STUDIES ON THE EFFECT OF PROGESTERONE ON THE REGULATION OF BODY TEMPERATURE AND REPRODUCTIVE RHYTHM IN FEMALE RATS

By

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I. INTRODUCTION

As a measure of studying the effect of progesterone on the reproductive rhythm, the parameters like body temperature and oestrus cycle are best suited that may give more information about the rhythmic changes that occur due to this ovarian hormone.

No previous work has been done regarding the effect of progesterone on the body temperature rhythm although other aspects like it's effect on ovulation and enzyme activity and histopathological studies have been done.

In more complex animals, a whole series of Interlocking and Synchronized events must follow one another if reproductive efficiency has to be attained. All animals evolved an intricate and interrelated system of checks and balances, a time clock which actually consists of two interlocking systems the Endocrine System and the Nervous System (A.V.Nalbandov, 1970).

The governing parts of the reproductive system are the glands, the hormones secreted by them, the end organs (Such as the ovaries and the uterus) that are acted upon by the hormones, and under certain circumstances the nervous system.

The endocrine function of the ovaries commences just before puberty and continues upto menopause. The hormones elaborated by the ovaries are progesterone, estrogen and relaxin.

The period from the begining of one heat to the next is called as "oestrus cycle" and is under the influence of the hormones progresterone and estrogen. This cycle may be influenced by several factors like temperature, light, nutritional status etc. Endocrinologically, oestrus is a period of high estrogen and progesterone activity.

The estrus cycle is commonly divided into four phases called prooestrus, oestrus, metoestrus and dioestrus. Based on the hormonal status of the animals this period can be classified into (i) Estrogenic phase consists of prooestrus and oestrus during which there will be increased amounts of estradiol in the circulation. (ii) Progestational or luteal phase-consists of metoestrus and dioestrus and here there will be increased levels of progresterone in the circulation.

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The cyclic alterations of the reproductive system are regulated by hormones from the anterior pituitary-gonadal axis. According to current concept a feed back mechanism operate where by the pituitary release of the FSH and LH is controlled by the levels of estrogen and progesterone in the circulation (Donnell Turner, 1967).

Within the four phases of the oestrus cycle, the body temperature is high only during the dioestrus stage (Indira and Gopal, 1981). So it is clear that the body temperature in the female rat is related to the oestrus cycle. The body temperature of the starved rat is also high at dioestrus but the oestrus rhythm is being disrupted. Therefore the optimum temperature is necessary to trigger the action of hormones as well as target organs.

The temperature of the body is influenced by an almost infinite number of factors (Brayans, 1951). The biological system of thermoregulation includes multiple sensors, multiple feed back loops and multiple outputs. (Hensel _et al_, 1973).

In homeotherms, the body temperature remain relatively constant, and this constancy is dependent on the function of the brain. The brain has a kind of built in information about the level at which temperature is to be

set and maintained. This level is called "Setpoint" and involves brain structures which determines the level of rhythmic variations in the body temperature.

The separate anterior and posterior centres in hypothalamus are concerned with temperature regulation. The posterior centre is temperature insensitive. The anterior centre responds mostly to its own temperature at this point deep within the brain. The direct thermogenesis without muscle contraction is not widespread, but is fcund in animals as varied in rats, and guineapigs. It usually occur in special brain adipose tissue and metabolism at this site is found to be controlled by hormones (Leon Gold Stein, 1977).

It is found that the formation of corporalutea at Metoestrus stage shows a very low progesterone level (Eto etal, 1962; Feder etal, 1969). This coincides with a low temperature at this stage. With the development during dioestrus, (Nalbandov, 1970) the corpus luteum is completely formed and the progesterone sceretion reaches the peak. So the body temperature reaches the peak. It is interesting to ncte that the rise of the body temperature of rats coincides with progesterone peak. (Indira and Gopal, 1981).

So the present investigation has been made to support this concept and also to know about the various other effects of progesterone. It is also found that there is an increase in the body temperature due to food intake (Indira and Gopal, 1981). So it is necessary to know whether progesterone has any role to play in the metabolism of the animals. Therefore the parameters like food intake and body weight are also included in this study. It is hoped that this line of investigation would give more information about the interactions of the ovarian hormone (progesterone) with the body temperature and reproductive rhythm.

Review of Literature

II. REVIEW OF LITERATURE

An understanding of the rhythmicity of the body temperature and reproductive rhythm and changes in these activities due to the exogenous sexual hormone (progesterone) which is the main aim of this work will help to understand more about the physiological role of the hormone and it's relationship with circadian hormonal effects.

II. 1. Circadian rhythms:

One of the characteristic feature of the nature is "rhythmicity". Summer follows winter, new moon follows old, day follows night "so do flux and reflux - the rhythm of change alternate and persist in everything under the Sky" (Thomas Hardy, 1886). The distance between the two identical positions of two waves is called a cycle or a period. As biological rhythms are not direct response to the environmental factors such as light, temperature and humidity, these factors have been referred to as 'Synchronizers' (Halberg, 1953) or 'clues' (Cloudsley-Thompson, 1952) terms which appear to correspond with the German Zeitgeber (Aschoff, 1954). The rapidity of the Synchronization may depend upon the intensity of the environmental "clues" (Johnson, 1939).

The rhythm of the 24 hour rotation of the earth which includes both the periods of light and darkness is called "circadian" - word derived from Latin ("Circa" - about, "Diem" - a day) and was coined by Franz - Halberg (1959). A circadian rhythm arises from an elaborate oscillatory organization residing in the living cell or organism. Besides indicating the time of the day it also participates in time keeping as man-made clocks do. Hence it is popularly known as "Biological clock". Several rhythmic function takes place within the body of living organism. The regulation of these functions, depends not only on factors outside the body but also on internal conditions which are constant (Aschoff, 1967; Wever, 1969 and 1971).

Light is found to be the dominant synchronizer of the rodent activities. If L-D cycle is shifted through 180, then the circadian rhythm will eventually but not immediately be inverted (Scheving et al, 1974). The expression "Biological clock" reminds of the fact that rhythmic functions of biological organisms are used as "time measuring devices" (similar to the swining of a pendulum). The rhythmic functions which can relatively be easily recorded are considered as the "hands" of the clocks and the mechanisms that activate these functions, i.e. the so called "wheels" are still to be identified (Bunning, 1973).

II. 2. Examples of biological rhythm:

Serum steroid rhythm (Scheving et al, 1974) which is one of the extensively studied hormonal rhythms in the rat and man is a good example of circadian rhythm. According to them, in the diurnally active man, serum steroid is secreted from the adrenal gland prior to awakening and reaches its peak shortly after he arises. In the nocturnally active rats, the peak occurs shortly before the active period begins (Halberg, 1973). Diurnal animals show a peak of activity at he beginning of the dark period. Subsidary peaks follow about the middle of the 24 hour period (Aschoff and Meyer - Lohmann, 1954). Menstrual cycle as its name implies is cyclic and during the different phases of this cycle. The hormones (Progesterone, estrogen), water and Nacl conntent show a marked but rhythmic variation (Savithri, 1979). The cyclic events in the ovary, uterus and the vagina are under the control of gonadotropins from the anterior pituitary. The hypothalamus plays a major role in the control of pituitary function what has been called as the "hypothalamic clock" which is in some way essential for the rhythmic release of gonadotropins which control the female sexual cycles (Rajathi, 1980). Circadian rhythms may be disrupted when the shift workers rotate from one work schedule to another (Turek Fred, 1986).

As a result of experiments involving injections of drugs, Everett and Sawyer (1950) have postulated that a mechanism, exhibiting a 24 hour periodicity of sensitivity, may control the release of ovulation inducing hormone in rats. They have found that nembutal (phenobarbital sodium- std dose 30mg/kg.in traperit) prevents the ovulatory activation of the hypophysis when administered at 14.00 hours during prooestrus persistence of graffian follicules for 2-3 days results from nembutal treatment on successive afternoons at the same critical hours on the second and third days, there has been need for a supplementary injection at 15-30 to 14.00 hours. If on any of these 3 days, treatment is discontinued or postponed until after 14.00 hours, pituitary activation promptly occurs followed by ovulation during the night. Similarly results are obtained with a mytal dial, barbital and phenobarbital. Blockade of lutenising hormone release by treatment with the barbiturates on successive days has disclosed a follicular cycle which terminated in atresid about 2 days before the onset of the next prooestrus. Everett and Sawyer have concluded that the 24 hour rhythm, although possibly a property of the lutenising hormone release- mechanism in other spontaneously ovulating forms as well as the rat, cannot be considered as an universal characteristic unit until a variety of animals has been investigated.

III. 3. Body temperature:

The body temperature is one of the parameters which is a very good example of a circadian pattern. The temperature of a living organism is a measure of the kinetic activity of its molecules and is proportional to the heat in the body. Studies on the regulation of the body temperature in the antelope ground squirrel has been caried out (Kramm, 1972). The regular variations in sleepiness, temperature and urinary flow might be ascribed "to an habitual hypothalamic rhythm, autochronous for a week atleast, but ultimately derived from external rhythms" (Mills, 1951). Male Japnese quail displayed day-night rhythms in their body temperature during the day than at night (Kavaliers, et al, 1991). In Djungarian hamster (dark active), the amount of activity/24 hour was significantly greater under temperature cycle than under constant temperature of 25°C (Tokara, et al, 1985). The food intake is found to have an influence in the body temperature. The day fed rats show a high average of the body temperature during day time in tune with the day feeding, and the night-fed rats show a similar trend during the night, in tune with the night feeding (Indira and Gopal, 1981).

The ability of the organisms to maintain homeostasis or stability is called homeothermy (Ashby, 1960 and 1966).

In homeotherms, this constancy is dependent on the functions of the brain. In the brain the "set pint" in which the temperature is set and maintained involves brain structures which determines the rhythmic variations in the body temperature.

In the hypothalamus, the anterior and posterior centres are concerned with temperature regulation. The posterior centre is temperature insensitive. The anterior centre responds mainly to its own temperature (Leon Goldstein, 1977). Stimulation of the medial - pre optic area inhibits the shivering induced by an anesthetic drug (Hemingway and Birzis, 1954). Hardy et al (1964) have found that there are also cold sensitive and heat-sensitive neurons in the pre-optic region. The pre-optic region is involved in heat-production mechanisms through its neural influences or endocrine function (Ramson et al, 1969). Comparison of the 24 hour overall mean body tempeature of the VMH lesioned rats with that of the control rats, reveals a higher amplitude and a raised level of the body temperature rhythm thro'out the 24 hour in the VMH lesioned rats. In tune with the incessant eating and higher metabolism, the body temperature remains high thro'out the day (Indira and Gopal, 1981). These results suggests that the ventro-medial hypothalamus is involved in the regulation

regulation of the body temperature. Ogata (1966) from various experiments, suggests that the other part of the central nervous system like hippocampus takes part in the regulation of body temperature against cold. He also pointed out that the amygdaloid complex also play a role in thermo regulation.

II. 4. Oestrus cycle as a "Biological clock"

The reporductive cycle which is also called as the oestrus cycle in the rat, occurs regularly with a short interval of 4 (or) 5 days (Long and Evans, 1922). The rhythmicity of the oestrus cycle is the consequence of reciprocal inter relationship between the gonad and the pituitary (Moore and price, 1932). The cyclic alterations of the reproductive system are regulated by hormones from anterior pituitary-gonadal axis. Such release of hormones is controlled by brain centres and hence these centres act as "Biological clock" (Bentley, 1976). The main eventin the oestrus cycle is ovulation which depends upon the release of gonadotrpins from the anterior pituitary. In rats, the surge of ovulation inducing hormone is controlled by a biological clock, having a circadian rhythm. Sometimes the cycle may be lengthened to 5 days. Vander schoot and Vilenbrock (1991) have suggested that the lengthening of the

oestrus cycle may be due to the activation of progrsterone by prolactin and is not due to late follicular maturation. It is found that the ovulation always occurs just after mid-night and (Armstrong and Kennedy, 1972) the short 4 or 5 days cycle in the rats is believed to be a consequence of a failure of corporalutea to become functional during the normal luteal phase (dioestrus) (Short and Austin, 1972). During proestrus, the levels of estrogen in the plasma reaches a peak, which stimulates the LH surge accompanied by FSH. The surge of gonadotropins occurs on the afternoon of proestrus and is followed by a marked surge of progesterone. Ovulation occurs a few hours after midnight on the day of estrus. Estrus lasts for about 9- 15 hours, cornified cells appear which were produced by the action of estrogens. The 3rd and 4th day of the cycle are termed dioestrus-I and dioestrus-II. There is limited secretary function by corpus luteum as evidenced by a slight increase in plasma progesterone. There is a transitional period between oestrus and dioestrus termed metoestrus. Due to this synchronized rhythmic activity the oestrus cycle is considered as a good example for the "Biological clock" (Turner et al, 1971). The LH surge occurs in an appropriate environment depnding upon resonance between neural and ovarian signals, the LH-surge the most dramatic change is emitted by the hypothamalus pituitary system (Fink, 1979) from a reinforcing cascade of events initiated by an increased secretion of estrogen. This ovarian signal is

positive i.e. it is the stimulatory effect (Knobil, 1974 Legan and Karsch, 1973; Fink, 1979). Lowest pulsatile release of LH was on oestrus and the largest is on dioestrus day first (Marco et al, 1989). In infantile rats an dynamic relationship exists between the hypothalamic Pituitary unit and the ovary. The effectiveness of estradiol as a -ve feed back signal for gonadotropin release is reduced in infantile rats due to the binding of the estradiol to α-fetoprotein, which renders the steroid biologically in active At the 2nd week of life the negative feed back control shifts from androgenic control to a estrogenic control. The tonic inhibition of gonadotropin release by estradiol requires the presence of progesterone. Thus the -ve feed back mechanism shifts from a estrogenic control to estrogen-progesterone control. Then the +ve feed begins only after 16 days and this reaches full capacity after 28 days of age (Andrews and William Walton, 1980). The pulsatile secretion of LH may enhance the pulsatile secretion of progesterone and estrogen, and changes in this secretion may affect central nervous system pituitary axis through a negative feed back, (Kajimura and Hideo, 1990). Ovulatory LH release can occur only during a relatively limited time of the day called "critical period" and is found to occur daily (Everett, 1961). The pattern and rhythm of the oestrus cycle can be changed and such changes can be effected by various factors. Groupism is shown to induce disruption of the oestrus cycle (Gangrade BIc and Dominic, 1990).

II. 5. Role of hormones on the body temperature and oestrus rhythm

In 1868, squire has published observations on fluctuations in the body temperature during the menstrual cycle. Continuos observations of the body temperature every half, one, two or three hour for days, weeks and through months have uncovered several areas of differences in the body temperature rhythm during the "Ovarian cycle" of the rat. Rats attain puberty at the age of 2-3 months. The cycle lasts for about 4 days. A wider range in setting between the maxima and minima is observed in females, as opposed to that of the males comparitively. This distinctiveness may be related to the wider setting of the hormonal interplay. From the observations of the oestrus cycle in the rats, the body temperature is found to be high only during dioestrus. In starved rats also the same effect was observed but the oestrus rhythm is found to be disrupted. So this indicates that the optimum temperature is necessary to trigger the action of hormones as well as the target organs to bring about the regular rhythm in the oestrus cycle (Indira and Gopal, 1981). The hormones that participate in the reproductive cycle are estrogen and progesterone. The chief sources of estrogen are the ovaries, placenta, adrenal cortex and testes. In non-pregnant mature women, it is secreted by the thecainterna cells of mature graffian follicle. (Hurkat and Mathur, 1976). The progestational hormane secreted by the corpus

luteum progesterone, was isolated in 1934. This is converted inside the body into pregnanediol (Carter et al, 1960), progesterone is secretd by intestitial cells. Both estrogen and progesterone are steroid hormones. It is necessary to know Whether progesterone has got any relation with body temperature. Rubenstein and Lindsley (1937) have demonstrated that it is the temperature raising effect of progesterone that causes the elevation of the body temperature during the later stages of ovulatory cycle. This has been confirmed by other workers (Palmer and Devilliers, 1939; Gareia and Rock, 1958). The temperature drop again in the metoestrus shows the degeneration of the corpus luteum and therefore the decreased production of progesterone. The levels of progesterone in the blood during menstrual cycle has been measured by various methods. Forbes (1950), by a bioassay method found that the free prgesterone appeared two days before, coincides with the increase in the body temperature and reaches a peak of 1.7 ug to 5.2 ug/ml around the 22nd day and then again decreases. It has been observed that progesterone secreted by the corpus luteum from 14th day onwards of the menstrual cycle are sufficient to increase the body temperature. Endroczi (1963) and Siteri et al (1968) have found the secretary rate of progesterone during the oestrus cycle. The secretion rate of progesterone at the morning of proestrus is 0.5 ± 0.2 ug/hr/ovary and on the evening of proestrus it is 4.4 ± 1.6. During oestrus it is about 1.3

± 0.4 and during metoestrus 2.2 ± 0.3 and 1.0 ± 0.3 at dioestrus. There was a high content of progesterone at the peripheral blood during the dioestrus and prooestrus stages. There was a low content of progesterone at oestrus and a slight increase at metoestrus (Teledgy, 1963). According to Leavit et al, (1970) and Lukaszewska et al (1970) in the rats, a second peak of plasma progesterone has been reported. But according to Roser et al (1969) the dioestrus peak is found to be higher than the proestrus. Hence the present study has been carried out.

It is also found that there may be decreased progesterone secretion in rats and guineapigs during the rapture of the follicle (Goldman et al, 1969 and Feder et al, 1968).

In pregnantcows progesterone is secreted either by curpus luteum located on the ovary or by placenta or by both. Estimation of progesterone in the blood or milk has helped in diagnosing pregnancy. By radio immuno assay technology it is found that the concentration of progesterone in milk is above 8 n.g/10ml, and it is a clear indication of pregancy. Levels less than 4ng/100ml indicates non-preganncy (Richard Masillamony, 1992).

It is found that there is an increase in the body temperature due to food in take (Indira and Gopal, 1981). So it is necessary to know whether progesterone has got any effect on the metabolism of the animal. It appears that increase in body temperature due to progesterone may be due to its metabolic influence. Katz and Kappas (1967) has shown that the thermogenic effect of progesterone may be due to its metabolite 3 , 20 pregnanediole. Inrodents, the appetite stimulating effect of progesterone maintains a positive energy balance which cause a significant increase in body weight (Harvey et al, 1967).

Though much work has been done in this aspect, the influence of progesterone on the integration of the body temperature rhythm and oestrus cycles and as well as on the metabolism has not been done and much information is not available regarding this aspects and hence the present study has been carried out.

Materials and Methods

III. MATERIALS AND METHODS

III. 1. Choice, Selection, Housing of animals:

Female albino rats of the same age group namely 2 - 3 months have been used for the experimentation. This age group is the suitable age group for the study of reproductive rhythm because in infantile rats, a negative estrogen feed back occurs. The inhibition of gonadotropin release by estrogen requires the presence of progesterone. After 16 days the estrogen negative feed back changes to estrogen-progesterone feed back. Then the positive feed back begins after 22 days and it attains it's full capacity only after one month. so rats attain puberty at about 40-45 days. So this age group is selected for the study.

Selection of the albinorat was due to the following reasons

a. It is a typical example of mammals having homeothermic regulation, well suited for this study.

b. It is easy to handle the animal.

c. Occurrence of the reproductive cycle is very short and rhythmic (occur at an intervel of 4 days)

The animals were housed and maintained in individual cages.

III. 2 Sex and number of the animals used:

Female albino rats of the age group 2-3 months were used. The number of animals used and the parameters on which the observation was made are as follows:

S.No.	Item	Group	Number of animals used	Parameters observed
1.	Control	I	4	1. Body temperature rhythm. 2. Oestrus cycle for a period of 12 days. 3. Body weight (once in 3 days) 4. Estimation of serum cholesterol at the end of the experiment (after 12 days) 5. Sacrificing the animal and noting the morphology of the reproductive system (Uterus)

1	2	3	4	5
2.	Experiment	II	6	1. Injection of the corticosteroid-progesterone for each animal. 2. Observing the body temperature rhythm for a period of 12 days. 3. Oestrus cycle for a period of 12 days. 4. Body weight (once in 3 days) 5. Estimation of serum cholesterol at the end of the experiment (after 12 days) 6. Sacrificing the animal and noting the morphology of the reproductive system (Uterus)

III. 3 Diet

The animals were fed on balanced diet which was prepared daily. The composition of the food was:

a.	Wheat flour	: 35 gms
b.	Bengal gram flour	: 10 "
c.	Green gram flour	: 15 "
d.	Whole milk	: 15 "
e.	Groundnut oil	: 10 "
f.	Greens	: 5 "
g.	Cod liver Oil	: One drop
h.	Yeast	: " gm

This balanced diet provides Protein 12.5% and 250 K. Cal.

The Wheat flour, Bengal gram flour and green gram flour were mixed with whole milk and water. To this the greens and also groundnut oil was added. This was cooked until this mixture attained to a semi solid consistancy. Yeast tablets were then powdered and a pinch of it was added and finally a drop of codliver oil was also added and served.

III. 4. Measurement of the body temperature:

Temperature is generally differentiated as "core" temperature and "Shell" temperature. Core temperature represents the temperature of the internal organs and shell temperature represents the skin temperature. Here an instant thermometer strip was used. So the shell temperature was selected.

III. 5. Instant thermometer:

The thermometer used in this study was "Hanimax instant thermometer". This was in the form of a strip on the strip, the readings were given both in faranheit (°F) and Centigrade (°C) (Fig-1). The farenheit readings were taken for the study. The strip was kept on the animal's body and held at it's both the ends (without touching the centre of the strip) (Fig.-2). The green colour indicated the correct reading. The strip was kept for two minutes until it showed green color at the corresponding reading. The same strip was used to record the temperature of all the animals care was taken to see that there should be atleast an intervel of two minutes between the successive readings of different animals because the color formed in one reading should disappear completely before the next reading. This was done in order to avoid minute errors in the readings.

III. 6. Time of measurement:

The body temperature readings were recorded at an intervel of two hours continuously for 24 hours. The observation was done for a period of about 12 days. The temperature readings were recorded at 07.00, 09.00, 10.00,

11.00, 13.00, 15.00, 17.00, 19.00, 21.00, 23.00, 01.00, 03.00 and 05.00 hours. The first reading was taken at 07.00 hours and thereafter at 2 hours intervel.

III. 7. Experiment:

Since the study is focussed on the effect of pprogesterone on the body temperature rhythm and reproductive cycle, the animals were divided into two groups.

In the first group, about 4 animals were used. All were females of the age group 2-3 months. The group served as the control. They had adlibitum feeding schedule by which food and water was made available at all times. All the animals were maintained under normal light-dark conditions. Temperature recordings were noted in these rats at an intervel of 2 hours continuously for 24 hours. Meanwhile, the vaginal cytology was also observed daily. This experiment was carried out for a period of 12 days. At the end of the experiment 1.00ml of blood was drawn from each animal and the estimation for the total serum cholesterol was done. Food intake of the animal was also observed daily. Correspondingly, the body weight of the animal was observed once in three days.

For investigations made on the influence of progesterone on the body temperature rhythm and oestrus cycle, a second group of animals were used. This group consisted of six animals. First 0.5ml of the hormone progesterone was injeted to each animal. This consisted of 12.5mg of progesterone. The injection was done intramuscularly. The animals had adlibitum feeding, and maintained under normal light-dark conditions. From the next day of injection onwards, the body temperature readings were observed at an intervel of 2 hours continuously for 24 hours. This experiment was carried out for a period of 12 days. The vaginal cytology of each rats were also observed daily. At the end of the experiment blood was drawn and the estimation for serum cholesterol was done. Also the food intake was observed daily. The body weights of the animals were taken once in three days.

III. 8. Estimation of serum cholesterol-Total

(Wybenga pileggi method)

Principle

Both free and esterified cholesterol reacts with hot ethyl acetate and sulpuric acid in the presence of ferric iron to give a purple coloured complex. This is measured at 520-540 nm (Green filter)

Reagents:

Reagent : 1. Cholesterol reagent

Reagent : 2. Standard.

Procedure

Took 3 test tubes marked as T (Test), S(Standard) and B(Blank). About 1.0ml of the blood was drawn from the animal and the serum was separated out. Then about 0.05ml of the serum was pipetted out into the tube with a mark as 'Test (T)'. About 0.05ml of cholesterol standard was pipetted out into the test tube with a mark as 'Standard (S)'. To the blank only 0.05ml of distilled water was added. Then to all the three tubes 5ml of cholesterol reagent was added, mixed well and kept in a boiling water bath for exactly 1½ minutes. They were cooled immediately for 5 minutes. Then the color developed was read at 520-540nm.

Calculation

The total cholesterol present in 100 ml of the given sample was caalculated by the following formula.

$\frac{T-B}{S-B}$ x 200 mg/100 ml sample

III. 9. Vaginal smear technique:

The Vaginal Smear Technique generally consists of gentle scrapping of the cells with a swab or collection by a pipette with a small amount of Saline water (Long and Evans, 1922). Swab method was followed in the present study.

A small cotton swab moistened with Saline Solution was used. This is done in order to prevent irritation to the animal. A fresh cotton swab was used everytime for each animal. The animal was held by the left hand, and the swab was inserted very gently (Fig.-3). With the gentle twist of the swab, the material at the vagina was collected and a Smear was prepared on a glass slide. The Smear was prepared daily between 10.00 A.M. to 11.00 A.M. because the vaginal morphology changes daily during this period. The vaginal matterial thus collected was smeared as a thin layer, on glass slides on which was marked the serial number of the rats and the date on which the smear was taken. Then the following steps were carried out.

a.	Smearing	Swab method
b.	Drying	Air drying
c.	Fixation	100% alcohol
d.	Staining	Ehrlich's hematoxylin
e.	Rinsing	With tap water
f.	Dehydration	90% alcohol and 70% alcohol (Graded alcohol series)

g.	Rinsing	With distilled water
h.	Staining	Eosin
i.	Rinsing	Tap water
j.	Dehydration	Graded alcohol series
k.	Cleaning	Xylol
l.	Drying	Air drying
m.	Mounting	DPX

The vaginal cytology of different stages is given in the following table.

Oestrus Stages	Sign Assigned	Vaginal Cytology	Duration	Ovarian Events
Metoestrus	'+'	Dominance of leucocytes with a few cornified epithelial cells	6 hours	Corpora lutea formed
Dioestrus	'2+'	Predominance of leucocytes with the polymorphic nucleus, with 1-2 epithelial cells	48 hours	Regression of corporalutea occurs
Prooestrus	'3+'	Predominantly epithelial cells	12 hours	Follicles grow fast
Oestrus	'4+'	Abundant larger flat, singly spaced anuclear cells in clumps and with curling at the edges appeared. The curled edge imparting to the cell the appearance of "Potato Chip"	30 hours	Ovulation occurs

III. 10. Statistical Analysis

In the present study, 't' test was used. Based on 't' values, difference between control and experimental are stated as significant or non-significant.

Figure - 1

Thermometer Strip /Showing Temperature Readings.

Note the green color at 100.4°F which indicates the correct reading.

Figure - 2

Method of taking the temperature reading.

Note the position of temperature strip on the body of rat where in the strip was held at it's both the ends without touching the centre or black part.

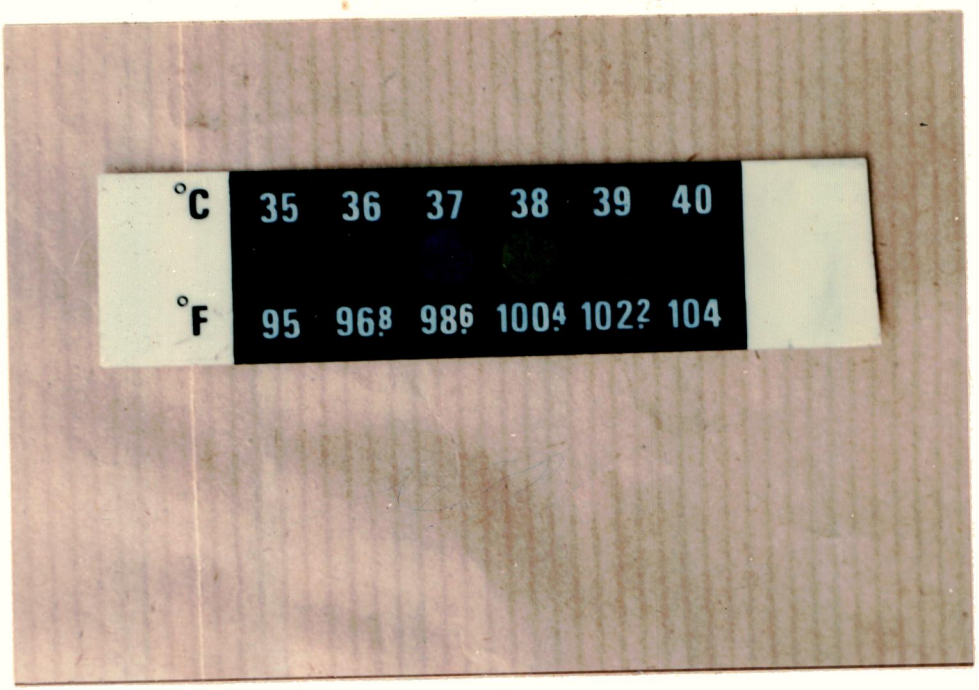


Fig - 1

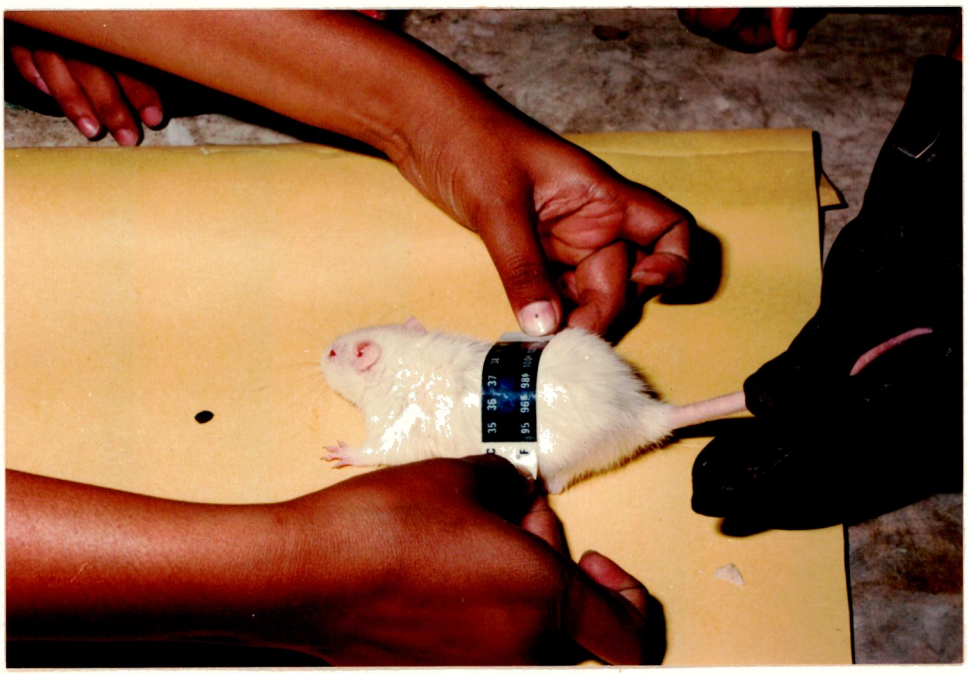


Fig - 2

Figure - 3

Method of Collection of Vaginal Smear.

The animal is held by lefthand and note the insertion of the cotton swab gently at the Vagina.

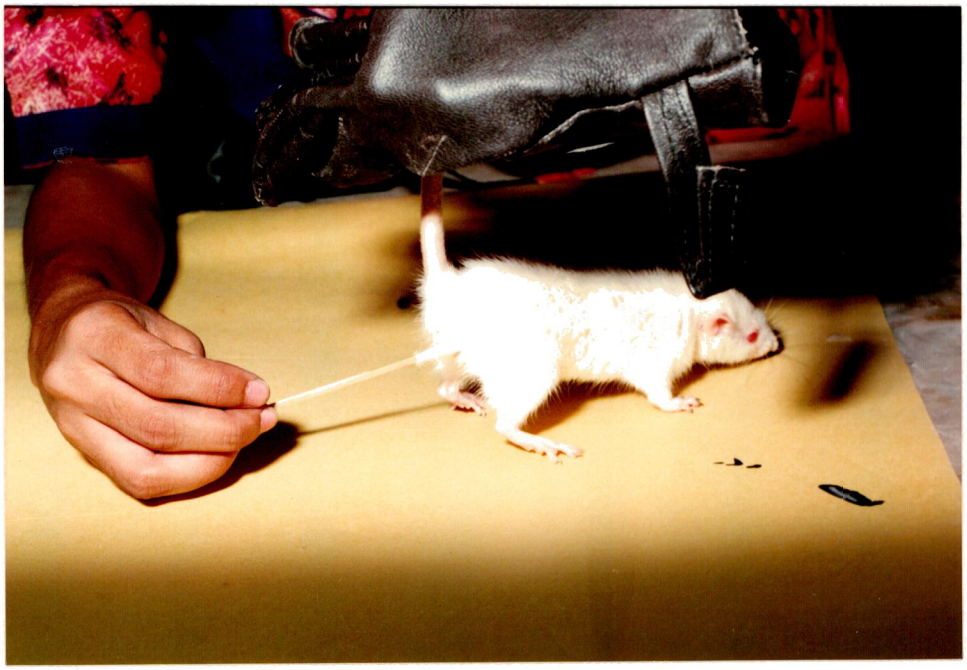


Fig - 3

Results

IV. RESULTS

IV. 1. The body temperature rhythm in control and experimental rats

The body temperature rhythm, which is one of the main parameters of the present study, was observed both in control and experimental rats. In order to find out the normal rhythmic pattern of body temperature in female albino rat s, sexually mature female rats of the age group 2-3 months were selected. The first group of 4 animals which served as control were taken for the study. Th rats were maintained under normal conditins of room temperature, exposed to normal conditios of light/dark (12L:12D), fed adlibitum, balanced diet and free access to water. The temperature readings were obtained continuosly for every 2 hours intervel for a period of 12 days. The pattern of the body temperature readings (Mean values) are shown in Table-I and Fig. 4 . The graph reveals a minima of 97.5 ± 0.1°F between 07.00 and 09.00 hours which is followed by a small rise of 99.1 ± 0.1°F during 19.00 hours, and reached a highest peak of 100.8 ± 0.1°F for the day during 01.00 hour; This rise in the body temperature at 01.00 hour in females which perhaps, is due to the involvement of oestrus cycle and is related to ovulation process in operation between 23.00 hours to 03.00 hours. This is followed by a

gradual decline of body temperature of 98.7 ± 0.2°F at 05.00 hours which is followed by 97.5 ± 0.1°F at 07.00 hours. Thus there is a rhythmic pattern of the body temperature found to occur in female albino rats. The day-wise mean value of the body temperature and the over all average obtained for control rats was found to be 98.8 ± 0.1°F as shown in table-II (Fig. 7). Every two hour recordings, recorded continuosly for 24 hours, for 12 days were pooled and computed to get the average for a single day and to obtain the overall average as shown in table-II. Comparison of mean values of body temperature (table-II) between successive days shows significant values.

The second group of six female rats after administration of progesterone (experimental) were maintained under standardised conditions such as natural light/dark (LD) conditions, room temperature of 28.0 ± 0.2°C, adlibitum balanced diet and free access to water. Like control rats, in experimental rats also, the body temperature readings were obtained at an intervel of 2 hours continuosly for 12 days. The day-wise pattern of the body temperature readings in experimental rats are shown in table-III and Fig.5 . The graph starts with a temperature of 99.1 ± 0.1°F at 07.00 hours and slowly increases and reaches its peak of 102.9 ± 0.1°F at 03.00

hours and gradually declines to a minimum of 99.1 ± 0.1°F at 07.00 hours. This shows that the body temperature occur rhythmically in experimental rats with the occurrence of the peak value at 03.00 hours unlike the control rats where the peak occurs at 01.00 hour (Fig.6). The difference in the peak body temperature between control (100.8 ± 0.1°F) and experimental rats (102.9 ± 0.1°F) is found to be 2.1°F. As in control rats, in the experimental rats also every two hour recordings obtained continuosly for 24 hours, were pooled to get the average for a single day and to obtain the overall average, which is 100.6 ± 0.1°F as shown in table-IV and Fig.7. Comparison of the day to day body temperature between the first day with the subsequent days in experimental rats reveals a significant variation in the body temperature (table-IV). The comparison of overall body temperature of the control and experimental rats is shown in table-V and Fig.7. Te bargraph shows the overall average of 100.6 ± 0.1°F in experimental rats which is significant when compared to control which was only 98.8 ± 0.1°F. This fig. also shows the comparison of the 12 days body temperature readings of the control and experimental rats besides the overall average . From this figure it is evident that there is a significant increase in the body temperature of experimental rats, compared to control rats.

The maximum body temperature readings obtained during each day were pooled to get the overall maximum body

temperature in control and experimental rats. Table-VI shows the comparison of the maximum body temperature in control and experimental rats. This shows a maximum of 101.1 ± 0.1°F in control rats, and a peak of 102.9 ± 0.1°F in experimental rats which is significantly higher (Fig 8). Table-VII shows the comparison of minima of the body temperature in control and experimental rats. This shows a minima of 97.3 ± 0.1°F in control rats, and 99.1 ± 0.1°F in experimental rats, which is significantly higher (Fig 9). From this, it is clear that the maxima and minima of body temperature recordings obtained are significantly higher.

From the above results, it is seen that there is a circadian rhythmic pattern of the body temperature existing both in control as well as in experimental rats. But the experimental rats shows a higher peak (102.9 ± 0.1°F)of body temperature compared to that of control rats (100.8 ± 0.1°F). Which might be due to the action of the hormone-progesterone. The mechanism of the role of hormone is discussed elaborately.

IV. 2. Relationship between body temperature and oestrus cycle in control and experimental rats

From the previous work on "Studies on the regulation of the body temperature rhythm in the rat" (Indira and Gopal, 1981), it is clear that there exists a correlation

between the body temperature and oestrus cycle in female rats. According to the work of Indira and Gopal, (1979) the body temperature reaches its peak and coincides with the dioestrus phase of oestrus cycle in female rats. During metoestrus stage where the corpusluteum degenerates tend to secrete only less amount of progesterone, and hence a decrease in body temperature and during dioestrus, corpus luteum is formed, which produces higher amounts of progesterone and hence aan increase in the body temperature. So it is clear that progesterone has relationship on body temperature and oestrus rhythm (Indira and Gopal, 1981). Hence the present study is carried out to analyze the hormonal role behind the body temperature and oestrus cycle.

The four stages of the oestrus cycle are identified daily bythe vaginal smear technique, both in the control and experimental rats. The body temperature readings obtained at every two hours continuosly for 12 days for specific phases of the oestrus cycle have been pooled and show in table-VIII. Fig.10 shows the comparison of the pattern of the body temperature readins obtained at every specific stages of the oestrus cycle in control rats. Thus the pattern of the body temperature for specific phases of oestrus cycle in control rats exhibit a temperature of 98.5 ± 0.3°F at metoestrus, 99.4 ± 0.2°F at dioestrus, 99.2 ± 0.3°F at prooestrus and 98.8 ± 0.2°F at

oestrus (Fig.11). From this, it is obvious that the body temperature at dioestrus is significantly higher when compared to other phases of oestrus cycle (Table-IX).

The maxima of the body temperature recording of control rats is shown in Fig.12a. Here one can observe that the maxima is high at dioestrus state with the temperature recordings of 101.8 ± 0.3°F whereas during metoestrus, the maxima of the body temperature is 100.7 ± 0.2°F, at proestrus 101.6 ± 0.3°F and 100.9 ± 0.1°F at oestrus. It can also be observed that the minima at dioestrus stage is significantly higher (98.0 ± 0.1°F) by comparison with the recordings at other stages of the oestrus cycle (Fig. 12b)(Table-XIII).

The four stages of the oestrus cycle are identified daily by the vaginal Smear technique in experimental rats also. The body temperature readings observed for every two hours continuosly for 12 days were pooled (table X) and plotted. This graph (Fig.13) shows a peak of 101.2 ± 0.4°F during dioesrus and 100.7 ± 0.4°F at proestrus and 100.4 ± 0.2°F at oestrus and 100.2 ± 0.2°F during metoestrus. From these recordings it is observed that at dioestrus stage the overall body temperature patternishigh significantly (table-XI). fig.14. It shows the action of progesterone. The bar graph shows the comparison of body

temperature at specific phases of oestrus stages in control and experimental rats. An important aspect note here is that the body temperature readings during oestrus phases shows significant increase in experimental rats when compared that of control rats. (fig - 15)

This is the reason why the body temperature pattern of the female rat is not the same on all 4 days of the oestrus cycle. It confirms that the body temperature pattern of the female rat is related to the oestrus cycle and which is reflected by the effect of progesterone.

The maxima of the body temperature recording of experimental rats is shown in (Fig.16 a). In this figure, one can notice a maxima of 103.5 ± 0.1°F at dioestrus, 103 ± 0.2°F during proestrus and 102.3 ± 0.1°F at oestrus and 102.2 ± 0.1°F at metoestrus (table-XII). It can also be observed that as in control rats, in experimental rats also. the minimum body temperature is found to be high at dioestrus with a value of 98.0 ± 0.1°F at dioestrus, and 97.6 ± 0.2°F during proestrus, and 97.3 ± 0.2°F at oestrus and 97.1 ± 0.1°F during metoestrus(Table XIII)(Fig.16b) Thus observations showed that there is a rise in the body temperature during dioestrus in control rats; This rise of dioestrus body temperature is also seen in experimental rats. This significant increase of body temperature rhythm coinciding with the dioestrus stage of the cycle, is discussed in the background of hormonal rhythm.

Rats attain puberty at the age of 30-45 days. The cycle lastsø, usually for 4 days in the rat (but in some 5 days). In the present series of experiments rats with only 4 days cycle have been used. That the occurrence of the oestrus cycle in the normal female rate is regular fig-17 with an equal frequency of 25% each stage is depicted in pie diagram (table-XIV, Fig.18.a).. But in experimental rats the frequency of the different phases of the oestrus cycle gets modulated as a result the cycle occurs fig-19 with unequal frequency of 26.3% metoestrus, 29.1% dioestrus and 22.2% each of prooestrus and oestrus respectively (Fig.18b).

The vaginal cytology of the control and experimental rats were compared. The following observations can be made out.

Table showing the comparison of the vaginal cytology of the different stages of oestrus cycle in control and experimental rats

Stages	Control	Experimental
1.Metoestrus '+'	Scattering of the leucocytes and epithetial cells with few cornified cells (Fig. 20a)	Clustering of the leucocytes, epithelial cells and cornified cells (Fig. 20b)
2.Dioestrus '2+'	Dominance of leucocytes with the polymorphic nucleus with few epithelial cells (Fig.21 a).	Predominantely clustering of the leucocytes with few epithelial cells. The leucocytes occur more in number when compared to control (Fig.21b).

1	2	3
3. Proestrus '3+'	Predominantely epithelial cells (Fig. 22a)	Occurrence of epithelial cells. But the epithelial cells occur more in number when compared to control (Fig.22 b)
4. Oestrus '4+'	High percentage of large, flat a nuclear cells in clumps with curled edges. The curled edges imparting to the cell the appearance of a potato chip (fig. 23a).	Scattering of the cornified cells. The cells are less in number compared to control (Fig. 23b).

Thus the kobvious differences observed in vaginal cytology between the control and experimental rats may be attributed to the effective setting of hormonal (Progesterone) interplay.

IV. 3. Food intake of control rats and experimental rats

The daily food intake of both control and expperimental rats were observed for 12 days. The data obtained was pooled and computed to get the mean food intake value. Table XV shows the comparison of food intake (g)/body weight/day of the control and experiental rats. The table shows a day by day increase in the food intake of experimental rats, compared to control rats. The mean value shows a tremendous increase in the food intake of experimental rats which is about 87.6 ± 2.7g/body

weight/day and is found to be significant when compared to that of control rats which shows a mean value of only 57.5 ± 0.7g/body weight/day (fig.25). This significant increase in food intake is attributed to the metabolic effect of progesterone. The mechanism of the action is discussed in detail.

IV. 4. Body weight/gm of the control and experimental rats

In tune with the food intake, the control and experimental rats showed an increase in the body weight. The pattern of the body weight for 12 days in control and experimental rats is shown in table-XVI (fig. 26). This table shows a significant increase in the body weight of experimental rats, at every 3 days intervels. The data obtained was pooled to get the mean body weight (gm) of control and experimental rats. The mean body weight shows a significant increase in experimental rats which is 116.8 ± 2.4 gm compared to control which is only 101.9 ± 0.66 gm. These investigations reveal that there is an interaction between the body temperature and food intake and in tune the body weight which is discussed in the metabolic role of the hormone progesterone.

IV. 5. Serum cholesterol/100 ml of the sample of control and experimental rats

Estimation of serum cholesterol/100 ml of the sample was done at the end of the experiment (after 12 days) in control and experimental rats. Table XVII shows the comparison of the serum cholesterol levels in control and experimental rats. From the table, it is clear that the experimental rats shows a highly significant increase in the bloo Cholesterol which is found to be 453.0 ± 0.5/100 ml of the sample, when compared to control which is only 231.4 ± 0.6/100 ml of the sample. The bar graph (Fig. 27) shows the comparison of the serum Cholesterol readings between control and experimental rats. From this, it is clear that the experimental rats developed hyperlipedemia which proves the physiological role of the hormone in cholesterol metabolism which is discussed.

TABLE - I

DAYWISE BODY TEMPERATURE READINGS (CONTROL RATS)

Days	A M				P M.						A M.		
	7 hrs.	9 hrs.	10 hrs.	11 hrs.	13 hrs.	15 hrs.	17 hrs.	19 hrs.	21 hrs.	23 hrs.	1 hr.	3 hrs.	5 hrs.
1st day	97.3	98.1	98.1	98.1	98.1	98.1	98.2	96.8	96.8	98.6	99.5	100.9	100.0
	± 0.3	±0.3	± 0.3	± 0.3	± 0.3	± 0.3	± 0.3	± 0.6	± 0.6	± 0.0	± 0.7	± 0.3	± 0.4
2nd day	97.3	97.7	98.1	98.1	98.6	98.6	98.6	100.0	100.4	100.9	100.9	100.4	98.6
	± 0.3	±0.2	± 0.3	± 0.3	± 0.0	± 0.0	± 0.6	± 0.4	± 0.0	± 0.3	± 0.3	± 0.6	± 0.0
3rd day	98.1	98.6	98.6	98.6	98.6	98.6	99.1	100.0	100.4	101.3	100.9	100.9	98.6
	± 0.3	± 0.0	± 0.0	± 0.0	0.0	0.0	± 0.4	± 0.4	± 0.2	± 0.3	± 0.3	± 0.3	± 0.0
4th day	98.1	98.6	98.6	98.6	98.6	98.6	98.6	99.1	100.4	100.4	100.9	101.8	101.3
	± 0.3	±0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.4	± 0.0	± 0.0	± 0.3	± 0.4	± 0.2
5th day	97.3	97.3	97.7	97.7	97.7	97.7	98.2	99.1	99.5	100.4	100.4	98.6	98.2
	± 0.3	±0.3	± 0.2	± 0.2	± 0.2	± 0.2	± 0.3	± 0.4	± 0.2	± 0.0	± 0.0	± 0.0	± 0.3
6th day	97.3	97.3	97.7	97.7	97.7	97.7	98.6	99.5	100.4	100.4	100.9	99.5	98.2
	± 0.3	±0.2	± 0.2	± 0.2	± 0.2	± 0.2	± 0.0	± 0.2	± 0.0	± 0.0	± 0.3	± 0.6	± 0.3
7th day	97.3	97.3	97.3	97.7	97.7	98.1	98.1	99.1	100.4	100.4	100.4	98.6	98.1
	± 0.3	±0.3	± 0.3	± 0.2	± 0.2	± 0.3	± 0.3	± 0.4	± 0.0	± 0.0	± 0.0	± 0.6	± 0.3
8th day	97.3	97.3	97.7	97.7	97.7	97.7	98.6	98.6	100.0	100.4	101.3	100.0	98.6
	± 0.3	±0.0	± 0.2	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.4	± 0.0	± 0.2	± 0.4	± 0.0
9th day	97.7	97.7	97.7	98.2	98.6	98.6	98.6	99.1	100.4	101.3	100.9	99.5	98.6
	± 0.0	±0.0	± 0.0	± 0.3	± 0.0	± 0.0	± 0.0	± 0.4	± 0.0	± 0.2	± 0.3	± 0.2	± 0.0
10th day	97.3	98.2	98.2	98.2	98.2	96.8	98.6	99.1	100.4	101.3	101.8	100.9	98.2
	± 0.3	±0.3	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.4	± 0.0	± 0.2	± 0.3	± 0.7	± 0.0
11th day	97.3	97.3	97.7	96.8	98.2	98.6	98.6	99.5	100.4	100.9	101.3	100.0	98.2
	± 0.3	±0.0	± 0.2	± 0.0	± 0.3	± 0.0	± 0.0	± 0.2	± 0.0	± 0.3	± 0.2	± 0.5	± 0.3
12th day	97.7	97.7	97.7	98.2	98.2	98.6	98.6	100.0	100.9	100.9	101.3	100.0	98.2
	± 0.2	±0.0	± 0.2	± 0.0	± 0.3	± 0.0	± 0.0	± 0.3	± 0.7	± 0.7	± 0.2	± 0.3	± 0.3
Mean	97.5	97.7	97.9	97.9	98.1	98.1	98.5	99.1	100.0	100.6	100.8	100.0	98.7
	± 0.1	±0.1	± 0.1	± 0.2	± 0.1	± 0.1	± 0.1	± 0.2	± 0.2	± 0.1	± 0.1	± 0.2	± 0.2

Thirteen observations each:

Mean ± S.E. are calculated for the body temperature readings observed for every two hours continuoulsy for 12 days for 4 rats.

TABLE II

COMPARISON OF EVERYDAY BODY TEMPERATURE RECORDINGS OF THE CONTROL RATS

Days	Mean ± S.E.(°F)	Comparison between Subsequent days and first day	't'	'p' value
1	98.4 ± 0.3	-	-	-
2	99.1 ± 0.3	1 and 2	1.7	* 0.05
3	99.4 ± 0.3	1 and 3	4.4	** 0.005
4	99.5 ± 0.3	1 and 4	2.9	** 0.005
5	98.4 ± 0.3	1 and 5	0.2	N.S.
6	98.6 ± 0.3	1 and 6	1.0	N.S.
7	98.5 ± 0.3	1 and 7	0.6	N.S.
8	98.6 ± 0.3	1 and 8	0.8	N.S.
9	98.9 ± 0.3	1 and 9	1.6	* 0.05
10	99.1 ± 0.4	1 and 10	1.5	* 0.05
11	98.6 ± 0.3	1 and 11	2.3	** 0.01
12	98.9 ± 0.3	1 and 12	2.2	* 0.025

Mean= 98.8 ± 0.1
(13 observations each)

** Significant at 1% level
* Significant at 5% level
N.S. Not Significant

Mean ± S.E. readings are calculated for every 2 hours recordings continuosly for 12 days for 4 rats.

TABLE -III

DAYWISE BODY TEMPERATURE READINGS (EXPERIMENTAL RATS)

Days	A M				P M						A M		
	7 hrs.	9 hrs.	10 hrs.	11 hrs.	13 hrs.	15 hrs.	17 hrs.	19 hrs.	21 hrs.	23 hrs.	1 hr.	3 hrs.	5 hrs.
1st day	98.9	98.9	98.9	99.2	99.8	100.4	100.4	100.4	101.5	101.3	102.5	102.8	101.0
	± 0.2	±0.2	± 0.2	± 0.3	± 0.3	± 0.0	± 0.0	± 0.0	± 0.5	± 0.5	± 0.2	± 0.3	± 0.3
2nd day	98.9	99.2	99.8	99.2	100.1	100.4	100.4	100.4	101.0	101.9	102.5	103.4	101.6
	± 0.2	±0.3	± 0.3	± 0.3	± 0.2	± 0.0	± 0.0	± 0.0	± 0.5	± 0.5	± 0.2	± 0.3	± 0.3
3rd day	99.5	99.5	99.5	100.1	100.1	100.4	100.4	100.4	101.0	102.2	102.5	103.1	101.9
	± 0.1	±0.1	± 0.1	± 0.2	± 0.2	± 0.0	± 0.0	± 0.0	± 0.5	± 0.6	± 0.2	± 0.5	± 0.4
4th day	98.9	99.5	99.5	99.8	99.5	99.8	99.8	99.8	102.2	102.2	102.8	102.8	102.8
	± 0.2	±0.1	± 0.1	± 0.2	± 0.1	± 0.2	± 0.2	± 0.2	± 0.6	± 0.6	± 0.3	± 0.3	± 0.3
5th day	99.2	99.5	99.5	99.8	100.1	100.4	100.4	100.4	101.6	102.5	102.8	103.1	101.9
	± 0.3	±0.1	± 0.1	± 0.2	± 0.2	± 0.4	± 0.4	± 0.4	± 0.5	± 0.5	± 0.3	± 0.5	± 0.4
6th day	99.2	99.2	99.2	99.2	99.2	100.1	100.1	100.1	101.0	101.3	102.5	102.8	101.0
	± 0.3	±0.3	± 0.3	± 0.3	± 0.3	± 0.2	± 0.2	± 0.2	± 0.5	± 0.5	± 0.2	± 0.3	± 0.3
7th day	99.5	99.5	99.5	100.4	100.4	100.7	100.7	100.7	101.9	102.8	102.8	103.7	102.8
	± 0.1	±0.1	± 0.1	± 0.4	± 0.4	± 0.2	± 0.2	± 0.2	± 0.6	± 0.3	± 0.3	± 0.4	± 0.3
8th day	99.2	99.8	99.2	99.2	99.5	100.7	100.7	100.7	101.0	101.3	102.5	102.8	101.6
	± 0.3	±0.3	± 0.3	± 0.3	± 0.1	± 0.2	± 0.2	± 0.2	± 0.5	± 0.5	± 0.2	± 0.3	± 0.5
9th day	99.2	98.9	98.6	99.5	99.5	100.4	100.4	100.4	100.4	101.3	102.2	102.5	101.0
	± 0.3	±0.2	± 0.0	± 0.1	± 0.1	± 0.0	± 0.0	± 0.0	± 0.0	± 0.1	± 0.2	± 0.2	± 0.3
10th day	98.9	99.5	99.8	99.5	99.5	100.1	100.1	100.1	101.3	102.2	102.5	102.5	101.6
	± 0.2	±0.1	± 0.3	± 0.1	± 0.1	± 0.2	± 0.2	± 0.2	± 0.5	± 0.6	± 0.2	± 0.2	± 0.3
11th day	98.9	99.5	98.9	99.2	99.8	100.4	100.4	100.4	101.6	102.2	102.8	102.8	101.9
	± 0.2	±0.1	± 0.1	± 0.3	± 0.3	± 0.4	± 0.4	± 0.4	± 0.5	± 0.5	± 0.3	± 0.3	± 0.5
12th day	99.8	100.1	99.2	99.5	99.5	101.0	101.0	101.0	101.9	102.5	102.8	102.8	102.2
	± 0.3	±0.2	± 0.3	± 0.1	± 0.1	± 0.3	± 0.3	± 0.3	± 0.6	± 0.5	± 0.3	± 0.3	± 0.6
Mean	99.1	99.4	99.3	99.5	99.7	100.3	100.4	100.4	101.3	101.9	102.6	102.9	101.7
	± 0.1	±0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1

Thirteen observations each:

Mean ± S.E. are calculated for the body temperature readings observed for every two hours continuoulsy for 12 days for 6 rats.

TABLE IV

COMPARISON OF EVERDAY BODY TEMPERATURE READINGS OF THE EXPERIMENTAL RATS

S.No.	Mean ± S.E (°F)	Comparison between subsequent days and first day	't'	'P' value
1.	100.4 ± 0.3	-	-	-
2.	100.6 ± 0.3	1 and 2	2.7	** 0.01
3.	100.8 ± 0.3	1 and 3	3.4	** 0.005
4.	100.7 ± 0.3	1 and 4	1.7	* 0.05
5.	100.8 ± 0.3	1 and 5	3.9	** 0.005
6.	100.3 ± 0.3	1 and 6	1.0	N.S
7.	101.1 ± 0.3	1 and 7	2.9	** 0.01
8.	100.6 ± 0.3	1 and 8	2.3	** 0.01
9.	100.3 ± 0.3	1 and 9	0.6	N.S
10.	100.5 ± 0.3	1 and 10	1.1	N.S
11.	100.6 ± 0.3	1 and 11	2.5	** 0.01
12.	101.0 ± 0.3	1 and 12	4.5	** 0.005
Mean ± S.E.	100.6 ± 0.1 (13 observations each)			

** Significant at 1% level

* Significant at 5% level

N.S - Not significant

Mean ± S.E. readings are calculated for every 2 hours recordings continuously for 12 days for 6 rats.

TABLE V

COMPARISON OF THE OVERALL BODY TEMPERATURE OF CONTROL AND EXPERIMENTAL RATS

S.No.	Comparison	Meant ± S.E (°F)	't' value
1.	Control	98.8 ± 0.1	*** 13.60
2.	Experimental	100.6 ± 0.1	

(13 observations each) *** Significant

TABLE VI

COMPARISION OF THE MAXIMA OF THE BODY TEMPERATURE IN CONTROL AND EXPERIMENTAL RATS

S.No.	Comparison	Maximum body temprature rature ± S.E.(°F)	't' value
1.	Control	101.1 ± 0.1	
2.	Experimental	102.9 ± 0.1	*** 11.30

13 observations each

*** Highly Significant

TABLE VII

COMPARISON OF THE MINIMA OF THE BODY TEMPERATURE IN CONTROL AND EXPERIMENTAL RATS

S.No.	Comparison	Minimum body Temperature rature ± S.E (°F)	't' value
1.	Control	97.3 ± 0.1	
2.	Experimental	99.1 ± 0.1	*** 11.59

13 observations each

*** Highly Significant

TABLE VIII

THE PATTERN OF THE BODY TEMPERATURE FOR SPECIFIC PHASES OF OESTRUS CYCLE (CONTROL RATS)

Time	Metoestrus (+) Mean ± S.E (°F)	Dioestrus (2+) Mean ± S.E (°F)	Proestrus (3+) Mean ± (°F)	Oestrus (4+) Mean ± S.E (°F)
7.00	97.1 ± 0.1 MN	98.0 ± 0.1	97.6 ± 0.2	97.3 ± 0.2
9.00	97.5 ± 0.1	98.0 ± 0.2	97.6 ± 0.2	97.5 ± 0.2
10.00	97.5 ± 0.1	98.2 ± 0.2	97.7 ± 0.2	98.0 ± 0.2
11.00	97.6 ± 0.2	98.2 ± 0.1	98.2 ± 0.1	98.0 ± 0.2
13.00	97.7 ± 0.2	98.3 ± 0.2	98.2 ± 0.2	98.4 ± 0.3
15.00	98.1 ± 0.1	98.6	98.3 ± 0.2	98.4 ± 0.2
17.00	98.2 ± 0.2	98.6	98.6	98.4 ± 0.2
19.00	98.2 ± 0.1	98.9 ± 0.2	98.9 ± 0.2	98.6
21.00	98.8 ± 0.3	99.7 ± 0.2	100.7 ± 0.2	99.0 ± 0.3
23.00	99.1 ± 0.3	100.7 ± 0.2	100.7 ± 0.2	100 ± 0.1
1.00	100.1 ± 0.2	101.5 ± 0.2	101.5 ± 0.2	100.9 ± 0.1
3.00	100.7 ± 0.2	100.8 ± 0.3	101.6 ± 0.3	100.4
5.00	99.2 ± 0.2	101.5 ± 0.2	100.4 ± 0.2	99.0 ± 0.2
Average	98.5 ± 0.3	99.4 ± 0.2	99.2 ± 0.3	98.8 ± 0.2

TABLE IX

DIOESTROUS HIGH BODY TEMPERATURE (CONTROL RATS)

S.No.	Phase of oestrus cycle	Mean ± S.E (°F)	Comparison of phases	'P' value
1.	Metoestrous	98.5 ± 0.3	-	-
2.	Dioestrous	99.4 ± 0.2	Dioestrous Vs Metoestrus	0.005
3.	Prooestrous	99.2 ± 0.3	Dioestrus Vs Proestrus	0.01
3.	Oestrous	98.8 ± 0.2	Dioestrus Vs Oestrus	0.005

13 Observations each.

Dioestrus body temperature compared with that of metoestrous, proestrous and oestrus stages. Note the significant increase in dioestrus when compared to other stages and also the order of significance.

TABLE X

THE PATTERN OF THE BODY TEMPERATURE RHYTHM FOR SPECIFIC PHASES OF OESTRUS CYCLE (EXPERIMENTAL RATS)

Time	Metoestrus (+) Mean ± S.E (°F)	Dioestrus (2+) Mean ± S.E (°F)	Proestrus (3+) Mean ± S.E (°F)	Oestrus (4+) Mean ± S.E (°F)
7.00	99.0 ± 0.2	99.5 ± 0.1	99.3 ± 0.1	99.1 ± 0.1
9.00	99.3 ± 0.2	99.5 ± 0.1	99.3 ± 0.2	99.3 ± 0.2
10.00	99.3 ± 0.2	99.5 ± 0.1	99.7 ± 0.2	99.5 ± 0.2
11.00	99.4 ± 0.2	99.5 ± 0.1	99.5 ± 0.2	99.5 ± 0.2
13.00	99.6 ± 0.2	100.4 ± 0.2	99.8 ± 0.2	99.8 ± 0.2
15.00	100.4	100.4 ± 0.2	100.6 ± 0.1	100.4
17.00	100.4	100.4 ± 0.2	100.6 ± 0.1	100.4
19.00	100.4	100.4 ± 0.2	100.6 ± 0.1	100.4
21.00	100.4	103.5 ± 0.1	100.6 ± 0.1	100.4
23.00	100.6	103.5 ± 0.1	102.3 ± 0.1	101.1 ± 0.2
1.00	102.2	103.5 ± 0.1	102.2	102.2
3.00	102.2	103.5 ± 0.1	103 ± 0.2	102.3 ± 0.1
5.00	100.6 ± 0.1	102.5 ± 0.1	102.1 ± 0.1	101 ± 0.2
Mean	100.2 ± 0.2	101.2 ± 0.4	100.7 ± 0.4	100.4 ± 0.2

TABLE XI

DIOESTROUS HIGH BODY TEMPERATURE (EXPERIMENTAL RATS)

S.No	Phases of oestrous cycle	Symbol used	Mean ± S.E(°F)	Comparison of Phases	'P' value
1.	Metoestrous	'+'	100.2 ± 0.2	-	-
2.	Dioestrous	'2+'	101.2 ± 0.4	Dioestrous Vs metoestrous	0.005
3.	Proestrous	'3+'	100.7 ± 0.4	Dioestrous Vs proestrous	0.025
4.	Destrous	'4+'	100.4 ± 0.2	Dioestrous Vs oestrous	0.010

13 Observations each

Note : Dioestrous body temperature compared with that of metoestrous, prooestrous, and oestrous stages of the experimental rats. There is significant increase at dioestrous when compared to other stages, and also the order of significance is shown.

TABLE XII

COMPARISION OF THE MAXIMA OF THE BODY TEMPERATURE DURING OESTROUS PHASES IN CONTROL AND EXPERIMENTAL RATS

S.No.	Oestrous phases	Symbol used	Maximum body temperature ± S.E (°F)		't' value
			Control	Experimental	
1.	Metoestrous	'+'	100.7 ± 0.2	102.2 ± 0	*** 9.896
2.	Dioestrous	'2+'	101.8 ± 0.3	103.5 ± 0.1	** 5.690
3.	Proestrous	'3+'	101.6 ± 0.3	103 ± 0.2	*** 7.628
4.	Oestrous	'4+'	100.9 ± 0.1	102.3 ± 0.1	***10.26

*** Highly Significant

** Significant at 1% level

The maximum body temperature obtained during different phases of oestrous cycle of control and experimental rats are compared. Note the difference to be significant. As in control, here also the dioestrous body temperature is high.

TABLE XIII

COMPARISON OF THE MINIMA OF THE BODY TEMPERATURE DURING OESTROUS PHASES IN CONTROL AND EXPERIMENTAL RATS

S.No	Oestrous phases	Symbol used	Minimum body temperature ± S.E (°F) Control	Experimental	't' value
1.	Metoestrous	'+'	97.1 ± 0.1	99.0 ± 0.2	*** 6.601
2.	Dioestrous	'2+'	98.0 ± 0.1	99.5 ± 0.1	*** 5.037
3.	Proestrous	'3+'	97.6 ± 0.2	99.3 ± 0.1	*** 5.493
4.	Oestrous	'4+'	97.3 ± 0.2	99.1 ± 0.1	*** 5.603

*** Highly Significant

The minimum body temperature obtained during different phases of oestrous cycle of control and experimental rats are compared. Note that the difference is significant. Here also the dioestrous body temperature is high.

TABLE XIV

FREQUENCY OF SPECIFIC STAGES OF OESTROUS CYCLE OF THE NORMAL RATS AND EXPERIMENTAL RATS

S.No	Nature of the experiment	% Frequency of oestrous phases			
		+	2+	3+	4+
1.	Control (12)	25	25	25	25
2.	Experimental (12)	26.3	29.1	22.2	22.2

Number of parenthesis indicates the number of observations.

TABLE - XV

FOOD INTAKE (g) BODY WEIGHT/DAY OF THE CONTROL AND EXPERIMENTAL RATS

Days	Control Mean ± S.E. (gm)	Experimental Mean ± S.E. (g)	
1	53.75 ± 2.0	59.1 ± 3.0	
2	53.75 ± 2.0	75.0 ± 2.0	
3	53.75 ± 2.0	79.1 ± 0.7	
4	56.25 ± 2.7	78.3 ± 2.2	
5.	57. 5 ± 2.1	86.6 ± 1.4	
6	57. 5 ± 2.1	88.3 ± 0.9	
7	60. 0 ± 1.7	93.3 ± 1.9	
8	58.75 ± 2.0	95.0 ± 1.6	
9	58.75 ± 2.0	97.5 ± 0.6	
10	60. 0 ± 1.7	99.1 ± 0.7	
11	60. 0 ± 1.7	101.6 ± 0.9	
12	60. 0 ± 1.7	103.3 ± 1.5	
Mean ± S.E.	57. 5 ± 1.7	87.6 ± 2.5	t=10.80 significant

The mean food intake (g)/body weight oof both control and experimental animals are calculated for 12 days. Note the significant increase in the food intake of experimental rats, when compared to control rats.

TABLE - XVI

BODY WEIGHT (gm) OF THE CONTROL AND EXPERIMENTAL RATS

Days	Control Mean ± S.E.(g)	Experimental Mean ± S.E.(°F)	't' value
3rd day	100 ± 1.7	110 ± 2.0	** 3.098
6th day	101.25 ± 1.1	115 ± 2.0	** 4.820
9th day	102.5 ± 0.6	119.2 ± 1.4	*** 7.473
12th day	103.75 ± 1.1	123.3 ± 1.5	*** 8.4777
Mean ± S.E. (g)	101. 9 ± 0.6	116.8 ± 2.4	

*** Significant

** Significant at 1% level

The body weights of the control and experimental animals were observed for once in three days for 12 days. Note the significant increase in the body weights of experimental rats when compared to control rats and also the levels of significance.

TABLE XVII

SERUM CHOLESTEROL/100ML OF THE SAMPLE OF THE CONTROL AND EXPERIMENTAL RATS

S.No.	Comparison	Mean/100 ml of the sample	't' Value
1	Control	231.4 ± 0.6	7.11***
2	Experimental	453.0 ± 0.5	

*** Significant

PATTERN OF THE BODY TEMPERATURE RHYTHM IN THE CONTROL RATS

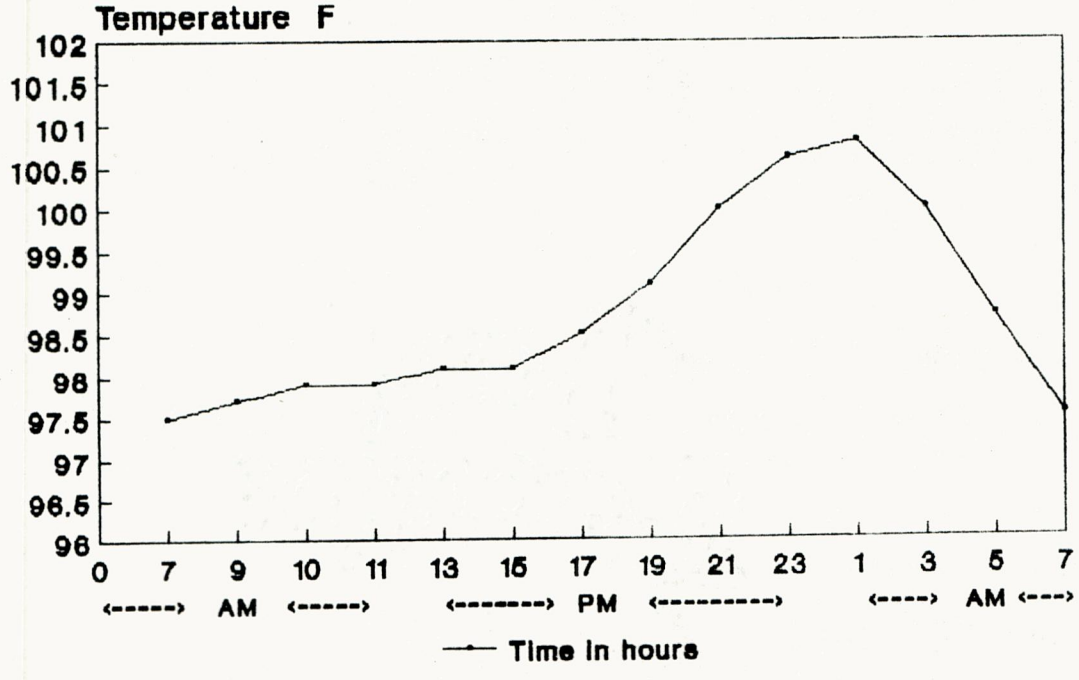


Fig 4

PATTERN OF THE BODY TEMPERATURE RHYTHM IN THE EXPERIMENTAL RATS

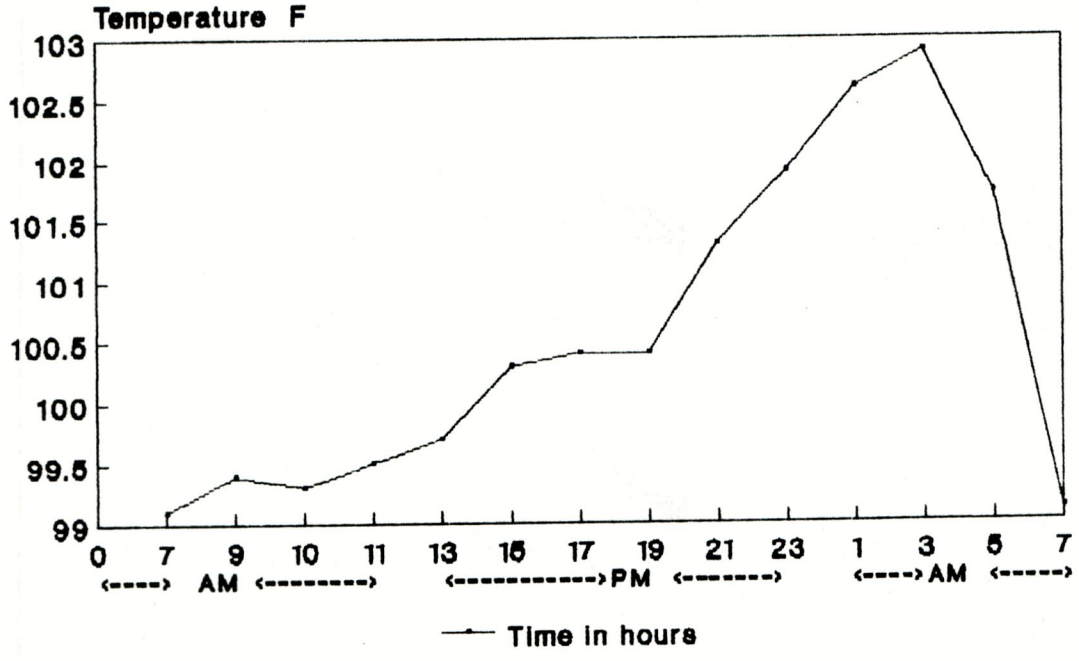


fig 5

COMPARISON OF THE BODY TEMPERATURE RHYTHM IN THE CONTROL AND EXPERIMENTAL RATS

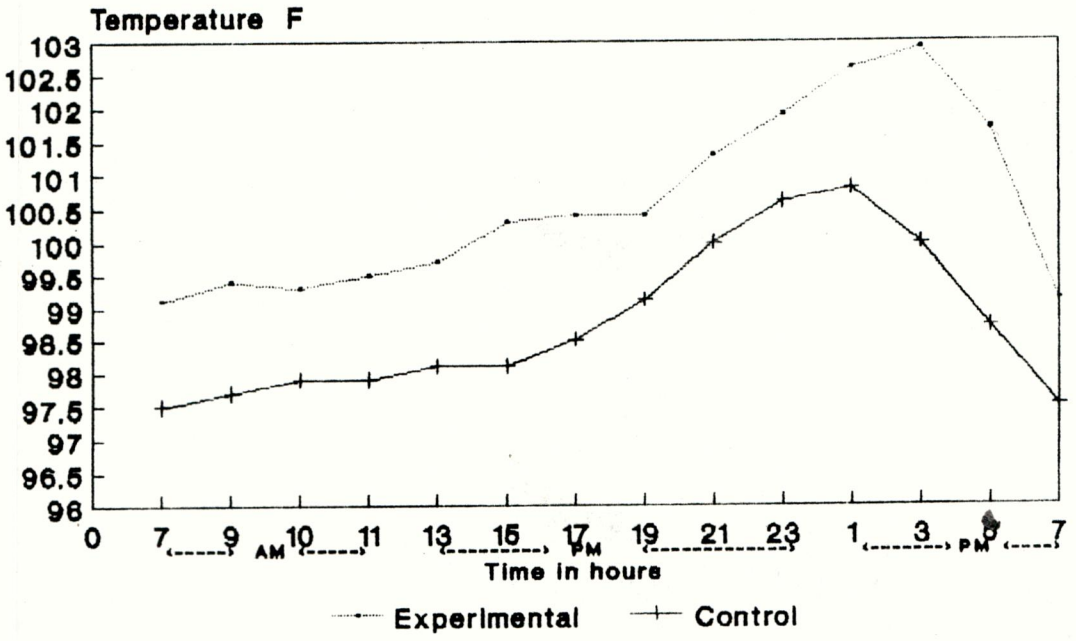


fig 6

BODY TEMPERATURE OF THE CONTROL AND EXPERIMENTAL RATS-DAYWISE AVERAGE

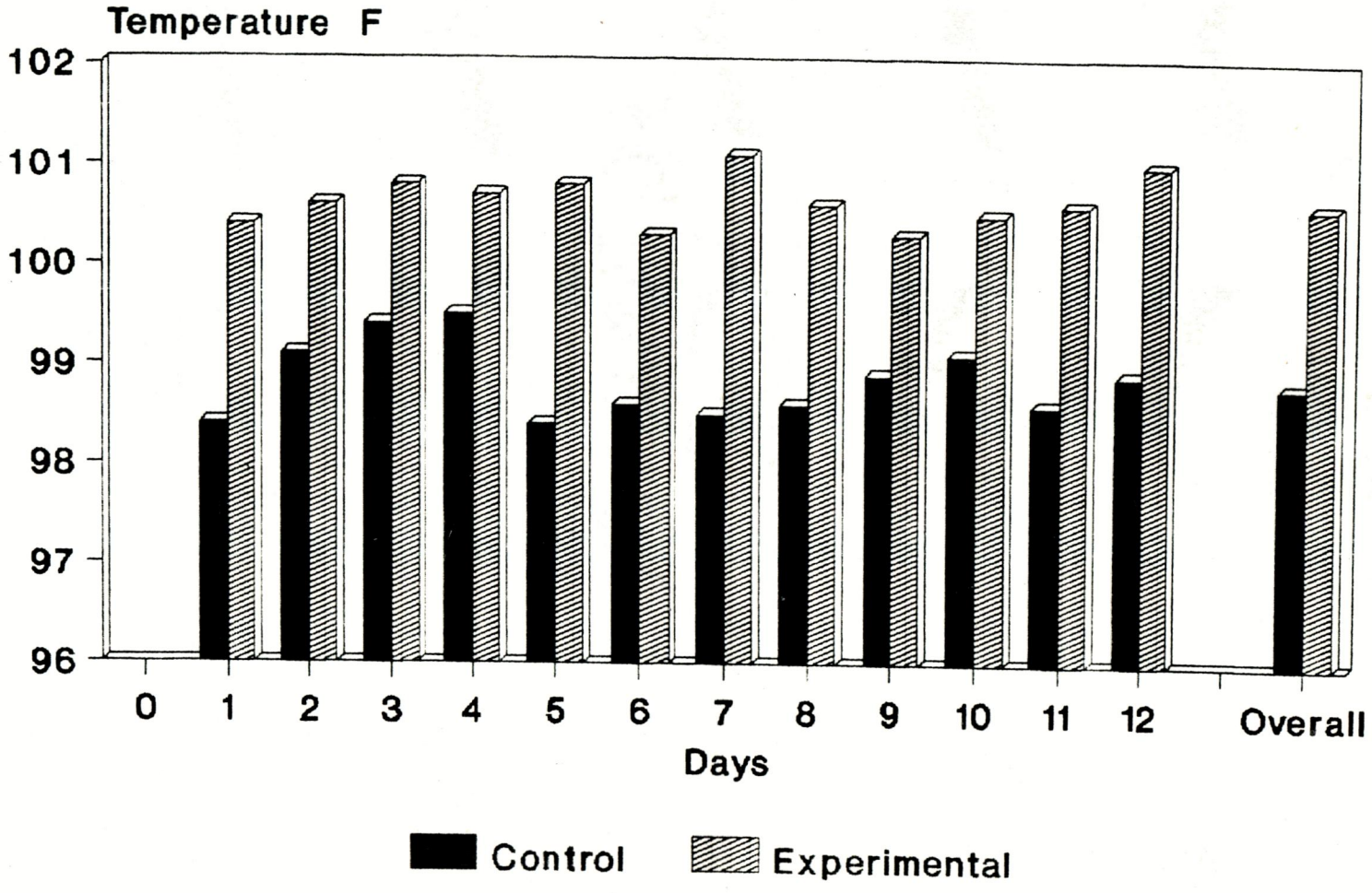


Fig 7

COMPARISON OF THE MAXIMA OF BODY TEMPERATURE IN CONTROL AND EXPERIMENTAL RATS

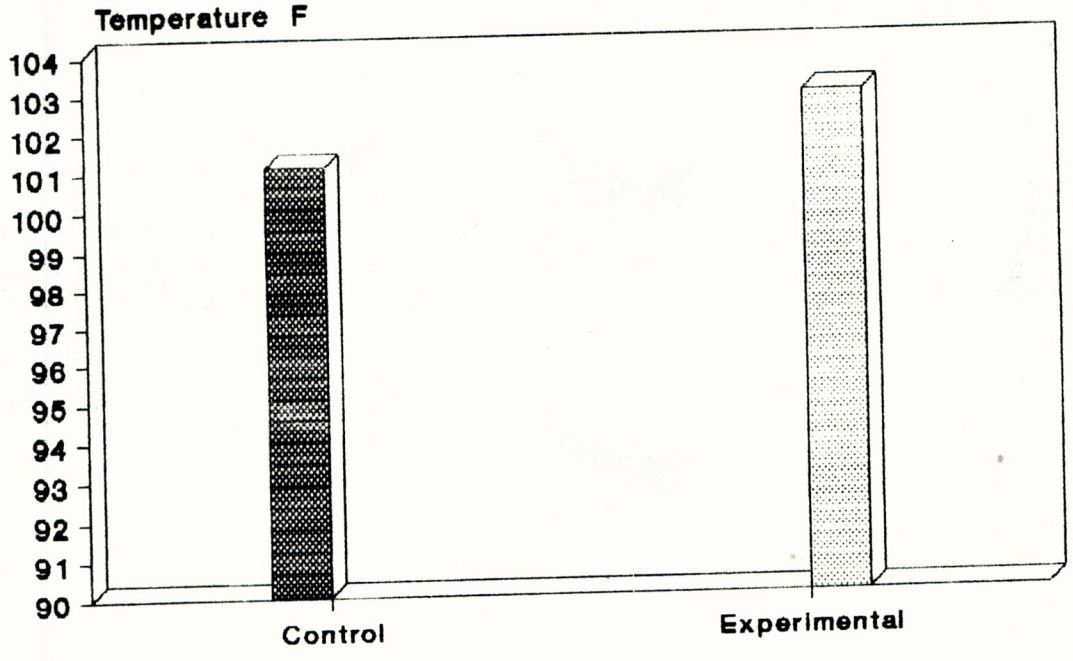


Fig 8

COMPARISON OF THE MINIMA OF BODY TEMPERATURE IN CONTROL AND EXPERIMENTAL RATS

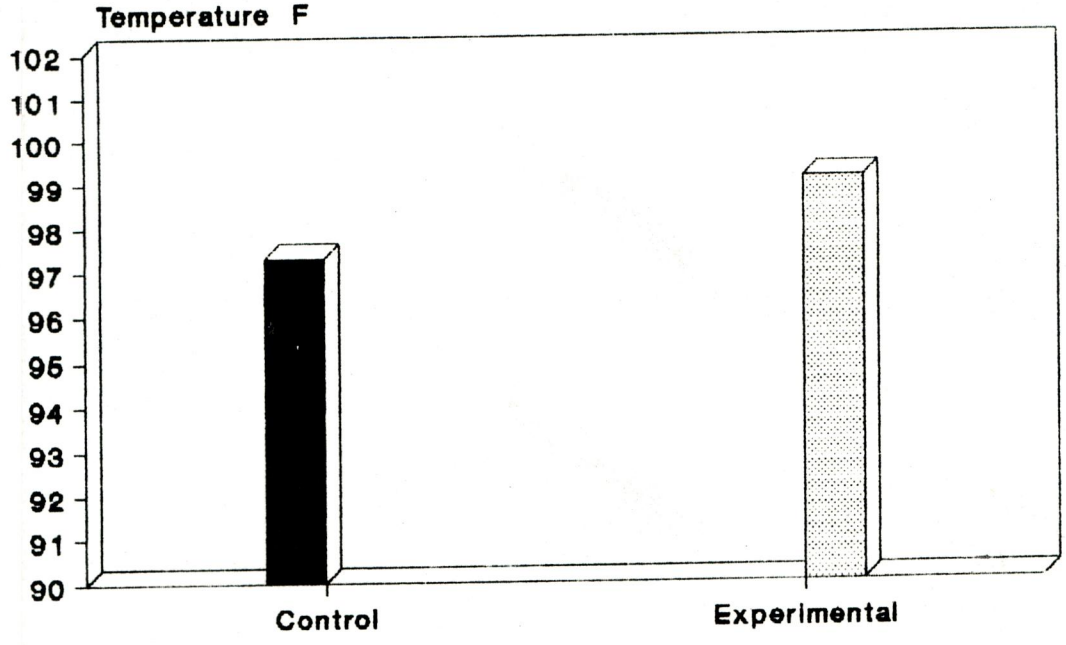


Fig 9

DIOESTRUS PEAK OF THE BODY TEMPERATURE OF CONTROL RATS-OVERALL MEAN VALUES-PHASEWISE

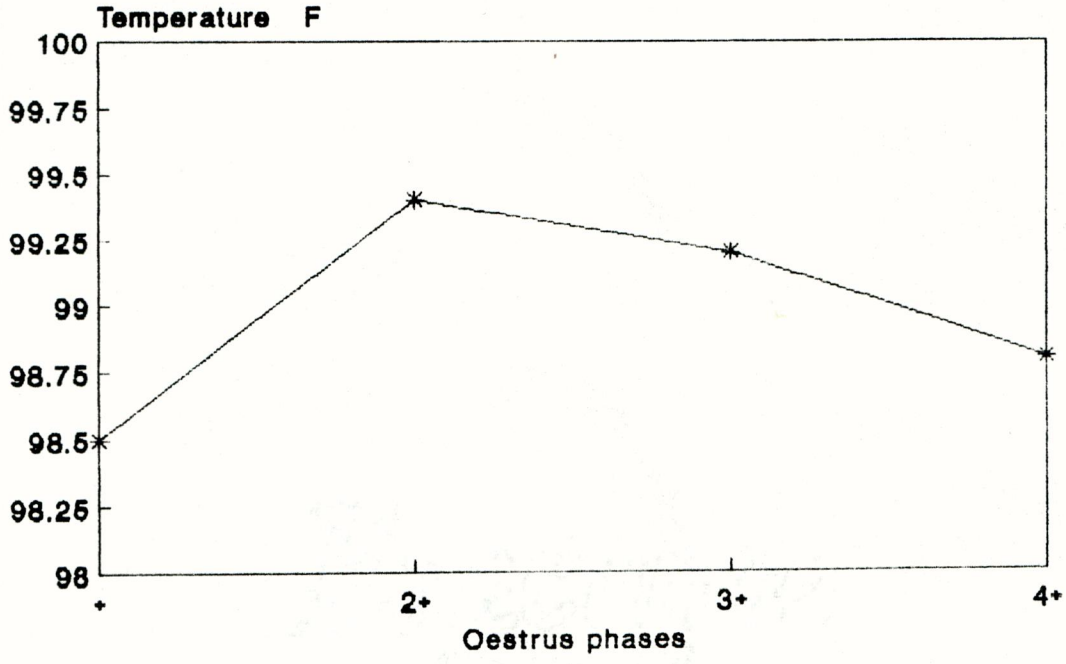
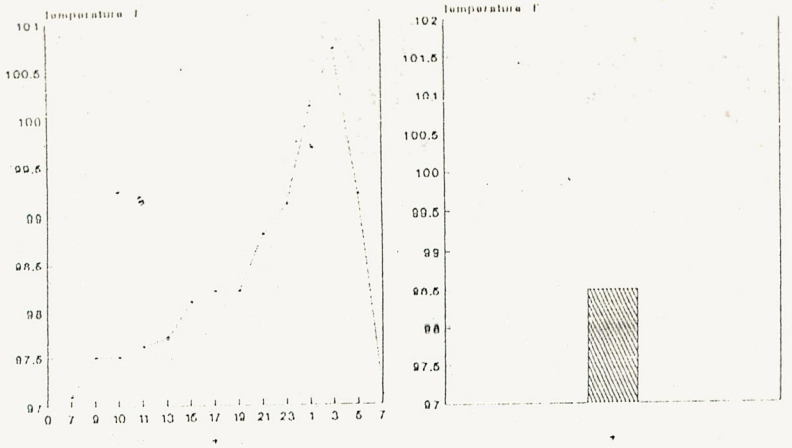


Fig 10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99.5
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98.5
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97.5
97
0 7 9 10 11 13 15 17 19 21 23 1 3 5 7
Temperature F
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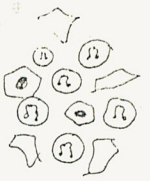
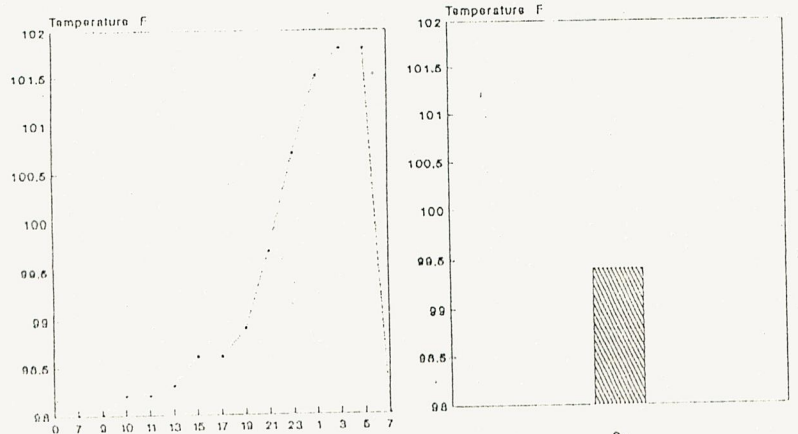
101.5
101
100.5
100
99.5
99
98.5
98
97.5
97




Temperature F
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101.5
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100.5
100
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99
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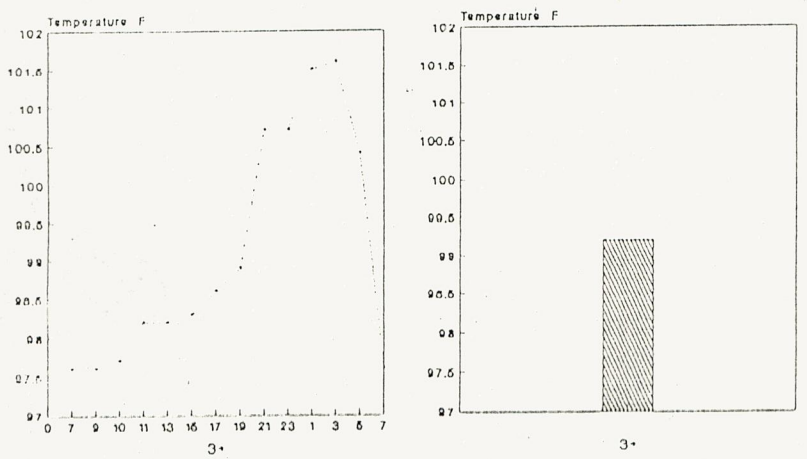
98
0 7 9 10 11 13 15 17 19 21 23 1 3 5 7
2•
Temperature F
102
101.5
101
100.5
100
99.5
99
98.5
98
2•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97.5
97
0 7 9 10 11 13 15 17 19 21 23 1 3 5 7
3•
Temperature F
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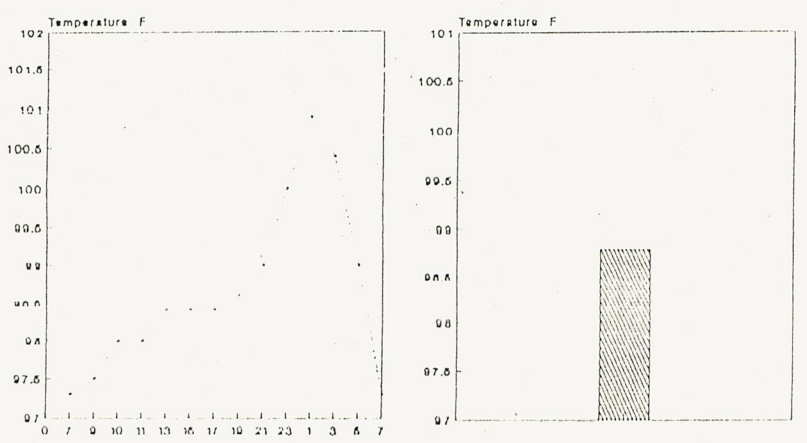
101.5
101
100.5
100
99.5
99
98.5
98
97.5
97
3•


Temperature F
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101.5
101
100.5
100
99.5
99
98.5
98
97.5
97
0 7 9 10 11 13 15 17 19 21 23 1 3 5 7
4•
Temperature F
101
100.5
100
99.5
99
98.5
98
97.5
97
4•

COMPARISON OF MAXIMA AND MINIMA OF THE BODY TEMPERATURE DURING OESTRUS PHASES (CONTROL)

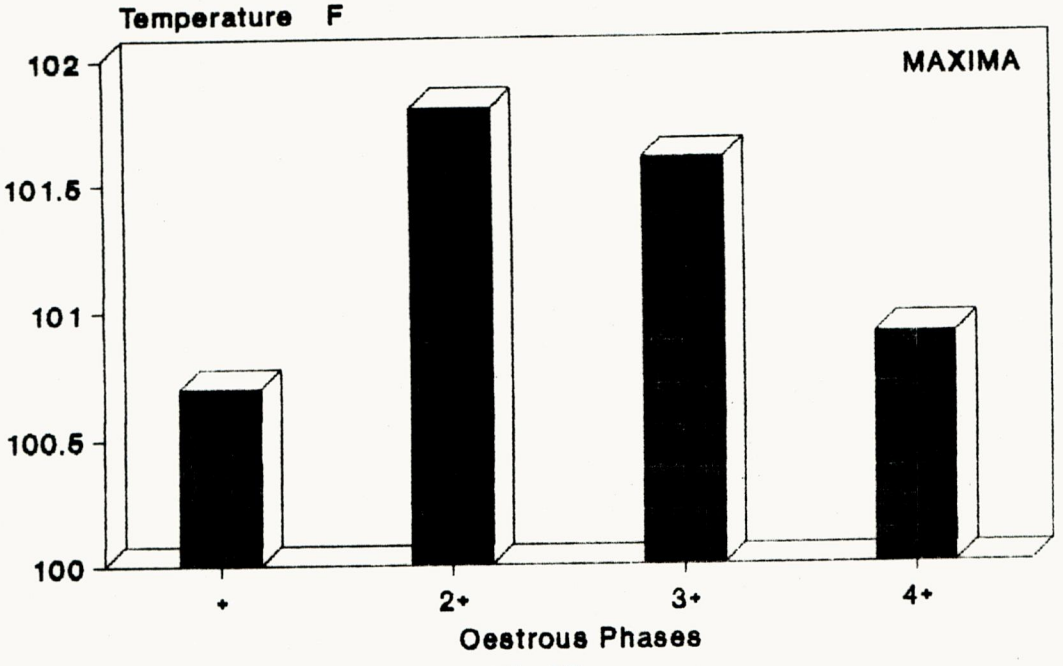


Fig-12a

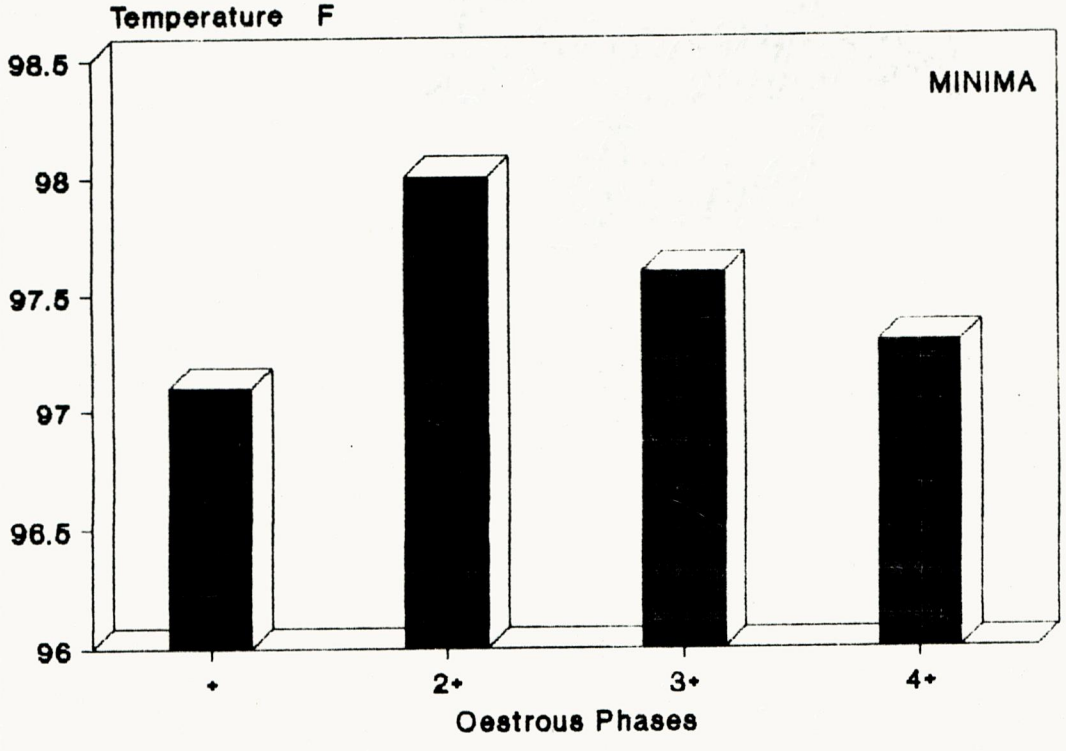


Fig 12 b

DIOESTRUS PEAK OF THE BODY TEMPERATURE OF EXPERIMENTAL RATS-OVERALL MEAN VALUES-PHASEWISE

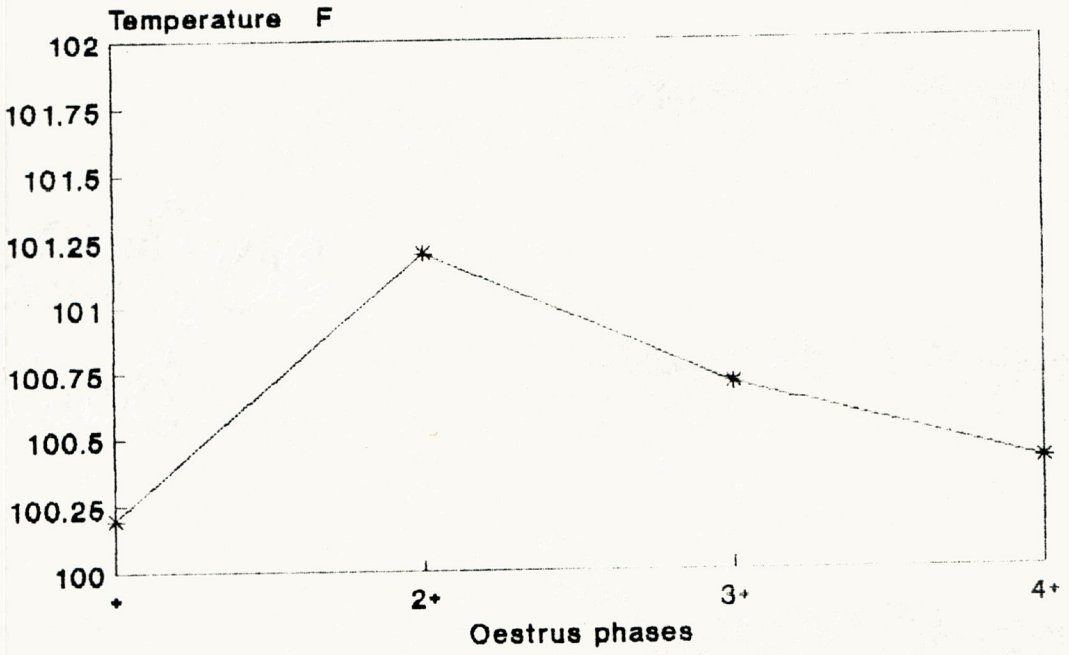
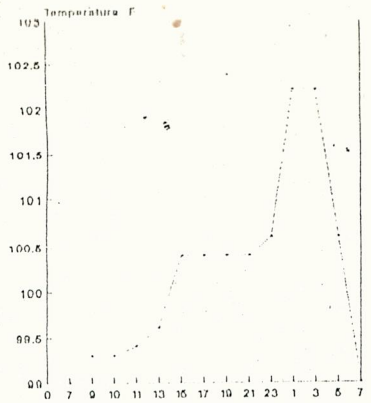


Fig 13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102
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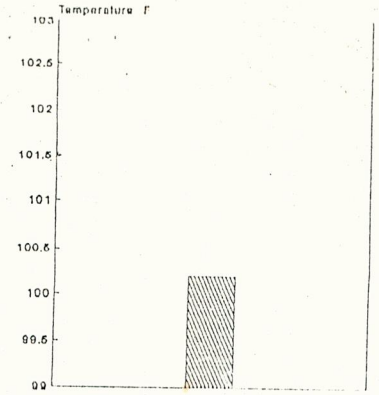
101
100.5
100
99.5
99
0 7 9 10 11 13 15 17 19 21 23 1 3 5 7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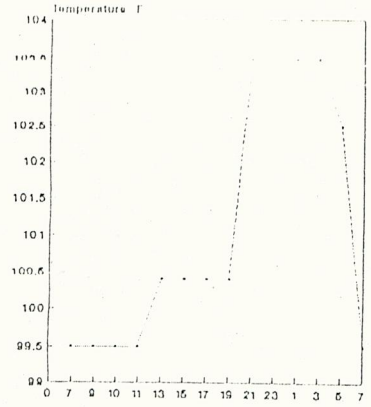
102
101.5
101
100.5
100
99.5
99


Temperature F
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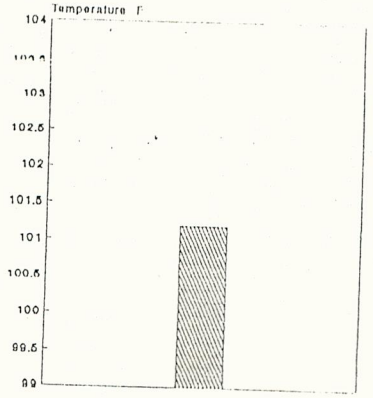
103.5
103
102.5
102
101.5
101
100.5
100
99.5
99
0 7 9 10 11 13 15 17 19 21 23 1 3 5 7

2•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103.5
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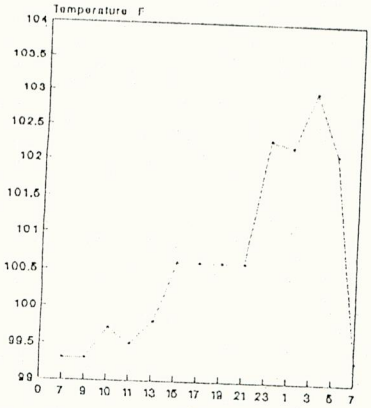
102.5
102
101.5
101
100.5
100
99.5
99

2•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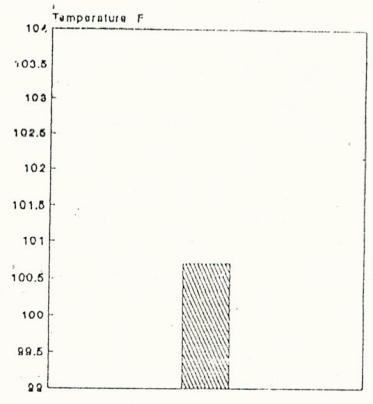
101
100.5
100
99.5
99
0 7 9 10 11 13 15 17 19 21 23 1 3 5 7

3•


Temperature F
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103
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101
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100
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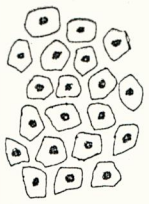
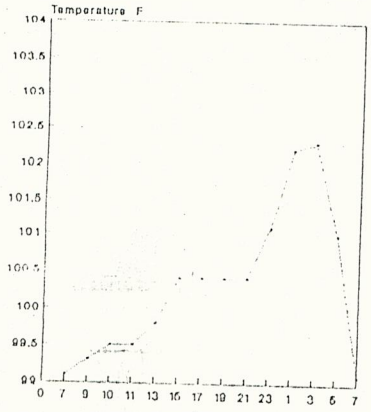
99

3•

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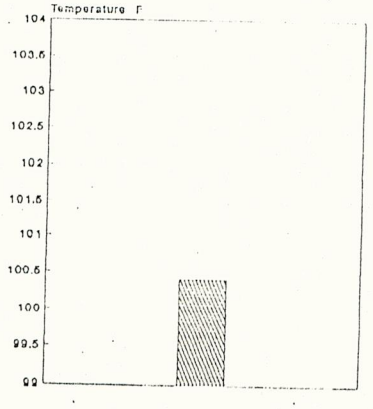
0 7 9 10 11 13 15 17 19 21 23 1 3 5 7

4•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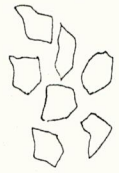
99

4•



COMPARISON OF BODY TEMPERATURE AT OESTRUS PHASES (CONTROL AND EXPERIMENTAL)

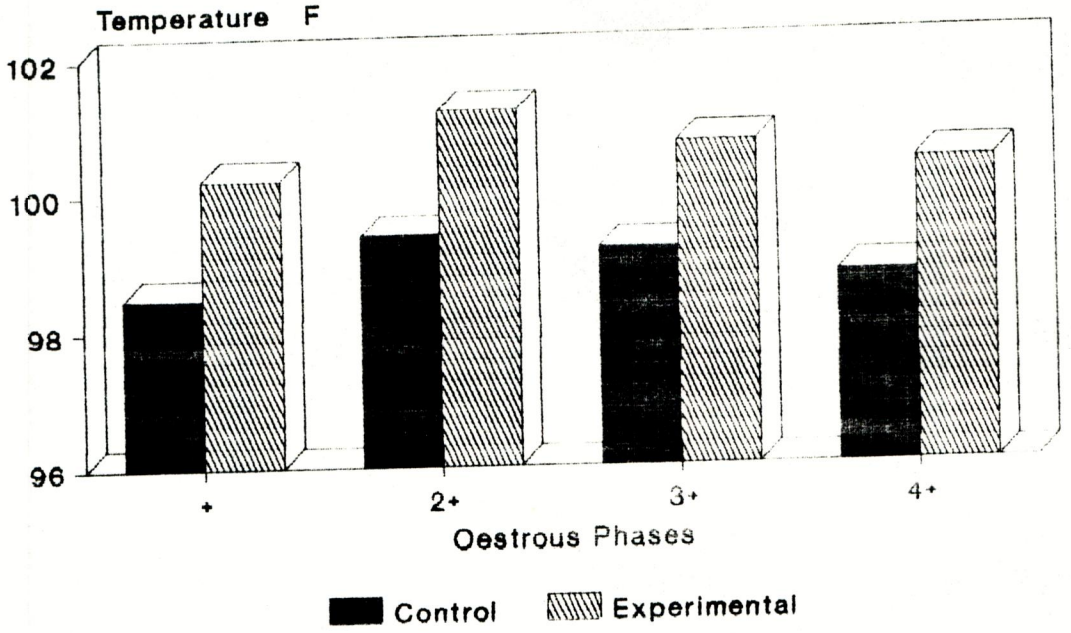


Fig 15

COMPARISON OF MAXIMA AND MINIMA OF THE BODY TEMPERATURE DURING OESTRUS PHASES (EXPERIMENTAL)

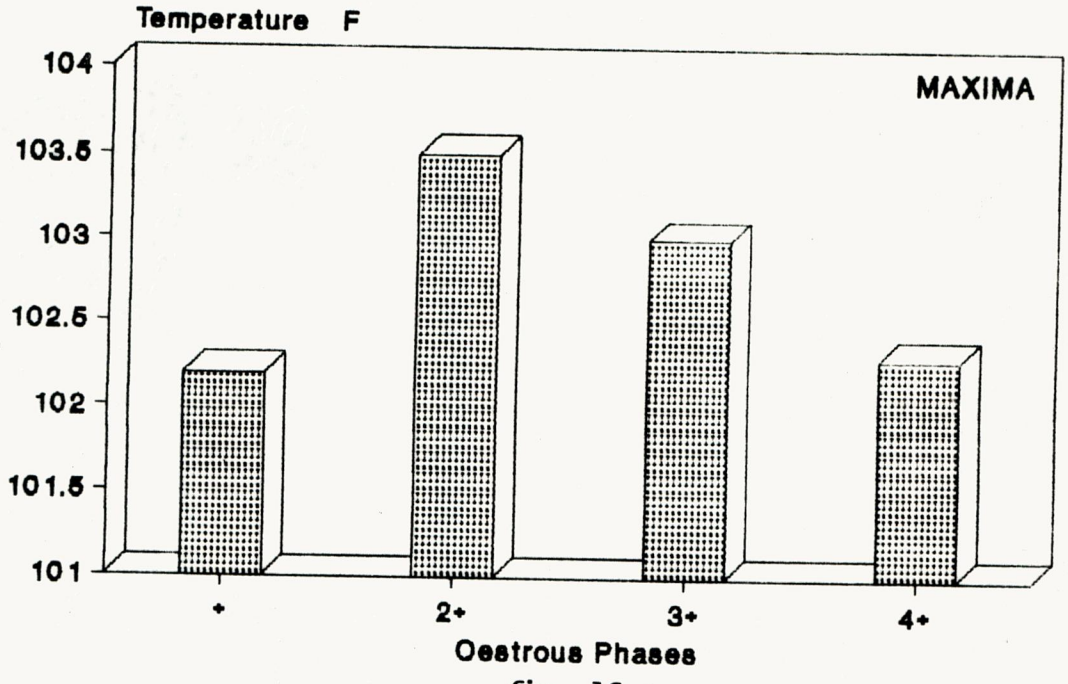


fig 16 a

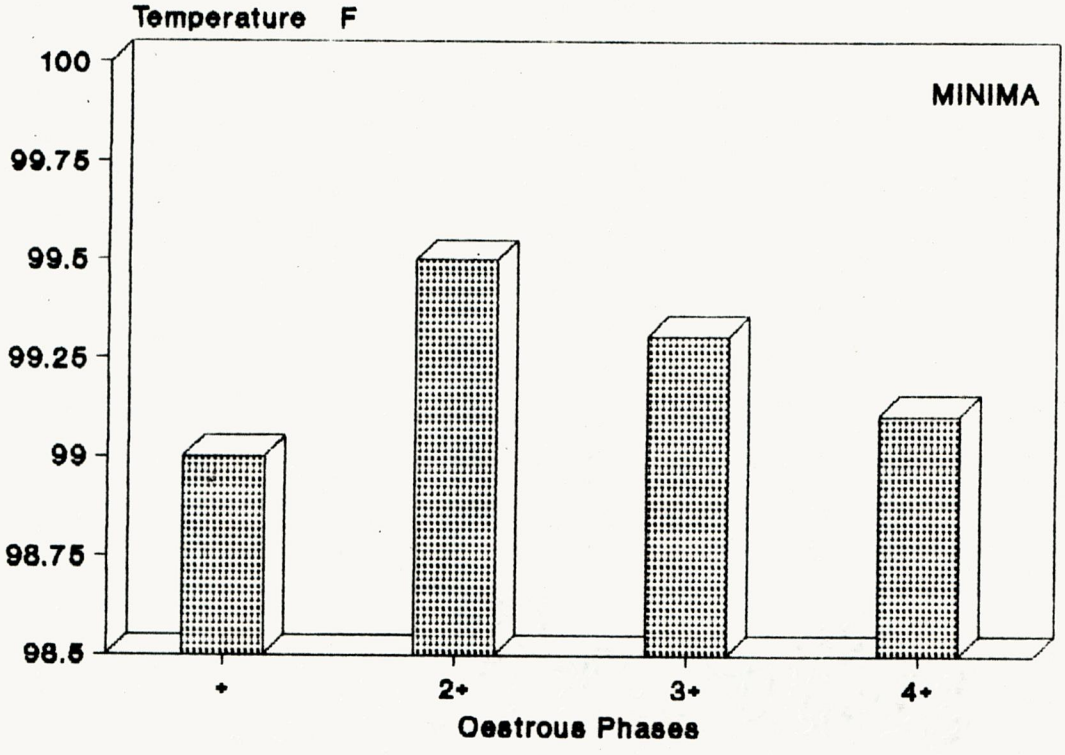
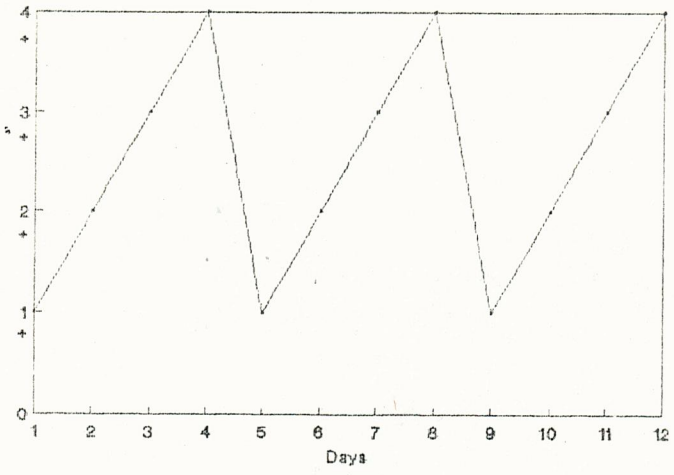


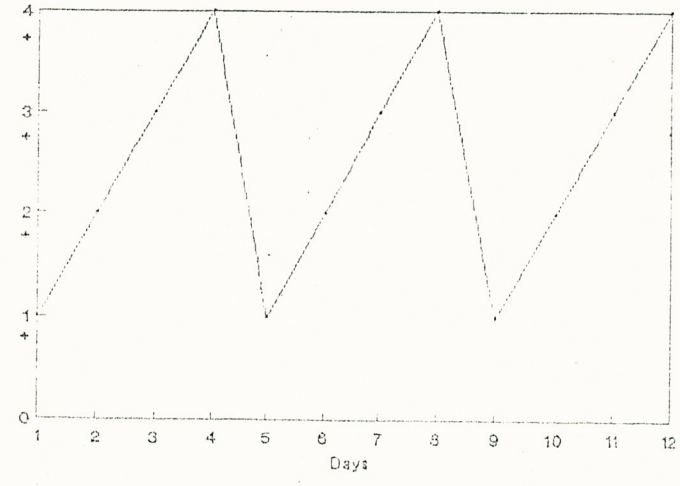
Fig 16 b

Figure - 17

Oestrus rhythm in control rats

Note the regular occurence of oestrus cycle in Control rats.


4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12
Days


4
3
2
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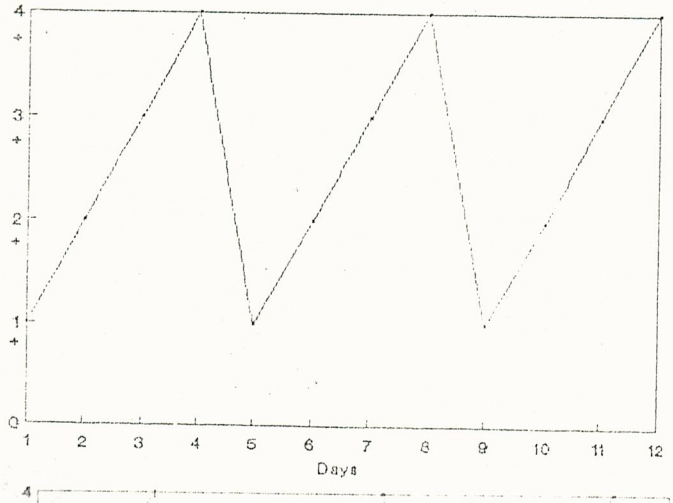
0
1 2 3 4 5 6 7 8 9 10 11 12
Days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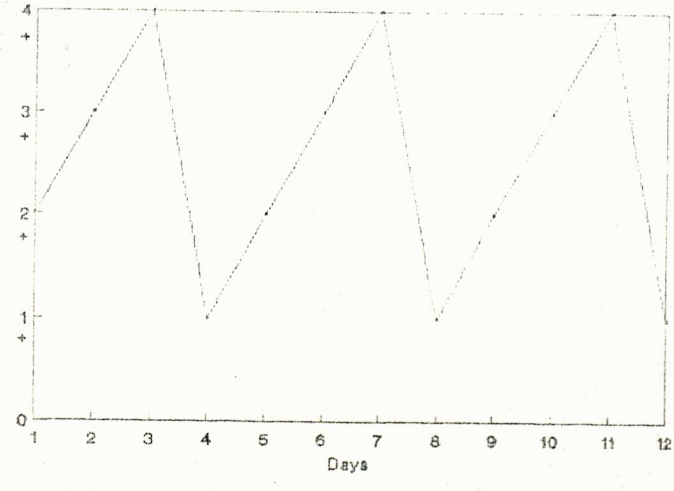
Days


4
3
2
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1 2 3 4 5 6 7 8 9 10 11 12
Days

FREQUENCY OF SPECIFIC STAGES OF OESTRUS CYCLE OF THE CONTROL AND EXPERIMENTAL RATS

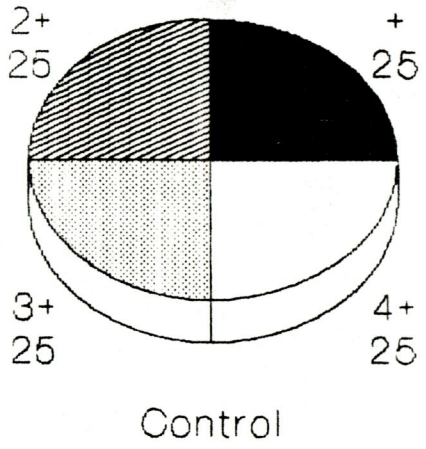


Fig 18 a

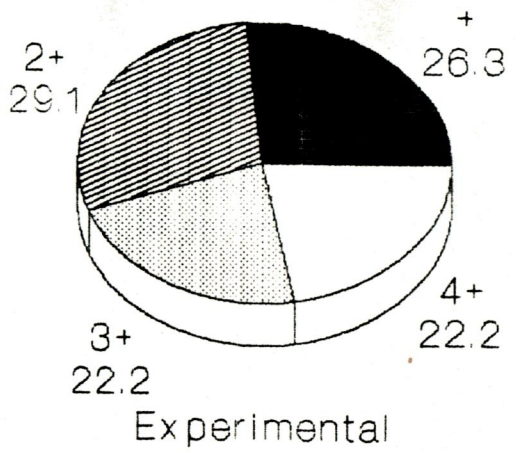


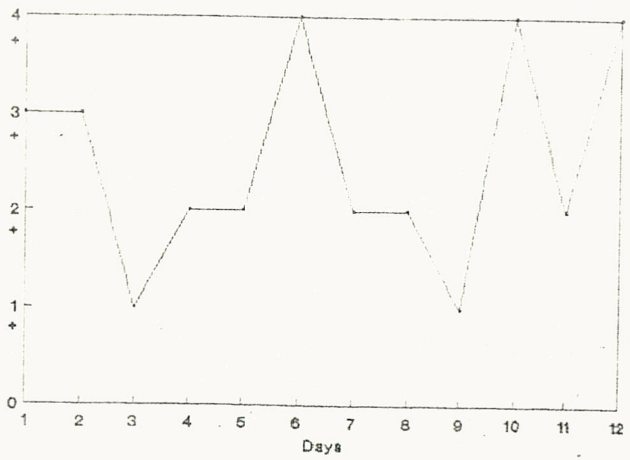
Fig 18 b

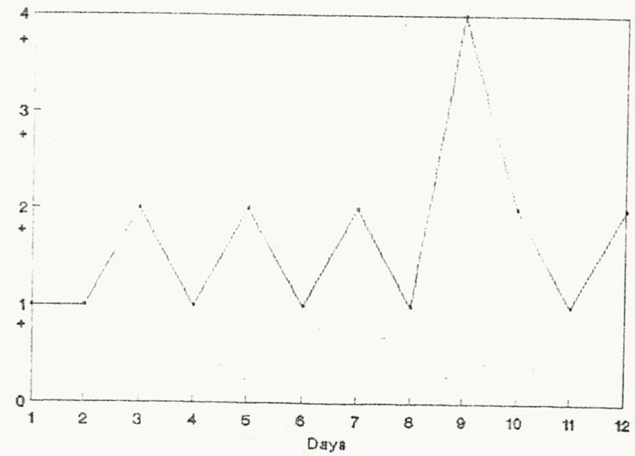
+ - Metoestrus

2+ Dioestrus

3+ Proestrus

4+ Destrus


0 1 2 3 4
1 2 3 4 5 6 7 8 9 10 11 12
Days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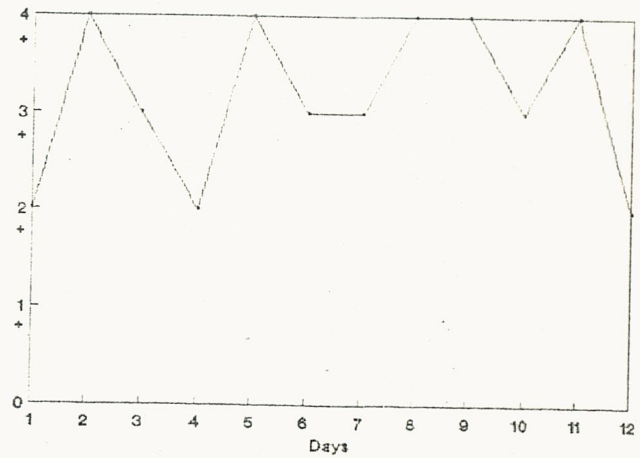
Days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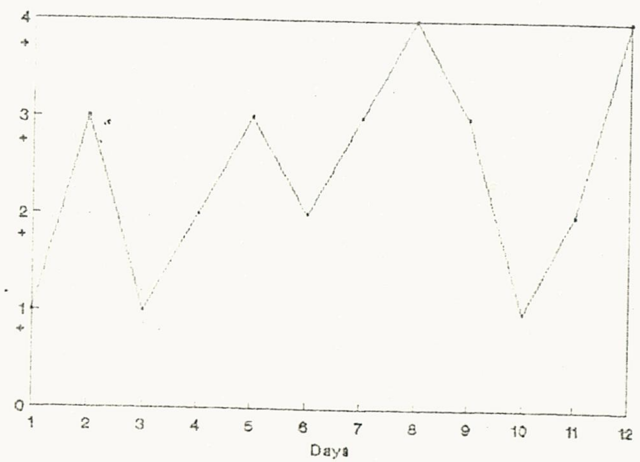
Days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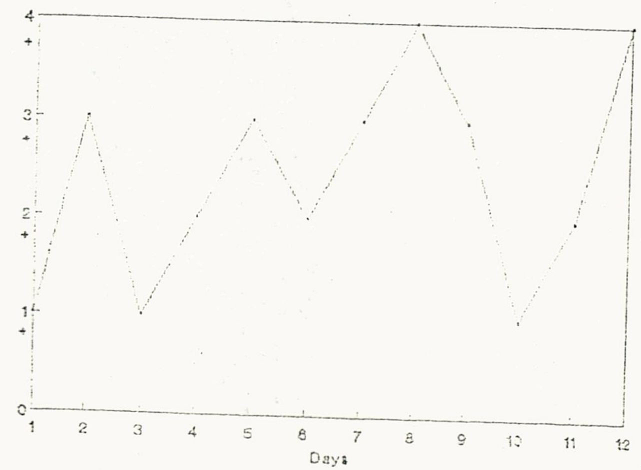
1 2 3 4 5 6 7 8 9 10 11 12
Days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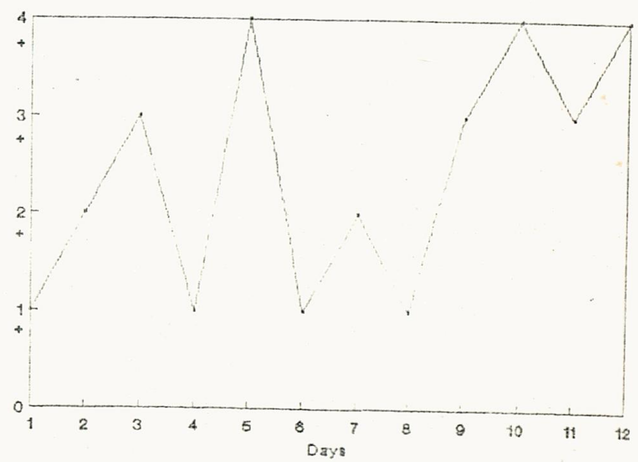
1 2 3 4 5 6 7 8 9 10 11 12
Days


0 1 2 3 4
1 2 3 4 5 6 7 8 9 10 11 12
Days

FOOD INTAKE (g)/BODY WEIGHT/DAY OF THE CONTROL AND EXPERIMENTAL RATS

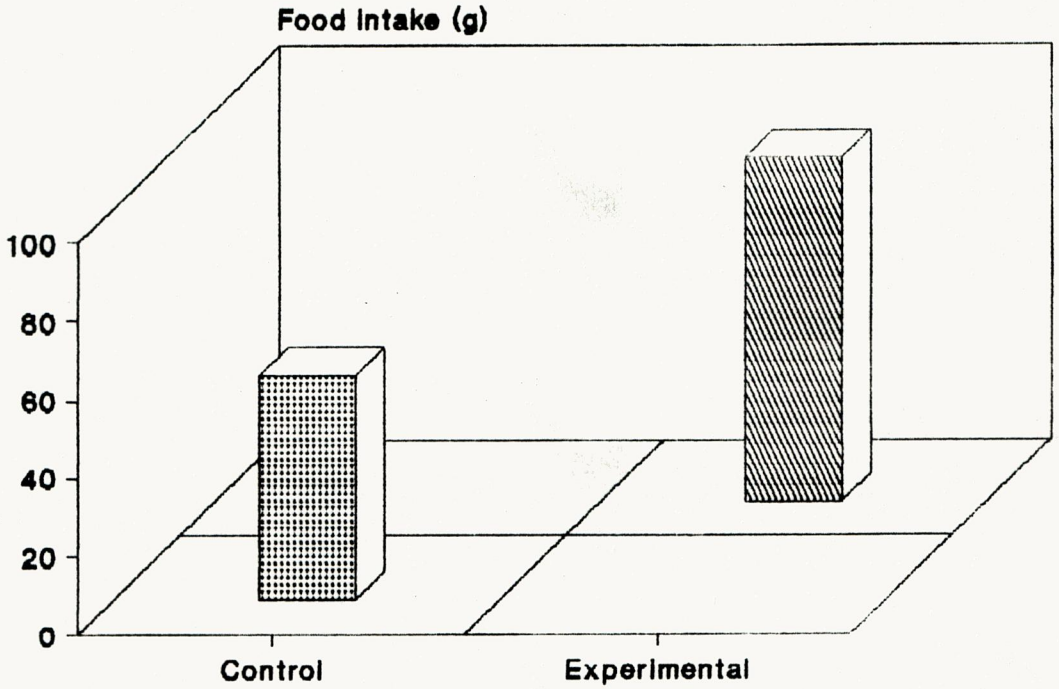


Fig 25

BODY WEIGHT (g) OF THE CONTROL AND EXPERIMENTAL RATS

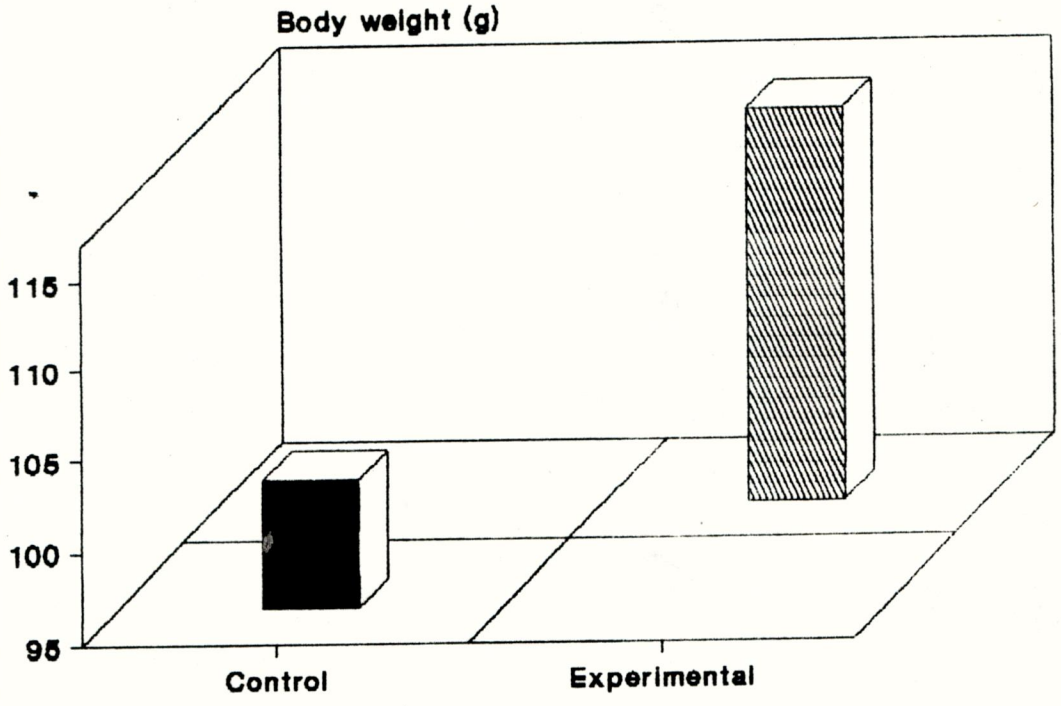


Fig 26

SERUM CHOLESTEROL/100 ml OF THE SAMPLE OF CONTROL AND EXPERIMENTAL RATS

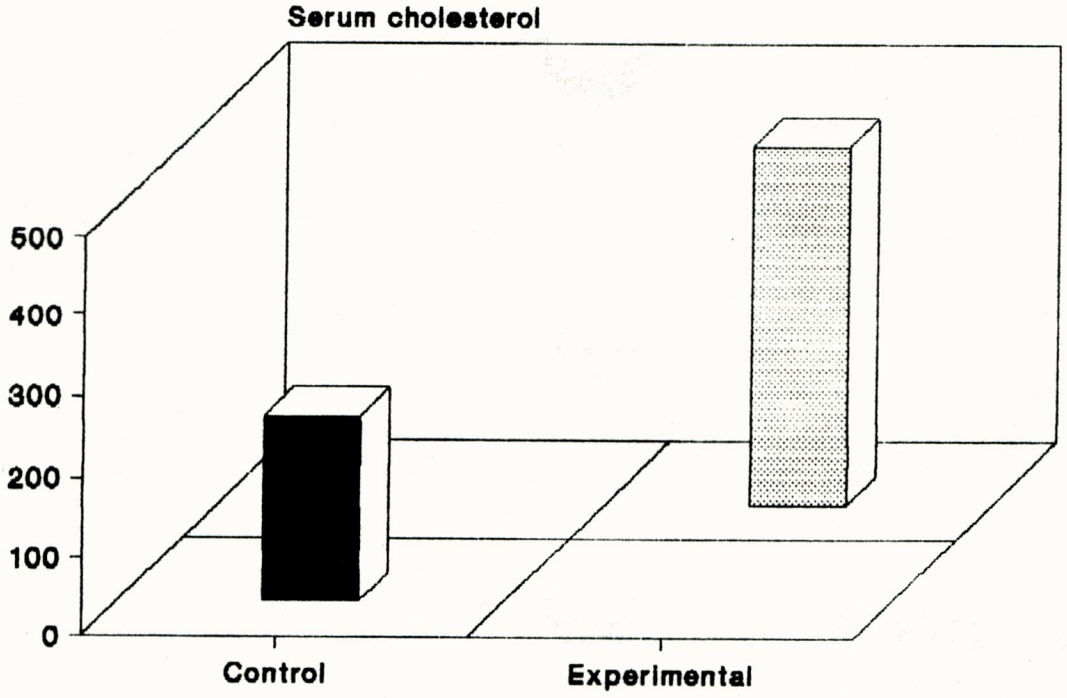


Fig 27

Figure - 20

20 a - Typical normal metoestrus smear.

Observe the scattered cornified cells and epithelial cellls.

20 b - Metoestrus smear of experimental rat.

Note the clustering of epithelial cells and cornified cells.

E - epithelial cells

C - Cornified cells.

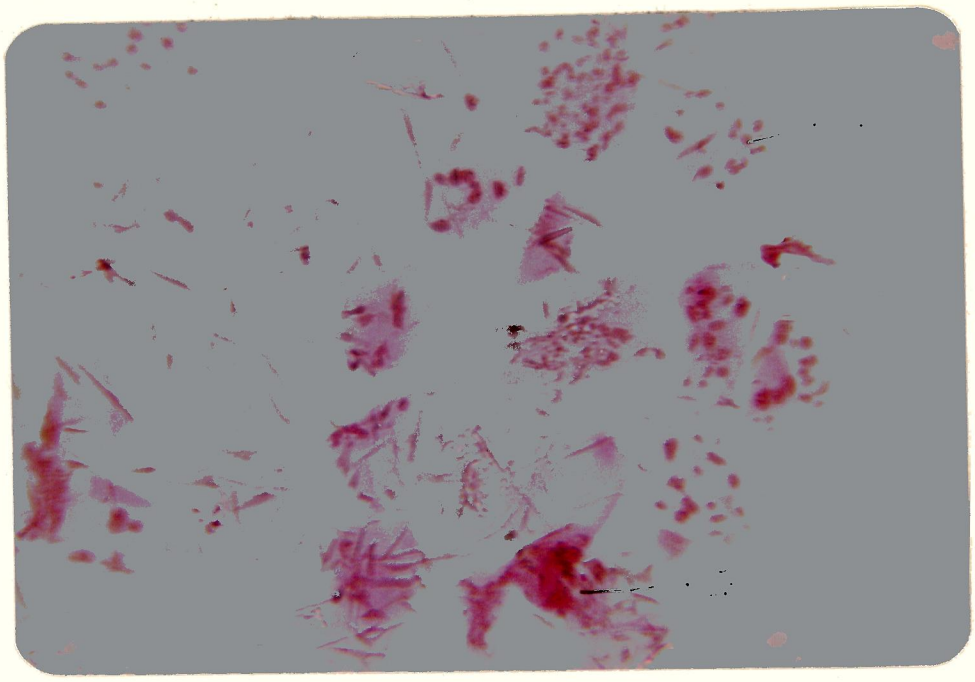


Fig - 20a

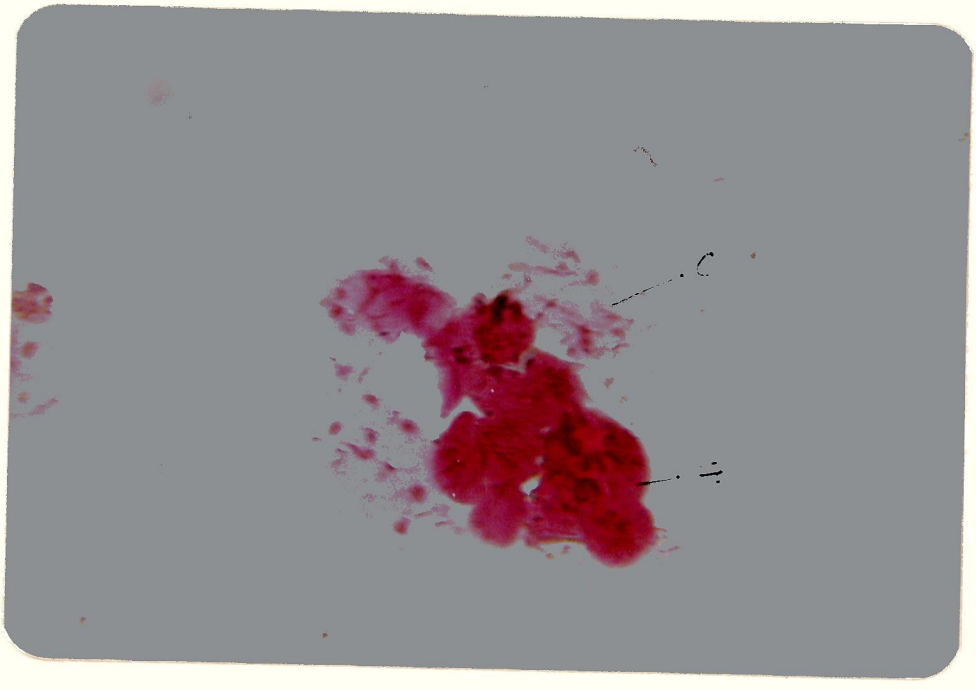


Fig - 20b

Figure - 21

21 a - Dioestrus smear of control rat.

Observrve the Dominance of leuccocytes with few epithelial cells.

21 b - Dioestrus smear of experimental rat.

Clustering of leucocytes with few epithelial cells. Note the occurrence of more leucocytes compared to control.

L - leucocytes

E - epithelial

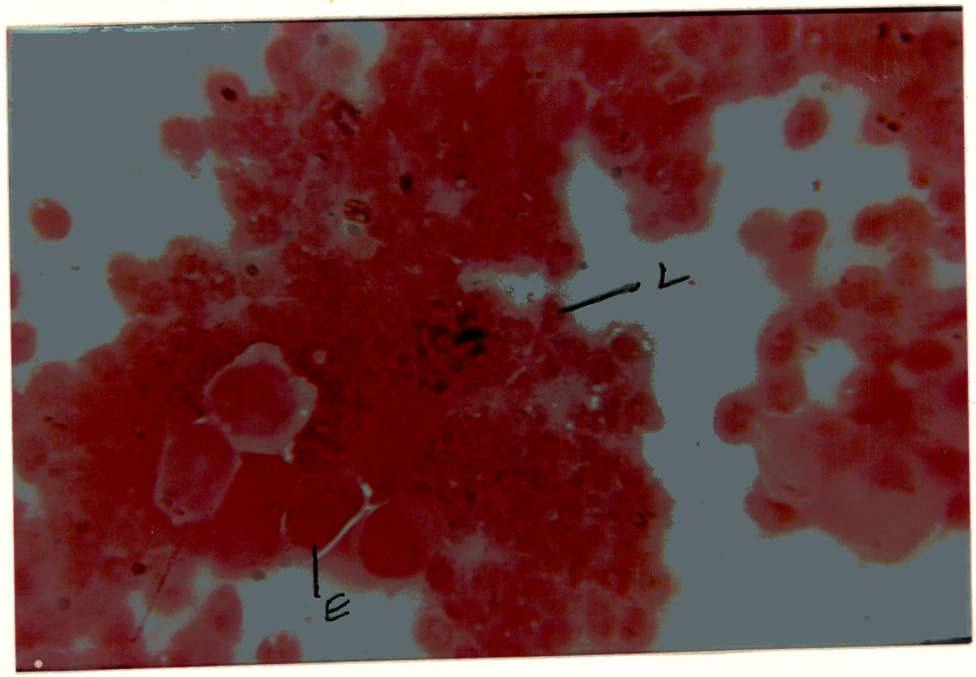


Fig - 21a

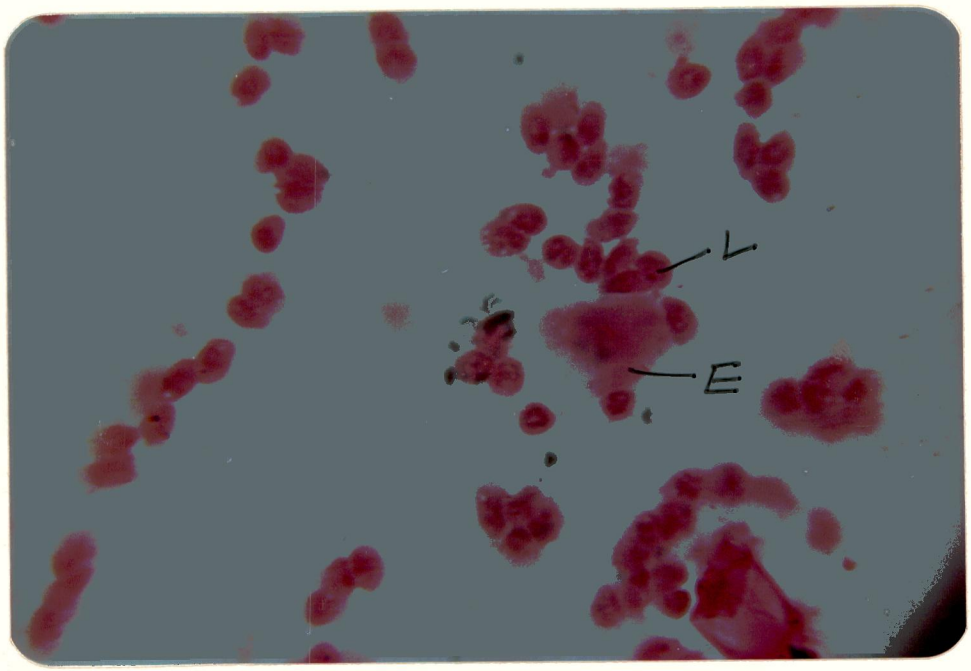


Fig - 21b

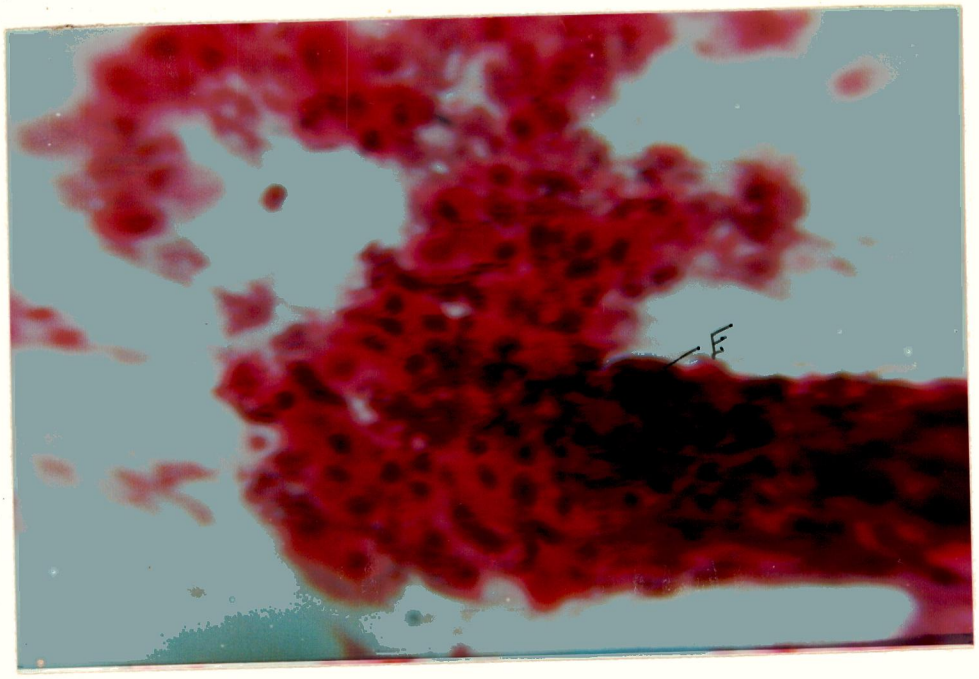


Fig - 22a

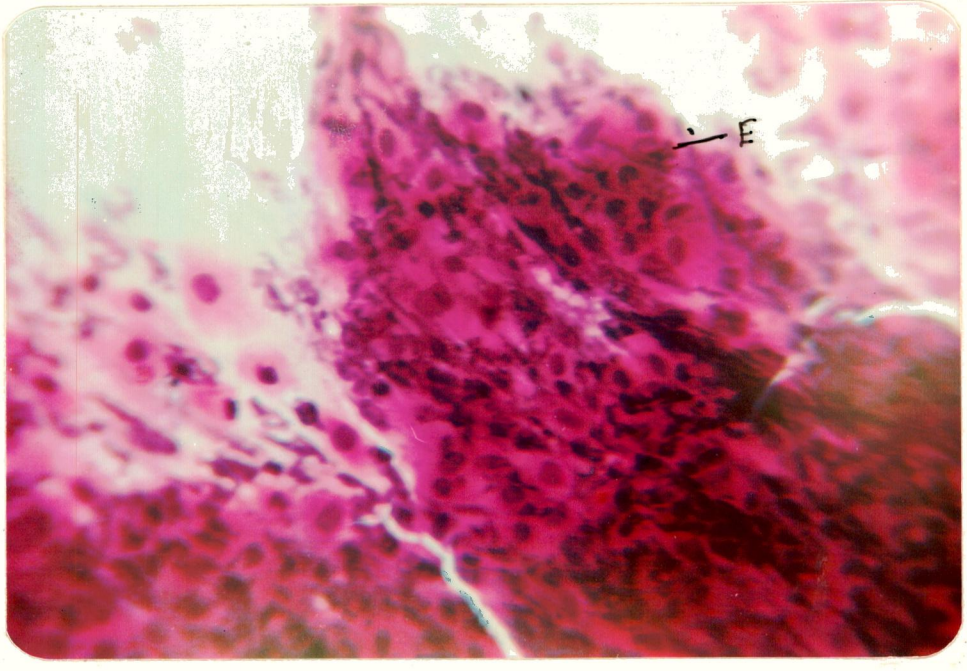


Fig - 22b

Figure - 23

23 a -- Typical oestrus smear of control rat.

Note the occurrence of high percentage of flat, anuclear cells, with the appearance of a potato chip.

23 b - Oestrus smear of experimental rat.

Note the scattering of the anuclear corfied cells. The cornified cells are less in number compared control.

C - Cornified cells.

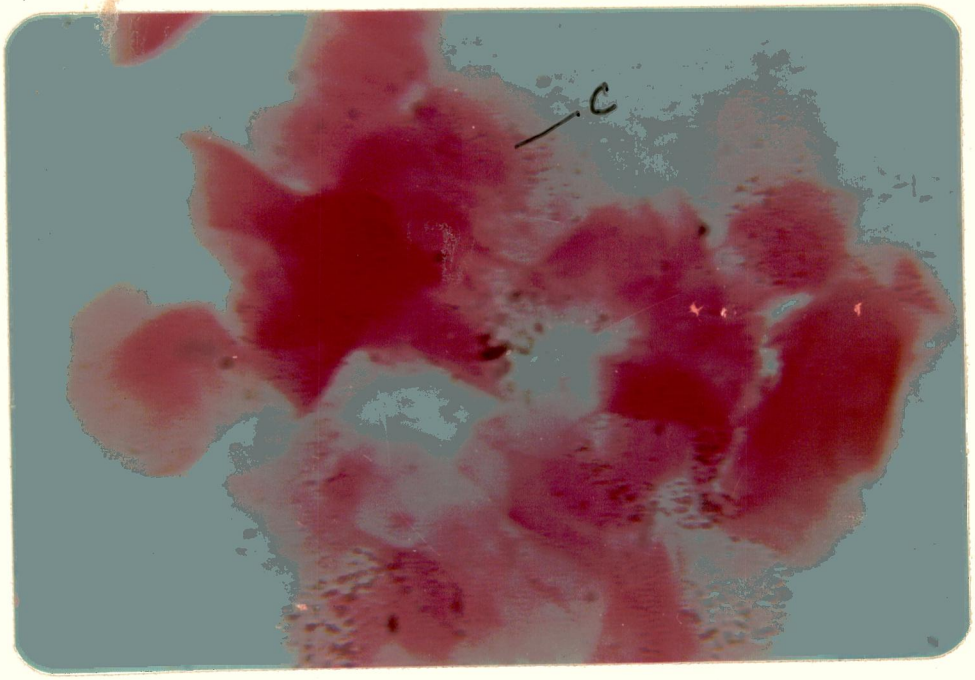


Fig - 23a

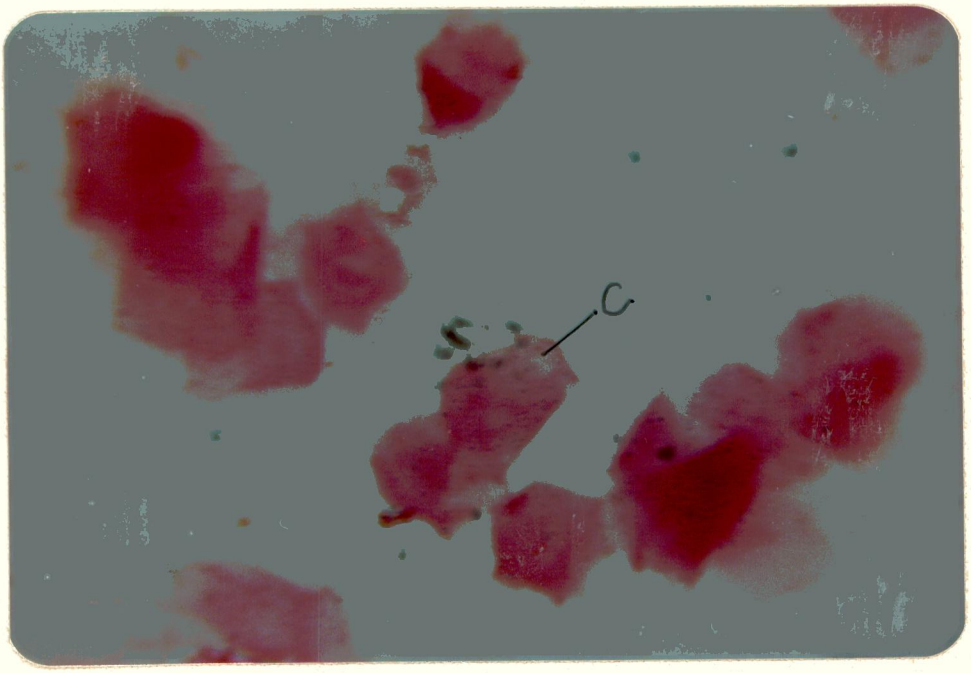
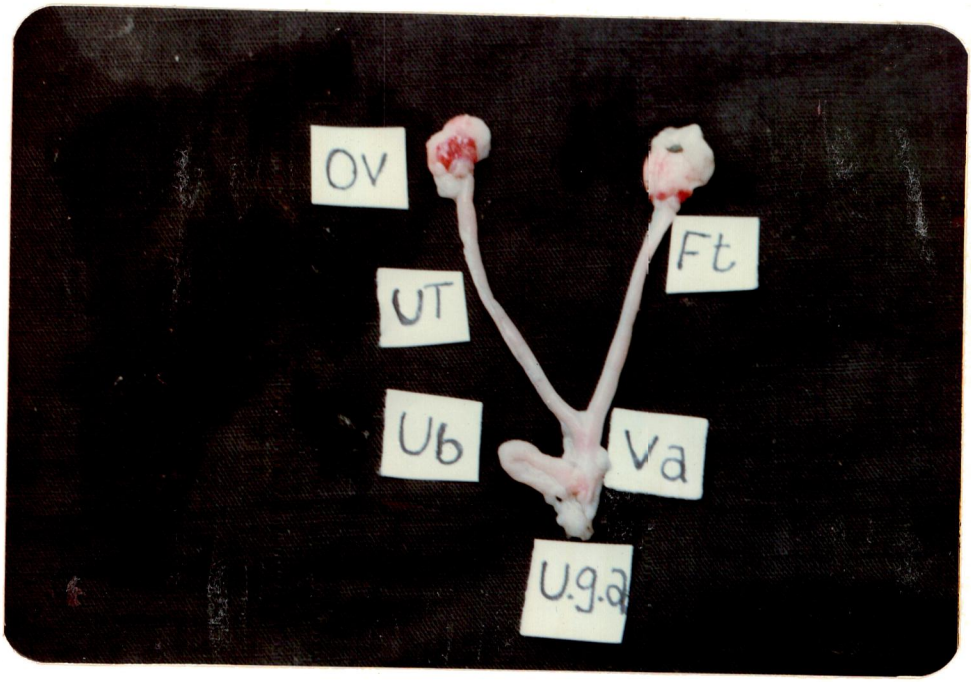


Fig - 23b

Figure - 24

24 a - Genetalia of the conntrol rats fed adlibitum and kept under normal L-D conditions. The uteri and ovaries are of normal in size. The ovaries are surrounded by fatty tissues giving a healthy look.

24 b - Genetalia of the experimental rat after giving injection of progesterone. The uterus shows slightly enlarged state. Compared to control and also the occurrence of more fatty tissues around the ovary.



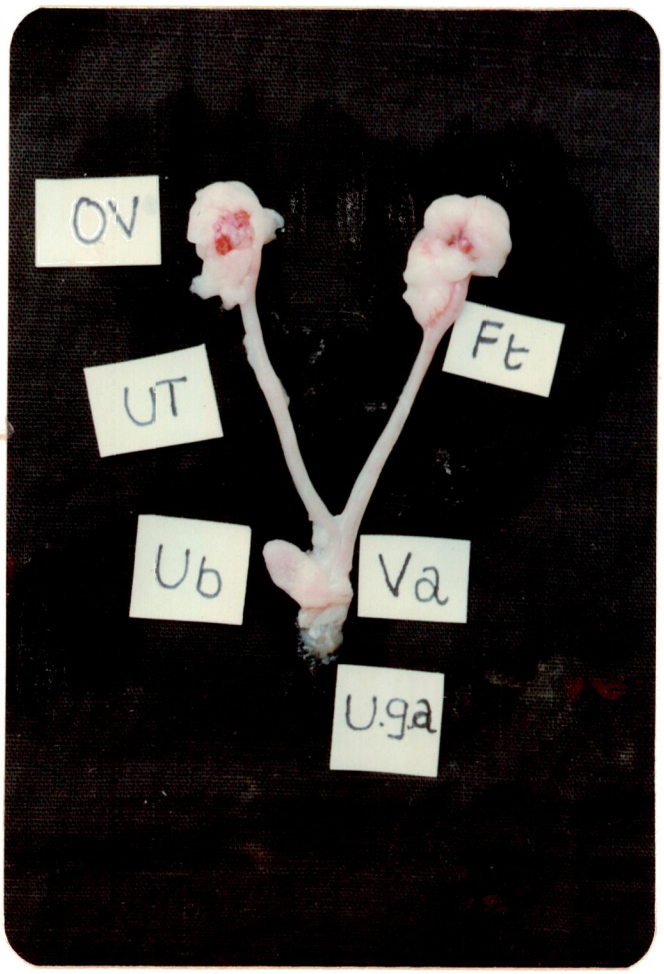


Fig - 24

Disscussion

V. DISCUSSIONS

V. 1. Body temperature rhythm

The present investigations reveal that there is a well defined rhythm for the body temperature that occur in a cyclic pattern in the female rats i.e the temperature increases gradually and regularly for every 24 hours from 07.00 to 01.00 hours. After that there is a slight increase in body temperature which reaches the peak at 23.00 and 01.00 hours. Again it decline between 07.00 and 09.00 hours. This cyclic rhythmic pattern of can be infleunced by several factors. The present study reveals clearly that the hormone is one of the factors among them. The results of the work show an increase and a higher amplitude in the body temperature of experimental female rats compared to that of control. Here also a well defined rhythm exists but the body temperature is found to be increased. The female experimental rats shows an enhanced peak of 102.9 ± 0.1°F when compared to that of control female rats where it is only 100.8 ± 0.1°F. Thus there is difference of about 2.1°F in the peak temperature between the control and experimental female rats. This temperature rise/correlated with an increase of the progesterone level which is obvious from the following discussion .

According to Uchida et al (1969), prgesterone concentration is higher than 20 - OH - P at dioestrus and lower than 20 - OH - P at proestrus and oestrus. Perhaps the higher level of concentration of progesterone and the administration of 0.5 ml of progesterone may be responsible for the difference of peak temperature of 2.1°F at the dioestrus stage as shown in the present study.

The Body temperature is found to be high only during dioestrus when corpus luteum secretesprogesterone and it declines at metoestrus when corpus luteum degenerates.

If it is so, the question naturally arises as whether there is any evidence to show the relationship between the ovarian hormones and the body temperature. This seems to be the case as seen from the earlier works (Indira & Gopal, 1980). This higher body temperature by progesterone seems to be due it's temperature raising effect (Rubenstein and Lindstey, 1937). This was further confirmed by palmer and Devillers (1939), Gareia and Rock (1958). According to Palmer et al the decline in body temperature at metoestrus indicates the degeneration of curpus luteum which causes decrease in body temperature. Progesterone is found to be thermogenic and causes an increase in basal metabolic rate in women (Barton et al,

1945). Daily administration of progesterone (1-10 mg) is shown to increase the rectal temperature in rats by 0.3 - 0.5° c (Freeman etal 1970).

According to Teledgy (1963), there is an increased secretion of progesterone at dioestrus (0.99-3.87 mg/100 mg ovarian tissue) and proestrus and a decreased secretion atoestrus and dioestrus (0.57-0.93 mg/100 mg). This higher concetration of progesterone at dioestrus suggests that the temperature elevation at dioestrus is due to the action of progesterone. So from this it is clear that the body temperature has a relationship with progesterone and the present work suggests that administration of progesterone causes an increase in body temperature compared to control rats i.e. in the body when the progesterone level exceeds, it shown it's effect by increasing the body temperature.

One of the most interesting observation in the present study is that the temperature raising effect of progesterone is dose dependent i.e. here 0.5 ml of the

hormone was used which consisted of 12.5 mg of progesterone. This effect could be observed only for 12 days. After 12 days when the temperature was observed on the 13th day it was found to be normal. Further more after 12 days the vaginal cytology was observed for 4 more days (one cycle) to confirm this. The cycle was found to occur regularly. So these results prove that the temperature raising effect of progesterone is dose dependent. So the injected dose could manifest it's effect only for 12 days. This is reflected in noting the effect only for 12 days. This is reflected in noting the morphology of the reproductive system (Fig.270, 276). The genetalia when observed after 3 days of injection, shows an enlarged uteri and bulged ovaries which reveals that the hormonal interplay. The uterus change must be due to the effect of the hormone on food intake and fat deposition.

V. 2. Effect of Progesterone on Oestrus Rhythm

Thermogenesis in the rat isi based on several physiological and biological, and is more complicated in the female rat because of the occurence of oestrus cycle and hormonal action (Indira & Gopal, 1979). The

present study reveals that the body temperature reaches its peak during dioestrus stage in control rats. This is due to the action of progesterone (Indira and Gopal, 1980). In experimental rats also, the body temperature reaches the peak only at dioestrus, and there is a overall elevation of body temperature when compared to that of control. This must be due to the influence of progesterone. The maxima and minima of the body temperature during the different phases of oestrus cycle is also found to be high in experimental rats compared to that of control rats. Rats ovulate spentaneously every 4 days. The 4^{th} day is called oestrus stage (4+) during which the vaginal cytology reveals cornified cells The cornification is due to the result of the internal event that had occcurred in the ovary. The stage preceeding this is called proestrus (3+). The stage preceeding proestrus is called dioestrus (2+) which occurs for 48 hours, during which the ovary grows. The preceeding phase is called metoestrus (+) which occurs for 6 hours. If the egg is not fertilized, the corpus luteum is formed which produces its own characteristic hormone - pregesterone. The start of formation of corpus luteum at metoestrus shows a low progesterone

and secretes progesterone which attains its peak level at dioestrus. This must be the reason for the occurrence of the peak body temperature which coincides with dioestrus stage. In the present study, the hormone has been injected which must have increased the level of pregesterone in the blood and this in turn has elevated not only the overall amplitude of the body temperature but also has increase the peak temperature at dioestrus.

In sterile old rats, the dioestrus peak of body temperature is not to be seen which otherwise occurs in fertile rats. This is due to the lack of progesterone, the rise of which is indicated a rise in the fetile rats (Kistner, 1972). So this concept also supports that progesterone has relationship with body temperature and oestrus rhythm and thus proves as a causative factor for the overal elevation of body temperature in experimental rats.

Unlike control rats, the ovestrus cyclke exhibit modulations with a slight variations in the frequencies of various stages of oestrus cycle. The

dioestrus (29.1%) and metoestrus (26.3%) was occuring mmore frequently than proestrus (22.2%) and oestrus (22.2%). This is because in control rats, the dioestrus and metoestrus stages are referred to as "progestational phases" during which there will be increased levels of progesterone in the circulating blood (Prasad and Sinha, 1985). So naturally, when progesterone is injected, in experimental rats, this level gets altered in the blood and hence the variation of frequency of occurrence among the four stages of oestrus cycle. It was shown by Sirois and Fortune (1990) that in progesterone injected animals, it prevented the return to the oestrus at the normal time and so the cycle length is increased. This is due to the prolactin which has an effect on the secretion of progesterone by corpus luteum (Sanchez - Criado and Vander School, 1991).

V. 3. Metabolic Effect of Progesterone

The current experiment prove that the food in take and reproductive cycle are additive factors that

influences the body temperature. In this section the influence of the food in take is discussed.

With regard to the influence of food in take on the body temperature in the female rats earlier investigation have shown that adlibitum fed rats exhibit, a set pattern of body temperature when compared to restricted feeding schedule (Indira and Gopal, 1992). So according to Indira and Gopal, food in take and it's metabolism is one of the additive factors for the body temperature.

Since the results of the present work also shows an increase in the body temperature and body weight due to food in take the question naturally arises whether pregesterone has any role to play in the metabolism of the animal. Indeed, it appears that the thermogenic influence of progesterone is partially due to its mmetabolic influence. The work of katz and kappas (1967) has shown that the thermogenic influence of progesterone is due to it's metabolite 3 , 20 , pregnandiol. In rodents it has been observed that the appetite stimulating influence of progesterone maintains positive

energy balance which in turn effect a very significant increase in body weight (Harvey etal, 1967). It is not known how exactly the progesterone effect it's metabolic influence to elevate the body temperature. However progesterone enhances the protein catabolism (Izoo etal, 1961). Thus progesterone may be influential in stimulating a greater food in take, which inturn might result in more fat deposition.

In the present work, this has been confirmed by nothing the morphology of genetal system where in the the uterus has shown an enlarged state compared to control. This might be due to more fat deposition. According to Everetts (1947) prolaction which produces cholesterol - a precursor of progesteroe gets stored up in corpus luteum . so this cholesterol by lutenising can be converted to progesterone. So it can be concluded that the stimulating influence of progesterone on the food in take and fat deposition may increase the basal metabolic rate which causes an increase in body temperature.

V. 4. Effect of Progesterone on Serum Cholesterol

Results of the present work reveals that progesterone causes an increase in the serum cholesterol compared to that of the control rats. This might be due to the effect of progesterone on increased food intake and more fat deposition. Rats administered with progesterone showed an increased hepatic cholesterogenesis, which is evidenced by increased activity of HMG-COA reductase and increase in corporation of labelled a cetate into liver cholesterol. Activity of the plasma LCAT which causes the transport of cholesterol from the tissues was found to be decreased (Sissan and Leelamma, 1991). This might be the reason for increased level of serum cholestrol in the female temperature rats when adiministered with progesterone.

Summary and Conclusion

VI SUMMARY AND CONCLUSIONS

SUMMARY

Body temperature in the rat is based on various physiological and biological facts, and is more complicated in female rats, because of the occurrence of oestrus cycle and hormonal action. Female rats that exhibit regular cycles of oestrus were screened and maintained under standardised conditions. They were fed adlibitum and exposed to normal light-dark (12 L : 12 D) conditions. Body temperature were observed for every two hours continuously around the clock for 12 days. Simultaneously vaginal Cytology was also observed thro' smear preparation. The summary of this work is as follows

VI. 1 The body temperature rhythm in control and experimental rats

(1) There is a rhythmic pattern of body temperature found to occur in female acbino rats both in control and experimental rats. In control the peak (100.8 ± °F) is found to occur at 01.00 hours, where as in experimental (102.9 ± °F) it occurs at 03.00 hours. (Table-I, Table-III, Fig-3).

(2). The overall average of the body temperature in experimental rats (100.6 ± 0.1 °F) is significantly higher than the control rats (98.8 ± 0. 1 °F) (Table-V, Fig- 7).

(3). The maxima of the body temperature when compared is significantly higher in experimental rats (102.9 ± 0.1 °F) than that of the control, where it is only 101..1 ± 0.1 °F (Table - VI, Fig-8).

(4). The minima of the body temperature also shows a significant value in experimental rats, with a value of 99.1 ± 0.1 °F compared to that of control. (97.3 ± 0.1 °F) (Table - VII, Fig- 9).

VI. 2. Body temperature and oestrus cycle in control and experimental rats.

(5). Comparison of the pattern of the body temperature recordings at every specific stages of oestrus cycle shows a higher peak of 99.4 ± 0.2 °F at dioestrus stage in control rats. (Table - IX, Fig-10).

(6). In experimental rats also comparison of the body temperature at every specific stages of the oestrus cycle reveal a peak of 101.2 ± 0.4 °F at dioestrus (Table - XI, Fig- 13) which is higher than the control rats (99.4 ± 0.2 °F).

(7). The maxima of the body temperature recordings of control rats reveals a maxima of 101.8 ± 0.3 °F at dioestrus, 101.6 ± 0.3 °F at proestrus, 100.9 ± 0.1 °F at oestrul and 100.7 ± 0.2 °F during metoestrus (Table-XII, Fig-12a).

(8). In experimental rats the maxima of the body temperature readings reveals a maxima of 103..5 ± 0.1 °F at dioestrus, and 103 ± 0.2 °F at proestrus, 102.3 ± 0.1 °F at oestrus and 102.2 ± 0 °F at metoestrus (Table-XII, Fig- 16a). But in experimental rats the values are highly significaant coompared to that of the control.

(9). The minima of the body temperature recordings of the control rats reveals a minima of 98.0 ± 0.1 °F at

dioestrus, and 97.6 ± 0.2 °F at proestrus, 97.3 ± 0.2 °F durring oestrus and 97.1 ± 0.1 °F at metoestrus (Table XIII, Fig- 12b).

(10). In experimental rats also, the minima is found to be higher at dioestrus (99.5 ± 0.1 °F) compared to other phases. But the values are significantly higher compared to that of control (Table - XIII, Fig-16b).

(11). The frequency of the oestrus phases is found to be regular in control rats (25% each) unlike the experimental rats, where the frequency of the different phases of oestrus cycle gets modulated, as a result the cycle occurs with an unequal frequency of 29.1 % dioestrus, 26.3 % metoestrus, 22.2 % each of proestrus and oestrus (Table - XIV, Fig- 18a,b). This is reflected in the vaginal cytology and in the morphology of the reproduction system of both control and experimental rats (Fig 24).

VI. 3. Food intake and body weight of control and experimental rats

(12). The experimental rats shows a significant increase in food intake (87.6 ± 2.7g/body weight/day). (Table - XV, Fig- 25). So correspondingly the body weight also shows a significant rise in experimental rats (116.8 ± 2.4g) compared to that of control rats (101.9 ± 0.6g) (Table - XVI, Fig-26).

VI. 4. Serum cholesterol in control and experimental rats

(13). The serum cholesterol when compared to control and experimental rats shows a significant increase in experimental rats with a value of 453.0 ± 0.5/100 ml of the sample, compared to that of the control where it is only 231.4 ± 0.6/100 ml of the sample.(Fig - 27)

CONCLUSION

The influence of progesterone on body temperature and reproductive cycle and the relationship amongst them reveals a greater understanding of the basal body temperature patterns in the control and experimental female rats. It helps as a confirmatory test to bring out the influence of progesterone on the modulation of the four phases of reproductive cycle besides pinpointing the preceedent time of ovluation and progesterone secretion by the ovary. Thus the present work throws some light on the rhythm method of birth control.

The significance of the present work is manifold, though the clinical and physiological implications appear to be some what oestric. Based on the significance of the present work, it is suggested that this study can be coupled with other parameters like light/dark (LD) schedule, different diets, restricted feeding schedule and other ovarian hormones so as to evolve a newer dimensions on the functioning of endocrine system in the physiology of mammals.

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