TABLE II. Characterization of the Virus Strain.

	"G" strain	"L" strain
Plaque size	small	large
Cytopathogenicity	rounding cells	cytolytic giant cells
Intranuclear inclusion	positive	positive
"Mad-itch" in rat	negative or weak	"
CAM	typical pocks	typical pocks
Hemolytic activity	negative	negative
Antiserum	neutralized	neutralized

ments revealed that the "L" strain uniformly produced symptoms but these were only rarely produced by the "G" strain even though the $TCID_{50}$ titers were approximately the same.

The characteristics of the 2 strains are summarized in Table II.

Discussion. The demonstration of 2 types of cytopathogenicity in monkey kidney cells infected with pseudorabies virus suggests either that the original stock virus contained 2 strains of virus particles which could be differentially selected by adaptation in monkey kidney cells or that mutation from the "G" to the "L" strain occurred in this host. At present it has not been possible to separate these 2 strains sufficiently cleanly to test them for genotypic characteristics by recombination experiments. The apparent correlation between type of cytopathogenicity and ability to cause symptoms in rats raises the possibility that the virus which affects cell membranes in tissue culture leading to lysis may have some action on the nerve membrane which in turn gives rise to the abnormal electrical responses described by Dempsher *et al.* This point is under further investigation.

Summary. During the course of adaptation of the egg-adapted pseudo-rabies virus to tissue culture of monkey kidney cells, 2 types of cytopathogenic variations were encountered which appeared to be induced by 2 different strains of virus. One strain produced rounding degeneration, and the other cytolytic giant cell formation. Descriptions are given of the growth curve, plaque type in tissue culture, and infectivity for rats of these 2 strains.

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Influence of Estrogens on Body Growth and Food Intake.* (23393)

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Estrogens have been shown by many workers to depress body weight in laboratory animals(1-5). Several lines of evidence, too extensive to review here, indicate that they may do this by influencing either the release or action of other hormones in the body. Heavy or prolonged treatment with estrogens also causes anorexia. The resulting inanition may be partially responsible for the effects sometimes attributed to hormonal alterations. In addition, inanition itself may result in hormonal imbalances(6,7,8), which resemble in some respects the effects brought about by estrogen treatment. Therefore, dietary restriction, whether imposed experimentally or

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occurring as a result of estrogen treatment, may act on growth directly as well as indirectly through alteration in endocrine functions.

Growth depression in rats, accompanied by voluntary restriction of food intake as a result of estrogen treatment has been most consistently demonstrated with stilbestrol. The depression of growth and food intake was proportional to the dosage of stilbestrol; growth depression could be duplicated by pair-feeding(1). After a single dose or repeated administration of estrogen, there is a negative nitrogen balance attributable to decreased intake(2,3). Estradiol, or the benzoate, 100 μg daily, has been reported to produce either no body weight depression(4) or depression without influence on food intake(1). It thus appeared that in rats the natural estrogens, represented by estradiol, were less effective than stilbestrol in depressing body growth, although the 2 compounds have approximately the same activity in the Allen-Doisy test(9).

Since the effects of estradiol on body growth and food intake reported in the literature have been quite variable, a range of doses was first explored to determine if a dose of this natural estrogen could be found that would produce statistically significant depression of growth and food intake. In later and more extensive experiments, growth rates of estradiol-treated rats were compared with those of pair-fed controls. Results indicate that estradiol produces a significant depression of growth, which can be readily duplicated by restricting food intake of the controls to that of the estrogen-treated partner.

Materials and methods. Male rats of the Sprague-Dawley strain,‡ weighing less than 80 g at the start of the experiments, were divided into groups of 3 to 4 animals and injected subcutaneously with estradiol, in 0.1 ml sesame oil, in doses of 1, 10, or 100 μg per rat daily, or with estradiol benzoate in doses of 2, 20, or 200 μg per rat every other day, control animals receiving equal volumes of the vehicle.§ The animals were housed in individual wire-bottomed cages at 70°F, and fed a diet of ground Ralston-Purina chow checkers. Food was presented in tared metal cups secured to the cages to eliminate tipping, and fitted with an anti-scatter cover.‖ When pair-feeding regimens were undertaken, each treated rat was matched with an untreated rat of similar body weight. The pair-fed control was presented throughout with the amount of food eaten *ad lib.* by the treated member during each previous 24-hour period. Records of body weight and food consumption were made daily each morning. Mean body weight and food consumption were calculated for each group, and the significance of the difference of the means tested by application of "Student's" t test.

Results. In preliminary experiments with estradiol, a significant depression of body growth, proportional to dose, was found for all groups after 30 days' treatment. The differences among the doses of hormone did not, however, prove significant. At 30 days a significant depression of food intake was also observed in the groups on the 2 highest doses, while a suggestive depression occurred with the lowest dose ($P>0.05<0.1$). Body weight depression was proportionately greater than food intake, the amount of the disproportion being directly related to the dose. When the control animals were pair-fed with rats treated with the highest dose of estradiol (100 μg daily), their growth rate tapered off to parallel that of the treated rats. Restoration of *ad libitum* feeding led to an immediate resumption of growth. Estradiol benzoate produced similar effects on growth, food intake, and efficiency, but they were prompter and more clear-cut than with estradiol. For this reason, all subsequent experiments were conducted with estradiol benzoate.

Growth rates of the estrogen-treated rats did not differ from those of the untreated, pair-fed controls, whether pair-feeding was begun at the start or in the middle of the experiment

‡ Purchased from the Charles River Breeding Laboratories.

§ Estradiol was obtained through the generosity of the Schering Corp., Bloomfield, N. J., the estradiol benzoate as a gift from Ciba Pharmaceutical Products, Summit, N. J.

‖ Purchased from Geo. H. Wahmann Manufacturing Co., Baltimore, Md.

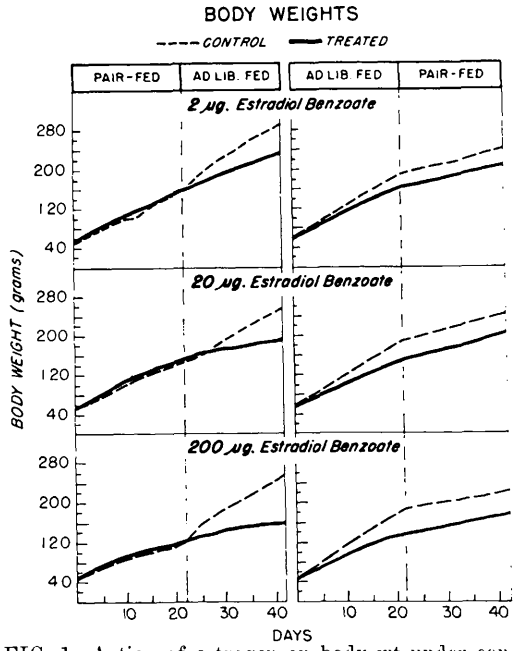

FIG. 1. Action of estrogen on body wt under conditions of *ad lib.* restricted pair-feeding.

(Fig. 1). Experiments in which the controls were initially fed *ad libitum* (right aspect, Fig. 1) demonstrated depression in body weight gain of the estrogen-treated rats proportional to the dosage of estrogen, which was significantly different from the controls with the 2 higher doses at the end of 3 weeks treatment. Placing the controls on restricted pair-feeding resulted in their growth rates paralleling those of the treated rats, at each level of estrogen dosage. Similarly, growth of the treated rats and their respective controls for each estrogen dosage were identical when pair-feeding was begun initially (left aspect, Fig. 1). When the controls were allowed to resume *ad libitum* feeding, their growth rates immediately accelerated, while those of the estrogen-treated rats remained unchanged. By the end of 3 weeks, the body weights of the estrogen-treated rats were now significantly less than those of the controls, and the extent of these differences was proportional to the dosage of estrogen. Voluntary food intake of the estrogen-treated animals was consistently less than that of the untreated controls eating *ad libitum* (Fig. 2). At 3 weeks, the differences were significant for the highest dose of estrogen for both initial and delayed *ad lib.* feeding, and for the intermediate dose for initial *ad lib.* feeding. Depression in body weight was proportionately more marked than that of food intake for the estrogen-treated groups compared to the controls fed *ad libitum,* as found previously with estradiol.

In one group of experiments, the relative influence of estrogen and of the pair-feeding restriction on gonads and sex accessories was also determined by weighing ventral prostate, seminal vesicles, and testes at the end of an experiment (Table I). As would be expected after chronic treatment, estrogen led to depression roughly proportional to dose for prostate and testes. While pair-feeding brought about depression of the weight of sex-accessories, it was proportionally not as great as that obtained with estrogen. Testicular weight, however, was not affected by restricted feeding.

Discussion. Estradiol and its benzoate in adequate doses brings about a profound depression in growth rate accompanied by voluntary restriction in food intake. The failure of similar doses of estradiol benzoate (100 μg

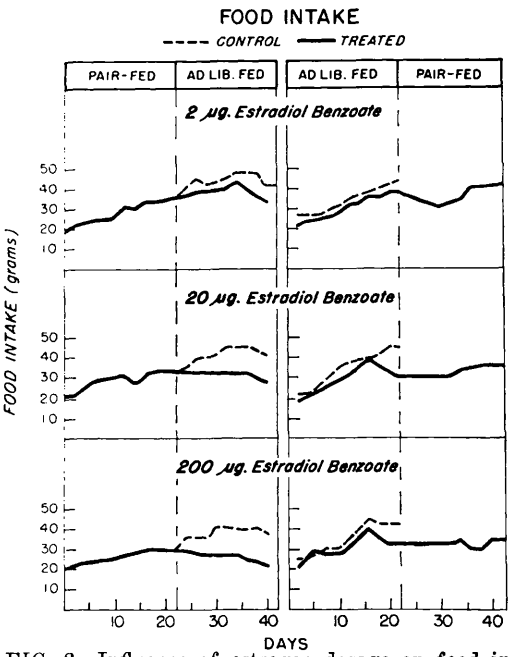

FIG. 2. Influence of estrogen dosage on food intake.

TABLE I. Effect of Estrogen or Pair-Feeding on Testes and Sex Accessories.

Group	Organ weights: Prostate mg absolute	Prostate Per 100 g body wt	Seminal vesicles mg absolute	Seminal vesicles Per 100 g body wt	Testes mg absolute	Testes Per 100 g body wt
Ad lib. controls	309	100.8	714	230	3100	978
2 μg estrogen	53	24.4	93	44.2	826	372
Pair-fed	195	71.8	553	206	3363	1345
20 μg estrogen	17	8.8	124	64.4	258	134
Pair-fed	166	66.6	311	123.3	3030	1200
200 μg estrogen	11	6.1	137	80.6	210	124
Pair-fed	202	88.2	539	232	2950	1285

every other day for 3 weeks) to influence body weight in 200 g male rats in the experiments of Lynch(4) may be due in part to the use of older, heavier animals, which are probably less sensitive to the growth-depressant action of this estrogen than younger, more rapidly-growing rats. Furthermore, when estrogen dosages are calculated on the basis of body weight, his dose is about equivalent to the intermediate dose used in the present experiments.

Differences in age, weight, and possibly sex may also explain the divergence of results obtained in the present experiments from those reported by Meites(1), who did not find any reduction in growth rate until after 30 days treatment and no decrease in food intake regardless of the length of treatment in 150 g female rats given 100 μg of estrone or estradiol daily. The nature of the diet, perhaps quite crucial, was not stated. Under the conditions of our experiments we could not confirm this author's conclusion that "growth reduction can be induced with natural estrogens without lowering daily food intake."

Both the present experiments with estradiol or estradiol benzoate, and the experiments of Meites(1) with diethylstilbestrol have shown that pair-feeding duplicates the effects of estrogens on body weight. Somewhat different results were obtained with larger doses of diethylstilbestrol by Glasser(2) who found that in 250-350 g adult male rats, fed a semi-synthetic diet containing 18% casein, daily injection for 3 weeks depressed average body weight 100 g, while pair-feeding depressed body weight only 55 g. Rats receiving stilbestrol showed at the same time a greater negative nitrogen balance than pair-fed controls. With further treatment, the depression of growth and food intake tapered off and return toward normal of the negative nitrogen balance occurred. A similar phenomenon was reported by Selye(5) who noted an initial weight loss during the first week followed by a slow gain in rats given excessive daily doses of estradiol or stilbestrol. This secondary recouping of weight loss, termed "adaptation" by Selye was not seen in the present experiments; this does not exclude the possibility that adaptation might have been detectable in nitrogen balance studies. No stimulation of body weight gain or increase in food utilization efficiency, similar to that reported for cattle(10,11) or sheep(12) treated with stilbestrol, has in our knowledge been reported to occur in small laboratory animals. The interpretation that voluntary restriction of food intake was chiefly responsible for the negative nitrogen balance in the experiments cited above(2,3) is strengthened by the present results with estradiol and those of Meites(1) with stilbestrol.

Although pair-feeding failed to reproduce the inhibitory action of estrogen on the hypophyseal-gonadal system, the fact that estrogen produces general effects on growth and appetite suggests that the effects of chronic estrogen treatment on other endocrine systems and skeletal growth should be reinvestigated in experiments using pair-fed controls.

Summary. The effects of estradiol and its benzoate ester on body weight and food consumption of immature male rats have been studied in experiments using controls which were either eating *ad lib.* or pair-fed to the

treated animals. In animals eating *ad lib.*, estrogen treatment for 3 weeks or more resulted in a depression of body weight accompanied by a voluntary restriction in food intake; body weight was more greatly depressed than food intake. Restriction of food intake in the controls to amounts eaten *ad lib.* by the treated animals duplicated the effects of the estrogen on depression of body weight, since the growth curves of pair-fed control and treated groups became parallel.

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Free Amino Acid Patterns of Certain Tissues from Potassium-Deficient Dogs.* (23394)

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Rats placed on potassium-deficient diets, with or without simultaneous administration of desoxycorticosterone acetate (DCA), exhibit typical changes of the electrolyte compositions of plasma and skeletal muscle(1,2). Thus, there is an increased plasma bicarbonate content and a lowered plasma chloride concentration in association with a loss of skeletal muscle potassium and an unequivalent gain of muscle sodium. Cooke *et al.*(3) developed the thesis that the unequivalent distribution of potassium and sodium is accompanied by a passage of hydrogen ions into the muscle cell, thus accounting for the alkalosis of the extracellular fluid. Dogs fed a potassium-deficient diet, with or without simultaneous administration of DCA, also exhibit a loss of skeletal muscle potassium and an unequivalent gain of sodium. However, the increased plasma bicarbonate content and fall of the plasma chloride concentration become conspicuous only with simultaneous dietary restriction of chloride(2,4).

Evidence was recently presented(5), on the basis of paper chromatography, that the amino acids lysine and arginine were increased in the skeletal muscle, diaphragm and kidneys from potassium-deficient rats, while simultaneously there appeared to be decreased concentration of the amino acids aspartic acid and glutamic acid in these same tissues. The suggestion was made that possibly the changes in the concentrations of basic and acidic amino acids in the skeletal muscle of the rat may compensate in part the discrepancy between the potassium loss and sodium gain. This view was supported by the observations of Eckel, Pope and Norris(6) that from 8-40% of the metallic cation deficit present in the potassium-depleted rat skeletal muscle could be accounted for by the amino acid lysine.

The results with rats prompted the present study of the free amino acid patterns of skeletal muscle, left ventricle and kidneys from potassium-deficient dogs. Special attention was given to potassium and sodium concen-

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