I The Effects of Feeding Diethylstilbesterol and a Forage Antiestrogen on the Reproduction of Female Mink. (*Mustela vison*).

II The Effects of Various Protein and Energy Levels on the Maintenance and Early Growth of Mink. (*Mustela vison*).

by

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ABSTRACT

In Part A of Study I, six groups of female standard mink were fed (once daily) either 5, 10, or 15 mcgm. of DES/mink/day or a forage antiestrogen extract at levels of 5, 10, or 15 gm. equivalent of alfalfa/mink/day. In Part B, seven groups of female pastel mink were fed (once daily) 15 mcgm. of DES/mink/day prior to and at various stages of the gestation period. Also, the antiestrogen extract was again fed (once daily) at levels of 5, 10, or 15 gm. equivalent of alfalfa to three groups of female pastel mink after implantation of the fertilized egg had occurred.

From the results of Part A and B of Study I it is evident that DES at levels as low as 5 to 15 mcgm/mink/day caused complete reproductive failure. At the 15 mcgm. level it is possible to prevent or disrupt pregnancy if the hormone is administered for at least one week during the period starting one week before breeding and ending 30 days prior to parturition. The forage antiestrogen at levels between 5 and 15 gm. equivalent of alfalfa reduced the number of kits per litter from 5.8 to 1.6. The feeding of various levels of DES or the antiestrogen extract did not adhere to a dose/response relationship either in interrupting pregnancy or reducing the number of kits per litter.

The DES, fed at levels of 5, 10, or 15 mcgm./mink/ day was a more potent antifertility agent than the forage antiestrogen fed at levels equivalent to 5, 10, or 15 gm. of alfalfa.

In Part A of Stufy II, 200 mature male and female mink of a variety of colour phases were divided into five groups. The groups were fed (twice daily) rations varying in crude protein (36.6 to 44.8%, on a d.m. basis) and gross fat (21.3 to 28.9%, on a d.m. basis). In Part B, 280 pastel and standard kits (6-7 wks. old) were fed (twice daily) rations varying in crude protein (39.0 to 54.3%, on a d.m. basis) and gross energy (5.20 to 5.52 Kcal/gm. dry feed).

From the results of Part A and B of Study II it is evident that rations containing 35 to 40% crude protein and 20 to 25% fat, on a dry matter basis, are satisfactory for the late growth and maintenance requirements of mink. The mean apparent digestibility coefficients obtained in these studies for dry matter, protein, fat, and energy are 66.38, 74.05, 87.20, and 73.74%, respectively. As the apparent digestible energy (A.D.E.) was raised from 353.4 Kcal/100 gm. dry matter to 426.0 Kcal/100 gm. and as the apparent digestible nitrogen (A.D.N.) level reached 4.980 gm/100 gm

dry matter, the early growth of both male and female mink was improved. Maximum early growth rate was obtained in kits when the ration contained 85.54 Kcal of A.D.E./gm A.D.N. (13.6 Kcal gross energy/gm gross protein). Also, increasing A.D.E. content in the diets had a definite protein-sparing effect. Maximum nitrogen retention was obtained when the diet contained 426.0 Kcal of A.D.E./100 gm. of dry feed. Finally the results suggest that the growing male kits (from 6-28 weeks of age) were capable of utilizing feeds more efficiently than female kits of the same age.

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I. Introduction

It has become evident that various hormones of animal and plant origin and certain synthetic hormones have a marked effect on fertility. Occasionally, in the past, the contamination of feeds by estrogens has caused reproductive disturbances resulting in a decrease in the number of litters in breeding colonies of laboratory animals.[142] In commercial enterprises, the area which has been most drastically affected by estrogen contamination of feeds is the mink industry. The synthetic estrogen, diethylstilbesterol (DES), for a number of years, caused marked reductions in the annual kit production across Canada and the United States. During the late 1950's, the indiscriminate use of DES as a "growth stimulant" resulted in the introduction of DES into mink feeds via pellet-inserted chicken heads,[105] poultry viscera or dried tankage,[105] and beef tripe from animals implanted with or fed DES prior to slaughter.[136]

Today, however, legislation at the turn of the decade has limited the use of DES in livestock feeding. As a result, less contamination of slaughterhouse by-products, especially in the poultry industry, has safeguarded against the major introductory routes of DES into mink rations. The drastic effects of estrogens on fertility, however, still causes concern that other possible routes of estrogen contamination exist.

II. Literature Review

A. Estrogens and Antiestrogens

Reproductive difficulties in animals grazing certain kinds of pastures were first noticed in Australia in connection with the increased use of subterranean clover (Trifolium subterranean) as a sheep pasture.[11] Since then, a number of estrogen-like compounds have been isolated from a variety of plants.[95,96]. The most significant plant estrogen is coumestrol isolated from ladino clover (Trifolium repens) by Bickoff, et al[14] in 1957. Kitts, et al[77,78] demonstrated that the level of estrogenic activity in certain legumes and grasses (e.g. alfalfa, ladino clover, and birdsfoot trefoil) was highest in spring and decreased toward autumn. Legg, et al [80] felt that the level of estrogenic activity may be associated in some way with the reproductive status of the plant material from which they were recovered.

In 1960, Bickoff, et al [15] found inhibitors to estrogen activity in some alfalfa samples. Adler,[1] in 1962 demonstrated that as well as possessing estrogenic properties, alfalfa also contained antiestrogenic properties. Ostrovsky and Kitts[97] have suggested that birdsfoot trefoil contains an antiestrogen similar to

that found in alfalfa. Cook and Kitts[33] have reported the presence of an antiestrogenic substance in yellow pine needles. In 1965, Adler[2] established the presence of estrogen inhibitory activity in oat hay (Avena satina) and fahli clover hay (Trifolium alexandrinum var. Fahli).

Inhibition of fertility by estrogens and anti-estrogens can be achieved by interfering with normal mechanisms at any one of several vulnerable points in the reproductive process.[114] These include impairment of gametogenesis by compounds having direct action on germ cells,[72,112] prevention of ovulation by administering steroids which suppress pituitary production of gonadotrophins,[111,13] interference with the estrogen/progesterone ratios essential for tubal transport and implantation of the fertilized ovum and destruction of ova and developing embryos with antimetabolites.

1) Suppression of Pituitary Gonadotrophins

The antifertility effects due to prevention of ovulation can be obtained by chemical antagonism to the secretion of gonadotrophins.[72] Compounds which can antagonize the secretion of gonadotrophins include estrogens, such as DES,[72] and norethynodrel;[112] androgens, such as ethinyl androstenediol 3-cyclohexy-lproprionate[13]; and the progestational steroids.[72]

The inhibition of the pituitary gonadotrophin secretion results in an impairment of follicular development and subsequent ovulation of the mature ovum.[103]

2) The effects of estrogens and antiestrogens on transport of the fertilized ovum.

a) Estrogens

Following the initial work of Parkes and Bellerby,[98] and Smith[130] with mice and rats respectively and Kelly[75] with guinea pigs, Burdick and Pincus[21] in 1934 suggested that the daily injection of an estrogen (eotrin) into mice and rabbits during the preimplantation period resulted in the retention of ova in the Fallopian tubes. It was felt that the retention of the ova was probably due to either a closure of the tubo-uterine junction or an alteration in the nature of the ciliary movement which subsequently resulted in the inhibition of pregnancy. Also, Burdick and Pincus[21] found that all ova, whether they descended into the uterus or remain in the tubes, showed definite signs of degeneration by the fourth day after copulation. In 1936, Whitney and Burdick[138] showed that the injection of estrogenic substances during the preimplantation period

caused retention of ova in the Fallopian tubes and originated the term "tube-locking" of ova. Whitney and Burdick[138] found that tube-locking occurred when low doses (5-10 R.U.[1]) of an estrogenic substance was injected into the rat, but suggested that with heavier doses of estrogens increased ova transport occurred due to increased ciliary motility. It was later demonstrated by Burdick and Whitney[23,24] that injections of 100 to 500 R.U. of an estrogenic substance accelerated the passage of fertilized ova through the Fallopian tubes of mice. Ova were frequently found in the uterus even in the two-celled stage, 30 to 40 hrs. after finding the vaginal plug. This is at least 30 hours sooner than usual. In 1938, Whitney and Burdick[139] obtained similar results after injecting rabbits with a single massive dose (5000 R.U.) of an estrogenic substance. Synthetic estrogens, such as DES, administered in early pregnancy also accelerated the rate of tubal passage.[22]

To investigate further the effects of estrogens on pregnancy, Dreisbach,[44] in 1959, attempted to find the smallest dose of hormone and the most

1. R.U. = rat unit.

appropriate time of administration which would produce alterations in tubal transport in the rat. He found that as little as 0.02 mg/kg. body weight was effective during the period of tubal transport and the period most sensitive to estrogen administration was the third day post-coitum. Also in 1959, Greenwald[58] studied the comparative effectiveness of estrogens in interrupting pregnancy in the rabbit. He found that estradiol benzoate was the most effective estrogen in interrupting pregnancy when compared with estrone and DES.

In 1961[59] and again in 1963[61] Greenwald, in his study of the transport of ova through the rabbit oviduct, reported results which were contrary to the tube-locking effect of estrogens in the rat reported by Whitney and Burdick[21] in 1936 and the acceleration effect on fertilized ova reported by Burdick and Whitney in 1937. Greenwald[59] reported that while the injection of 250 µg of estradiol resulted in the tube retention of ova, 25 µg of the same compound accelerated ova transport into the uterus; many of the ova were then expelled into the vagina. Also, Greenwald[59] found that when the ova were retained in the tube by the injection of a large

dose of estrogen, the ova were blocked, not at the tubo-uterine junction as reported earlier,[21] but at the beginning of the isthmus or ampullary-isthmus junction. Later in 1961, Greenwald[60] found that after a single injection of estradiol cyclopentylpropionate, on the morning sperm were found in the vagina, tubal and uterine motility were increased so that the fertilized eggs were expelled from the uterus by 48 hrs. post-coitum. Deanesly,[40] in 1963, concurrently found that low doses of estradiol benzoate, given immediately after mating caused premature expulsion of fertilized eggs from the Fallopian tubes in the guinea pig. He suggested that the term "tube-locking" should be dropped. In 1964, Banik and Pincus[10] showed that as much as 20 µg/rat of estrone administered on Day 1 of pregnancy expells the eggs from the tube into the uterus in about 20-24 hours on Day 2. There was no evidence that a large quantity of estrone (250 µg/rat) blocks the fertilized egg at the utero-tubal junction. Also in 1964, Harper,[64] using autoradiographic techniques to trace the movement of "artificial eggs" (radioactive spheres), demonstrated that estradiol benzoate, in the dose administered (0.5 µg), had an accelerating effect on the passage of the spheres through the tube, and also

through the uterus. It was observed, however, that despite the accelerating effect of the estrogen a certain percentage of spheres were trapped at the junction of the ampulla and the isthmus. Recently, in 1968, Humphrey and Martin [71] presented experiments in which one level of estradiol-17β (0.4 μg/day) caused both retention and loss of ova in mice. Retention did not only involve blockage at the utero-tubal junction, since ova were also located in the ampulla. Later in 1968, Humphrey[70] demonstrated that sphincters at the ampulla-isthmus and isthmo-uterine (utero-tubal) junctions are capable of delaying fertilized ova during their sojourn through the Fallopian tubes.

b) Antiestrogens

Although not as extensively studied as the estrogens, both the natural and synthetic antiestrogens are capable of interfering with pregnancy by altering the rate of tubal transport of the fertilized ovum. It was first reported by Burdick, Emmerson and Whitney,[20] in 1940 that testosterone propionate injected in adequate amounts into mice will cause the retention of blastocysts in the oviducts. The results showed that 2.0 mg. daily was the liminal amount of testosterone propionate necessary to cause retention of blastocysts in the tubes beyond 100 hrs. after the formation of the vaginal plug. In 1961, Greenwald[59] with a single injection of progesterone caused some acceleration in ova transport. In only one instance were

ova recovered from the uterus at 48 hrs. after administration of 25 mg. of progesterone. Acceleration of ova was also obtained with the injection of similar amounts of progestin.[59]

In 1964, Harper,[64] found that progesterone delayed movement of the radioactive spheres through the ampulla very markedly, but if the spheres reached the isthmus they passed into the uterus on all occasions. Chang[30] in 1964 and Chang and Yanagimachi[31] in 1965 showed that a number of antiestrogens interfered with ovum transport in rabbits, causing acceleration in some and retention in others. In 1968, Humphrey and Martin[71] demonstrated that DMS[1.] (100μg/day), MER-25[2.] (2.0 mg/day) and MRL-37[3.] (2.0 mg/day) administered subcutaneously on Days 1, 2 and 3 post-coitum to female mice resulted in retention of ova in the oviduct. As well, the antiestrogens delayed the development of the retained ova and ova were prominently recovered from the tube in the morula rather than the blastula stage.

Although antiestrogens are capable of disrupting the passage of fertilized ova through the Fallopian tubes, they are even more effective as anti-implantation agents. In 1959, Dreisbach[44] reported that pregnant rats were most

1. DMS = dimethylstilbesterol
2. MER-25 = 1-(p-2-diethylamino-ethoxyphenyl)-1-phenyl-2-anisyl ethanol
3. MRL-37 = corresponding ethane of MER-25

sensitive to antiestrogen administration thus differs from the time when estrogens produce their greatest effect, that is when the ova are in the Fallopian tubes.

c) Mechanism of Action:

i) Estrogens

With regards to tube-locking, it is presently felt that the ampullary-isthmic junction rather than the utero-tubal junction is the primary block to egg transport in the rabbit.[61] According to Wintenberger-Torrès[141] the major block to egg transport in the ewe occurs at this junction. But Greenwald[61] feels that the utero-tubal junction is probably the primary barrier in some species.

It is important to comprehend the nature of the blocking mechanisms not only to understand how large doses of estrogenic compounds[61] act to retain ova in the tube, but also to determine how low levels of estrogens prematurely advance the time of opening of the ampullary-isthmic junction.[61] The mechanism of action may also explain why Whitney and Burdick[138] found that low doses of an estrogen resulted in tube-locking and large doses caused ova acceleration; results which are contradictory to the more recent reports.

The first theory proposed was by Hartman[65] in 1939. Hartman suggested that the administration of an estrogen caused an alteration in the estrogen-progesterone balance,

which caused a variation in the rate and amplitude of muscle activity in the tube and in turn the rate of passage of the ova.

A second theory was proposed by Alden[5] in 1942. From his observations by transillumination, Alden felt that while ciliary action was the primary force in effecting the entrance of the egg into and through the cephalic ampulla, action of the tubal musculature was the controlling factor governing further advancement. The administration of an estrogen, therefore, would result in the closure of the lumen sphincter at the site of the block due to the contraction of muscle layers.

Black and Asdell[16,17] later proposed a third theory to account for the retention of the fertilized ova. They suggested that there was a slight or complete absence of activity of the circular muscle at the site of the blocked ova. Also movement induced by the circular muscle is sufficient to account for transport of ova through all of the oviduct except the isthmus portion immediately anterior to the tubo-uterine junction.[16] The absence of circular muscle activity at the uterine end of the oviduct may partially account for ovum delay in this portion of the tube.[17] Black and Asdell[17] have attempted to demonstrate the presence of edema in the tubo-uterine junction, but

these attempts have failed. They feel, however, that the possibility still exists that it is present since the degree of edema would not have to be great to be effective in closing the tube.

Edgar and Asdell,[46] in 1960, from their study of the valve-like action of the utero-tubal junction in the ewe, were able to propose a fourth theory. Evidence they presented indicates that estrogens cause edema on the subserosa and muscle coats of the wall of the utero-tubal junction of the ewe. It seems likely that the edema causes an increase in the degree of flexure of the tubal wall in the immediate area and the two phenomena contribute, along with the narrow lumen and the folding of the tubal lining to the valve-like action of the utero-tubal junction.[46] In support of this theory, Voyes, et al[93] found it increasingly difficult to force fluid from the ampulla through the utero-tubal junction as the dosage of estrogen was increased.

Finally a fifth theory was proposed by Wintenburger-Torrès[141] in 1961 which suggests that strong antiperistaltic contractions of the isthmus maintain the egg in the ampulla and prevent any further progression down the uterine tube.

At present there is little evidence to support any of these theories. It would appear, however, that the injection of estrogen interferes with progesterone dominated mechanisms.[23] Greenwald [57] in 1957 demonstrated that mucin, a mucopolysaccharide, secreted by the tubal epithelim onto the ova is inhibited by the administration of estradiol in rabbits. It is not known, however, whether or not the

reduced mucin layer plays a significant role in the retention of fertilized ova.

In conclusion ovum transport is aided by the contractile activity of the tube which is co-ordinated by anatomical and physiological mechanisms. Many of the mechanisms of reception and transport of ova are markedly affected by the complex actions and interactions between the ovarian steroids and pituitary hormones, the sympathetic nervous system and the adrenal steroids.[63] Any abnormal alterations in hormone ratios, therefore, would cause alterations in both reception and transport of the fertilized ova.

ii) Antiestrogens

To determine the mechanisms by which antiestrogens interrupt pregnancy, it was first necessary to determine whether these compounds were acting either by virtue of their estrogenic or antiestrogenic properties. It was shown by Martin, et al[87] in 1960 that antiestrogens are also estrogenic (or pro-estrogenic) in high doses. Martin and his colleagues did, however, show that differences exist between the relative potency of dimethylstilbesterol, the most potent anti-strogen tested and estradiol as anti-fertility agents and their relative potency as estrogens. Also, differences existed between their capacities to interrupt pregnancy in single doses. It was therefore concluded that DMS, at least, probably acts by virtue of its

antiestrogenic properties. In the same year, Emmens, et al[51] found that when injected together, DMS did not antagonize theanti-fertility action of estradiol. This was regarded as very good evidence that DMS is, in fact, acting as a proestrogen in preventing fertility. In 1963, Martin, et al[8] demonstrated that a number of synthetic anti-estrogens interrupted early pregnancy in mice. It was still not clear, however, whether they did so by reason of their anti-estrogenic or proestrogenic activity since, in the series tested, both properties were highly correlated with the effectiveness of the compound in interrupting pregnancy. Again in 1964, Emmens, et al[52] while studying the estrogenic and antiestrogenic activity of compounds related to DES, found a high correlation between proestrogenic, anti-estrogenic and anti-fertility potencies in these compounds. It was still hazardous, however, from the evidence presented, to ascribe the anti-fertility action of antiestrogens to either their proestrogenic or antiestrogenic properties.

Up until this point the majority of the evidence presented was interpreted in favour of DMS acting as an anti-fertility agent by virtue of its antiestrogenic activity. However, in 1964, Stone and Emmers,[133] found that sensitivity to single injections of both estradiol and DMS was highest approximately 72 hrs. after mating and had decreased after 96 hrs. In view of the similar action of the two compounds and the lack of consistent antagonism when

injected together, it was suggested that the anti-fertility action of DMS in the mouse was due to its pro-estrogenic activity. In a second fertility study, Stone and Emmens[134] found that a single, simultaneous injection of DMS and estradiol to 60 hrs. pseudopregnant rats showed only an additive effect in inhibiting deciduoma formation and suggested that DMS was acting as a proestrogen rather than an antiestrogen. In 1965, Emmens,[49,50] by further detailed analysis of the activities of DMS by different routes of administration in the rat and mouse, decided that in the case of that particular compound the anti-fertility effect is very probably due to its proestrogenic action. However, among the non-steroids, some, including MRL-37 and MER-25 may have anti-fertility effects unrelated or incompletely related to estrogenic activity and possibly related to antiestrogenic activity.[50] Finally, in 1968, Humphrey and Martin[71] presented results which confirm the view that most antiestrogens are acting by virtue of their proestrogenic activity.

The effects of estrogens and antiestrogens on implantation.

The ability of estrogens and anti-estrogens to interrupt pregnancy, administered after the ovum has normally entered the uterus, has been well described in the literature. In 1938, Parkes, et al[99] demonstrated that oral administration of estrogens is effective in preventing implantation of the blastocyst. DES, prepared by Dodds, Goldberg, Lawson and Robinson[42], the biological properties of which have been described by Dodds, Lawson and Noble,[43] is reported highly active by mouth. Oral administration of DES is highly effective in preventing implantation in rats at levels as low as 5 to 16µg.[99]

D'Amour, et al,[36] in 1933, presented evidence indicating that appropriate dosages of estrin, administered during the early stages of gestation, prevented implantation in the rat and that this effect was not overcome by simultaneous injection of an estrogen antagonist, such as progesterone. In 1934, D'Amour and Gustavson[37] suggested that the action of estrin might be a direct one, affecting the uterine mucosa in such a manner astto prevent implantation.

Greenwald,[57] in 1957, has reported that the administration of estrogens in the rabbit 72 hrs. post-coitum involves an alteration in the progesterone dominated uterine environment prevents the implantation of ova. Segal and Nelson[114] reported that the anti-estrogen MER-25 can also interfere with the progestational support of the endometrium, which results in the reduced development of implantation sites.

Dreisback[31,44] found that as little as 0.1 mg/kg. body weight of estrone, injected subcutaneously, was necessary to prevent implantation in the rat when the ova were in the uterus. Fowler and Edwards,[113] in 1960, found that mice, injected 7 days after mating with 1.0 µg. of estradiol benzoate or progesterone had no implanted embryos on the day of autopsy. Cochrane and Shackelford[32] attempted to hasten ova implantation in mink by daily injections of a combination of 2.4 mg. of progesterone and 24 µg. of estradiol benzoate, beginning 7 to 22 days post-coitum. The hormonal treatment caused most females to be barren. In the animals which did produce kits, the administration of the exogenous estrogen alone or in combination with progesterone, resulted in reduced litter size and caused further delay of ovo-implantation.

The progestational proliferation or preparation of the endometrium, necessary for implantation of eggs, depends upon a delicate balance between estrogens and progesterone.[9] It has been adequately reported that both exogenous estrogens,[113] and anti-estrogens[51,45] interfere with the formation of deciduoma prior to implantation of the fertilized egg.

At physiological levels, the estrogens are essential for implantation to occur. Nutting and Meyer,[94] in 1964, reported that the optimum amount of estrone required for inducing implantation at the normal time appears to be between 0.3 and 1.0 µg. daily. If the level of estrone is below a threshold level a delay in nidation occurs, but if the estrone level is above the threshold level, nidation is induced.[94] Progesterone appears to be vitally concerned with maintaining the viability of the blastocyst during the extended preimplantation period.[88] Estrogens play an active part in determining whether implantation will occur,

but only if relatively large quantities of progesterone are available to ensure the presence of viable blastocysts.[88] Humphrey,[69] in 1967, Smith and Biggers[129] and Smith,[127, 128] in 1968, have shown that in the absence of estrogens or progesterone implantation will not occur, but when a uterus is primed with progesterone, the injection of sufficient amounts of an estrogen (0.024 µg. estradiol[69], 0.5 µg. 17β-estradiol benzoate[129]) will result in implantation of the fertilized ovum.

Mechanism of Action

In order to understand the mechanism by which estrogens and antiestrogens interfere with implantation, the suggested mechanism of ovum implantation must first be discussed. In 1952, Shelesnyak[116] reported that antihistamines, such as diphenhydramine or ephedrine hydrochloride, inhibited the formation of deciduoma when introduced as a pellet or in solution into the uterine lumen. Later, Shelesnyak,[117] in 1955, and Carlsen, et al.,[29] in 1961, also showed that antihistamines inhibited deciduoma formation, and that this inhibition was reversed by the concurrent administration of progesterone. These results suggested that antihistamines disturb the critical estrogen/progesterone balance for the induction and growth of the decidual cell reaction. In 1957, Shelesnyak[118] postulated that histamine by some means was released from the blastocyst and that histamine, the deciduoma inducing factor, plus progesterone operating on a progravid uterus are required for successful decidua

development. In 1958, Johnson and Shelesnyak[73] and Spaziani and Szego[131] demonstrated that the histamine was not produced by the blastocyst, but was produced by the uterus and that the release of the uterine histamine is under estrogenic control. In 1959, Shelesnyak[119,120,121] demonstrated that estradiol, estrone and estriol cause the disappearance of mast cells from the uterine endometrium thus suggesting a reduction in uterine histamine. Shelesnyak also suggested that a histamine-releasing-factor was involved, liberated by estrogens in the decidua cell reaction.[79] In 1962, Shelesnyak,[122] therefore, suggested that the normal stimulus for implantation is a surge of estrogens which release histamine from the mast cells.

Shelesnyak, et. al.[123] have shown that an antiestrogen (MER-25), administered septematically, inhibits nidation presumably by antagonizing the estrogen surge. Also, early injection of an estrogen or antiestrogen[46,55] could possibly antagonize the progestational state of the uterus making it insensitive to the surge of estrogen and thus, a decrease in implantation sites. Yochim and DeFeo[143,144] have suggested that nidation is dependent on fairly narrow limits of estrogen and progesterone concentration and any change in the circulating hormone level would result in the interruption of pregnancy. Martin[85] has shown that the histamine-estrogen relationship is indicative to the uterus, for histamine release is not involved in other estrogen sensitive tissues, such as those found in the vagina.

4. The effects of estrogens and antiestrogens on pregnancy after implantation has occurred.

Pregnancy can be interrupted after the completion of implantation by the administration of either estrogens or antiestrogens. It has been noted, however, ever since the early works of Smith[130] in 1926, Kelly[75], in 1931 and D'Amour, et. al.[36] in 1933, that as gestation proceeds, greater quantities of estrogens and antiestrogens are required to interrupt pregnancy[47]. Levin, et. al.[82] found that estrogens administered in relatively small doses, over a period of 3 days, during the second half of pregnancy, in rats, did not adversely affect either the pregnancy or length of gestation.

In 1938, Parker, et. al.[99] found that rabbits given 0.5 mg. or more of ethinylestradiol, an orally active estrogen, 9 to 10 days after mating resulted in resorption of all embryos. Also, rabbits receiving 1.0 mg. of DES on the 13, 14 and 15 days were found to contain 10 embryos, all being reabsorbed. Conversely, a similar dose on the 11, 12, and 13 day and half this dose on the 10, 11 and 12 day in other rabbits did not disturb pregnancy. In 1939, Heckel and Allen[66] found that the administration of estrogen early after implantation resulted in resorption of the rabbit fetuses, but if administered between the twentieth to twenty-seventh day, of the 32 day gestation period, spontaneous abortion occurred. Huggett and Pritchard[68] in 1945, found that after the twelfth day of pregnancy, with involution of decidua and the establishment of the allantoic circulation, low doses of estrone and

DMS can no longer cause fetal death in rats. Jost[74] in 1945, caused resorption and abortion in mice by the injection of 5.0 mg. of testosterone proprionate on the eighth day of pregnancy, and Courrier and Jost[35] found that the death of rabbit fetuses occurred when 20 mg. of testosterone proprionate was given daily to the mother from 16 to 21 days of gestation. Velardo, et. al.,[137] in 1956, found that estriol and certain of the C_{21}-metabolites related to progesterone (e.g. pregnane-3-~~ol~~-20-~~ol~~-diol and pregnanedione), administered in later pregnancy to rats, resulted in early abortions, some resorption of fetuses and reduced litter sizes. Velardo and his colleagues suggest that early abortion can be ascribed primarily either to inadequacy of the implantation site or to abnormalities of the embryo, or perhaps a combination of the two[137]. In 1960, Fowler and Edwards[56] demonstrated that mice, when injected with progesterone or estradiol benzoate in late pregnancy, resulted in high fetal mortality.

As well as the animal estrogens and antiestrogens, plant estrogens and antiestrogens can result in reproductive disorders in late pregnancy. MacDonald[84] in 1952, demonstrated that the ingestion of pine needles or pine buds, was the causative agent of abortion and the birth of weak beef calves. In 1964, Cook and Kitts[33] attributed the deleterious effects of the pine needle to its antiestrogenic content. Allen and Kitts[6] in 1961, extracted

the estrogenic components from pine needles which were then incorporated into a ration and fed to mice after theoretical implantation had occurred. When autopsied, many of the embryos had been completely resorbed.

5. Directoeffects of estrogens and antiestrogens on ova and embryos.

It has been shown that the natural estrogens[104] and progesterone[140] in excessive amounts are neither directly toxic to the ova nor are they essential for normal cleavage up to the blastula stage. Burdick, et. al.,[25] found that after estrogen injection, the normal round blastocyst stage, in which the trophoblast cells are in contact with the zona pellucida, is usually attained by the developing tubal ova. In 1942, Alden[3,4] concluded that no special uterine environment is necessary for production of the blastocyst, nor are ovarian secretions necessary for the early stages of the blastocyst formation. In fact, mouse eggs have shown development when placed into the anterior chamber of the eye.[110]

Antimetabolites, however, such as 6 diazo 5 oxo L-norleucine,[111] and synthetic estrogens and antiestrogens, such as DES[9] and MER-25[115] are cytotoxic to the developing ova. Even after implantation, compounds such as norethynodrel[39] and others can induce extence fetal destruction. It is felt that cytotoxic effects of the estrogens and antiestrogens, however, is not due to their respective hormonal activities per se.[76]

III Materials and Methods

PART A: Feeding Trial

A study extending from April 3, 1967 to May 19, 1967 was designed to observe the effects of daily feeding of DES and a forage antiestrogen extract on female mink during pregnancy. The two hormones were not mixed into the daily ranch ration until implantation of the fertilized egg was assumed to have occurred in the female. Commercial powdered DES was used, while the antiestrogen was extracted from dehydrated ground alfalfa leaf-meal. DES was incorporated into the mink ration, so that the animals received 5, 10, or 15 mcg. per day. The antiestrogen extract was incorporated into the diet so that the mink consumed 5, 10, or 15, grams equivalent of alfalfa per day.

1. Materials

a. Into a completely randomized design, thirty standard female mink were distributed into 6 groups with 5 animals per group.

b. Housing - All animals were housed at the U.B.C. mink unit in individual wire cages (17" x 15" x 15"). A wooden next box (8.5" x 7" x 7.5") was attached to one end of the cage and straw was used as bedding.

2. Methods

a. Feeding - The 6 experimental rations were mixed once every second day. Sufficient amounts of each ration were mixed and weighed into equal portions to permit daily feeding of the rations. The second day's feed was kept refrigerated until fed. To reduce the possibility of DES or antiestrogen contamination in the feed for the remainder of the U.B.C. herd

(the control groups), all mixing was done at a separate location distant to where the control feed was mixed. A wooden cover was placed over the feed on top of the experimental cages to prevent further contamination by physical transfer of feed to the surrounding cages. Water was fed ad libitum.

b. Extraction of antiestrogen - The extraction of the alfalfa antiestrogen was a modification of the method developed by Luizzo, et. al.[83] (See Appendix I). All alfalfa extractions were stored under refrigeration until mixed into the ranch ration.

c. Antiestrogen activity - The hormonal activity of the antiestrogen was determined using the Astwood, six-hour bioassay[8]. (See Appendix II)

d. Oral activity of DES - DES was fed to immature female rats for one week. At the end of this period the animals were sacrificed and the uteri were dissected free, trimmed of fat and weighed. The uterine response is expressed as a percentage of body weight.

e. Statistical Analysis:- Treatment effects were analysed using the Analysis of Variance[132].

PART B: Feeding Trial

On March 4, 1968 a second experiment was initiated to study the effects of DES fed at various stages of pregnancy. The experiment extended until May 11, 1968. Fifteen mcgms. of DES was fed to 6 groups of female mink according to the following scheme:

Group I For one week pretupping.

Group II From breeding to implantation.

Group III From implantation to the end of gestation.

Group IV From one week pretupping to implantation.

Group V From breeding to the end of gestation.

and Group VI From one week pretupping to the end of gestation.

The alfalfa antiestrogen extract was fed to 3 groups of mink after implantation had occurred at levels as described in Part A of this study.

1. Materials

a. Animals - In the DES study, 70 pastel female mink were distributed randomely into 7 groups with 10 animals per group. The controls received no DES. In the antiestrogen study, 20 pastel mink were randomized into 4 groups with 5 animals per group. One group, the controls, received no antiestrogen.

b. Housing - All animals were housed as described in Part A of this study.

2. Methods

a. Feeding - All mixing and feeding of the experimental rations were performed according to the procedure described in Part A of this study.

b. Extraction of antiestrogen - See part A of this study.

c. Antiestrogen and DES activity - See Part A of this study.

d. Statistical Analysis - See Part A of this study.

IV Results and Discussion

The estrogenic activity of the DES and the antiestrogenic activity of the alfalfa extract used in Part A and B of Study I were tested before each experiment was initiated. The results of the alfalfa extract bioassays are given (Tables I and II). The uterine response to the hormonal injections is expressed as a percentage of body weight to adjust for the variations in body size. It was found, by means of an Analysis of Variance, that the differences between the average uterine weights in the three treatments of the first bioassay (Table I) were significant ($P \leq 0.025$). The results of the second bioassay (Part B) were also significant, but to a higher degree ($P < 0.005$). In the bioassay for Part A the average uterine weight as a percent body weight in group III (estradiol-17β + C.E.C.) was lower than the control group. This suggests that the alfalfa antiestrogen not only acted antagonistically to the estradiol 17-β, but caused a dehydration of the uterus, which may be of some significance in its action as an antifertility agent. From the results, therefore, in Tables I and II, it can be said that the alfalfa extract was significantly antiestrogenic.

The oral activity of the DES was also tested. The same source of DES was used for Parts A and B of this study, therefore only one bioassay was done. The results presented in Table III show that the oral administration of DES to immature, ovariectomized Wistar rats caused a significant ($P < 0.05$) increase in uterine weight. Over the range studied, each increase in the level of DES fed resulted in a significant ($P < 0.05$) increase in uterine weight. A plot of the data (Fig. I) shows a typical linear dose/response relationship between the

TABLE I

Antiestrogenic Activity of the Alfalfa Extract Used in Study I Part A.

Group	Body Weight (gms)	Uterine Weight (mgm)	Uterine Weight (% body wt.)	Average Uterine Weight ± S.E.[a] (% body wt.)
I Controls 0.2 ml. physiological saline (0.9% saline) per rat.				
	79.1	21.1	0.0267	
	82.4	23.8	0.0289	
	70.4	22.0	0.0313	0.0325 ± 0.0026
	78.5	27.7	0.0353	
	80.3	32.4	0.0403	
II 0.025 µg Estradiol 17-β in 0.2 ml 0.9% saline per rat.				
	77.4	32.8	0.0424	
	85.6	28.1	0.0328	
	82.7	36.0	0.0435	0.0382 ± 0.0026
	85.8	26.5	0.0309	
	72.5	30.1	0.0415	
III 0.025 µ g Estradiol 17-β + CEC (10 gm D.M./rat) in 0.2 ml 0.9% saline				
	77.4	20.4	0.0264	
	68.1	16.9	0.0248	
	83.6	23.5	0.0281	0.0271 ± 0.0019
	90.3	22.7	0.0251	
	83.2	26.0	0.0313	

[a]S.E. = Standard Error

TABLE II

Antiestrogenic Activity of the Alfalfa Extract used in Study I Part B.

Group	Body Weight (gm)	Uterine Weight (mgm)	Uterine Weight (% Body Wt.)	Average Uterine Wt. ± S.E.[a] (% Body Wt.)
I	Controls 0.2 ml physiological saline (0.9% saline)			
	59.8	22.0	0.0368	
	60.6	16.0	0.0264	
	67.8	25.0	0.0369	0.0320 ± 0.0026
	75.0	23.0	0.0307	
	57.2	22.0	0.0385	
	61.5	14.0	0.0228	
II	0.025 µg Estradiol 17-β in 0.2 ml 0.9% saline			
	63.7	32.0	0.0502	
	68.3	34.0	0.0498	
	69.7	25.0	0.0359	0.0451 ± 0.0021
	53.7	24.0	0.0447	
	73.0	34.0	0.0466	
	74.5	32.5	0.0436	
III	0.025 µg Estradiol 17-β + C.E.C. (10 gm D.M./rat) in 0.2 ml 0.9% saline			
	59.9	22.0	0.0367	
	63.0	19.0	0.0302	
	52.2	21.0	0.0402	0.0337 ± 0.0016
	51.1	16.5	0.0323	
	50.2	15.0	0.0299	
	47.1	15.5	0.0329	

[a] S.E. = Standard Error

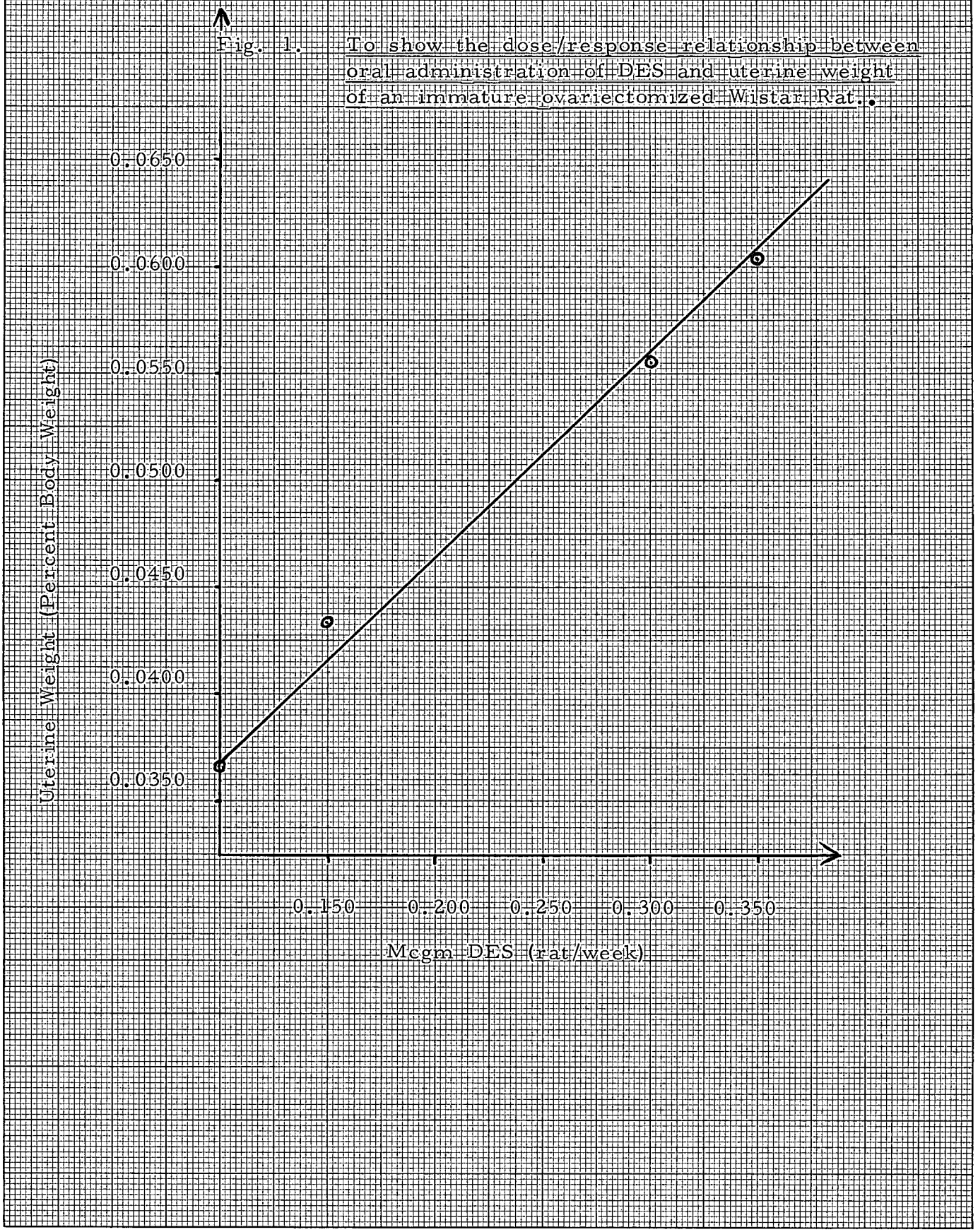
TABLE III

The Estrogenic Activity of DES Administered Orally to Weanling Female Wistar Rats.

Group	Body Weight (gm)	Uterine Weight (mgm)	Uterine Weight (% Body Wt.)	Average Uterine Wt. ± S.E.[a] (% Body Wt.)
I	Controls: 0 mcgm DES/rat/week.			
	57.3	25.6	0.0447	
	68.2	33.8	0.0496	
	71.4	18.9	0.0265	0.0366 ± 0.0038
	72.0	23.0	0.0319	
	53.3	20.8	0.0390	
	68.1	19.1	0.0280	
II	0.150 mcgm DES/rat/week			
	72.1	30.2	0.0419	
	66.6	22.1	0.0332	
	52.4	28.5	0.0544	0.0434 ± 0.0049
	63.0	18.4	0.0292	
	67.0	40.8	0.0609	
	58.4	23.8	0.0408	
III	0.300 mcgm DES/rat/week			
	67.3	43.0	0.0639	
	60.1	38.1	0.0634	
	60.4	27.9	0.0462	0.0551 ± 0.0031
	64.6	35.9	0.0556	
	56.8	30.9	0.0544	
	70.2	33.1	0.0472	
IV	0.350 DES/rat/week			
	61.7	45.8	0.0742	
	52.0	38.5	0.0740	
	61.6	28.8	0.0468	0.0602 ± 0.0067
	71.5	40.0	0.0559	
	62.4	46.2	0.0740	
	59.0	21.5	0.0364	

[a] S.E. = Standard Error

Fig. 1. To show the dose/response relationship between oral administration of DES and uterine weight of an immature ovariectomized Wistar Rat.



level of DES fed and the uterine response. The response can only be said to be linear within the range studied, because further increases in the level of DES fed would eventually show a curvilinear relationship as a maximal uterine response was approached[8].

In Part A of this study the DES and antiestrogen were not fed to the mink until implantation had occurred. The length of gestation in the mink varies from 42 to 79 days with an average of 51 days[38]. The embryos implant about 28 to 30 days before parturition[54]. The DES and antiestrogen, therefore, were not incorporated into the mink's diet until 30 days after coitus. It was assumed that implantation had occurred in all animals by this time.

A definite decrease in the number of kits per female occurred when DES or the antiestrogen was incorporated into the diet (see Table IV). The levels of 5, 10, and 15 mcg. DES or the plant antiestrogen at levels of 5, 10, and 15 gm. equivalent of alfalfa were not sufficient to produce a complete reproductive failure in any one group. In each group, however, some females were barren at the end of the gestation period. It has been demonstrated many times that as gestation proceeds greater quantities of estrogenic and antiestrogenic compounds are required to interrupt pregnancy and for some animals in this study the levels of hormones used were not sufficient to cause complete reproductive failures. Other factors which would contribute to variations in the severity of hormonal administration, include:
(1) individual sensitivity to hormonal actions, (2) variations in the stage of gestation, and (3) variations in feed intake which would cause a variation in the level of hormonal intake.

TABLE IV

Reproductive Performance of Mink Receiving DES or Antiestrogen after Ova Implantation - Part A

Group	DES Level (mcg/mink/day)	No. of Females Mated	No. of Litters Born	Total Kits Born	No. Kits Per Female
I	5	5	3	7	1.4
II	10	5	2	8	1.6
III	15	5	4	15	3.0
	Antiestrogen Level[1] (gm/mink/day)				
I	5	5	3	15	3.0
II	10	5	3	14	2.8
III	15	5	2	8	1.6
Control	0	169	154	612	3.6

[1]Antiestrogen extract incorporated in equivalent of grams of alfalfa

In 1960, Travis[136], in a similar study of DES feeding, found almost complete failure of the normal reproductive processes. As well as a lowered kit production, Travis found reabsorption of litters, lower kit weights and greater kit mortality in mink receiving the DES. Spontaneous abortion was neither reported by Travis[136] nor observed in the present study.

Part B of this study was initiated the following breeding season (March, 1968). It was thought possible, after studying the data of the previous experiment (Part A), that a dose/response relationship could exist between the level of hormone administered and kit production. No such relationship was evident in the DES groups of Part A, but there is a possibility of such a relationship in the antiestrogen groups. The antiestrogen experiment was, therefore, repeated the following breeding season and to gain further knowledge of the effects of DES on reproduction, 15 mcg. of DES was fed at various stages of pregnancy. The results of Part B are given in Table V.

The feeding of DES during any of the various stages of pregnancy described was catastrophic (Table V). In Group III of the DES experiment, one female died before the completion of the study. Upon necropsy, 6 fetuses were found in utero, with 2 fetuses partially reabsorbed. None of the other animals were sacrificed for necropsy.

Feeding DES one week before breeding (Group I, IV, and VI) prevents ovulation by chemically antagonizing the secretion of the gonadotrophins[72]. The inhibition of the gonadotrophins prevents oogenesis and subsequent ovulation of the ovum[112]. When DES is fed immediately after mating (Groups II and V the hormone disrupts the

transport of the ova through the Fallopian tubes. It has been recently suggested by Humphrey[70], that low doses of an estrogen can cause both tube-locking of the ova at the isthmo-uterine or ampulla-isthmus junction as well as acceleration of the tubal ova. If the fertilized ova reach the uterus normally, the feeding of DES at this time would disrupt the hormonal concert associated with pregnancy and prevent implantation. If DES is not fed until the ova are implanted (Group III), reabsorption or spontaneous abortion of the fetuses may result.

In the antiestrogen study of Part B, it can be seen from the results in Table V, that a reduction in productivity occurred when the antiestrogen was incorporated into the ration. The results, however, suggest that no dose/response relationship exists between the level of antiestrogen fed and the number of kits born per female. In 1962, Emmens and Finn[53] could not find any dose/response relationship between progesterone or ethyl-19-nortestosterone and litter size by either local or parenteral administration in pregnant rats and mice.

From the results of Study I it is evident that DES at levels as low as 5 to 15 mcgm /mink/day can cause complete reproductive failure. At the 15 mcgm. level it is possible to prevent or disrupt pregnancy if the hormone is administered for at least one week from one week before breeding to 30 days before parturition. The forage antiestrogen can also reduce the productivity of the mink even when fed after implantation has occurred. It is not as effective, however, as an antifertility agent as is DES. The present experiments show that the alfalfa antiestrogen, concentrated into an extract, is capable of interrupting pregnancy, however its concentration in the plant itself may be so low

TABLE V

Reproductive Performance of Mink Receiving DES at Various Stages of Pregnancy or Antiestrogen after Implantation

Group	DES Level (mcg/mink/day)	No. of Females Mated	No. of Litters Born	Total Kits Born	No. of Kits per Female
I	15	10	1[a]	1	0.1
II	15	10	1	1	0.1
III	15	10	1[b]	4	0.4
IV	15	10	1	1	0.1
V	15	10	0	0	0
VI	15	10	0	0	0
Control	0	10	7	49	4.9
	Antiestrogen Level[c] (gm/mink/day)				
I	5	5	3	15	3.0
II	10	5	5	18	3.6
III	15	5	4	21	4.2
Control	0	5	5	29	5.8

[a] One kit born dead

[b] One animal in Group III died and upon necropsy, 6 fetuses were found in utero

[c] Antiestrogen extract incorporated in equivalents of grains of alfalfa

that the antiestrogen level in a mink ration from natural sources is of no significance.

V Conclusions

1. The daily feeding of 5, 10 or 15 mcg. of DES for one week, to female mink, at any stage of the reproductive process (from one week before breeding to immediately after implantation) can cause complete reproductive failure.

2. The feeding of a forage (alfalfa) antiestrogen extract at levels equivalent to 5, 10 or 15 gm. of alfalfa to pregnant mink after implantation has occurred will result in a fewer number of kits per litter than mink not receiving the antiestrogen extract.

3. The feeding of various levels of DES or the antiestrogen extract does not adhere to a dose/response relationship either in interrupting pregnancy or reducing the number of kits per female.

4. DES, fed at levels of 5, 10 or 15 mcgm. per mink per day is a more potent antifertility agent than the forage antiestrogen fed at levels equivalent to 5, 10 or 15 gm. of alfalfa.

STUDY II

The Effects of Various Protein and Energy Levels on the Maintenance and Early Growth of Mink, (*Mustela vison*).

PART A. Maintenance

PART B. Early Growth

I. Introduction:

Among the various nutritional interrelationships affecting the dynamic state of the complex organism, the relationship between protein metabolism and energy has been the most extensively investigated. Protein has two functions within the animal body. Firstly, it is essential for the synthesis of body tissues and secondly, it is a source of energy. However, it must be remembered that if the amino acid constituents of the fed protein are used for tissue synthesis, they are not supplying energy for metabolic processes; and if they are supplying energy, they are not available for tissue synthesis.[101]

It is well established that the dietary energy level has a profound effect on food consumption. Sibbald, et al,[124] have shown that 74% of the variation in food consumption in rats is associated with the apparent digestible energy content of the ration. Within physiological limits, the need to satisfy energy requirements evidently provides the primary stimulus to food intake.[89] It is understandable, therefore, that the dietary requirements for nutrients and the efficiency of nutrient utilization are closely related to the intake of energy.

The primary need of the body is for energy, and its priority over the need for protein synthesis should be assumed, since synthesis is an energy requiring process.[101] The primary function of protein, tissue synthesis, can take place only if the energy needs of the organism are provided for.[101]

II. Literature Review

A. Relationship of protein utilization to energy intake.

1) The effects of constant protein levels with varying energy intake.

Protein utilization in an adult individual on an adequate protein intake and nitrogen equilibrium will respond to changes in the energy content of the diet.[101] From studies on undernutrition it is apparent that withdrawal of energy in the form of either carbohydrate or fat results in a decrease in nitrogen retention[18] evident by an impaired nitrogen balance.[90] Animals restricted in energy intake below a maintenance level fall into a negative nitrogen balance and the degree of negativity is directly related to the severity of the caloric restriction.[27] With sufficient fat and labile protein stores, the increase in nitrogen excretion on a caloric deficient diet is a result of the animal catabolizing its labile protein reserves to provide sufficient energy for the essential anabolic functions.[109] During the initial stages of caloric restriction, the nitrogen balance index, which isaa function of the nitrogen retained in the body of the animal and is defined as the rate of change of nitrogen balance with respect to absorbed nitrogen, is unchanged.[107] If the degree of caloric depletion is not too marked, the initial rapid loss of body nitrogen tapers off, which indicates that animals are capable ofaadapting to caloric restrictions by reducing their catabolic activity.[89] In the growing animal, Bosshardt[19] estimated that a 34 percent decrease in energy intake results in a 14 percent decrease in energy expenditure. This shows that

in the growing animal on a constant protein intake energy conservation is accomplished by a reduction in basal metabolism.[19] If the caloric restriction is marked, however, the animal becomes severely depleted in fat and tissue protein reserves and the nitrogen balance index is reduced[109] and the catabolism of tissues is again increased eventually culminating in death of the animal.[89]

The resistance to caloric restriction is correlated, in part, with the magnitude of the protein stores and the caloric reserves of the body.[107] Rosenthal and Allison[109] found that there were differential changes in body tissues in animals on caloric deficient diets. This demonstrated that some organs, such as the liver, were more labile to neutral lipid and protein depletion than other organs, such as the heart. The response, therefore, to a caloric restriction is a function of the physiological state of the animal as well as the nature of the diet.[109]

Energy has a sparing effect on protein netabolism. As caloric intake increases with protein intake constant, protein utilization increases to a maximum, beyond which no additional protein utilization occurs.[101] Calloway and Spector[26] found that for young essentially normal active men, in negative nitrogen balance, when no protein is fed, the protein deficit can be maximally reduced by supplying about 700 non-protein calories. No significant protein-sparing is achieved by intakes as high as 2000 Kcal in the absence of protein.[26] When the diet provides adequate amounts of protein, increments in energy intake, produced by adding carbohydrate or fat to a sub-maintenance diet, causes a linear

improvement in nitrogen balance through equilibrium to a considerable nitrogen retention.[92] Protein metabolism must thus be in a state of dynamic equilibrium with energy intake, even when the animal is in nitrogen equilibrium.[92] Munro and Naismith[92] also found that the total amount of protein contained in the liver responded to increasing energy intake in a linear fashion. Changes in energy intake caused a smaller percentage change in the nitrogen content of the carcass than in the nitrogen content of the liver.[92]

Once a certain energy intake has been reached on a protein-free diet additional increases in energy intake will not lead to further reduction in nitrogen output.[92] This means that when the supply of amino acids circulating to the tissues comes solely from endogenous sources, this becomes a limiting factor in the rate of protein synthesis at quite low levels of energy intake.[92] When the diet supplies adequate protein this limitation is no longer present.[92] However, the major factor limiting growth through out a wide range of caloric intakes is still the amount of protein ingested.[19] During pregnancy, Pike, et al[102] have shown that in the rat the limiting factor of nitrogen retention during organogenesis was the supply of non-protein calories, rather than the level of protein. However, during the period of rapid fetal growth calories are no longer the factor limiting nitrogen retention; rather, it is the level of nitrogen in the diet that is imposing the limitation.[102] It is possible that there is a caloric intake for each protein intake, the perfect balance between protein and calories resulting in an adequate development of body mass.[109]

2) The effects of constant energy levels with varying protein intake.

Although the efficiency of protein utilization, in conditions of calorie undernutrition, is governed largely by the extent of the caloric restriction; within limits, increasing amounts of protein can be utilized by the body if the protein intake is increased while the non-protein caloric content of the ration is kept constant.[19] As the protein increases with caloric intake constant, protein utilization rises sharply to a peak rate and the rise becomes progressively less.[101] Rosenthal[107] found that dogs fed restricted diets utilized nitrogen in a normal fashion when the protein content was relatively low. Additional dietary protein resulted in a small positive nitrogen balance which could not be increased further no matter how much protein is included in the diet.[107]

The net protein utilization, however, decreases linearly as the percentage of protein calories in the diet is increased, and the rate of decrease is a characteristic of each protein.[89] Calloway and Spector[28] have shown that the nitrogen utilization in rats diminished from approximately 55 to 17 percent when the level of dietary nitrogen increased from 75 to 604 mg. daily. Thus indicating an increased use of dietary protein for energy.

B. The effects of carbohydrate and fat on protein utilization.

Although the caloric intake exerts a significant effect on protein utilization, the difference in response to carbohydrate and to fat feeding clearly indicates that carbohydrate also exerts an effect separate from its calorigenic function.[101] On diets that are adequate in protein and energy, replacement of carbohydrate calories by fat calories produces a transient increase in nitrogen excretion which then declines.[101] Furthermore, in complete protein starvation carbohydrate, but not fat, has the property of sparing body protein.[19] Munro and Naismith[92] have shown that in rats on a protein-free diet, the addition of fat to bring the energy intake up from 900 to 1700 Kcal/m^2 failed to influence nitrogen balance. The addition of carbohydrate produced some improvement up to 1200 Kcal/m^2, but not thereafter.[92] In the case of the rat, the separation of the time of feeding dietary protein and carbohydrate (but not fat) has an adverse effect on nitrogen balance, which is of short duration.[90] These observations may be taken as indicating that carbohydrate does play a part in protein metabolism which cannot be taken by fat.

Munro, et al[91] have suggested that the mechanism through which carbohydrate has an effect of protein utilization may be related to the fact that dietary carbohydrate, but not fat, causes a temporary fall in plasma amino acids and the deposition of amino acids in the muscle. The mechanism causing the shift of amino acids into muscle has been attributed to the action of insulin.[91]

As a result of the reduced plasma amino acid level, the supply of amino acids to other tissues is curtailed and with two consequences:

(a) Urea production by the liver is reduced (protein-sparing action), and,

(b) Tissues other than muscle have a dimished supply of amino acids for protein syntheses.[91]

Whether the insulin accelerates the transport of amino acids across the muscle cell membrane making the amino acids available to the protein synthetic mechanism within the cell or whether the effect is on the polypeptide synthesis within the cell has not been clarified.[101]

III Materials and Methods

PART A: Feeding Trial

An experiment, extending from November 3, 1966 to December 7, 1966 was designed using a simple randomized block design to study the effects of feeding regular ranch rations varying on the level of protein and energy during the non-critical period of feeding. The protein and energy content of the rations were regulated by altering the protein and energy content of the cereal portion.

1. Materials

a. Animals - Two hundred mature male and female mink were divided into 5 groups with 20 males and 20 females per group. Due to the restricted availability of any one colour phase of mink, a variety of colour phased animals were used. An attempt, therefore, was made to distribute the different colour phases equally within each group to equalize any colour phase interaction between the groups.

b. Housing - All animals were individually housed in wire cages (17" x 15" x 15") equiped with a wooden nest box (8.5" x 7" x 7.5") and a water cup. All cages were adequately sheltered at the U.B.C. mink unit.

c. Feed - Each of the 5 groups received similar rations,(Table VI) which varied only in the cereal mix used. The cereal mixes (Tables VII and VIII) incorporated into the rations differed in level of protein and energy, namely in the form of fat. All rations were mixed and fed daily

TABLE VI

GENERAL COMPOSITION OF RATIONS

Constituent	Amount (Per Cent)	Price (¢/lb) Control	M-1A	M-1B	M-1C	M-1D
Cereal Mix[1]	25.8	6.86	7.68	8.34	7.08	6.49
Fish Scraps	25.8		3.5			
Chicken Wastes	25.8		4.5			
Horse Meat	17.5		11.0			
Liver	4.1		10.0			
Brewer's Yeast	1.0		19.5			
Vitamin Premix[2]	80 gms/100lbs of ration -		120.0			
Salt Mix	20 gms/100lbs of ration -		3.1			
Water	15lbs. of H_2O added per 85lbs. of above mixed ingredients.					

Cost of Ration (c/lb) (as fed)	Control	M-1A	M-1B	M-1C	M-1D
	5.90	6.08	6.23	5.96	5.81

1. See Tables VII and VIII.

2. Per gram : Vitamin A 550 I.U., Vitamin D 110 I.U., Vitamin E (d,alpha tocoperyl acetate) 0.51 I.U., Citrus Bioflavonoid compound 3.5 mg., Methionine 6.3 mg., Choline 6.3 mg., Inositol 2.1 mg., Thiamine HCl 0.1 mg., Riboflavin 0.3 mg., Pyridoxine 0.03 mg., Vitamin B_{12} 0.4 mg., Niacin 1.2 mg., d, pantothenic acid 0.1 mg., Tricalcium phosphate 48.0 mg., potassium bi-phosphate 22.0 mg., Sodium chloride 17.7 mg.

TABLE VII

THE COMPOSITION OF THE CONTROL CEREAL OF PART A

Constituent	Amount (lbs)
Corn Meal	350
Ground Barley	200
Ground Wheat	335
Bran	100
Shorts	100
Soybean Meal (44%)	330
Fishmeal (71%)	60
Meatmeal (50%)	285
Vita Grass	40
Tomatoe Pomace	50
Brewer's Yeast	40
Distillers' Dried Solubles	40
Stabalized Fat	55
Salt	10
Vitamin Premix	5

TABLE VIII

THE COMPOSITION OF EXPERIMENTAL CEREAL MIXES OF PART A

Constituents	Amounts M-1A (lbs)	M-1B (lbs)	M-1C (lbs)	M-1D (lbs)
Ground Wheat	300	300	525	650
Ground Oats	300	300	525	650
Brewers' Yeast	60	60	40	40
Salt	20	20	20	20
Herring Meal	600	600	500	500
Soybean Oil Meal	500	500		
Blood Meal	80			
Meat Meal	100		100	100
Bone Meal	40	40	40	40
Stabalized Fat		180	250	
Vitamin Premix[1]	5	5	5	5

1. Dry Vitamin A 1,000,000 I.U., Vitamin D 240,000 I.U., Vitamin E 28 gms., Thiamine mononitrate 2 gms., Riboflavin 7 gms., Niacin 12 gms., Pyridoxine 5 gms., Biotin 0.1 gms., Folic Acid 1.5 gms., Manfanese Sulfate 18.0 gms., Dacal 6.0 lbs., Alfalfa leafmeal 93.0 lbs.

in the afternoon. The raw meats were stored at a temperature of approximately -20°C. Twenty-four hours prior to mixing, the daily allotments of meat were removed from the freezer and allowed to thaw. Water was supplied ad libitum.

2. Methods

a. Animals - The day prior to the feeding of the experimental rations, all mink were individually weighed and their body weights recorded. At the completion of the experiment all mink were sacrificed by an interperitoneal injection of 1 ml. of nicotine sulfate (20% solution) and weighed.

b. Feeds - The percentage dry matter, protein and ether extract of the cereals and total rations were determined using the official A.O.A.C. methods[67].

c. Statistical Analysis - Initial body weight, final body weight, and body weight gain were analysed using the Analysis of Variance and Duncan's New Multiple Range Test[132].

PART B

1. Feeding Trial

To gain additional information on the protein/Calorie requirements of mink, an experiment, extending from July 8, 1967 to October 31, 1967 was designed (simple randomized block design) to study the effects of feeding rations of varying protein and energy levels to mink kits

during the critical period of feeding. The protein and energy content of the total rations were again regulated by using cereal mixes with different protein and energy contents.

a. Materials

i. Animals - Two hundred and eighty pastel and standard kits were randomly distributed into 7 groups with 40 kits per group. Twenty males and twenty females were in each group.

ii. Housing - All animals were housed in cages as described in Part A of this study. Due to the lack of space, however, one-half of the animals on experiment had to be housed two per cage. The nest boxes were removed from the cages on approximately August 1, 1967.

iii. Feed - The cereal mixes were incorporated into rations identical in composition to the rations given in Part A (Table VI) of this study. The cereal mixes (Table IX) varied in protein and energy content as in the previous experiment, however, in this study the protein and calorie density of the rations were increased to allow for the increase nutritive demands of growth. The preparation and mixing of the experimental rations is described in Part A of this study.

b. Methods

Data collection, feed and statistical analysis are described in Part A of this study. One method included was the gross energy determination of the total rations and cereal mixes using a Parr oxygen bomb calorimeter. Thermethod is described in the Parr technical manual No. 130[100].

TABLE IX

THE COMPOSITION OF EXPERIMENTAL CEREAL MIXES OF PART B

Constituent	Amounts M-2A lbs.	M-2B lbs.	M-2C lbs.	M-2D lbs.	M-2E lbs.	M-2F lbs.	DS-67 lbs.
Ground Wheat	420	370	250	445	430	170	450
Ground Oats	525	370	250	550	500	500	450
Brewers' Yeast	50	35	50	50	50	50	50
Salt	20	20	20	20	20	20	20
Herring Meal (71%)	300	725	800	500	540	465	550
Soybean Oil Meal (48.5%)	425	250	350	250	250	200	250
Meatmeal	90	90	140	140	100	360	90
Bonemeal	55	40	40	40	40	100	40
Stabalized Fat	115	100	100	5	70	135	100
Vitamin Premix[1]	5	5	5	5	5	5	5

1. Per Ton : Vitamin A 4,800,000 I.U., Vitamin D 240,00 I.U., Vitamin E 28.0 gm., Thiamine 3.2 gm., Riboflavine 5.4 gm., Pantothenie acid 22.4 gm., Nicotine Acid 27.5 gm., Pyridoxine 3.2 gm., Folic acid 0.5 gm.

2. Digestibility Trials

Two digestibility trials, each of a 7 day duration, were carried out on the rations used in Part B of Study II. The total collection method was used with the adaptation period lasting 3 days and the collection period continued for 4 days. Each day of the collection period the feces and urine excreted and feed consumed were recorded. The feces was collected on a wire screen and the urine in a 125 ml. flask containing 2 ml. of 50% H_2SO_4. The urine and feces were pooled for analysis.

a. Materials

i. Animals - In each trial, 7 male and 7 female pastel (Trial 1) or Standard (Trial 2) adult mink were transferred to a digestibility cage. This was designated as Day 1 of the digestibility trial. The animals were weighed before and after the 7 day adaption and collection period.

ii. Digestibility Cages - The cages used were constructed to the specifications as outlined by Sinclair and Evans[125]. Before each digestibility trial the cages were thoroughly cleaned with detergent and hot water and scrubbed with a wire brush.

iii. Feeds - The frozen meats used in the rations were allowed to thaw for twenty-four hours prior to grinding. To ensure uniformity of the feed and also to prevent the mink from carrying large pieces of feed away from the feeding area, the meats were put through a grinder twice. Sufficient quantities of the rations were mixed for the entire trial on Day 1 and stored under refrigeration until fed. Each ration was fed to one male and one female mink.

b. Methods

All nitrogen, ether extract and dry matter determinations were done using the official A.O.A.C. procedures[67]. Gross energy of the feeds and feces were determined by oxygen bomb calorimetry[100].

IV. Results and Discussion

The mean initial, final, and body weight gains of the animals used in Part A of Study II are shown in Table X. A complete record of initial and final body weights is given in Appendix III. For the purpose of this experiment the criterion for a maintenance ration was defined as that ration which, when fed ad libitum, caused neither a significant increase nor decrease in initial body weight. The levels of protein and fat of the various rations are given in Table XI. For purposes of clarity, the four experimental rations are classified as follows: a) low protein/low fat, M-1D; b) low protein/high fat, M-1C; c) high protein/low fat, M-1A; and d) high protein/high fat, M-1B. The ration classified as the control was the U.B.C. ranch ration (1960 to 1966) and was employed in this experiment for the purpose of comparison.

The ration M-1C (low protein/high fat and ration M-1B (high protein/high fat) supported greater weight gains to give significantly

Table X. Mean Initial, Final and Body Weight Gains of Study II, Part A

Ration	Initial Body Wt. (gm.)	Final Body Wt. (gm.)	Body Wt. Gain (gm.)
Control	1203	1218	15
M-1A	1233	1311	79
M-1B	1263	1339	76
M-1C	1260	1338	79
M-1D	1220	1218	-2

TABLE XI. Proximate Analysis of Rations[a] for Study II, Part A.

Ration	Cereal Mix			Total Ration		
	Percent Dry Matter	Percent Protein (D.M. Basis)	Percent Ether Extract (D.M. Basis)	Percent Dry Matter	Percent Protein (D.M. Basis)	Percent Ether Extract (D.M. Basis)
Control	88.56	27.02	10.19	30.75	43.03	23.76
M-1A	84.13	40.96	7.42	34.62	44.15	24.20
M-1B	88.46	37.27	19.87	35.27	44.75	28.87
M-1C	84.02	26.05	19.05	33.47	40.57	27.17
M-1D	83.68	26.71	11.08	32.00	36.64	21.25

[a] All values are averages of triplicate determinations.

greater ($P < 0.05$) final body weights over the control and M-1D rations, of which ration M-1D is low in protein and fat. Ration M-1A (high protein/low fat) did not produce final body weights which differed significantly ($P < 0.05$) from either rations M-1D and control or M-1B and M-1C.

The M-1D ration appears to be the most satisfactory ration to maintain mink near a constant body weight. This would suggest that rations containing approximately 36 percent protein and 21 percent fat, on a dry matter basis, are satisfactory for the late growth requirements of mink or over the maintenance period. Rations higher in protein or fat content would subsequently be higher energy rations and as a result, because the animals are in the latter stages of growth, would deposit

excessive amounts of body fat, a condition undesirable for either proper pelt processing or breeding purposes.

In Part B of this study a more detailed investigation was conducted to determine the protein and energy requirements of mink during their growing period. The proximate analyses of the 7 rations are shown in Table XII. The percent protein and fat (ether extract) on a dry matter basis ranged from 38.9 to 54.3 percent and 18.3 to 22.8 percent, respectively. The mean initial body weight, final body weight and body weight gains are given in Table XIII and calculated from the values in Appendix IV. From an analysis of variance it was found that the initial body weights of the mink used in this study

Table XII. Proximate Analysis of Rations[a] for Study II, Part B

	Cereal Mix			Total Ration		
Ration	Percent Dry Matter	Percent Protein (D.M. Basis)	Percent Ether Extract	Percent Dry Matter	Percent Protein (D.M Basis)	Percent Ether Extract
Control	92.18	36.70	12.53	37.10	42.42	20.55
M-2A	92.16	32.11	9.99	39.93	38.95	18.27
M-2B	91.64	42.09	10.78	35.43	46.01	18.38
M-2C	90.85	47.46	13.26	34.87	54.31	18.94
M-2D	92.31	40.49	10.50	37.92	48.10	20.38
M-2E	91.65	37.51	9.53	37.80	45.01	19.46
M-2F	90.23	39.18	14.27	34.99	47.87	22.78

[a] Each value is the mean value from triplicate determinations

Table XIII. Mean Initial, Final, and Body Weight Gains of Study II, Part B

Ration	Initial Body Wt.[a] (gm.)	Final Body Wt. (gm.)	Body Wt. Gain (gm.)
Control	553.6	1160	606.7
M-2A	569.0	1052	482.7
M-2B	582.9	1124	541.9
M-2C	583.4	1098	514.2
M-2D	667.3	1098	430.8
M-2E	627.7	1016	389.1
M-2F	660.3	1119	459.3

[a] Significantly different ($P < 0.01$)

were significantly different ($P<0.01$). The final and initial body weights were therefore tested using the Analysis of Covariance (62). A significant difference ($P< 0.01$) in final body weight was obtained by this analysis. The ration which caused the greatest gains in weight was the control ration resulting in significantly greater final body weights. The final body weights produced by rations M-2B, M-2C, M-2D and M-2F were not significant ($P< 0.05$). Ration M-2A did not produce a final body weight significantly different ($P< 0.05$) from that produced by ration M-2E, but this final body weight was significantly different ($P < 0.05$) from the final body weights produced by the other rations.

The detailed analysis of the digestibility studies and nitrogen retention calculations are given in Appendices V and VI. Summaries of the digestibility trials are given in Tables XIV and XV.

It would be hazardous to suggest from the limited replications in the digestibility trials that any one ration was superior due to a larger apparent digestibility coefficient. The data in Tables XIV and XV do show, however, definite ranges in apparent digestible dry matter, nitrogen, ether extract and energy coefficients. In both trials the apparent dry matter digestibility of the 7 rations averaged between 61.2 and 71.6%; the percent apparent digestible nitrogen (A.D.N.) averaged between 70.1 and 78.1, the apparent ether extract digestibility averaged between 83.0 and 91.4% and the apparent digestible energy (A.D.E.) varied between 69.6 and 77.9% of the gross energy. It has been repeatedly shown that mink utilize fat efficiently and that fats are digested more efficiently than protein (81). The apparent digestibility coefficients for fat and protein have been reported slightly higher in early growth mink rations than those obtained in this study. For example, Roberts and Kirk (106) obtained apparent digestibility coefficients for fat and protein of 97.96 and 74.82%, respectively. Bernard, et al. (12) obtained values for fat and crude protein (chief source raw horse meat) of 93.0 and 87.0%, respectively. Leoschke (81) corrected the apparent digestibility of fat for the metabolic fat present in the faeces of an early growth ration and obtained a true digestibility coefficient for fat (beef tallow) of 91.9%. The average apparent digestible dry matter in this

Table XIV. Summary of Digestibility Trial I[a]

Ration	Percent D.M. Digestibility Male	Female	Percent Nitrogen Digestibility Male	Female	Percent Ether Extract Digestibility Male	Female	Percent Digestible Energy Male	Female
Control (mean)[b]	66.08	68.20 (69.76)	72.00	82.67 (78.1)	77.21	86.80 (86.81)	67.82	81.01 (76.96)
M-2A (mean)	56.14	72.45 (61.26)	69.12	78.42 (70.1)	82.61	93.73 (86.51)	65.17	77.75 (69.6)
M-2B (mean)	68.24	73.11 (71.6)	73.93	79.68 (76.91)	88.26	92.40 (90.10)	73.96	82.61 (77.91)
M-2C (mean)	66.21	67.44 (68.7)	79.55	80.41 (76.81)	72.50	94.59 (87.2)	74.86	79.01 (76.6)
M-2D (mean)	68.10	66.82 (69.81)	72.45	84.86 (77.10)	61.73	92.39 (83.0)	73.61	83.49 (77.91)
M-2E (mean)	63.90	70.78 (69.31)	64.95	81.44 (72.6)	90.20	88.49 (91.4)	65.07	81.68 (76.04)
M-2F (mean)	69.66	65.08 (62.71)	81.76	76.47 (71.6)	71.13	90.09 (85.5)	82.55	78.15 (75.01)

a. All values are apparent digestibilities

b. Mean of digestibility trial I and II

From the data presented in Tables XIII and XVI, it is evident that as the A.D.E. is raised from 353.4 Kcal/100 gm. dry matter to 426.0 Kcal/1oo gm. and as the A.D.N. level reaches 4.980 gm./100 gm. dry matter the early growth of both male and female mink is improved. Maximum performance was obtained in the mink when the ration contained 426.0 Kcal A.D.E./100 gm. dry matter and 4.980 gm. A.D.N./100 gm. dry matter. Sinclair, et. al. (126), in 1962, reported similar results to those obtained in this study. He found that diets high in energy (5.23 Kcal of gross energy/gm; 4.18 Kcal of A.D.E./gm.) were superior to low energy (4.57 Kcal of gross energy/gm; 3.43 Kcal of A.D.E./gm.) diets in promoting early growth and enhancing final body weights in male and female mink.

Table XVI. The Apparent Digestible Energy : Apparent Digestible Nitrogen Ratio for the Experimental Rations as Determined from Digestibility Trial II

Ration	Gross Energy (Kcal/100 gm. feed)	Apparent Digestible Energy (Kcal/100 gm. feed)	Total Nitrogen (gm./100 feed)	Gross Cal:Protein Ratio (Kcal/gm.)	Apparent Digestible Nitrogen (gm./100 gm. feed)	A.D.E. : A.D.N. Ratio (Kcal/gm.)
M-2A	522.2	353.4	5.520	15.14	3.455	102.29
M-2B	525.4	407.2	6.914	12.16	5.327	76.44
M-2C	552.1	420.6	7.061	12.51	5.199	80.90
M-2D	526.0	406.1	6.221	13.53	4.699	86.42
M-2E	527.1	414.9	6.955	12.13	5.002	82.95
M-2F	520.0	362.5	7.004	11.88	4.482	80.88
Control	535.9	426.0	6.322	13.56	4.980	85.54

Also, diets of high crude protein (4.912 gm. gross N/100 gm; 3.952 gm. A.D.N./100 gm.) were superior to those of low crude protein content (3.760 gm. gross N/100 gm; 2.673 gm. A.D.N./100 gm.) in ability to promote early growth and to enhance final body weights of both sexes.

Sinclair, et. al. (126) also found that the percent digestible nitrogen was curvi-linear related to the A.D.E. : A.D.N. ratio and appeared to be maximal when the diet contained 140 Kcal of A.D.E./gm. A.D.N. The results obtained in this study do not show a curvilinear relationship between percent A.D.N. and the A.D.E. : A.D.N. ratio (Fig. II); however, a maximum response was obtained when the diet contained 85.54 Kcal of A.D.E./gm. A.D.N. (13.6 Kcal gross energy/gm. gross protein).

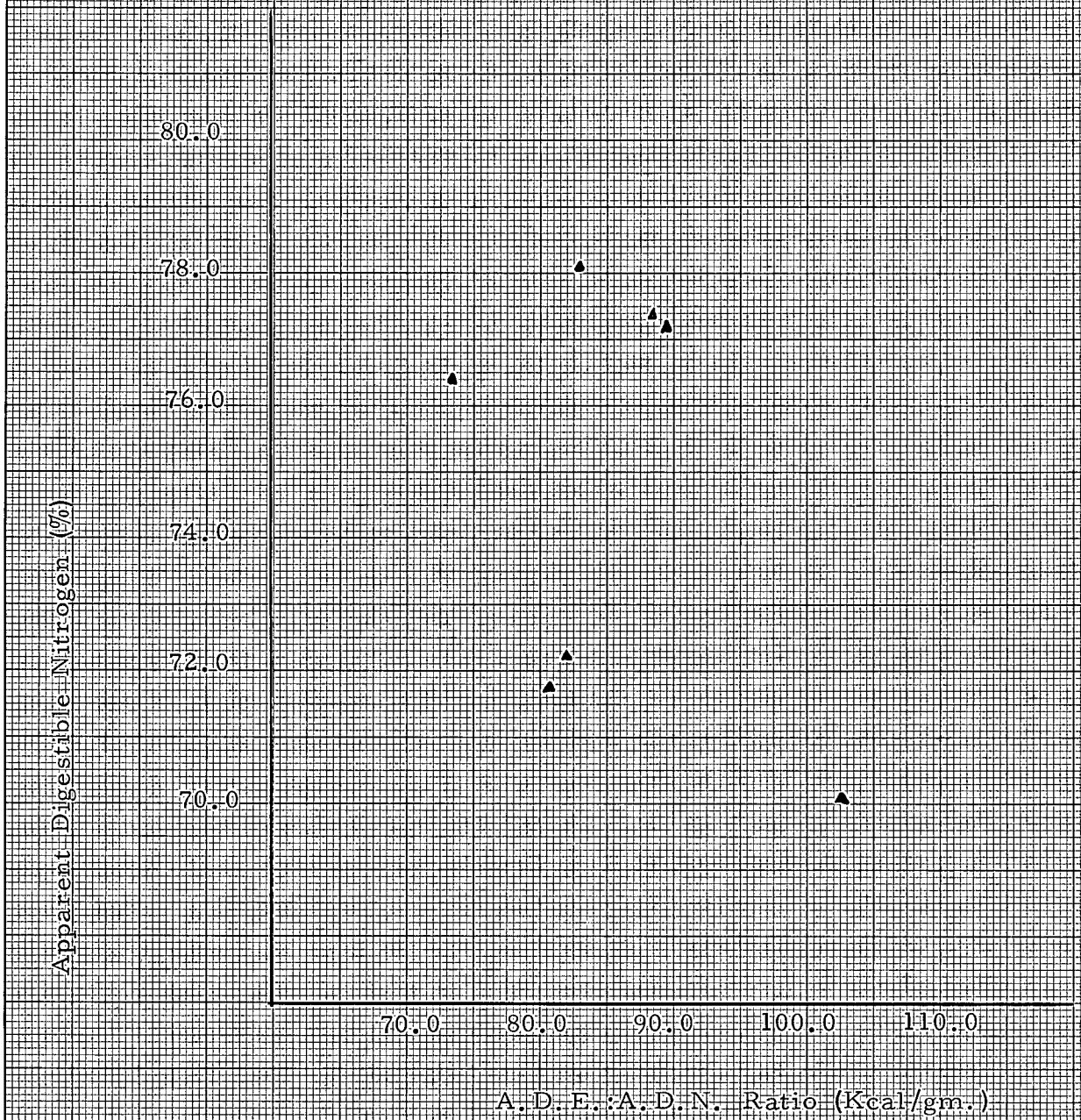
Although a narrow range of A.D.E. : A.D.N. ratios were studied, results from the nitrogen balance studies (Appendices VI) indicate that the A.D.E. content of the diet had a definite protein-sparing effect (see Table XVII). Maximum nitrogen retention was obtained when the

Table XVII. The protein-sparing effect of increasing apparent digestible energy[a]

Ration	A.D.E. (Kcal/100 gm.)	Average Nitrogen Retained (g.m.)
Control	426.0	27.7
C	420.6	11.7
E	414.9	13.4
B	407.2	14.0
D	406.1	13.9
F	362.5	10.0
A	353.4	9.3

a. All values are averages of 2 replications as determined from digestibility trial II.

Fig. II. Relationship between Percent A.C.N. and the A.D.E.:A.D.N. Ratio.



ration contained 426.0 Kcal of A.D.E./100 gm. of dry feed and as the energy level of the ration was reduced more protein was metabolized as a source of energy, indicated by the reduced amount of nitrogen retained.

V. Conclusions

1. Rations containing 35 to 40% crude protein and 20 to 25% fat, on a dry matter basis, are satisfactory for the late growth and maintenance requirements of mink.
2. The mean apparent digestibility coefficients obtained in these studies for dry matter, protein, fat, and energy are 66.38, 74.05, 87.20, and 73.74%, respectively.
3. It is suggested that growing male kits (from 6-28 weeks of age) are capable of utilizing feeds more efficiently than female kits of the same age.
4. As the A.D.E. is raised from 353.4 Kcal/100 gm. dry matter to 426.0 Kcal/100 gm. and as the A.D.N. level reaches 4.980 gm./100 gm. dry matter the early growth of both male and female mink is improved.
5. A maximum early growth rate was obtained in kits when the ration contained 85.54 Kcal of A.D.E./gm. A.D.N. (13.6 Kcal gross energy/gm. gross protein).
6. Increasing the A.D.E. content of the diets had a definite protein-sparing effect. Maximum nitrogen retention was obtained when the ration contained 426.0 Kcal of A.D.E./100 gm. of dry feed.

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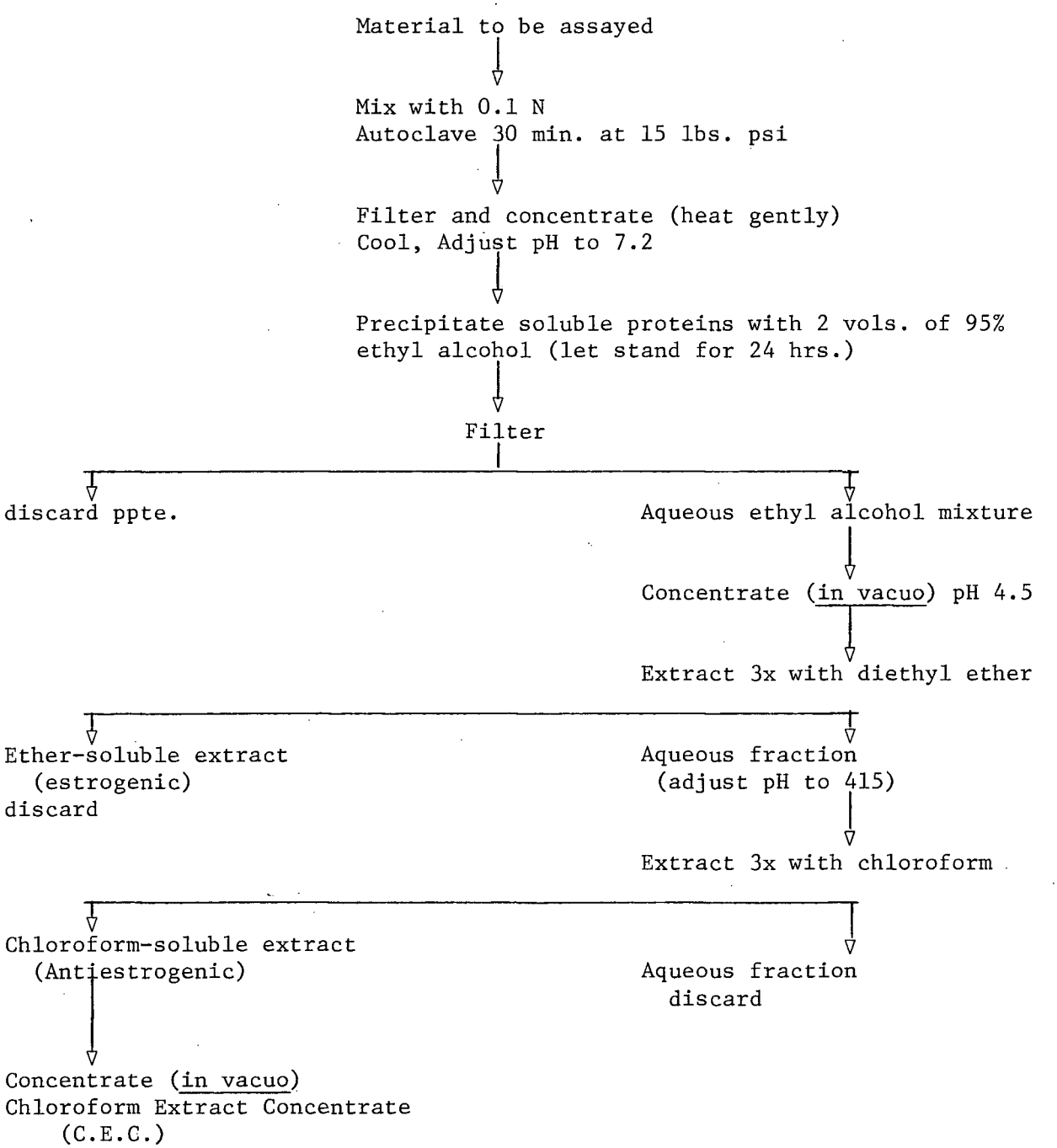
XII APPENDICES

APPENDIX I

ANTIESTROGEN EXTRACTION



APPENDIX II

Bioassay of Estrogenic and Antiestrogenic Compounds

A. Ovariectomy : rat [12]

The animal is placed in a jar and anesthesia is induced with ether. When the animal has ceased to move or struggle, it is removed and placed on an operating board. Hair may or may not be clipped from the site of operation. Usually anesthesia is continued with an ether cone.

The area is cleaned with alcohol and an incision from 1.0 to 1.5 cm. long is made in the skin midway between the last rib and the knee about 1 cm. lateral to the spinal muscles.

A second incision is made through the muscle layer and into the peritoneal cavity. If the incision is made correctly, the ovary will be seen immediately underneath, embedded in a mass of fat. The fat is withdrawn, and the ovary is separated and held with a hemostat. It may then be cut away and the uterus returned to the peritoneal cavity. The incision is then closed by means of a surgical clamp. The ovary on the opposite side may then be removed through a separate incision.

B. Bioassay

Replacement therapy with estrogens restores the reproductive tract of ovariectomized animals to the precastrate state. The degree of growth of the uterus under such therapy is proportional to the dose hormone administered within limits. Thus, the increase

in uterine weight serves as a convenient bioassay for estrogens. Several compounds inhibit the action of estrogens on the reproductive tract and can therefore be tested for their antiestrogenic properties with a similar bioassay as that used for the estrogens.

Immature rats, weighing approximately 60 gm. and which have been ovariectomized two days previously, are distributed into the required number of groups. All injections are made subcutaneously. The control group is injected with physiological saline (0.9% NaCl). The animals are killed with ether 6 hours after injection; the uteri are removed, trimmed of fat, and weighed rapidly on a torsion balance to the nearest 0.1 mg. The uteri are expressed as percent body weight.

APPENDIX III.

INITIAL & FINAL BODY WEIGHTS OF STUDY II, PART A

CONTROL				M-1A				M-1B				M-1C				M-1D			
An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)
STANDARDS				STANDARDS				STANDARDS				STANDARDS				STANDARDS			
X33	M	1450	1440	X154	M	1670	1627	X274	M	1540	1598	DM33	M	2060	2155	X142	M	1740	1757
X44	M	1220	1813	X13	M	1530	1512	X288	M	1200	1418	X164	M	1200	1182	X172	M	1470	1372
X183	M	1570	1563	X37	M	1660	1689	X223	M	2080	2154	DM20	M	1490	1544	X192	M	1380	1444
X296	M	1950	1969	X38	M	1710	1875	X281	M	1980	2250	W14	M	1380	1395	X194	M	1420	1563
X112	M	1720	1397	X152	M	1510	1650	X255	M	1750	1754	W13	M	1720	1821	X195	M	1720	1854
WHITE AND PASTEL				STANDARD AND WHITE				WHITES				WHITES				WHITES			
X27	M	1460	1490	X42	M	1740	1812	X293	M	820	1810	DM31	M	1650	1646	X3	M	1990	2044
X85	M	1730	1669	X299	M	2030	2025	X298	M	1850	2063	DM29	M	1780	2480	X300	M	1810	1751
X75	M	1280	1285	X291	M	1460	1687	X214	M	1560	1774	W20	M	1620	1686	X61	M	1350	1470
X122	M	1350	1146	X306	M	1280	1213	W18	M	1670	1858	W19	M	1700	1739	DM15	M	1490	1495
X107	M	1450	1332	X300	M	2030	2009	W16	M	1910	2056	DM34	M	1820	1753	L134	M	1890	2060
PASTELS				PASTELS				PASTELS				PASTELS				PASTELS			
X81	M	1230	1285	X12	M	1340	1544	X62	M	1540	1538	X187	M	1840	2038	X101	M	1820	2081
X65	M	1480	1318	X11	M	1380	1436	X63	M	1500	1520	X186	M	1920	1762	X59	M	1130	1137
X65	M	1490	1516	X229	M	1760	1882	X50	M	1520	1591	W11	M	1710	2006	X83	M	1280	1394
X66	M	1550	1509	X117	M	1250	1269	X49	M	1250	1154	W9	M	1280	1412	X130	M	1900	2017
X71	M	1670	1669	X58	M	1220	1179	X102	M	1520	1586	DM11	M	1320	1386	X67	M	1670	1636

APPENDIX III (CONT.)

CONTROL				M-1A				M-1B				M-1C				M-1D			
An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)
GREY BLUE & STANDARD				GREY BLUE & PASTEL				GREY BLUE & PASTEL				GREY BLUE & PASTEL				GREY BLUE & PASTEL			
X185	M	1580	1569	X128	M	1280	1166	X217	M	2750	2013	DM16	M	1210	1398	W2	M	1300	1268
X7	M	1350	1297	X127	M	1610	1595	X144	M	1800	1857	L140	M	1750	1938	X248	M	1570	1535
X242	M	1490	1584	X138	M	1310	1363	X105	M	1690	1838	DM13	M	1840	1920	X52	M	1720	1717
X216	M	1800	2081	X311	M	1908	2104	X108	M	1590	1667	DM10	M	1360	1604	X113	M	1540	1006
X32	M	1900	1991	X312	M	1720	1971	X126	M	1210	1425	DM9	M	1570	1725	X100	M	1470	1667
STANDARDS				STANDARDS				STANDARDS				STANDARDS				STANDARDS			
DF8	F	1010	988	DF104	F	890	986	DF11	F	940	960	DF106	F	1040	1164	DF2	F	1020	1020
DF109	F	810	943	DF81	F	900	914	DF31	F	1040	1097	DF55	F	1150	1125	49	F	920	945
DF10	F	970	1059	DF54	F	960	996	DF4	F	1070	1169	DF80	F	910	1015	DF1	F	720	429
DF79	F	860	928	DF52	F	950	1125	DF74	F	1060	1108	DF59	F	850	853	DF99	F	800	863
DF12	F	850	849	52	F	780	956	DF28	F	870	863	DF57	F	670	611	DF30	F	920	1027
WHITES				WHITES				WHITES				WHITES				WHITES			
DF15	F	980	1015	E84	F	700	796	DF17	F	840	967	DF42	F	930	921	E112	F	720	704
DF138	F	740	653	E95	F	810	1051	DF48	F	950	944	DF47	F	820	840	E98	F	990	914
E80	F	1150	1131	DF116	F	850	955	51	F	890	1073	DF117	F	870	967	E87	F	800	835
DF141	F	1060	1141	DF44	F	770	962	DF115	F	640	619	DF21	F	1030	1264	E78	F	1020	1013
E82	F	890	921	DF175	F	930	1003	DF89	F	890	910	D174	F	820	979	E73	F	910	976

APPENDIX III (CONT.)

CONTROL				M-1A				M-1B				M-1C				M-1D			
An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)	An. No.	Sex	Int. Wt. (gm)	Final Wt. (gm)
PASTELS				PASTELS				PASTELS				PASTELS				PASTELS			
DF236	F	810	776	W106	F	920	1108	DF189	F	920	982	DF166	F	860	777	DF132	F	760	794
DF240	F	770	825	W97	F	810	880	DF187	F	760	736	DF212	F	900	768	DF152	F	900	979
DF73	F	695	664	W91	F	1080	1159	DF148	F	970	1115	DF131	F	1000	1151	DF230	F	840	814
DF128	F	860	926	W76	F	1060	1218	DF184	F	870	956	DF231	F	740	750	DF151	F	740	680
DF229	F	840	892	W86	F	880	928	DF185	F	800	944	DF239	F	930	1010	DF135	F	600	359
GREY BLUE & WHITE				GREY BLUE & WHITE				GREY BLUE & WHITE				GREY BLUE & WHITE				GREY BLUE & WHITE			
DF200	F	680	652	DF225	F	770	707	DF87	F	770	782	DF40	F	860	892	DF118	F	920	699
DF111	F	810	780	E96	F	900	1017	DF86	F	1140	1123	DF37	F	940	921	DF97	F	980	996
DF96	F	730	756	W15	F	1020	1246	DF71	F	760	640	DF22	F	960	895	DF61	F	860	814
DF65	F	830	848	E103	F	1060	1006	DF119	F.	800	855	DF147	F	1050	1011	50	F	780	799
DF62	F	1040	1061	E104	F	880	848	DF216	F	840	806	DF223	F	880	1025	DF69	F	920	781

A P P E N D I X I V

Initial and Final Body Weights of Study II, Part B

Ration		DS-67				M-2A		
Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)	Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)
1	Y227	F	597	762	31	Y627	F	460	678
2	Y619	F	370	864	32	Y626	F	497	700
3	Y225	F	570	1091	33	Y654	F	544	770
4	Y185	F	629	1061	34	Y655	F	578	857
5	Y603	F	467	781	35	Y51	F	563	580
6	Y52	F	535	687	36	Y354	F	530	747
7	Y662	F	438	899	37	Y50	F	582	605
8	Y634	F	515	814	38	Y40	F	531	755
9	Y74	F	668	776	39	Y39	F	491	800
10	Y628	F	484	870	40	Y3	F	560	746
11	Y180	F	538	793	41	Y656	F	488	658
	Y81	F	525	834		Y657	F	583	992
12	Y621	F	459	866	42	Y641	F	427	606
	Y622	F	424	686		Y642	F	420	687
13	Y29	F	548	950	43	Y637	F	367	596
	Y33	F	584	1100		Y638	F	372	550
14	Y581	F	436	653	44	Y644	F	466	781
	Y582	F	452	687		Y645	F	466	734
15	Y357	F	460	817	45	Y630	F	496	978
	Y358	F	436	736		Y631	F	456	1035
16	Y186	M	751	1930	46	Y8	M	820	1725
17	Y226	M	625	1529	47	Y629	M	642	1438

Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)	Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)
18	Y184	M	548	1495	48	Y136	M	674	1652
19	Y159	M	524	1428	49	Y368	M	561	1445
20	Y127	M	620	1503	50	Y143	M	474	1073
21	Y623	M	412	1143	51	Y643	M	517	1216
22	Y79	M	668	1630	52	Y644	M	563	1121
23	Y633	M	494	1660	53	Y652	M	600	1091
24	Y170	M	590	1673	54	Y663	M	473	1295
25	Y593	M	604	1558	55	Y653	M	668	996
26	Y583	M	668	1677	56	Y646	M	496	1117
	Y584	M	737	1510		Y647	M	540	1293
27	Y223	M	622	1273	57	Y18	M	695	1484
	Y224	M	594	1306		Y19	M	698	1296
28	Y179	M	415	1159	58	Y21	M	645	1357
	Y180	M	613	1225		Y22	M	724	1566
29	Y113	M	378	800	59	Y4	M	739	1444
	Y114	M	606	1313		Y13	M	660	1247
30	Y48	M	781	1931	60	Y31	M	778	1343
	Y49	M	758	1942		Y32	M	917	2014
Ration		M-2B					M-2C		
61	Y353	F	542	832	91	Y373	F	529	1072
62	Y640	F	489	554	92	Y372	F	432	885
63	Y639	F	476	628	93	Y401	F	530	888
64	Y632	F	497	1035	94	Y411	F	457	886
65	Y602	F	496	845	95	Y481	F	491	786

Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)	Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)
66	Y601	F	559	945	96	Y517	F	550	870
67	Y600	F	524	821	97	Y271	F	456	717
68	Y586	F	480	832	98	Y279	F	485	1030
69	Y47	F	556	979	99	Y278	F	515	1052
70	Y15	F	598	798	100	Y460	F	415	734
71	Y598	F	517	849	101	Y479	F	458	748
	Y599	F	494	704		Y480	F	516	856
72	Y594	F	455	851	102	Y515	F	482	720
	Y595	F	492	650		Y516	F	477	875
73	Y591	F	535	808	103	Y269	F	505	954
	Y592	F	465	808		Y270	F	414	795
74	Y45	F	501	769	104	Y293	F	521	854
	Y46	F	627	1070		Y294	F	524	963
75	Y11	F	574	884	105	Y431	F	453	657
	Y12	F	450	714		Y432	F	465	746
76	Y363	M	616	1390	106	Y435	M	626	1305
77	Y648	M	512	1222	107	Y487	M	678	1210
78	Y369	M	529	1882	108	Y486	M	618	1225
79	Y590	M	624	1550	109	Y520	M	743	1472
80	Y589	M	618	1502	110	Y312	M	629	1460
81	Y20	M	700	1387	111	Y560	M	740	1460
82	Y10	M	895	1651	112	Y36	M	909	2094
83	Y9	M	736	1336	113	Y44	M	695	1622
84	Y14	M	952	1367	114	Y43	M	633	1190

Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)	Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)
85	Y123	M	700	1537	115	Y299	M	616	1229
86	Y359	M	629	1268	116	Y518	M	658	897
	Y360	M	583	1383		Y519	M	745	1440
87	Y361	M	629	1480	117	Y297	M	634	1008
	Y362	M	619	1156		Y298	M	716	1197
88	Y658	M	705	1436	118	Y484	M	602	1022
	Y659	M	867	1796		Y485	M	694	1515
89	Y617	M	478	1293	119	Y433	M	630	1425
	Y618	M	497	1398		Y434	M	656	1415
90	Y587	M	539	1258	120	Y558	M	692	1306
	Y588	M	566	1330		Y559	M	745	1322
Ration			M-2D					M-2E	
121	Y422	F	523	864	151	Y414	F	470	729
122	Y471	F	554	765	152	Y415	F	507	719
123	Y548	F	556	705	153	Y416	F	500	755
124	Y335	F	535	786	154	Y417	F	455	632
125	Y300	F	595	848	155	Y418	F	497	635
126	Y379	F	445	580	156	Y427	F	520	797
127	Y288	F	511	886	157	Y247	F	362	465
128	Y260	F	640	817	158	Y428	F	530	774
129	Y253	F	621	1095	159	Y237	F	604	897
130	Y233	F	561	745	160	Y511	F	532	856
131	Y350	F	515	575	161	Y37	F	530	753
	Y351	F	555	470		Y38	F	535	623

Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)	Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)
132	Y348	F	506	645	162	Y624	F	535	844
	Y349	F	560	885		Y625	F	490	777
133	Y276	F	547	873	163	Y235	F	528	747
	Y277	F	457	758		Y236	F	525	802
134	Y505	F	568	702	164	Y561	F	625	820
	Y506	F	624	872		Y562	F	687	865
135	Y279	F	523	997	165	Y239	F	577	735
	Y280	F	399	740		Y497	F	435	612
136	Y541	M	767	1592	166	Y470	M	676	1103
137	Y352	M	710	1585	167	Y496	M	852	1546
138	Y403	M	940	1606	168	Y576	M	834	1495
139	Y419	M	617	1147	169	Y72	M	720	1155
140	Y340	M	480	897	170	Y238	M	746	1469
141	Y334	M	906	1632	171	Y447	M	666	1510
142	Y550	M	808	1462	172	Y240	M	816	1434
143	Y305	M	640	1212	173	Y579	M	603	1473
144	Y336	M	836	1600	174	Y234	M	888	1560
145	Y553	M	600	1300	175	Y465	M	717	1343
146	Y399	M	743	1198	176	Y41	M	800	1186
	Y400	M	840	1294		Y42	M	730	1100
147	Y507	M	775	1119	177	Y310	M	550	689
	Y508	M	797	1595		Y311	M	590	739
148	Y301	M	898	1502	178	Y248	M	626	1374
	Y302	M	947	1389		Y254	M	744	1427

Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)	Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)
149	Y28	M	848	1248	179	Y429	M	835	1683
	Y30	M	895	1443		Y430	M	674	1129
150	Y34	M	854	1625	180	Y295	M	768	1243
	Y35	M	996	1869		Y296	M	828	1174
Ration		M-2F							
181	Y585	F	475	723					
182	Y494	F	565	729					
183	Y578	F	506	700					
184	Y577	F	451	694					
185	Y462	F	560	515					
186	Y461	F	475	784					
187	Y495	F	638	817					
188	Y370	F	552	902					
189	Y241	F	573	804					
190	Y378	F	544	613					
191	Y249	F	565	852					
	Y250	F	564	850					
192	Y1	F	568	620					
	Y2	F	696	653					
193	Y251	F	490	817					
	Y252	F	530	872					
194	Y286	F	436	726					
	Y287	F	527	831					
195	Y303	F	581	882					
	Y304	F	577	857					

Cage No.	An. No.	Sex	Int. Wt. (gm.)	Final Wt. (gm.)
196	Y242	M	785	2028
197	Y668	M	470	1535
198	Y261	M	958	1372
199	Y263	M	947	1453
200	Y620	M	568	1028
201	Y463	M	591	1430
202	Y259	M	796	1383
203	Y16	M	908	1712
204	Y262	M	918	1393
205	Y73	M	873	1475
206	Y65	M	833	1537
	Y66	M	898	1581
207	Y285	M	664	1388
	Y284	M	822	1396
208	Y490	M	799	1517
	Y491	M	777	1420
209	Y282	M	714	1297
	Y283	M	820	1138
210	Y492	M	689	1429
	Y493	M	720	2026

A P P E N D I X V

Digestibility Trial I

Apparent Digestibility Dry Matter

Ration	Total Feed Consumption (gms.)		Total Faeces Excreted (gms.)		Percent D.M. Feed	Percent D.M. Faeces		Total D.M. Consumed (gms.)		Total D.M. Excreted (gms.)		A.D.M. (gms.)		Percent D.M. Digestibility	
	Males	Females	Males	Females		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
M2-A	1134	622	564	203	39.93	35.21	33.71	452.81	248.36	198.58	68.43	254.23	179.93	56.14	72.45
M2-B	956	405	353	103	35.43	30.47	38.44	338.71	143.49	107.56	38.59	231.15	104.90	68.24	73.11
M2-C	681	521	238	166	34.87	33.71	35.63	237.46	181.67	80.23	59.15	157.23	122.52	66.21	67.44
M2-D	673	512	285	127	37.92	28.56	50.72	255.20	194.15	81.40	64.41	173.80	129.74	68.10	66.82
M2-E	521	435	258	122	37.80	27.56	39.38	196.94	164.43	71.10	48.04	125.84	116.39	63.90	70.78
M2-F	872	546	233	184	34.99	39.73	36.26	305.11	191.05	92.57	66.72	212.54	124.33	69.66	65.08
Control	788	440	363	132	37.10	27.32	38.68	292.35	163.24	99.17	51.06	193.18	112.18	66.08	68.20

Digestibility Trial I

Apparent Digestible Nitrogen

Ration	Total Feed Consumption (gms.)		Total Faeces Excreted		Percent Nitrogen Feed	Faeces		Total Nitrogen Consumed (gms.)		Total Nitrogen Excreted (gms.)		Nitrogen (gms.)		Percent Nitrogen Digestibility	
	Males	Females	Males	Females		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
M2-A	1134	622	564	203	6.2317	3.8688	4.1214	70.667	38.761	21.820	8.366	48.847	30.395	69.12	78.42
M2-B	956	405	353	103	7.3620	5.1986	5.8816	70.381	29.816	18.351	6.058	52.030	23.758	73.93	79.68
M2-C	681	521	238	166	8.6889	5.0844	5.3422	59.171	45.269	12.101	8.868	47.070	36.401	79.55	80.41
M2-D	673	512	285	127	7.6954	5.0071	4.6981	51.790	39.400	14.270	5.967	37.520	33.433	72.45	84.86
M2-E	521	435	258	122	7.2009	5.0969	4.7645	37.517	31.324	13.150	5.813	24.367	25.511	64.95	81.44
M2-F	872	546	233	184	7.6599	5.2286	5.3472	66.794	41.823	12.183	9.839	54.611	31.984	81.76	76.47
Control	788	440	363	132	6.7881	4.1253	3.9220	53.490	29.868	14.975	5.177	38.515	24.691	72.00	82.67

APPENDIX V (CONTD)

Digestibility Trial I

Apparent Digestible Ether Extract

Ration	Total Feed Consumption (gm.)		Total Faeces Excreted (gm.)		% Ether Extract			Total Fats Consumed (gm.)		Total Fats Excreted (gm.)		Ether Extract (gm.)		Percent Ether Extract Digestibility	
					Feed	Faeces									
	Males	Females	Males	Females		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
M2-A	1134	622	564	203	18.2740	8.3723	7.3908	207.23	113.66	47.23	15.00	160.00	98.66	77.21	86.80
M2-B	956	405	353	103	18.3785	8.6536	4.5343	175.70	74.43	30.55	4.67	145.15	69.76	82.61	93.73
M2-C	681	521	238	166	18.9370	6.3598	4.5188	128.96	98.66	15.14	7.50	113.82	91.16	88.26	92.40
M2-D	673	512	285	127	20.3758	13.2306	4.4409	137.13	104.32	37.71	5.64	99.42	98.68	72.50	94.59
M2-E	521	435	258	122	19.4598	15.0401	5.2756	101.39	84.65	38.80	6.44	62.59	78.21	61.73	92.39
M2-F	872	546	233	184	27.7775	8.3507	7.7840	198.62	124.37	19.46	14.32	179.16	110.05	90.20	88.49
Control	788	440	363	132	20.5512	12.8800	6.7892	161.94	90.43	46.75	8.96	115.19	81.47	71.13	90.09

APPENDIX V (CONTD)

Digestibility Trial I

Apparent Digestible Energy

Ration	Total Feed Consumption (gm.)		Total Faeces Excreted		Caloric density (Cal.)			Total Energy Consumed (KCal.)		Total Energy Excreted (KCal.)		Energy (KCal.)		Percent Dig. Energy	
					Feed	Faeces									
	Males	Females	Males	Females		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
M2-A	1134	622	564	203	5.2634	3.6857	3.5887	5969.04	3274.02	2078.73	728.51	3890.31	2545.51	65.17	77.75
M2-B	956	405	353	103	5.0656	3.5722	3.4640	4842.71	2051.57	1260.99	356.79	3581.72	1694.78	73.96	82.61
M2-C	681	521	238	166	5.0281	3.6163	3.3118	3424.14	2619.64	860.68	549.76	2563.46	2069.88	74.86	79.01
M2-D	673	512	285	127	5.0741	3.1622	3.3780	3414.87	2597.94	901.23	429.01	2513.64	2168.93	73.61	83.49
M2-E	521	435	258	122	5.0614	3.5702	3.3068	2636.99	2201.71	921.11	403.43	1715.88	1798.28	65.07	81.68
M2-F	872	546	233	184	5.0465	3.2954	3.2722	4400.55	2755.39	767.83	602.08	3632.72	2153.31	82.55	78.15
Control	788	440	363	132	5.1772	3.6167	3.2778	4079.63	2277.97	1312.86	432.67	2766.77	1845.30	67.82	81.01

APPENDIX V (CONTD)

Digestibility Trial I

Nitrogen Retention

Ration	Total Nitrogen Consumed (gm.)		Total Faecal Nitrogen (gm.)		Average Concentration Urinary N. (mgN/ml.)		Total Urine Excreted (ml.)		Total Urinary Nitrogen (gm.)		Total Nitrogen Excreted (gm.)		Nitrogen Balance (gm.)	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
M-2A	70.67	38.76	21.82	8.37	43.52	34.37	389.5	198.0	16.95	6.81	38.77	15.18	+31.90	+23.58
M-2B	70.38	29.82	18.35	6.06	40.19	34.77	385.5	152.0	15.49	5.29	33.84	11.35	+36.54	+18.47
M-2C	59.17	45.27	12.10	8.87	29.74	34.33	394.5	218.0	11.73	7.48	23.83	16.35	+35.34	+28.92
M-2D	51.79	39.40	14.27	5.97	41.96	37.20	255.5	194.0	10.72	7.22	24.99	13.19	+26.80	+26.21
M-2E	37.52	31.32	13.15	5.81	41.00	32.99	217.0	164.0	9.90	5.41	23.05	11.22	+14.47	+16.85
M-2F	66.79	41.82	12.18	9.84	39.92	30.64	300.5	209.0	12.00	6.40	24.18	16.24	+42.61	25.58
Control	53.49	29.87	14.98	5.18	31.85	27.73	339.5	187.5	10.81	5.20	25.79	10.38	+27.70	+19.49

APPENDIX VI

Digestibility Trial II

Apparent Digestible Dry Matter

Ration	Total Feed Consumption (gms.)		Total Faeces Excreted (gms.)		Percent D.M. Feed	Percent D.M. Faeces		Total D.M. Consumed (gms.)		Total D.M. Excreted (gms.)		A.D.M. (gms.)		Percent D.M. Digestibility	
	Males	Females	Males	Females	%	Males %	Females %	Males	Females	Males	Females	Males	Females	Males	Females
M2A	300	570	133	270	39.86	30.32	33.32	90.96	227.20	40.33	89.96	50.63	137.24	55.66	60.40
M2B	472	291	146	98	38.19	34.48	30.66	180.26	111.13	50.34	30.05	129.92	81.08	72.07	72.96
M2C	483	331	159	131	41.27	34.50	32.68	199.33	136.60	54.86	42.81	144.47	93.79	72.48	68.66
M2D	676	603	203	213	38.09	34.68	30.57	257.49	229.68	70.40	65.11	187.09	164.57	72.66	71.65
M2E	482	561	143	183	38.00	32.53	37.49	183.16	213.18	46.52	68.61	136.64	144.57	74.60	67.82
M2F	605	206	285	85	38.11	34.34	38.26	230.57	87.51	97.87	32.52	132.70	45.99	57.55	58.58
Control	1052	592	348	171	36.79	30.64	35.78	387.03	217.80	106.63	61.18	280.40	156.62	72.45	71.91

APPENDIX VI (Contd.)

Digestibility Trial II

Apparent Digestible Nitrogen.

Ration	Total Feed Consumption (gms.)		Total Faeces Excreted (gms.)		Percent Nitrogen Feed	Faeces		Total Nitrogen Consumed (gm)		Total Nitrogen Excreted (gm)		Nitrogen (gm)		Percent Nitrogen Digestibility	
	Males	Females	Males	Females		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
M2A	300	570	133	270	5.5201	4.0400	4.0420	16.56	31.47	5.37	10.91	11.19	20.56	67.55	65.32
M2B	472	291	146	98	6.9138	4.6189	5.1780	32.63	20.12	6.74	5.07	25.89	15.05	79.33	74.78
M2C	483	331	159	131	7.0612	4.7581	5.4641	34.11	23.37	7.57	7.16	26.54	16.22	77.89	69.37
M2D	676	603	203	213	6.2212	4.2046	3.4085	42.06	25.35	8.54	7.26	33.52	18.09	79.71	71.37
M2E	482	561	143	183	6.9545	4.3091	4.9898	33.52	24.17	6.16	9.13	27.36	15.04	81.62	62.23
M2F	605	206	285	85	7.0044	6.3902	4.4968	42.38	13.16	18.21	3.82	24.17	9.34	57.02	70.97
Control	1052	592	348	171	6.3220	4.0911	4.6084	66.51	37.43	14.24	7.88	52.27	29.55	78.59	78.95

APPENDIX VI (Contd)

Digestibility Trial II

Apparent Digestible Ether Extract

Ration	Total Feed Consumption (gms)		Total Faeces Excreted (gms)		Percent Ether Extract			Total Fats Consumed (gms)		Total Fats Excreted (gms)		Ether Extract (gms)		Digest. E.E. %	
					Feed	Faeces									
	Males	Females	Males	Females	%	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
M2-A	300	570	133	270	27.6196	8.2374	9.9047	82.86	157.43	10.96	26.74	71.90	130.69	86.77	83.01
M2-B	472	291	146	98	24.6411	5.1281	10.1018	116.31	71.71	7.49	9090	108.82	61.81	93.56	86.19
M2-C	483	331	159	131	24.5268	4.4388	7.6408	118.46	81.18	7.06	10.01	111.40	71.17	94.04	87.67
M2-D	676	603	203	213	24.7850	6.8621	9.7553	167.55	149.45	13.93	20.78	153.62	128.67	91.69	86.10
M2-E	482	561	143	183	24.1943	3.4682	6.5267	116.62	135.73	4.96	11.94	111.66	123.79	95.75	91.20
M2-F	605	206	285	85	24.6602	5.2335	5.5846	149.19	50.80	14.92	4.75	134.27	46.05	90.00	90.65
Control	1052	592	348	171	24.4532	7.6919	5.5401	257.25	144.76	26.77	9.47	230.48	135.29	89.59	93.46

Appendix VI (Contd) - Digestibility Trial II

Apparent Digestible Energy

Ration	Total Feed Consumption (gm)		Total Faeces Excreted (gm)		Caloric Density Kcal / gm Feed Faeces			Total Energy Consumed (Kcals)		Total Energy Excreted (Kcal)		Δ Energy (Kcals)		Digestible Energy %	
	Males	Fem-ales	Males	Fem-ales		Males	Fem-ales	Males	Fem-ales	Males	Fem-ales	Males	Fem-ales	Males	Fem-ales
M2-A	360	570	133	270	5.2223	3.6339	3.7264	1566.69	2976.71	483.31	1006.13	1083.38	1970.58	69.15	66.20
M2-B	472	291	146	98	5.2538	3.6151	3.6993	2479.79	1528.86	527.80	362.53	1951.99	1166.33	78.72	76.29
M2-C	483	331	159	131	5.5210	3.6719	3.5902	2666.64	1827.46	583.83	470.32	2082.81	1357.13	78.11	74.26
M2-D	676	603	203	213	5.2604	3.6952	3.6499	3556.03	3172.02	750.13	777.43	2805.90	2394.59	78.91	75.49
M2-E	482	561	143	183	5.2709	3.6122	3.5957	2540.57	2956.97	516.54	658.01	2024.03	2298.96	79.67	77.75
M2-F	605	206	285	85	5.1897	3.6725	3.4270	3139.77	1069.08	1046.66	291.30	2093.11	777.78	66.66	72.75
Control	1052	592	348	171	5.3590	3.6500	3.4281	5637.67	3172.53	1270.20	586.21	4367.47	2586.32	77.47	81.52

APPENDIX VI (Contd)

Digestibility Trial II - Nitrogen Retention.

Ration	Total N Consumed (gm)		Total Fecal Nitrogen (gm)		Average Concentration Urinary N (mgN/ml)		Total Urine Excreted (ml)		Total Urinary Nitrogen (gm)		Total N Excreted (gm)		Nitrogen Balance (gm)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
M-2A	16.56	31.47	5.37	10.91	31.40	44.52	165.5	180.0	5.20	8.01	10.57	18.92	+5.99	+12.55
M-2B	32.63	20.12	6.74	5.07	46.13	40.79	169.5	129.5	7.82	5.28	14.56	10.35	+18.07	+ 9.77
M-2C	34.11	23.37	7.57	7.16	42.81	39.80	253.5	171.5	10.85	6.83	18.42	13.99	+15.69	+ 7.68
M-2D	42.06	25.35	8.54	7.26	46.22	38.56	295.0	266.0	13.63	10.26	22.17	17.52	+19.89	+ 7.83
M-2E	33.52	24.17	6.16	9.13	35.36	41.99	235.0	176.5	8.33	7.41	14.49	16.54	+19.03	+ 7.73
M-2F	42.38	13.16	18.21	3.82	37.53	39.80	229.5	126.5	8.61	5.03	26.82	8.85	+15.56	+ 4.31
Control	66.51	37.43	14.24	7.88	38.36	37.21	455.5	241.0	17.47	8.97	31.71	16.85	+34.80	+20.58

APPENDIX VII

Analysis of Variance Tables.

Study I

A. Bioassay 1 : Estradiol and C.E.C.

Source	DF	Sum Sq.	Mean Sq.	F
Trts.	2	3.0702×10^{-4}	1.5351×10^{-4}	6.48
Error	12	2.8420×10^{-4}	2.3683×10^{-5}	
Total	14	5.9122×10^{-4}		

B. Bioassay 2 : Estradiol and C.E.C.

Source	DF	Sum Sq.	Mean Sq.	F
Trts.	2	6.1120×10^{-4}	3.0560×10^{-4}	10.79
Error	15	4.2492×10^{-4}	2.8328×10^{-5}	
Total	17	1.0361×10^{-3}		

C. Bioassay of DES.

Source	DF	Sum Sq.	Mean Sq.	F
Trt.	3	1.054×10^{-2}	3.513×10^{-3}	24.83
Error	20	2.820×10^{-3}	1.415×10^{-4}	
Total	23	1.337×10^{-2}		

STUDY II

PART A.

1. Initial Body Weight

Source	DF	Sum Sq.	Mean Sq.	F
Ration	4	111120	27780	0.61
Sex	1	24691000	24691000	
R-S	4	454920	11373	
Error	190	8586200	45191	
Total	199	33434000		

2. Gain

Source	DF	Sum Sq.	Mean Sq.	F
Ration	4	210710	52678	2.26
Sex	1	43395	43395	
R-S	4	97700	24425	
Error	190	4424000	23284	
Total	199	4775800		

3. Final Body Weight

Source	DF	Sum Sq.	Mean Sq.	F
Ration	4	619050	154760	2.85
Sex	1	27096000	27096000	
R-S	4	274480	68619	
Error	190	10333000	54384	
Total	199	58322000		

PART B.

1. Initial Body Weight

Source	DF	Sum Sq.	Mean Sq.	F
Ration	6	493510	82252	10.09
Sex	1	2326300	2326300	285.51
RxS	6	202730	33789	4.15
Cage	1	167000	167.00	0.02
CxR	6	45976	7662.7	0.94
CxS	1	16386	16386	2.01
RxSxC	6	163110	27186	3.34
Error	252	2053300	8147.9	
Total	279	5301500		

2. Gain

Source	DF	Sum Sq.	Mean Sq.	F
Ration	6	1263900	210650	7.84
Sex	1	12755000	12755000	474.72
RxS	6	530100	88350	3.29
Cage	1	129950	129950	4.84
CxR	6	301750	50292	1.87
CxS	1	163980	163980	6.10
RxCxS	6	51761	8626.9	0.32
Error	252	6771000	26869	
Total	279	21968000		

3. Final Body Weight

Source	DF	Sum Sq.	Mean Sq.	F
Ration	6	549860	91644	244
Sex	1	25990000	25990000	691.07
RxS	6	320740	53457	1.42
Cage	1	121760	121760	3.24
CxR	6	326840	54473	1.45
CxS	1	75928	75928	2.02
RxCxS	6	208880	34813	0.93
Error	252	9477400	37609	
Total	279	37072000		

Analysis of Covariance. Study II. Part B.

Source	d.f.	SSx	SP	SSy	SS'y	MS'y	F
Trts.	39	845520	495465	42513335	-29557767	-760455	12.08
Error	239	42076681	49022592	42071512	-15043604	-62944	
Total	278	42922201	49518057	42513335	-14614163		