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THE EFFECT OF DIETARY PROTEIN AND LIGHT CYCLE ON SEXUAL MATURATION: AN INTERACTION STUDY

by

Lorraine Heidecker

A dissertation submitted to the Graduate Faculty in Anthropology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

THE EFFECT OF DIETARY PROTEIN AND LIGHT CYCLE ON SEXUAL MATURATION: AN INTERACTION STUDY

by

Lorraine Heidecker

Adviser: Professor Edward E. Hunt, Jr.

Field data and experimental evidence published in the recent literature indicate that the amout of dietary protein received by a young animal influences its growth rate. This is especially true when the diet contains lower amounts of protein than the animal requires for growth, resulting in retardation of growth and maturation.

Similarly, it has been discovered that the amount of light stimulus received by an animal in a 24-hour period also influences the rate at which an animal will mature, with increased light stimulus appearing to accelerate maturation.

The experiment described in this study was designed to test the amount of interaction between these two variables in influencing the growth and sexual maturation of female mice.

Pregnant, near-term, mice were placed in boxes whose light was controlled to receive 0, 6, 12, 18, 24, or "natural" hours of light per day. Their litters were sexed at birth and

the females were retained. At twenty-one days, the females were weaned and weighed. Within each box the weanlings were assigned to diet groups containing either 0%, 6%, 12%, 24%, or 50% protein. Each animal was examined daily for vaginal opening, at which time the animal was weighed, and daily vaginal smears were taken for 14 to 28 days. The animals were sacrificed at 51 days, weighed, and measured. Several organs were dissected out and weighed.

The results showed that both dietary protein and light influenced the growth and sexual maturation of the animals and, in most of the data sets observed, there was interaction between the two variables. Dietary protein had the strongest influence over growth and maturation rate but its effects were mediated by the number of hours of light per day received by the animals.

The results suggest that the action of these two environmental variables on the organism follows a pathway that will not allow the effects of both to be cumulative. This indicates that both may affect a common site which is in some way limited in its ability to influence gonadal maturation.

The fact that these two environmental variables interfere with each other in their effects on the organism gives a possible explanation for the lack of any clearly definable clines in menarcheal age among human populations. It is suggested that the data gathered on human populations in the

field cannot be meaningfully analyzed until we understand more completely the effects of all environmental variables on maturation rate and the interactive effect these variables may have on each other.

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INTRODUCTION

In most of the world's populations, menarche, or the onset of the menses, marks the transition from child to woman. It is generally accepted as an outward sign that the female is now ready for childbearing and is in many cultures accompanied by ritual and ceremony.

Due to its dramatic nature, this phenomenon has undergone considerable study. When the data are analyzed, the result is largely a feeling of bewilderment. Human populations vary considerably in the mean age at which a girl achieves menarche, and it is not possible to arrange them into a neat pattern no matter how the data are manipulated.

In recent years it has become clear to investigators that many environmental variables may affect the rate of sexual maturation in the female. The effect of such variables as diet (Kralj-Cercek, 1956 and Tanner, 1965), climate (Barnett and Coleman, 1959), number of hours of sun or artificial light (Browman, 1937; Zacharias and Wurtman, 1964), and even the psychological "set" of the individual (Chattapahdyay and Khullar, 1969) have been investigated and set forth as causal in influencing the age of menarche on both an individual and a populational basis.

If all of these factors, varying almost infinitely around the globe, can be said to influence the onset of the

menses, it is small wonder that a highly confusing picture of varying menarcheal ages results. Yet another aspect of the problem that few workers have investigated is the degree of interaction between these factors. Which affects the organism most strongly? which least strongly? Can the effect of one influence or even negate the effect of another?

It is the intent of this study to begin work on this last problem; to investigate, under controlled laboratory conditions, the interaction between the amount of dietary protein and the number of hours of light per day on sexual maturation in the female mouse.

THE EFFECT OF PROTEIN ON MATURATION

All living tissue is composed largely of protein, composed in most mammals of 22 amino acids. In order for a young animal to grow and mature, its daily diet must provide a sufficient amount of protein to supply the demands of newly forming tissue in a metabolically active body in addition to sufficient calories for the body's energy needs. In humans, a certain proportion of the protein must contain the eight "essential" amino acids, isoleucine, leucine, lysine, methionine, phynylalinine, threonine, tryptophan, and valine. These are the amino acids that the body either cannot manufacture at all or manufactures in quantities insufficient for the body's needs. In addition, a young child must receive a certain amount of arginine and histadine although these are not required by the adult (Chaney and Ross, 1971).

Ingested protein is digested and hydrolyzed and then absorbed through the gut wall into the blood stream. There, it undergoes partial combustion into glycogen and body protein depending on the needs of the body. Normally, a large part of ingested protein is burnt to provide energy. To a great degree, then, the amount of protein needed by the body depends on the total number of calories of other foodstuffs consumed (I. G. Macy, 1946). Protein, as such, cannot be stored by the body. Unused, absorbed nitrogen will be voided

in the urine and unabsorbed protein in the feces (Chaney and Ross, 1971).

Protein content is usually measured by its nitrogen content. Hence, a measurement of ingested and/or excreted protein is usually given in terms of grams of nitrogen. Fecal nitrogen comes from undigested food, cellular debris from the gastrointestinal tract, secretions of the intestine and the bodies of symbiotic bacteria in the intestine (I. G. Macy, 1946). Usually approximately 10 per cent of the total nitrogen ingested is excreted as unabsorbed in the feces, but this is not a constant and there is currently no way of determining what percentage of fecal nitrogen is unabsorbed, ingested nitrogen (Hegsted, 1964; I. G. Macy, 1946).

A certain proportion of nitrogen is also excreted in perspiration. Experiments have shown that this can amount to a considerable quantity of nitrogen which is not compensated for by a decrease in urine and feces loss (Consolazio et al., 1963). The techniques for establishing this loss, however, are complex and most workers have disregarded this amount as minimal in their calculations (Food and Nutrition Bureau, 1959).

If the amount of protein, or of any one of the essential amino acids supplied, is insufficient for the needs of the child, a retardation of growth can result in the skeleton and all the body organs. A similar situation can result if the total caloric intake of the child is insufficient. In such a case the protein, as well as the fats and carbohydrates

in the diet will be burned to meet the body's energy requirements. It will not matter in such a case that the protein ingested is of high quality as it will not be used to replenish the supply of free amino acids in the constituents of the body cells (Cussworth et al., 1959; Sukhatma, 1970).

In the growing child the normal condition is one of nitrogen retention rather than equilibrium.[1] The amount of protein needed for this condition depends on the other dietary constituents. As indicated above, the energy requirements of the body have precedence and protein will be used for this should fats or carbohydrates be unavailable.

Observations made on human populations indicate that, during conditions of starvation or severe malnutrition, children appear to be retarded in skeletal growth, motor skills, and neural development. Body weight is also affected; however, it is noted that both body weight and height differences can be "made up" if the conditions of starvation do not last too long. Recovery on an adequate diet can be rapid (Chase and Martin, 1970; Keys et al., 1950). Under long-term food restriction, such as was found in the United States during the depression or in Europe during World War I, the result appears to be a depressed adult height for individuals raised under starvation conditions (Keys et al., 1950).

Under such conditions it is difficult to ascertain what the effects of restriction of protein alone or the

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[1] A condition in which the nitrogen intake is equal to the nitrogen excreted (Food and Nut. Bureau, 1959).

feeding of incomplete protein can do to the development of a child.[2] It is only when the protein lack becomes severe and the child develops Kwashiorkor or other protein deficiency syndromes that our information becomes more complete. Hence, again we have a difficulty, as a child with Kwashiorkor is usually also suffering from severe caloric malnutrition. This is common in parts of the world where a large part of the dietary protein is obtained from a vegetable source. When a child is weaned his gut cannot yet efficiently process the food that his parents eat and he cannot extract from it that marginally sufficient amount of nutritional material it provides (Food and Nut. Bureau, 1959; Keys et al., 1950).

Experimental data, largely obtained from rats, are more precise. When an animal is raised on a diet deficient in protein, a slowing down or stoppage of longitudinal growth will occur and the animal may lose weight (Food and Nut. Bureau, 1959). In young animals, a low protein diet will also result in a drop in the food intake and caution must be taken to separate out the effects of protein deficiency from caloric insufficiency.

Experiments on pigs fed protein-deficient diets showed retarded longitudinal long bone growth with X-rays showing structural changes in cartilage and bony trabeculae of the long bone (Chaney and Ross, 1971). The formation of intramembranous bone as well as endochondral bone is also slowed

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[2]Protein lacking one or more of the eight essential amino acids.

or stopped and the entire skeleton becomes rarefied (Food and Nut. Bureau, 1959).

If protein is deficient, the hypophysis and thyroid will atrophy (Keys et al., 1958) and the spleen and lymph nodes also will become smaller in size (Fallis, 1958). It has been reported that the adrenal cortex will increase in width (De Costa and Clayton, 1952) although similar result has been reported by Tepperman et al. (1943) for extremely high protein diets of 55 to 70 per cent. In both cases, the increased size of the adrenal cortex may be a reaction to a prolonged stressful situation and not a direct result of amounts of dietary protein. An animal raised on such a low protein diet is less resistant to infections (Chaney and Ross, 1971) and to starvation (Boyd et al., 1970).

The gonads respond to low protein availability by a curious combination of hypertrophy and atrophy. In the adult animal reproductive activity may be reduced or may vanish altogether. The testes are precociously enlarged in the juvenile, and often make up a larger proportion of the body weight than they do in an adequately fed animal. The seminiferous tubules develop, but the interstitial cells do not, and no sperm is produced.

In the ovaries a similar phenomenon is observed, with heavier ovaries in proportion to body weight with active follicles but no evidence of corpora lutea (Widdowson et al., 1964). The time of vaginal canalization is also extended and the oestrous cycle appears to be irregular for a longer

period of time (Nakagawa and Masna, 1971). In addition, vaginal opening, first oestrous, mating behavior, and attainment of full fertility are strung out over a longer time period in experimental than in control groups (Kennedy and Mitra, 1963).

Noting that both human children and young animals on a calorie-poor diet showed delayed growth and later maturation, many workers have assumed that, if a calorically adequate diet of fats and carbohydrates is provided, the more high quality protein that is provided, the faster the young animal will mature and the healthier he will be. Experimental work on this aspect of maturation, however, appears to contradict this assumption.

If the protein intake of a normal infant is raised above that found in human breast milk, so-called "chemical maturation" can be produced (Holt et al., 1966). However, tests made on the red blood cells of infants on high protein diets merely showed a higher than normal concentration of "free" (that is, "unused") amino acids in the cells rather than a higher quantity of manufactured protein (Cussworth et al., 1959). This placed the value of artificially increasing the amount of protein in the diet above that found in human milk in doubt. It may be that the child will mature functionally only at his own rate. This could be a reason why, in comparison with other animals, human breast milk is relatively low in protein (Holt, 1966). The only situation in which elevated protein intake has been found to be

beneficial is in the case of the premature infant, whose protein requirements are higher than those of the full-term child (Syderman et al., 1958).

Experimentally, it has been found that as the protein intake of the weanling rat is accelerated, so is his growth rate up to a maximum level. Increasing protein beyond that level results in a drop of growth rate with a smaller maximal growth achieved. In the casein-fed rat, the optimum level is attained when the protein constituent of the diet falls between 30 and 35 per cent (Holt et al., 1966).

In a series of experiments on the effects of starvation, rats fed a diet of 27 per cent protein were most resistant to later starvation, living an average of 14 days after removal of food, whereas those animals accustomed to an 8 per cent protein diet lived an average of only 7 days (Boyd et al., 1970).

A diet high in protein places considerable load on the kidneys and can produce hypertrophy of that organ. Such a diet increases the risk of dehydration in infants and substantially increases the adults' susceptibility to water withdrawal (Chaney and Ross, 1971). Hypertrophy of the liver can also occur, although this has been interpreted as a response to overfunction (Addis and Walter, 1939). As mentioned above, hypertrophy of the adrenal cortex can also occur.

It is reported (Aschkenazy-Leula and Aschkenazy, 1947) that high protein diets of 80 to 90 per cent can increase the

production of pituitary gonadotropins and can induce prolonged periods of oestrous in the rat.

Information regarding the long-term effects of high protein diets on humans is almost totally lacking. The amount of protein that would lead to maximum growth in the human is a matter of conjecture (Holt et al., 1966). It is to be remembered that the protein requirements of an individual may depend on both genetic and environmental factors (Wohl and Goodhart, 1964) and may vary from individual to individual. In the laboratory, these factors can be controlled to a fine degree but the variability in energy expenditure and diet found in human populations, as well as the genetic variability in the species, suggests we can never do more than approximate the human protein optimum.

In brief, the material presented indicates the effect of protein in adequate, inadequate, and overadequate amounts on the organism is both widespread and far from completely understood.

Even though its precise effects are not clear, however, it is obvious that the amounts of protein supplied to the growing organism can profoundly affect the rate of growth and maturation. Many workers, noting the apparent effects of diet on skeletal age, growth rate, and the onset of adolescence (Tanner, 1962), have concluded that differences in the amounts of dietary protein result in differing rates of sexual maturation (Bjolen and Bentzon, 1968).

EFFECT OF LIGHT ON MATURATION RATE

In recent years it has become clear that the amount and intensity of light received by the young mammal have an effect on its growth rate, and its gonadal maturation rate.

The mechanism that controlled this phenomenon was for many years unclear but work done largely in the last two decades indicates it is the mysterious pineal gland that, under the influence of environmental light rhythms, secretes various hormones that appear to control several aspects of maturation.

The pineal gland is attached by a stalk to the rear of the roof of the diencephalon. In lower vertebrates, it is a saccular structure containing photoreceptors similar in structure to retinal cones (Wurtman, Axelrod, and Kelly, 1968). In mammals it is a solid organ containing cells largely of a secretory nature whose innervation appears to come largely or even exclusively from the sympathetic nervous system (Ariens-Koppers, 1965).

The neural connection between the eyes and the pineal gland has not yet been found. That the influence is neural and not due to light reaching the gland through the skull has been shown by the cessation of light-influenced secretory activity in the pineal after blinding of the animal (Wurtman et al., 1964a). A suggested pathway is diagrammed below.

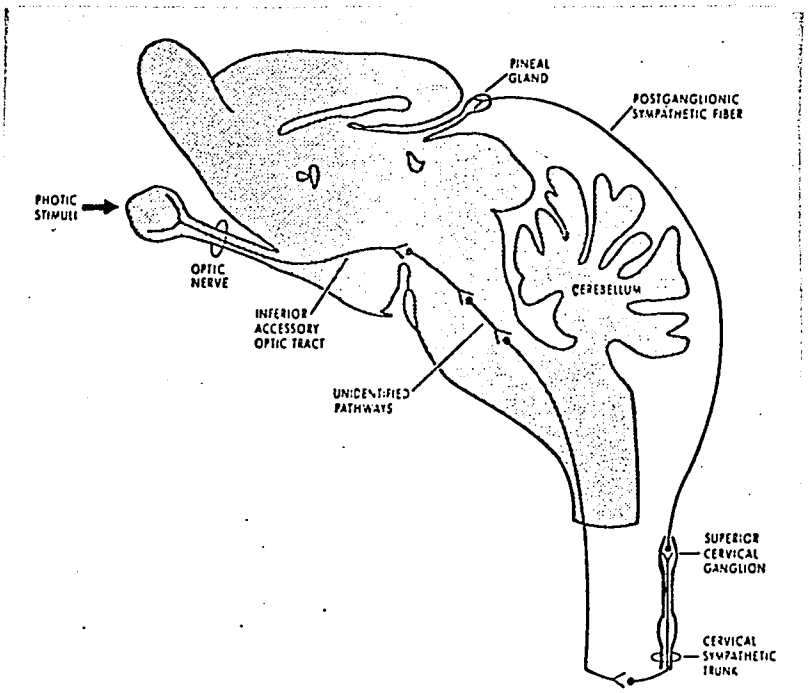

Fig. 1. Neural connections between the eyes and the pineal gland. Axons of retinal ganglion cells traverse the optic nerve, decussate within the optic chiasm and enter the inferior accessory optic tract, at least in the rat [MOORE *et al.*, 1968]. This tract accompanies the medial forebrain bundle through the lateral hypothalamus to the midbrain. Neural connections, probably multisynaptic, between the midbrain and the intermediolateral cell column of the upper thoracic cord have not been identified. Preganglionic sympathetic fibers originating within the thoracic cord carry information to the superior cervical ganglia; at least some of the postganglionic fibers terminate directly upon pineal parenchymal cells.

Figure 1

(Reiter and Fraschini, 1969)

Although this is an admittedly cumbersome path, according to work currently in progress it appears to be a correct one. The neural connections that have not been identified are those between the midbrain and the intermedio-lateral cell column of the upper thoracic cord, as indicated in the diagram.

A fine analysis of the anatomy of the pineal has yet to be completed. However, Wurtman, Axelrod, and Kelly (1968)

continually refer to the relationship between the saccular and parenchymatous (solid) type of pineal glands as a phylogenetic one with the saccular form occurring first and the solid form developing from such a gland later. They also stress the possibility that secretory processes may be taking place in the sacculated type of gland and that sensory information gathering may occur in the solid glands even though the bulk of their structures do not appear suited to these purposes.

The mammalian pineal gland secretes several substances, primary among which is the hormone melatonin. This is secreted by the pineal and released into the blood stream as long as light stimulus received from the environment and transmitted to the gland via the sympathetic nerves is minimal or absent. When the light stimulus is increased, the production of melatonin ceases.

That this information is transmitted via sympathetic nerves to the pineal was shown by an experiment in which the nerves to the pineal were cut as they enter and exit from the superior cervical ganglia. Following such an operation, HIOMT[3] activity could no longer be altered by exposure to light or darkness (Wurtman et al., 1964b).

Experiments have shown (Fiske et al., 1960) that the pineal glands of rats housed in continual light for six to

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[3]Hydroxyindole-O-Methyl-Transferase, an enzyme present in the pineal which acts as a catalyst in the final step of the manufacture of melatonin (Wurtman, Axelrod, and Kelly, 1968).

twenty-five weeks are approximately 25 per cent lighter than are those of rats kept in continual darkness or of control animals. A similar study has shown that circadian cycles of serotinin (a melatonin precursor) are established by the third day of life in rats kept in constant darkness or under controlled light conditions but do not occur in rats kept under continual light (Ilnerova, 1971).

It has been known for some time that the amount of light received by an animal can influence the development of the gonads. If rats are exposed to continual light, the result is a depression in the age of vaginal introitus in rodents and an increase in the growth rate of the ovaries (Fiske, 1941). In addition, the enlarged ovaries release significant amounts of estrogen, resulting in an enlarged uterus and a high percentage (80%) of vaginal smears showing cornification of the vaginal mucosa (Browman, 1937).

It has also been shown that the persistent vaginal cornification occurring in rats exposed to constant light could be reversed by treating the animals with bovine pineal extract (Jochle, 1956).

This series of experiments suggested that perhaps light acted on the ovary of the rat by suppressing the synthesis or release of a gonad-inhibiting substance from the pineal gland.

To test this hypothesis, a series of experiments was performed (Wurtman et al., 1961). First, pinealectomized and sham-operated animals were placed in continual light or

continual darkness for 80 days. These animals were then killed and the ovaries weighed. It was found that both pinealectomy and constant light produced an increase in the weight of the ovaries of the animals but that their effect was not cumulative. This suggested that both operated through the same pathway. Normal animals were then placed in conditions of continual light or darkness and both were given bovine pineal extract. The result was an inhibition of the increase in ovary weight expected under conditions of constant light.

These results were supported by a separate set of experiments (Relkin, 1971) in which four groups of rats were raised under conditions of normal light. Group 1 was pinealectomized, group two had anterior basal hypothalamic lesions, group three had bilateral amygdaloid lesions, and groups four were sham operated. All were sacrificed on the day of vaginal opening. Groups one and two were comparable to each other, with vaginal opening occurring within 32-33 days, whereas groups three and four did not show vaginal opening until 41-42 days.

It was thus concluded that the pineal did contain a factor that inhibited gonadal activity in the rat and that the formation and release of this factor was related to environmental light.

It remained now to isolate the factor and determine its relationship to environmental light.

It has been mentioned earlier that melatonin is one of the primary substances found in the pineal gland. This substance was injected subcutaneously into female rats that had been kept in continuous light for four weeks and whose vaginal smears exhibited approximately 85 per cent oestrous. A single injection of 10 micrograms caused the incidence of oestrous to fall to 45 per cent, which is a normal figure. Injection of melatonin also reduced gonadal weight and function in animals kept under normal light conditions (Wurtman et al., 1963).

These experiments, coupled with the one mentioned above regarding the innervation of the pineal, led Wurtman and his colleagues to the conclusion that the pineal gland acted as a neuro-endocrine transducer, acting to convert a sympathetic nervous input to a hormonal output (Wurtman, Axelrod, and Kelly, 1968) and that environmental light acted to suppress the formation of melatonin and thus hasten gonadal maturation.

The work done by Wurtman, Axelrod, and their many collaborators strongly suggests it is the pineal gland that is responsible for the effects that light or its absence appears to have on gonadal maturation.

If female rats, or any polyoestrous nocturnal animals, are exposed to continual light, several specific changes occur in the reproductive system. Early vaginal opening will occur (Fiske, 1941) and the entire reproductive system will hypertrophy. The gonads will enlarge and secrete large

amounts of estrogen although corpora lutea will be largely absent and ovulation will not occur (Wurtman, Axelrod, and Kelly, 1968; Krisna Murtly and Russfield, 1970). The uterus will hypertrophy and there will be an increase in the cornification of the vaginal epithelium to the extent that a very high percentage (as much as 85%) of daily vaginal smears will be classified as "oestrous" (Krisna Murtly and Russfield, 1970; Browman, 1937; Wurtman et al., 1963).

These reports of hypertrophy of the reproductive tract as a result of pinealectomy or constant light have been questioned by Reiter and Fraschini (1969). They feel it is more accurate to call this "premature development" or "transient hypertrophy" as the adult animal does not have abnormally large gonads.

If the illumination is continuous for several months, the vaginal epithelium of hamsters becomes permanently cornified (Kent et al., 1968). This has been observed in rats also although eventually the ovaries decrease in size, the uterus atrophies, and the vaginal smears cease to show constant oestrous (Maric et al., 1965).

The effects of continual darkness or blindness on the gonads has also been investigated. In rats, blinding or continual darkness appears to have little effect on the gonads of the female, although it appears to inhibit gonadal activity and development in the male (Itoh et al., 1962; Wurtman, Axelrod, and Kelly, 1968; Sorrentino et al., 1970; Takahashi et al., 1971). However, blinding will reverse the

"persistent oestrous" observed in female rats due to early injection of testosterone propionate. Ablation of the pineal in such animals will reverse this effect (Reiter, 1969).

If hamsters are kept on "short days" in which only one or two hours of light are permitted in a 24-hour cycle, the result is a depression of the maturation rate (Hoffman and Reiter, 1965, 1966). This is contrary to the "no effect" reported in rats between controls and animals kept in total darkness and adds a new dimension to the problem. As both are nocturnal animals, perhaps there is a specific difference, or perhaps the very short light stimulus "primes the pump," as it were, and triggers the antigonadotropic activity of the pineal.

Support for this suggestion is found in Relkin (1968), who feels the first twenty days of life are critical in establishing pineal function. He feels alternating light/dark cycles are essential during this period if subsequent light deprivation is to inhibit the reproductive system and delay vaginal introitus in the female rat.

An objection to this has been raised by Reiter and Fraschini (1969), who point out that under wild conditions both rats and hamsters raise the young in the continuous darkness of the nest for the first few weeks of life.

It is their suggestion that the regularity of laboratory lighting is responsible for the relationship between maturation and light cycling remaining undiscovered for so

long. They feel (Reiter, 1969) that the pineal gland is still capable of influencing gonadal function even if the experimental conditions are imposed at birth.

In human subjects, however, darkness does appear to affect the gonads. In a study done in 1964 (Zacharias and Wurtman, 1964), groups of normally sighted term and premature girls were compared with girls that had been blinded at birth or within one year after birth. It was found that the blind girls experienced menarche as much as a year earlier than the sighted group, with the effect being most pronounced among those girls with no light perception at all.

A similar study compared two groups of girls, one with minimal or no light perception and one with shadow vision. Here again, the groups with poorest vision experienced menarche earlier, with a mean of 12.0 years as compared to 12.8 in the group with shadow vision (Magee et al., 1970).

It appears that not only the presence or absence of light but also the intensity of light affects gonadal maturation in experimental animals.

Two groups of mice were raised with the amount of light received by each controlled with drapes. Even though both received the same number of hours of light per day, the group receiving a lower light intensity reached first oestrous, as set by vaginal smear, approximately 7.6 days later than the higher light intensity group (Williams, 1969). It was also found that rats and golden hamsters kept on the lower levels of cage racks away from the artificial light

source in the laboratory were receiving considerably less light than those at higher levels. When these intensities were measured, it was found hamsters receiving light below 60 lux either showed a prolonged oestrous cycle or were anoestrous. Among rats, animals kept at 30 and 60 lux showed vaginal introitus an average of three days later than animals raised at higher light intensities (Weihe et al., 1969).

These results suggest that there is a difference between diurnal and nocturnal animals in the effects of light or its absence on the gonads. However, it has not discouraged some workers from suggesting that the current trend in lowered menarcheal age (Tanner, 1968) can be attributed to the increasing use of artificial light (Jafary, Kahn, and Jafary, 1970).

Needless to say, this is an interesting speculation but a bit premature. Such an hypothesis would require considerably more experimental evidence from diurnal animals and a fine analysis of the specific light intensities that affect the gonads in nocturnal and diurnal species.

The effect of light on gonadal maturation, perhaps mediated by the activity of the pineal gland, seems to be one that is in process of being thoroughly investigated. The effect of light on body growth is less well investigated but merits discussion here as there appears to be a connection between body weight and/or body length on the timing of sexual maturation in the female (Frisch and Revelle, 1970; Engel, Crafts, and Zeithaml, 1937).

It has been known for some time that well nourished children do not grow steadily throughout the year but that both height and weight increases show definite seasonal peaks and valleys. In the northern hemisphere the greatest height increase occurs during the spring, peaking in March, April, and May, while fall is the time of greatest weight increase, with peaks in September, October, and November (Tanner, 1962).

That this may have something to do with the amount of light received by the growing child was first noticed in a study done in 1929 by G. Nylin, who noted the autumn weight increase could be obliterated and turned into a second height increase by exposing children to a sun lamp for a certain number of hours of light per day. Further support was gained for the theory by the observation that the times of maximal height and weight gains were exactly reversed in southern hemisphere children to correspond with their spring and fall periods (Fitt, 1941).

Work done in experimental animals indicates that animals blinded or raised under conditions of complete darkness have a depressed growth rate and retarded body size when compared with normal controls (Wurtman, 1967). This effect can be reversed by removal of the pineal gland (Reiter et al., 1969c). If the pineal is removed in sighted rats kept under normal lighting conditions, the result is an accelerated growth rate (Malm et al., 1959).

When similar information is sought for human subjects, it is found that the spring height spurt observed in normal children does not occur in blind or partially sighted children. In a group of 116 such children, the times of maximum height increase were distributed throughout the year with no discernible peaks or valleys (Marshall and Swan, 1971).

Here again, light intensity appears to be a factor as the most rapid growth was observed in rat litters raised in light intensities of 30 lux while retarded growth was noted in animals kept at 1,000 lux (Weihe et al., 1969). Similar results were obtained by raising seven groups of male rats under seven different lighting conditions--complete darkness, 12 hours dark/light at 100 m., and continuous illumination at .01, .1, 1.0, 10, and 100 ml. While the results were not an exact fit, the body weight of the animals at 102 days from birth was roughly an increasing function of rearing luminescence (Lockhart, 1963).

The results of these experiments and observations, although confusing and in some cases apparently contradictory, at least indicate that the amount of light received by an organism does affect its growth rate; perhaps both body length and weight.

What the mechanism is we cannot be sure until further experimental work is done and more careful records are kept as to exactly what "increased" or "retarded" growth entails. We cannot yet be sure the pineal is involved at all or, if the pineal is involved, whether it secretes a hormone that

acts directly on body growth or by suppressing or encouraging the secretion of pituitary hormones (Reiter et al., 1968c).

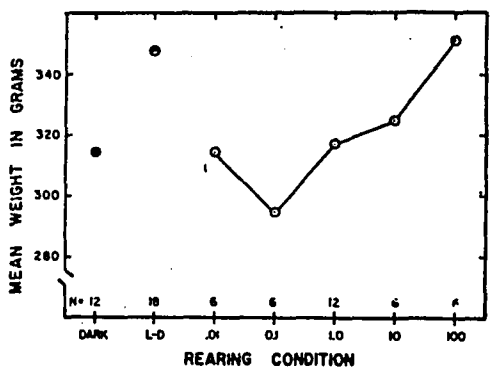

FIG. 1. Mean body weight at 102 days of age as a function of rearing condition.

Figure 2

(Lockhart, 1963)

Little work has been done on the effects of the pineal on the pituitary; however, some reports indicate pinealectomy causes pituitary hypertrophy or hyperactivity of the gland (Girod et al., 1963; Thieblot, 1965; Moszkowska, 1965). This could explain the acceleration of maturation caused by pinealectomy as well as the effect ablation of the gland appears to have on growth. It has been noted that pinealectomy does appear to increase the amount of gonadotropin in the adenohypophysis although no one, it appears, has yet checked the effects pinealectomy has on GH production.

The connection between light and the rates of growth and sexual maturation in mammals appears to be well established. Its role in human populations has been suspected for some time as studies going back as early as 1934 indicated a consistent seasonal difference in the onset of the menses in groups of European girls with a certain amount of variation between rural and urban groups (Valsik, 1965).

The work outlined in this chapter confirms this connection and indicates that here also is an environmental variable that should be carefully considered when attempting to determine the reasons for maturational age in a non-experimental population. The degree to which this factor varies in human groups is, of course, considerable, depending not only on the latitude but on the degree of urbanization and the availability of electric power for lighting.

So far, no work has been done on the effects of various kinds of light on maturation and growth. Current workers appear to consider all light, natural or artificial, to be equally influential. It remains for the future to determine the effect, if any, of various light wave lengths, including the "invisible" ones.

MENARCHEAL AGE IN WORLD POPULATIONS

The previous sections of this dissertation have dealt with experimental evidence that dietary protein and the number of hours of light per day can influence the maturation rate of laboratory animals. When we turn to human populations, however, we find difficulty in correlating the differences observed in mean age of menarche with either of the above environmental variables. Only in conditions of starvation or extreme protein deficiency is the retardation of development severe enough to be directly connected with the lack of proper nourishment. One finds the literature full of references to "starvation edema" and a wealth of information on the diagnosis and treatment of kwashiorkor but little material that attempts to correlate the amount of dietary protein with growth or maturation rates in populations that are not starving or exhibiting gross pathologies.

One exception here is a study done by Frisch and Revelle (1970) in which the height and weight at menarche were determined in three groups of girls. It is their suggestion that a critical body weight may trigger three major events of adolescence: initiation of the weight growth spurt, the rate of weight gain, and menarche. They base this on the observation in the groups studied that early- and late-maturing girls exhibit different growth rates and

achieve menarche at different heights but that in all cases the mean weight was the same--approximately 86 pounds.

These authors feel the attainment of critical body weight may cause a change in the metabolic rate, reducing the sensitivity of the hypothalamus to oestrogens and altering the ovarian/hypothalamic feedback system, thus triggering menarche. A similar relationship could alter the production of GH and thus affect the growth spurt.

A similar result was reported by Engel, Crafts, and Zeithaml (1937), who noted that in rats the occurrence of first oestrous is closely correlated with reaching a length of 160-170 millimeters, regardless of age. They also report a tendency for the body weights of animals at first oestrous to be similar but with a larger range and a more variable standard deviation than was observed for length.

It is generally accepted that a "better" diet can, within genetically imposed limits, influence the rate at which a child gains weight. If the hypothesis of Frisch and Revelle is valid, it can go a long way toward explaining the secular trends in menarcheal age and some populational differences in this trend. However, we are still left with the unanswered question of what constitutes a "better" diet.

Even less information is available regarding the effects of light on human populations. Except for the studies mentioned above dealing with blind children, no studies have attempted to correlate maturation rates with

degree of light stimulus received. Such data as exist concentrate mainly on determining the mean menarcheal age of girls in various populations. These studies usually include little or no information as to the diets or amount of light stimulus under which the girls are raised. Even with this minimal amount of information, however, certain consistent differences between groups have been recognized.

Donovan and Van der Werf Ten Bosch (1965) note that, aside from genetically determined factors, the age of menarche can be influenced by climate, stress, season, nutrition, socioeconomic factors, and temperature. Clinically they note stimulation of the uterus, irradiation of the ovaries, neuropharmacological agents, disease, and electroshock all influence the time of menarche.

Valsik (1965) and Valsik and Veli (1964) have noted that in Europe menarche is more likely to occur in the winter in large cities, with a peak in December and January, whereas more rural areas show a peak in early summer. A large-scale study made during 1963 and 1964 by a team of Polish and Czech anthropologists showed rural populations in the Danubian lowlands showed two peaks, one in mid-winter and one in summer, with the summer peak being the larger. As the study moved upland, however, the pattern changed, with a maximum winter peak similar to the pattern shown for large cities. Valsik also mentions the pattern changes depending on whether the girl experiences "early" (before 11 years, 9 months),

"average" (between 11 years, 9 months, and 13 years, 10 months), or "late" (after 13 years, 9 months) menarche. Early maturers tend to experience menarche during the first quarter of the year; average maturers tend to show two peaks, one in January and one in August, with a minimum during April and October; while late maturers tend to peak during the fall with a low in March and April.

The age at which the individual will reach menarche is also influenced by external conditions. This was already noted in 1789 when Buffon (quoted in Valsik, 1965) indicated that city girls achieved menarche before rural and rich girls before poor. Since that time, several studies have confirmed this observation. One recent study was done among girls in Southern India (Medhaven, 1965). Here the mean menarcheal age was found to be lower for urban girls, 55 to 70 per cent of whom were members of a high socioeconomic group, as opposed to the rural group, 90 per cent of whom belonged to a lower group. A similar study, done by Valsik in 1964 on a large sample of Polish and Czech girls, noted town girls reaching menarche two to three years before country girls, with the earliest menarche appearing among daughters of clerical workers and the latest among children of agricultural workers. The dichotomy between urban and rural populations is explained as influenced by socioeconomic status.

It has been thought that climate played a definite role in the achievement of menarche, with girls in the

tropics maturing much earlier than those in the Arctic; however, studies made on this subject tend to indicate this is not the case.

This was first noted in the 1840's by J. Robertson (quoted in Bojlen and Bentzon, 1968). Working with a questionnaire, he collected information on mean age of menarche in Labrador, India, the West Indies, and several European locations. Although his sample sizes were often far from what are now considered adequate and the large error involved in recollected age of menarche may bias his data, his results were interesting. He found a range of from 16.4 years for Barbados to 13.2 years for Calcutta with no cline demonstrable that could be correlated with climate. He concluded that climate has little, if any, influence on menarcheal age.

Robertson's work was largely ignored, but subsequent studies, especially those made during the past twenty years, tend to support his conclusions.

Table I lists several studies made in various areas of the world. It is to be noted the bulk of this data was collected after 1950. Although a great number of earlier studies exist, the results they give tend to be less reliable due to the size of the sample, which was often under 100 individuals, and their heavy reliance on "recollected" menarcheal age and questionnaires. It has been shown (Damon et al., 1969) that women often do not accurately recall the age at which they first menstruated, often raising the date

from one-quarter to one-half year. It is for this reason that such studies were not included in this table. The studies listed were, for the most part, done on large groups of school girls with the information collected by direct interview. The table is arranged in order of approximate latitude, with the northern groups heading the list.

If this material is placed on a graph, no pattern results. The arranging of the data by climate type, as is done in Graph 1, with the polar climates to the left and the tropical climates to the right, produces no recognizable curve.

If one rearranges the data, as is done in Graph 2, so the mean menarcheal ages form a smooth curve from highest to lowest, no pattern can be discerned in the climate types.

In 1961, Foll interviewed 1,150 Assamese girls and 704 Burmese girls. Both groups were represented as being members of a high socioeconomic group. He found a similar mean menarcheal age for both groups, in spite of the fact that they live in different climatic zones. He compared his results to figures obtained by other workers in Nigeria, Central India, and Ceylon and also concluded that climate played no part in determining the age of menarche.

A similar conclusion is reached by Wilson and Sutherland (1953), who review the existing data on menarche in tropical countries and state that menarche may occur early or late in the tropics and is not determined solely by climate.

Here I must note that the term "climate," as used in these studies, is impossible to define precisely even when broken down as closely as is done in Table II (Goode's World Atlas, 1960). There are many variables involved in the term "climate": number of hours in the daylight period and seasonal variation in same, degree and frequency of cloud cover, mean annual temperature and yearly temperature range, number of feet above sea level, pollutants in the atmosphere, humidity, vegetation. This list covers only a few of the myriad environmental variables that are sheltered under the term. Considering this plethora of variables, it is small wonder that no "climatically determined" clines have been observed in human populations.

A study done by Chattapadhyay and Khullar (1969) listing 34 studies on menarcheal age among various Indian populations revealed few interpopulation differences, but did note a slightly lower age of menarche among girls of Eastern India as compared to the rest of the country. They suggested this may have been due to the "progress of civilization," noting that most authors agree girls of higher socioeconomic groups have a lower menarcheal age and implying that "civilization" may result in an improved diet. They also noted the data suggest climate does not seem to have much effect on the age of menarche.

What may be indicated by these studies is some support for the theory that "better-nourished" girls seem to achieve

menarche earlier. In 1943, Kark (1956) established that better-nourished Southen Bantu girls reached menarche an average of one year earlier than Northern Bantu girls, even though the climate occupied by the Southern group was more temperate. In a similar study on 1,259 Indian girls in Africa, Kark found upper class girls achieving menarche 5.3 months earlier than lower class girls. A study done in Roumania (Stukovsky et al., 1967) on 581 girls indicates menarche occurred later by 2.1 months for each sibling the girl had. It was the opinion of the authors that this reflected the level of care the child received and the amount of food available, reasoning that a large family would be able to provide less food and less individual care per child.

A general secular trend of about four months per decade toward earlier menarche (see Figure 4) has been attributed to an increase in urbanization and a general improvement--in Europe, at least--of the standard of living. Tanner supports this theory (1968) by noting that in Britain over the last century girls have experienced earlier menarche at a rate of three to four months per decade, which can be correlated with a similar increase in height and weight. A similar trend has been noted in the Soviet Union (Vlastovsky, 1966), where the mean age in Leningrad has fallen from 14.2 in 1927-30 to 12.11 in 1959. Similar trends have been noted in Melbourne, Australia (Towns et al., 1966) and in Saratow (Vlastovsky, 1966).

A rather extensive survey of this problem compares the mean menarcheal ages of large samples of girls in various parts of Britain and Oslo, Norway, and their fluctuation over a period of 117 years (Brown, 1966). As his chart below indicates, there does appear to be a tendency for the mean age of menarche to decrease although in all cases the range is roughly the same.

TABLE I

PERCENTAGE OF GIRLS MENSTRUATING BY A GIVEN BIRTHDAY

Birthday	1845	1890	1928	1949	1949	1952	1962	1962
	Birmingham	Sheffield	Oslo	Oxford 1	Oxford 2	Oslo	Scotland	Yorkshire West Riding
9	—	—	—	—	—	—	—	—
10	0·2	0·4	—	0·2	—	0·4	—	0·3
11	0·5	1·7	0·3	0·6	1·0	2·0	5·0	3·9
12	2·9	6·3	2·5	5·6	7·5	12·3	17·2	20·0
13	10·3	16·9	15·4	24·8	29·6	41·2	44·6	55·3
14	24·2	40·5	44·8	64·6	63·3	74·7	79·8	84·9
15	45·1	59·1	74·4	90·8	89·4	93·2	95·9	96·9
16	63·6	72·6	95·5	95·5	95·4	97·1	99·7	99·4
17	80·4	86·1	98·3	100·0	98·2	96·3	100·0	99·9
18	91·2	90·7	98·9	—	100·0	95·1	—	100·0
19	98·1	96·2	100·0	—	—	94·3	—	—
20	99·7	97·5	—	—	—	—	—	—
21	100·0	98·3	—	—	—	—	—	—
22	—	99·2	—	—	—	—	—	—
23	—	99·6	—	—	—	—	—	—
No. of Girls	623	237	9,169	572	766	11,618	369	685
Average Age (yrs) ..	15·3	14·8	14·2	13·6*	13·6*	13·4	13·1	12·9
Range (yrs)	11	14+	8	7	7	10+	6	8
Lower Limit	9	9	10	9	10	9	10	9
Upper Limit	20	23+	18	16	17	19+	16	17

* Median score

Figure 3

(Brown, 1966)

How long this trend will continue is open to question, and may depend on whether or not standards of living continue to change or stabilize. A study done in 1966 (Poppleton and Brown) on 685 girls in the West Riding School District of England indicated the menarcheal age in that region has

remained stable since 1949. The scope of this study is very limited, however, and gives no information as to the general stability of living standards in that region during the same period. A similar situation appears to exist in India, where the mean menarcheal age has remained relatively unchanged since 1849 (Bojlen and Bentzon, 1968), reflecting the unchanged diet and standard of living of the population.

Few precise studies have been made toward determining the exact influence of diet and socioeconomic status on menarcheal age. Such information as exists is contained in general surveys of menarcheal age for girls of this or that area and is given as an hypothetical explanation for differences between the means of various groups. I could find little information to support such an hypothesis except for nutritional studies which mention increased growth and general maturation for "better-nourished" children (Tanner, 1962). No one quotes any studies which would indicate exactly what "better nourishment" entails or how it could influence age of menarche. Several studies do mention earlier maturation for girls of higher socioeconomic groups, with the implication that such girls eat better, but no analysis of diet for these groups is included and, without such studies, any cross-cultural comparisons would be difficult.

The data presented in this section support the statement made earlier that the world-wide picture of menarcheal age, although reflecting the decreasing secular trend, is a

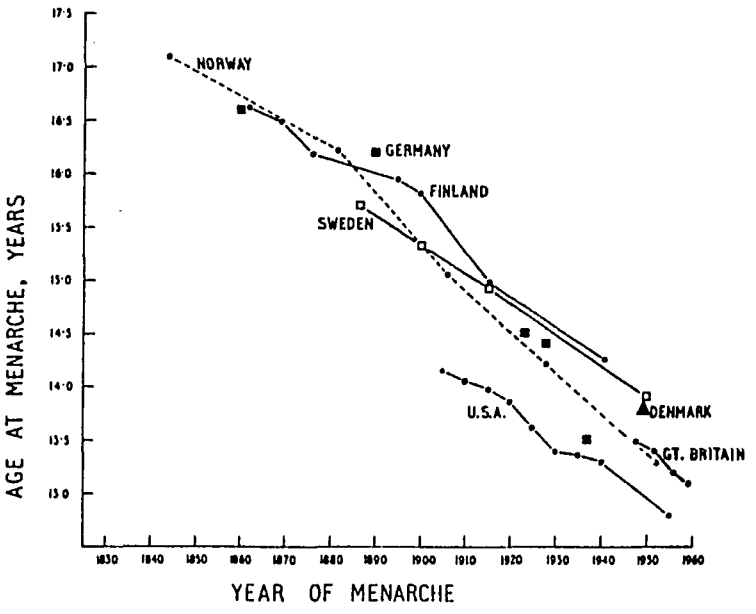

FIG. 31. Secular trend in age at menarche 1830-1960. Sources of data as follows:
Finland: 1862-1915, hospital patients Helsinki, from Malmio (1919) and Vara (1943); 1941, medical practice and health visitor inquiries, all Finland, age at interrogation 17 to 27 only, from Simell (1952).
Sweden: 1886-1915 hospital patients, Lund and Stockholm, from Essen-Möller (quoted in Lenner, 1944), Lundh (1925), Samuelson (1942) and Lenner (1944) (hospital data of last two pooled for value at 1915); 1950, schoolchildren, estimated from data of Romanus (1952).
Norway: 1844-81, from Backman (1948); 1907 Oslo hospital patients, from Skerlj (1939); 1928-52 Oslo schoolchildren, data of Schiötz (1930), and Kiil (1953) fitted by probits.
Germany: 1860-1928 hospital patients various towns, successively from Schlichting (1880), Heyn (1920) and Schaeffer (1908) pooled, Risopoulos (1936), Scheibner (1938); 1937, schoolchildren S.W. Germany, probits fitted to data of Ley (1938). See also values reported in Backman (1948) and Wallau (1952).
Great Britain: 1948-60 schoolchildren, probits, successively S. England, from Wilson & Sutherland (1950b), Edinburgh from Provis & Ellis (1955), Bristol from Wofinden & Smallwood (1958), London from Scott (1961).
U.S.A.: 1905-40 University of South Carolina entrants, from Mills (1950), 1960 estimated, see text.
Denmark: 1950, Copenhagen schoolchildren, probits, from Bojlén, Rasch & Weis-Bentzon (1954).
Values are plotted at year in which the average menarche took place, i.e. in 'recollected-age' data if average menarche of 40-year-olds interrogated in 1900 was 15 years, this is plotted at 1875. This places old data on same age scale as modern probit data. Where age of interrogation is not recorded an estimated amount has been subtracted according to nature of population studied (primiparae, etc.). Grouping errors have been corrected where necessary (i.e. '13-year-olds' centred at 13·5 years, not 13, as in some of older literature).

Figure 4

(Tanner, 1962)

highly variable one and does not appear to correlate closely with fluctuations in any one environmental variable. It also points to the lack, in large part, of truly accurate data regarding the individuals involved in any one study. This is not the fault of the investigator, but of the situation. It would be extremely difficult for a single worker or even a team to keep careful track of the exact exposure of each member of a human group to any one of a number of environmental variables. Only constant surveillance would allow the accurate recording of even one variable--say protein intake, for example--to be made. This is a condition few individuals would tolerate, especially for the length of time necessary for a nutrition study.

It is for this reason we must turn to the laboratory for answers. We cannot hope to make sense of the changing pattern presented by human populations until we understand more fully the effects of all influencing factors and the degree of interaction among them.

It is toward this end that the following experiment was designed. Dietary protein and the light cycle were selected as the two variables to be studied as they appear to be the most extensively documented areas believed to influence the rate of sexual maturation, and because of the recent interest in the effects of light on the pineal gland stimulated by the work of Wurtman and his colleagues. The

independent effects of light and of protein upon growth and maturation have been established. It is the primary concern of this study to examine the degree of interaction between these two variables and the effect of that interaction on various phenomena generally accepted as parameters of growth, nutritional status, and sexual maturation.

METHODS

Sixty-two, near-term CF1 mice were randomly assigned to one of six light groups: 0, 6, 12, 18, or 24 hours of light per twenty-four hour period plus a naturally lit box. Each female was caged in a single 7 x 11 x 4½ inch Carworth Iso-Cage.

At birth each litter was sexed and the males removed. In no case were more than eight or fewer than five permitted to remain with one mother. Extra females were discarded to minimize the possibility of a disturbed female destroying the litter.[4] Each mother, fed Purina Standard lab chow, was permitted to nurse her litter for 21 days. At weaning, the litters within each light group were numbered, weighed, and randomly assigned to a diet group.[5]

Each light group occupied a 3 x 4 foot covered box two feet deep constructed of plywood. Each box had all seams and edges sealed with caulking compound and opaque black friction tape, and each was checked to be sure it was completely light

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[4]The pregnant females were placed in the light boxes prior to the birth of the actual experimental animals so the litters would be exposed to the various light conditions during the 21-day "critical period" proposed by Relkin (1968).

[5]The animals were numbered by means of a head-back-tail code marked in picric acid on the animal's fur. This technique was used rather than ear punching or toe clipping, to avoid any unnecessary trauma to the animal.

tight when the lid was shut. Air was circulated through the boxes by means of a small, shaded pole blower and vented through specially constructed vents that were rendered light-tight through a series of four baffles that permitted air to pass through. To further minimize the possibility of light leakage through reflection, the vents were finished in matte black inside and out. The opening made for the blower was similarly baffled to permit air but no light to pass in. After installation of these fixtures, the boxes were again checked to be sure they were light-tight (see Plate II).

Four of the boxes had two 25" long fluorescent fixtures holding 20-watt, daylight bulbs mounted at each end of the inside of the box. When lit, these fixtures provided a light of 65 foot-candle brightness when measured in the center of the floor of each box with a standard photographic light meter. It should be understood that the actual light intensity within the box was somewhat higher with the lid closed, due to reflection from the lid. The fifth box was not lit.

The sixth box was covered with a clear plastic lid. This box received illumination through the lid from the laboratory light and the daylight through the windows. The light intensity in this box was also measured at 65 foot-candles during the daylight hours with the laboratory light on.

Twenty-four hour timer switches were mounted on four of the boxes to permit accurate regulation of the number of hours of light per day.

Each box was capable of housing ten of the Iso-Cages, although such a large number was rarely kept in one box for any length of time. To be sure all cages received an equal amount of illumination, they were rotated on the following pattern every five days:

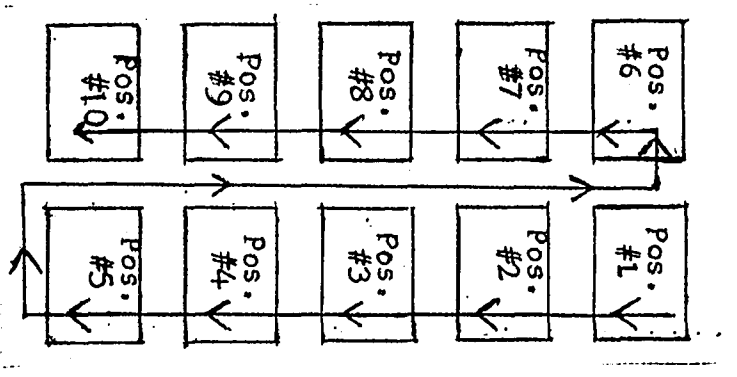

Figure 5

As the experiment lasted approximately 45 to 50 days for each litter, this placed each animal in positions 1 through 10 for the same length of time.

The experiment ran for several months, from November through July, at approximately 42° north latitude. This resulted in considerable seasonal fluctuation in the "naturally" lit box with the clear plastic lid. This fluctuation is not quantifiable.

The diets for each group were identical in all respects, except for the relative amounts of protein and carbohydrate they contained. Each diet contained 4 per cent Nutritional Biochemicals (Chagrin Falls, Ohio) Salt Mixture #XIV (offered by the manufacturer as containing all essential salts to a rodent diet); 10 per cent corn oil; and, in quantities of 15 grams per 1,000 milliliters of mixture, Nutritional Biochemicals Vitamin Diet Fortification Mixture, considered to be a complete vitamin supplement. The amount of vitamins in the 0 and 6 per cent protein diets was doubled. In these diets the animals would be expected to eat less (Keys et al., 1950; Kinzey, 1970) and doubling the vitamin content decreased the possibility of skewed results due to vitamin deficiency.

The relative amounts of protein and carbohydrates in the diets varied as indicated in Table III.

The diets, mixed in a powdered form, were placed in feeding dishes and offered ad libitum to the weanling mice. The dishes were cleaned and new food supplied every other day to avoid excessive contamination by urine and feces. Cages were cleaned twice a week. All cage handling was carried on during the "light" period of each box. The animals in the total darkness box were serviced in dim light.

The weanling females were checked daily for vaginal opening. When this was achieved, the animal was weighed and

the date noted. From that day, daily vaginal smears were taken from each animal for 14 days.

The smears were taken by the introduction of a sterile platinum wire loop into the vaginal opening to collect a sample of the vaginal secretions and epithelium. The loop was then rinsed in a drop of tap water on slide, the smear fixed with a coating of Spray-Cyte and stored for staining.[6]

The vaginal smears were taken daily for 21 days in half the experimental animals. This was later reduced to 14 days as the slides began to indicate that permanent cornification of the vaginal epithelium occurred in most of the animals after two weeks of daily smears.[7]

Half these slides were stained with a 5% solution of methylene blue and allowed to air dry before reading. The remaining slides were stained by the standard Papanicolaou technique. All slides were examined under a microscope and note made for each animal of the date of the first "adult"

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[6]Spray-Cyte, manufactured by Clay-Adams, Parsippany, New Jersey, is a mixture of polyethylene glycol and isopropyl alcohol which, when sprayed on a slide, fixes the smear and prepares it for staining.

[7]At the time, this was thought to be a result of the smearing technique. Some workers have reported permanent cornification of the vaginal epithelium due to daily vaginal smears (Emerg and Schwerd, 1936). It was to avoid this condition that the wet-lavage technique was developed (Vandenburgh, 1969). Other workers, however, have reported permanent cornification of the vaginal epithelium attributed to constant illumination (Browman, 1937). It is not possible at this time to determine which of the above factors may be responsible for the persistent cornification observed here.

smear, defined as the first smear showing more than a sketchy amount of cellular material; and the date of first oestrous, taken as the first day in which the slide showed clumps of cornified epithelial cells.

Thirty days after vaginal opening, the animals were sacrificed by ether overdose and weighed. Immediately after sacrifice, the atlas-anus length of the animal was taken with a vernier caliper and the following organs were dissected out: adrenals, ovaries, uterus, spleen, liver, and pituitary gland. The wet weight of these organs was taken on a Mettler scale and the organs deposited in FAA solution for histological analysis.

The histology of these organs revealed no pathologies or congenital abnormalities which would affect the results of this experiment. A complete histological analysis of the organs is still in progress and will be reported on in a later paper.

At the conclusion of the experiment, the data listed in Table V had been determined for each animal in the experiment, a total of 149 animals. (This figure does not include the animals who died in the course of the experiment, but only those on whom a complete set of data was obtained.) Each observation was made to determine the effect of the two variables on specific day counts and weight gains that had been shown by other workers to indicate sexual maturity or to trigger sexual maturity. The organ weights were taken to

determine the effect of the variables on these organs that would reflect most strongly changes in diet, sress, light stimulation, and the rate of sexual maturation.

The material was reviewed and all data for each animal on whom all 16 observations had not been made were eliminated. The result was a constant number of animals in each cell of the experiment that did not change from one set of data to the other. The number of animals in each cell of the experiment and the totals for each group are listed in Table VII.

This table lists six light groups and five diet groups, but in the statistical analysis of the data only five light groups and four diet groups are included. The "natural" light box received a fluctuating amount of light, depending on the season and how much the room was in use. This fluctuation is not quantifiable; therefore, the data obtained from these animals will be discussed separately. As listed in Table XIV, a T Test was done in this last group comparing the various diets with the 25 per cent diet, which contains the same protein/carbohydrate ratio as standard lab chow. Diet #1, which contained 0 per cent protein, experienced 100 per cent mortality in the first two weeks after weaning and is therefore also not included in the analysis.

To test the extent of interaction between the two variables, the remaining data were subjected to a two-factor, fixed effect, completely randomized model of Analysis of

Variance, with an unequal number of observations in each cell.

The operations around which variance analysis is based center on an examination of the variability among groups in such a way as to ascertain whether the magnitude of that variability is sufficiently small to be attributed to sampling variation. The mechanics of this examination hinge on the theory that, if the null hypothesis (an assumption that nothing significant is occurring) is true, the variability of scores within the various groups and the variability among whole groups should both be proportional to the variability contained within the population from which the groups were taken. Thus, within-group variability is used as an index of the amount of population scatter "expected" as a result of sampling variation. The amount of variability observed among groups is then compared to determine whether its magnitude is small enough to be consistent with sampling variation. If the null hypothesis is true, these two separately derived estimates of population variation should be the same and F should approach a value about equal to one. If the null hypothesis is not true, significant differences among groups can be detected if the estimate of population variance based on scatter proves to be too large to be compatible with the estimate derived from within-group scatter. This can be determined by looking in a probability table of F at an appropriate p level (Senter, 1969).

The model used in this case can be written as follows:

$$yijk = \mu + \delta_i + \beta_j + \gamma_{ij} + \iota_{ijk}$$

$$\begin{cases} i = 1,2,3,4 \text{ (diet groups)} \\ j = 1,2,3,4,5 \text{ (light groups)} \\ k = 1,2..... \text{ kij} \end{cases}$$

In this equation, the errors ι_{ijk} are independent variables from a population assumed to be normal with 0 and variance σ^2. Yijk represents the result of the $\underline{jk}^{th}$ observation for the $\underline{i}^{th}$ diet and the $\underline{j}^{th}$ light group. The effects of the $\underline{i}^{th}$ diet are represented by δ_i. Effects of the $\underline{j}^{th}$ light group are represented by β_j, with γ_{ij} representing the effect due to interaction between the $\underline{i}^{th}$ diet and the $\underline{j}^{th}$ light group. Kij represents the number of observations in the (i,j) cell of the experiment.

The theoretical background for performing analysis of variance when there is an unequal number of observations can be found in Scheffé (1959). In such a situation, although an ANOVA table of sums of squares is not available, the theory and equations leading to the F test for interactions and the F tests for main effects are available and have been utilized in this analysis. In such an analysis, estimates for the means and the sum of squares are unique and are developed by Scheffé's procedure.

RESULTS

The results of this analysis indicate that in many of the observed data sets there is interaction between the two variables. This interaction is not cumulative, but reveals instead a pattern showing interference of one variable with the effects of the other. The outcome can be to obscure the effect of one variable.

Mortality in this experiment is limited entirely to the 0% and 6% protein diet groups (see Table VI). As mentioned in the preceding section, the 5% diet group experienced 100% mortality in the first two weeks after weaning, and the deaths in the 6% diet group also occurred in that period. None of these animals achieved vaginal opening. There were no deaths in the high protein diet groups.

As Table VI indicates, mortality in the various light groups follows no particular pattern. The deaths are evenly distributed from group to group, with no significant differences between them.

Three sets of data taken from this experiment involved the number of days necessary for the appearance of certain landmarks of sexual maturation.

The number of days from weaning to vaginal opening were counted and when ANOVA was applied it was found that, although light appears to have no effect on the number of

days necessary for the appearance of this maturational landmark, this variable does interact with diet in producing the final outcome.

In the "natural" light group, increasing amounts of dietary protein definitely depresses the age of vaginal opening; however, it appears this depression can be at least partially offset by either increasing or decreasing the amount of light received by the animal.

This variation is especially noticeable in the 6% diet group (see Graph #19). In this group, the values are far higher than those of the other diet groups under 6 and 12 hours of light; however, they are lowest of all under 0 and 24 hours of light. Such a pattern is less clearly seen in the other diet groups, although, in most, the latest maturation occurs in the 12-hour light groups, decreasing with either more or less light per 24-hour period.

The optimal combination for earliest maturation appears to be 6 hours of light with a high protein diet (25% or 50%); the least favorable combination, 6/12 hours of light at a low protein diet (6%).

No significant interaction was noted between the two variables in either the number of days from vaginal opening to Oestrous I or the number of days from vaginal opening to the first adult smear. Neither light nor diet was a significant variable in either data set.

In the five data sets tabulating weights and weight gains, light has no significant effect, diet is significant in all but the weight gain from vaginal opening to sacrifice, and there is significant interaction between the two variables in all data sets.

Increasing protein appears to increase the weight at vaginal opening and, as the graph of the cell means indicates, (#20), light appears to act, at least in part, by increasing the weight of vaginal opening with both an increasing and decreasing number of hours of light per day. All groups show depressed weight gain at 12 hours of light/day, with increased weight gains in both increasing and decreasing light/day groups. This pattern is broken only in the 0 hours light group, where both the 25% and the 6% diet groups show a sharp drop in mean weight, and in the 24-hour light group, where the 50% diet also shows a weight decrease.

Interaction also occurs between the two variables in the data set charting weight gain from weaning to vaginal opening. Again, increased protein increases the weight gain in all but the 25% diet group. This group scored lower than the 12% diet group under all but 18 hours of light. In this data set it is the 6-hour light group which served as a central point. All diets show increasing weight gains with more or less light per day, except for the 6% group, where the opposite occurs. It can be said here that increased light acts to increase weight in all but the 6% diet group.

A similar pattern is seen in the graph for Total Weight Gain (#23), although here it is less clearly defined. Increased protein results in higher weight gains, but the effect of light is hard to see. All but the 6% diet groups show a decrease in weight gain under 6 hours of light per day, with means increasing with more and less light. The 6% group is again the opposite, with the largest weight gains under 6 hours of light.

Weight at sacrifice shows an increase in weight with increasing amounts of protein, and light appears to affect each diet group differently. The 50% protein diet shows a steady decrease in weight with increasing light, from a high point under 0 hours of light per day to 18 hours of light. Then there is a sharp jump in the 24-hour group back to a level somewhat higher than the means for the 0 light group. The 25% group has its lowest weights at 12 hours light per day and a steady increase in weight with increasing light from that point. The 12% group has a high point at 12 hours and a decrease in both light directions. The 6% group almost exactly agrees with this, with its highest point also at 12 hours.

The curves for the 6% and 12% diets and the 25% and 50% diets resemble each other, and each pair appears to exhibit a curve that opposes the curve of the other.

Weight gain from vaginal opening to sacrifice shows an increase with increased protein. Here, light interacts in

all but the 25% diet group by increasing the weight gain to the 12 hours light group and shows decreasing weight gain as the hours of light per day continue to increase. The 25% diet group opposes this pattern, showing a sharp decline in weight gains from 0 to 12 hours of light per day and then an increase to the 24-hour light period.

In body length at sacrifice, there is an increase in length with higher protein diets. Light and protein interact in the 6% and 50% protein diets by causing an initial increase in length and then a decline, while the 12% and 25% diets show a trend toward length increase with increased light. In the first two diet groups, then, increased light appears to oppose the effect of increased protein, while in the second two diet groups increased light enhances the effect of increased protein.

Light has no effect on the gross weight at sacrifice of any of the organs observed. Diet does appear to have a significant effect on gross weights. This is to be expected, as diet was significant in influencing the gross weight and weight gains of the animals.

The figures obtained for the adrenal and pituitary glands show no significance for diet and no interaction between the two variables. Light is significant only for the adrenal gland, and in this case the heaviest glands occur in the 6-hour light group.

DISCUSSION

As both variables included in this experiment had previously been demonstrated to affect maturation rates, it was expected that the various light and diet groups would show differences. What was also expected, however, was that the two variables would be cumulative in their effects. That is, it was expected that more light and less protein would show results similar to less light and more protein. Instead, the two variables interacted with each other in such a way as to indicate that there were certain optimal light/protein combinations that produced very positive results, with all other light/protein combinations producing less positive effects. This optimal combination varied from group to group and was not always easily predictable.

As the ANOVA tables show, in most of the data sets light does not appear to be significant, but there is significant interaction occurring between the two variables. The non-significance of light is peculiar here as other experimenters have shown that varying the number of hours of light per day received by experimental animals does affect the rate of sexual maturation in female rodents (Fiske, 1941; Wurtman, 1967; Williams, 1969). However, looking at the interaction figures in the various cells of each data set, it can be

determined where the greatest amounts of positive and negative interaction are occurring between light and diet.

It can be concluded that, in this experiment, the effect of diet is so strong that it obscures the effects of light. The interaction between the two is revealed only by ANOVA.

It should be remembered, in this discussion, that this experiment was performed on an animal that is nocturnal in its natural state. It has not, to my knowledge, been determined whether the effects of light on nocturnal and diurnal animals are similar. That is, does an increase in light stimulus result in accelerated sexual maturation in both groups? If this is the case, we may reasonably expect other mammalian populations to react in a similar fashion to variation in light stimuli and dietary protein.

That the effect of increased light on nocturnal rodents does result in more rapid sexual maturation and higher fertility is indicated by the worke of Weihe, Schidloin, and Strittmutter (1969), who noted that rats and hamsters kept in cages on the top shelves of cage racks and therefore receiving considerably more light than those kept on lower shelves, matured faster and conceived more often.

As mentioned in the previous section, mortality in this experiment was limited entirely to the 0% and 6% diet groups. This may have resulted in a slight skewing of the results due to selection in the lower protein diets for

animals that could survive to maturity on less protein. No such pressures were placed on the higher protein diet groups, all of whom were abundantly supplied with protein.

As reference to Table VI will indicate, deaths were fairly evenly distributed among the light groups and in most cases the mean weights at weaning of the animals who did not survive were below the weights of those that did. It may be concluded, then, that survival of the young mouse after weaning at 21 days depends on a combination of having achieved a critical weight and receiving sufficient protein.

As stated above, there is significant interaction between the two variables affecting age at vaginal opening. As graph #19 indicates, the earliest maturational age means observed were, as expected, in the 50% and 25% protein diets. In all but the 6% protein diet groups, however, the earliest maturation occurred under 6 hours of light per day. This appeared to be the optimal lighting condition for early maturation.

The 6% diet group reacted in a completely opposite fashion. In this diet group, the earliest maturation occurred at 0 hours of light per day and rose drastically in the 6-hour light group. Only in this 6% diet group did increasing light result in earlier maturation, agreeing with Fiske (1941) and others who reported earlier maturation in animals exposed to constant light, and with Hoffman and Reiter (1965), who reported depressed maturation rates in

hamsters kept on "short days" of 2 hours of light per 24.

Fiske did not test varying protein levels and it may be seen that in the 25% diet, which approximates the amount found in standard lab chow, animals kept under 12 hours light/day did mature more slowly than animals kept in continual light.

If these were the only observations made, one could conclude that more light led to earlier maturation; however, the presence of the other light and diet groups proves this to be an erroneous assumption.

The data also suggest that, in this 6% diet group at least, there is a difference between fluctuating and non-fluctuating light rhythms in their effects on the organism. It can be noted that the greatest amounts of negative interaction between the variables occur in this diet group under 0 and 24 hours of light, while the greatest positive interaction occurs in this same diet group at 6 and 12 hours of light. A similar pattern does not occur in the other light groups; however, the 6% diet group appears to be particularly sensitive to light fluctuation, and this may bear further investigation.

When the diets are considered separately, it can be seen that one of the effects of increased protein on the groups is to decrease the range of dates of vaginal opening from 47 in the 6% protein diet through 26 in the 12% diet and from 14 in the 25% diet to 11 in the 50% protein group (see

graph #10). These results agree with the observations of Kennedy and Mitra (1963), who noted that the achievement of full fertility, vaginal opening, and first oestrous was strung out over a longer period of time in animals fed a low protein diet. Similar results, noting irregular oestrous cycles, were also reported by Nakagawa and Masana (1971).

It should be noted here that the mortality in the 6% diet group in this experiment was 17.2%. It has been suggested elsewhere in this paper that this group of animals experienced selective pressures favoring those who could survive on less protein. The result of such selection could lead to a group of animals who can thrive on minimal amounts of protein. Combine these animals with animals whose need for protein is closer to the average and whose maturation can be delayed by a lack of sufficient protein, and a broad flat curve such as is seen in graph #10 could result.

These results indicate there are certain optimal combinations of light and protein which will result in early vaginal opening and other combinations which will delay vaginal opening. The effect of any particular combination is not predictable along any set trend or pattern. If we do not consider the 6% protein diet group, we can say that increased protein depresses the age of vaginal opening, and that the most rapid maturation in all diet groups occurs under conditions of 6 hours of light per 24-hour period. What is clear is the outcome of each light/diet combination is due to an

interaction of the two variables and not to the influence of one or the other.

No significant figures were obtained in measuring the number of days from vaginal opening to oestrum I or from the number of days from vaginal opening to the first adult smear. This does not agree with the results of Nakagawa and Masana, mentioned above, who reported an increase in the range of the occurrence of first oestrous under conditions of low dietary protein.

In this study, the date of oestrum I was taken as the first day on which the slide showed a substantial amount of cornified epithelial cells. Usually the first smear, taken on the day of vaginal opening, showed a few scattered cornified cells, but this was usually followed by a time lag longer than would normally be expected in a normal cycle before such cells appeared again. The smears taken for a day or so after vaginal opening generally contained scattered and sketchy cellular material and sometimes none at all. It may be that the appearance of a few cornified cells in the smear on the day of vaginal opening is the result of the same elevation in blood steroid levels responsible for the opening. If this is so, we could assume slight initial cornification and then a gap of a day or so before normal cycling begins. This would agree with the observed data; however, another study would be necessary to establish such a connection. The day of the first "adult" smear was taken as the first day on

which a substantial amount of cellular material was visible on the slide. What constituted a "substantial" amount varied, of course, from animal to animal. In many cases, both the above events occurred simultaneously, indicating the animal may respond to adult levels of steroid hormones by immediately going into oestrous.

In most cases, after approximately 15 days of daily vaginal smears the slide began to show repetitive cornification of the vaginal epithelium. This was taken to be a result of the vaginal smear techniques as this problem had been reported by other workers using a cotton swab to obtain vaginal smears from rats (Emerg and Schwerd, 1936). However, Browman (1937) has reported exposure to continual light resulted in 80% of vaginal smears in a large group of females showing cornification of the vaginal mucosa. Similar results were reported by Krisna Murtly and Russfield (1970) and by Doone (1971). Doone suggests that this constant oestrous syndrome can be explained by a hypersecretion of FSH and LH which he found to be constant in rats exposed to continual illumination.

It is not clear at the present if there is a pattern in the constant cornification of the vaginal epithelium observed in this study that could be attributed to the effects of light.

Weight at vaginal opening showed significant interaction between the two variables at the 1% level. Diet is

significant, with a shift toward higher weight with increasing protein. This does not agree with the material presented by Fresch and Revelle (1970) and by Monteiero and Falconer (1966), which suggested sexual maturation occurred when triggered by the achievement of a critical weight. However, data presented by Vandenburgh (Vandenburgh et al., 1972) disagree with these authors and agree with the results presented here.

In this data set, the highest weights occurred in total darkness in the 50% protein group, while the same light group had the lowest weight in the 25% diet group. This last cell, with a mean of 13.2, was comparable to the 6% diet mean of 13.3 in the same light group. If the 0 light group is omitted, the curves of all the diet groups show a decreased weight gain at 12 hours of light. This would indicate that this light/dark rhythm is least favorable for producing heavier mice at vaginal opening no matter what the protein content of the diet.

A somewhat similar pattern is seen in the graph of cell means for weight gain from weaning to vaginal opening (#20) and, to a lesser degree, in the cell mean graphs for Total Weight Gain (#23). In the former, the 6-hour light group appears to be a balance point. The lowest weight gains are in this group regardless of diet and increase with increasing light or with no light. The 6% diet group does now conform to this pattern, however, and is in fact almost

exactly opposed, with the highest weight gains at 6 hours of light and decreasing gains with more and less light.

The cell mean graph of total weight gain is somewhat like the above. The 6% diet group curve is totally opposed to those of the other diet groups, with the greatest weight at 6 hours of light and smaller gains with more and less light. The "low point" of the other diet curves is, however, not clearly defined.

In the graph for Weight at Sacrifice (#24), we can see the pattern of the curves does not closely resemble any of the other weight gain graphs. If we compare it to the graph of Total Weight Gain (#23), we see that the 6% diet group curves are similar, with the lowest weights in the 0- and 24-hour light group and a central peak in the 12-hour group. The 12% diet also shows the highest weight in the 12-hour group and these curves are also similar in the two graphs. The 25% and 50% diet curves resemble to a greater extent the curves for these same diet groups in graph #21, but the fit is not a good one.

Light appears to affect the lower protein diets and higher protein diets differently. The lower protein diets show the highest weights in the 12-hour light group and then a decrease with increasing light. The 50% diet shows a steady decrease in weight with increasing light, then a sharp weight increase in the 24-hour group. The 25% group is most erratic, first increasing from 0 to 6 hours of light and then

decreasing to its lowest point at 12 hours of light. From there, the increase in weight is marked, and exceeds the 50% diet group at 24 hours.

There is no overall pattern for this group which describes the direction of interaction of light and diet. Instead, the effect of light appears to differ, depending on the amount of protein in the diet. Low protein diets react favorably to small amounts of light and less favorably as the light increases past the 12-hour/day mark. On higher protein diets the opposite occurs.

It can be noted here that the curve for the 25% protein group from 6 hours to 24 hours is very similar to that shown by Lockhart (1963), who raised rats fed standard lab chow under varying light intensities on a 12-hour light/dark schedule. The similarity may indicate a valid comparison could be made between varying light intensities and varying hours of light at the same intensity in their influence on weight at maturity. The determination of the relationship would require additional study.

When we look at the weight gain from vaginal opening to sacrifice (graph #22), we begin to see why the previous data sets are unclear. Here the greatest weight gain is seen in the 12% diet group under 12 hours of light and the least in the 50% group under 18 hours of light. The 50% diet group curve is, in fact, low in this data set in all light groups. This is in partial agreement with Holt (Holt et al., 1966),

who notes that animals on extremely high protein diets (over 50%) appear to have reduced growth rates. It may also be that the curve for the 50% group is low due to these animals having achieved their maximal growth earlier in their maturation period due to the availability of adequate protein.

In this data set, as in others, the 12-hour light group appears to act as a central point for the curves. It is the high point in the 6% and 12% and 50% curves and the low point in the 12% curve.

In general, the effects of the two variables on weight gain appear to be an increased weight under increased dietary protein, with light acting to increase the weight and the weight gains as the photoperiod increases in length from 6 or 12 hours to 24.

The variations seen in the data sets charting weights and weight gains reflect the varying growth rates of animals at different times in their life cycle. During these variations in growth rate, it appears that two variables, each influencing the growth rate, fluctuate in the expression of that effect at different times. Thus, deprivation of protein prior to vaginal opening may have a far greater effect on growth and weight gains than a similar deprivation after vaginal opening. Similarly, an animal who has shown accelerated growth due to high protein levels may show an earlier cessation of that growth than an animal whose growth has been retarded by a low-protein diet. Add to this the light

variable, which also affects the growth curve, and the pattern will become even more complex, as shown.

In the graph of cell means for Atlas-Anus Length at Sacrifice, we see that the four diet group curves show very little overlap and that the effect of increased protein appears to be an increase in body length. As in the weight at sacrifice, the 12% diet curves lie mostly above that of the 25% protein diet in all light groups. Light reinforces the effect of protein, at least in part, in all but the 6% diet group. (The extreme drop in the 50% diet/24-hour light cell can be attributed to two animals who were extremely stunted. Without these two animals, the size decrease for this cell is still present, but is much less pronounced.) If a "balance" point is sought in this data set, it seems to be in the 6-hour light group with increasing or decreasing length moving away from that point in all diet groups.

Only the larger organs showed interaction at a significant level between the two variables. Both liver and spleen showed diet to be significant at the 1% level. This agrees with studies done reporting hypertrophy of the liver in diets containing high percentages of protein. It is noted, however, that this increased size could be a result of increased liver function (Addis and Walter, 1939). Falls also reports smaller spleens on protein deficient diets (1958).

The cell mean graphs for both of the above organs (#28 and #29) show that the 6% protein diet group curve

falls far below those of the other diet groups, but the curves are generally similar for all diet groups. Most of them peak in either the 6- or 12-hour light group, fall sharply with increasing light, and then rise slightly in the 24-hour light group. Although the graphs are not a perfect fit to those charting weight gains, it is suggested that they reflect weight gains.

In the weight at sacrifice of the ovaries, we see that diet does significantly influence the weight of the organ, with the most dramatic differences occurring between the 6% and higher protein diets, all of which show similar curves in the same range. There is a slight tendency to lighter ovarian weights with increasing light, but this tendency is not significant. This does not agree with Widdowson (Widdowson et al., 1964), who reported precociously enlarged ovaries with no corpora leutea in females fed low protein diets. Other workers have reported an increase in ovarian weight under conditions of constant light (Wurtman et al., 1961). I did not observe such a response, and my results agree with those of Reiter and Fraschini (1961), who reported no abnormally large gonads under continual light.

The size of the uterus is related to ovarian activity and also shows diet as the significant variable. As in the raw data graphs of ovarian weight (#15), the major shift in this group is most pronounced between the 6% protein diet and higher protein groups. Interaction between the two variables

is present at the 5% level, with light appearing to depress uterine weight. Ovarian weight also decreased with increased light, and the combination may indicate less activity of these glands; however, such a statement would require more precise monitoring of gland activity under varying conditions of light. In most of the diet groups there is a drop in uterine weight in the 0- and 24-hour light groups, although this is not clearly seen in the graph of cell means (#27).

The data set of adrenal weight at sacrifice shows no interaction between the variables but is the only set in which light is significant and diet not. This disagrees with studies that report increase in adrenal size with increase of dietary protein (De Costa and Clayton, 1952; Tepperman et al., 1963). In this data set the raw data graphs show a slight tendency for the weight of the gland to increase under 6 hours of light while remaining fairly constant in the other groups. It is customary to attribute an increase in adrenal weight to a stress condition, resulting in increased adrenalin production and an increase in the size of the adrenal medulla. We should not rule out the possibility that this weight increase here is the result of increased cortical activity; however, it is more likely to be due to stress. The frequency with which the 6-hour light group is mentioned as a group in which a major amount of interaction is occurring in preceding data sets should be remembered here. It may be that the 6-hour light group does place more

strain on the animal than the other groups and that this is reflected by the greater adrenal weight in this light group.

Neither light nor diet is significant in the weight of the pituitary gland at sacrifice and there does not appear to be any significant interaction between the two variables. The raw data graphs of the diet groups (#14) do seem to show a certain shift toward heavier glands with a higher protein diet, but the range is very small--.5 to .3 mg. This increase can be noticed in all but the 0- and 24-hour light groups. I found no evidence of hypophyseal atrophy in the lower protein diets as reported by Keys et al. (1950) and Fallis (1958).

This supports the work done which indicates this "master gland" may have some sort of first claim over nutrients coming into the organism. As the single gland that monitors and controls so many of the body's functions, it is essential to the organism that the pituitary function at peak efficiency. It appears that the pituitary will receive its optimal requirements even if other body systems must be deprived to do so. A similar situation has been described for the brain (Riesenfeld, 1967). In this study, rats were raised under conditions of extreme starvation. Body weight differences between starved animals and normal controls showed the starved group weighing over 80% less. The brain in both groups, however, remained almost entirely unaltered

in size. As the pituitary is part of the brain, we can expect it to respond in a similar fashion.

In general, the data presented in Table XIV follow the pattern expected in an experiment designed to test the effect of increased dietary protein on maturation. We see significance between the 25% and 6% protein diets in Weight at Vaginal Opening, Weight at Sacrifice, and Atlas-Anus Length at Sacrifice, which last also shows significance between the 25% and 12% protein diets. In most of the above-mentioned data sets, the means show an increase in weight gain and length increase with increased dietary protein.

Significance at the 1% level is seen between the 25% and 6% protein diets and between the 25% and 12% in Number of Days--Weaning to Vaginal Opening. The number of days necessary for vaginal opening shows a sharp decrease as one goes from the 6% to the 25% protein diets and then increases slightly again as the dietary protein increases to 50%.

Organ weights at sacrifice show a similar pattern, with significance in all but Uterus and Spleen weights between the 6% and 25% diet groups and between the 12% and 25% protein group in Ovary and Liver weights. Again the means show a tendency to an increase in weight with an increase in dietary protein.

In general, the effects of increased dietary protein in this "natural" light box group appear to be to increase growth and weight gains both before and after vaginal opening,

increase gross organ weight, and shorten the time necessary for vaginal opening. There are no surprises here. These results confirm those reported by earlier workers.

In many of the raw data graphs of the effects of light it can be seen that the 0- and 24-hour group curves resemble each other, while the 6-, 12-, and 18-hour groups form a separate cluster with a tendency to grade in one direction or another. Similarly, it should be noted here how frequently the curves in the graphs of divers cell means have a "central point" in the 6- or 12-hour group, indicating a very strong reaction, either positive or negative, to that particular light stimulus. These tendencies do not give "significant" results when subjected to statistical analysis, but the patterns are too persistent to be totally ignored. It is possible that there is a difference in the response of the organism to fluctuating vs. non-fluctuating light stimuli. The similarity of many of the light group curves in raw data graphs between 0 and 24 hours of light seems to show the organism is responding equally to both--that is, it is the rhythms of photoperiods, or rather the lack of rhythm, that is influencing the outcome. This suggests that some further investigation should be done of the differences in reaction of animal groups to fluctuating and non-fluctuating light.

The results of this experiment indicate that the two variables interacted to affect the organism, but not always in an easily predictable way. In most of the observations

made, it is the amount of dietary protein that appears to have the strongest effect, with the hours of light/24 hours mediating that effect. This mediation can, in some light/protein combinations, reinforce the effects of dietary protein and, in others, interfere with them. It is not always possible to determine the direction of this interaction. Other experimental work mentioned in this paper indicates that the rate of sexual maturation increases with increasing number of hours of light. In this experiment, the presence of the second variable obscures the effect of light.

Most important to this study was the effect of the variable in the length of time from weaning (a constant at 21 days) to vaginal opening. ANOVA showed significant interaction at the 1% level between the two variables for this data set, indicating that varying combinations of light/day and dietary protein can influence the rate of sexual maturation of an animal. This supports the suggestion made earlier in this paper that the age at which the female mouse achieves sexual maturity is controlled by a combination of environmental variables.

These results indicate that any analysis of the effects of one or another environmental variable on the rate of sexual maturation in the female must take into consideration the context of that variable. If several factors are experimentally proven to affect maturation, all must

be taken into consideration when making observations on "natural" populations.

It is highly probable that it is just such interaction of the effects of several environmental variables that results in the lack of a well-defined "cline" of any sort in the menarcheal ages of human populations. Two populations that receive the same amount of light per day may not receive similar protein amounts, and so on. The world-wide trend to earlier menarche is doubtless attributable to increased high quality protein in the diet, but the interaction of this factor with the other variables still will not allow a neat pattern of menarcheal age in world populations to be seen.

More work obviously needs to be done. As mentioned earlier, the effects on the organism of light intensities, varying photoperiods, varying light rhythms, and light wave lengths all must be explored further before we have proper information of even this one environmental variable. The import of this study, however, is clear. There is interaction between environmental variables--in this case light and dietary protein. This interaction will influence the maturation rate of the female mouse. Some light/protein combinations reinforce each other well and produce more rapid maturation than other combinations. This study indicates that such optimal combinations are not always of the highest protein and longest light period, but those combinations the body's own physiology uses most efficiently.

CONCLUSION

This experiment indicates that there is interaction between light and diet which influences the rate of maturation of the female mouse. The most effective light/protein combination of those tested was 50% under 6 hours of light per 24-hour period. This combination produced the earliest maturation, with a cell mean of 10 days after weaning.

This increased maturation rate was not mirrored by increased weight gains at vaginal opening, thus disproving the suggestion that sexual maturation is triggered by achievement of critical weight. It is true, however, that the 50%/6-hour cell showed a high (though not the highest) weight gain at sacrifice and that the same cell showed the highest adrenal weights at sacrifice, indicating what may be a stress condition.

In terms of the general effect of the interaction, we see that increased protein does result in earlier maturation and that the general effect of increased light is to reinforce the effect of increased protein. The results of the interaction of the two, however, do not cause a smooth decrease in maturational age with increased light and protein. Some light periods seem to be more amenable to earlier vaginal opening than others--specifically, 6 and 18 hours of light per 24-hour period.

This is not true for all diet groups, however, nor do all diet groups respond to all light groups the same way. The response of the 6% diet group, for example, is most erratic, providing the earliest vaginal opening of all in the 0- and 24-hour light groups, and very late vaginal opening in the 6- and 12-hour light groups.

The experiment results also hint at a difference in reaction of the organism to an absence of light rhythms.

These results indicate that any projection of the anticipated rate of sexual maturation in the female mouse must consider that rate as a product of the interaction of light and dietary protein. Both act as significant factors.

That this interaction was missed for so long can be attributed to the constancy of light/dark periods in most laboratories and to the fact that light, studied alone, does not produce significant results under conditions of varying protein.

If this same effect is true for human populations, it explains the discrepancies found in comparing maturational rates and menarcheal ages between human groups. When one seeks to chart human groups in terms of one or another of these variables, the overall picture is unclear because these environmental variables are interacting with one another. We could, then, say that the observed age of menarche is nothing more nor less than the resultant of a series of forces acting on the organism. For us to formulate a meaningful picture of

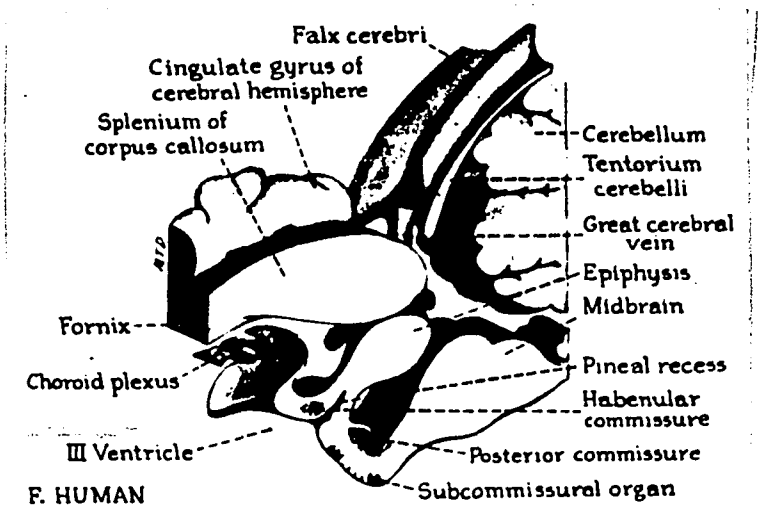
variation in maturation rates in the human species, it will be necessary for us to acquire a more complete knowledge of the environmental variables that affect female sexual maturation and a better understanding of how these variables interact in the organism.

Given the infinite variability of light and dietary protein in world populations, this would seem an impossible task, but this is a beginning. The interaction indicated here to exist between light and protein in influencing the rate of sexual maturation is another link in a long chain of facts that will ultimately lead to a good understanding of what controls maturation.

PLATES

PLATE I

The Human Pineal Gland



Diagrammatic representation depicting longitudinal slice from the diencephalic roof region of the brain. The facing surface of the slice has been taken at or near the median sagittal plane of the brain. Overlying cranial and/or integumental components are shown, particularly in relation to several pineal derivatives. This diagram serves to illustrate the various pineal components, their innervation, their vascularization, and their relationship to other diencephalic circumventricular organs.

(Wurtman, Axelrod, and Kelly, 1968)

PLATE II

Box Unit

A. Cutaway detail of box lid, showing overlapping lip.

B. Enlarged cutaway view of air vent, showing baffles to permit air flow and prevent light.

C. Plan view of one light box, showing placement of:

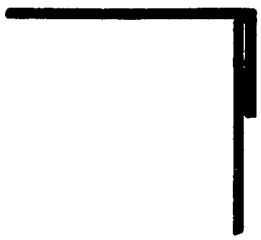
   a. Vent

   b. Lights

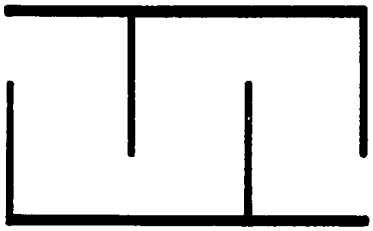
   c. Timer switch

   d. Shaded pole blower

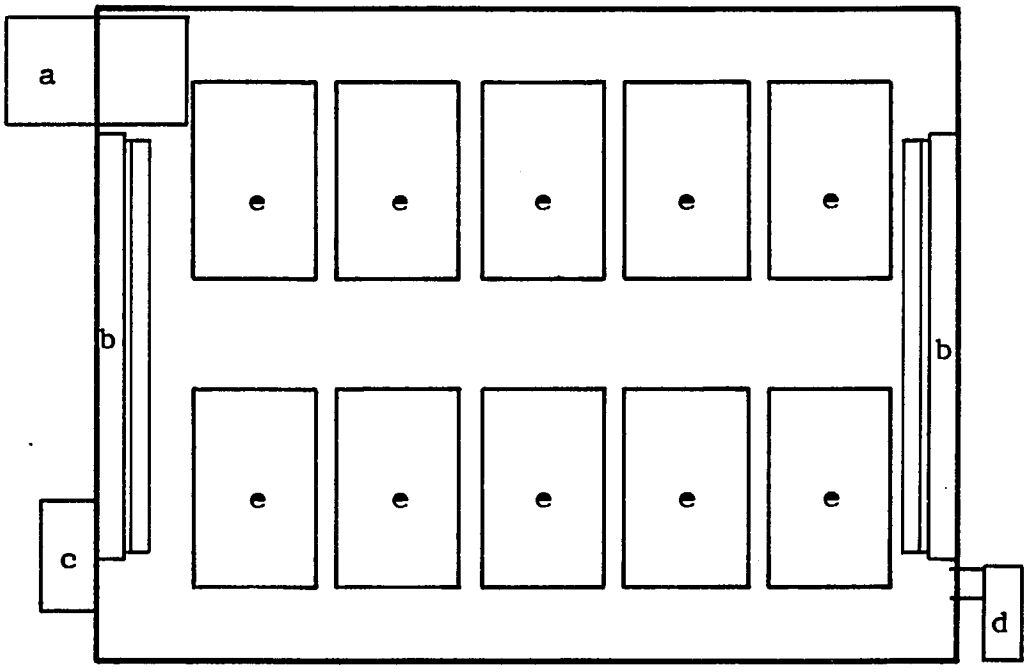
   e. Cages



A.



B.



C.

PLATE II

TABLES

TABLE I

Mean Menarcheal Ages in World Populations

Location	Sample Size	Mean Age of Menarche
Pt. Barrow, Alaska (Levine, 1953)	122	14.42
Warsaw, Poland (Milicer and Szcyatka, 1966)	3,918	13.01
Wroclow, Poland (Zukowski et al., 1964)	1,621	12.60
West Riding, England (Poppleton & Brown, 1966)	685	13.4
Southern England (Wilson and Sutherland, 1953)	2,590	13.49
Constanzia, Roumania (Stukovsky et al., 1967)	581	13.47
Florence, Italy (Boutourline-Young et al., 1963	111	12.22
Malta (French, 1964)	662	13.20
Tel Aviv, Israel (Ber and Breciner, 1964)	500	13.03
Havana, Cuba (Pospislova et al., 1965)	1,560	12.98
Hong Kong, China (Lee, Chang, and Chan, 1963)	3,278	12.51
Assam (Foll, 1961)	1,150	13.21
Burma (Foll, 1961)	704	13.25
Central India (Thompson, 1952	411	14.65
Lagos, West Africa (Ellis, 1950)	142	14.3
N. Nigeria (Wilson and Sutherland, 1953)	74	14.3
Eastern Nigeria (Tanner and O'Keefe, 1962)	346	14.1

TABLE I (Continued)

Location	Sample Size	Mean Age of Menarche
Ceylon		
Rural	296	14.39
Urban (Wilson and Sutherland, 1953)	844	12.84
Surinam (Doornbos, Jonnes, and Visser, 1960)	53	13.1
Rio de Janeiro, Brazil (Eveleth, 1966)	198	12.60
South Africa (Kark, 1956)	1,259	13.60
Melbourne, Australia (Towns et al., 1966)	975	13.2

Table II

World Climatic Regions

Types and Subtypes	
A. Tropical forest climates; coolest month above 64.4°F	a. Warmest month above 71.6°F
	b. Warmest month below 71.6°F
B. Dry climates: BS--Steppe or semiarid BW--Desert or arid	c. Less than four months over 50°F
C. Mesothermal forest climates: coldest month above 32°F but below 64.4°F, warmest month above 50°F	d. Same as c but coldest month below -36.4°F
	e. Constantly moist, rainfall all through year
D. Microthermal, snow-forest climates; coldest month below 32°F, warmest month above 50°F	h. Hot and dry, all months above 32°F
	k. Cold and dry, at least one month below 32°F
E. Polar climates; warmest month below 50°F ET--Tundra; warmest month below 50°F but above 32°F EF--Perpetual frost; all months below 32°F	m. Monsoon rain, short dry season but total rainfall sufficient to support rain forest
	n. Frequent fog
	n'. Infrequent fog but high humidity and low rainfall
	s. Dry season in summer
	w. Dry season in winter

Goode's World Atlas, 1960, ed. E. B. Espenshade, pp. 8-9.

TABLE III

Relative Amounts of Protein and Carbohydrate in Diets #1 Through #5

Diet #1	0% protein 86% starch
Diet #1	6% protein 80% starch
Diet #3	12% protein 74% starch
Diet #4	25% protein 61% starch
Diet #5	50% protein 36% starch

In all diets the protein used was Nutritional Biochemicals Vitamin Free Casein. The carbohydrate was corn starch.

TABLE IV

Number of Hours of Light per Twenty-four Hour Period

Box #1	0 hours (total darkness)
Box #2	6 hours
Box #3	12 hours
Box #4	18 hours
Box #5	24 hours (no dark period, continual light)
Box #6	Natural light (same amount as received in lab through windows and ceiling lights)

TABLE V

Data Obtained for Each Animal

Weight at weaning
Weight at vaginal opening
Number of days--weaning to vaginal opening
Weight gain--weaning to vaginal opening
Weight at sacrifice
Weight gain--vaginal opening to sacrifice
Total weight gain--weaning to sacrifice
Atlas-anus length at sacrifice
Number of days--vaginal opening to Oestrum I
Number of days--vaginal opening to first adult smear
Adrenal weight at sacrifice
Uterus weight at sacrifice
Ovary weight at sacrifice
Spleen weight at sacrifice
Liver weight at sacrifice
Pituitary weight at sacrifice

TABLE VI

Mortality Figures

Diet Groups

Diet #1--0% Protein: 100% mortality
28 deaths out of 28 animals
Average weight at weaning 7.52 grams
Range 4.5 - 10.5 grams

Diet #2--6% Protein: 17.2% mortality
10 deaths out of 58 animals
Average weight at weaning
(a) Dead group 6.8 grams
Range 5 - 12 grams
(b) Live group 10.37 grams
Range 2.5 - 15.0 grams

Diet #3--12% Protein: 0% mortality
0 deaths out of 31 animals
Average weight at weaning 7.47 grams
Range 4 - 10 grams

Diet #4--25% Protein: 0% mortality
0 deaths out of 38 animals
Average weight at weaning 9.12 grams
Range 6.5 - 12.5 grams

Diet #5--50% Protein: 0% mortality
0 deaths out of 32 animals
Average weight at weaning 8.1 grams
Range 6 - 14 grams

Light Groups

Darkness: 25.7% mortality
9 deaths out of 35 animals
Average weight at weaning
(a) Dead group 7.75 grams
Range 6 - 12 grams
(b) Live group 9.52 grams
Range 7 - 13 grams

TABLE VI (Continued)

Light Groups (Continued)

Six hours light: 18.1% mortality
4 deaths out of 22 animals
Average weight at weaning
(a) Dead group 8.88 grams
Range 8.5 - 9.5 grams
(b) Live group 8.55 grams
Range 4 - 15 grams

Twelve hours light: 30.0% mortality
9 deaths out of 30 animals
Average weight at weaning
(a) Dead group 6.33 grams
Range 5 - 7 grams
(b) Live group 8.55 grams
Range 5 - 15 grams

Eighteen hours light: 16.6% mortality
5 deaths out of 30 animals
Average weight at weaning
(a) Dead group 7.33 grams
Range 4.5 - 9.5 grams
(b) Live group 8.45 grams
Range 6 - 14 grams

Twenty-four hours light: 15.8% mortality
6 deaths out of 38 animals
Average weight at weaning
(a) Dead group 8.10 grams
Range 5 - 10.5 grams
(b) Live group 9.56 grams
Range 5 - 15 grams

Control light: 15.6% mortality
5 deaths out of 32 animals
Average weight at weaning
(a) Dead group 5.60 grams
Range 4 - 6.5 grams

(b) Live group 8.46 grams
Range 2.5 - 11.5 grams

TABLE VI (Continued)

Light Hours	% of Protein in Diet					
	0%	6%	12%	25%	50%	Total
0	4	5	0	0	0	9
6	4	0	0	0	0	4
12	5	4	0	0	0	9
18	5	0	0	0	0	5
24	6	0	0	0	0	6
Natural	4	1	0	0	0	5
Total	28	10	0	0	0	38

TABLE VII

Number of Animals in Each Cell of the Experiment

Light Hours	% of Protein in Diet				
	6%	12%	25%	50%	Totals
0	9	6	6	5	26
6	4	6	4	4	18
12	6	5	5	5	21
18	7	5	7	6	25
24	15	5	7	5	32
Natural	7	4	9	7	27
Total	48	31	38	32	N = 149

TABLE VIII

Results of Variance Analysis

	Diet	Light	Inter-action
Day Counts			
Weaning to Vaginal Opening	5.37*	2.32	2.35*
Vaginal Opening to Oestrum I	0.19	0.60	0.90
Vaginal Opening to First Adult Smear	1.51	1.09	0.72
Weights, Weight Gains, and Length			
Weight at Vaginal Opening	17.28*	1.09	2.62*
Weight Gain--Weaning to Vaginal Opening	31.41*	2.73+	2.02+
Weight at Sacrifice	21.92*	1.60	3.67*
Weight Gain--Vaginal Opening to Sacrifice	3.03	1.20	2.66*
Total Weight Gain--Weaning to Sacrifice	27.81*	0.54	2.81*
Atlas-Anus Length at Sacrifice	3.10	1.24	3.47*
Gland and Organ Weight at Sacrifice			
Adrenal	0.36	5.06*	0.99
Pituitary	2.06	0.19	1.20
Ovary	17.78*	3.16	1.22
Uterus	13.83*	0.22	2.18+
Spleen	19.87*	1.84	2.48*
Liver	12.09*	2.50	2.21*

* indicates significance at the 1% level.

+ indicates significance at the 5% level.

Note: In the following ANOVA tables,

A = Diet, B = Light.

TABLE IX

Number of Days--Weaning to Vaginal Opening

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	962.86	3	320.95	5.37
B	556.60	4	139.15	2.32
AB	1,692.78	12	141.07	2.35
Error	6,099.58	102	59.80	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	12.89	1.79	-6.9	23.67	4.38	4.7
6	29.25	4.48	9.8	14.17	1.96	-4.5
12	29.67	7.27	5.1	22.40	3.30	-1.4
18	15.71	5.21	-2.6	17.00	1.82	-0.4
24	13.40	3.60	-5.4	19.80	1.66	1.8
Total Group	16.21			19.37		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
14.00	1.79	0.2	15.40	1.61	2.0	16.12
11.25	1.67	-2.2	10.00	1.54	-3.0	15.94
17.80	1.99	-0.8	15.40	1.84	-2.8	21.71
14.43	1.86	2.1	12.83	1.86	0.9	14.92
13.57	1.90	0.7	15.40	1.91	3.0	14.75
14.28			13.92			

TABLE X

Number of Days--Vaginal Opening to Oestrum I

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	3.68	3	1.29	0.19
B	14.82	4	3.71	0.60
AB	66.94	12	5.58	0.90
Error	629.85	102	6.18	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	2.33	0.57	-1.5	3.83	1.23	0.3
6	4.00	1.32	0.9	2.67	0.77	-0.2
12	2.67	0.43	-0.1	1.60	0.48	-0.9
18	4.43	1.40	0.9	3.80	2.12	0.5
24	3.07	0.77	-0.1	3.40	0.73	0.4
Total Group	3.05			3.07		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
3.83	1.56	0.4	4.20	1.27	0.9	3.39
1.25	0.55	-1.5	3.50	1.35	0.9	2.83
3.60	0.91	1.2	2.20	0.90	-0.1	2.52
2.86	0.65	-0.4	2.17	0.71	-0.9	3.32
3.29	0.96	0.4	2.00	1.08	-0.7	3.00
3.07			2.76			

TABLE XI

Number of Days--Vaginal Opening to First Adult Smear

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	14.50	3	4.83	1.51
B	13.98	4	3.50	1.09
AB	27.91	12	2.33	0.72
Error	325.99	102	3.20	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	2.33	0.60	-0.2	2.17	0.38	0.2
6	2.25	0.53	-0.9	2.00	0.56	-0.7
12	2.67	0.60	0.6	1.00	0.38	-0.6
18	2.14	0.74	-0.2	2.40	1.05	0.6
24	2.80	0.71	0.6	2.20	0.44	0.5
Total Group	2.43			1.96		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
3.00	0.71	-0.0	2.60	0.33	0.0	2.50
4.25	1.66	0.6	4.25	0.88	1.0	3.06
2.80	0.54	0.2	2.00	0.87	-0.2	2.14
2.71	0.58	-0.1	2.17	0.74	-0.2	2.36
2.14	0.71	-0.6	1.60	0.95	-0.6	2.38
2.86				2.44		

TABLE XII

Weight at Vaginal Opening (in gms)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	219.40	3	73.13	17.29
B	18.46	4	4.62	1.09
AB	133.18	12	11.10	2.62
Error	431.60	102	4.23	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	13.28	1.65	-0.6	16.75	1.55	1.3
6	14.25	1.53	-0.1	14.75	1.76	-1.2
12	13.83	1.65	0.6	14.50	1.44	-0.3
18	14.21	1.51	0.1	15.30	1.52	-0.3
24	14.37	1.59	0.3	16.50	1.56	0.6
Total Group	13.99			15.57		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
13.17	1.64	-2.7	19.90	1.45	2.1	15.33
17.00	1.47	0.7	19.00	1.51	0.7	16.08
15.40	1.47	0.2	16.60	1.43	-0.5	15.02
16.43	1.77	0.4	17.75	1.64	-0.2	15.90
17.71	1.68	1.4	16.20	1.79	-2.0	15.70
15.97			17.84			

TABLE XIII

Weight Gain--Weaning to Vaginal Opening (in gms)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	516.25	3	172.08	31.41
B	59.97	4	14.99	2.73
AB	132.76	12	11.06	2.02
Error	558.80	102	5.48	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	1.72	0.87	-1.6	8.00	0.89	-1.1
6	5.50	1.37	3.1	5.58	1.32	-0.5
12	2.50	1.13	-0.4	7.30	1.60	-0.0
18	3.93	1.22	-0.3	7.70	1.22	1.0
24	3.47	0.87	-0.9	9.70	1.14	0.6
Total Group						

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
5.67	1.22	-1.1	11.00	1.20	2.1	5.87
5.38	0.94	-0.5	6.63	1.23	-1.4	5.75
6.30	1.06	-0.0	8.60	0.84	0.2	6.00
8.71	1.02	1.0	9.83	1.09	0.0	7.44
8.43	0.94	0.6	9.00	1.06	-0.9	6.39
7.14			9.14			

TABLE XIV

Total Weight Gain--Weaning to Sacrifice (in gms)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	908.19	3	302.73	27.81
B	23.31	4	5.83	0.54
AB	367.72	12	30.64	2.81
Error	1,110.31	102	10.89	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	4.83	1.78	-1.9	11.91	1.80	-1.0
6	9.63	1.55	3.7	11.42	1.12	-0.7
12	6.75	2.13	0.2	15.20	2.45	2.5
18	6.43	1.70	0.2	13.10	1.04	0.7
24	5.10	1.47	-2.2	12.10	1.27	-1.4
Total Group	6.55			12.67		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
13.50	1.87	1.2	15.20	1.52	1.8	10.46
9.63	1.24	-1.9	11.63	1.37	-1.0	10.67
9.00	1.04	-3.1	13.60	1.53	0.4	10.93
13.43	1.57	1.6	10.42	1.36	-2.5	10.68
15.07	1.11	2.2	15.30	1.13	1.3	9.97
12.55			13.18			

TABLE XV

Weight Gain--Vaginal Opening to Sacrifice (in gms)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	73.14	3	24.38	3.03
B	38.85	4	9.71	1.20
AB	256.68	12	21.39	2.66
Error	819.68	102	8.04	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	3.33	1.42	0.1	3.83	1.28	-1.3
6	4.13	1.52	0.5	5.83	1.23	0.3
12	4.25	1.47	0.5	7.90	1.06	2.2
18	2.50	1.16	0.4	5.40	1.00	1.4
24	1.50	1.11	-1.5	2.40	0.41	-2.5
Total Group	3.99			5.06		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
6.42	0.86	1.4	4.20	0.45	-0.1	4.33
4.25	0.93	-1.2	5.00	0.96	0.3	4.92
2.70	1.04	-2.9	5.00	1.31	0.2	4.93
4.72	1.03	0.8	0.58	0.85	-2.6	3.24
6.64	0.93	1.8	6.30	1.00	2.2	3.52
5.12			4.04			

TABLE XVI

Weight at Sacrifice (in gms)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	426.45	3	142.15	21.91
B	41.61	4	10.40	1.60
AB	285.71	12	23.81	3.67
Error	661.42	102	6.48	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	16.50	2.14	-0.6	20.58	2.28	-0.1
6	18.00	2.11	0.1	20.58	1.88	-0.9
12	18.08	2.32	1.1	22.40	1.94	1.8
18	16.71	2.15	0.7	20.70	1.92	1.1
24	15.89	1.98	-1.4	18.90	1.86	-2.0
Total Group	17.04			20.63		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
19.42	1.79	-1.5	24.10	1.86	2.1	19.58
21.50	1.89	-0.2	23.75	1.97	0.9	20.92
18.30	2.12	-2.5	21.60	2.13	-0.3	20.00
20.46	2.09	1.0	18.00	1.89	-2.9	18.98
24.36	1.86	3.2	22.50	1.81	0.2	19.25
21.05			21.76			

TABLE XVII

Atlas-Anus Length at Sacrifice (in mm)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	349.83	3	116.61	3.10
B	186.16	4	46.54	1.24
AB	1,565.27	12	13.02	3.47
Error	3,832.83	102	37.58	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	61.81	6.01	-0.5	66.50	6.13	0.0
6	64.60	6.16	1.1	64.70	5.97	-3.0
12	63.42	6.06	0.1	65.64	5.93	-1.8
18	60.83	6.04	-2.3	67.70	5.99	0.4
24	61.60	6.00	1.5	68.76	5.95	4.4
Total Group	62.45			66.58		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
63.30	5.88	-2.9	68.72	6.04	3.3	64.57
66.73	5.90	-0.6	69.03	6.13	2.5	66.09
64.58	6.05	-2.6	70.62	5.97	4.2	65.94
66.58	5.90	-0.4	68.60	6.03	2.4	65.68
70.44	5.94	0.5	50.82	13.06	-12.4	62.96
66.51				65.54		

TABLE XVIII

Adrenal Weight at Sacrifice (in mgs)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	4.39	3	1.46	0.36
B	81.15	4	20.29	5.06
AB	37.70	12	3.14	0.99
Error	408.19	102	4.00	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	7.49	1.15	1.2	6.15	1.41	-0.5
6	8.03	1.20	-0.6	8.07	1.05	-0.8
12	6.77	0.72	0.5	7.04	1.52	0.5
18	5.79	0.80	-0.4	6.92	1.24	0.4
24	6.50	0.79	-0.7	7.88	1.30	0.4
Total Group	7.00			7.20		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
6.40	0.83	-0.1	6.28	0.93	-0.6	6.69
9.08	1.26	0.3	10.23	1.08	1.1	8.76
5.78	0.70	-0.6	6.30	0.66	-0.4	6.50
7.13	1.18	0.7	6.12	0.81	-0.7	6.47
7.23	0.92	-0.2	8.34	0.79	0.6	7.16
7.04			7.30			

TABLE XIX

Pituitary Weight at Sacrifice (in mgs)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	5.19	3	1.73	2.06
B	0.60	4	0.15	0.19
AB	12.13	12	1.01	1.20
Error	85.70	102	0.84	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	2.37	0.99	0.9	1.68	0.25	-0.0
6	1.10	0.26	-0.3	1.52	0.19	-0.0
12	1.30	0.19	-0.1	1.58	0.22	-0.0
18	1.07	0.21	-0.2	1.62	0.25	0.1
24	1.03	0.17	-0.3	1.48	0.21	-0.1
Total Group	1.62			1.58		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
1.67	0.17	-0.3	1.44	0.23	-0.6	1.87
1.90	0.16	0.1	2.16	0.18	0.2	1.64
1.74	0.22	-0.1	2.12	0.21	0.2	1.67
1.73	0.18	-0.0	1.88	0.21	0.1	1.56
2.16	0.30	0.3	1.90	0.38	0.0	1.48
1.85			1.88			

TABLE XX

Ovary Weight at Sacrifice (in mgs)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	546.98	3	182.33	17.78
B	129.74	4	32.44	3.16
AB	150.37	12	12.53	1.22
Error	1,045.88	102	10.25	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	5.30	1.18	1.3	9.20	1.87	0.6
6	7.73	2.48	0.4	9.98	1.29	-1.9
12	5.35	1.22	0.4	11.00	1.42	1.4
18	3.17	0.85	-1.1	8.24	1.62	-0.7
24	4.19	0.86	-0.9	10.38	2.13	0.6
Total Group	7.90			9.75		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
7.52	0.96	-0.9	8.36	1.87	-1.0	7.30
11.65	1.59	-0.1	14.20	1.47	1.6	10.79
7.90	1.82	-1.5	9.98	1.81	-0.3	8.41
11.21	2.46	2.5	8.88	1.38	-0.7	7.81
9.40	0.88	-0.1	10.84	1.84	0.4	7.30
9.50			10.45			

TABLE XXI

Uterus Weight at Sacrifice (in mgs)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	64,402.81	3	21,467.60	13.83
B	1,416.14	4	354.04	0.22
AB	40,687.21	12	3,390.60	2.18
Error	158,324.93	102	1,552.21	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	40.13	5.38	1.6	99.52	32.09	7.4
6	49.03	21.05	6.4	116.25	24.18	20.0
12	36.47	11.05	6.5	86.86	8.48	2.3
18	31.07	19.99	-4.5	80.94	25.58	-8.2
24	26.95	7.63	-9.9	68.94	15.49	-21.5
Total Group	36.73			91.79		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
121.13	18.59	29.4	49.12	8.69	-38.4	74.26
70.55	17.69	-25.1	90.28	12.69	-1.3	85.38
63.18	9.39	-20.9	92.00	17.38	12.1	68.33
116.07	21.99	27.4	69.75	10.91	-14.7	74.13
79.24	12.39	-10.8	128.02	22.83	42.2	60.74
92.83			85.01			

TABLE XXII

Spleen Weight at Sacrifice (in mgs)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	21,121.51	3	7,040.50	19.87
B	2,615.74	4	653.94	1.84
AB	10,532.83	12	877.74	2.48
Error	36,150.25	102	354.41	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	48.13	9.38	1.5	72.32	9.78	1.3
6	44.08	13.12	-9.9	79.52	9.31	1.1
12	55.73	10.22	11.0	89.98	9.96	20.8
18	39.23	6.68	0.6	58.38	9.81	-4.7
24	39.43	6.96	-3.2	48.62	7.67	-18.5
Total Group	45.32			70.22		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
79.17	7.44	-0.1	74.26	6.61	-2.7	65.90
89.78	8.59	3.2	90.08	15.29	5.7	76.27
54.60	7.70	-22.7	65.86	8.36	-9.1	66.03
74.71	11.25	3.5	69.60	15.32	0.6	60.28
91.38	10.32	16.1	78.64	8.01	5.6	58.36
78.27			74.87			

TABLE XXIII

Liver Weight at Sacrifice (in mgs)

ANOVA TABLE

Source	ss	d.f.	MS	F
Treatment				
A	4,911,771.32	3	1,637,257.11	12.09
B	1,354,115.36	4	338,528.84	2.50
AB	3,597,996.23	12	299,833.02	2.21
Error	13,807,752.90	102	135,370.13	

DATA SET

Diet	6%			12%		
	Mean	s.e.	Int.	Mean	s.e.	Int.
Light						
0	839.99	119.55	-104.1	1,250.00	141.13	-43.7
6	1,222.88	131.31	151.4	1,292.17	133.83	-129.0
12	1,203.10	174.42	146.8	1,812.00	685.25	406.0
18	707.04	130.07	-56.6	1,013.96	221.33	-99.3
24	830.01	124.67	-137.5	1,181.98	115.14	-134.0
Total Group	1,209.04			1,307.14		

25%			50%			Total Group
Mean	s.e.	Int.	Mean	s.e.	Int.	
1,310.57	114.76	-12.4	1,661.16	126.40	160.2	1,201.12
1,455.63	119.99	5.3	1,600.78	122.95	-27.6	1,381.67
983.90	178.93	-451.3	1,511.60	128.79	-101.6	1,369.34
1,401.34	151.70	258.8	1,217.63	156.44	-102.9	1,085.37
1,545.91	143.10	199.5	1,596.30	123.34	71.9	1,161.34
1,352.97			1,502.17			

TABLE XXIV

"Natural" Light Group

Diet	Means				"t" Test		
	6%	12%	25%	50%	25% & 6%	25% & 12%	25% & 50%
Weight Gains							
Weight at Vaginal Opening	13.44	14.36	16.38	17.16	3.17*	1.83	0.70
Gain--Weaning to Vaginal Opening	4.21	7.43	6.62	9.62	1.84	0.78	2.05
Weight at Sacrifice	18.06	19.93	21.08	20.75	2.48+	0.85	0.30
Gain--Vaginal Opening to Sacrifice	4.63	5.57	4.58	3.71	0.04	0.73	0.78
Total Gain--Weaning to Sacrifice	9.00	13.00	11.21	13.00	1.73	1.60	1.43
Atlas-Anus Length at Sacrifice	62.74	62.57	67.31	67.26	2.81+	3.20*	0.05
Day Counts							
Weaning to Vaginal Opening	31.00	20.43	15.14	16.54	2.70+	2.68+	1.02
Vaginal Opening to Oestrum I	2.63	1.14	2.57	3.46	0.78	2.56	1.08
Vaginal Opening to First Adult Smear	4.43	1.33	3.14	3.69	0.27	2.80+	1.19

TABLE XXIV (Continued)

Diet	Means				"t" Test		
	6%	12%	25%	50%	25% & 6%	25% & 12%	25% & 50%
Organ Weight at Sacrifice							
Adrenal	6.61	6.81	8.74	8.80	2.12+	1.93	0.75
Pituitary	1.09	1.27	1.62	1.65	3.54*	1.93	0.15
Ovary	4.11	4.63	9.37	8.85	5.02*	3.48*	0.34
Uterus	64.54	58.56	71.85	77.01	0.32	0.87	0.28
Spleen	52.19	64.57	56.06	57.45	0.66	1.36	0.26
Liver	1,133.26	1,302.26	1,590.26	1,437.04	4.27*	2.41+	1.28
Number of Animals in Each Group	7	4	9	7			

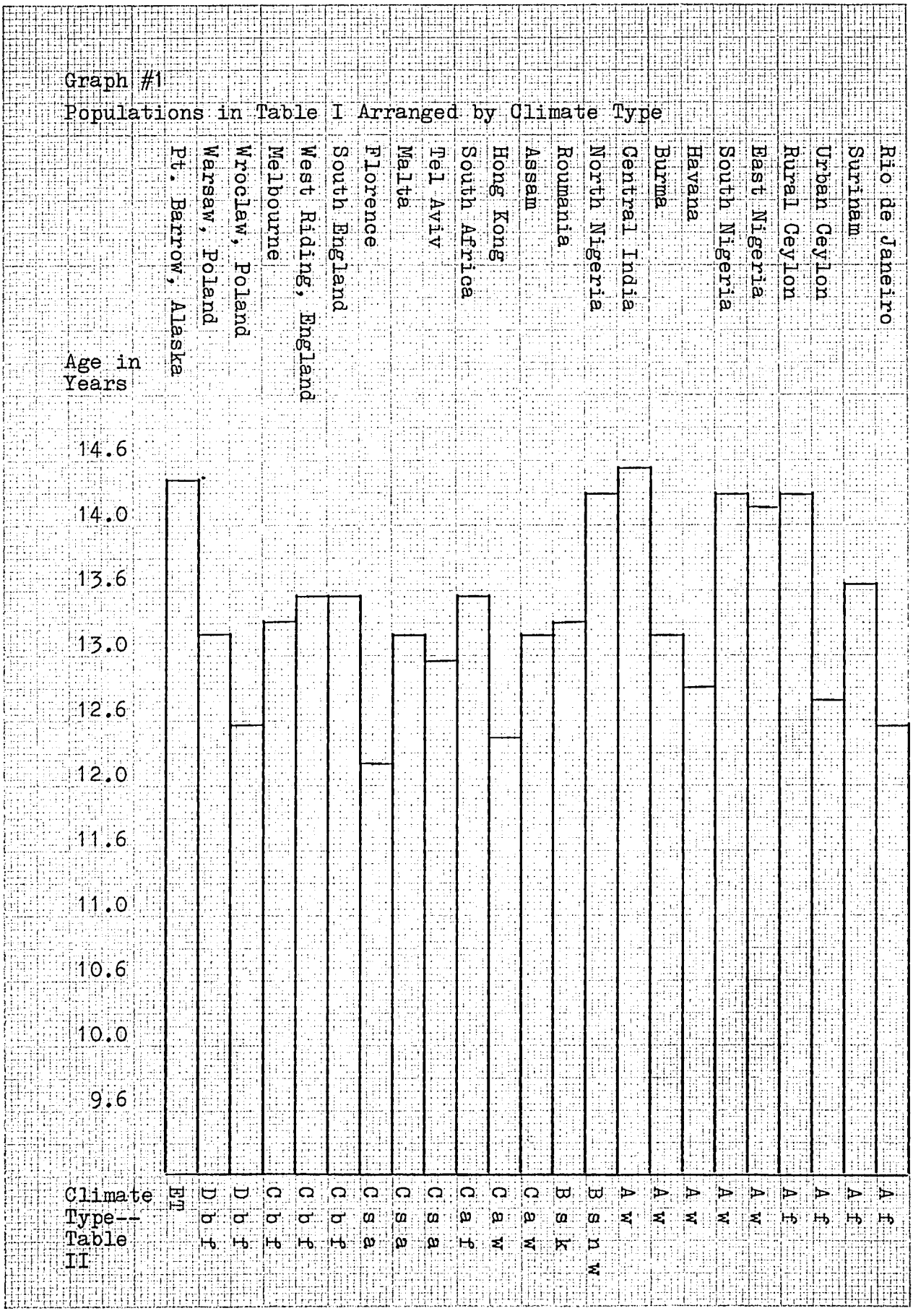
* indicates significance at the 1% level.

+ indicates significance at the 5% level.

GRAPHS

Graph #1

Populations in Table I Arranged by Climate Type

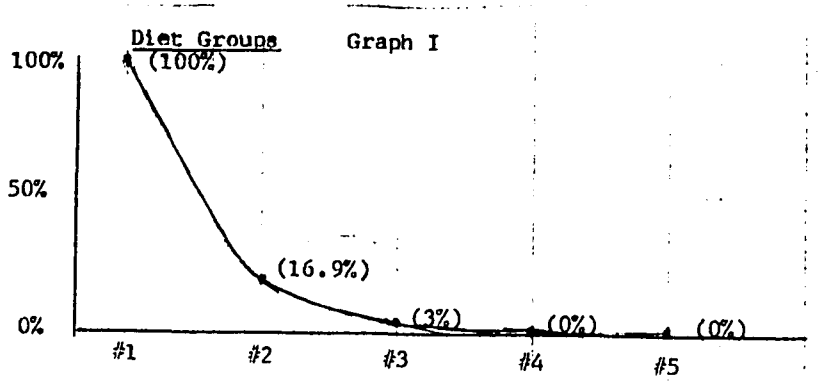


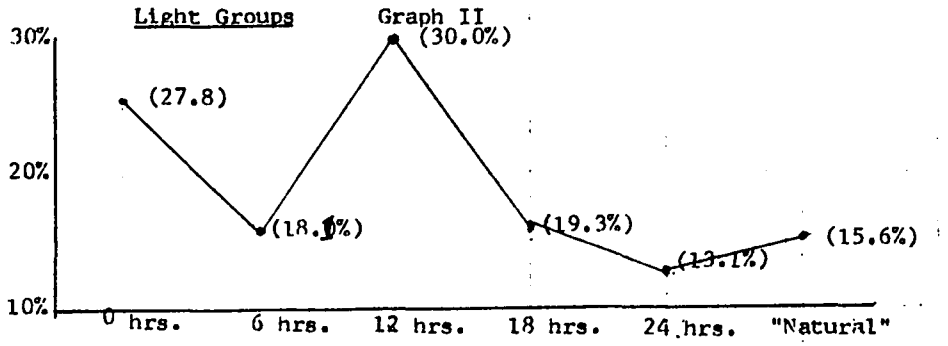
Graph #2

Populations in Table I Arranged by Menarcheal Age

Graph # 3

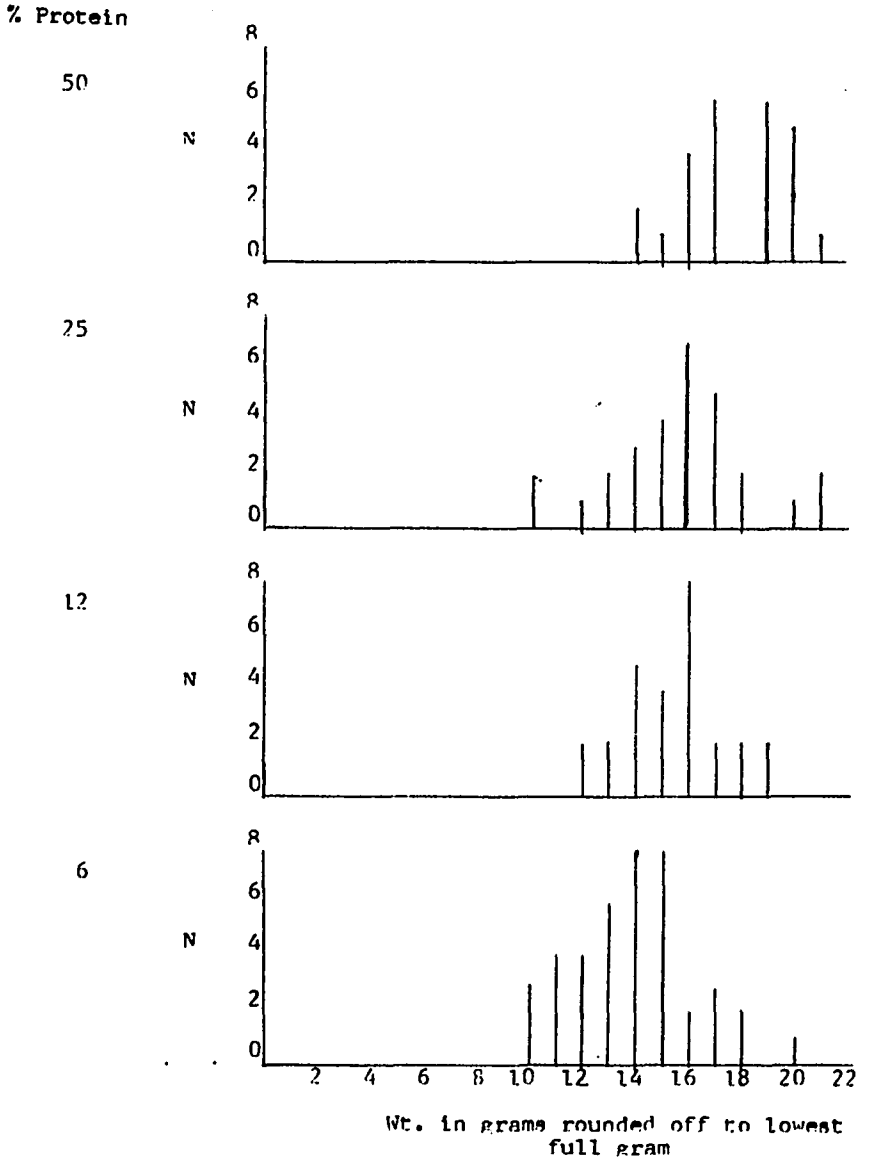
Mortality Figures





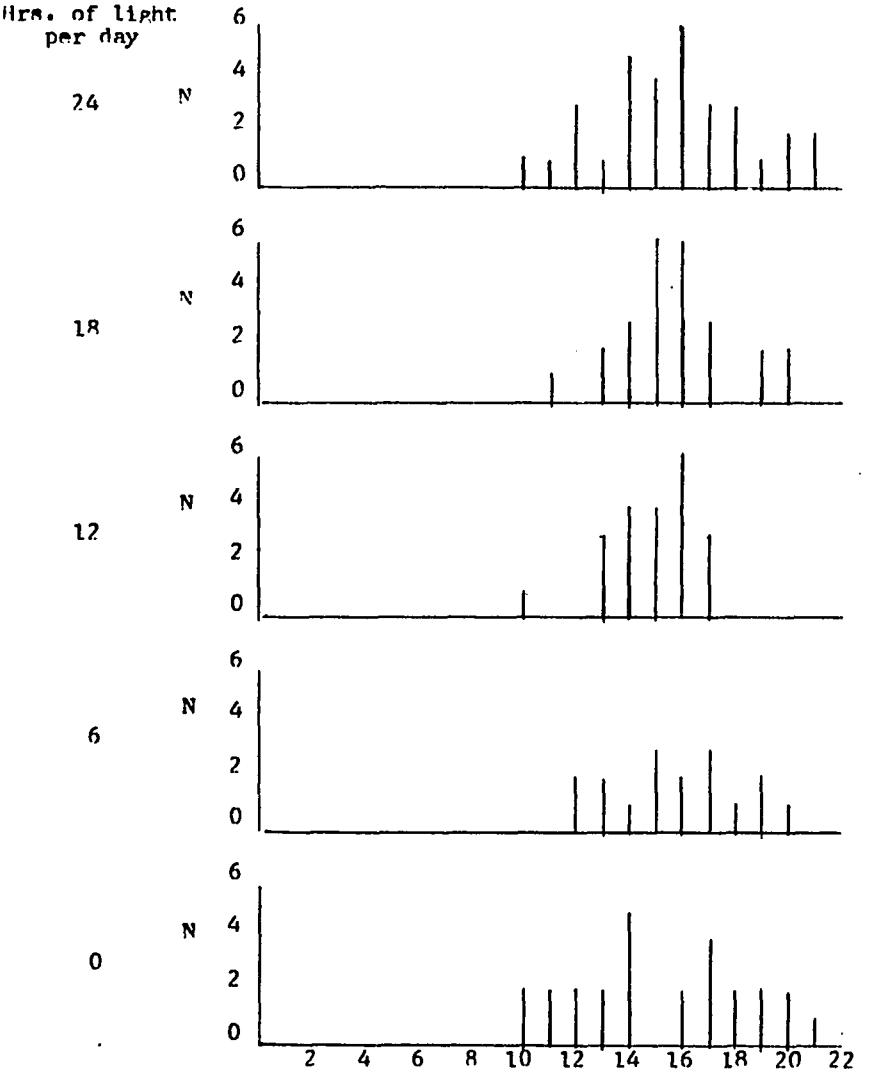
Graph # 4 a

Weight at Vaginal opening



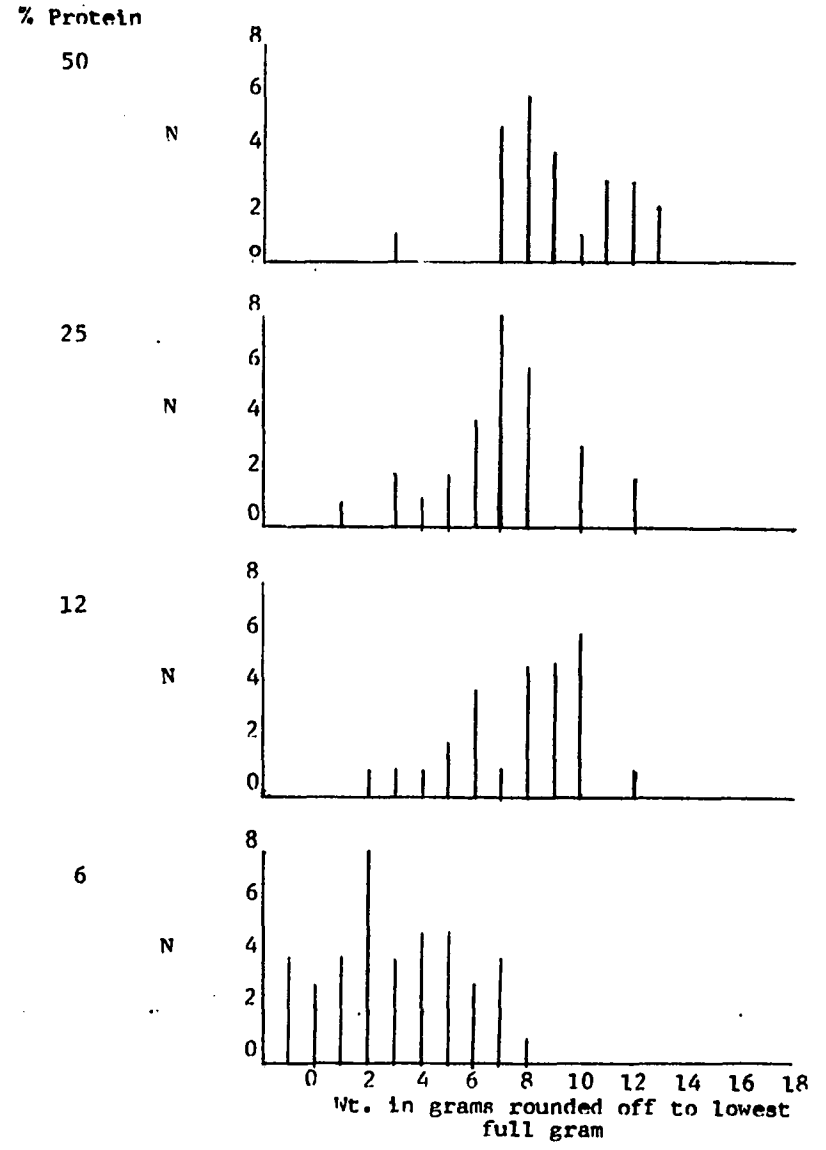
Graph # 4 b

Weight at Vaginal Opening



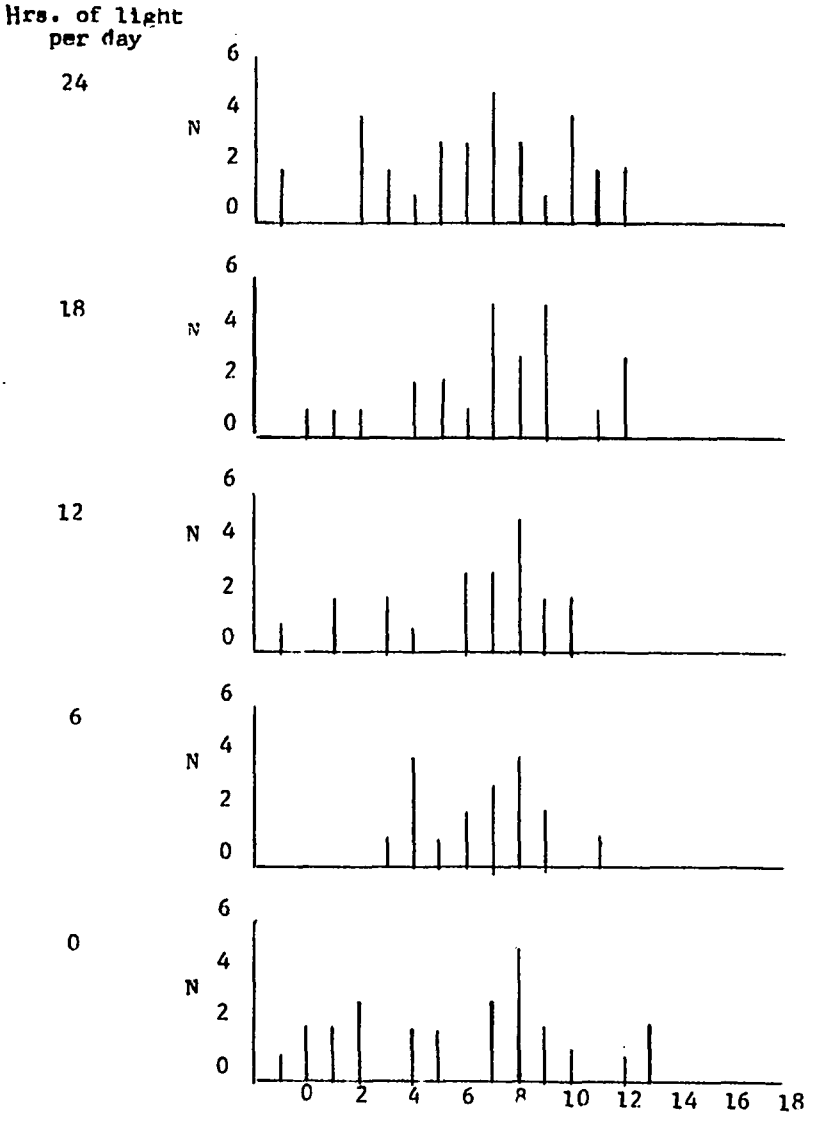
Graph # 5 a

Weight Gain - Weaning to Vaginal Opening



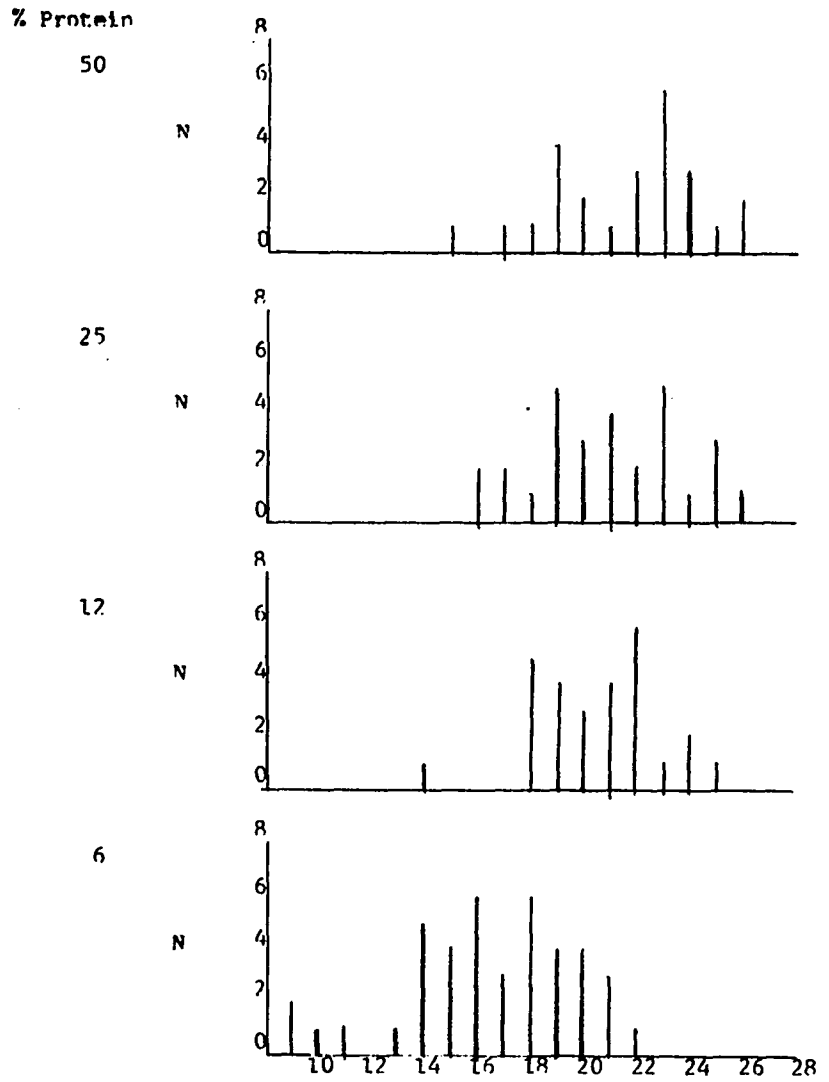
Graph #5 b

Weight Gain - Weaning to Vaginal Opening



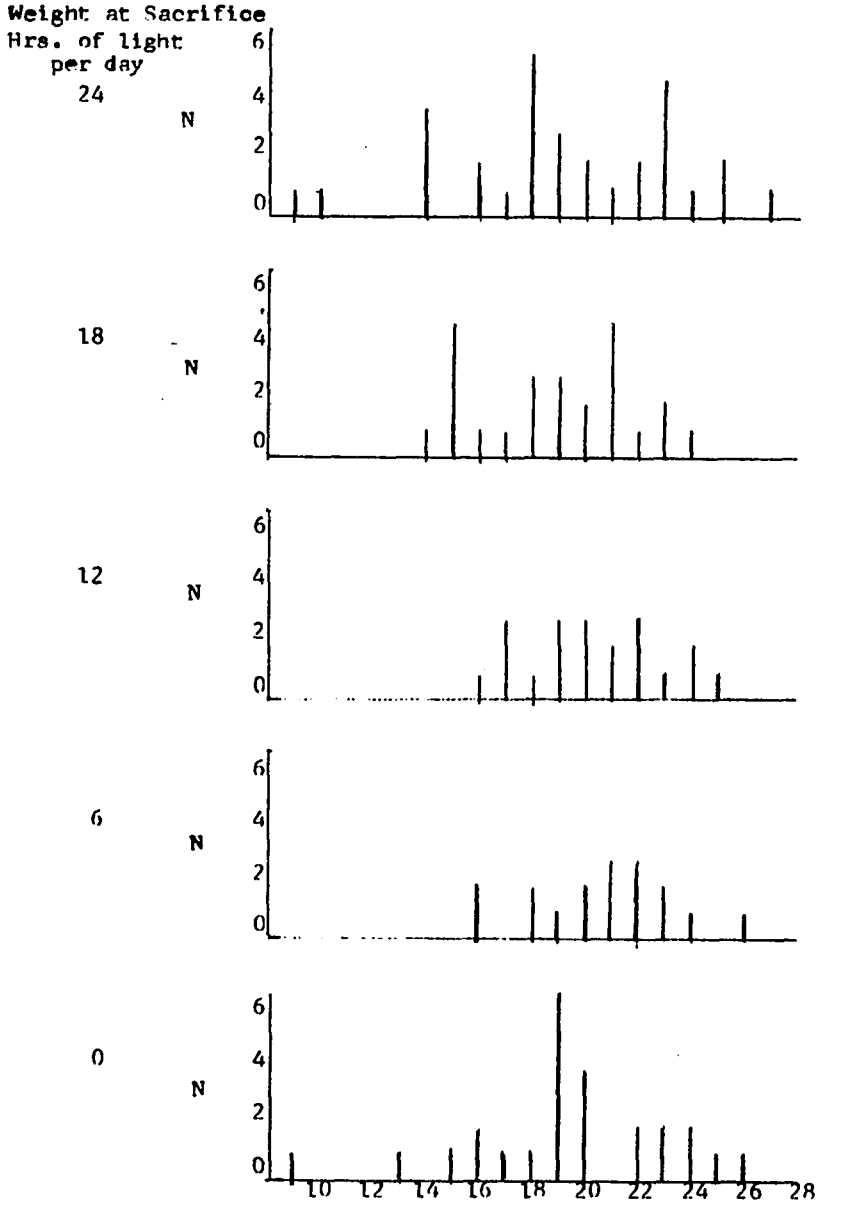
Graph # 6 a

Weight at Sacrifice



Graph # 6 b

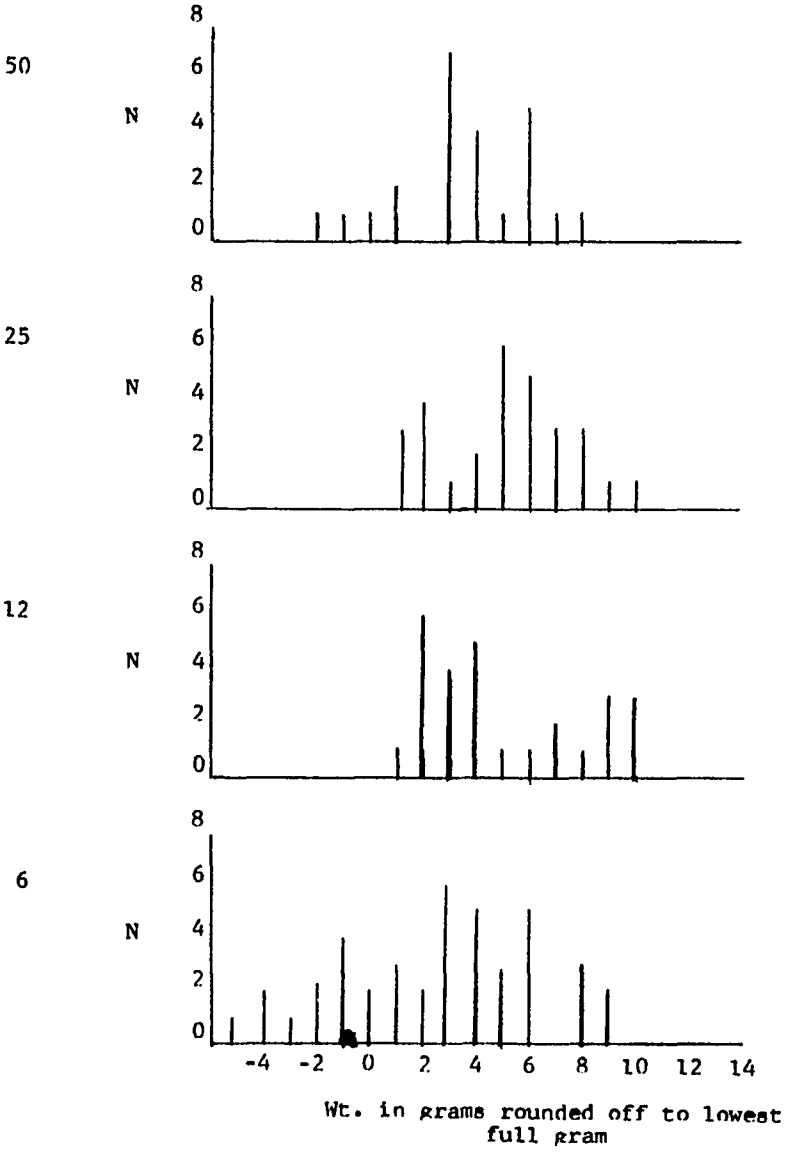
Weight at Sacrifice



Graph # 7 a

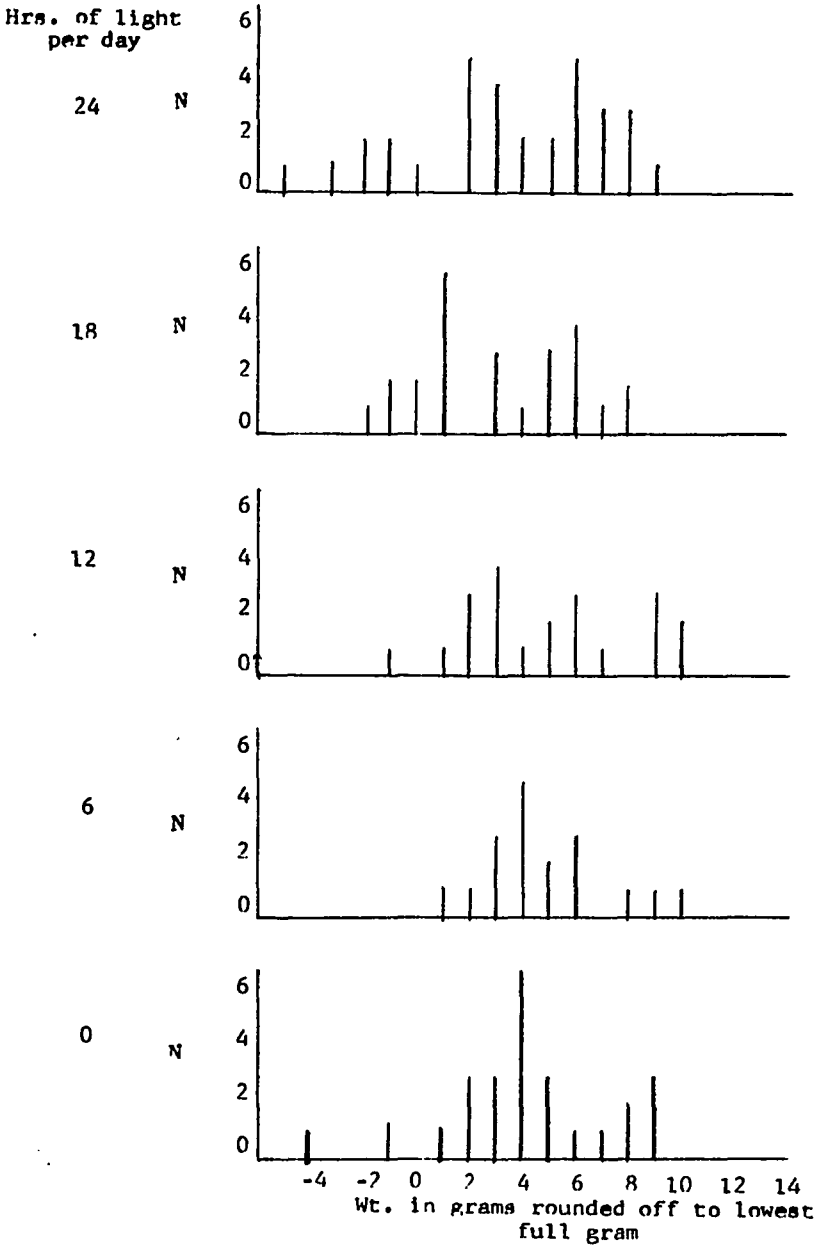
Weight Gain - Vaginal Opening to Sacrifice

% Protein



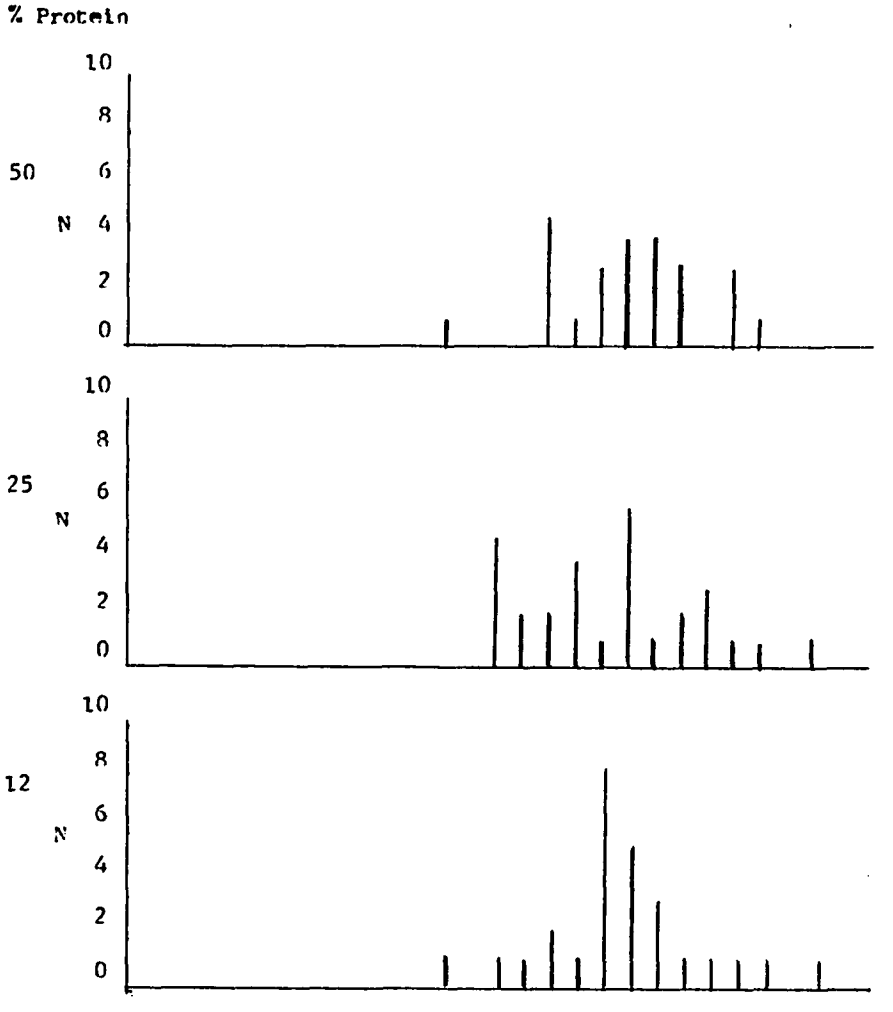
Graph # 7 b

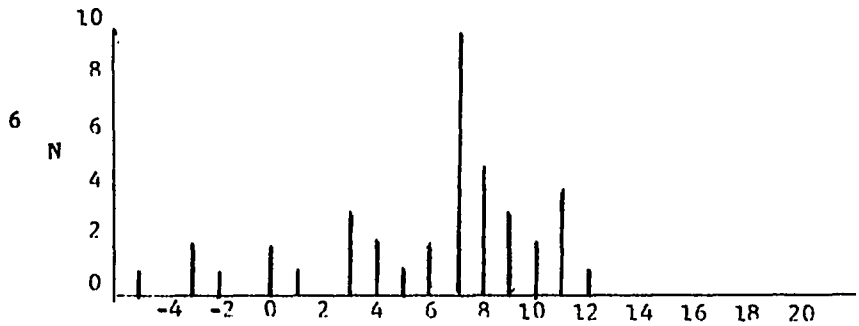
Weight Gain - Vaginal Opening to Sacrifice



Graph # 8 a

Total Weight Gain - Weaning to Sacrifice

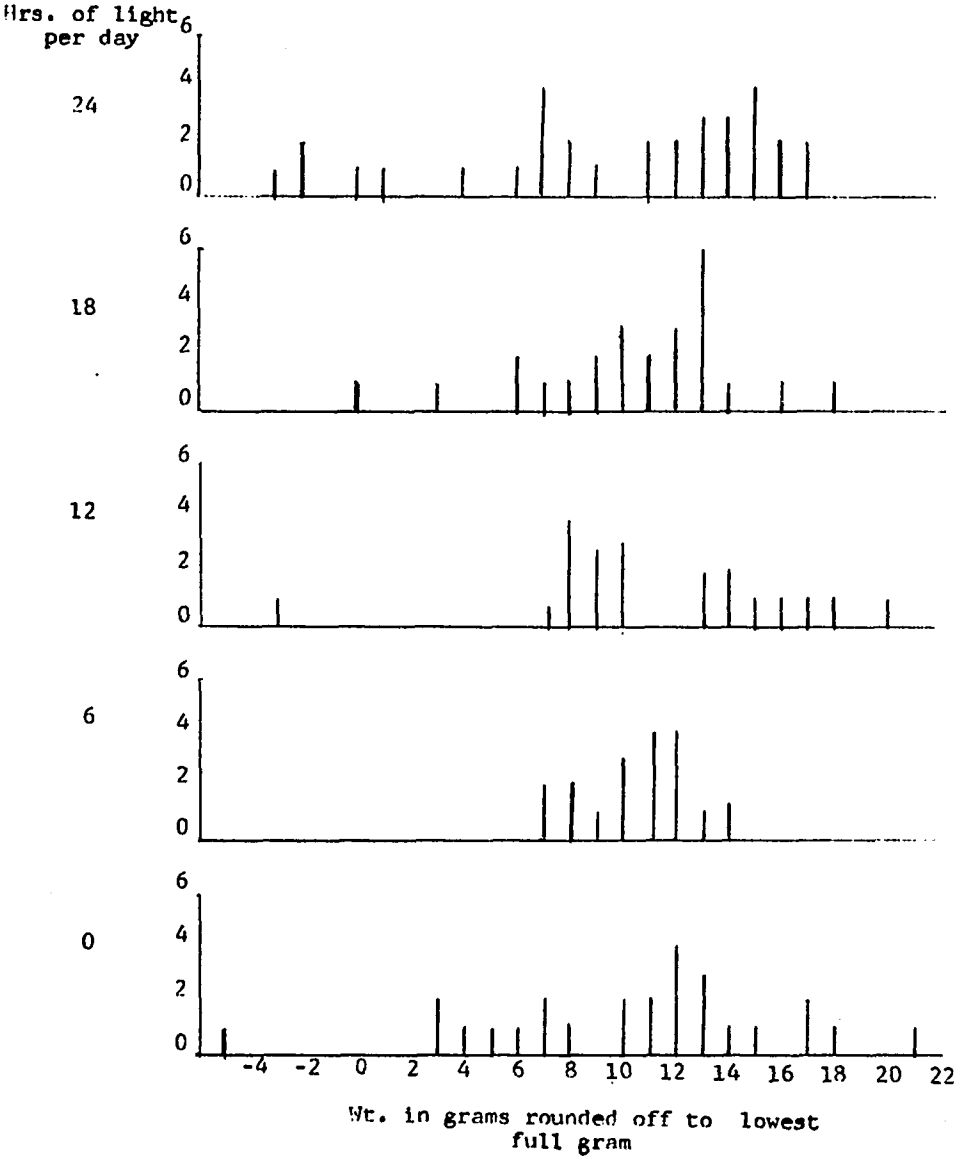




Wt. in grams rounded off to lowest full gram

Graph # 8 b

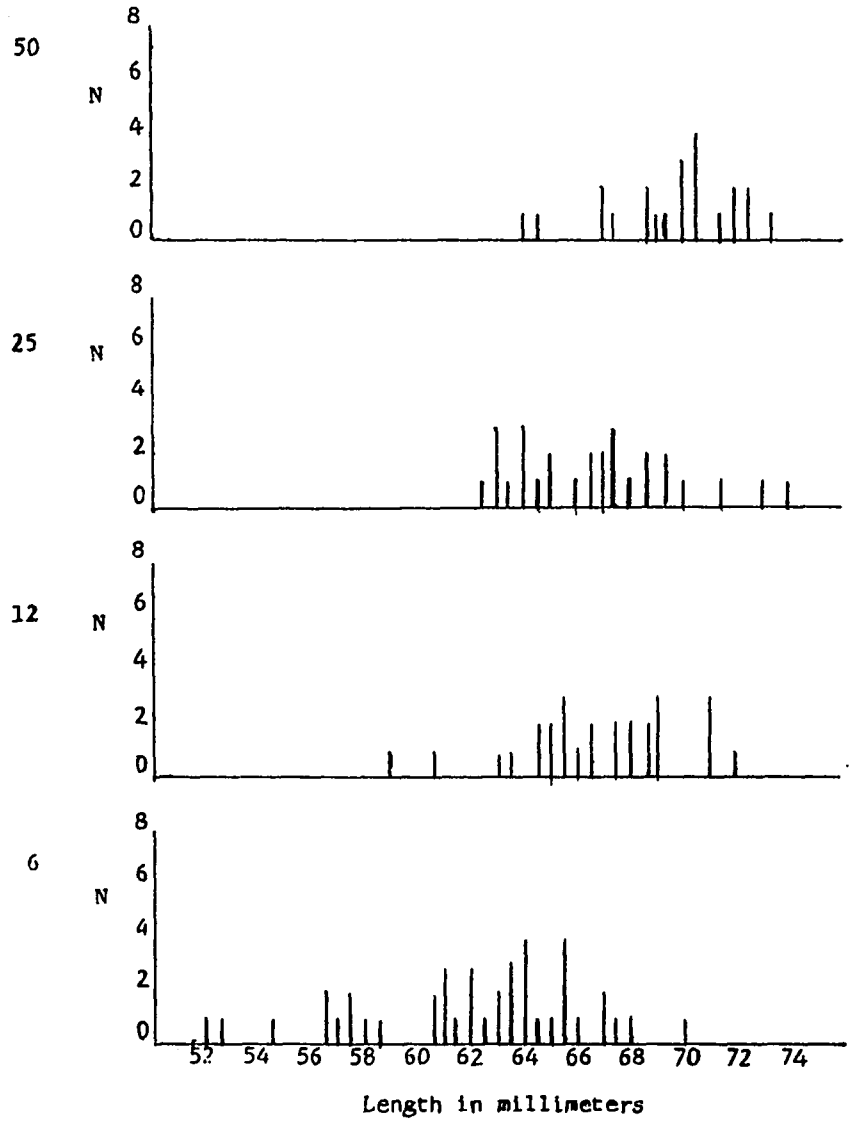
Total Weight Gain - Weaning to Sacrifice



Graph # 9 a

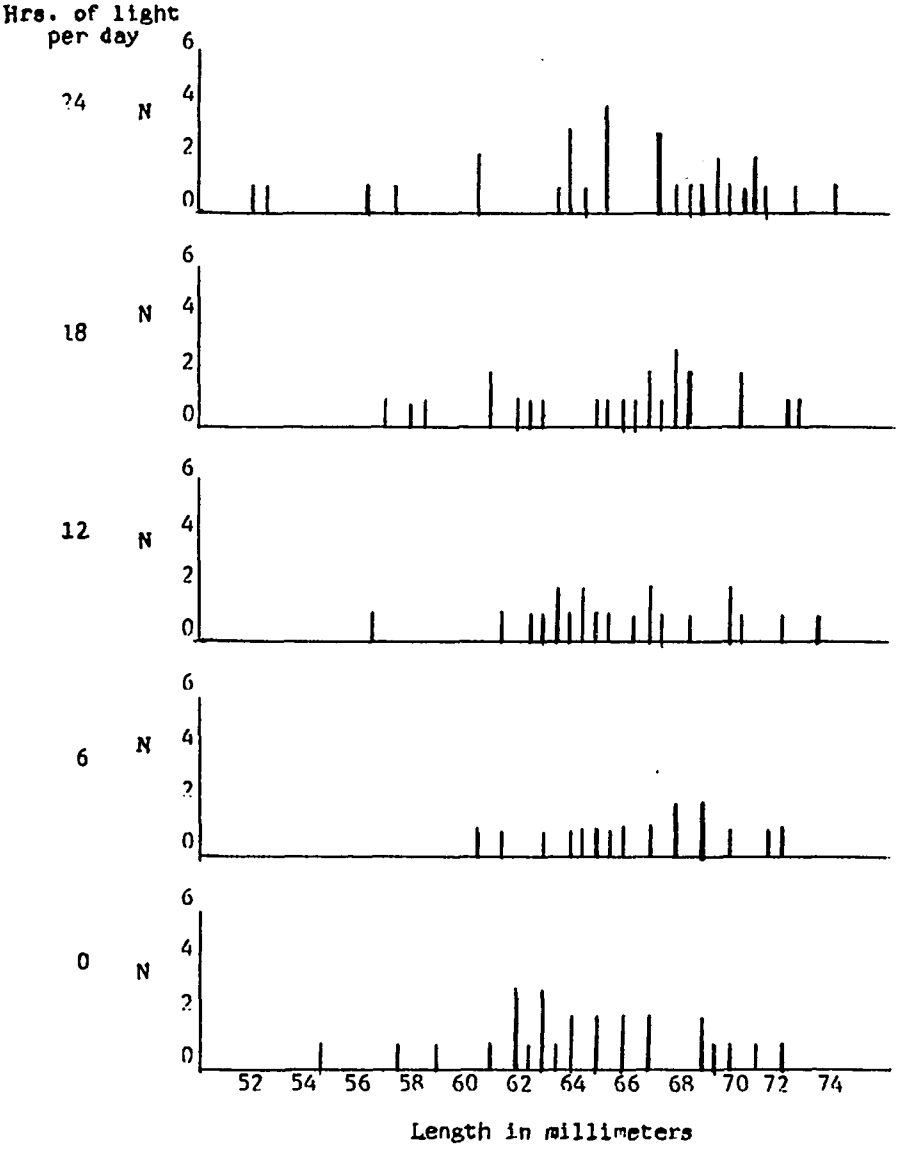
Atlas-Anus Length at Sacrifice

% Protein



Graph # 9 b

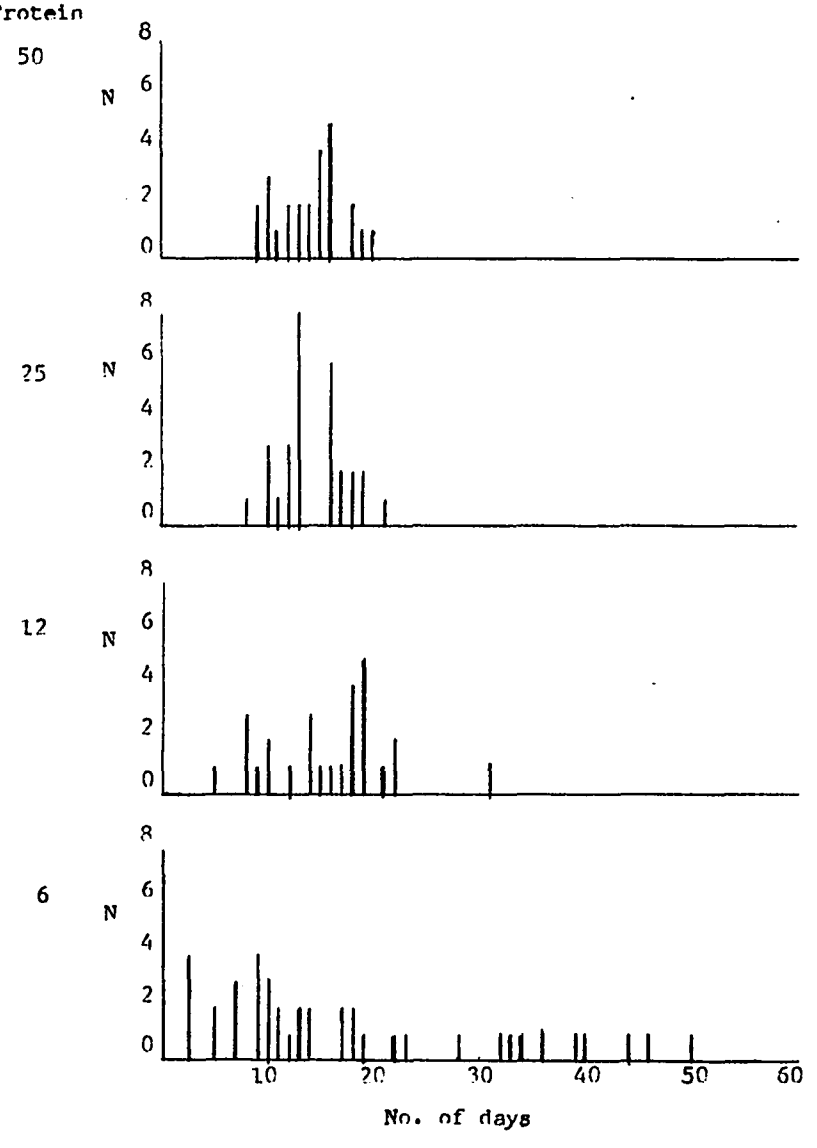
Atlas-Anus Length at Sacrifice



Graph # 10 a

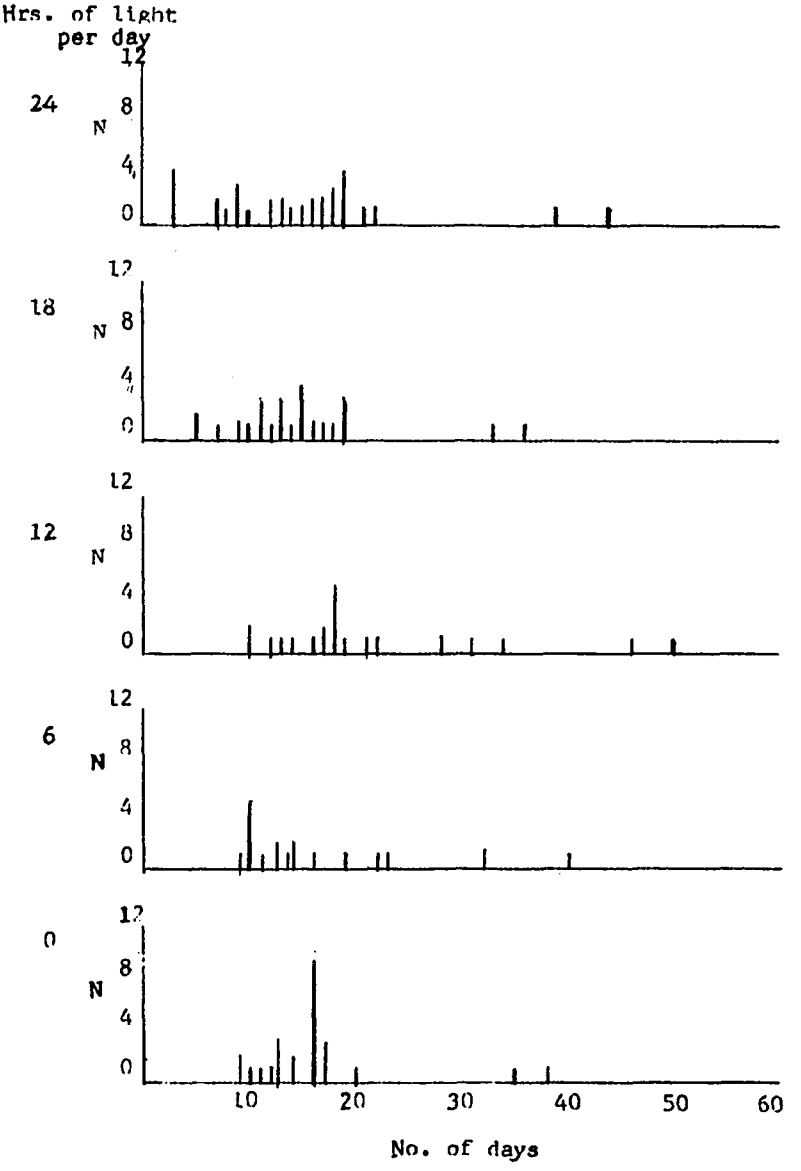
Number of Days Weaning to Vaginal Opening

% Protein



Graph # 10 b

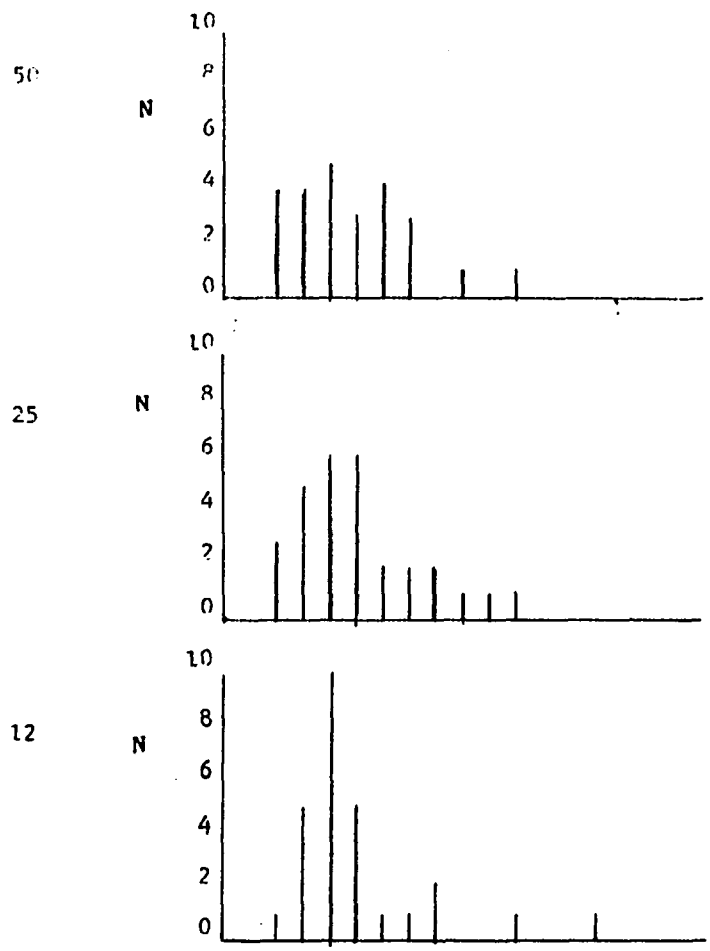
Number of Days Weaning to Vaginal Opening

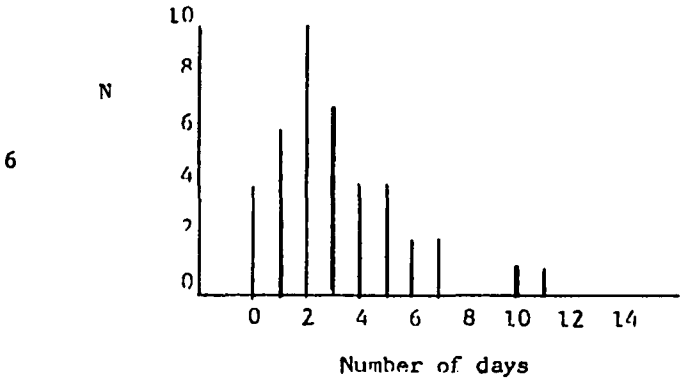


Graph # 11 a

Number of Days Vaginal Opening to Oestrum I

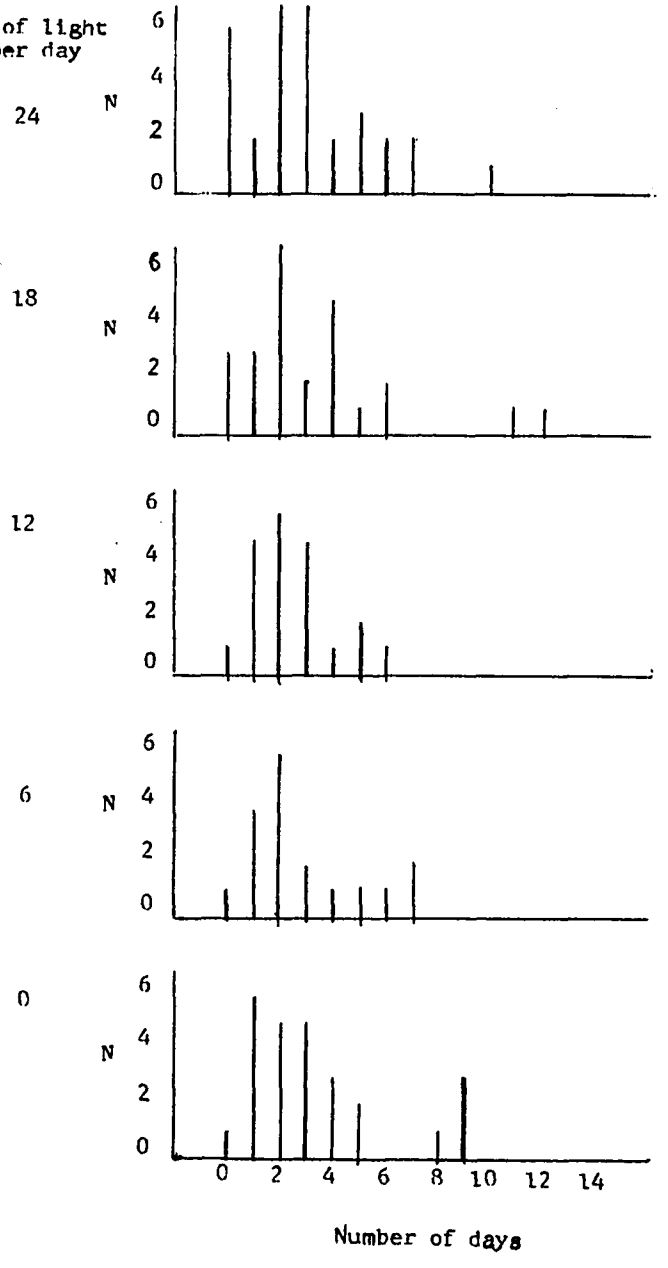
% Protein




10
8
N
6
4
2
0
6
0 2 4 6 8 10 12 14
Number of days

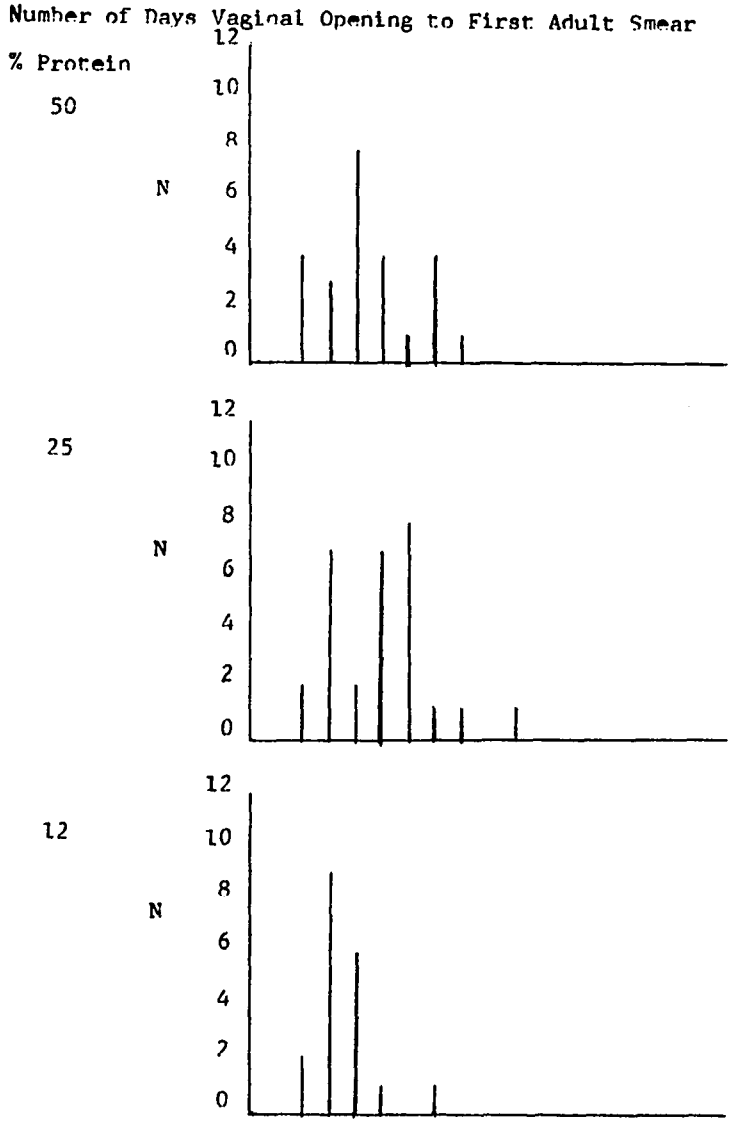
Graph # 11 b

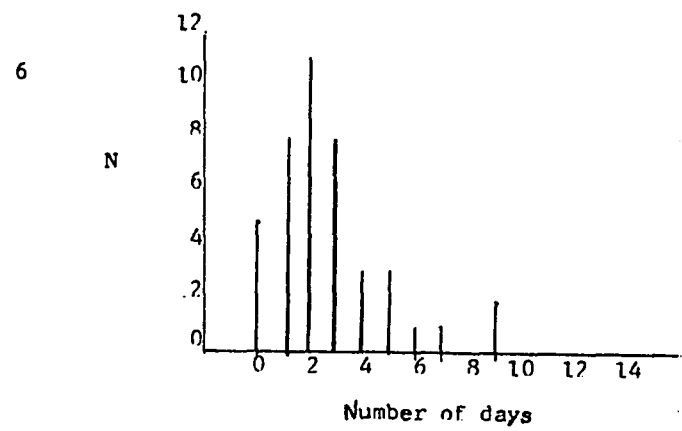
Number of Days Vaginal Opening to Oestrum I



Graph # 12 a

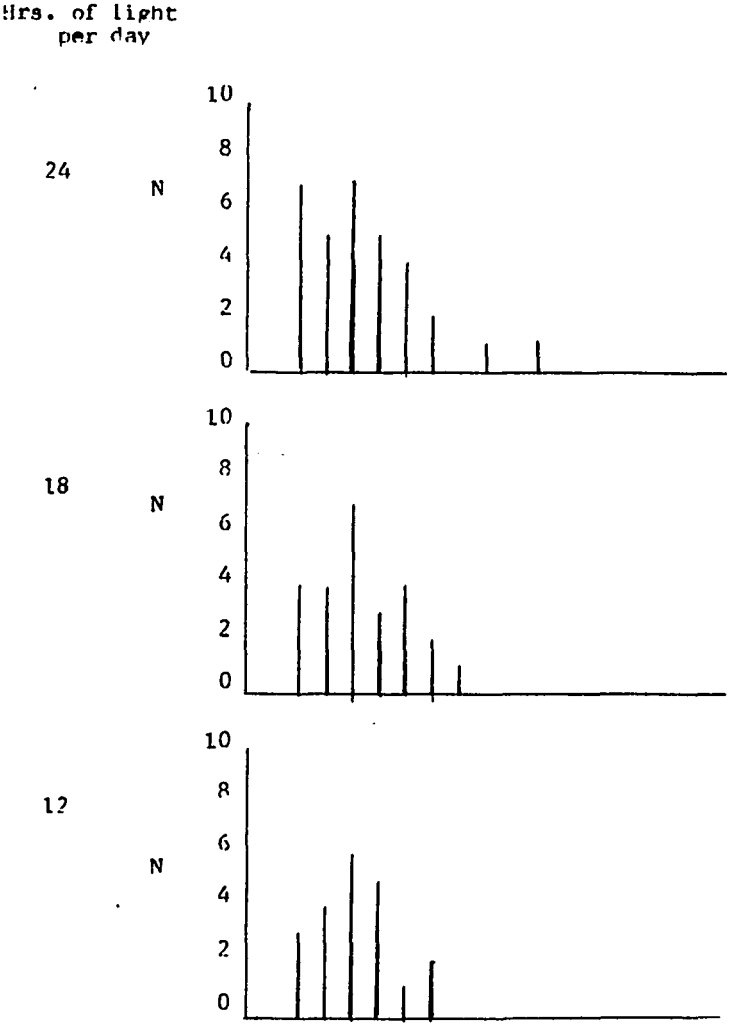
Number of Days Vaginal Opening to First Adult Smear

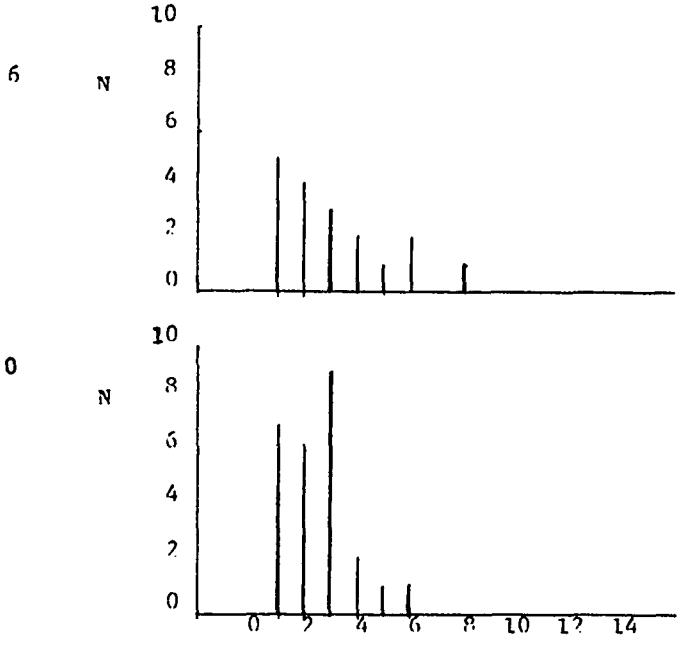



6
N
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
Number of days

Graph # 12 b

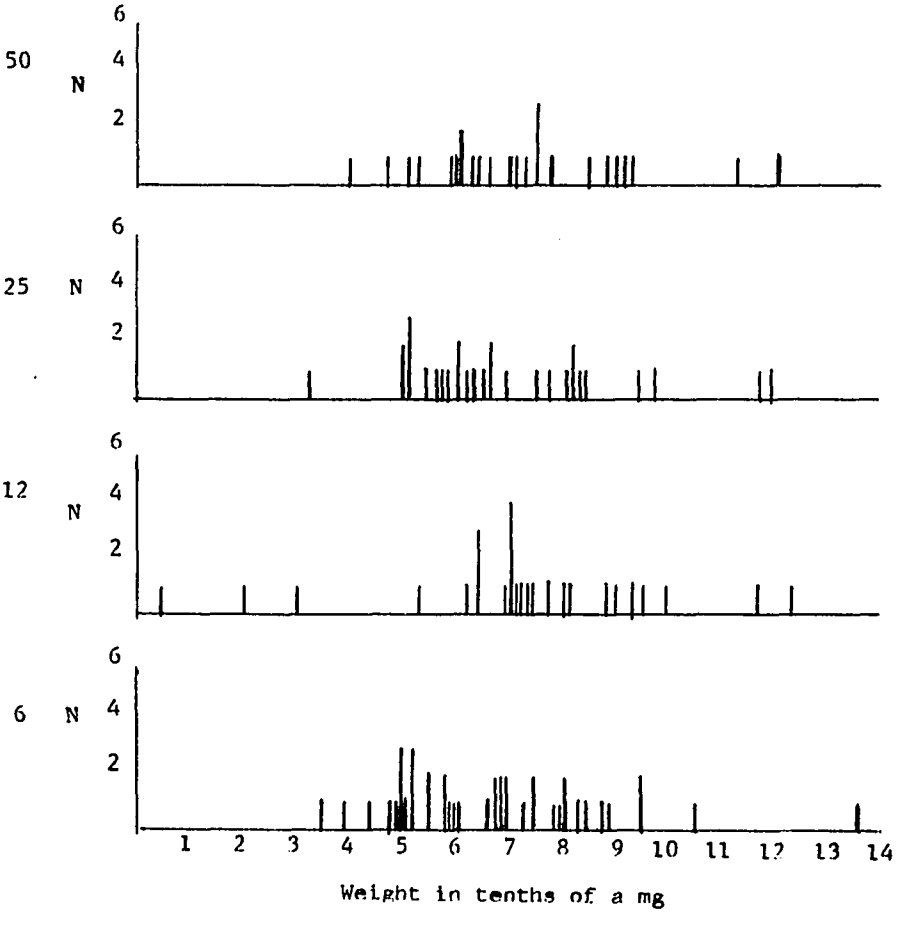
Number of Days Vaginal Opening to First Adult Smear




6
N
10
8
6
4
2
0
0
N
10
8
6
4
2
0
0
2
4
6
8
10
12
14
Number of days

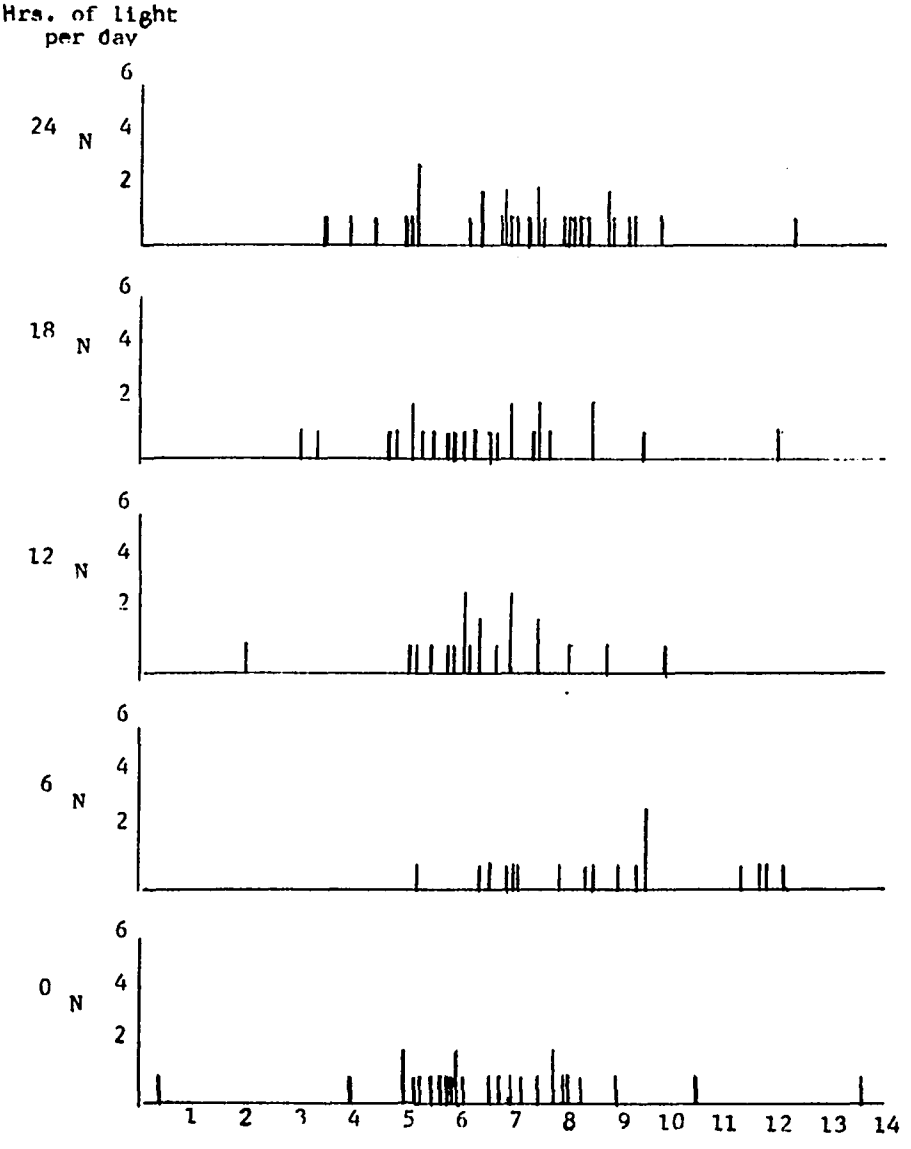
Graph # 13 a
Adrenal Weight at Sacrifice

% Protein



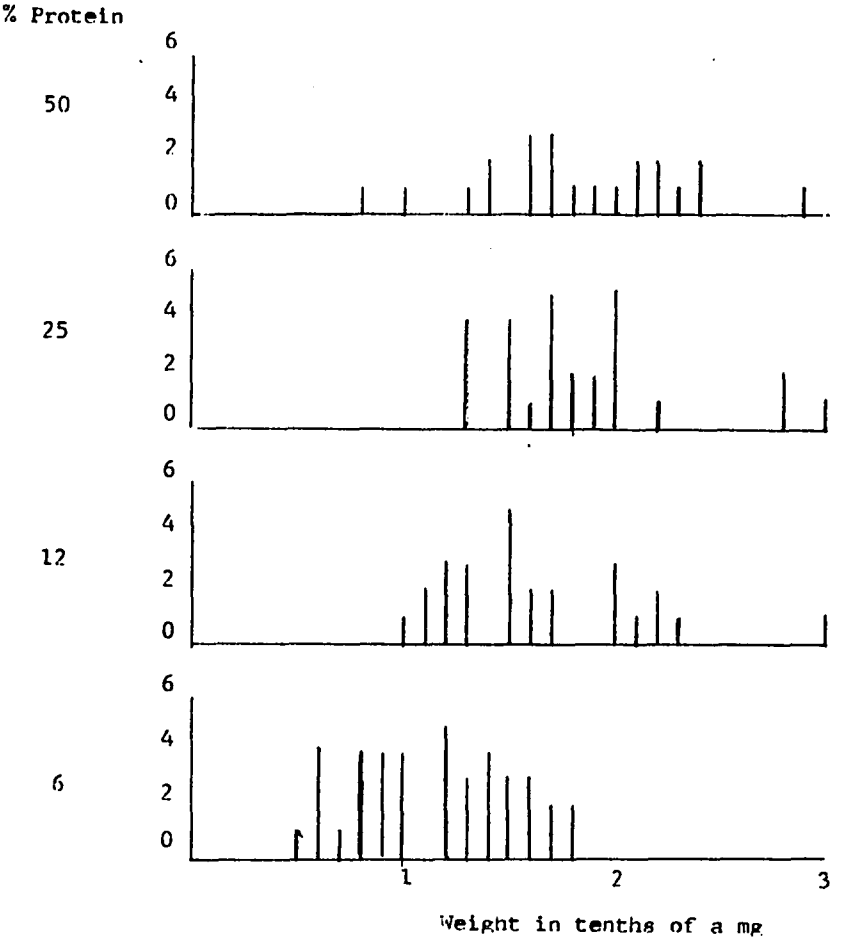
Graph # 13 b

Adrenal Weight at Sacrifice



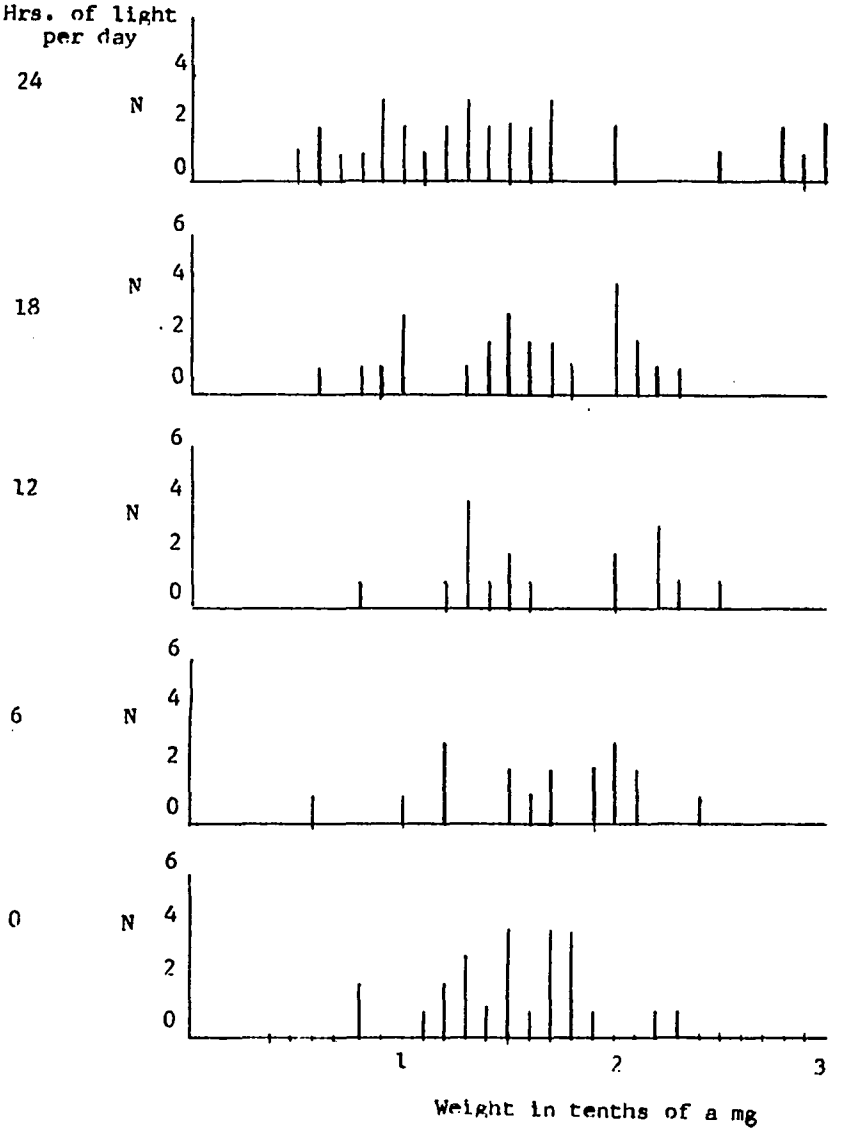
Graph # 14 a

Pituitary Weight at Sacrifice



Graph # 14 b

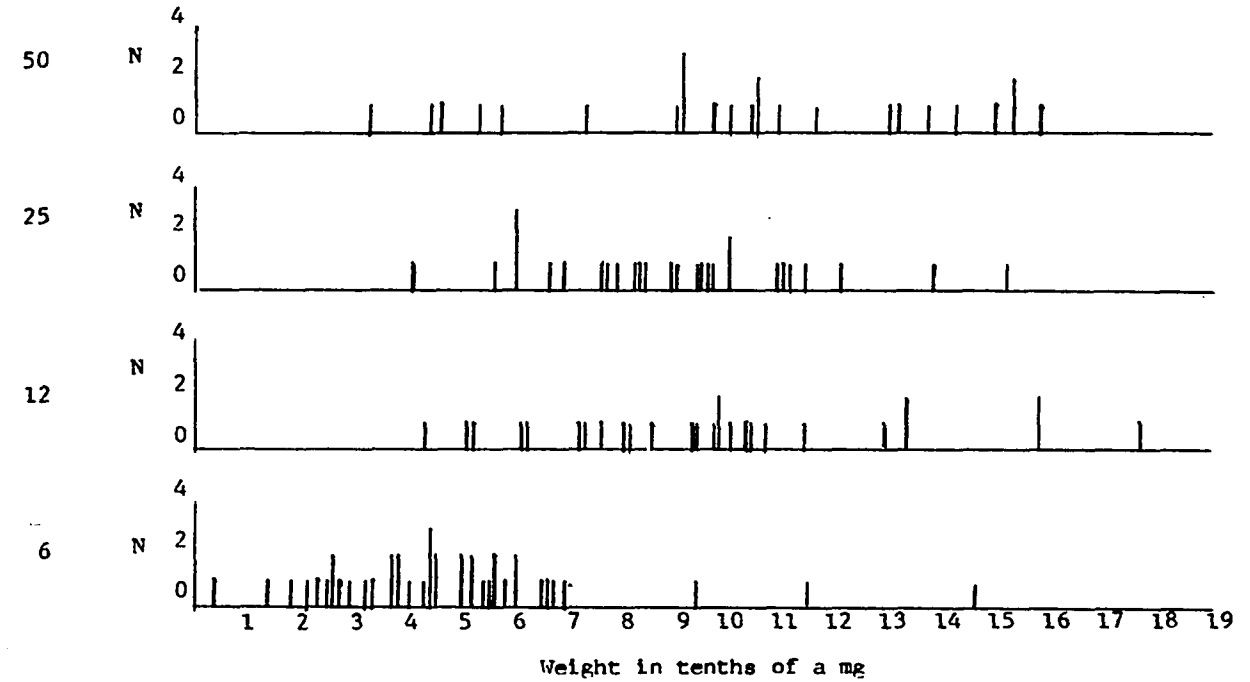
Pituitary Weight at Sacrifice



Graph # 15 a

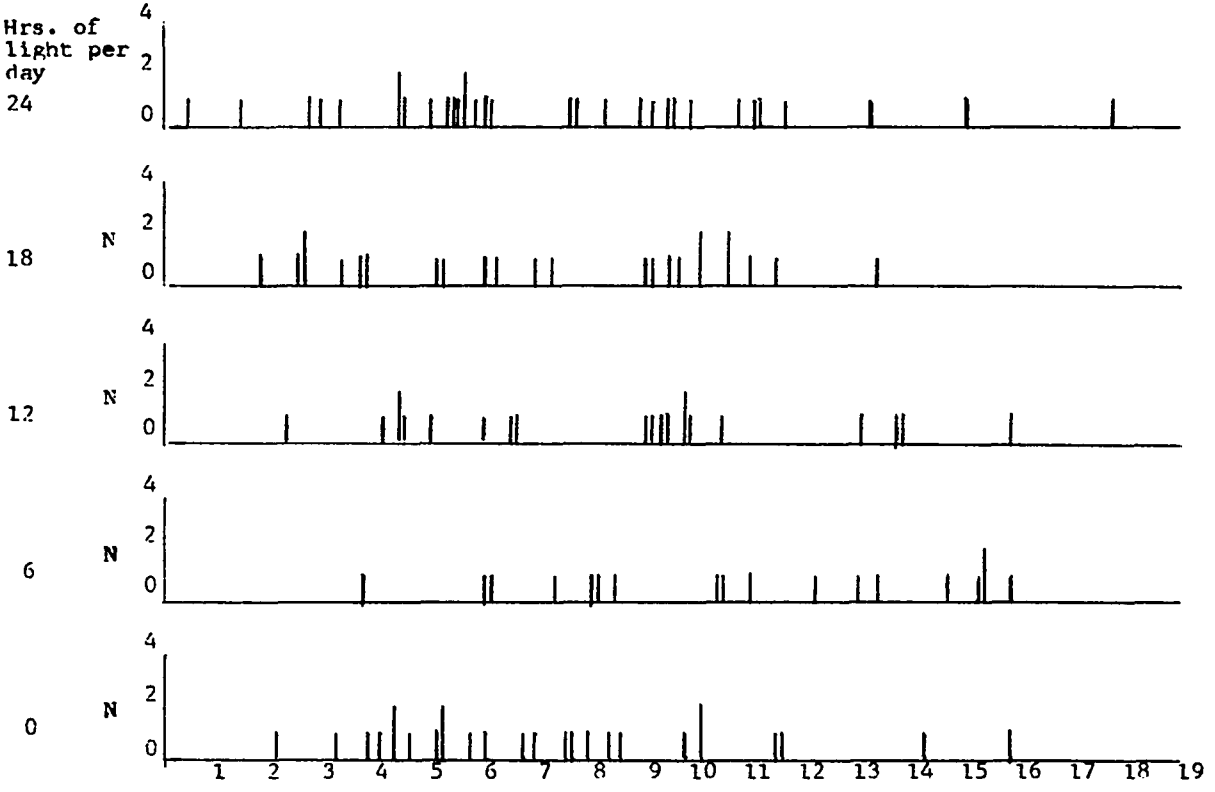
Ovary Weight at Sacrifice

% Protein



Graph # 15 b

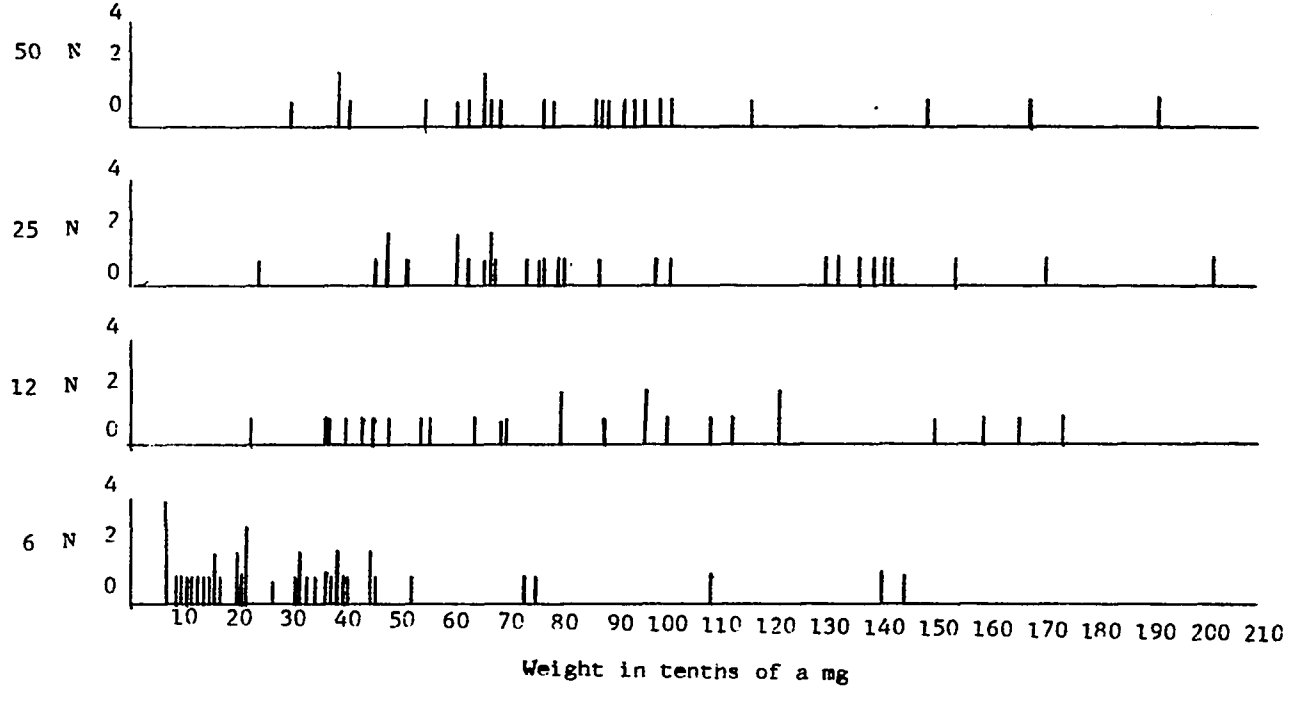
Ovary Weight at Sacrifice



Graph # 16 a

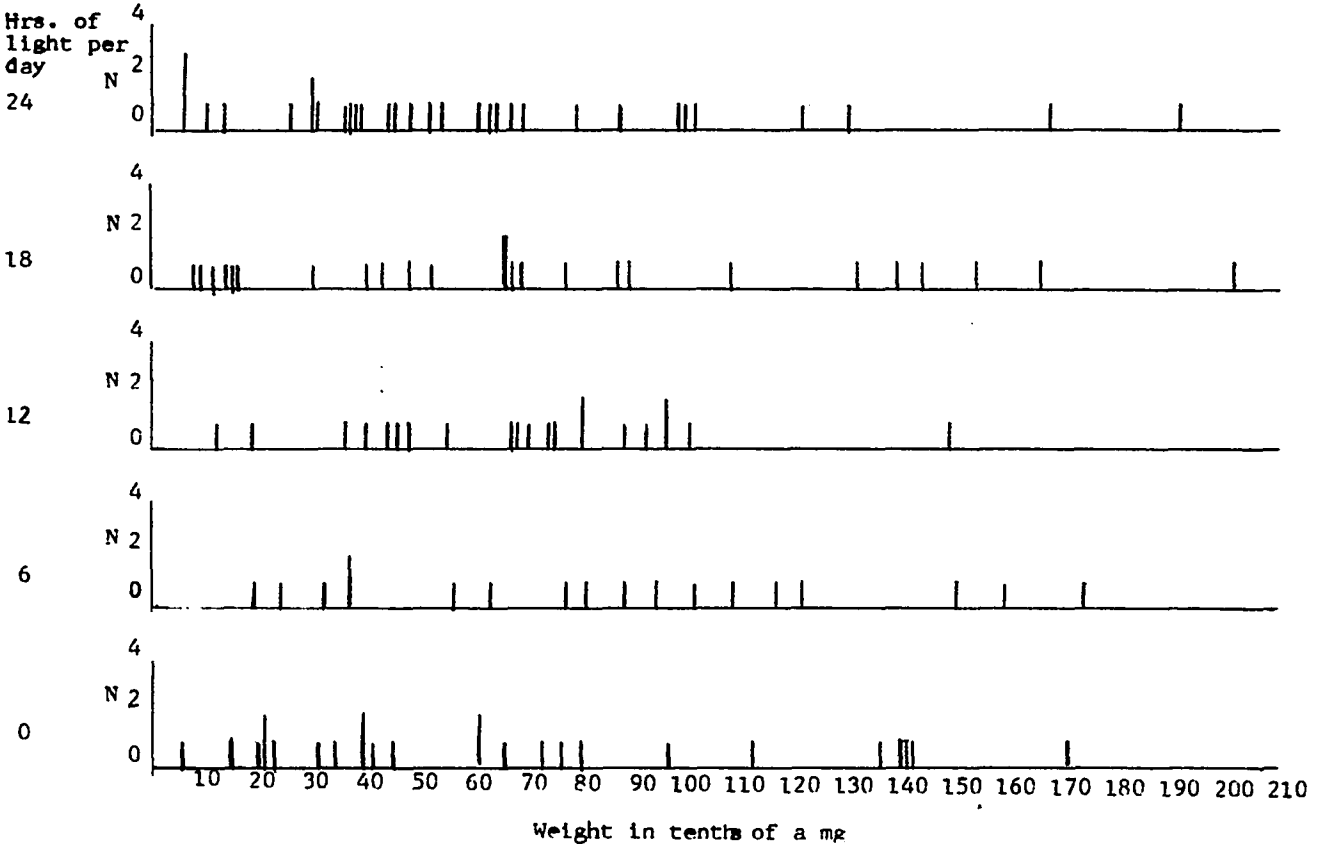
Uterus Weight at Sacrifice

% Protein



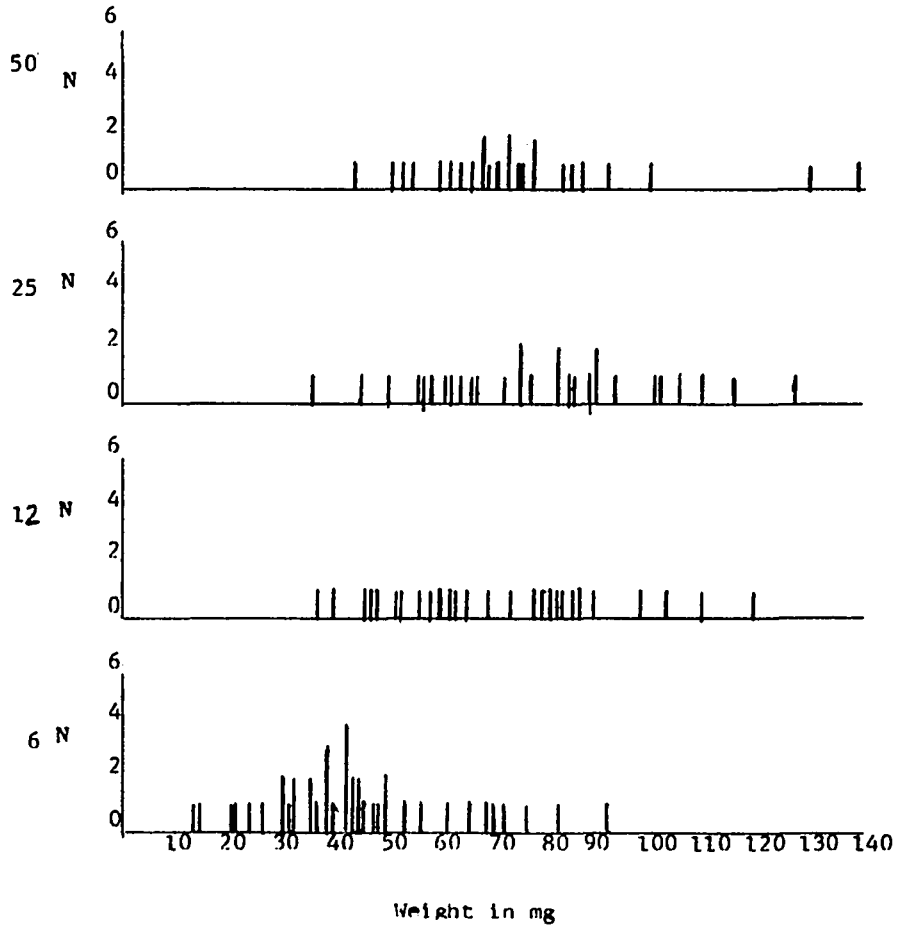
Graph # 16 b

Uterus Weight at Sacrifice



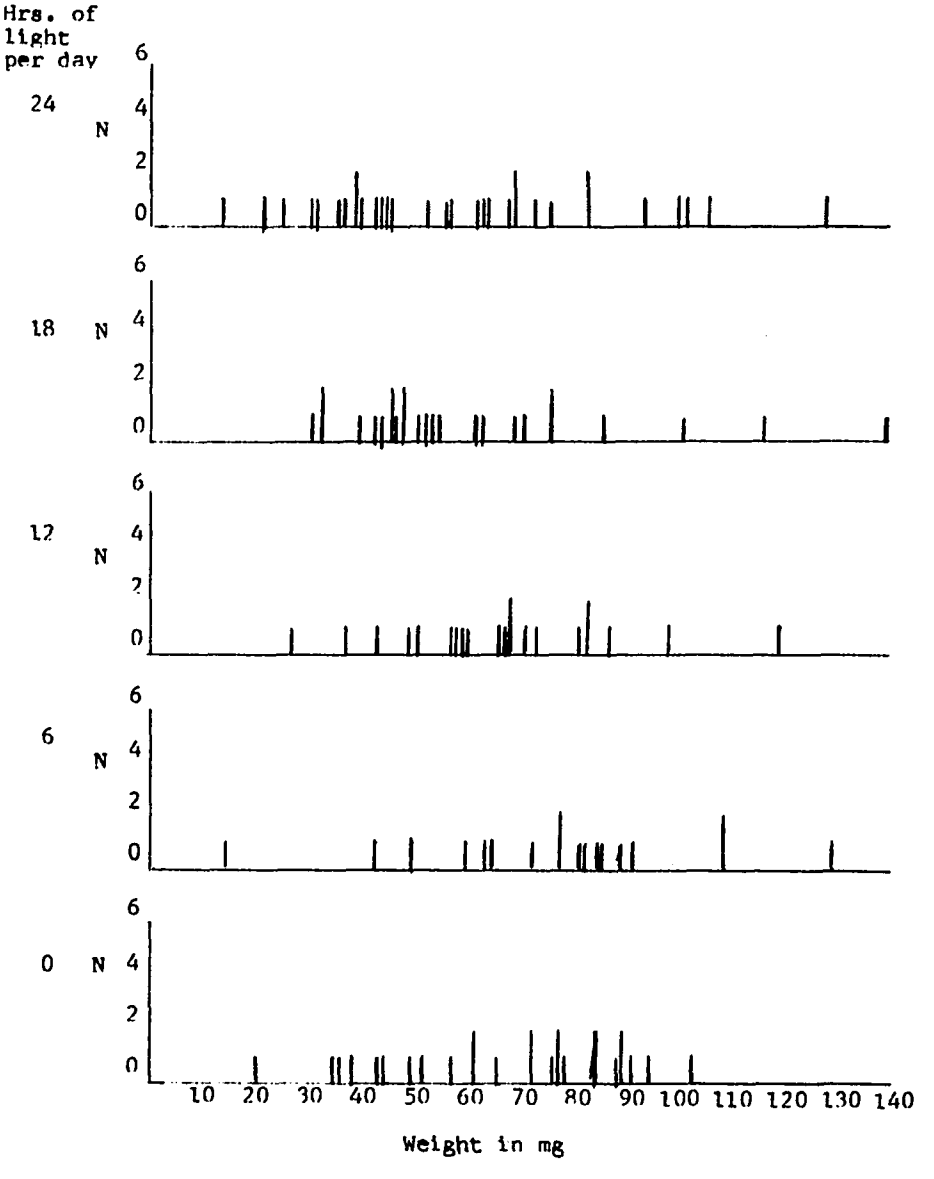
Graph # 17a

Spleen Weight at Sacrifice



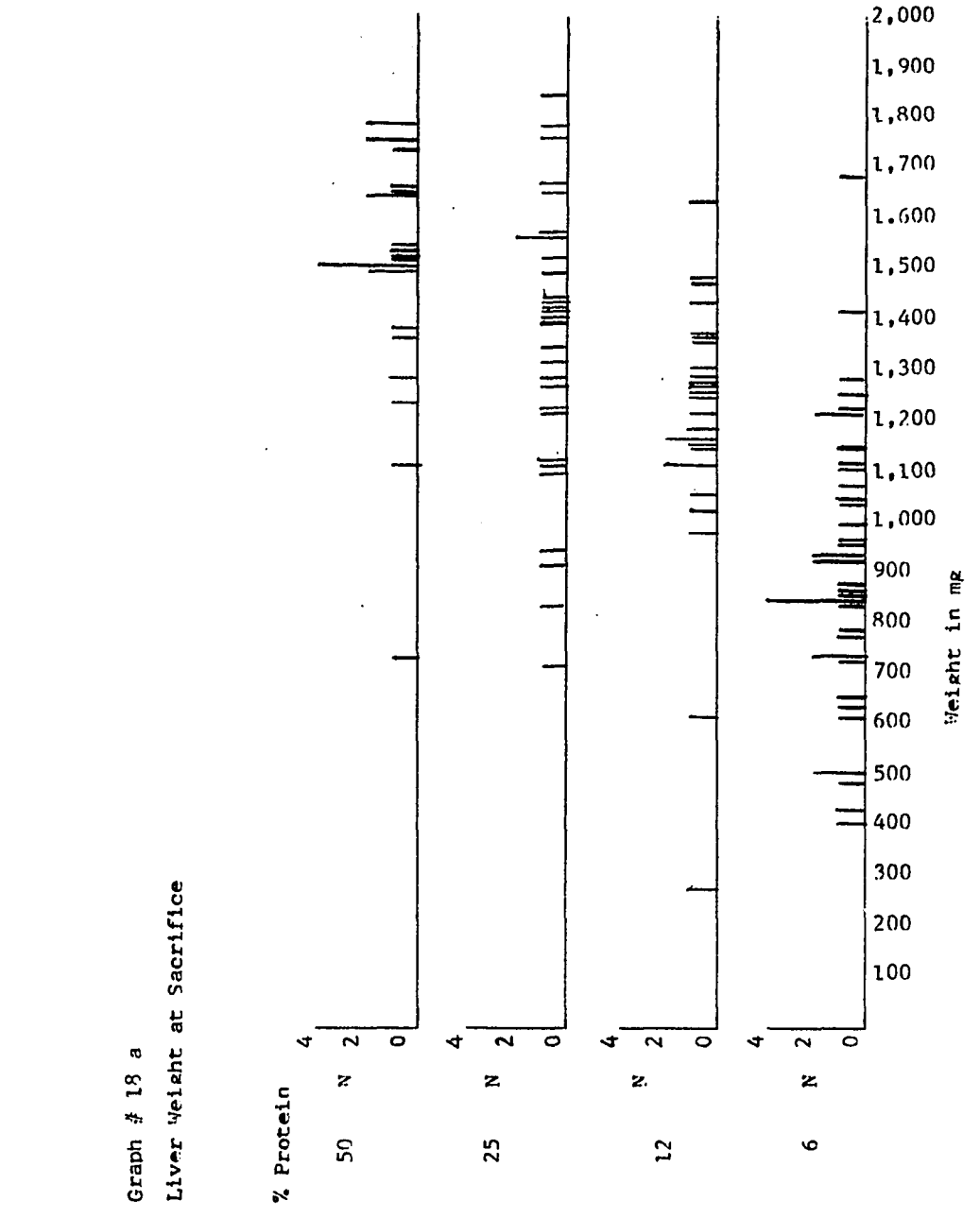
Graph # 17 b

Spleen Weight at Sacrifice



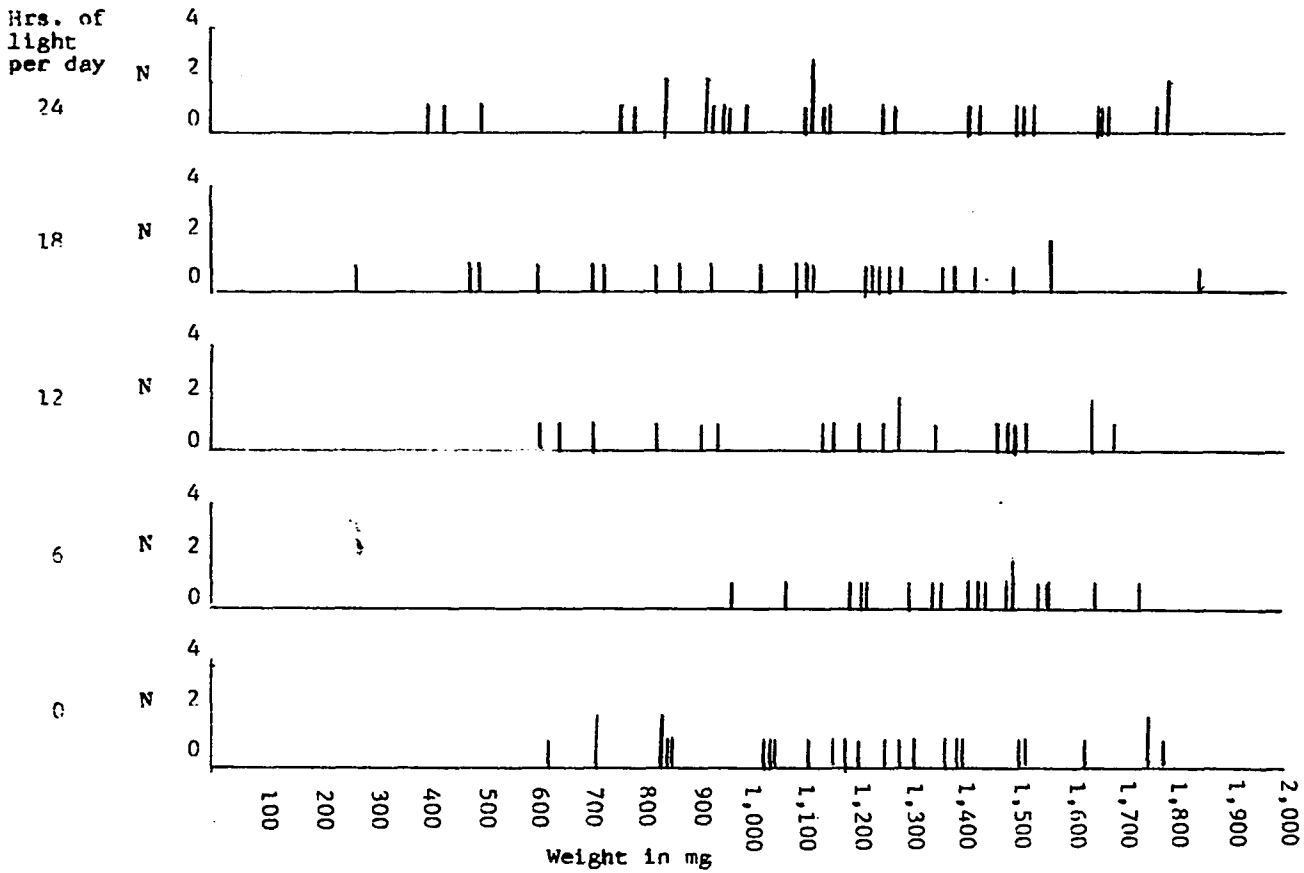
Graph # 18 a

Liver Weight at Sacrifice

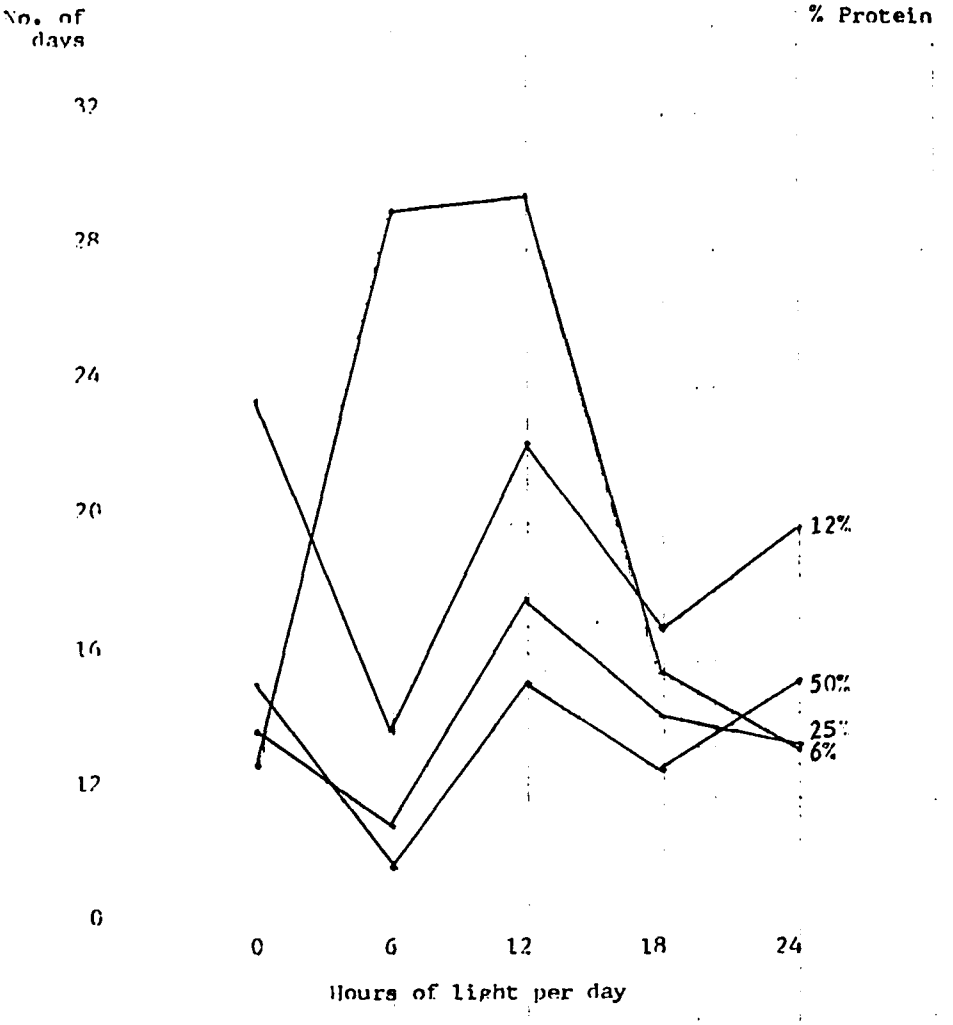


Graph # 18 b

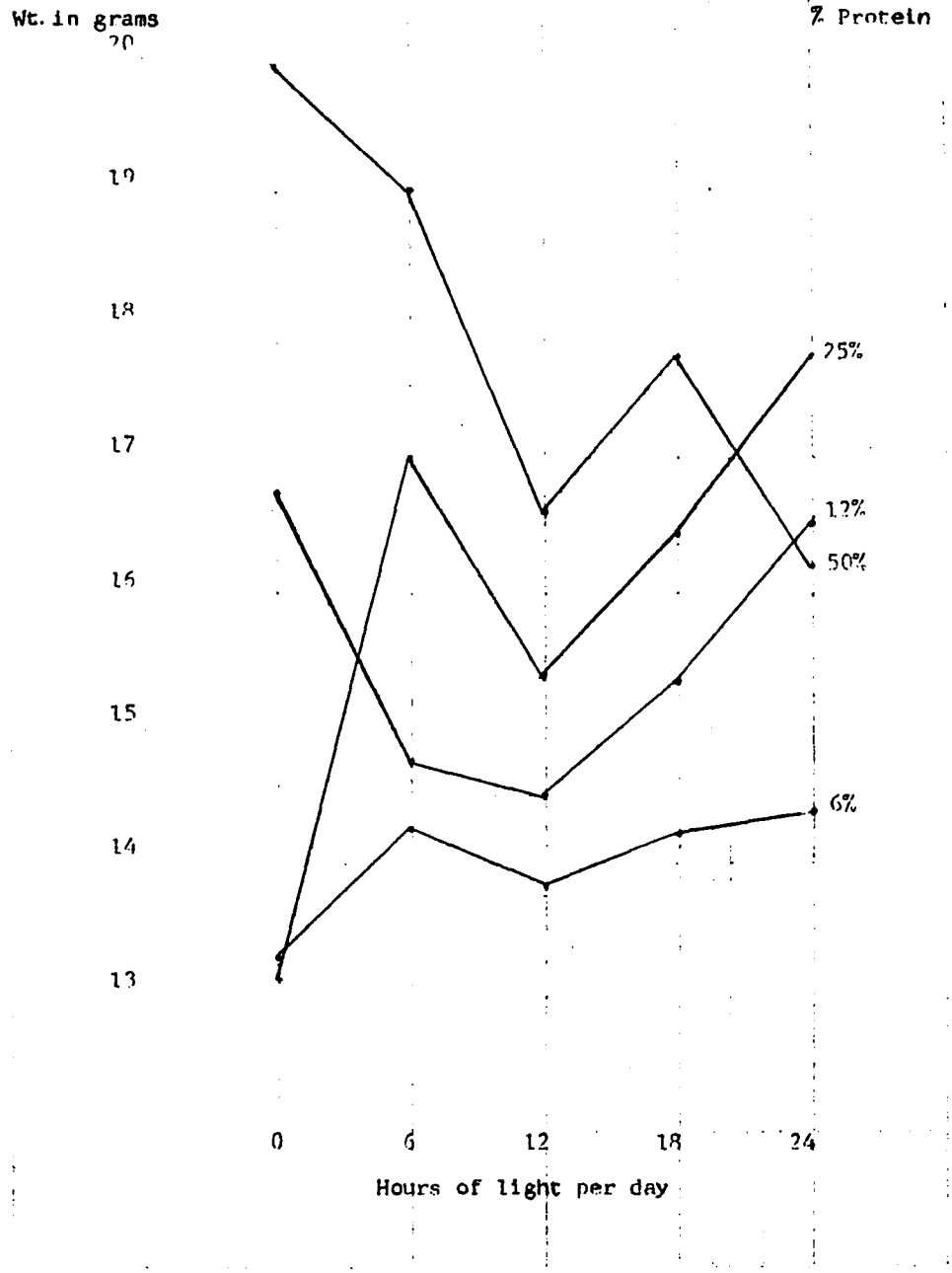
Liver Weight at Sacrifice

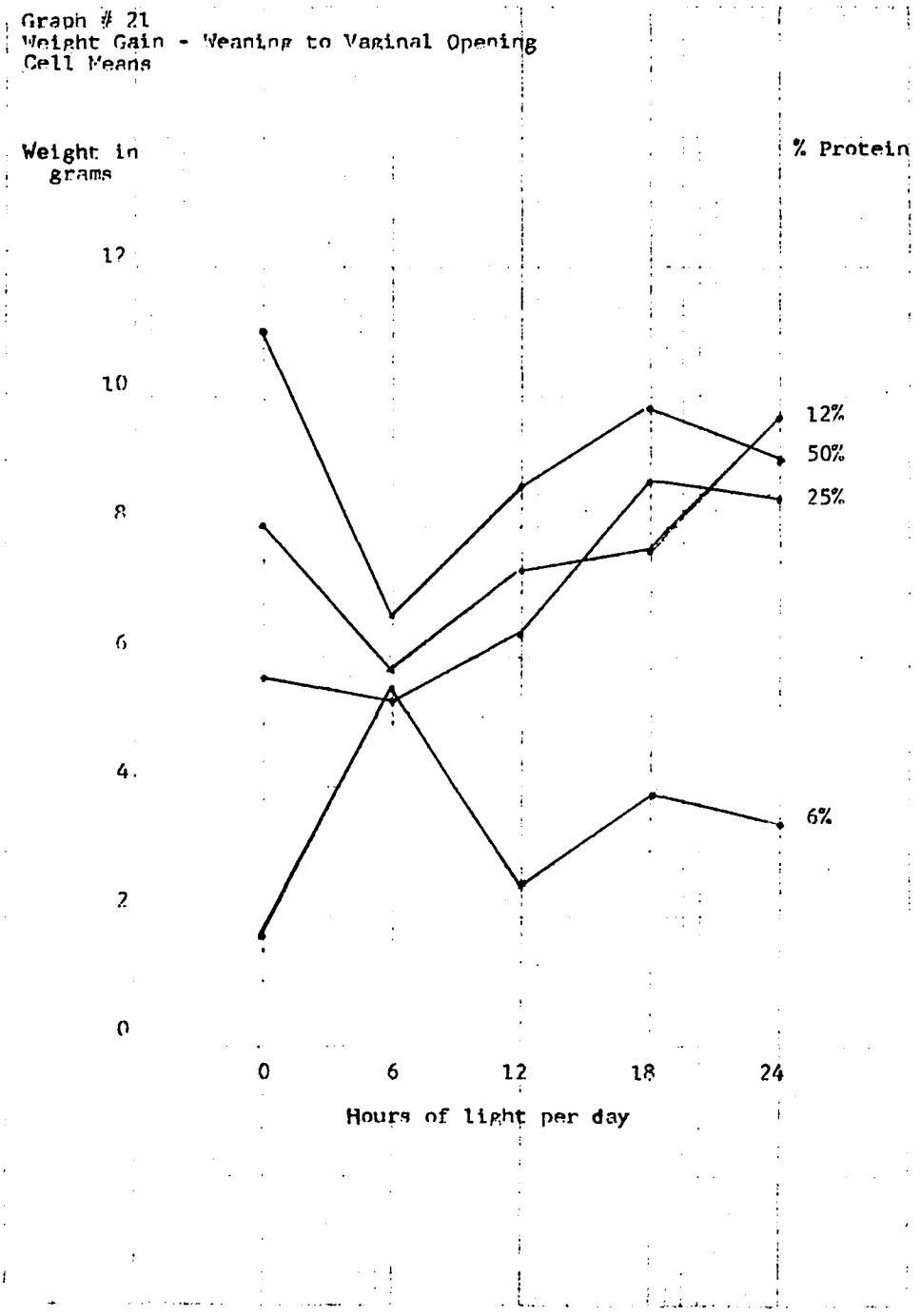


Graph # 19
Number of Days - Weaning to Vaginal Opening
Cell Means

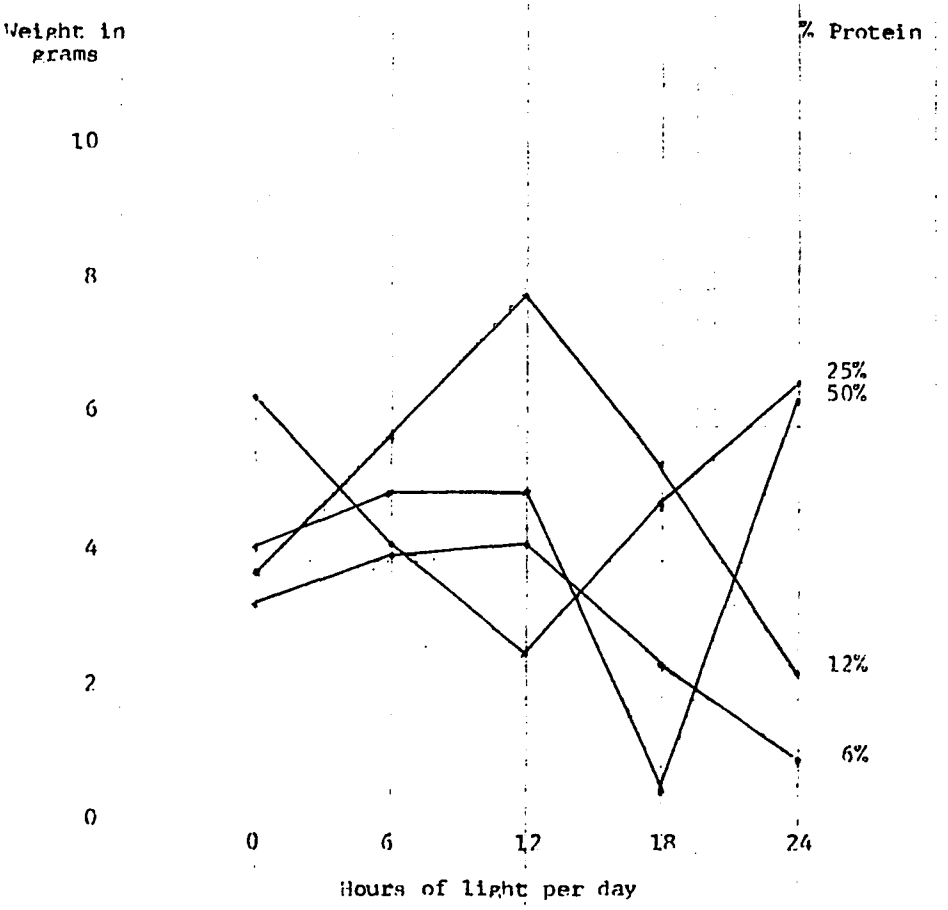


Graph # 20
Weight at Vaginal Opening
Cell Means

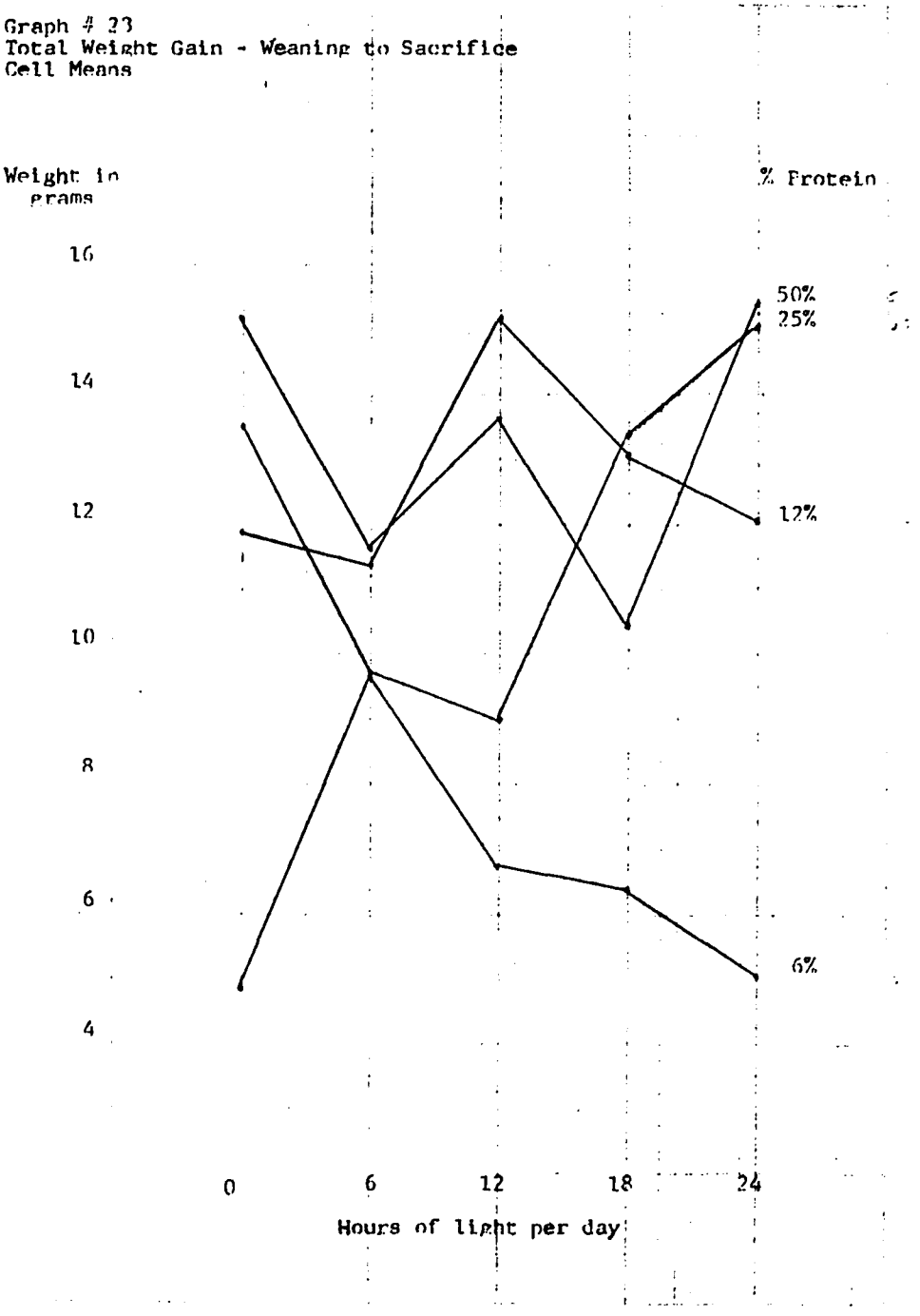


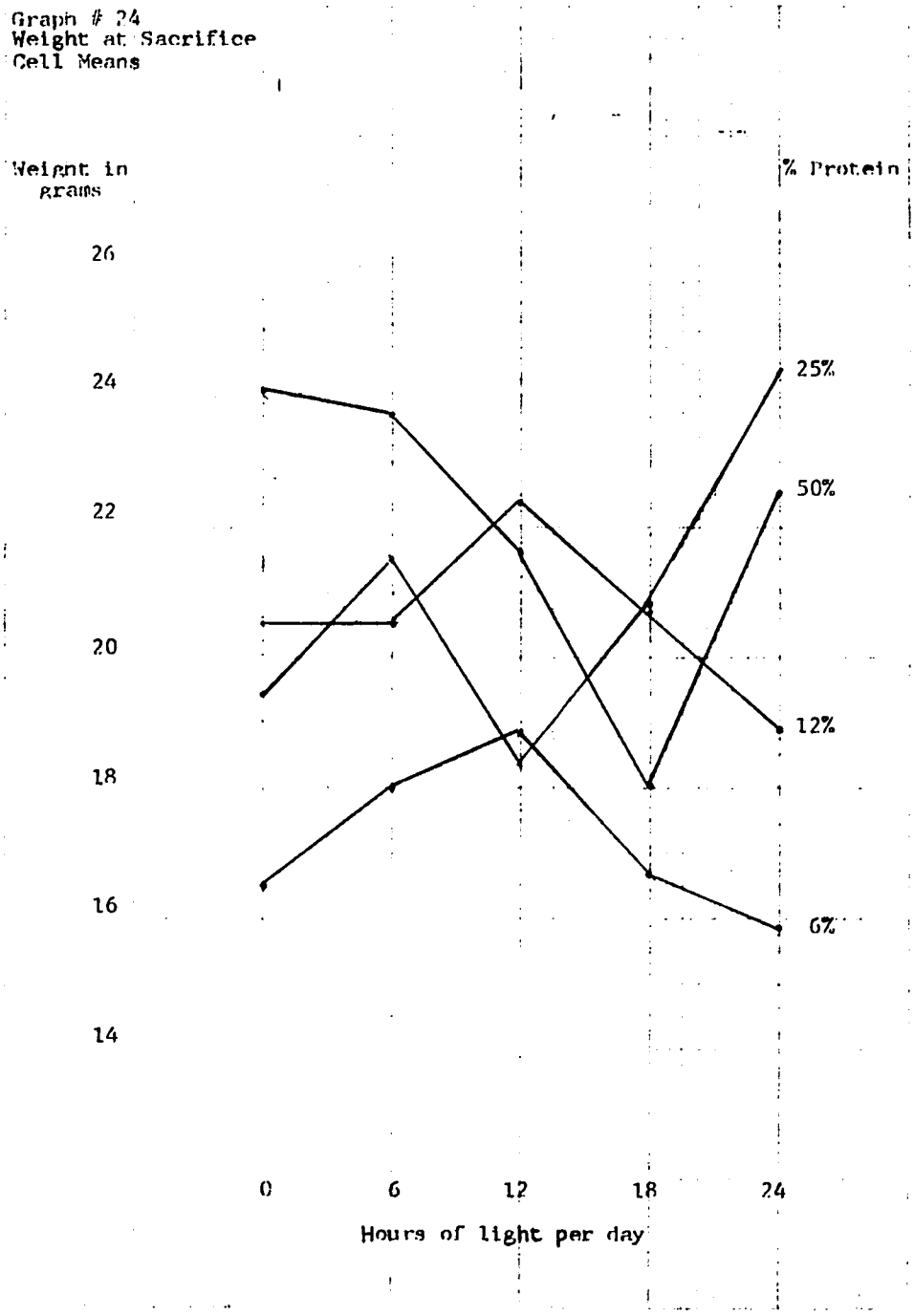

Graph # 21
Weight Gain - Weaning to Vaginal Opening
Cell Means
Weight in grams
% Protein
12
10
8
6
4
2
0
12%
50%
25%
6%
0
6
12
18
24
Hours of light per day

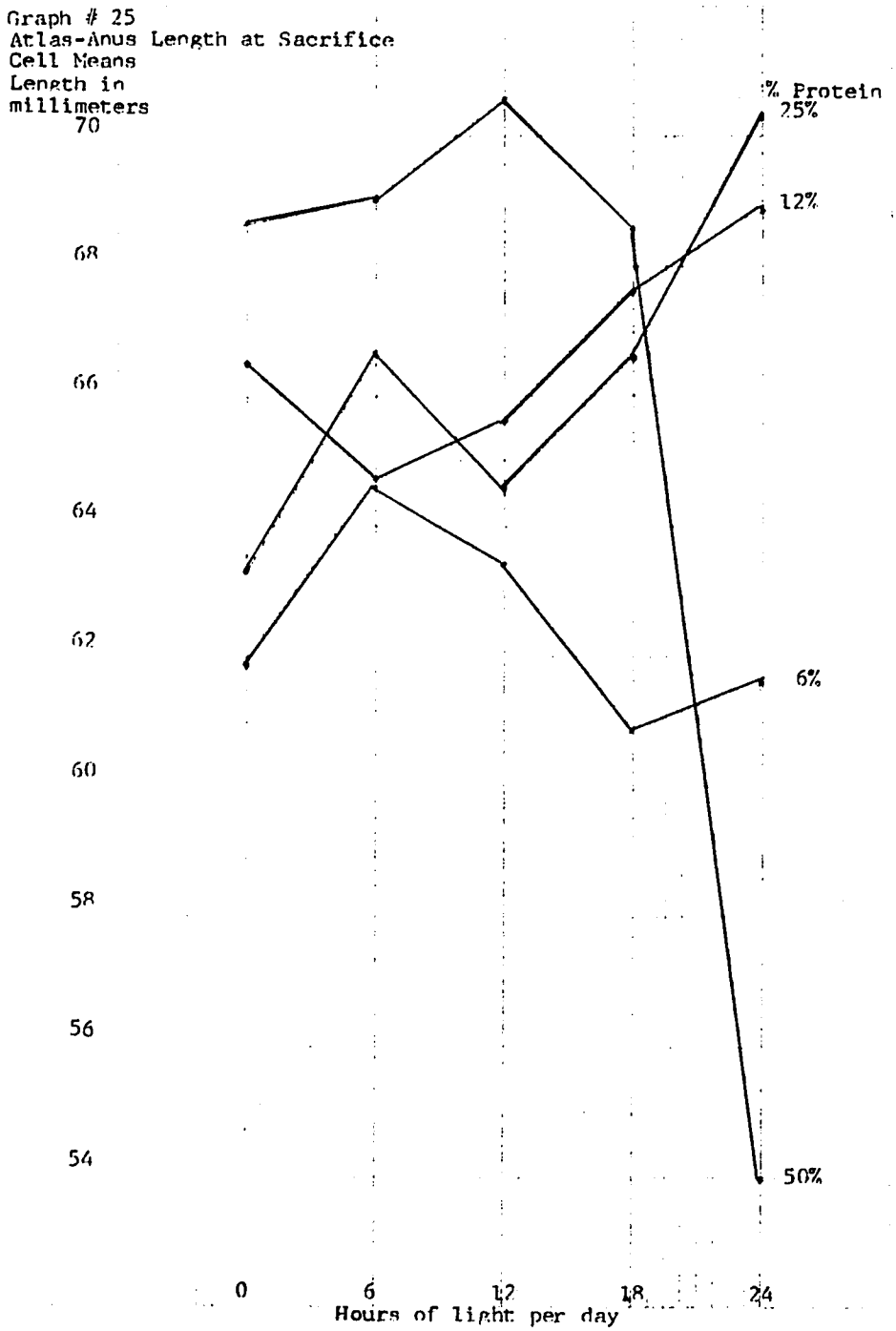
Graph # 22
Weight Gain - Vaginal Opening to Sacrifice
Cell Means

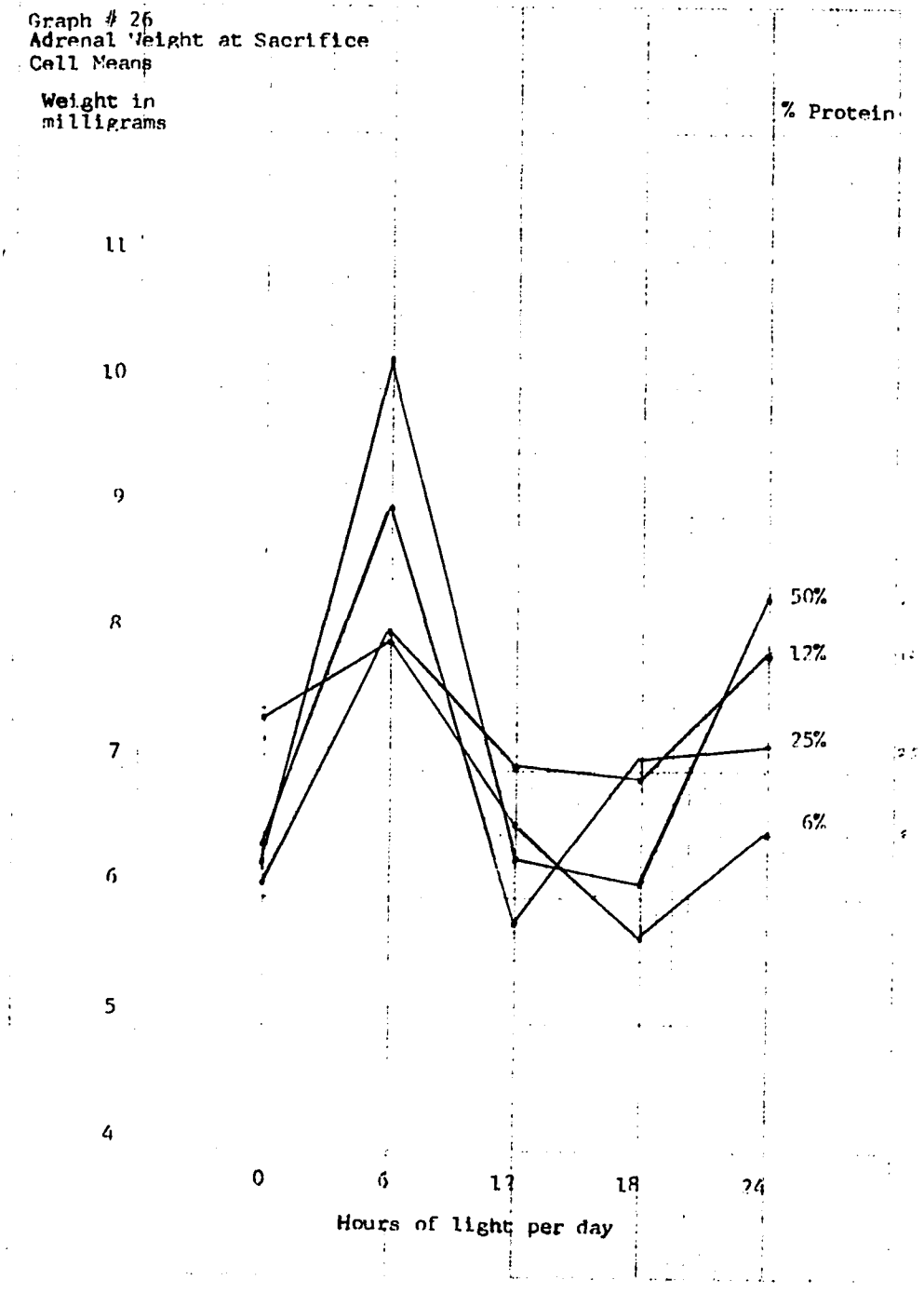


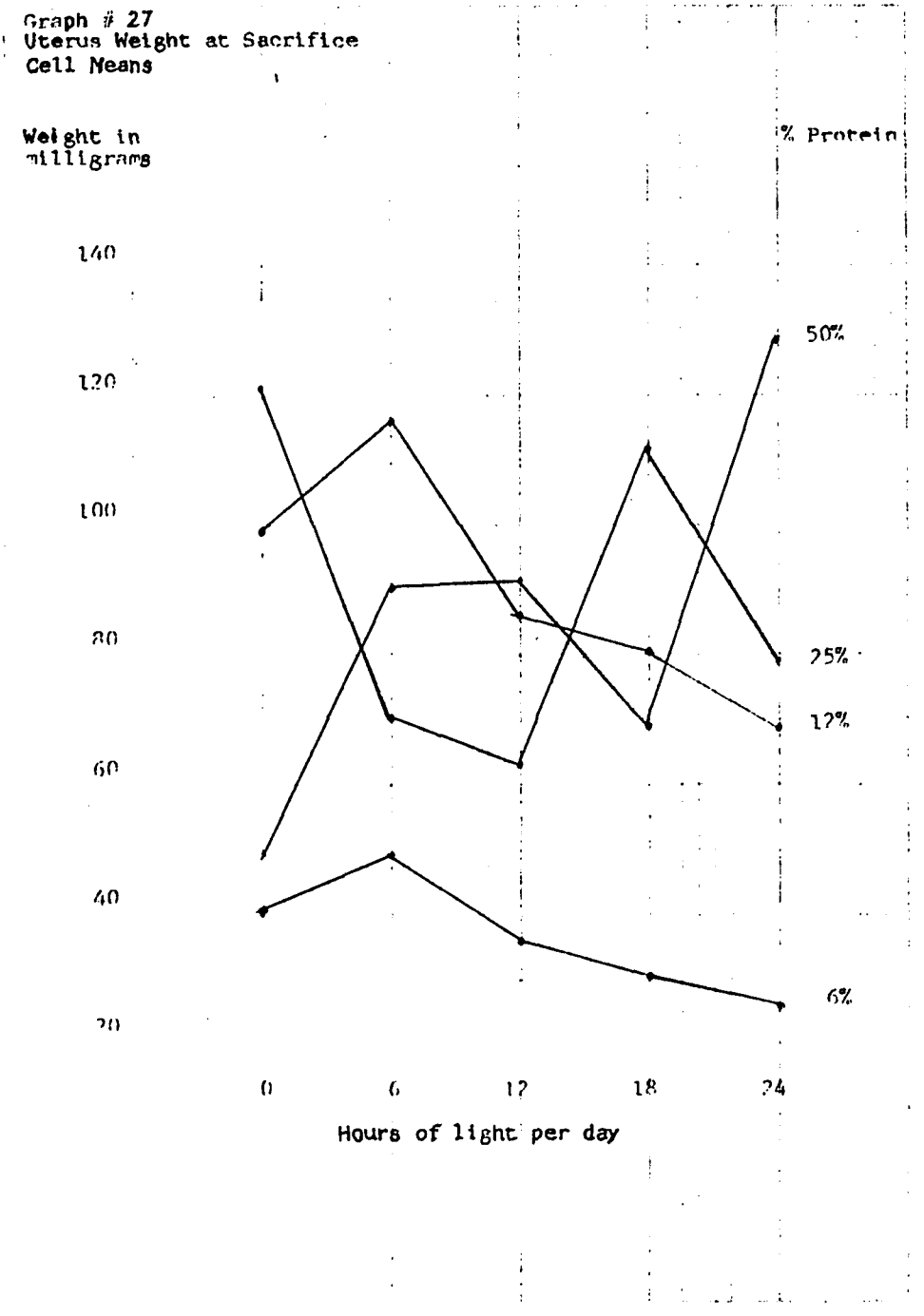
Graph # 23
Total Weight Gain - Weaning to Sacrifice
Cell Means



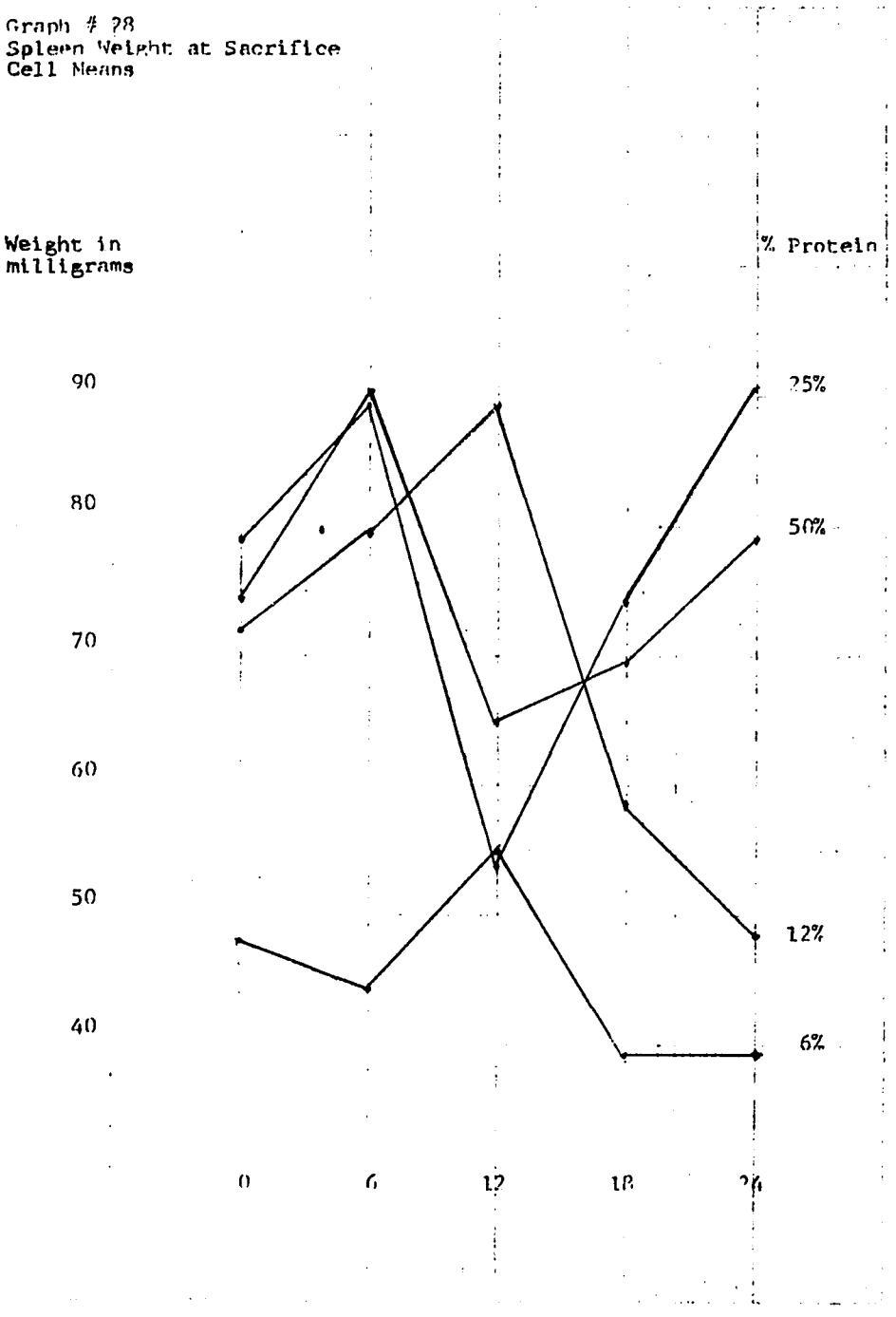

Graph # 24
Weight at Sacrifice
Cell Means
Weight in grams
% Protein
26
24
22
20
18
16
14
25%
50%
12%
6%
0
6
12
18
24
Hours of light per day

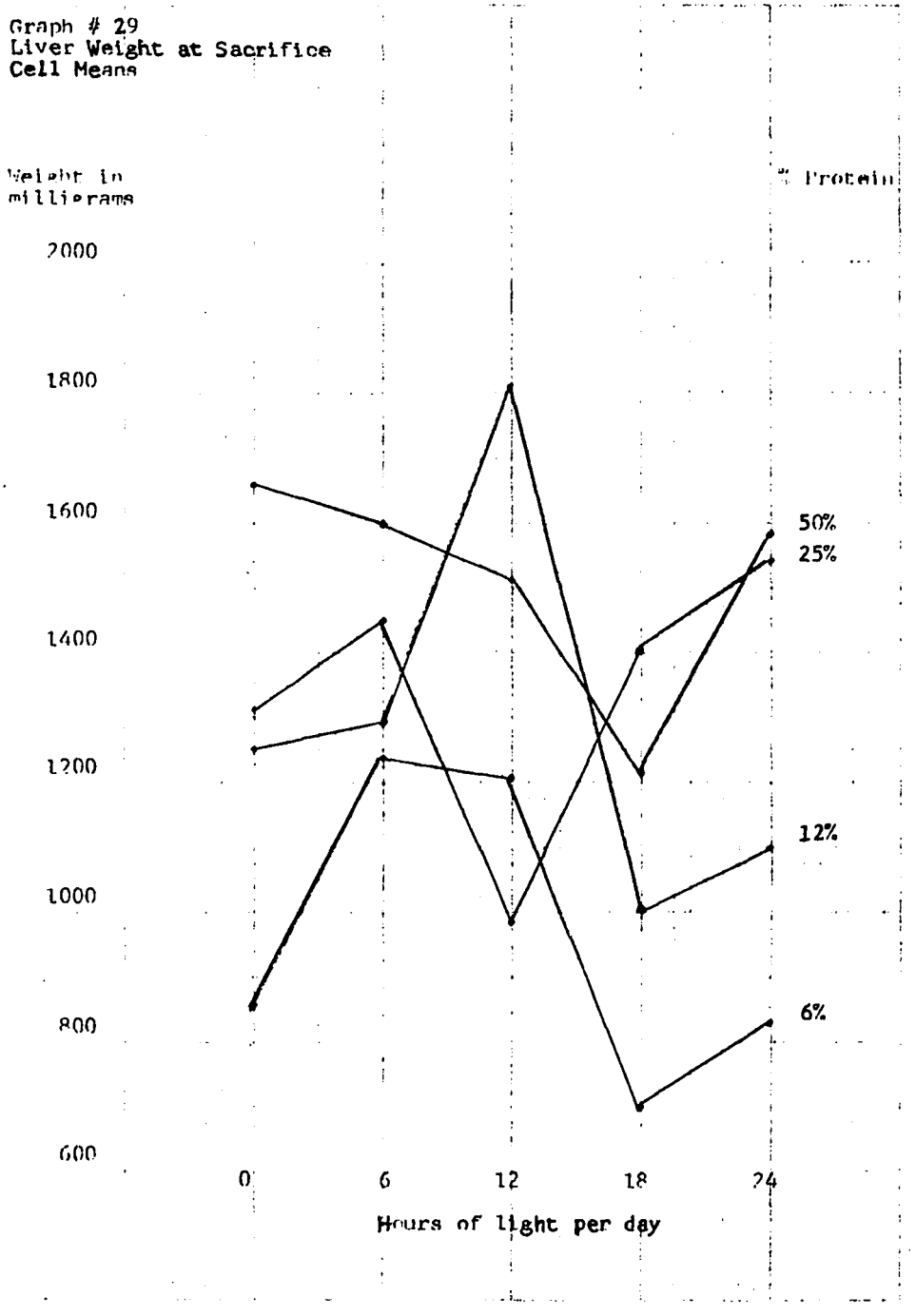

Graph # 25
Atlas-Anus Length at Sacrifice
Cell Means
Length in
millimeters
70
68
66
64
62
60
58
56
54
% Protein
25%
12%
6%
50%
0
6
12
18
24
Hours of light per day


Graph # 26
Adrenal Weight at Sacrifice
Cell Means
Weight in milligrams
% Protein
11
10
9
8
7
6
5
4
50%
12%
25%
6%
0
6
12
18
24
Hours of light per day


Graph # 27
Uterus Weight at Sacrifice
Cell Means
Weight in milligrams
% Protein
140
120
100
80
60
40
20
50%
25%
12%
6%
0
6
12
18
24
Hours of light per day

Graph # 28
Spleen Weight at Sacrifice
Cell Means




Graph # 29
Liver Weight at Sacrifice
Cell Means
Weight in milligrams
% Protein
2000
1800
1600
1400
1200
1000
800
600
50%
25%
12%
6%
0
6
12
18
24
Hours of light per day

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