SOME PHYSIOLOGICAL EFFECTS OF ESTROGENS UPON RUMINANTS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The effects of stilbestrol in promoting rate of gain, feed consumption, and efficiency of feed utilization in livestock have been demonstrated by many investigators. Thus far, little attempt has been made to establish the mechanism or the site(s) of the action of estrogens in ruminants. The influence of estrogens upon nitrogen metabolism and upon mineral behavior, in particular calcium and phosphorus retention, has been well established. However, correlation between data obtained from balance trials and the physiological performance of animals treated with estrogens has not been attempted.

It is recognized that stilbestrol exerts a profound physiological effect upon many classes of animals, particularly in its interrelationship with the endocrine system. Few attempts have been made to study the effects of stilbestrol upon the endocrine system of cattle and sheep. Because of species differences in response to estrogens, inferences drawn from the use of laboratory animals with respect to hormonal interrelationships may not be applicable for interpreting the response obtained with ruminants.

Current developments in radioisotope procedures with farm animals and the availability of a wide range of isotopes has made feasible intensive investigation of the physiological behavior of animals in response to a great variety of dietary and environmental conditions. It was considered that this study, utilizing radioisotopes as well as

conventional experiments, would permit a clarification of the behavior of estrogens in ruminants and would facilitate a critical evaluation of the response reported for farm animals under the influence of estrogens.

These investigations were designed for the purpose of determining the following: (1) the effects of stilbestrol upon total body fluids and upon the physiological behavior of calcium and phosphorus in ruminants, (2) the site(s) of action of stilbestrol in stimulating rate of gain, feed consumption, and efficiency of feed utilization in ruminants, and (3) the effects of stilbestrol on certain endocrine organs and their products.

REVIEW OF LITERATURE

The value of diethylstilbestrol, hereafter referred to as stilbestrol, a synthetic, female-like sex hormone, in promoting rate of gain and improving feed efficiency has been shown by many workers. Dinusson et al. (1950) demonstrated that subcutaneous implantations in beef heifers significantly increased rate of gain, feed consumption, and feed efficiency. Andrews et al. (1949) reported that subcutaneous implantation of stilbestrol pellets increased the rate of gain and feed efficiency of feeder lambs. Since this early work, the stimulating effects of stilbestrol in fattening ruminants have been amply demonstrated.

Burroughs et al. (1954) first demonstrated that orally administered stilbestrol was effective in increasing weight gains and efficiency of feed utilization in fattening steers. These workers stated that oral administration overcame the undesirable side effects associated with implantation, eliminated the human hazard connected with residual estrogen from the implanted pellet, and provided a means to control more closely the amount of estrogen taken into the system.

Since these data were first published, they have been confirmed at a number of agricultural experiment stations. Thus far, however, little progress has been made in establishing the mechanism of action in ruminant animals.

Species Differences: Study of the mechanism of action of stilbestrol is complicated by a species difference which apparently exists in the response to estrogens. In contrast to an increased rate of gain noted in ruminants under estrogenic influence, swine apparently show no growth response to estrogens as measured by weight increase (Dinusson et al., 1951; Woehling et al., 1951; Pearson et al., 1952; and Beeson et al., 1955).

The growth of laboratory animals under estrogenic influence is markedly depressed. Deanesley and Parks (1941) found that large doses of estrogens implanted in young male rats caused an immediate retardation of growth. However, growth did not cease until a weight of 120-130 grams was reached. Richards and Kueter (1941) administered 2 milligram implants of stilbestrol to young male and female rats and 10-20 milligram implants to adult female rats. They found that growth in the young animals was markedly inhibited, while the adult females lost weight initially, but began to gain after a short interval. Meites (1949) injected stilbestrol into albino rats at daily levels of 0.001, 0.01, and 0.1 milligram per animal. All levels of stilbestrol depressed growth and intake of food and water. The author concluded that the inhibition of growth was due primarily to appetite depression. Korenchevsky and Denison (1934) found that large daily doses of estrone depressed appetite and gain in weight of rats. Light and Tornaben (1953a, 1953b), after applying ointment containing 30 micrograms of estradiol to weanling male and female rats, found, in contrast to the work of Meites (1949), that estrogens induced a period of extreme energy expenditure which caused an increase in appetite for food and water but

permitted only sub-normal growth. Gardner and Pfeiffer (1943) observed that the oral administration of 50-250 micrograms of stilbestrol greatly reduced the rate of growth in young rats. They found, in addition, that estrogens decreased rate of gain in young guinea pigs.

Effects on Metabolism: Although work with albino rats is conflicting as to the effect of estrogens on appetite, Dinusson et al. (1950) and Perry et al. (1955) with fattening beef cattle, and O'Mary et al. (1952) with fattening lambs reported that stilbestrol, either implanted or orally administered, increased daily feed consumption.

In metabolism trials with lambs, Whitehair et al. (1953) found that stilbestrol implanted subcutaneously had little effect on total digestibility of the ration, but significantly increased calcium, phosphorus, and nitrogen retention. Jordan (1953) also found no difference in digestibility of feed nutrients by fattening lambs, but nitrogen retention was increased approximately 30 percent as a result of stilbestrol implantation. Brooks et al. (1954) demonstrated that high levels of stilbestrol fed to sheep (10-20 milligrams per head daily) significantly increased cellulose and protein digestibility, but the level of estrogen fed was too high to be of practical importance. Clegg (1952) and Clegg and Cole (1954) obtained an increase in nitrogen retention in steers implanted with stilbestrol which was almost double that of the controls. Klosterman et al. (1955) fed three different levels of protein, obtained by the daily addition of 1.5 pounds, 0.75 pounds, or no soybean oil meal to a fattening ration, to steers receiving stilbestrol either orally or by implants. They reported highly significant differences in average daily gain between steers fed various

levels of protein and between control and treated steers. The response in daily rate of gain from stilbestrol was 0.51, 0.36, and 0.03 pounds when the three levels of protein were included in the ration. Schilling and Laszlo (1950) administered estrogen therapy to an elderly male suffering prostatic carcinoma and observed no appreciable changes in nitrogen or phosphorus retention. However, a positive calcium balance achieved on a low calcium diet was accredited to an initial decline in urinary calcium excretion and a subsequent reduction in fecal calcium excretion. Kochakian (1946) in reviewing the protein anabolic effects of the steroid hormones, stated that the estrogens increased nitrogen retention in dogs and that stilbestrol therapy in human females with Cushing's syndrome decreased nitrogen excretion. Roberts and Szego (1953) stated that the growth which occurs in the female reproductive structures in response to estrogen stimulation has been characterized by the deposition of protein as well as water and electrolytes.

Govaerts et al. (1951) performed balance studies on pigeons utilizing calcium-45 and alpha-estradiol dipropionate. They found that daily injections of the estrogen decreased calcium excretion and increased overall calcium retention. Under estrogenic influence, calcium had a biological half-life of 75 days as compared to 45 days in the controls. Norman and Mittler (1948) found that estrogens administered to weanling rats stunted the growth of these animals but increased the density of the bone shaft, accelerated epiphyseal closure, and increased the percentage of bone ash. Support for these data is found in the work of Day and Follis (1941) who observed that estradiol benzoate injected into rats 30-650 days of age caused a decrease in the

destruction of bony trabeculae immediately beneath the cartilage shaft junction and a decrease in osteoblastic activity at the cartilage shaft junction. This increased the density of the epiphysis and apparently accounted for the increased bone ash concentration. However, total bone was decreased due to decreased growth in bone length. Budy et al. (1952) found that the length and density of the long bones of young growing mice was increased in response to moderate doses of estrogens. This action was confined to a specific effect upon one part of the growth apparatus; i.e. interference with the resorption of spongy bone in the metaphysis. Since the estrogenic effect on the bones of rats was limited to that part of the growth apparatus concerned only with endochondral ossification, no similar action upon intramembranous ossification occurred. Similarly, Edgren and Calhoun (1956) reported that injections of diethylstilbestrol, estradiol, or estrone into mice stimulated increases in the density of the femora of the treated animals, presumably by causing proliferation of the medullary bone. In this respect, estrone appeared to be the most potent and stilbestrol the least potent. In contrast to the foregoing, Urist et al. (1948) stated that, in guinea pigs, hamsters, rabbits, cats, and dogs, there was no apparent specific effect of estrogens upon bone formation.

Effect upon Carcass Analysis and Body Composition: Little critical work has been performed concerning the influence of estrogens on skeletal development and body composition in large animals. O'Mary et al. (1952), in analyzing carcasses from wether and ewe lambs that had received stilbestrol implants, found a greater amount of total bone in the treated lambs. Wilkinson et al. (1955) observed non-significant

differences in percentage bone ash between treated lambs and their controls, while Clegg and Carroll (1956) obtained no differences in percentage bone between steers that had received stilbestrol implants and their controls.

Cahill et al. (1956) reported that stilbestrol tended to induce deposition of fat in bulls, but retarded fat deposition in steers. O'Mary et al. (1952), after implanting lambs with 12 milligram pellets of stilbestrol, found a smaller amount of external fat, a highly significant increase in moisture content of the external fat, a greater amount of bone and connective tissue, and no difference in the amount of muscle and internal fat. Wilkinson et al. (1955) obtained essentially the same results with full-fed or limited-fed fattening lambs, but observed no differences in the percentage water in the eye muscle or percentage ash in the shank bone. Clegg and Carroll (1953) studied a seven-rib cut from carcasses of steers receiving stilbestrol implants and their controls. The data revealed no differences in percentage water, but there was a greater eye muscle area, a greater percentage of lean and a smaller percentage of ether extract and fat in the treated animals. In later work, Clegg and Carroll (1956) utilized the twelfth-rib cut from steers and heifers which had received stilbestrol implants. Treatment caused decreased fat deposition and increased protein anabolism, did not affect percentage bone nor percentage moisture in steers, but caused a significant increase in percentage moisture in heifer carcasses.

Carcass analysis, however, where minimal sample areas are employed, has proven to be a crude method of evaluating the physiological actions

of estrogens. The limited data suggest, in beef cattle at least, that there is an increase in total lean tissue. This is in agreement with balance studies which show a great increase in nitrogen retention under estrogenic influence. Data which indicate no difference in percentage bone in stilbestrol-treated animals should be interpreted with caution when compared with data which indicate increased calcium and phosphorus retention. Analyses for percentage moisture have indicated little effect of stilbestrol upon water retention, although, in certain instances, there has been increased moisture reported as determined by carcass analysis.

Roberts and Szego (1953) have reviewed steroid interactions in the metabolism of reproductive target organs. They found that true growth of the structures of the female reproductive tract, induced by endogenous hormonal stimulation or by estrogen administration was preceded by an influx of water and electrolytes into the tissues involved. Although these data were concerned with smooth muscle tissue, there is the possibility that estrogens would have similar effects on striated muscle tissue.

Paschkis *et al.* (1954) have indicated that estrogens exert a slight effect on retention of salt and water in the tissues. Burrows (1949) stated that estrogens markedly increased retention of sodium, chloride, and water with a gain in body weight and blood pressure. In this respect, Furth and Sobel (1946) determined blood volume in mice bearing transplanted granulosa-cell tumors which are estrogen producing. They observed an increase in blood volume of one to five times that of the normal mice. However, Genzell and Sjostrand (1956) reported no effect

upon blood volume as a result of injecting estrogens into normal and castrate rats. Thorn and Engel (1938) found that injection of estrogens into normal dogs was followed by decreased excretion of inorganic phosphorus and sodium which would indicate an accompanying increase in water retention.

These data point to hydration of the tissues as one of the effects of estrogen administration and must be considered in determining the mechanism of action of stilbestrol in ruminants. The work of Perry et al. (1955) and Reynolds et al. (1955) indicated that for the first month following the initiation of estrogen feeding to steers, there was a period of extreme weight gain following which the effect of the estrogen was gradually diminished. It was possible that estrogens induced a period of greatly increased anabolism after which the animals became adapted to treatment. However, it was also possible that estrogens induced a period of increased fluid retention followed by a gradual adaptation to the treatment.

Hormonal Interrelationships: The demonstrated action of stilbestrol as it affects nitrogen and mineral metabolism in ruminants strongly suggests pituitary influence, although there is the possibility that the hormone acts directly upon other endocrine organs. That estrogens exert an influence upon the anterior pituitary is well established. Spencer et al. (1932) and Reece and Leonard (1939) attributed growth inhibition in estrogen-treated rats to a depression in the growth stimulating power of the hypophysis. Deanesley (1939) implanted estrone or estradiol into male rats and mice and observed an inhibition of pituitary function as well as a marked depression of growth, loss of fertility,

shrinkage of gonads and accessory organs, and enlargement of the pituitary and adrenals. Richards and Kueter (1941) attributed the growth depression observed when young rats were given large doses of stilbestrol to an inhibition of pituitary growth hormone.

Depression of growth in laboratory animals has also been attributed to stimulation of the adrenal cortex. Ellison and Burch (1936) reported that a number of estrogens, in sufficient quantities, caused hypertrophy of the pituitary and adrenals in the castrate rat. Korenchevsky and Denison (1934) found that large doses of estrone in normal rats depressed development of the sexual organs and induced hypertrophy of the adrenals and hypophysis. Allen and Bern (1942) noted that estrogens increased the size of the adrenal cortex in the guinea pig and Deanesley (1939) observed enlargement of the adrenals in estrogen-treated rats.

The thyroid has also been implicated in the response to estrogens although the data are somewhat conflicting. Victor and Andersen (1938) concluded that the increased metabolism of the liver of spayed rats injected with estrogens resulted from the action of the estrogen on the anterior pituitary which caused an increase in thyroid activity. Meites and Turner (1948), however, found that estrogens decreased the pituitary production of the thyroid stimulating hormone in male rabbits. Feldman (1956) reported that the administration of estrogens for short periods of time to intact male and female rats, castrated male, and hypophysectomized female rats induced increased thyroid uptake of I-131. Prolonged injection of estrogens prevented increased uptake or depressed the usual uptake of I-131. The administration of

estrogens did not affect thyroid size. Soliman and Reineke (1954) reported that estrogens administered in small doses consistently increased thyroidal iodine collection. In later work Soliman and Reineke (1956) demonstrated that a functional pituitary was necessary for this action to occur.

Paschkis et al. (1954) surveyed the effects of estrogens on the endocrine system. They stated that prolonged administration of large doses of estrogens produced dwarfism apparently resulting from inhibition of the growth hormone. In some species, estrogens caused increased adrenal weight, but this difference was not consistent. There has been no conclusive evidence that estrogens administered in physiologic doses affected thyroid function. In high dosages or after prolonged administration, estrogens appeared to depress thyroid function and lower the basal metabolic rate. This effect was probably mediated by changes in the thyroid stimulating hormone.

Research on the effects of estrogens upon the endocrine system of large animals is extremely limited. Cahill et al. (1956) found that stilbestrol implants in steers resulted in significantly heavier adrenal glands and slightly heavier pituitaries. Clegg and Cole (1954) implanted stilbestrol into steers and heifers and studied the effect on endocrine weights, ACTH, and growth hormone content of the pituitary. In the treated animals, the pituitary and adrenals were significantly larger than in the controls, but the growth hormone and ACTH content per gram of total organ of the treated steers were not significantly different from the controls. However, the growth hormone content of the hypophysis of treated heifers was twice as great as the controls.

Although the effects of stilbestrol upon calcium, phosphorus, and nitrogen retention have been demonstrated, further work is needed to determine the underlying mechanisms of these processes. In view of the effects of estrogens upon fluid uptake of certain tissues in laboratory animals, critical studies should also be made to determine whether the large increases in initial rate of gain which have been reported might be correlated with increases in total body fluids.

It has been shown that estrogens have a marked effect upon certain endocrine organs in laboratory animals as well as ruminant animals. In ruminants the effects of stilbestrol implants upon the endocrine system of steers has been studied to some extent. There is a need for investigations of the effects of orally administered stilbestrol in ruminants as well as a more detailed evaluation of the actions of estrogens upon endocrine function.

EXPERIMENT I

EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON CALCIUM AND PHOSPHORUS METABOLISM IN LAMBS

It has been shown that stilbestrol administration results in increased calcium and phosphorus retention in lambs, however, the underlying mechanism has not been demonstrated. This experiment was designed to determine the effects of orally administered stilbestrol upon absorption of calcium and phosphorus from the gastrointestinal tract and upon the loss of calcium and phosphorus from body sources.

Experimental

Procedures with Animals: Eighteen crossbred spring wether lambs of known breeding were used in an experiment conducted at the University of Tennessee-Atomic Energy Commission Agricultural Research Program, Oak Ridge, Tennessee, during the summer and fall of 1956. Twelve of the lambs, averaging approximately 70 pounds in weight, were used during the summer period (May 21-August 18) and six of the lambs, late spring lambs averaging approximately 80 pounds in weight, were used during the fall period (September 7-November 12). Immediately after the animals were brought in from pasture they were shorn and drenched with phenothiazine and placed on UT-AEC ration H-1 which consisted of yellow corn (75 percent), alfalfa meal (15 percent), soybean oil

meal (10 percent), and irradiated yeast (10 grams per cwt.), with orchard grass hay ad lib., until they had become accustomed to the ration and surroundings.

At the beginning of the summer period, the twelve lambs were divided at random into two groups of six lambs each. One group was placed in the metabolism racks for a short orientation period, followed by a four-day preliminary period, during which time the lambs received 1.0 pound of UT-AEC ration H-1 and an equal amount of poor quality orchard grass hay. This ration was fed during the entire experimental period and was calculated to provide 3-4 grams of calcium and 2.5 grams of phosphorus per head daily. At the end of the preliminary period, the lambs were divided at random into two groups of three animals each. One group continued to receive the basal ration while the other group received the basal ration plus 2 milligrams of stilbestrol per head daily. At this point, one animal in each group, selected at random, was injected intravenously with 1 millicurie of calcium-45 and 1 millicurie of phosphorus-32, administered concurrently, and for comparison two animals in each group received the same amount of radioactivity administered orally by means of a stomach tube. A concurrent 7-day chemical and radiochemical blood study and balance was then conducted. At the conclusion of this trial, the second group of six lambs, which had been on the basal ration, were similarly treated. During the summer period, three trials were conducted with both groups at approximately three-week intervals. In the intervals between balance trials, all lambs continued to receive their same experimental rations. At the beginning of the second and third trials, the orientation period was omitted, but a four-day preliminary period was included before the

collection period. Immediately before each trial, all lambs were weighed without shrink. These weights were used for subsequent calculations.

During the fall period the lambs were handled in a similar manner, except that the experimental ration consisted of 1.5 pounds of UT-AEC ration H-1 and 1.0 pound of mixed orchard grass-alfalfa hay per head daily which provided an intake of approximately 5 grams of calcium and approximately 3.5 grams of phosphorus per head daily. During the second trial of the fall period all lambs were dosed orally with the appropriate radioisotope. For the final balance an additional lamb, which received the appropriate experimental ration for the entire period, was added to each group. These lambs received calcium-45 only, injected intravenously.

Procedures for handling the animals and for the quantitative administration of radioisotopes have been described in detail by Hansard (1951) and Hansard et al. (1951).

Isotope Administration: The radiocalcium was prepared as the chloride and was diluted for use to an appropriate volume with sterile saline. Immediately before dosing, the pH was adjusted to 6.0-7.0 to prevent precipitation upon standing. Radiophosphorus was prepared as phosphoric acid in weak hydrochloric acid solution and was neutralized with dilute ammonium hydroxide immediately before administration.

For intravenous administration a No. 12 bleeding needle was inserted into the jugular vein and a small polyethylene catheter inserted through the needle and into the vein. A syringe adapted with a short length of gum rubber tubing and filled with heparinized normal saline was attached to the catheter with a No. 20 hypodermic needle. The dose,

contained in a small tuberculin syringe, was introduced into the tubing and washed quantitatively into the bloodstream by simultaneous administration of the heparinized normal saline solution. A reference standard was prepared from an aliquot of the injection solution for later reference.

For oral administration the activity was administered quantitatively by washing the dose down as described for intravenous administration, using a carefully inserted stomach tube.

Sampling of Blood, Excreta, and Feeds: Blood samples were taken routinely during the experimental period by jugular puncture. During each balance study 10-12 blood samples were taken from each animal in order to determine the effect of treatment upon the disappearance rate of the administered isotope. The blood was collected in heparinized centrifuge tubes and centrifuged as soon as possible after collection at 1500 revolutions per minute for 30 minutes. Hematocrit readings were taken for calculation of total blood activity and the plasma was decanted and stored under refrigeration until chemical and radiochemical analyses could be made.

During the balance trials daily quantitative collections of urine and feces were made. Representative samples were taken from each daily collection and placed in separate containers for individual analysis. Ration constituents and weighback were carefully sampled for analysis and subsequent intake calculations. All urine and fecal samples were refrigerated until total calcium, phosphorus, and radiochemical determinations could be made.

Methods of Analysis: In investigations involving labeled elements, it is essential to have both chemical and radiochemical determinations for interpretation of the physiological behavior of the stable element. It was desirable, therefore, to integrate these analyses in order to minimize and simplify the steps necessary for routine determinations on large numbers of samples.

Analysis for Calcium: Calcium-45 has been reported as having no gamma radiation and a weak beta emission (0.26 mev). Because of this low beta energy and concomitant self-absorption, it was desirable to have a minimum mass in the sample to be measured and it was necessary that suitable corrections be applied. All radioactive samples were compared with a standard prepared from a known aliquot of the original dosing solution which was evaporated to dryness in a stainless-steel cup and which was assumed to have no mass. Self-absorption corrections based on the sample mass weight were applied to all samples using values reported by Comar et al. (1951).

Blood Plasma: Heparinized blood was centrifuged for 30 minutes at 1500 revolutions per minute, the hemtocrit reading was taken, and the plasma was drawn off. A 3 milliliter aliquot was taken from each sample, placed in a 40 milliliter centrifuge tube, and analyzed for total calcium by the method of Clark and Collip (1925). Following titration, the sample was prepared for radioassay of calcium-45 by adding a solution containing 8 milligrams of calcium as a carrier and then 3 milliliters of saturated ammonium oxalate. The solution was then neutralized with 1:1 ammonium hydroxide and returned to pH 6.0 with 1:1 acetic acid. After standing overnight, the solution was

centrifuged and the precipitate washed and collected in a plastic tube and metal cup assembly. This technique has been described in detail by Comar et al. (1951). Briefly, the tube-and-cup assembly consisted of a tapered-end polyethylene cylinder with a stainless-steel Tracerlab cup forming the bottom. The cup was pressed securely over the end of the fitted tube until the assembly was water-tight. The calcium oxalate precipitate was then washed quantitatively into the tube and the assembly placed in a 50 milliliter centrifuge carrier. To equalize centrifugal pressure and to insure no leakage, water was placed in the carrier to a height equal to that of the fluid in the plastic tube. Following centrifugation at 1500 revolutions per minute, the supernatant was drawn off and the cups removed and dried to a constant weight in a circulating oven at approximately 60° C. The samples were then measured for radioactivity by conventional methods using a thin mica window Geiger-Mueller Tube connected to a scaler unit.

Feces: Duplicate samples of 20-30 grams of fresh feces were dried overnight at 100° C and ashed in a muffle furnace at 600° C until a white ash was obtained. The ash was dissolved in 6N HCL and made to a volume of 25 milliliters with re-distilled water. After the solution was mixed thoroughly, the insoluble residue was allowed to settle and duplicate 3 milliliter aliquots taken for analysis. The calcium was precipitated by adding 3 milliliters of saturated ammonium oxalate, then, by neutralizing the HCL with 1:1 ammonium hydroxide, and by adjusting the solution to a final pH of approximately 6.0 using 1:1 acetic acid as a buffer. After the solution was allowed to stand overnight, it was centrifuged, and the precipitate washed and transferred

quantitatively into a tared cup as described for plasma calcium. The total calcium and the correction for self-absorption were calculated from the mass weight of the oxalate in the dry cup.

Urine: Twenty gram samples of fresh urine were ashed according to the procedure described for feces. The ash was dissolved in 6N HCL and diluted to 25 milliliters with re-distilled water. Duplicate 10 milliliter aliquots were taken and precipitated as for fecal calcium analysis. Total calcium was determined by the titrimetric method of Clark and Collip (1925). For radioassay, 8 milligrams of calcium carrier were added and the calcium was precipitated for counting in the cup assembly as described for plasma and feces.

Feed Samples: Ten to twenty gram aliquots were taken in triplicate and chemical analysis was performed on 0.5 ml aliquots of the acid solution as described for blood plasma and urine.

Analysis for Phosphorus: Phosphorus-32 is reported to have a 1.71 mev beta emission. In this study the phosphorus-32 was administered concurrently with the calcium-45. To eliminate the necessity of chemical separation, the difference in beta energies of the two isotopes was utilized. Absorption data have indicated that an aluminum absorber having a mass of 55 milligrams per square centimeter would absorb all of the calcium-45 beta particles but would reduce the phosphorus-32 contribution by a factor of only 1.5 (Comar et al., 1951). In view of this, all samples assayed for phosphorus-32 were counted through such an absorber and the activity was compared to a standard prepared from the original dosing solution which was also counted through the absorber. In counting standards, the volume used was

equal to the volume of the sample counted to eliminate the necessity of correcting for self-absorption.

Blood Plasma: One milliliter of heparinized plasma was added to 4 milliliters of 5 percent trichloroacetic acid in a small centrifuge tube and mixed thoroughly. The solution was centrifuged and a 2 milliliter aliquot was analyzed for total inorganic phosphorus by the method of Fiske and Subbarow (1925). Radioassay for phosphorus-32 was performed by counting a 4-5 milliliter aliquot of plasma in a Petri dish using a thin mica window Geiger-Mueller counter connected to a scaler.

Feces: Duplicate aliquots of the same acid solution prepared for calcium analysis, generally 0.01 milliliter, were analyzed for phosphorus by the method of Fiske and Subbarow (1925). For radioassay, 10 milliliters of this solution were counted in a Petri dish as described for plasma.

Urine: Duplicate 0.2 milliliter aliquots of the solution of ash dissolved in acid were analyzed for total inorganic phosphorus as previously cited. For radioassay, 10 milliliters of fresh urine were counted in a Petri dish as described for plasma and for feces.

Feed Samples: Duplicate aliquots each representing a volume of 0.01 milliliter of the solution of ash dissolved in acid were analyzed for phosphorus as previously cited.

Methods for Calculation of Data: The comparative balance method of Hansard et al. (1954) and the isotope dilution procedure described by Visek et al. (1953) and modified by Hansard et al. (1957) to permit a single intravenous dose of radioactivity, were used concurrently to

measure fecal endogenous losses of both calcium and phosphorus in these trials.

Endogenous fecal calcium or phosphorus (E) was determined from the average specific activity values for the feces (SA_f) and plasma (SA_p) on the last two days of the trial as follows:

$$E = \frac{SA_f}{SA_p} \text{ X Daily Fecal Excretion.}$$

The results were then expressed in milligrams per kilogram of body weight.

The comparative balance procedure is well-adapted to the study of factors affecting mineral behavior in that it allows a direct determination of absorption as well as the estimation of endogenous fecal losses which may be used to correct conventional balance data to give true digestibility values. The 7-day isotope excretion data from animals receiving a single oral dose and that from similar animals receiving a single intravenous dose of the appropriate radioactivity permitted calculation of the percentage of dietary calcium or phosphorus that was absorbed (A) as follows:

$$A = \frac{100 - \text{Fecal Isotope from Oral Dose}}{1 - \text{Fecal Isotope from I.V. Dose.}}$$

The percentage of fecal endogenous calcium or phosphorus (P_e) was then calculated as described by Hansard (1956):

$$P_e = A - (100 - \text{Percent Fecal Mineral}).$$

The daily endogenous fecal calcium or phosphorus was then expressed as follows:

$$\text{Grams Endogenous} = \frac{P_e \text{ X Daily Intake}}{100}$$

For a convenient unit of expression, fecal endogenous calcium or phosphorus was expressed as milligrams per kilogram of body weight.

By solving the foregoing equations, the conventional balance data were corrected for endogenous calcium or phosphorus losses to give the true digestibility values thusly:

$$\text{True Digestibility} = 100 - \left[\frac{(\text{Fecal Mineral} - \text{Daily Gms. Endog. Min.}) \cdot 100}{\text{Daily Intake of Mineral}}\right]$$

All data were analyzed statistically using the analysis of variance method of Snedecor (1956).

Results and Discussion

Effects upon Calcium Metabolism: A summary of the calcium intake and excretion values for both the summer and fall trials is presented in Table 1. Average calcium retention for lambs receiving the basal ration was 0.09 grams per head daily, while the stilbestrol-fed lambs averaged 0.57 grams per head daily, a difference that was highly significant (P .002). The increased calcium retention due to the influence of orally administered stilbestrol was in general agreement with earlier work of Whitehair et al. (1953) in which stilbestrol was implanted.

Radioactivity in the total blood as a function of time following a single dose of orally or intravenously administered calcium-45, graphically illustrated in figure 1, was calculated from the blood concentrations and the blood volume values as described by Hansard et al. (1953). The curves were plotted from the average radiocalcium concentration values in the periodic blood samples obtained during the entire

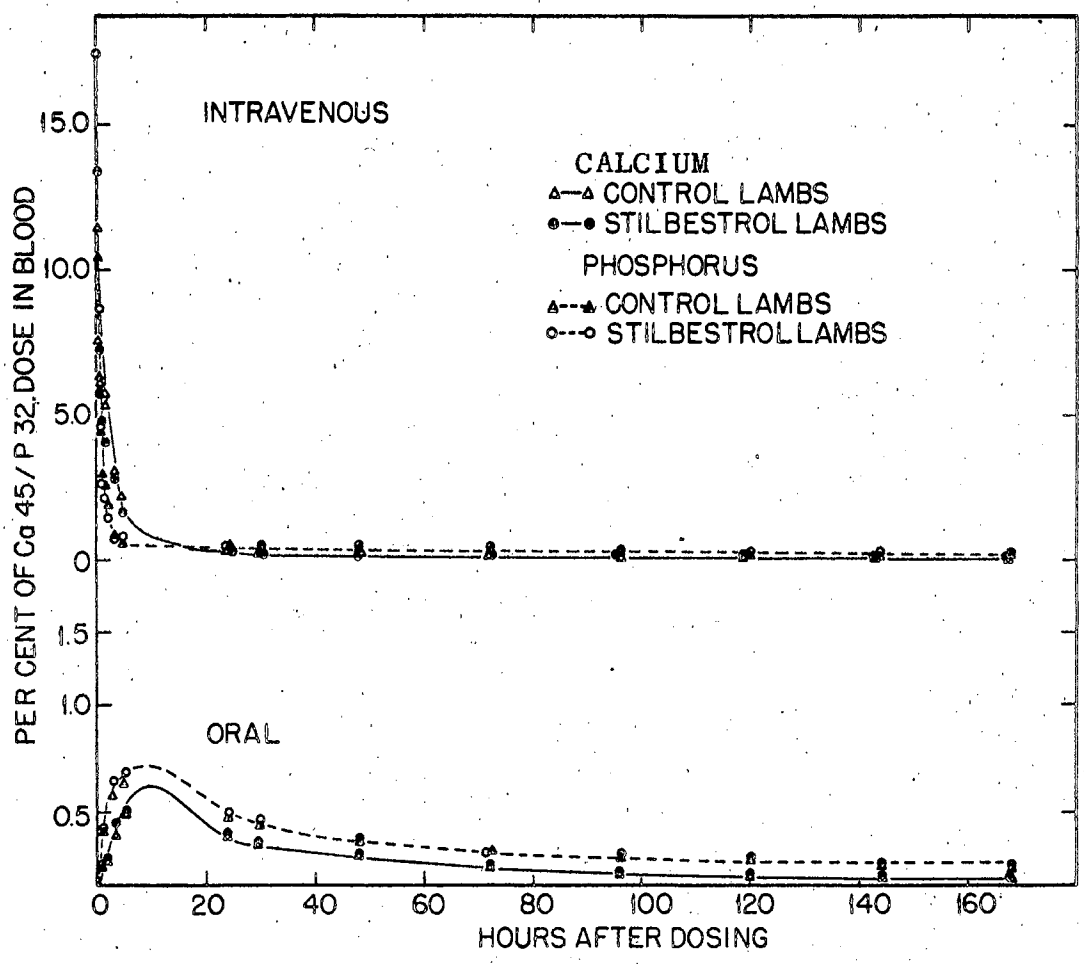

Figure 1. Calcium-45 and phosphorus-32 in the total blood of control or stilbestrol-fed wether lambs as a function of time, following oral or intravenous administration.

experimental study. The disappearance of intravenously administered calcium-45 from the blood follows a characteristic curve. Similar curves have been presented for rabbits by Thomas et al. (1952) and for cattle by Hansard et al. (1954a). These workers indicated that blood calcium-45 disappearance curves in young cattle are represented by four exponential terms and have related the rate process for each term with a definite physiological process. From these equations it was possible to calculate the rate of exchange of calcium between the plasma and the extra-vascular compartments. During the first few hours after injection, the dose was being rapidly transferred to the bones and other tissue. The latter part of the curve represents a period of equilibrium between the blood, the tissues and bones, and the gastrointestinal tract; an equilibrium characterized by a different concentration gradient in each area.

Figure 1 also shows the amount of calcium-45 in the blood as a function of time after ingestion. The calcium-45 as it enters the blood by absorption is removed at the same rate as the intravenously administered isotope. Therefore, the oral curve reflects the difference between absorption from the gastrointestinal tract and removal from the blood. After approximately 100 hours, however, the blood level of calcium-45 is governed primarily by the exchange rate between bone and plasma.

Although these curves reflect the behavior of the current dietary calcium intake only, they may be used to interpret the movement of calcium during the experimental period. By inspection it is apparent that stilbestrol was not effective in altering the rate of exchange

of calcium between the bones, tissues, blood, and gastrointestinal tract.

Figure 2 illustrates the rate of fecal excretion of a single dose of the orally or intravenously administered calcium-45. The accumulative curve, representing the net excretion of intravenously administered calcium-45 after all physiological processes have occurred, is indicative of the fecal endogenous calcium fraction. By the definition to be used hereafter the term "fecal endogenous" will apply to any calcium or phosphorus appearing in the feces which has passed through the plasma pool. From these curves, a marked reduction in the loss of calcium-45 from body sources is noted in the stilbestrol-fed animals.

The relatively steep initial slope of the oral curve (during the first 48 hours) shown in the same figure reflects the excretion of unabsorbed, or exogenous, calcium-45. Conversely it also gives some indication of the absorption of calcium-45 from the tract. From the curve it is apparent that stilbestrol exerts little influence upon the absorption of calcium. The divergence of the two curves following the absorptive phase, however, indicates that stilbestrol reduced the excretion of calcium derived from body sources and is in agreement with the calculated values (Table 1) from the chemical balance studies.

It must be borne in mind that the behavior of the single dose of radioactivity is not directly comparable to the behavior of dietary calcium over a period of time. Rather, it reflects the behavior of a unit amount of dietary calcium ingested at a given time and is affected directly by the current calcium status and the nutritional history of

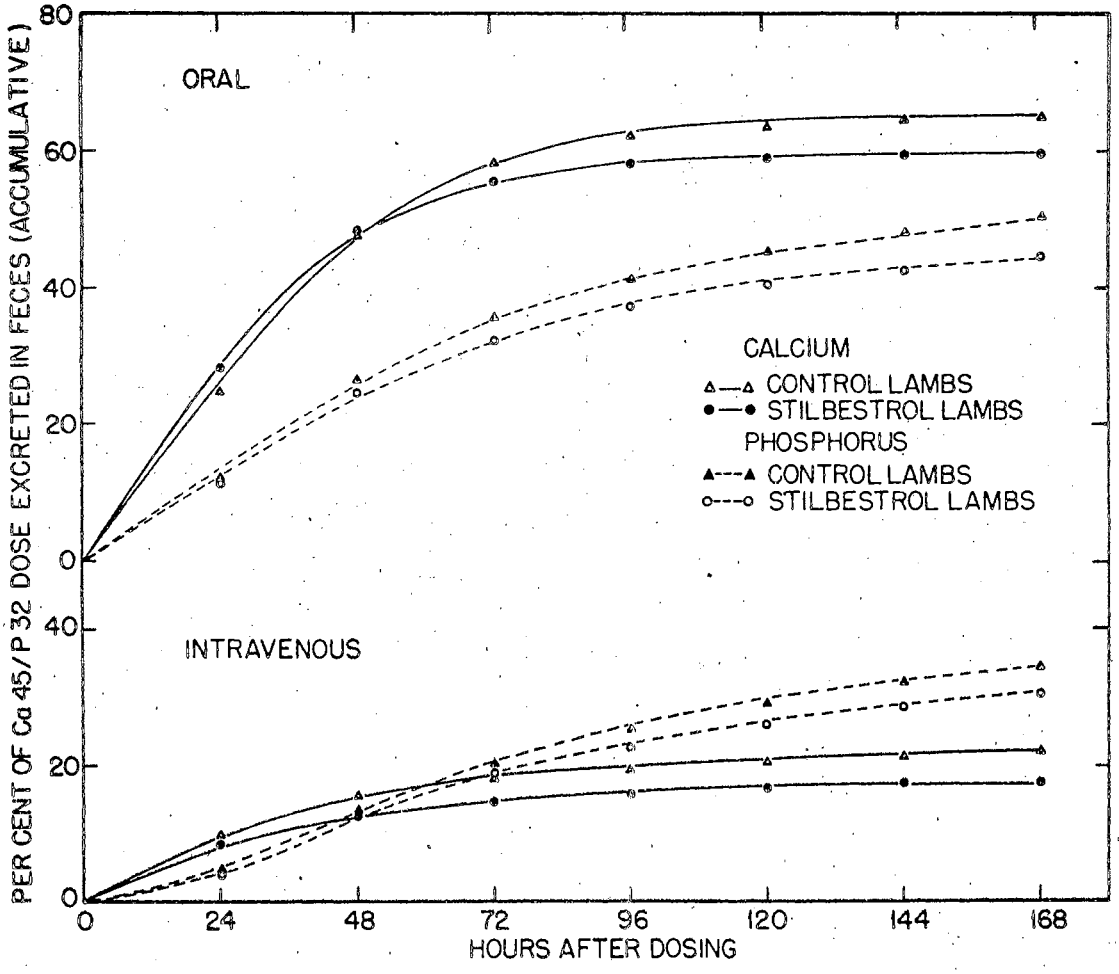

Figure 2. Accumulative fecal excretion of orally and intravenously administered calcium-45 and phosphorus-32 of control or stilbestrol-fed wether lambs.

the animal (Hansard et al., 1951b). In order to interpret the effects of treatment upon calcium metabolism, radiochemical data must be correlated with conventional balance data.

The effects of ingested stilbestrol upon calcium absorption and excretion are summarized in Table 1. Of the intravenously administered dose of calcium-45, 22.1 percent appeared in the feces of the control lambs as contrasted to 18.0 percent for the stilbestrol-fed lambs, a difference approaching statistical significance ($P < .09$). These data indicate that those animals receiving stilbestrol utilized absorbed calcium more efficiently than did their controls. Of the orally administered calcium-45, the control lambs excreted 65.5 percent in the feces as compared to 60.0 percent by the treated lambs. This difference also approached significance ($P = .10$). Whether stilbestrol promoted increased re-absorption within the gastrointestinal tract or decreased re-excretion of the absorbed calcium, however, will require further study.

Apparent digestibility of calcium was 4.3 percent for the control lambs and 15.2 percent for the stilbestrol-fed lambs, a difference which was highly significant ($P < .005$). By combining chemical and radiochemical balance data, the percent of fecal endogenous calcium was calculated using the comparative balance procedure of Hansard et al. (1954b). Fecal endogenous calcium, expressed as milligrams per kilogram of body weight per day, was then calculated. Daily fecal endogenous calcium excretion amounted to 43.9 milligrams per kilogram for the control lambs as contrasted to 34.7 milligrams per kilogram for the lambs receiving stilbestrol; the difference being significant ($P < .05$).

TABLE 1 EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON CALCIUM ABSORPTION AND EXCRETION IN LAMBS

	Basal	Stilbestrol
Number of Animals[1]	9	9
Number of Trials	3	3
Average Weight of Animals (Kg.)	38.3	39.9
Chemical Balance Data, Daily Gms.:		
Intake	4.10	4.16
Feces	3.93	3.52
Urine	.08	.07
Net Retention	+ .09	+ .57
Radiochemical Data:		
Percent Ca-45 in Feces:		
Intravenously Administered	22.1	18.0
Orally Administered	65.5	60.0
Fecal Endogenous Loss:[2]		
Isotope Dilution Technique	41.6	34.7
Comparative Balance Technique	43.9	34.7
Apparent Digestibility (%)	4.3	15.2
True Digestibility (%)[3]	45.1	48.6

[1]In the final trial of the fall period an additional animal was included in the calcium balance for each group.

[2]Expressed as milligrams per kilogram of body weight per day.

[3]Calculated as $100 - \frac{(\text{Fecal Ca} - \text{Fecal Endogenous Ca})\ 100.}{\text{Ca Intake}}$

Concurrently with the comparative balance procedure, fecal endogenous calcium excretion was estimated by the isotope dilution technique described by Hansard et al. (1957), in which values from the lambs dosed intravenously were utilized. Insufficient animals were used for a completely valid estimate of endogenous loss by this technique, however, the data demonstrate the close agreement which might be attained using the two methods separately or concurrently (Comar et al., 1953). By the isotope dilution procedure, fecal endogenous calcium excretion was calculated to be 41.6 milligrams per kilogram of body weight for the control lambs and 34.7 milligrams per kilogram of body weight for the stilbestrol-fed lambs. The difference between the two groups was not significant, apparently due to more variation between samples than was obtained using the comparative balance procedure. Nevertheless, there was a consistent reduction in the net fecal endogenous calcium loss of the stilbestrol-fed lambs throughout all trials.

With data for fecal endogenous loss, true digestibility was calculated by substituting in the conventional formula (see footnote 3, Table 1). For calculation of true digestibility, the fecal endogenous values derived from the comparative balance technique were used. Under the conditions of this experiment, true digestibility of calcium was not significantly different for the control lambs (45.1 percent) as compared to the stilbestrol-fed lambs (48.6 percent).

From this trial, it is apparent that the increased retention of calcium under estrogenic influence was more a function of decreased calcium loss from body stores than of absorption per se. Inasmuch

as urinary excretion of calcium was negligible throughout all periods, the reduction in rate of turnover was a result of an inhibition of fecal endogenous calcium excretion. Thus it appears that once dietary calcium reaches the plasma pool, stilbestrol enhances the retention and utilization of the mineral in some way.

Effects upon Phosphorus Metabolism: A summary of the phosphorus intake and excretion values for the combined summer and fall balance trials is presented in Table 2. Average phosphorus retention for the control lambs was -0.25 grams per head daily, and for the stilbestrol-fed lambs; 0.05 grams per head daily. The highly significant ($P < .004$) increase in phosphorus retention, as with calcium, agrees with the results reported by Whitehair et al. (1953).

The rate of disappearance of a single dose of orally or intravenously administered phosphorus-32 from the blood is shown in figure 1. Essentially, these curves were not different from those plotted for calcium-45, varying only in the slightly higher concentration of phosphorus-32 in the blood at any given time. In many instances, the physiological forces which affect blood calcium may also affect blood phosphorus. It appears from these data that stilbestrol did not alter the rate of movement of phosphorus between the bones, tissue, blood, and gastrointestinal tract.

Figure 2 represents the rate of fecal excretion of a single dose of orally or intravenously administered phosphorus-32. The accumulative excretion curve for the intravenously administered dose as a

TABLE 2 EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON PHOSPHORUS ABSORPTION AND EXCRETION IN LAMBS

	Basal	Stilbestrol
Number of Animals	9	9
Number of Trials	3	3
Average Weight of Animals (Kg.)	38.0	39.5
Chemical Balance Data, Daily Gms.:		
Intake	2.82	2.94
Feces	2.98	2.79
Urine	0.09	0.10
Net Retention	- .25	+ .05
Radiochemical Data:		
Percent P-32 in Feces:		
Intravenously Administered	35.5	31.2
Orally Administered	51.1	45.1
Fecal Endogenous Loss:[1]		
Isotope Dilution Technique	51.2	48.4
Comparative Balance Technique	61.9	57.7
Apparent Digestibility (%)	- 5.7	4.8
True Digestibility (%)[2]	77.7	82.7

[1]Expressed as milligrams per kilogram of body weight per day.

[2]Calculated as $100 - \frac{(\text{Fecal P} - \text{Fecal Endogenous P})\ 100.}{\text{P Intake}}$

function of time indicates that stilbestrol induced a moderate reduction in the fecal endogenous excretion of phosphorus-32.

The initial slope of the curve representing excretion of the orally administered phosphorus-32 reflects exogenous radiophosphorus. Unlike calcium-45, the divergence of the curves indicates that stilbestrol promoted absorption from the gastrointestinal tract. Continued divergence of the curves following the absorptive period demonstrate the role of estrogens in reducing the loss of phosphorus from body stores.

When considering excretion patterns, it must be pointed out that, like calcium-45, the behavior of a single dose of radiophosphorus may not be directly correlated with the physiological activity of dietary phosphorus over a period of time. However, the same factors affecting the movement of dietary calcium (current status and nutritional history of the animal) also affect the behavior of dietary phosphorus. Although inferences may be drawn from a consideration of the kinetics of radiophosphorus, to interpret the effects of treatment upon phosphorus metabolism, the results must be correlated with conventional balance data.

The effects of stilbestrol upon phosphorus absorption and excretion are shown in Table 2. Of the intravenously administered phosphorus-32, the control lambs excreted 35.5 percent in the feces as compared to 31.2 percent by the stilbestrol-fed lambs, a difference that was highly significant ($P < .001$). As with the calcium excretion values, the data indicate increased efficiency of utilization of the absorbed phosphorus. The control lambs excreted 51.1 percent

of the orally administered phosphorus in the feces contrasted with 45.1 percent excreted by the treated lambs ($P < .025$).

Apparent digestibility of phosphorus was significantly in favor of the stilbestrol-fed lambs, amounting to 4.8 percent for the treated lambs as compared to - 5.7 percent for those receiving the basal ration ($P < .005$). By utilizing both chemical and radiochemical balance data as previously discussed, fecal endogenous phosphorus was calculated to be 61.9 milligrams per kilogram for the control lambs, which was not significantly different from the 57.7 milligrams per kilogram of body weight per day for the stillbestrol-fed lambs. By the isotope dilution technique (Hansard et al., 1957) a non-significant difference of 51.2 milligrams per kilogram of body weight per day was obtained for the control lambs as compared with 48.4 milligrams per kilogram of body weight for the lambs receiving stilbestrol.

True digestibility values for dietary phosphorus, calculated by the comparative balance technique, were 77.7 percent for the control lambs as compared to 82.7 percent for the stilbestrol-fed lambs. This approached significance ($P = .06$).

Stilbestrol apparently increased phosphorus retention, primarily, through its effect upon absorption. However, the slight but non-significant decrease in fecal endogenous phosphorus must also be considered, since urinary phosphorus losses were not affected by treatment.

Because stilbestrol increased phosphorus absorption with no apparent effect upon calcium absorption, it may be postulated that the first manifestation of the physiological activity of stilbestrol with respect to mineral metabolism is increased absorption of phosphorus

from the gastrointestinal tract. This may be responsible, in part, for the reduction of fecal endogenous calcium since calcium may enter into a combination with the phosphorus in the plasma pool or at the site of calcification, thus augmenting transport and laydown in the bone.

However, the slight decrease in fecal endogenous phosphorus indicates that, under estrogenic influence, the body itself was actively conserving minerals. If this is true, then the explanation of the behavior of calcium is not entirely adequate. Since fecal endogenous calcium and phosphorus were both affected to some extent, the bones themselves may have been actively involved in the conservation of calcium, the magnitude of which was regulated by stilbestrol. As a result, the reduction in fecal endogenous calcium must be interpreted as a result of a combination of the factors discussed.

EXPERIMENT II

EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON GROWTH IN LAMBS

The effects of stilbestrol upon calcium, phosphorus, and nitrogen retention may be correlated with reports showing increased lean tissue and total bone, but no difference in percentage bone, to indicate that stilbestrol has a growth stimulating property. However, no investigations have indicated specifically that stilbestrol increases the growth rate in ruminants. This experiment was conducted to determine the effects of stilbestrol upon skeletal growth in lambs.

Experimental

In an experiment conducted at the University of Tennessee-Atomic Energy Commission Agricultural Research Program, Oak Ridge, Tennessee, ten crossbred spring wether lambs, averaging approximately 78 pounds in weight, were selected at random from a uniform group of lambs from the experimental flock and placed in drylot. Immediately after coming off pasture all lambs were shorn and drenched with phenothiazine and placed on UT-AEC ration H-1 until they had become accustomed to the experimental ration. The experiment was initiated when it was felt that the lambs were consuming enough feed to permit rapid growth. At this point the wethers were divided at random into two groups of five lambs each, and each animal was dosed with a single tracer dose of 500 microcuries of calcium-45, administered intramuscularly, to

mark the bones for radioautograph studies. One group continued to receive the basal ration, while the remaining lambs received the basal ration to which had been added 2 milligrams of stilbestrol per pound of feed. Throughout the experimental period, the lambs consumed approximately 2.0 pounds of the concentrate per head daily, with orchard grass hay being fed ad lib. The lambs were weighed without shrink at the beginning and at the end of the experimental period.

At the end of a 75-day feeding period the lambs were sacrificed and the right femur bone of each removed for preparation of radioautographs. Radioautographs were prepared essentially by the method described by Lotz et al. (1951). The freshly excised bone was cleaned of excess tissue and frozen. While still frozen thin sagittal sections were cut from each bone using an adapted band-saw. Extraneous residue, bone dust, fat, muscle tissue, and connective tissue was carefully removed and each section was monitored with a count-rate meter to determine the dose concentration in the section. Two or three sections from each bone were placed on sheets of Kodak, double-coated, No-Screen X-ray film in a special film holder. A second sheet of X-ray film was placed on top of each section, the film-holder was then placed in a press, and the film was exposed for approximately two weeks depending upon the dose concentration. Following exposure the film was developed by routine procedures for study and measurements.

Figure 3 represents typical radioautographs prepared from the femurs of the lambs. When animals are dosed with calcium-45, the isotope is rapidly deposited in the sub-epiphyseal area directly beneath the epiphyseal plate and remains there until removed by growth and/or

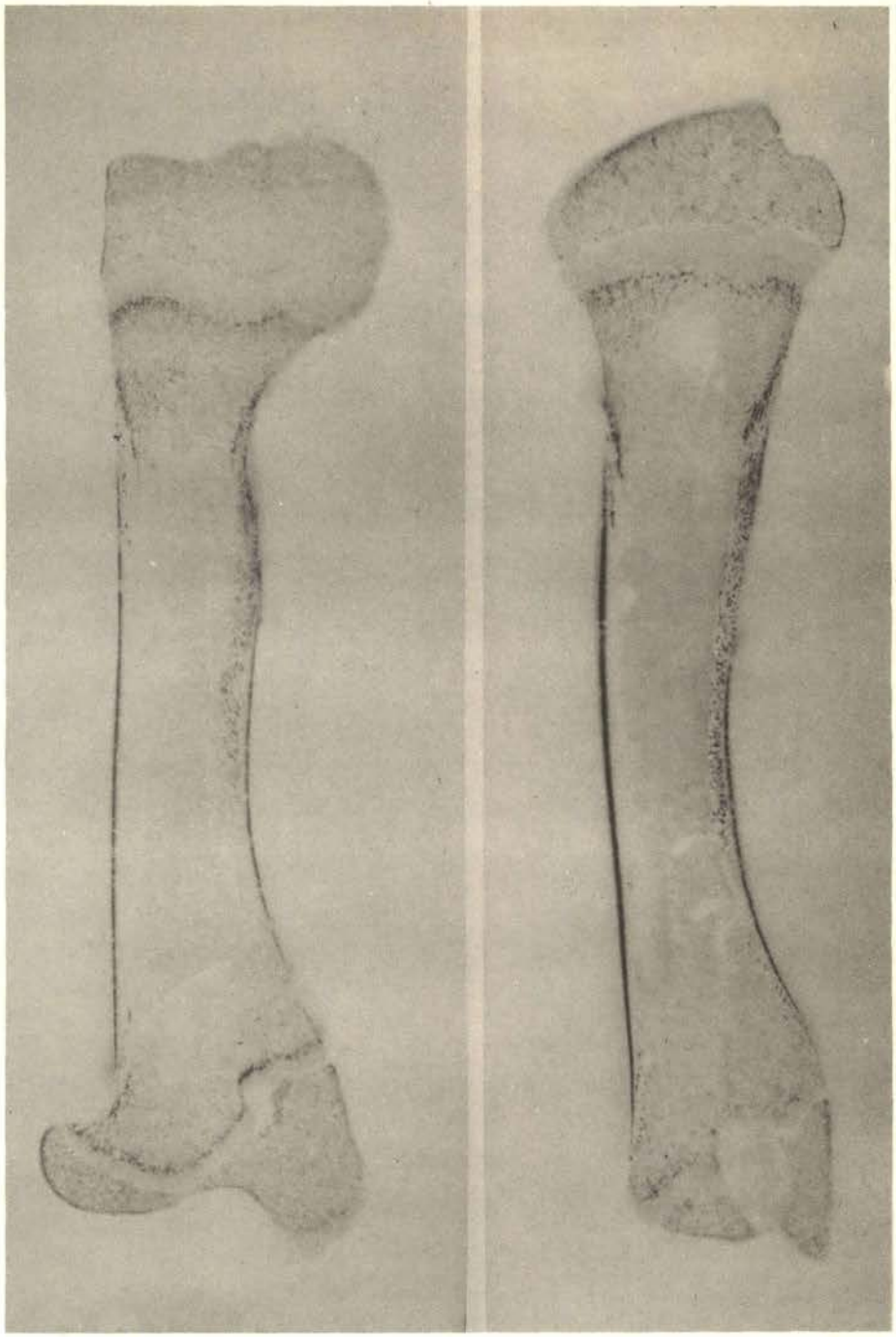

Figure 3. Radioautographs of sagittal sections of femur bones from control (left) and stilbestrol-fed (right) wethers demonstrating the growth effects.

exchange (Hansard et al., 1951b). It thus provides a good "marker" to be used for bone growth measurement. In the radioautographs obtained, the actual bone laydown during the 75-day feeding period is represented by the prominent gray area in the epiphyseal region, a result of diffusion of the isotope through the area and the exposure of the film in the vicinity of the radioactivity. This prominent growth area was easily measured with precision calipers. Measurements of each femur were made on four to six exposures representing two or three bone sections, and the results averaged to give the centimeters of bone growth.

Results and Discussion

Weight gains and bone growth data are presented in Table 3. Average daily gains for the control lambs were 0.41 pounds and for the stilbestrol-fed lambs, 0.47 pounds. This difference approached significance ($P = .10$). At the proximal end of the femur an average of 0.28 centimeters of bone growth occurred in the control lambs as compared to 0.32 centimeters of growth in the treated lambs, a difference that was not significant. Measurements at the distal end of each femur indicated 0.53 centimeters of bone growth in the control lambs and 0.63 centimeters of growth in the stilbestrol-fed lambs, a difference that was highly significant ($P < .005$). It is interesting to note that the same percentage difference in growth occurred at the proximal and distal end of the bones of each group.

When the bone growth from the control and the treated groups was compared on the basis of centimeters per unit of gain in weight, there

TABLE 3 EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON GROWTH IN LAMBS

	Basal	Stilbestrol
Number of Animals	5	5
Weight Gain (Lbs.)[1]	30.4	34.8
Average Daily Gain (Lbs.)	0.41	0.47
Bone Growth of Femur (Cm):		
Proximal End	0.28	0.32
Distal End	0.53	0.63
Bone Growth of Femur (Cm/Lb. Gain x 10^3):		
Proximal End	9.4	9.1
Distal End	17.9	18.2

[1]Total gain over a 75-day feeding period.

were no differences between lots. Thus bone growth at the proximal end (expressed in the table as centimeters per pound of gain X 10^3) was 9.4 and 9.1, respectively, and at the distal end, 17.9 and 18.2, respectively.

The data support the idea that the primary effect of stilbestrol in ruminants is that of growth stimulation. The amount of total bone in the treated lambs was increased, in agreement with the findings of O'Mary et al. (1952). However, when expressed as a percentage of body weight, there was no increase in bone as compared to other tissue. The results of Wilkinson et al. (1955) with lambs and Clegg and Carroll (1956) with cattle support this observation.

EXPERIMENT III

EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON TOTAL BODY WATER AND BLOOD VOLUME IN LAMBS

Estrogens have been shown to increase fluid uptake in certain tissues in laboratory animals. It was thought that the early increase in rate of gain reported in beef cattle receiving stilbestrol might be due in part to increases in total body fluids. This experiment was initiated to determine the effects of dietary stilbestrol upon total body water and blood volume in ruminants.

Experimental

The ten lambs utilized for bone growth studies (Experiment II) were also employed in these investigations. For initial measurements of total blood volume and body water, determinations were made a few days prior to the start of the feeding period. An intermediate measurement was made approximately five weeks later, and the final determination was made immediately before the animals were sacrificed.

For blood volume determinations, the method of Hansard et al. (1953), modified by the use of chromium-51, was used. The animals were weighed and approximately 10 milliliters of blood were drawn from each into a sterile heparinized flask. Approximately 100 microcuries of chromium-51, as sodium Cr-51-chromate, were added to each sample, and the blood was incubated in a constant temperature water bath for

one hour at 37°C to facilitate cellular uptake of the radiochromium. The samples were placed in calibrated centrifuge tubes and centrifuged for 20 minutes at 1500 revolutions per minute, following which hematocrit readings were taken and the plasma drawn off. The red blood cells were then carefully suspended in 50 milliliters of ice-cold sterile physiological saline and centrifuged again for 10 minutes. The red blood cells were washed three times in this manner to remove any plasma containing residual chromium-51. Following the final washing the red blood cells were re-suspended in sterile saline at room temperature and restored to the volume of the original blood sample. One milliliter aliquots of each sample were removed for use as a dosing standard, and the remainder of the sample was immediately injected into the animal from which it was originally drawn, using the techniques for isotope administration previously described. At intervals of 15 minutes, 30 minutes, and 1 hour after dosing, blood samples were taken and radioactivity measurements made on 4 milliliter aliquots of whole blood using a well-type scintillation counter connected to a conventional scaler unit. Radioactivity in these samples was compared to that of the dosing standard for calculations. The activity of each sample was plotted against time on semi-logarithmic paper and a line was extrapolated to zero time by method of least squares to estimate the dose concentration at that point. Total blood volume was then estimated thus:

$$\text{Blood Volume (ml.)} = \frac{\text{Total counts per minute in administered dose}}{\text{Total counts per minute/milliliter of sample.}}$$

Percent blood volume was then determined:

$$\text{Blood Volume (\%)} = \frac{\text{Blood Volume (ml.)}}{\text{Body Wt. (gms.)}} \times 100.$$

For the second and third blood volume determinations, pre-samples were taken and counted for residual radioactivity. Subsequent sample counts were corrected using these values.

For total body water determinations, the method of Hansard et al. (1957) was employed. Following an overnight shrink, the animals were weighed, a blood sample taken for estimation of residual radioactivity, and approximately 100 microcuries of 4-iodo-I^{131}-antipyrine were injected using methods previously described. At intervals of 15 minutes, 1, 2, 3, and 5 hours, blood samples were taken. The blood samples were centrifuged and 4 milliliter aliquots of plasma were removed for counting in a well-type scintillation counter connected to a conventional scaler unit. The radioactivity in the plasma samples was corrected to account for residual radioactivity and the counts compared to the radioactivity in a standard prepared from the original dosing solution. The activity of each sample was plotted against time on semi-logarithmic paper and a line was extrapolated to zero time. Total body water was then estimated as follows:

$$\text{Total Body Water (ml.)} = \frac{\text{Total counts per minute in administered dose}}{\text{Total counts per minute/milliliter of sample.}}$$

Percent body water was estimated as follows:

$$\text{Total Body Water (\%)} = \frac{\text{Total Body Water (ml.)}}{\text{Body Wt. (gms.)}} \times 100.$$

Results and Discussion

Average values for three determinations of total body water and total blood volume are shown in Table 4. For the initial, intermediate, and final determinations, total body water averaged 54.8, 57.8, and 58.5 percent in the control lambs and 58.8, 57.5, and 58.4 percent in the stilbestrol-fed lambs. Values for blood volume for corresponding periods were 6.5, 6.8, and 6.5 percent for the control lambs and 6.0, 7.1, and 6.4 percent for the treated lambs.

From the data it seems apparent that the weight increase derived from stilbestrol administration was not from tissue hydration or increased blood volume. The actions of estrogens upon smooth muscle which caused an initial influx of water and electrolytes, described by Roberts and Szego (1953), are apparently not the same with respect to skeletal muscle. If the large initial increase in weight gains in steers reported by Perry et al. (1955) and Reynolds et al. (1955) was not due to an influx of water into the tissues, obviously, the early effects of stilbestrol are manifested in greatly increased anabolism, following which the animals become adapted to hormone administration and the effect diminishes.

Before positive conclusions may be drawn as to the lack of effect of stilbestrol upon body fluids, however, it must be considered that, in the animals used, although there was an appreciable percentage increase in total gain due to treatment, the actual difference in pounds of gain was relatively small. Any data, obtained through the use of dilution techniques such as those employed in this trial, must be interpreted with the knowledge that such techniques may not be sensitive

TABLE 4 EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON TOTAL BODY WATER AND BLOOD VOLUME IN LAMBS

	Basal	Stilbestrol
Number of Animals	5	5
Average Weight (Lbs.):		
Initial (5/7/56)	78.0	79.6
Final (7/24/56)	108.4	114.4
Total Body Water (Percent):		
Initial (5/10/56)	54.8	58.8
Intermediate (6/13/56)	57.8	57.5
Final (7/24/56)	58.5	58.4
Total Blood Volume (Percent):		
Initial (5/7/56)	6.5	6.0
Intermediate (6/21/56)	6.8	7.1
Final (7/21/56)	6.5	6.4

enough to detect such small total differences. Perhaps by using larger experimental animals, the same percentage increase in weight might produce differences in body fluids that could be more easily detected by the methods employed if such differences exist.

EXPERIMENT IV

EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON CERTAIN ENDOCRINE ORGANS IN LAMBS AND STEERS

Research concerned with the effects of stilbestrol upon the endocrine system of ruminants has been extremely limited and has been conducted with estrogen implants only. This experiment was conducted to determine the effects of dietary stilbestrol upon the endocrine system of sheep and cattle, in particular upon levels of certain pituitary hormones and upon adrenal function as indicated by adrenal cholesterol.

Experimental

Pituitary and adrenal glands were obtained at sacrifice from the ten lambs described previously (Experiment II). After removal they were immediately trimmed free of extraneous material, blotted dry, weighed on a precision balance, and frozen until further studies could be made. In addition, similar organs were removed from nineteen steers at the end of a 160-day fattening trial conducted at Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma. Of these steers, ten received a basal ration and nine received the basal ration plus 10 milligrams of stilbestrol per head daily mixed with the protein supplement. The tissues removed were immediately frozen until used for

further study. At this time the glands were thawed, trimmed free of any extraneous material, blotted and weighed.

Each anterior pituitary was pressed between two glass plates and dried over concentrated sulphuric acid in a vacuum desiccator. When dry, the material was ground to a fine powder with a mortar and pestle. All residue, such as connective tissue, which would not reduce to a powder was carefully removed and the powder weighed. The powder from each of the experimental groups was pooled in four weighted lots and stored in a desiccator for biological assay. When ready for use weighed portions of each group were suspended in a known volume of physiological saline for injection.

For assay of growth hormone, gonadotropic hormones and adrenocorticotropic hormone, hypophysectomized immature female rats were employed. These animals, weighing approximately 50 grams, were used 14 days following hypophysectomy. Assay for thyrotropic hormone and adrenocorticotropic hormone was performed using day-old White Leghorn cockerels.

Growth Hormone Assay: Seventy-five hypophysectomized immature female rats were allotted at random into eight groups and maintained on a commercial rat ration supplemented with dog food, fresh oranges, fresh milk, moist bread, and fresh potatoes. Thirteen rats were placed in each of four groups to receive injections of pituitary powder, six rats were placed in each of three groups representing three levels of purified growth hormone, and five rats were retained as negative controls. Within the pituitary groups each rat received 500 micrograms of pituitary powder intraperitoneally daily for five days. The positive

controls received 20, 100, or 300 micrograms of purified growth hormone daily for five days and the negative controls were injected daily with sterile normal saline during the period. Eighteen to twenty-four hours following the last injection, the surviving rats were sacrificed. The response to the growth hormone was measured using as a criterion the increase in width of the epiphyseal cartilage of the tibia, a procedure commonly referred to as the "tibia test", described by Greenspan et al. (1949). The right tibia was removed at autopsy, dissected free of soft tissue and split in the mid-sagittal plane by means of a sharp razor blade. The bone halves were fixed in 10 percent neutral formalin, washed in water for 30 minutes, immersed in acetone for approximately 1 hour, and then washed in running tap water for 30 minutes. They were then placed in freshly prepared 2 percent silver nitrate for 2 minutes, rinsed in water, and exposed to a strong light while under water until the calcified portions appeared dark brown. They were then immersed in 10 percent sodium thiosulphate for 30 seconds, washed in running water for 30 minutes, and stored in 80 percent ethanol until measured. The width of the uncalcified epiphyseal cartilage at the distal end of the tibia was measured using a dissecting microscope fitted with a micrometer eyepiece calibrated with a stage micrometer to permit measurements in microns. Four to six readings were taken on each bone and the results averaged.

Gonadotropic Hormone Assay: The assay procedure used was essentially that of Evans et al. (1940) and Heller et al. (1938). In this study the hypophysectomized immature female rats used for growth hormone assay were utilized with the exception of those receiving purified

growth hormone. At sacrifice, the ovaries and uteri were dissected out, trimmed free of extraneous tissue under a dissecting microscope, blotted lightly, and weighed on a precision balance. Prior to weighing the capsule was removed from the ovaries and the fluid removed from the uteri.

Adrenocorticotropic Hormone Assay: The techniques used for ACTH assay were those of Bates et al. (1940), with baby chicks and Simpson et al. (1943), with hypophysectomized immature female rats. The rats used were those described in the two preceding assays. In addition 80 one-day-old White Leghorn cockerels were divided at random into five groups, two pens per group, with a total of sixteen chicks per group. The chicks in four groups received daily intraperitoneal injections of 300 micrograms of the appropriate pituitary powder for five days, and those in the fifth group received injections of sterile saline. All chicks were sacrificed the day following the last injection. In order to facilitate autopsy, one pen in each group received initial injections and were sacrificed a day later than the remaining pen. At autopsy, the adrenals were removed, trimmed under a dissecting microscope, blotted lightly and weighed on a precision balance.

Thyrotropic Hormone Assay: The procedure used for thyrotropin assay was essentially that of Bergman and Turner (1939). The chicks described in the previous assay were each injected with 2 microcuries of iodine-131 on the last day of the injection period. Thyroidal activity was determined by measurement of radioiodine uptake as described by Rawson and McArthur (1947). At sacrifice, the thyroids

were dissected out, trimmed under a dissecting microscope, blotted lightly, and weighed on a precision balance. They were then transferred to a small test tube and radioactivity measured in a well-type scintillation counter connected to a conventional scaler unit. The radioactivity concentration in the chick thyroids was compared to a standard made from the original dosing solution and the percentage uptake of the administered dose was computed for each thyroid.

In addition to pituitary assays cholesterol levels were determined in adrenals from control and treated steers and lambs. The positive relationship existing between adrenal function and adrenal cholesterol has been pointed out by Sayers and Sayers (1948). Zaffaroni et al. (1951) have presented evidence that cholesterol was transformed by the adrenal gland into adrenocortical steroids, therefore cholesterol concentration should reflect cortical activity.

Five to six small sections weighing a total of approximately 1.5 gms. were cut from each pair of adrenal glands and macerated with sterile sand with a mortar and pestle. The sample was extracted with repeated washings of glacial acetic acid, brought to a volume of 50 milliliters with glacial acetic acid, and appropriate aliquots, taken in duplicate, analyzed for total cholesterol by the method of Zlatkis et al. (1953). A standard curve representing analysis for known amounts of pure cholesterol was prepared, and the milligrams of cholesterol in the adrenals from each animal were calculated.

Results and Discussion

Fresh weights of the pituitary and adrenal glands from control and treated lambs and steers are shown in Tables 5 and 6. In addition, percentage moisture for the pituitaries and levels of adrenal cholesterol (expressed as milligrams per gram of fresh tissue x 10^2) are presented. Pituitary weights for the control and stilbestrol-fed lambs were significantly different, averaging 0.61 grams and 0.81 grams, respectively ($P < .02$). For the control and treated steers, the difference in pituitary weights; 1.33 grams compared to 1.59 grams; approached significance ($P < .06$). Brody (1945) has cited evidence of a definite positive logarithmic relationship between body weight and pituitary weight. Pituitary weights expressed as grams per hundred pounds of body weight were significantly different for the control lambs, with an average pituitary weight of 0.56 grams per hundred pounds body weight, and the stilbestrol-fed lambs, with an average pituitary weight of 0.71 grams per hundred pounds body weight ($P < .02$). In converting the steer pituitary weights to this unit, the difference between the control and treated groups; .15 grams as compared to .17 grams per hundred pounds of body weight; was not significant. There were no differences in percentage moisture of the pituitaries in any of the groups. Thus the control and treated lambs averaged 86.4 percent and 86.1 percent, respectively, while the control and treated steers averaged 85.3 percent and 85.9 percent, respectively.

If it is assumed that sheep and cattle react similarly to estrogen administration, certain interpretations of these data are possible. The work of Perry *et al.* (1955) and Reynolds *et al.* (1955) with

TABLE 5 EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON PITUITARY WEIGHT AND PERCENTAGE MOISTURE OF PITUITARIES IN LAMBS AND STEER CALVES

	Basal		Stilbestrol	
	Lambs	Steers	Lambs	Steers
Number of Animals	5	10	5	9
Average Gland Weight (Gms.)	0.61	1.33	0.81	1.59
Average Gland Weight (Gms./Cwt. Body Wt.)	0.56	0.15	0.71	0.17
Average Percent Moisture	86.40	85.30	86.10	85.90

steers gave clear indication that the early phases of stilbestrol administration are marked by an initial period of extreme anabolic activity followed by gradually diminishing effects until the hormone-fed cattle were gaining no faster than their controls. The steers in the present experiment were slaughtered at the completion of a 160-day feeding period. The pituitary weights of the treated animals, per hundred pounds of body weight, were only slightly greater than those of the controls.

The lambs, however, were slaughtered at the end of a 75-day feeding trial, at which time stilbestrol may have been actively promoting the anabolic processes characteristic of the hormone. Because the pituitaries of the stilbestrol-fed lambs, per hundred pounds of body weight, were significantly larger than their controls, it may be inferred that, after a period of time under the influence of estrogens, the physiological functions responsible for the increased anabolic processes adapted to the hormone and regressed to normal or near normal

activity. This postulation would account for the relative differences in the behavior of the pituitaries in the two species.

The percentage moisture figures reveal that the increase in pituitary weight, in the lambs at least, was not due to a large influx of water. Rather the increase in size might have been due to hypertrophy and/or hyperplasia either of which would tend to indicate increased activity on the part of the organ.

TABLE 6 EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON ADRENAL WEIGHT AND CHOLESTEROL CONTENT IN LAMBS AND STEER CALVES

	Basal		Stilbestrol	
	Lambs	Steers	Lambs	Steers
Number of Animals	5	10	5	9
Average Total Gland Weight (Gms.)	2.84	10.84	3.20	11.98
Average Total Gland Weight (Gms./Cwt. Body Wt.)	2.62	1.26	2.80	1.26
Cholesterol Content (Mgs/Gm. X 10^2)	7.1	8.7	8.1	9.4

Fresh adrenal weights for both steers and lambs disclose non-significant differences due to treatment. Thus adrenal weights for the control and treated steers were 10.84 grams and 11.98 grams, respectively, and for the control and treated lambs were 2.84 grams and 3.20 grams, respectively. Conversion of adrenal weights to grams per hundred pounds of body weight gave values of 1.26 for both the control and treated steers, and 2.62 and 2.80 for the control and treated lambs, respectively. Christian (1953) has reported a definite logarithmic relationship between body weight and adrenal size. In light of this,

it is apparent that adrenal size was not affected by treatment beyond its effect upon the growth of the animals involved.

Adrenal cholesterol content was determined as a measure of the activity of the adrenal cortex. The adrenal cholesterol content of the control lambs was 0.071 milligrams per gram of fresh tissue, significantly less than the 0.081 milligrams per gram in the stilbestrol-fed lambs ($P < .025$). In the control and treated steers, adrenal cholesterol content was 0.087 milligrams per gram and 0.094 milligrams per gram, respectively, a difference that was not significant. Apparently the adrenals of the stilbestrol-fed lambs were stimulated to a rate of secretion which was above normal. With respect to the steers, the same theory of adaptation to continued treatment previously cited may be advanced to explain the observed insignificant difference in adrenal activity.

Supra-normal levels of adrenal activity are not consistent with the increased retention of nitrogen due to stilbestrol, indicative of protein anabolism, reported by many workers using sheep and cattle (Whitehair et al. 1953; Jordan, 1953; Clegg, 1952; and Clegg and Cole, 1954). Engel (1951) stated that the action of the adrenal cortex in nitrogen metabolism was predominantly at the level of the whole protein; thus, serving to facilitate the mobilization of amino acids, presumably by promoting protein catabolism and inhibiting protein anabolism. This is in agreement with the generally accepted role of one group of cortical hormones. Certainly the increased secretion of cortical hormones is difficult to reconcile with the anabolic processes occurring under the influence of stilbestrol.

A hypothesis may be advanced which would possibly account for the increased adrenal cortical activity without implicating the adrenals in the growth response to stilbestrol. Sayers and Sayers (1948) noted that, with a gradual change in environment, such as the stress of pregnancy and other causes, the cholesterol level of the adrenals dropped initially, then gradually increased to a level which was higher than normal. A gradual increase in the size of the glands occurred and it was apparent that new secretory units could be constructed at a rate sufficient to meet these increasing demands. In this connection Randall and Graubard (1940) found that in the latter stages of pregnancy in rabbits, hypertrophy of the adrenal gland was accompanied by an increase in the absolute amounts of cholesterol. The increased estrogen levels of pregnancy might be correlated with stilbestrol administration to explain the observed increase in adrenal cholesterol.

However, a second hypothesis might implicate the adrenal corticoids in the favorable response to stilbestrol. It is generally accepted that, in addition to the true adrenal cortical hormones, the cortex also secretes androgens and estrogens. If stilbestrol administration caused an estrogen imbalance, the adrenal cortex might attempt to balance the over-supply of estrogens by increased production of androgens. This might account for increased adrenal cholesterol. Clegg and Cole (1954), after observing the actions of stilbestrol-implanted steers in the feed lot typical of the behavior of bulls, postulated that the protein-anabolic effects attributed to stilbestrol were actually due to the action of androgens, which if true might confirm the theory of an estrogen imbalance. However, Beeson et al. (1956)

and Andrews et al. (1954) have reported that neither oral nor implanted testosterone improved rate of gain or feed efficiency in beef steers. Thus, although the action of stilbestrol upon the adrenals might be as described, it appears unlikely that the anabolic effects of the estrogen were actually due to androgenic secretion, unless an androgen having anabolic properties were synthesized under the influence of stilbestrol.

Results of pituitary assays for growth hormone, thyrotropic hormone, gonadotropic hormones, and adrenocorticotropic hormone are presented in Tables 7, 8, 9, 10, and 11.

The growth hormone assay data (Table 7) were expressed in terms of width of the epiphyseal cartilage of the tibia of hypophysectomized rats. The width of this cartilage averaged 76.9, 126.1, 121.9, 111.7, and 126.4 microns for the hypophysectomized rats receiving injections of saline, control steer pituitary, stilbestrol-fed steer pituitary, control lamb pituitary, or stilbestrol-fed lamb pituitary, respectively. The difference between the saline-injected rats and those animals receiving lamb or steer pituitary powder was highly significant ($P < .001$). Assays of the steer pituitaries for growth hormone were not significantly different. Assay for growth hormone content of the lamb pituitaries, however, resulted in significantly different epiphyseal measurements between the rats receiving control and treated pituitary powder ($P < .02$).

The level of any pituitary hormone in the gland itself could be the result of increased secretory activity or impaired release. Because of this, it is difficult to derive positive conclusions from pituitary

TABLE 7 PITUITARY GROWTH HORMONE ASSAY USING HYPOPHYSECTOMIZED IMMATURE FEMALE RATS

Treatment	No. of Rats	Total Material Injected Per Rat (mgs.)	Final Wt. of Rats (Gms.)	Wt. Increase (Gms.)	Width of Epiphyseal Cartilage (Microns)
Pituitary Powder					
Lambs					
Control	10	2.5	55.5	3.1	111.7 ± 2.8[1]
Stilbestrol-fed	7	2.5	57.4	4.3	126.4 ± 5.2
Steers					
Control	10	2.5	60.5	1.1	126.1 ± 2.5
Stilbestrol-fed	7	2.5	57.8	3.5	121.0 ± 6.8
Growth Hormone	5	0.1	57.0	5.2	119.6 ± 6.6
Growth Hormone	5	0.5	59.8	5.9	133.9 ± 4.0
Growth Hormone	5	1.5	60.6	6.2	181.6 ± 25.7
Hypoph. Control	4	Saline	52.2	.7	76.9 ± 3.4

[1]Mean ± standard error of the mean.

assays. However, with the data presented, certain inferences are possible.

From data presented in Experiment II, it was concluded that stilbestrol feeding increased the growth rate of lambs. The assay data, if correlated with the increased growth of the animals from which the glands were taken, indicate that, per gram of pituitary tissue, there was increased production of growth hormone. However, there is no evidence to indicate that the entire growth response was due to increased growth hormone secretion.

Before positive conclusions may be drawn, the absence of any difference in the growth hormone content of the control and treated steer pituitaries must be explained. The adaptation theory advanced for pituitary size, if valid, might also apply when considering pituitary function. Thus, it may be postulated that, initially, there was an increase in pituitary size and function under estrogenic influence. After a long period of stilbestrol administration, however, the physiological mechanisms responsible for the increased activity might have become adapted to estrogenic stimulation and pituitary functioning reverted to a level approaching normal.

Thyrotropic hormone assays (Table 8) using day-old chicks were conducted using increase in thyroid weight and percentage uptake of intraperitoneally administered iodine-131 as criteria. Differences in thyroid weights between chicks receiving injections of saline compared to pituitary powder and between chicks injected with pituitary material from control compared to treated lambs or steers were not significant. Thus, the thyroids from saline-injected chicks averaged

TABLE 8 PITUITARY THYROTROPIC HORMONE ASSAY USING DAY-OLD CHICKS

Source of Pituitary	No. of Chicks	Total Material Injected Per Chick (Mgs.)	Final Wt. of Chicks (Gms.)	Thyroid Wts. Mgs.	Thyroid Wts. Mgs/100 Gms.	% Uptake I-131
Lambs						
Control	13	1.5	53.1	4.6	8.9 ± .9[1]	7.78 ± 1.1[1]
Stilbestrol-fed	15	1.5	51.5	4.9	9.8 ± .7	7.09 ± 1.0
Steers						
Control	15	1.5	49.6	5.2	10.7 ± 1.0	5.54 ± 1.1
Stilbestrol-fed	12	1.5	52.5	4.6	8.9 ± .8	8.02 ± 1.3
Control	12	Saline	48.3	3.9	8.0 ± .8	3.69 ± 1.0

TABLE 9 PITUITARY GONADOTROPIC HORMONE ASSAY USING HYPOPHYSECTOMIZED IMMATURE FEMALE RATS

Source of Pituitary	No. of Rats	Total Material Injected Per Rat (Mgs.)	Final Wt. of Rats (Gms.)	Ovarian Wts. Mgs.	Ovarian Wts. Mgs/100 Gms.	Uterine Wts. Mgs.	Uterine Wts. Mgs/100 Gms.
Lambs							
Control	9	2.5	55.5	5.5	9.6 ± 1.2	49.4	87.8 ± 7.8[1]
Stilbestrol-fed	7	2.5	57.4	5.6	10.0 ± 1.3	29.6	51.2 ± 3.7
Steers							
Control	10	2.5	60.5	8.0	13.2 ± 1.2	35.2	58.2 ± 3.6
Stilbestrol-fed	9	2.5	57.8	3.0	5.2 ± .6	31.5	54.1 ± 5.2
Hypoph. Control	4	Saline	52.2	2.0	3.7 ± 1.0	11.0	21.2 ± 1.8

[1]Mean ± standard error of the mean.

8.0 milligrams per hundred grams of body weight; the thyroids of the control and stilbestrol-fed lamb pituitary group, 8.9 and 9.8 milligrams, respectively; and those of the control and stilbestrol-fed steer groups, 10.7 and 8.9 milligrams, respectively.

On the basis of percent thyroidal uptake of administered radioiodine the chicks injected with saline, control and stilbestrol-fed lamb pituitary, and control and stilbestrol-fed steer pituitary averaged 3.69, 7.78, 7.08, 5.54, and 8.02, respectively. The difference between the saline-injected chicks and those receiving lamb pituitary was significant ($P < .02$). In comparing the uptake of saline-injected chicks with those receiving steer pituitary, the difference approached significance ($P = .07$). However, the differences between neither the lamb groups nor the steer groups were significant.

These results indicate that stilbestrol feeding had no effect upon thyrotropin production by the pituitaries of either lambs or steers.

Gonadotropic hormone assay data for steer and lamb pituitaries are presented in Table 9. Ovarian weights of the test animals, expressed as milligrams per hundred grams of body weight, averaged 3.7 milligrams for the hypophysectomized controls, 9.6 and 10.0 milligrams for those receiving pituitary powder from the control and treated lamb groups, respectively, and 13.2 and 5.2 milligrams for the control and treated steer groups, respectively. A comparison between the hypophysectomized controls and both the lamb and steer groups indicated highly significant differences ($P < .001$). The difference in the steer groups was also highly significant ($P < .001$).

Uterine weights for the hypophysectomized control rats averaged 21.2 milligrams per hundred grams of body weight as compared to 87.8

and 51.2 milligrams for the control and stilbestrol lamb groups, respectively, and 58.2 and 54.1 milligrams for the control and treated steer groups, respectively. The values for the hypophysectomized controls were significantly lower than either the lamb or steer pituitary assay data ($P < .001$). As contrasted to ovarian weights, the difference in uterine weights within the lamb group was highly significant ($P < .001$), while no difference was observed between the two steer groups.

The action of stilbestrol upon gonadotropic potency of the assayed pituitaries produced expected results; when the blood levels of estrogen increased as a result of stilbestrol administration pituitary production of gonadotropins decreased. It is highly questionable, however, that alteration of pituitary gonadotropin levels would be involved in the growth response from stilbestrol. It is of interest, however, that pituitary powder from stilbestrol-fed steers affected ovarian weights in the test animals, whereas pituitary powder from stilbestrol-fed lambs affected uterine weights. Although it is not possible to correlate pituitary gonadotropin content with the growth response from stilbestrol, these data do demonstrate that stilbestrol has a systemic effect in both steers and lambs.

Data from adrenocorticotropic hormone assays for sheep and steer pituitaries using hypophysectomized rats appear in Table 10, while assay data using day-old chicks appear in Table 11. In the hypophysectomized rat assay the adrenals of the hypophysectomized controls averaged 10.2 milligrams per hundred grams of body weight, those from rats receiving control and treated lamb pituitary averaged 12.2 and

TABLE 10 PITUITARY ADRENOCORTICOTROPIC HORMONE ASSAY USING HYPOPHYSECTOMIZED IMMATURE FEMALE RATS

Source of Pituitary	No. of Rats	Total Material Injected Per Rat (Mgs.)	Final Wt. of Rats (Gms.)	Wt. of Adrenals Mgs.	Wt. of Adrenals Mgs/100 Gms.
Lambs					
Control	9	2.5	55.5	6.9	12.2 ± 2.5[1]
Stilbestrol-fed	7	2.5	57.4	7.2	12.7 ± 4.8
Steers					
Control	10	2.5	60.5	7.6	12.6 ± .7
Stilbestrol-fed	8	2.5	57.8	7.0	12.1 ± 1.7
Hypoph. Control	4	Saline	52.2	5.3	10.2 ± 2.6

[1]Mean ± standard error of the mean.

TABLE 11 PITUITARY ADRENOCORTICOTROPIC HORMONE ASSAY USING DAY-OLD CHICKS

Source of Pituitary	No. of Chicks	Total Material Injected Per Chicks (Mgs.)	Final Wt. of Chicks (Gms.)	Wt. of Adrenals Mgs.	Wt. of Adrenals Mgs/100 Gms.
Lambs					
Control	13	1.5	53.1	12.8	24.0 ± 1.3[1]
Stilbestrol-fed	15	1.5	51.5	11.2	21.9 ± 1.4
Steers					
Control	15	1.5	49.6	12.7	26.3 ± 1.9
Stilbestrol-fed	12	1.5	52.5	12.4	23.8 ± 1.6
Control	14	Saline	49.9	10.7	22.1 ± 2.3

[1]Mean ± standard error of the mean.

12.7 milligrams, respectively, while those from animals receiving control or treated steer pituitary averaged 12.6 and 12.1 milligrams, respectively.

In the chick assay, the adrenal glands of the saline-injected chicks averaged 22.1 milligrams per hundred grams of body weight; the control and stilbestrol-fed lamb groups averaged 24.0 and 21.9 milligrams, respectively; and the control and stilbestrol-fed steer groups averaged 26.3 and 23.8 milligrams, respectively.

From these data it is apparent that stilbestrol feeding had little effect upon ACTH secretion or storage by the pituitary per gram of tissue. These results are in agreement with data reported by Clegg and Cole (1954). Since the pituitaries from the stilbestrol-fed animals were larger, there was probably more total ACTH present but this higher level was only reflected in an increase in adrenal size which was consistent with the difference in size between the control and stilbestrol-fed animals.

SUMMARY

Investigations were conducted with the following objectives: (1) to study the effects of stilbestrol upon total body fluids and upon the physiological behavior of calcium and phosphorus in ruminants, (2) to determine the site(s) of action of stilbestrol in stimulating rate of gain, feed consumption, and efficiency of feed utilization in ruminants, and (3) to study the effects of stilbestrol on certain endocrine organs and their products.

To study the physiological behavior of calcium and phosphorus as influenced by orally administered stilbestrol, chemical and radiochemical balances and blood studies were conducted with wether lambs. Total body water was determined in wether lambs using 4-iodo-I^{131}-antipyrine and blood volume was determined by the use of chromium-51-labeled red blood cells.

Effects of stilbestrol upon growth in lambs were studied through the use of radioautographs of sagittal sections of femurs from lambs which had received marker doses of calcium-45. Pituitary assays using tissue from stilbestrol-fed lambs and steers and their controls were conducted using hypophysectomized immature female rats for growth hormone, gonadotropin, and adrenocorticotropin assays and day-old chicks for thyrotropin and adrenocorticotropin assays. In addition, adrenals from both lambs and steers were analyzed for total cholesterol to measure the effects of stilbestrol upon adrenal functioning.

The results indicated that stilbestrol feeding significantly increased bone growth in lambs. When bone growth was correlated with weight gain, there were no differences between the control and treated lambs indicating that stilbestrol exerts its effect as a growth stimulant.

Stilbestrol feeding resulted in a marked reduction in fecal endogenous calcium, but only a slight reduction in fecal endogenous phosphorus. At the same time phosphorus absorption was increased but calcium absorption was apparently not influenced as a result of estrogen administration.

No apparent effect upon total body water or blood volume could be demonstrated in lambs fed stilbestrol.

Stilbestrol feeding significantly increased pituitary weights in steers and lambs and markedly increased adrenal weights. When correlated with body weight, the stilbestrol-fed lamb pituitaries were significantly larger than their controls, but the difference in the steer group was not significant. When adrenal weights were correlated with body weight, there were no differences in either the lamb or steer groups.

Adrenal cholesterol levels, indicative of cortical function, were significantly higher in the stilbestrol-fed lambs, but differences between the steer groups were not significant.

Stilbestrol feeding resulted in a significant increase in pituitary growth hormone in lambs but little difference in steers. Gonadotropin levels were significantly reduced in both lambs and steers as a result of estrogen feeding, but hormone administration apparently had little influence upon levels of pituitary ACTH and thyrotropin.

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A P P E N D I X

TABLE 12 CHEMICAL BALANCE DATA WITH LAMBS - PERIOD I - SUMMER 1956[1]

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
5/21/56-	Stilbestrol	691	76	34.50	3.45	2.46	2.66	2.48	.09	.12	2.75	2.60	+ .71	- .15
		644	86	39.04	3.50	2.48	4.26	3.78	.12	.10	4.38	3.88	- .87	-1.42
5/28/56		613	81	36.77	2.79	2.14	2.03	2.23	.06	.12	2.09	2.35	+ .69	- .21
		Ave.	81.0	36.77	3.20	2.36	2.98	2.83	.09	.11	3.07	2.95	+ .13	- .59
5/29/56-	Stilbestrol	641	73	33.14	3.75	3.31	3.38	3.54	.05	.05	3.43	3.59	+ .31	- .28
		660	84	38.14	3.63	3.24	2.99	2.94	.05	.06	3.04	3.00	+ .60	+ .23
6/5/56		634	77	34.96	3.54	3.17	2.92	3.22	.05	.05	2.97	3.27	+ .58	- .10
		Ave.	78.0	35.41	3.64	3.24	3.10	3.24	.05	.05	3.14	3.29	+ .50	- .05
5/21/56-	Basal	662	76	34.50	2.76	2.16	2.90	3.13	.07	.08	2.97	3.21	- .20	-1.05
		648	75	34.05	3.04	2.29	3.09	3.47	.11	.10	3.19	3.57	- .16	-1.25
5/28/56		617	81	36.77	2.83	2.19	2.47	1.94	.06	.09	2.53	2.03	+ .30	+ .15
		Ave.	77.3	35.10	2.87	2.22	2.82	2.84	.08	.09	2.90	2.93	- .02	- .72
5/29/56-	Basal	659	68	30.87	3.03	2.75	2.54	3.53	.09	.05	2.63	3.59	+ .41	- .84
		628	67	30.42	2.82	2.57	2.62	3.47	.12	.05	2.75	3.52	+ .08	- .94
6/5/56		606	101	45.85	2.47	2.35	2.71	3.22	.12	.07	2.83	3.44	- .40	- .95
		Ave.	78.6	35.76	2.76	2.56	2.62	3.41	.11	.06	2.74	3.51	+ .03	- .91

[1]Data Expressed as grams per day.

TABLE 13 CHEMICAL BALANCE DATA WITH LAMBS - PERIOD II - SUMMER 1956[1]

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
6/15/56-	Stilbestrol	644	92	41.75	3.16	3.41	3.76	3.23	.07	.10	3.83	3.33	- .67	+ .08
		613	84	38.12	3.27	3.51	2.87	2.74	.08	.12	2.95	2.86	+ .33	+ .66
6/22/56		691	82	37.21	3.14	3.37	3.47	3.23	.08	.16	3.55	3.39	- .42	+ .02
		Ave.	86.0	39.03	3.19	3.43	3.36	3.06	.08	.13	3.44	3.19	- .26	+ .24
6/25/56-	Stilbestrol	660	94	42.66	3.29	2.99	2.58	2.38	.04	.11	2.62	2.49	+ .67	+ .51
		634	84	38.12	3.33	3.02	2.94	2.66	.05	.09	2.99	2.75	+ .35	+ .27
7/2/56		641	85	38.57	3.33	3.02	2.75	2.67	.04	.10	2.79	2.77	+ .54	+ .25
		Ave.	87.7	39.78	3.31	3.01	2.76	2.57	.04	.10	2.80	2.67	+ .52	+ .34
6/15/56-	Basal	617	82	37.21	2.93	3.37	3.66	3.15	.05	.07	3.71	3.22	- .79	+ .15
		648	78	36.30	2.60	3.03	3.04	2.85	.04	.12	3.09	2.97	- .48	+ .06
6/22/56		662	74	33.58	2.89	3.33	3.23	2.65	.08	.08	3.31	2.74	- .40	+ .59
		Ave.	78.0	36.30	2.81	3.25	3.31	2.89	.06	.09	3.37	2.98	- .56	+ .27
6/25/56-	Basal	628	75	34.03	3.44	2.88	3.19	2.72	.05	.11	3.24	2.80	+ .20	+ .08
		659	79	35.85	3.40	2.86	3.36	2.90	.04	.09	3.41	2.99	- .00	- .14
7/2/56		606	103	46.74	3.42	2.81	3.70	2.99	.06	.10	3.76	3.10	- .34	- .23
		Ave.	85.7	38.87	3.42	2.87	3.41	2.87	.05	.10	3.47	2.96	- .05	- .09

[1]Data Expressed as Grams Per Day.

TABLE 14 CHEMICAL BALANCE DATA WITH LAMBS - PERIOD III - SUMMER 1956[1]

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
7/10/56-	Stilbestrol	691	94	42.66	5.43	2.61	4.51	2.92	.09	.14	4.60	3.06	+ .83	- .44
		613	93	42.20	5.43	2.61	4.05	2.64	.04	.08	4.09	2.72	+1.34	- .11
7/17/56		644	98	44.72	5.43	2.61	3.78	2.22	.08	.15	3.86	2.37	+1.57	+ .24
		Ave.	95.0	43.19	5.43	2.61	4.11	2.59	.07	.12	4.19	2.72	+1.25	- .10
7/11/56-	Stilbestrol	634	88	39.93	5.43	2.61	3.73	2.58	.09	.12	3.82	2.70	+1.61	- .09
		641	86	39.03		2.61		3.03		.09		3.12		- .51
7/18/56		660	99	44.93	5.43	2.61	3.48	3.03	.06	.08	3.55	3.11	+1.88	- .49
		Ave.	91.0	41.29	5.43	2.61	3.61	2.88	.08	.10	3.68	2.98	+1.75	- .36
7/10/56-	Basal	648	93	42.20	5.36	2.68	4.42	3.39	.06	.09	4.48	3.48	+ .88	- .80
		662	81	36.76	4.14	2.31	3.55	2.77	.06	.06	3.61	2.84	+ .53	- .53
7/17/56		617	81	36.76	5.36	2.68	5.58	3.34	.05	.08	5.63	3.41	- .27	- .74
		Ave.	85.0	38.57	4.96	2.55	4.52	3.17	.06	.08	4.57	3.24	+ .38	- .69
7/11/56-	Basal	628	79	35.85	5.36	2.68	3.87	2.87	.09	.11	3.96	2.97	+1.41	- .29
		659	77	34.94	5.36	2.68	4.32	2.91	.09	.08	4.41	2.99	+ .95	- .32
7/18/56		606	107	48.56	5.36	2.68	4.44	2.74	.17	.13	4.61	2.87	+ .75	- .20
		Ave.	84.3	39.78	5.36	2.68	4.21	2.84	.12	.11	4.33	2.95	+1.07	- .27

[1]Data Expressed as Grams Per Day.

TABLE 15 CHEMICAL BALANCE DATA WITH LAMBS - PERIOD I - FALL 1956[1]

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
9/7/56-	Stilbestrol	729	78	35.40	2.88	2.56	2.85	2.33	.03	.02	2.89	2.35	- .01	+ .21
		631	74	33.58	2.85	2.55	2.97	1.91	.12	.05	3.08	1.96	- .23	+ .59
9/14/56		706	87	39.48	3.07	2.64	3.62	2.77	.05	.05	3.67	2.82	- .60	- .18
		Ave.	79.7	36.15	2.93	2.59	3.15	2.34	.07	.04	3.21	2.38	- .28	+ .21
9/7/56-	Basal	840	81	36.76	2.95	2.50	3.93	2.79	.02	.05	3.96	2.84	-1.00	- .34
		839	78	35.40	3.26	2.62	4.42	3.06	.15	.05	4.57	3.12	-1.31	- .49
9/14/56		728	75	34.04	3.12	2.57	4.13	2.35	.07	.08	4.20	2.43	-1.08	+ .13
		Ave.	78.0	35.40	3.11	2.56	4.16	2.74	.08	.06	4.24	2.80	-1.13	- .23

TABLE 16 CHEMICAL BALANCE DATA WITH LAMBS - PERIOD II - FALL 1956[1]

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
10/1/56-	Stilbestrol	706	94	42.66	3.95	2.78	3.97	2.57	.05	.08	4.02	2.64	- .07	+ .13
		631	81	36.76	5.23	3.17	4.77	2.48	.09	.11	4.86	2.59	+ .36	+ .59
10/8/56		729	90	40.84	4.51	2.95	3.79	2.70	.06	.06	3.86	2.76	+ .66	+ .19
		Ave.	88.3	40.09	4.56	2.97	4.18	2.58	.07	.08	4.25	2.66	+ .32	+ .30
10/1/56-	Basal	840	91	41.29	4.32	2.82	4.44	2.86	.02	.05	4.46	2.91	- .14	- .09
		728	84	38.12	5.68	3.24	5.28	2.88	.13	.10	5.42	2.98	+ .26	+ .26
10/8/56		839	89	40.39	5.85	3.29	5.58	3.06	.16	.09	5.75	3.15	+ .11	+ .14
		Ave.	88.0	39.93	5.29	3.12	5.10	2.93	.11	.08	5.21	3.01	+ .08	+ .11

[1]Data Expressed as Grams Per day.

TABLE 17 CHEMICAL BALANCE DATA WITH LAMBS - PERIOD III - FALL 1956[1]

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
11/5/56-	Stilbestrol	838	115	52.21	5.74		3.84		.05		3.89		+1.84	
		706	107	45.58	5.67	3.69	5.07	3.54	.12	.17	5.18	3.71	+ .49	- .02
11/12/56		631	93	42.22	5.63	3.67	4.40	2.98	.13	.22	4.54	3.20	+1.09	+ .48
		729	94	42.68	5.37	3.59	3.71	2.67	.08	.12	3.79	2.79	+1.58	+ .79
		Ave.	102.2	46.42	5.60	3.65	4.26	3.06	.09	.17	4.35	3.23	+1.25	+ .42
11/5/56-	Basal	692	104	47.21	5.37		5.32		.16		5.48		- .11	
		840	99	44.95	5.92	3.59	4.48	3.17	.03	.13	4.51	3.29	+1.41	+ .30
11/12/56		728	93	42.22	5.96	3.60	5.03	3.11	.12	.23	5.15	3.34	+ .80	+ .26
		839	91	41.31	5.95	3.60	4.74	3.24	.07	.22	4.81	3.46	+1.14	+ .15
		Ave.	96.8	43.92	5.80	3.60	4.89	3.17	.09	.19	4.99	3.36	+ .81	+ .23

[1]Data Expressed as Grams Per Day.

TABLE 18 RADIOCALCIUM BALANCE DATA WITH LAMBS - PERIOD I - SUMMER 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
5/21/56-	Stilbestrol	691	76	34.50	IV	19.3	2.21	4.65	47.53	37.08	36.6	37.1
		644	86	39.04	OS	64.6						33.3
5/28/56		613	81	36.77	OS	62.2						28.1
		Ave.	81.0	36.77								32.8
5/29/56-	Stilbestrol	641	73	33.14	IV	13.55	1.44	4.85	29.69	28.98	30.3	32.9
		660	84	38.14	OS	51.55						27.6
6/5/56		634	77	34.96	OS	72.42						29.4
		Ave.	78.0	35.41								30.0
5/21/56-	Basal	662	76	34.50	IV	28.9	3.91	6.94	56.34	53.80	47.3	43.1
		648	75	34.05	OS	58.7						48.0
5/28/56		617	81	36.77	OS	62.0						41.4
		Ave.	77.3	35.10								44.2
5/29/56-	Basal	659	68	30.87	IV	16.42	2.00	5.46	36.63	44.02	30.1	43.3
		628	67	30.42	OS	66.51						40.9
6/5/56		606	101	45.85	OS	51.46						23.4
		Ave.	78.6	35.76								35.9

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

TABLE 19 RADIOCALCIUM BALANCE DATA WITH LAMBS - PERIOD II - SUMMER 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
6/15/56-	Stilbestrol	644	92	41.75	IV	16.48	1.60	7.60	21.05	45.76	19.0	34.7
		613	84	38.12	OS							39.3
6/22/56		691	82	37.21	OS	66.43						38.4
		Ave.	86.0	39.03								37.5
6/25/56-	Stilbestrol	660	94	42.66	IV	13.17	1.88	2.73	68.86	34.05	41.6	26.3
		634	84	38.12	OS	55.00						29.7
7/2/56		641	85	38.57	OS	33.40						29.4
		Ave.	87.7	39.78								28.5
6/15/56-	Basal	617	82	37.21	IV	19.16	1.79	6.71	26.68	68.71	26.3	54.0
		648	78	36.30	OS	59.02						49.3
6/22/56		662	74	33.58	OS							59.1
		Ave.	78.0	36.30							24.8	54.1
6/25/56-	Basal	628	75	34.03	IV	16.95	1.52	2.13	71.36	53.07	67.0	53.6
		659	79	35.85	OS	38.30						50.3
7/2/56		606	103	46.74	OS	73.40						38.9
		Ave.	85.7	38.87								47.6

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

TABLE 20 RADIOCALCIUM BALANCE DATA WITH LAMBS - PERIOD III - SUMMER 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
7/10/56-	Stilbestrol	691	94	42.66	IV	16.70	1.37	4.38	31.28	29.49	33.0	37.6
		613	93	42.20	OS	59.65						38.0
7/17/56		644	98	44.72	OS	50.82						35.9
		Ave.	95.0	43.19								37.2
7/11/56-	Stilbestrol	634	88	39.93	IV	14.42	1.30	3.96	32.83	28.88	30.7	39.3
		641	86	39.03	OS							
7/18/56		660	99	44.93	OS	46.55						34.9
		Ave.	91.0	41.29								37.1
7/10/56-	Basal	648	93	42.20	IV	22.97	1.71	5.15	33.20	36.96	34.7	47.0
		662	81	36.76	OS	70.44						41.7
7/17/56		617	81	36.76	OS	59.42						53.9
		Ave.	85.0	38.57								47.5
7/11/56-	Basal	628	79	35.85	IV	18.51	1.48	5.05	29.30	20.37	31.6	30.4
		659	77	34.94	OS	65.95						31.3
7/18/56		606	107	48.56	OS	65.82						22.4
		Ave.	84.3	39.78								28.0

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

TABLE 21 RADIOCALCIUM BALANCE DATA WITH LAMBS - PERIOD I - FALL 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
9/7/56-	Stilbestrol	729	78	35.40	IV	20.21	2.35	5.10	46.07	45.60	37.1	37.0
		631	74	33.58	OS	75.09						38.7
9/14/56		706	87	39.48	OS	65.50						35.4
		Ave.	79.7	36.15								37.0
9/7/56-	Basal	840	81	36.76	IV	23.85	2.02	5.20	38.84	54.52	41.6	43.9
		839	78	35.40	OS	89.29						50.1
9/14/56		728	75	34.04	OS	82.64						50.0
		Ave.	78.0	35.40								48.0

TABLE 22 RADIOCALCIUM BALANCE DATA WITH LAMBS - PERIOD II - FALL 1956[2]

10/1/56-	Stilbestrol	706	94	42.66	OS	60.23				45.06		41.7
		631	81	36.76	OS	57.27						64.0
10/8/56		729	90	40.84	OS	60.80						49.9
		Ave.	88.3	40.09								51.9
10/1/56-	Basal	840	91	41.29	OS	57.26				45.56		47.9
		728	84	38.12	OS	65.16						67.9
10/8/56		839	89	40.39	OS	68.62						66.0
		Ave.	88.0	39.93								60.6

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

[2]Value for Percent of the I.V. Dose in Feces Obtained from Final Balance (Period III).

TABLE 23 RADIOCALCIUM BALANCE DATA WITH LAMBS - PERIOD III - FALL 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
11/5/56-	Stilbestrol	838	115	52.21	IV	15.75	1.12	2.48	45.16	17.19	34.6	18.9
		706	107	48.58	IV	32.50	2.11	4.32	48.84		47.0	20.0
11/12/56		631	93	42.22	OS	75.97						22.9
		729	94	42.68	OS	62.50						21.6
		Ave.	102.2	46.42								20.9
11/5/56-	Basal	692	104	47.21	IV	24.20	1.25	3.12	40.06	22.05	47.4	25.1
		840	99	44.95	OS	70.65						29.0
11/12/56		728	93	42.22	OS	73.47						31.1
		839	91	41.31	IV	27.90	2.16	4.89	44.17		48.3	31.7
		Ave.	96.8	43.92								29.2

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

TABLE 24 RADIOPHOSPHORUS BALANCE DATA WITH LAMBS - PERIOD I - SUMMER 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
5/21/56-	Stilbestrol	691	76	34.50	IV	27.80	7.54	11.98	62.94	93.15	45.3	66.3
		644	86	39.04	OS	62.60						59.1
5/28/56		613	81	36.77	OS	33.00						54.1
		Ave.	81.0	36.77								59.8
5/29/56-	Stilbestrol	641	73	33.14	IV	32.08	6.14	14.50	42.34	77.68	45.3	77.7
		660	84	38.14	OS	40.15						66.0
6/5/56		634	77	34.96	OS	54.12						70.4
		Ave.	78.0	35.41								71.4
5/21/56-	Basal	662	76	34.50	IV	35.20	8.50	13.70	62.04	98.90	56.1	63.9
		648	75	34.05	OS	64.90						66.6
5/28/56		617	81	36.77	OS	43.20						59.0
		Ave.	77.3	35.10								63.2
5/29/56-	Basal	659	68	30.87	IV	29.82	6.51	12.87	50.58	110.42	57.9	98.1
		628	67	30.42	OS	53.62						93.3
6/5/56		606	101	45.85	OS	38.27						56.6
		Ave.	78.6	35.76								82.7

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

TABLE 25 RADIOPHOSPHORUS BALANCE DATA WITH LAMBS - PERIOD II - SUMMER 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
6/15/56-	Stilbestrol	644	92	41.75	IV	26.81	7.96	11.76	67.79	70.78	52.4	57.9
		613	84	38.12	OS	27.55						65.1
6/22/56		691	82	37.21	OS	53.28						64.1
		Ave.	86.0	39.03								62.4
6/25/56-	Stilbestrol	660	94	42.66	IV	22.47	10.73	14.50	74.00	60.96	41.3	42.9
		634	84	38.12	OS	41.90						48.3
7/2/56		641	85	38.57	OS	40.60						47.7
		Ave.	87.7	39.78								46.3
6/15/56-	Basal	617	82	37.21	IV	34.44	7.45	12.43	59.94	56.00	47.4	50.7
		648	78	36.30	OS	49.61						46.9
6/22/56		662	74	33.58	OS	62.58						55.6
		Ave.	78.0	36.30							45.3	51.1
6/25/56-	Basal	628	75	34.03	IV	31.94	8.35	14.50	57.59	74.85	46.1	63.4
		659	79	35.85	OS	48.80						59.6
7/2/56		606	103	46.74	OS	49.50						46.0
		Ave.	85.7	38.87								56.3

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

TABLE 26 RADIOPHOSPHORUS BALANCE DATA WITH LAMBS - PERIOD III - SUMMER 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
7/10/56-	Stilbestrol	691	94	42.66	IV	35.72	10.03	22.56	44.46	87.59	30.4	67.3
		613	93	42.20	OS	44.23						63.9
7/18/56		644	98	44.72	OS	42.17						49.0
		Ave.	95.0	43.19								60.1
7/11/56-	Stilbestrol	634	88	39.93	IV	33.13	10.16	13.40	75.82	90.93	49.0	59.6
		641	86	39.03	OS	47.15						60.9
7/18/56		660	99	44.93	OS	44.91						52.9
		Ave.	91.0	41.29								57.8
7/10/56-	Basal	648	93	42.20	IV	37.25	10.53	25.07	42.00	96.74	33.7	61.3
		662	81	36.76	OS	47.33						60.7
7/17/56		617	81	36.76	OS	62.10						70.4
		Ave.	85.0	38.57								64.1
7/11/56-	Basal	628	79	35.85	IV	41.67	11.34	15.78	71.86	87.20	57.4	65.1
		659	77	34.94	OS	49.68						66.9
7/18/56		606	107	48.56	OS	55.02						48.1
		Ave.	84.3	39.78								60.0

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

TABLE 27 RADIOPHOSPHORUS BALANCE DATA WITH LAMBS - PERIOD I - FALL 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
9/7/56-	Stilbestrol	729	78	35.40	IV	34.60	10.06	10.32	97.48	67.51	64.1	48.9
		631	74	33.58	OS	47.85						50.1
9/14/56		706	87	39.48	OS	51.23						45.1
		Ave.	79.7	36.15								48.0
9/7/56-	Basal	840	81	36.76	IV	36.00	8.38	13.32	62.91	75.87	47.9	51.6
		839	78	35.40	OS	61.26						56.1
9/14/56		728	75	34.04	OS	47.85						57.3
		Ave.	78.0	35.40								55.0

TABLE 28 RADIOPHOSPHORUS BALANCE DATA WITH LAMBS - PERIOD II - FALL 1956[2]

10/1/56-	Stilbestrol	706	94	42.66	OS	44.86				73.20		47.6
		631	81	36.76	OS	45.88						63.1
10/8/56		729	90	40.84	OS	45.30						52.8
		Ave.	88.3	40.09								54.5
10/1/56-	Basal	840	91	41.29	OS	43.45				77.49		53.1
		728	84	38.12	OS	49.70						66.1
10/8/56		839	89	40.39	OS	51.54						63.4
		Ave.	88.0	39.93								60.9

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

[2]Value for Percent of the I.V. Dose in Feces Obtained from Final Balance (Period III).

TABLE 29 RADIOPHOSPHORUS BALANCE DATA WITH LAMBS - PERIOD III - FALL 1956

Date	Treatment	Lamb No.	Body Wt. Lbs.	Body Wt. Kg.	Method of Administration	Percent Dose in Feces[1]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endog. Iso.Dil.	Percent Fecal Endog. Comp.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Iso.Dil.	Mgs. Fecal Endog. Per Kg. Body Wt. Per Day Comp.
11/5/56-	Stilbestrol									71.42		
		706	107	48.58	IV	36.61	6.59	6.94	94.96		69.1	54.3
11/12/56		631	93	42.22	OS	47.00						62.1
		729	94	42.68	OS	42.11						60.0
		Ave.	98.0	44.49								58.8
11/5/56-	Basal									74.97		
		840	99	44.95	OS	45.69						59.9
11/12/56		728	93	42.22	OS	46.60						64.0
		839	91	41.31	IV	37.96	7.85	9.78	80.27		63.0	65.4
		Ave.	94.3	42.83								63.1

[1]Percent of the Administered Dose Excreted During the 7-Day Balance.

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