Figure C₁**

**Caput**

- EP + S₂

**Cauda**

- EP + S₂

- % Motility

- Hours: 1, 2

**Figure D**

**Figure D₁**
CAPUT

EP + S₃

% MOTILITY

HOURS

FIGURE E

CAUDA

EP + S₃

% MOTILITY

HOURS

FIGURE E₁

CAPUT

EP + S₄

% MOTILITY

HOURS

FIGURE F

CAUDA

EP + S₄

% MOTILITY

HOURS

FIGURE F₁
FIGURE K

FIGURE K₁

FIGURE L

FIGURE L₁
Summary

In Experiment 3B 'sperm motility' (Table 9) was considered as a parameter of fertility. The influence of the samples $S_1$ to $S_6$ on sperm motility was noted in rats rendered experimentally subfertile by estradiol propionate which diminished sperm motility (Group 33) significantly -- on the 30th (last) day of the treatment.

It was found that the samples $S_4$, $S_2$, $S_5$ and $S_6$ brought about significant increase in per cent sperm motility in adult male albino rats both in normal subjects (Group 44) treated with the samples $S_1$ to $S_6$, individually, and in estradiol propionate - treated ones (Group 333). Samples $S_3$ caused significant decrease in per cent sperm motility which showed its contributory action to the estradiol propionate - induced diminished sperm motility, and hence to subfertility.

The reversal of estradiol propionate-lowered sperm motility (Group 333) by the samples $S_1$, $S_2$, $S_4$, $S_5$ and $S_6$ was considered as a significant profertility action. In Group 44, samples $S_1$, $S_2$, $S_4$, $S_5$ and $S_6$ were also found to have brought about a significant increase in sperm motility exhibiting their individual profertility action, free of interference of estradiol propionate. Similarly, sample $S_3$ was found to have exerted a significant sperm motility-lowering effect independent of estradiol propionate, thus discounting on possibility of occurrence of synergism.
REFERENCES:


REFERENCES CONTD. :


REFERENCES CONTD.


FRUCTOSE
ESTIMATION
ROLE OF FRUCTOSE IN FERTILITY IN MALES

It has been almost half a century since Mc Carthy et al. 1 first noted the presence of a reducing sugar in semen which they believed was glucose. Subsequent observations confirmed their findings. 2-4 However, it was Mann 5, 6 who proved that the seminal sugar was fructose and not glucose and that it was elaborated by the seminal vesicles under the influence of testosterone. Since then, numerous investigators have estimated the fructose content of semen and have tried to correlate it either with the sperm count or with the metabolic activity of spermatozoa, or their motility.

Site of formation

There are considerable quantitative differences between the fructose content of various species: man, bull, ram, boar, stallion, goat, opossum, rabbit, guinea pig, rat, mouse and hamster (Mann, 1949). 7 The reason for this conspicuous species differences in the concentration of fructose as well as the individual fluctuations, is the fact that fructose is a product not of testes, but of the male accessory organs of reproduction, principally seminal vesicles (Mann, 1946b). 8 Naturally, the highly variable anatomic characteristics of these glands such as their size, actual storage capacity, and secretory activity are important factors which determine the final output of fructose in the
ejaculate.

All these considerations are pertinent to studies of human semen because of the exceptionally large individual variations in the secretory function of the seminal vesicles and their rather small storage capacity which explains why the collection of consecutive ejaculates within a few days, usually yields samples with a conspicuously low level of fructose. It appears that a time interval of about two days is required to replenish the store of fructose in the vesicular secretion of man. Unlike in certain mammals, the human seminal vesicle and vas deferens open into a urethra through a common channel known as the ejaculatory duct. Consequently, any obstruction at the level of the ejaculatory ducts will prevent both fructose and spermatozoa from reaching the urethral canal. The fact has been aptly chosen as an aid to medical diagnosis by Young (1948, 1949) who described the case of a man in whom repeated semen analysis failed to detect fructose or sperm, although testicular biopsy revealed normal spermatogenesis; the case has been diagnosed as congenital bilateral aplasia of the vasa deferentia.
It must also be mentioned that though the seminal vesicles are the main source of fructose in the higher mammals, an additional amount of the seminal sugar is derived from the ampullar glands (Mann, 1948a), and in some animals also from certain other glands. Thus in the rabbit, fructose was located in the glandula vesicularis (a structure corresponding to seminal vesicles) and in the ampullae, as well as in the prostate (Davies and Mann, 1947b). In rat the seminal vesicles are free from fructose altogether; instead, fructose is found in the dorso-lateral prostate and in the so-called coagulating gland, a small organ immediately adjacent to the seminal vesicles proper, with which it shares a common peritoneal sheath (Humphrey and Mann, 1948, 1949).

Since fructose is produced by the accessory glands, and not the testes, it is not surprising that in whole fresh semen there is no direct proportion between fructose and sperm density. On the contrary, both in man and in domestic animals, an inverse ratio between fructose and sperm concentration in semen is frequently met with; the simplest explanation is that in a dense sample of semen the space occupied by the sperm cells is relatively larger, and the volume taken up by the fluid portion, i.e. the fructose-containing seminal plasma, correspondingly less.
This factor has a direct bearing on the interpretations of laboratory examinations concerned with semen and male fertility or sterility. It explains for instance, why a semen sample with high content of fructose need not necessarily be one of good sperm quality, and furthermore, why it is possible to come across samples with a high fructose content but of low sperm density. In fact some of our highest fructose values recorded so far, were encountered in semen of vasectomised and thus completely azoospermic, individuals.

Seminal fructose as an indicator of male sex hormone activity

The "fructose test", originally described by Mann and Parsons (1947)\textsuperscript{15} and subsequently developed by Mann, Davies and Humphrey (1949)\textsuperscript{16} Mann, Lutwak and Price (1948)\textsuperscript{17} and Mann and Parsons (1950)\textsuperscript{18}, is founded on the observation that the capacity of the accessory organs to produce fructose and, thereby, the actual level of fructose in the seminal plasma, reflects in a faithful manner the degree of testicular hormone activity in the male, and in this way provides an accurate indicator of endocrine testicular function. In experiments on rats and rabbits it was shown that seminal fructose disappears completely within two
weeks after castration and also that the postcastrate fall in the level of fructose can be prevented, or, if already developed, fully restored, by the implantation of testosterone.

The test can be carried out in two ways, by the chemical analysis of the seminal fluid collected from an intact animal by means of an artificial vagina, or by analysis of accessory sex organs of reproduction obtained from the experimental animal by dissection. The first method gives an opportunity to observe in the same animal the time sequence of changes brought about by castration and hormone treatment and eliminates the sacrifice of the experimental animal. In the second procedure, on the other hand, the test can be used for a quantitative assay of male sex hormone activity in the whole body, isolated tissues, body fluids and hormone preparations.

Endocrinological problems, in the recent years, were studied with the aid of "fructose test", applied either alone or in conjugation with "citric acid test", which depends on the relationship between the secretion of citric acid by some of the sex accessory organs and the male sex hormone activity.
In young rabbits (Davies and Mann, 1947b) \(^1\), rats (Mann, Lutwak-Mann and Price, 1948) \(^2\), and other mammals, fructose and citric acid appear in the accessory glands at an early age, before there is any evidence of active spermatogenesis; since the secretion of both these substances depends on the presence of male sex hormone, it must be concluded that the hormone begins to function in the male body well in advance of the actual spermatogenesis. Thus for instance, in bull-calves appreciable amounts of fructose are found in the vesicular secretion already at the age of about four months, whereas the first mature spermatozoa appear nearly eight months later.

One cannot, of course, rule out the possibility that the testicular hormone is active in the bull-calf even before the age of four months, but if so, then either its concentration is too small to produce a distinct response in the accessory organs, or else its action is countered by some other factors.
Effect of testosterone on the appearance of fructose in castrated animals

The following experiment was carried out by Mann, Davies and Humphrey (1949) at the Agricultural Research Council Field Station at Crompton, in Berkshire. Six bull calves were used. These were castrated when one to two weeks old, i.e. at an age prior to the appearance of fructose in the seminal glands. Several months later two of the calves received subcutaneous implants of 0.5g pellets of pure testosterone, whereas the remaining four were left untreated. After another four weeks all six animals were sacrificed and their seminal glands dissected out, weighed and examined both chemically and histologically. The unused portions of the hormone pellets were recovered from the subcutaneous tissue of the two hormone-treated calves; their weights were 0.344g and 0.338g respectively, showing that the quantities of testosterone absorbed per month per animal were 0.156g and 0.162g, respectively. Chemical analysis revealed the presence of considerable amounts of fructose in the seminal glands in response to four weeks hormone treatment (51 mg fructose per 100g tissue or 5.3 mg fructose per total gland), as against a negligible fructose content in the untreated castrates (8 mg per 100g or 0.25 mg per total gland).
In comparison with and in contrast to the marked chemical difference, the evidence for the functional recovery in the seminal glands, as assessed by the histological examination, was practically imperceptible. In this way it was evident that the early effects of testosterone treatment can be established far more convincingly than the large percentage-increase in the fructose content of the seminal gland secretion than by means of histological methods which at this stage failed to show significant changes in the glandular tissue.

An investigation concerned with the response to testosterone was also made by Rudolph and Samuels (1949) on rats. In castrated rats a significant increase in the fructose content of accessory organs was noticed already ten hours after the injection of 1mg. testosterone propionate. In bulls, fructose disappeared from ejaculates within two weeks after castration, led to a rapid return of fructose production to the pre-castrate level; yet, in spite of the fully restored fructose level, such seminal plasma, when added to washed spermatozoa obtained from a normal bull, was unable to support sperm metabolism to the same extent as plasma from normal, i.e. non-castrated animals.

An interesting example of the application of the fru-
ctose test to problems of infertility in man has been provided by a study of four eunuchoid patients who responded to androgenic treatment with a highly significant elevation of fructose in semen (Ladau and Loughead, 1951).²²

It seems probable that the fluctuations of fructose level in the semen of normal individuals may also be due, in part at least, to some periodic changes in the activity of the testicular hormone in the male body. Normal rats, injected with large doses of the male hormone invariably react by an increased level of fructose formation well above the non-treated controls. The effect is particularly striking with breeds of animals which exhibit a relatively low physiological level of fructose formation. In this connection, however, it is interesting to note that when injections of large doses of testosterone propionate in normal rats are continued to excess, e.g. 200µg daily for forty days, the state of administration in the accessory organs is accompanied by the marked decline in the size of the testes; after seven weeks of such treatment the reduction in the weight of the testes is nearly 50% (Mann and Parsons, 1950).²³ Injections of excessive doses of androgens are well known to produce harmful effects on the spermatogenesis
in animals and in man (Moore, 1939; Mc Cullagh and Mc Gurl, 1939; Heckel, 1951)\textsuperscript{24,25,26}.

In normal bulls, a dose of 100mg testosterone propionate, repeated three times weekly for six weeks, appears to produce a very slight increase in the level of fructose in semen (Gassuer, Hill and Sulzberger, 1952).\textsuperscript{27} However, according to another report, sexual excitation prior to service has stimulating effect on the output of fructose in bull semen (Branton, D Arensbourg and Johnson, 1952).\textsuperscript{28}

\textbf{Hormone-induced formation of fructose in subcutaneous transplants from accessory organs:}

Once the dependence of seminal fructose upon the activation of male sex hormone had been established it was possible to enquire into the mechanism of this hormonal relationship. One of the problems to settle was the extent to which the process of fructose generation in accessory glands depends upon the preservation of intact vascular and neural links. Insight into this matter was gained by the technique of subcutaneous transplantation, when it was demonstrated very clearly that small fragments of rat
coagulating gland, about 1mg in weight, transplanted subcutaneously into normal adult male hosts, given well and showed after some weeks of subcutaneous development of a high content of fructose. Following castration of the hosts, the transplants lost their ability to form fructose but this was promptly restored by treatment with testosterone propionate. Perhaps, the most remarkable fact in these experiments was that the growth of the grafts and their chemical secretory function occurred not only in male but also in female hosts provided with testosterone (Lutwak-Mann, Mann and Price, 1949).29

Thus for the first time the effect of male sex hormone on fructose secretion was demonstrated in tissue fragments dissected from the male accessory sex organs and developing in complete isolation from the rest of the male generative system. Actually, the transplant had an even higher fructose content than the corresponding intact glands of the graft-bearing hosts, because unlike intact glands, the grafts lack a secretory outlet.
Role of hypophysis in the formation of fructose

The endocrine influence of the testes on the formation of fructose in accessory organs is integrated closely with the functioning of the anterior pituitary gland. Hypophysectomy, like gonadectomy, invariably results in rapid decline in the level of fructose in the seminal plasma (Mann and Parsons, 1950). In the rabbit, for instance, a three to four weeks period after castration or hypophysectomy alike, usually leads to complete disappearance of fructose an ejaculate collected by means of an artificial vagina three weeks after the operation contains no more than 20μg fructose, as compared to 500 to 1000μg, before the operation. Both castrated as well as hypophysectomised animals promptly respond to subcutaneous implantation or injection of testosterone with renewed secretion of fructose. The same happens if instead of testosterone pregnant mare serum gonadotropin is injected into a hypophysectomised animal.

Effect of malnutrition on the fructose content:

Lutwak-Mann and Mann (1950a, b, 1951) applied chemical methods to the study of chemical changes brought about in the secretory function of rat accessory organs by vitamin B deficiency and inanition, and found that in rats maintained for four weeks on a deficient diet the content of fructose and citric acid in the accessory glands was reduced to a
castrate level. By treatment with testosterone propionate (0.2mg daily for one week) or with chorionic gonadotropin (200 units every other day for two weeks), the secretory activity of the glands could be completely restored. Mann and Walton (1953) made a study of the effect of underfeeding on the genital functions in the bull and found that, in contrast to the testes, the secretory function of the male accessory glands was markedly affected by understanding.

The enzymic mechanism of fructose formation:
Mann and Lutwak (1948, 1951 a,b) showed details of the mechanism whereby glucose is converted in the accessory gland tissue to fructose in some in vitro experiments; these revealed that small amounts of fructose are formed as a result of incubation of minced accessory gland tissues with glucose, and that these tissues possess the entire enzymic system which can convert glucose to fructose.

In the semen there are present in high concentration enzymes which belong to the group of phosphatases and include the alkaline phosphatase; the latter capable of splitting a number of phosphohexoses, including 6-phosphofructose, 1-phosphofructose and 1:6-diphosphofructose, to phosphoric acid and free fructose. The alkaline phosphatase found in semen is derived from several accessory organs of reproduction but its principal source in higher animals is the seminal vesicle. Owing to this fact, the usual channels of carbohydrate metabolism are diverted in the vesicular tissue: phosphofructoses are not metabolised to lactic acid.
as would be the case e.g. in muscle, but are dephosphorylated instead, so that free fructose is formed.

Tissue slices from the rat coagulating gland can glycolyse anaerobically glucose at a much higher rate than fructose; this, in turn, may be due to the ability of the tissue to re-phosphorylate more effectively glucose to 6-phosphoglucone, than fructose to 6-phosphofructose. Such evidence is at present available, derived from both in vivo and in vitro experiments, indicates that the enzymic reactions involved in conversion of blood glucose to seminal fructose are as follows:

```
BLOOD GLUCOSE
   ↓
GLYCOGEN
   ↓
1-PHOSPHOGLUCOSE
   ↓
6-PHOSPHOGLUCOSE
   ↓ PHOSPHOHEXOSE ISOMERASE
     ↓ SEMINATE FRUCTOSE
GLUCOKINASE ALKALINE PHOSPHATASE
   ↓
GLUCOSE
```

```
   ↓ ACIDIC PHOSPHATASE
8-PHOSPHOGLUCOSE
```
Clinical studies of Fructose:

C. Shirren (1963)\textsuperscript{37} carried out biochemical and morphological examinations on human semen on more than 2,000 patients at a fertility clinic. He found that the fertility of human semen depends on the presence of normal spermatozoa; centrifuged cell-free plasma shows no fructolysis.

The relationship between fructose content and the sperm count of the semen has been investigated. It was found that the absolute fructose concentration was inversely proportional to the number of spermatozoa. The normal fructose value was found to be 1,200 to 4,500\(\mu\)g/ml; values below 1,200\(\mu\)g/ml were considered to be pathological due to inflammation of the seminal vesicles or to androgen deficiency.

The variation in fructose content in normal patient (1,200 to 4,500\(\mu\)g/ml) was never found to be 1,200\(\mu\)g/ml. The fructose concentration was diminished following abstinence of 8-14 days or more.

The average value is higher in groups of aged patients. Particular attention was given to the group of patients with "normospermia" with postpubescent interstitial-cell insufficiency in this investigation. The patients are infertile but after treatment with methyltestosterone or chorionic gonadotropin the fructose value returned to normal
and patients became fertile.

Shirren concludes his study with emphasis on importance of fructose test in the evaluation of fertility. He says that on the basis of the preceding experimental and diagnostic findings, a modern fertility examination must be considered incomplete with the mere morphological examination of the ejaculate; on the contrary a biochemical analysis of the semen is a necessity in order to exclude a postpubescent interstitial-cell deficiency.

The diagnosis and treatment of male infertility remains quite a complicated, confusing problem for clinicians. Laboratory evaluation of male infertility has depended upon counts, viability, morphology, and the degree of activity of spermatozoa. Such studies cannot assess the role of seminal plasma in reduced fertility potential. Therefore, it seems desirable to investigate the physiologic and biochemical aspects of the plasma as related to such problem.

Moon and Bunge (1968) directed their investigation to the fructose level in semen samples from patients who presented as having possible male infertility.

Of male patients coming to the infertility clinic, 112 produced semen samples by masturbation after a minimum of 3 days' continence. Routine examination included the noting of volume, liquefaction time, count in millions
per milliliter, viability, and abnormal forms. Activity was judged subjectively. The fructose content and fructolysis were performed by the method of Mann within 33 min. after submission of the sample. Of the men, 44 were studied at least twice and in some instances 3 samples were examined. Each sample was centrifuged for 20 min. at 1800 rpm and frozen for future study.

It should be emphasized that the classification of semen quality by counts, etc., is an arbitrary one, albeit it based upon many years of observation. Normal semen was categorized as having the following characteristics:

1. Consistent sperm counts above 20 million per milliliter, with good activity.
2. Viability counts of 60%
3. Abnormally formed sperm below 30%
4. Good liquefaction within 30 min.
5. Total volume of semen above 1.0 ml.

Abnormal semen does not meet any of these requirements.

Fructose Value in Normal Semen

In table 1, sperm counts are classified into 3 groups:
1. 20-40 million per milliliter; (2) 40-60 million; and (3) over 60 million. The initial fructose levels are recorded to ascertain any correlation between these observation. The level of fructose in 61 samples was widely distributed from 84 to 636 mg./100 ml. of semen. The mean value was 222 mg./
100 ml. There is no statistical difference between sperm concentration and fructose level, although there is some tendency for lower sperm counts to show a slightly higher initial fructose level.

**TABLE-1. Fructose Values in Normal Semen**

<table>
<thead>
<tr>
<th>Patients (No.)</th>
<th>Sperm count (million/ml)</th>
<th>Fructose</th>
<th>Total ejaculatory fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Avg. (mg./100ml.)</td>
</tr>
<tr>
<td>16</td>
<td>20-40</td>
<td>141-636</td>
<td>259</td>
</tr>
<tr>
<td>18</td>
<td>40-60</td>
<td>84-360</td>
<td>213</td>
</tr>
<tr>
<td>27</td>
<td>60</td>
<td>90-390</td>
<td>206</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>84-636</td>
<td>222</td>
</tr>
</tbody>
</table>

**TABLE-2. Fructose Values in Abnormal Semen**

<table>
<thead>
<tr>
<th>Patients (No.)</th>
<th>Semen quality</th>
<th>Fructose</th>
<th>Total ejaculatory fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Avg. (mg./100ml.)</td>
</tr>
<tr>
<td>25</td>
<td>Oligospermia*</td>
<td>59-330</td>
<td>211</td>
</tr>
<tr>
<td>5</td>
<td>Azoospermia</td>
<td>114-411</td>
<td>193</td>
</tr>
<tr>
<td>8</td>
<td>Postvasectomy azoospermia</td>
<td>156-396</td>
<td>278</td>
</tr>
<tr>
<td>10</td>
<td>Low viability**</td>
<td>60-314</td>
<td>213</td>
</tr>
<tr>
<td>3</td>
<td>Markedly low viability***</td>
<td>22-36</td>
<td>29</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>22-411</td>
<td>209</td>
</tr>
</tbody>
</table>

* Below 20 M/ml.; ** Viability below 60% to 30%; *** Viability below 30%.
Initial Fructose Value in Abnormal Semen

As stated, abnormal semen did not meet any of the requirements stated for normal semen. For example, a specimen consisting of a volume of 1 ml., containing less than 20 million spermatozoa per milliliter with 30% viability and 40% abnormal forms, and possessing poor activity, was classified as abnormal. Table 2 records fructose levels in abnormal semen sample characterized as oligospermia (Below 20 million per milliliter); azoospermia; postvasectomy azoospermia; low viability (below 60%); markedly low viability. (below 30% or total number of viable spermatozoa below 40 million).

Oligospermia, azoospermia, postvasectomy azoospermia and low viability groups show the average level of fructose is not statistically different from the normal group. Generally, there is some tendency to higher fructose level in the postvasectomy azoospermia group, but it is of no great magnitude. In the markedly low viability group, 3 patients have shown a consistent low value of fructose (statistically different from the other groups).
Experiment 3-E:

Fructose Estimation:

Apart from its value in the metabolism of spermatozoa, the estimation of seminal fructose is becoming increasingly important for assessing semen quality in artificial insemination in the diagnosis of infertility (Mann, 1948 and 1952)\(^{38,39}\).

Fructose is formed from blood glucose by the accessory sexual organs, of which the seminal vesicles are usually the most important in this regard (Mann, 1954)\(^{20}\). However, in rat the seminal vesicles are free from fructose, altogether; instead, fructose is found in the dorsolateral prostate and in the coagulating gland, a small organ, immediately adjacent to the seminal vesicles proper, with which it shares a common peritoneal sheath (Humphrey and Mann, 1948, 1949)\(^{13,14}\). Fructose estimations (Experiment 3E (1)) have been carried out in the present work in the adult male albino rats rendered functionally sterile by microdose of estradiol propionate (10\(\mu\)g/rat/day; i.m.). Activity of the test samples \(S_1\) to \(S_6\) were noted in the coagulating gland after a period of treatment of 30 days, to note whether the samples brought about resumption to normal, as in controls, of fructose levels lowered by estradiol propionate in rendering them functionally sterile.

Estimation of fructose in the coagulating gland of rat:

Fructose estimations were carried out on the excised coagulating gland soaked between filter papers, from the dissected rats of groups I to IV (Table 14) after 30 days of treatment of estrogen-treated rats. Care was taken to freeze the coagulating
gland to prevent any in-vitro fructolysis, and brought out just when fructose was to be determined.

For fructose estimation tissue homogenate of coagulating gland was prepared and it was deproteinised by zinc sulphate. Alcoholic resorcinol was used for colour development. Fructose was quantified uv spectrophotometrically at 490 nm with the help of a standard curve for which known concentrations were prepared.

Procedure:

1. Took (20μg/ml) tissue homogenate of coagulating gland in glass-distilled water. Two ml of this homogenate was taken.

2. Added 1 ml of ZnSO₄ and 1 ml of 0.5N NaOH, mixed it well.

3. Centrifuged and separated out supernatant in a test tube.

4. Took (a) 2 ml of supernatant (bland); (b) 2 ml distilled water and (c) fructose standard: 2 ml of standard fructose containing 0.1 mg/ml.

5. Added 2 ml of alcoholic resorcinol (0.1%) - in all the three (a), (b) and (c).

6. Added 6 ml of 30% HCl to all the three. Shook vigourously all the three.

7. Placed in water bath at 80°C for 8-10 minutes.

8. Cooled in running tap water.

9. Read at 490 nm using Shimadzu double-beam spectrophotometer, uv-190.
**Blank:**

For blank '2ml' of distilled water was taken.

**Fructose standard:**

For standard fructose solution 0.1 mg/ml of fructose was taken.

**Stock solution:**

Stock solution was prepared by dissolving 50 mg of fructose in glass distilled water.

**Working solution:**

One ml of solution was made upto 100 ml to have a final concentration of 50 μg/ml = 0.05 mg.

**Acid solution:**

30% HCl = 82.5 ml acid-made up with 100 ml of water.

**Deproteinising agent:**

10% (w/v) ZnSO₄·7H₂O and 0.5 N NaOH. Two ml of this solution was used for analysis.

**Calculation:**

\[
\frac{DU}{DS} \times \text{Quantity in standard (mg)} \times \text{mg fructose/g of coagulating gland.}
\]

**DU = Absorbance**

**DS = Absorbance of standard.**
**Experiment 3E**

**Table 11:**

**Effect of the Plant Compounds on Fructose Content in Coagulating Gland of Estradiol Propionate - Treated (Chemically Hypophysectomised or Functionally Sterilised) Rats.**

*Period of treatment = 30 days*

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Fructose Content (mg/g) in Coagulating Gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Controls</td>
<td>10</td>
<td>$0.99 \pm 0.03^*$</td>
</tr>
<tr>
<td>II</td>
<td>Estradiol Propionate (EP) (10μg/rat/day, i.m.)</td>
<td>10</td>
<td>$0.15 \pm 0.004$</td>
</tr>
<tr>
<td>$A_1$</td>
<td>EP+S₁</td>
<td>10</td>
<td>$0.84 \pm 0.002$</td>
</tr>
<tr>
<td>$B_1$</td>
<td>EP+S₂</td>
<td>10</td>
<td>$0.77 \pm 0.003$</td>
</tr>
<tr>
<td>$C_1$</td>
<td>EP+S₃</td>
<td>10</td>
<td>$0.73 \pm 0.007$</td>
</tr>
<tr>
<td>$D_1$</td>
<td>EP+S₄</td>
<td>10</td>
<td>$0.82 \pm 0.002$</td>
</tr>
<tr>
<td>$E_1$</td>
<td>EP+S₅</td>
<td>10</td>
<td>$0.73 \pm 0.004$</td>
</tr>
<tr>
<td>$F_1$</td>
<td>EP+S₆</td>
<td>10</td>
<td>$0.79 \pm 0.07$</td>
</tr>
<tr>
<td>A₂</td>
<td>S₁</td>
<td>10</td>
<td>$0.03 \pm 0.03$</td>
</tr>
<tr>
<td>B₂</td>
<td>S₂</td>
<td>10</td>
<td>$0.98 \pm 0.004$</td>
</tr>
<tr>
<td>$C₂$</td>
<td>S₃</td>
<td>10</td>
<td>$0.48 \pm 0.002$</td>
</tr>
<tr>
<td>$D₂$</td>
<td>S₄</td>
<td>10</td>
<td>$0.08 \pm 0.02$</td>
</tr>
<tr>
<td>$E₂$</td>
<td>S₅</td>
<td>10</td>
<td>$0.97 \pm 0.01$</td>
</tr>
<tr>
<td>$F₂$</td>
<td>S₆</td>
<td>10</td>
<td>$1.06 \pm 0.03$</td>
</tr>
</tbody>
</table>

*Mean ± S.E.*
$P$ values:

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Fructose Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>I vs II</td>
<td>$P \leq 0.01$</td>
</tr>
<tr>
<td>$A_1$ ($S_1$)</td>
<td>$P \leq 0.01$</td>
</tr>
<tr>
<td>$B_1$ ($S_2$)</td>
<td>$P \leq 0.05$</td>
</tr>
<tr>
<td>II vs III</td>
<td>$P \leq 0.2$</td>
</tr>
<tr>
<td>$C_1$ ($S_3$)</td>
<td>$P \leq 0.01$</td>
</tr>
<tr>
<td>$D_1$ ($S_4$)</td>
<td>$P \leq 0.2$</td>
</tr>
<tr>
<td>$E_1$ ($S_5$)</td>
<td>$P \leq 0.2$</td>
</tr>
<tr>
<td>$F_1$ ($S_6$)</td>
<td>$P \leq 0.01$</td>
</tr>
</tbody>
</table>

| III vs IV     | $P \leq 0.05$    |
| $A_2$ ($S_1$) | $P \leq 0.05$    |
| $B_2$ ($S_2$) | $P \leq 0.2$     |
| IV vs V       | $P \leq 0.05$    |
| $C_2$ ($S_3$) | $P \leq 0.01$    |
| $D_2$ ($S_4$) | $P \leq 0.2$     |
| $E_2$ ($S_5$) | $P \leq 0.2$     |
| $F_2$ ($S_6$) | $P \leq 0.05$    |
**Figure 0**  Fructose Standard Curve
Summary:

In the Experiment 3F (Table 11) the samples S1, S4 and S6 were found to have brought about significant increment in fructose content in the coagulating gland of adult male albino rats both in normal subjects (treated individually with the samples S1, S4 and S6 only) and in estradiol-propionate-treated ones (Group III) following the last (30th) day of the experiment.

Samples S2 and S5 effected insignificant changes in per cent motility in both Group III and Group IV rats, whereas sample S3 caused significant decrease in per cent motility which suggested its contributory action to estrogen-induced diminished sperm motility - and indicated subfertility when the significant fall of fructose in the Group III and IV was compared with that of Group I (Controls).
REFERENCES


7. MANN, T. (1949). Advances in Enzymology. 9, 481

REFERENCES CONT'D:


REFERENCES CONT'D:


REFERENCES CONT'D.


GLYCERYLPHOSPHOCHOLINE
ESTIMATION
GLYCERYLPHOSPHORYLCHOLINE AND PHOSPHORYLCHOLINE

The nature of the phosphorus compounds in semen which yield choline after ejaculation, was investigated by Lundquist (1946, 1947a, b)\textsuperscript{1, 2} and by Diament, Kahane and Levy (1952).\textsuperscript{3}

In human semen deproteinized freshly with trichloroacetic acid, the Danish investigator found 110 mg. acid-soluble P/100 ml., including 10 mg./100 ml. of inorganic phosphate. On neutralization with barium hydroxide and precipitation with 2 vol. of ethanol, he recovered 60-70% of the phosphorus in the filtrate and from this he obtained by precipitation with mercuric chloride a fraction containing nitrogen and phosphorus in a ratio of approximately 1 : 1. The phosphorus compound thus separated was found to be very resistant to acid hydrolysis and no choline was set free from it after an hour's hydrolysis with n-H\textsubscript{2}SO\textsubscript{4}(100\textdegree), long enough for glycerylphosphorylcholine, to release all its choline in a free form. On the other hand, under the influence of the prostatic secretion the compound yielded equivalent amounts of choline and inorganic phosphate. All these facts pointed to the identity of the compound with phosphorylcholine, a substance previously isolated from beef liver (Inukai and Nakahara, 1935).\textsuperscript{4} Lundquist sought to obtain proof by preparing the calcium salt; this he found to be identical with the calcium salt of pure phosphorylcholine, C\textsubscript{5}H\textsubscript{13}O\textsubscript{4}NPClCa.4H\textsubscript{2}O, obtained synthetically by the method of Plimmer and Burch (1937).\textsuperscript{5}
Phosphorylcholine

The distribution of phosphorylcholine in the human reproductive organs has not been investigated in detail, but Huggins and Johnson (1933)\textsuperscript{6} have good evidence that the bulk of the phosphorus present in the human seminal plasma is derived from the vesicular secretion. From this Lundquist inferred that phosphorylcholine is formed in the seminal vesicles, and that the dephosphorylation is initiated at ejaculation, as a result of contact between the prostatic secretion which contributes the 'acid' phosphatase, and the vesicular secretion which provides the substrate; the optimum pH for the dephosphorylation of phosphorylcholine by the prostatic phosphatase measured in acetate buffer solutions is about 6.3 (Lundquist, 1947a, b).\textsuperscript{7} It is of some interest to recall here the claim put forward by Kutscher and Sieg (1950)\textsuperscript{8} that preparations of both the 'acid' and the 'alkaline' phosphatases contain pyrophosphorylcholine as a characteristic constituent. However, Roche and his colleagues (1952)\textsuperscript{9} were unable to detect any cophosphatase activity in pure, synthetically prepared pyrophosphorylcholine.
The possibility that compounds other than phosphorylcholine may act as precursors of free choline in semen was indicated already by the earlier observation of Kahane and Levy that the quantity of choline liberated after ejaculation exceeds considerably the simultaneously formed inorganic phosphate. Following up this observation, the French investigators accomplished in 1952 the isolation of a second natural precursor of choline, namely glycercylphosphorylcholine, from the seminal vesicle secretion of rats; the isolation and identification was performed as a ferric chloride compound (Diament, Kahane and Levy 1952, 1953).\textsuperscript{10,11} A similar result was obtained by Lundquist (1953)\textsuperscript{a,b,12,13} from his studies on the secretions of the seminal vesicles in rat and guinea-pig and the glandula vesicularis of rabbit.

\[\text{Glycercylphosphorylcholine}\]
Physiological function of free and bound choline

The occurrence of choline, phosphorylcholine and glyceryl-
phosphorylcholine in semen and in the accessory secretions
naturally rises the problem of their physiological function.
One possibility which merits serious attention, is that these
compounds may be bound up specifically with the metabolism of
phospholipids in either the male accessory organs or in the
spermatozoa.

The general importance of choline in the lipid metabolism of
animals was first brought to light in 1932 when Best and his
co-worker\(^{14,15}\) demonstrated that the appearance of the 'fatty
livers' in rats fed a choline-deficient, high-fat diet, could
be prevented by dietary supplements of choline. Researches
which followed established two principal functions of choline,
the lipotropic activity and the stimulating action on the
turnover of phospholipids. In 1939, du Vigneaud\(^{26}\) and his
co-workers discovered that choline is an important dietary
source of methyl groups for the living animal, and this led
to the recognition of choline as a participant in transme-
thylation processes. These three fundamental functions
probably represent the clue to the understanding of the
manifold symptoms associated with choline deficiency.
Among the various manifestations of choline deficiency
those concerned with reproduction are particularly striking;
choline is known, for example, to be essential for egg production in the chicken, as well as for normal lactation and nutrition in rats.

The role of choline in transmethylation is linked with the presence of the trimethyl quaternary nitrogen. It is worth noting, however, that while the phenomenon of transmethylation is common to a whole group of compounds bearing labile methyl groups, the lipotropic activity is restricted to choline and a few closely related derivatives. One of the lipotropically active derivatives is phosphorylcholine (Welch and Welch, 1948), and there is some evidence that the incorporation of choline into phospholipids proceeds via phosphorylcholine (Wittenberg and Kornberg, 1953).

A further possibility regarding the function of choline in semen is that choline and its derivatives belong to a group of substances endowed with well-defined pharmacological properties, and it is not improbable that the base itself or one of its compounds may exert some pharmacodynamic effects either on the spermatozoa or, perhaps, on some parts of the male or female reproductive tract. When assayed by Goldblatt (1935b) on the m. rectus abdominis of the frog, 1 ml. human seminal plasma exhibited roughly the same activity as 1 μg. acetylcholine. There is, however, no chemical evidence to show that the substance in seminal plasma, responsible for this activity is in fact, acetylcholine.
Choline

Compared with other animal tissues and body fluids, semen ranks as one of the richest sources of choline. It owes its high choline content to the seminal plasma and not to spermatozoa as such. In rat, Fletcher, Best and Solandt (1935)\textsuperscript{20} found the following distribution of total choline (mg./100 g.): seminal fluid 514, brain 325, liver 260, pancreas 232, stomach 152, uterus 74, fat 23, blood 22. The composition is similar in other species, including man, where values exceeding 2000 mg./100 ml. semen have been observed. This may explain a statement by Marcille (1931)\textsuperscript{21} that a positive Florence reaction can be obtained with dried human semen even when it is diluted with 1000 parts of water. However, there is no general agreement about the sensitivity of Florence's reaction. In fact, many investigators have criticized the reaction, mainly because the same specimen of semen will occasionally give a negative reaction at first, and a positive result later. This peculiar behaviour of human semen was elucidated by Zahane and Levy (1936, 1937)\textsuperscript{22,23} who discovered that human semen examined immediately after ejaculation contains practically no free choline, but that choline accumulates in semen gradually on
standing, as illustrated by the following experiment: from 3.5 ml. semen mixed with 20 ml. water, consecutive 2 ml. samples were withdrawn and deproteinized by boiling with 9 ml. ethanol for 2 minutes; the quantitatively collected filtrates were evaporated, the residues extracted with dry ether and redissolved in water; choline was precipitated from the aqueous extracts with the Reineckstreeagent and determined bromometrically. Results showed a sharp increase in the choline content of human semen during the first hour of incubation, and the relatively slow accumulation during the next 47 hours; the terminal decline is probably due to bacterial contamination.

Enzymic liberation of choline from precursors in semen

Following up their observation that choline accumulates in semen only after the ejaculation, Kahane and Levy demonstrated the presence in fresh semen of a 'precursor de la choline' which yields free choline as a result of hydrolysis which takes place in semen on standing. Apart from the seminal plasma itself, they found the choline precursor in various reproductive organs, including the testis of bull, boar, ram, stallion, rabbit and guinea-pig, the seminal vesicle of stallion and guinea-pig, and the epididymis of boar and ram, but not the prostate of dog, stallion or ram. However, the prostate, particularly that of dog, was found to be rich in the enzyme
which splits off choline from the precursor. In a series of studies, Kahane and Levy (1938, 1945, 1949) have shown that the precursor is a water-soluble compound (choline hydrosoluble combinee) which behaves like glycerylphosphorylcholine, and yields on incubation with prostatic extracts a mixture of free choline and inorganic phosphate; the quantity, however, of liberated choline was found to be far in excess of the simultaneously appearing inorganic phosphate.
Experiment 3-7:

Estimation of Glycerolphosphocholine in the Epididymides of Rat.

Since the discovery that glycerol increases the revival of deep-frozen spermatozoa it had become widely used in artificial insemination (Emmens and Blackshaw, 1956)\textsuperscript{27} and later it had been shown that ram and bull spermatozoa can metabolise glycerol under anaerobic conditions with the formation of fructose and lactic acid (White, 1956, 1957)\textsuperscript{28,29}.

Choline, phosphorylcholine and glycerylphosphorylcholine occur in semen and accessory secretions and are believed to be bound up specifically with the metabolism of phospholipides in the male accessory organs or in the spermatozoa.\textsuperscript{30}

The method for estimation of glycerol employs spectrophotometry. Glycerol which is estimated by this method\textsuperscript{31}, occurs in semen and accessory gland, epididymis - as a complex 'glycerylphosphocholine'. Glycerol was estimated by Ryley (1955). This method is essentially a modification of the technique of Lambert and Neish (1950)\textsuperscript{32}, in which glycerol is oxidised to formaldehyde by periodate and the excess periodate destroyed by arsenite. The formaldehyde is then estimated colorimetrically by the formation of a complex with chromotropic acid.

This method has proved useful in determining the rate of breakdown of glycerol when added to washed spermatozoal suspensions on sex accessory gland tissue homogenates, and is extended here to the estimation of glycerylphosphocholine epididymal tissue homogenates of rat.
Glycerylphosphocholine (GPC) estimations were carried out on the excised caput and cauda epididymides, soaked between filter papers, of rats of groups I-III of the estrogen-treated rats, (dissected after 30 days of treatment), subsequently treated with the samples S1 to S6; group IV rats were treated with samples S1 to S6 alone. Care was taken to freeze the epididymides of each individual, to prevent in vitro changes in GPC levels, and were analysed in a quick succession for GPC content by the procedure as follows:

Procedure:

1. Caput and cauda epididymides homogenates were prepared in 3 ml of distilled water containing quantities of glycerylphosphocholine between 60 mg and 90 mg/ml for its estimation.

2. To this 3.0 ml homogenate 0.5 ml of 5% ZnSO4, 7H2O and 0.5 ml of 0.1N NaOH were added, shaken and centrifuged.

3. Of this extract 2.7 ml was shaken with 0.3 ml of 20% CuSO4 and 0.40 gm Ca(OH)2 and left for 30 minutes and then centrifuged.

4. To the 1.5 ml supernatant of this centrifuged mixture, 1.5 ml was taken with 0.5 ml of 0-1M sodium metaperiodate and shaken and left for exactly 5 minutes.

5. Then added 0.5 ml of 13% sodium arsenite and left it exactly for 10 minutes (some turbidity and yellow colour appeared which faded away after some time).

6. Made up the volume to 10 ml with distilled water (6.5 ml of distilled water was to be added).
7. Took 0.5 ml of supernatant and 5 ml of chromatropic acid.
8. The sample was put in boiled water for exactly 30 minutes.
9. Cooled it and read it at 580 nm using Shimadzu double-beam spectrophotometer - uv 190.

Standard solution:

For preparing standard solution 1.5 ml was taken from the mixture in Step (4) containing 100 microgram of glycerol.

Stock Standard Solution:

Stock standard solution of GPC is 3 mg/ml glycerol

= 300 microgram/ml

Working standard:

Working standard = 66.66 microgram/ml

Took 22.22 ml of the stock standard and made it to 100 ml with distilled water. Aliquots and distilled water mixed in the ratios that yielded the series of ten varying between 10 and 100 microgram of glycerol.

Calculations:

In order to express the results as GPC per mg tissue, glycerol values thus obtained from standard curve were multiplied by 5.7.

Therefore, GPC per mg tissue

\[
= \frac{\text{Concentration in } \mu \text{g} \times 5.7}{3 \times \text{wt of tissue}}
\]
Table 12:

Effect of *Mucuna pruriens* Baker Seed Compounds on Glycerylphosphocholine Content of Epididymis (Caput and Cauda) of Estradiol Propionate - Treated ('Functionally' or 'Reversibly' Sterilised Rats):

**Period of treatment - 30 days**

<table>
<thead>
<tr>
<th>Groups No.</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Glycerylphosphocholine in Epididymis (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caput</td>
</tr>
<tr>
<td>I</td>
<td>Controls</td>
<td>10</td>
<td>$8.48 \pm 0.14^*$</td>
</tr>
<tr>
<td>II</td>
<td>Estradiol Propionate (EP) (10µg/rat/day, i.m.)</td>
<td>10</td>
<td>$3.64 \pm 0.77$</td>
</tr>
<tr>
<td></td>
<td>$A_1 \text{ EP} + S_1$</td>
<td>10</td>
<td>$8.18 \pm 0.43$</td>
</tr>
<tr>
<td></td>
<td>$B_1 \text{ EP} + S_2$</td>
<td>10</td>
<td>$8.27 \pm 0.22$</td>
</tr>
<tr>
<td>III</td>
<td>$C_1 \text{ EP} + S_3$</td>
<td>10</td>
<td>$2.92 \pm 0.64$</td>
</tr>
<tr>
<td></td>
<td>$D_1 \text{ EP} + S_4$</td>
<td>10</td>
<td>$8.32 \pm 0.48$</td>
</tr>
<tr>
<td></td>
<td>$E_1 \text{ EP} + S_5$</td>
<td>10</td>
<td>$8.75 \pm 0.61$</td>
</tr>
<tr>
<td></td>
<td>$F_1 \text{ EP} + S_6$</td>
<td>10</td>
<td>$8.36 \pm 0.73$</td>
</tr>
<tr>
<td>IV</td>
<td>$A_2 \text{ S_1}$</td>
<td>10</td>
<td>$8.88 \pm 0.65$</td>
</tr>
<tr>
<td></td>
<td>$B_2 \text{ S_2}$</td>
<td>10</td>
<td>$8.86 \pm 0.73$</td>
</tr>
<tr>
<td></td>
<td>$C_2 \text{ S_3}$</td>
<td>10</td>
<td>$2.98 \pm 0.53$</td>
</tr>
<tr>
<td></td>
<td>$D_2 \text{ S_4}$</td>
<td>10</td>
<td>$8.92 \pm 0.77$</td>
</tr>
<tr>
<td></td>
<td>$E_2 \text{ S_5}$</td>
<td>10</td>
<td>$9.16 \pm 0.38$</td>
</tr>
<tr>
<td></td>
<td>$F_2 \text{ S_6}$</td>
<td>10</td>
<td>$8.64 \pm 0.92$</td>
</tr>
</tbody>
</table>

* Mean $\pm$ S.E.
**P**. **values** :

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Epididymis</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caput</td>
<td>Cauda</td>
<td></td>
</tr>
<tr>
<td>J vs II</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.01 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( A_1 )</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td></td>
<td>( B_1 )</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>II vs III</td>
<td>( C_1 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td></td>
<td>( D_1 )</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td></td>
<td>( E_1 )</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td></td>
<td>( F_1 )</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td></td>
<td>( A_2 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td></td>
<td>( B_2 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>J vs IV</td>
<td>( C_2 )</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td></td>
<td>( D_2 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td></td>
<td>( E_2 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td></td>
<td>( F_2 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
</tr>
</tbody>
</table>
Figure P. Glycerylphosphocholine standard curve.
Summary:

In Experiment 37 (Table 12), the effects of the samples S₁ to S₆ on the glycerylphosphocholine (GPC) content observed in the epididymis of the 'functionally sterilised' (estradiol propionate (EP) - treated) rats (Group III) and in normal rats (Group IV) were compared with those observed in the rats of Group I and II.

In Groups III and IV, samples S₁, S₂, S₄, S₅ and S₆ were found to produce significant increase in GPC content in caput, corpus and cauda portions of epididymis; sample S₃ reduced the GPC content significantly.

The significant increase of GPC content indicated reversal of estradiol propionate (EP) - induced diminished GPC level, that is recovery from diminished fertility by restoration of GPC levels toward normality.

The significant decrease of GPC content effected by S₅ indicated contribution to EP - induced fall in GPC or diminished fertility.
REFERENCES:


