MAY, 1948 RESEARCH BULLETIN 416

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
E. A. TROWBRIDGE, *Director*

STUDIES CONCERNING THE INDUCTION AND MAINTENANCE OF LACTATION

II. The Normal Maintenance and Experimental Inhibition and Augmentation of Lactation

Joseph Meites and C. W. Turner

Publication authorized May 11, 1948
COLUMBIA, MISSOURI

TABLE OF CONTENTS

ACKNOWLEDGMENT

This study has been aided in part by grants from the Committee of Scientific Research, and the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

The writers wish to express their appreciation also to the following firms for their generous donations of products used in these investigations: Lederle Laboratory (thiouracil); George A. Breon and Co. (dipropionate of diethylstilbestrol); The Wm. S. Merrell Co. (hexestrol); Wallace and Tiernan Products, Inc. (monomestrol); Schieffelin and Co. (benzestrol); and Cerophyl Labs., Inc. (cerogras and protamone).

STUDIES CONCERNING THE INDUCTION AND MAINTENANCE OF LACTATION

II. The Normal Maintenance and Experimental Inhibition and Augmentation of Lactation

Joseph Meites and C. W. Turner

INTRODUCTION

The previous bulletin dealt with the mechanism controlling the initiation of lactation at parturition. This bulletin brings together data on the factors influencing the secretion rate of pituitary lactogen as correlated with the rise and decline of milk production after parturition.

After maximum milk production has been attained, milk secretion declines at variable rates in individual animals. A few dairy cows continue to secrete milk at a high level for many months. Most cows, however, decline at a rather constant rate and go dry in 10 or 12 months in spite of good feeding and management.

In the light of the increasing knowledge of the hormones regulating milk secretion, the question arises as to the possibility of preventing the decline in lactation after maximum production has been reached. The stimulus of milking does not prevent the usual decline in production.

Administration of estrogen initiates lactation in virgin or sterile cows and goats, but estrogens generally have been considered inhibitory once lactation is established. As a result of the research reported here, it seems probable that the action of large doses of estrogen in inhibiting established lactation may be mediated through a depression of certain metabolic processes, which overshadow the beneficial effects of estrogen on the lactogenic hormone and the mammary gland. However, small amounts of estrogen, particularly when given in combination with thyroid preparation, offer possibilities for augmenting milk production. Combinations of these two kinds of hormones were tested in goats with encouraging results.

For twenty years, the hormones influencing the growth of the udder and the initiation and maintenance of lactation have been studied to determine their role during each phase of this process. With the discovery of cheap orally active hormones, the next decade may see the development of methods by which these hormones can be introduced into the ration of dairy animals to stimulate and maintain higher than normal lactation levels for long periods.

I. FACTORS RESPONSIBLE FOR THE NORMAL POSTPARTUM MAINTENANCE OF LACTATION

A. Review

The theory has been advanced that estrogen is the factor which stimulates the secretion of lactogenic hormone by the pituitary at about the time of parturition and thereby initiates milk secretion (Meites and Turner, 1942a, 1947a). Following the onset of lactation, estrogen apparently is no longer necessary, since postpartum ovariectomy in the rat (Kuramitsu and Loeb, 1921), guinea pig (Turner and Gomez, 1936), and cow (Turner, 1933) does not interfere with lactation.

The normal postpartum lactation span is believed to be maintained by the suckling stimulus (Selye, Collip and Thomson, 1934; Selye and McKeown, 1934a,b) and the withdrawal of milk from the mammary

gland (Kuramitsu and Loeb, 1921; Hesselberg and Loeb, 1937). The former suggested that suckling stimulated the secretion and discharge of lactogenic hormone by the pituitary.

Reece and Turner (1937) and Holst and Turner (1939) demonstrated that suckling caused a discharge of lactogen from the pituitaries of rats, guinea pigs, and rabbits. Suckling also preserves the integrity of the mammary tissue (Turner and Reineke, 1936; Williams, 1941), and Hooker and Williams (1941) believe this is mediated through the action of lactogenic hormone on lobule-alveolar cells.

How suckling influences the anterior pituitary to secrete and discharge lactogenic hormone is not definitely known. It seems probable that the effect of nursing is not exerted indirectly through other endocrine glands. It has been established that lactation can be maintained in the absence of the ovaries and thyroids. The adrenals and the posterior pituitary must be eliminated as possible intermediaries, since adrenal cortical extracts and pituitrin have no effect on the pituitary lactogen content. It seems most probable that the effect of nursing on the anterior pituitary is mediated directly through the cerebro-spinal axis (Inglebrecht, 1935).

B. Experimental

1. RELATION OF LITTER SIZE TO PITUITARY LACTOGEN CONTENT OF NURSING RABBITS. - What effect does the extent or intensity of nursing have on the lactogenic hormone during the course of lactation? Desclin (1945) reported a reduction in litter size did not appreciably affect the cytology of the pituitary. Cole (1933) noted that some of the mammary glands of the mouse often showed involutional changes when a small litter was suckling. If the litters of parturient rabbits were reduced to two young each, there would be present a number of mammary glands in which milk stagnation, resorption, and involution would be in progress. Would such a condition tend to suppress the lactogen secretion of the pituitary?

Litters of ten New Zealand White rabbits were reduced to two each on the fifth postpartum day, while 11 control rabbits were permitted to keep their entire litters of 5 to 11 young. The mothers of both groups were sacrificed on the 20th day after parturition.

Control rabbits contained an average of 0.9 Reece-Turner lactogen units per pituitary on a body weight basis whereas experimental rabbits contained an average of 0.8 units (Table 1). The difference is

Table 1. Relation of Litter Size to Pituitary Lactogen Content of Nursing Rabbits

Group	No. of Rabbits	Av. Body Weight gms.	Av. Pit. Weight mgs.	Av. R.-T. Lactogen Units		
				Per Pit.	Per mg. Pit.	Per 100 gms. B.W.
Controls	11	3360	42.20	30.1	0.7	0.9
Experimental	10	3617	41.03	27.1	0.7	0.8

(Meites, Bergman and Turner, 1941)

not significant. The size of the nursing litter, therefore, does not influence the lactogen content of the pituitaries of postpartum rabbits.

2. EFFECTS OF SUCKLING AND NON-SUCKLING ON THE PITUITARY LACTOGEN CONTENT AND MILK SECRETION. - Since some lactation is usually initiated in most animals just before parturition, the

suckling stimulus is unnecessary for the onset of secretory activity. Reece and Turner (1937) found that the lactogen content of the pituitaries of rats on the second postpartum day rose just as much in non-suckled as in suckled animals. However, in rats the pituitary lactogen content reaches a peak by the second postpartum day, while in rabbits the peak is attained on the fifth day.

The object of these experiments was to determine (1) whether the act of nursing played a part in the postpartum rise of the lactogen content of the pituitary and milk yield of rabbits, and (2) to what extent the absence of nursing reduced the content of pituitary lactogen and milk yield in postpartum rats and rabbits. How long would it take the postpartum level of pituitary lactogen to return to the prepartum level in the absence of suckling?

Six parturient control rats were permitted to keep their entire litters. The litters of six other rats were permitted to drop through a wire floor at the time of parturition and were never suckled. Both groups of rats were sacrificed on the seventh postpartum day.

The rabbits which were not to be suckled were placed in sloping wire-bottom cages, thus permitting the litters to drop out of the cages at birth. The parturition date of each rabbit was recorded and at intervals of 2, 5, 10, or 20 days after parturition both suckled and non-suckled rabbits were killed. The litters of the suckled rabbits were removed from the cages fifteen hours before killing the mothers to avoid the immediate effect of nursing in causing a partial discharge of lactogenic hormone from the pituitary.

The results show that on the seventh postpartum day the six suckled rats contained an average of 5.9 R.T. lactogen units per pituitary on a body weight basis. Their mammary glands were widely distended with milk (Table 2). In contrast, the six mother rats which were not suckled for the seven days contained an average of only 2.3 units of lactogen.

Table 2. Effects of Suckling and Non-Suckling on the Pituitary Lactogen Content of Postpartum Rats and Rabbits

Series	Species	No. of Animals	Days Post-partum	Av. Body Wt.	Av. Pit. Wt.	Av. R.-T. Lactogen Units			Av. % Difference between Nursed and Non-Nursed	Av. Mammary Secretion
						Per pit.	Per mg. pit.	Per 100 gm. body		
				gms.	mgs.					
Suckled	Rat	6	7	182	14.08	10.8	0.8	5.9	+156	++++
Non-Suckled	"	6	7	217	10.13	5.1	0.5	2.3		-
Suckled	Rabbit	9	2	3487	40.18	22.0	0.5	0.6	- 17	++
Non-Suckled	"	5	2	3133	38.73	24.0	0.6	0.8		++
Suckled	"	7	5	3327	45.98	60.0	1.3	1.8	+ 87	++++
Non-Suckled	"	5	5	3723	37.60	36.0	1.0	1.0		+++
Suckled	"	5	10	2890	43.92	36.0	0.8	1.2	+ 55	++++
Non-Suckled	"	5	10	2684	35.07	21.6	0.6	0.8		++
Suckled	"	31	20	3605	42.41	28.1	0.7	0.8	+ 64	+++
Non-Suckled	"	5	20	2963	31.83	14.0	0.4	0.5		+

(Meites and Turner, 1942b)

This represents a decrease of about 50 per cent from the control level, and is practically as low as in nonparturient estrous rats. No milk was visible in the mammary glands of these rats.

In the rabbits, the lactogen content of the pituitaries of both suckled and non-suckled rabbits rose to about the same level on the second day after parturition (Fig. 1). Milk secretion had not become fully established in either group.

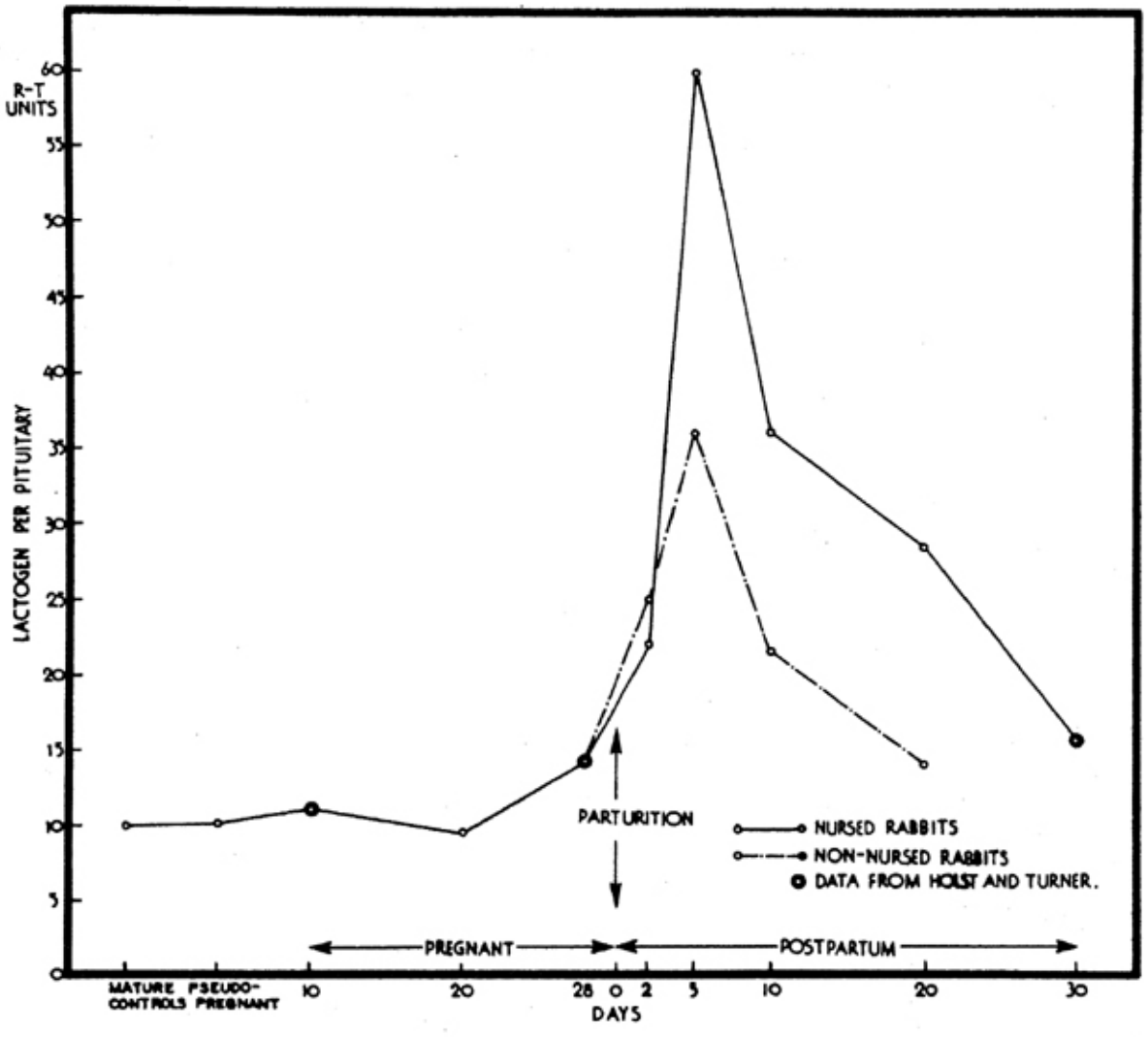

Fig. 1. Curve showing the effects of suckling and non-suckling on the lactogen content of the rabbit pituitary after parturition. The superiority of the suckled rabbits in pituitary lactogen is paralleled by the equal superiority in milk secretion of these animals.

At five days postpartum, the lactogenic hormone reached a peak in both groups, but the suckled rabbits had about 87 per cent more hormone per pituitary on a body weight basis than the non-suckled. There was definitely more milk in the mammary glands of the suckled rabbits, showing that the suckling stimulus is necessary to bring the pituitary lactogen content and milk secretion to the highest level in rabbits.

After the fifth day, the lactogenic hormone declined in both groups. Non-suckled rabbits always had less hormone and less mammary secretion. By the 20th day postpartum, the pituitary lactogen was reduced to the prepartum level in the non-suckled rabbits, and milk was seen only in the large ducts of the shrunken mammary glands.

The data of Hammond (1925) on the effects of suckling and non-suckling on the weight of the mammary glands of postpartum rabbits parallel these results. He found that the glands of suckled rabbits were consistently heavier than in non-suckled rabbits.

It will be noted that the pituitaries of the suckled rats and rabbits, with the possible exception of the two-day postpartum rabbits, were consistently heavier than in the non-suckled animals.

The importance of suckling for maintaining lactation is supported by this study. In the rat, the absence of suckling for the first week after parturition resulted in a fall of the lactogen content of the pituitary to the estrous level and in disappearance of mammary secretion. In parturient rabbits, the act of suckling plays a role in the initiation as well as the maintenance of lactation, since in this species maximum lactogen in the pituitary and maximum milk secretion cannot be attained without suckling.

3. EFFECT OF THIOURACIL OR STARVATION ON THE PITUITARY LACTOGEN CONTENT AND MILK SECRETION OF POSTPARTUM RATS. - Thiouracil administration to young female rats produced a reduction in the lactogen content of the pituitary (Meites and Turner, 1947b). Undernutrition or starvation decreases the secretion of several pituitary hormones, a condition Mulinos and Pomerantz (1940) termed "pseudo-hypophysectomy". Would thiouracil feeding or starvation reduce the pituitary lactogen content during lactation?

Twenty-four rats were divided into three groups of eight each on the day of parturition. One group served as a control, another received 0.1 per cent thiouracil in the feed, and the third received water but no feed from the 10th to the 14th postpartum day. The litters were uniformly reduced to six each on the third postpartum day. Both mothers and litters were weighed on the 1st, 3rd, 7th, 10th and 14th day after parturition. On the 14th day the litters were removed and after an elapse of four hours to standardize the suckling influence, the mothers were killed.

The average lactogen content in the pituitaries of the eight control rats was five R.T. units (Table 3). The mammary glands were widely distended with milk, and the young made normal weight gains. Thiouracil treated rats contained less lactogen in their pituitaries, 3.9 R.T. units, than the controls. Their mammary glands were not as full of milk, and the young did not make the same body weight gains as the young of the control rats (Fig. 2).

Body weight gains of the young from the thiouracil treated mother rats may not be a true index of their milk consumption, since Hughes (1944) and Monroe and Turner (1946) demonstrated that thiouracil fed to mother rats is transmitted through the milk to the suckling litters.

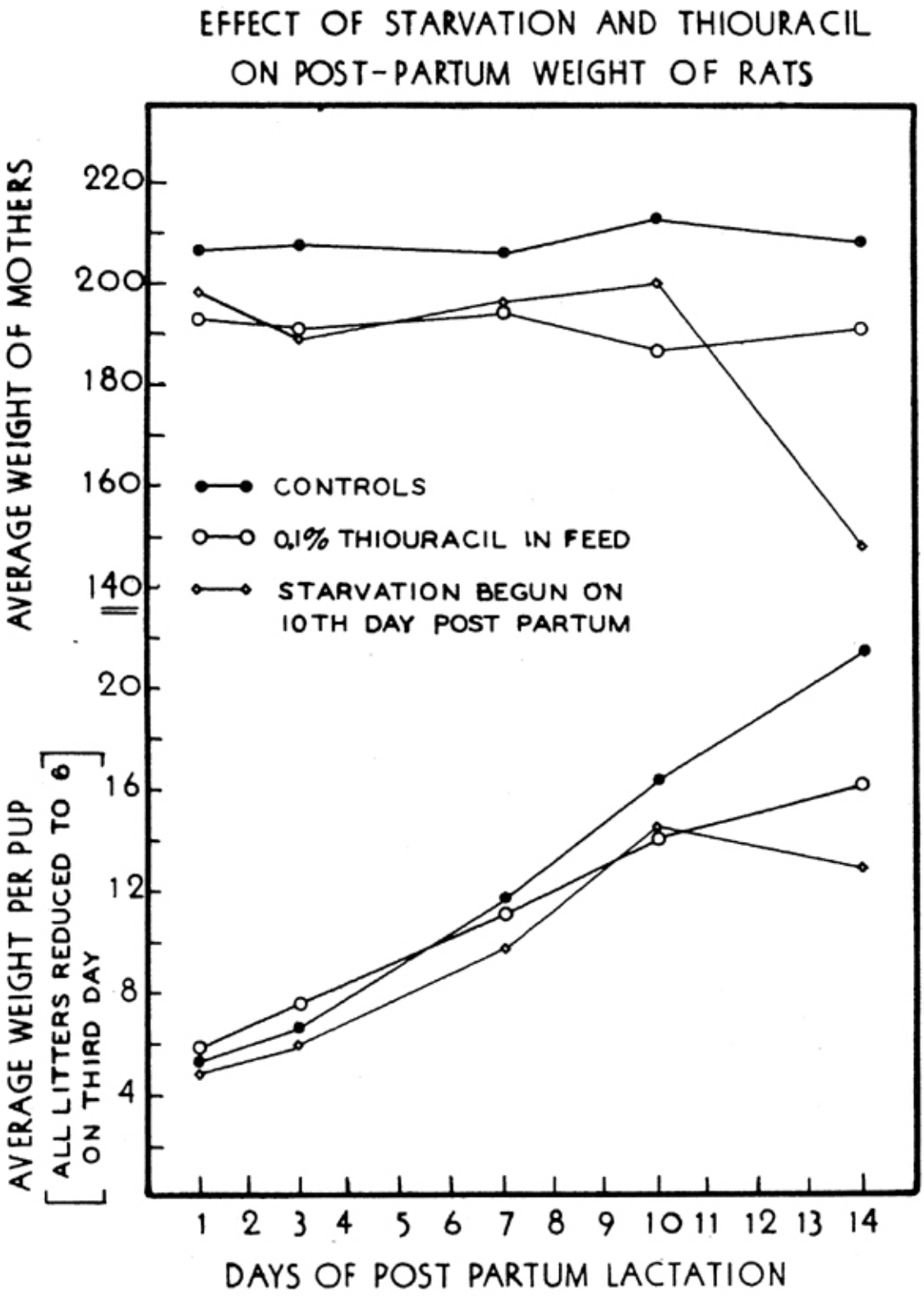

Fig. 2. Effect of starvation and thiouracil on postpartum body weights of mother rats and their young. Note the gradual decrease in growth rate of the young from thiouracil treated mothers, and the decrease in body weight of the rats starved between the 10th and 14th days after parturition.

Table 3. Effects of Thiouracil and Starvation on the Pituitary Lactogen Content and Milk Secretion of Parturient Rats

No. of Rats	Treatment (14 days)	Av. Body Wt. on Postpartum Day, gms.					Av. Pit. Wt. mgs.	Av. Thyroid Weight mgs.	Av. R.-T. Lactogen Units			Av. Mammary Secretion
		1	3	7	10	14			Per pit.	Per mg. pit.	Per 100 gms. body weight	
8	Controls						11.1	19.4	10.4	0.9	5.0	+3.7
	Mothers	207	208	207	213	209						
	Litters	5.3	6.7	11.7	16.4	21.6						
8	0.1% Thiouracil						9.5	49.6	7.5	0.8	3.9	+2.8
	Mothers	193	191	196	187	191						
	Litters	5.9	7.6	11.1	14.1	16.3						
8	No Feed on Days 10-14						7.4	12.4	5.1	0.7	3.4	-
	Mothers	199	190	196	201	149						
	Litters	4.9	6.0	9.8	14.5	13.0						

The former worker showed that thiouracil can retard growth when administered to newly born rats.

Monroe and Turner (1946) reported that thiouracil fed to lactating mother rats did not reduce the growth rate of the litters for the first 15 days after parturition. However, in their experiment the mother rats had their entire litters. There were more fatalities among the young of the thiouracil treated rats, suggesting that the surviving young may have obtained more milk.

The mother rats starved from the 10th to the 14th day postpartum contained considerably less lactogen in their pituitaries than the control rats, and no mammary secretion was evident. The starved rats lost an average of 52 grams in body weight, and this was reflected in reduced pituitary and thyroid weight. The young also lost weight during the four days in contrast to the gain made by the young of the control rats during this period. Only 5 young from the original total of 48 were dead on the 14th day.

It would appear from this study that the induction of hypothyroidism in lactating rats by the administration of thiouracil results in a moderate reduction in pituitary lactogen content and milk secretion. This agrees with the reports of the effects of thyroidectomy on lactation in rats by Folley (1938), Folley et al. (1943), Preheim (1940) and Karnofsky (1942). Only Nelson et al. (1937, 1939) failed to find any significant alteration in lactation following thyroidectomy in rats. In the goat and cow, thyroidectomy causes a reduction in milk yield, and Schultze and Turner (1945) reported that thiourea and thiouracil administration exert a like effect in these animals.

Brody and his students (1945) demonstrated that starvation results in a rapid decline in the milk yield of rats, goats, and cows. They found that within 10 hours after the food was removed from mother rats, milk production dropped to an insignificant level. Most of the suckling baby rats died of starvation within four days. Thus the failure in lactation which occurs during starvation may be caused partly by a decrease in secretion of pituitary lactogen. The effects of starvation on the pituitary lactogen content, milk secretion, body weight, and pituitary

and thyroid weight appear in a remarkably short time. This illustrates the profound interaction between food intake and hormone secretion levels.

II. THE EXPERIMENTAL INHIBITION OF ESTABLISHED LACTATION

A. Review

Estrogens are capable of exerting anomalous effects on lactation. Administration of certain dosages of estrogen may initiate milk secretion in virgin or parous, non-lactating animals, while higher dosages of estrogen may depress established lactation. Similar results were reported with certain androgens (for review see Meites and Turner, 1942a; Mixner, Meites and Turner, 1944; Folley, 1941, 1944).

The importance of the dosage of estrogen required to depress lactation was emphasized by Mixner et al. (1944). They demonstrated that while 0.25 milligrams of diethylstilbestrol was effective in initiating lactation in goats, dosages varying from one to four milligrams were progressively lactation-depressing in lactating goats. Four times as much diethylstilbestrol was required to inhibit lactation as compared to the optimal amount for initiating lactation.

The presence or absence of the ovaries seems to modify the lactation-inhibiting effect of estrogens in rats. Anselmino and Hoffmann (1936), Folley and Kon (1938), Edelman and Gaunt (1940) and Barsantini et al. (1946) reported that the suppression of lactation with estrogens was more effective in intact than in ovariectomized rats. The latter investigators also found that when estrogen was given in combination with progesterone to ovariectomized rats, lactation was more effectively inhibited than with estrogen alone. Progesterone alone had no inhibiting effect on established lactation even when administered in large amounts. The validity of these observations for other species is not known. The parturient rat and mouse are the only mammals known possessing corpora lutea of lactation.

If estrogen does not suppress established lactation by inhibiting the secretion of lactogenic hormone by the pituitary, through what other mechanisms may this be possible? Meites and Turner (1942a) suggested that estrogen may inhibit the secretion by the pituitary of hormones other than lactogen, particularly those affecting metabolic processes. The injection of increasing levels of estrone to male rabbits may depress the thyrotrophin content of the pituitary (Meites and Turner, 1947a) resulting in a decreased secretion of thyroid hormone, which reduces general body metabolism. Gessler (1936) and Sherwood (1938), showed that estrogens can effect a considerable reduction in the basal metabolic rate of guinea pigs and rats.

Mixner et al. (1944) suggested that the inhibiting effects of estrogen on lactation may involve the adrenal cortex. Estrogen can induce enlargement and increased secretion by the adrenal cortex, an effect which is mediated through the pituitary (Tepperman et al., 1943). An augmented secretion of certain adrenal cortical hormones would increase the rate of protein catabolism, with the production of glucose. This would have an unfavorable influence on milk production since the nitrogenous precursors of milk protein would be reduced.

Certain lines of evidence indicate that some of the inhibitory effects of estrogen on metabolism may not be mediated through the pituitary. Noble (1939a) and Reece and Leonard (1944) reported that the

inhibitory effects of estrogen on body growth may be direct. The latter noted that estrogen reduced growth or caused a loss in body weight of hypophysectomized rats, and that estrogen could also inhibit the effect of the growth hormone in these animals. Various important enzyme systems in the body may also be adversely affected by estrogens. McShan and Meyer (1946) found that diethylstilbestrol, hexestrol, and dienestrol were effective inhibitors of the succinoxidase system.

Part of the lactation-inhibiting effect of estrogen may be mediated through the nervous system. Turner (1939) states that the administration of estrogen might produce an exceedingly nervous condition associated with the production of "heat". Hain (1935) observed that after the administration of estrogen to lactating mother rats for five or six days, they deserted their nests and showed no further interest in their young. Weichert et al. (1942) similarly noted the loss of maternal instinct in mother rats injected with estrogens.

B. Experimental

1. EFFECT OF LARGE DOSAGES OF DIETHYLSTILBESTROL AND TESTOSTERONE PROPIONATE ON THE PITUITARY LACTOGEN CONTENT AND MILK SECRETION IN RATS. - Injection of large dosages of diethylstilbestrol into male guinea pigs does not reduce, but causes a small increase in the pituitary lactogen content (Meites and Turner, 1947a). Would large dosages of diethylstilbestrol and testosterone propionate suppress the lactogenic hormone and lactation in the postpartum rat?

Eighteen parturient rats were divided into three equal groups. Six rats served as controls and received no treatment; six were injected subcutaneously with two milligrams of diethylstilbestrol daily (approximately equal to 100,000 I.U. of estrone) for the first six days postpartum; and the remaining six were injected with two milligrams of testosterone propionate daily for the first six postpartum days. All were killed on the seventh day. The size of each litter was determined on the day of birth and on the seventh day, and their stomachs were examined for the presence of milk.

The control rats contained an average of 5.9 R.T. lactogen units per pituitary on a body weight basis (Table 4). Their mammary glands were fully distended with milk, and all the young were alive. The diethylstilbestrol treated rats contained an average of 8.0 R.T. lactogen units per pituitary on a body weight basis, which represents an increase of 35 per cent. The mammary glands of this group contained slightly less milk than the control rats, and 38 per cent of the young were dead by the seventh day. All the living young were found to have some milk in their stomachs. The pituitaries of the testosterone propionate treated rats showed no change in lactogen content on a body weight basis, but a slight increase was indicated per milligram of pituitary tissue. Their mammary glands were rated slightly below the control rats, and 11 per cent of the young were dead by the seventh postpartum day.

These results show that large dosages of diethylstilbestrol or testosterone propionate do not inhibit the production of lactogenic hormone by the pituitary. Although mammary secretion was slightly less than in the control rats, it is doubtful that this was responsible for the death of the young.

Table 4. Effect of Diethylstilbestrol and Testosterone Propionate on the Pituitary Lactogen Content and Milk Secretion of Seven Day Postpartum Rats

No. of Rats	Total Amt. of Hormone Inj. per Rat	Av. Body Weight gms.	Av. Pit. Weight mgs.	Av. R.-T. Lactogen Units per pit.	per mg. pit.	per 100 gms. body weight	Av. % of Young Dead on 7th Day	Av. Mammary Secretion
6	Controls	182	14.08	10.7	0.7	5.9	0	++++
6	Diethylstilbestrol 12 mgs.	176	14.12	14.2	1.0	8.0	38	+++
6	Testosterone Propionate, 12 mgs.	202	13.46	12.2	0.9	6.0	11	+++

(Meites and Turner, 1942a)

2. A COMPARISON OF THE POTENCY OF VARIOUS ORALLY ADMINISTERED ESTROGENS IN INHIBITING LACTATION IN GOATS. - Mixner, Meites and Turner (1944) investigated the inhibiting action of injected and orally administered diethylstilbestrol in lactating goats. They found the degree of inhibition was roughly proportional to the dosage of hormone given (Fig. 3). When administered orally, it required approximately 100 times as much hormone to depress lactation as when injected.

The purpose of this experiment was to determine which of the several available synthetic estrogens were most effective orally in inhibiting established lactation in goats. This information would be of value in inducing milk secretion in virgin or nonparous goats and cows through feeding the most potent hormones.

Six grade Toggenburg goats in the declining phase of lactation were used. They were paired into three groups of two each, and were injected during the summer and early fall of 1946. A mixed grain ration and alfalfa or lespedeza hay were fed, and the goats were milked twice daily.

The following hormones were administered orally by capsule: diethylstilbestrol (DES), dimethyl ether of diethylstilbestrol (DMEDES), dipropionate of diethylstilbestrol (DPDES), hexestrol, benzestrol and monomestrol. The hormones were given for either ten or five successive days to two goats each, and the goats were then allowed ten days for recuperation before they were treated again. The potency of each hormone was determined by comparing the average daily milk production in each goat for the three days prior to administering the capsules, with the lowest daily milk yield attained during or a few days after the last capsule had been given.

The relative effectiveness of each of these hormones in the individual goats may be seen in Table 5. In goats 438 and 304, 50 mgs. of DMEDES or 200 mgs. of monomestrol were completely without effect. Hexestrol at a 200 mg. level proved to be more potent than 400 mgs. of diethylstilbestrol. In goats 156 and 800, 100 mgs. of DMEDES caused a 26 and 52 per cent drop in milk yield, while 200 mgs. of hexestrol brought about the complete inhibition of milk secretion.

In goats 279 and 308, 200 mgs. of DMEDES effected a 31 and 77 per cent drop in milk yield, whereas the same amount of hexestrol elicited

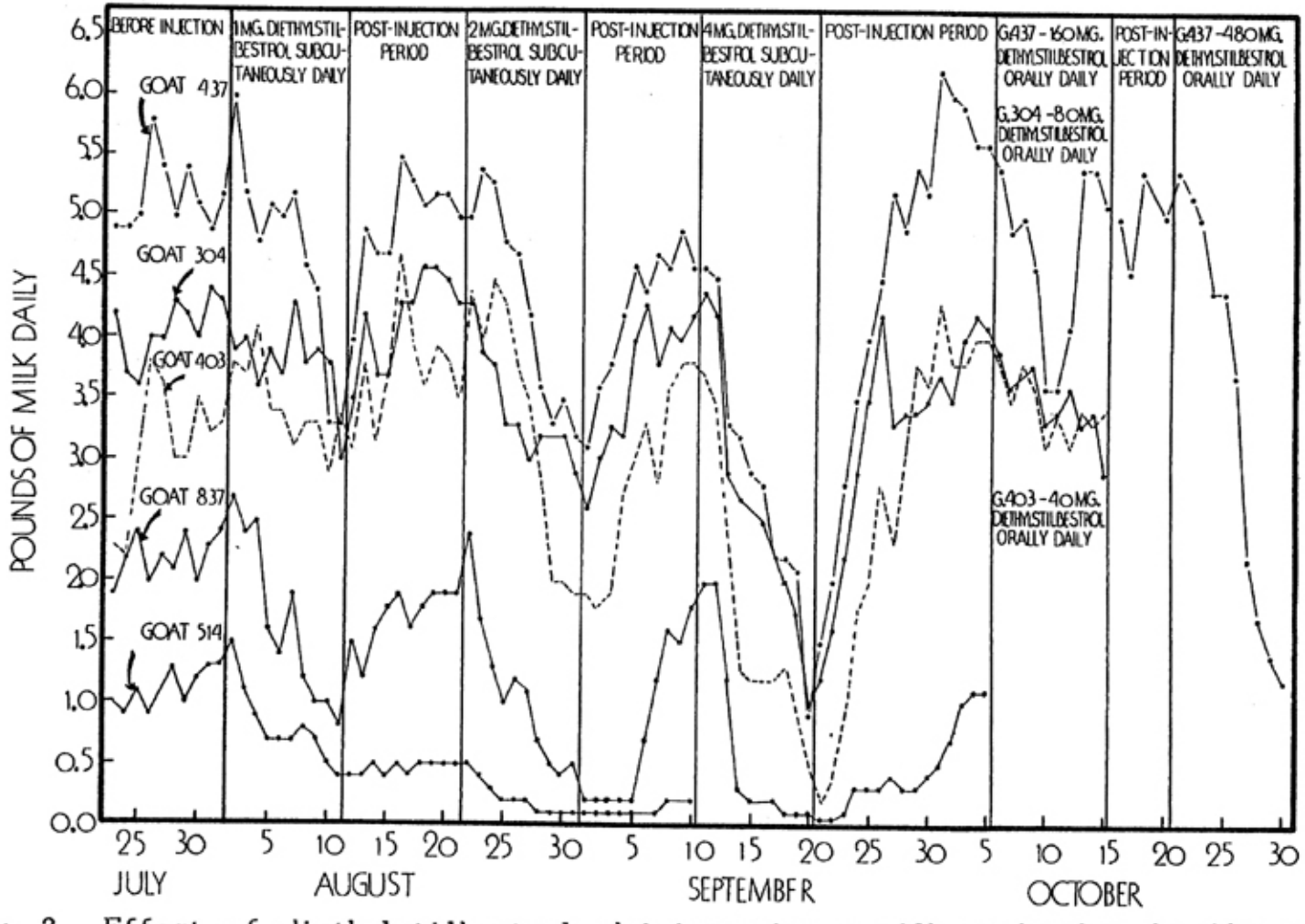

Fig. 3. Effect of diethylstilbestrol administration on milk production in five parous goats. Note the depression in milk yield coincident with the injection of 1.0 mg., 2.0 mg. and 4.0 mg. of diethylstilbestrol daily for ten-day periods, respectively. Also note the depression in milk production starting October 6, following the oral administration of diethylstilbestrol.

TABLE 5. THE RELATIVE POTENCIES OF VARIOUS ORALLY ADMINISTERED ESTROGENS IN DEPRESSING LACTATION IN INDIVIDUAL GOATS

Goat No.	Trial No.	Hormone	Length of Treatment	Daily Milk Yield		Percent Change
				Av. 3 days before treatment	Lowest Yield During or After Treatment	
			days	lbs.	lbs.	
438	1	50 mgs. DMEDES	10	4.3	4.7 (10 day av.)	+ 9
"	2	" " "	10	4.3	4.1 (10 day av.)	- 4
"	3	200 mgs. Monomestrol	10	3.7	4.1 (10 day av.)	+10
"	4	400 mgs. DES	5	3.6	2.2	-39
"	5	200 mgs. Hexestrol	5	2.8	1.4	-50
304	1	50 mgs. DMEDES	10	3.2	3.2 (10 day av.)	0
"	2	" " "	10	3.3	3.5 (10 day av.)	+ 7
"	3	200 mgs. Monomestrol	10	3.1	3.3 (10 day av.)	+ 7
"	4	400 mgs. DES	5	3.1	2.4	-22
"	5	200 mgs. Hexestrol	5	2.5	1.1	-56
156	1	100 mgs. DMEDES	10	4.4	2.1	-52
"	2	" " "	10	3.0	2.2	-26
"	3	200 mgs. Hexestrol	10	2.4	0.0	-100
880	1	100 mgs. DMEDES	10	3.4	1.6	-52
"	2	" " "	10	2.6	1.5	-42
"	3	200 mgs. Hexestrol	10	2.3	0.0	-100
279	1	200 mgs. DMEDES	10	4.5	3.1	-31
"	2	" " "	10	3.6	1.9	-47
"	3	200 mgs. DPDES	10	2.5	2.5	0
"	4	200 mgs. Benzestrol	5	3.5	2.6	-25
"	5	400 mgs. DMEDES	5	2.6	1.1	-57
"	6	200 mgs. Hexestrol	5	2.5	0.8	-68
108	1	200 mgs. DMEDES	10	4.0	1.5	-62
"	2	" " "	10	2.7	0.6	-77
"	3	200 mgs. DPDES	10	1.7	1.2	-29
"	4	200 mgs. Benzestrol	5	2.7	1.8	-33
"	5	400 mgs. DMEDES	5	2.0	0.2	-90

a 68 per cent reduction. The dipropionate of diethylstilbestrol was relatively ineffective at the 200 mg. level, and benzestrol was only moderately effective at this level. Administration of 400 mgs. of DMEDES daily for five days was no more effective than 200 mgs. of hexestrol given for the same period.

In Table 6, the six synthetic hormones were rated for their effectiveness in depressing lactation as follows: hexestrol, DMEDES, benzestrol, dipropionate of diethylstilbestrol, diethylstilbestrol, monomestrol. Diethylstilbestrol was rated above monomestrol because Mixner et al. (1944) showed that the oral administration by capsule of 160 mgs. of this hormone was partially effective in inhibiting lactation, whereas 200 mgs. of monomestrol was totally ineffective.

3. THE INFLUENCE OF DIET ON THE ABILITY OF HEXESTROL TO DEPRESS LACTATION. - Since hexestrol was the most potent of the various orally administered estrogens in depressing lactation, it was used to determine the possible effects of estrogen on some blood and urine constituents.

Two goats, 304 and 438 were placed on specially built metabolism cages. To prevent scattering of hay in the cages, the ration included one-half "cerogras" (a dehydrated and finely ground cereal grass rich in vitamins) in the regular grain mix, and freshly cut green grass instead of hay.

Since a gradual depression of milk secretion was desired the goats were started at 50 mgs. of hexestrol daily by capsule for five days.

TABLE 6. THE RELATIVE POTENCIES OF VARIOUS ORALLY ADMINISTERED ESTROGENS IN DEPRESSING LACTATION IN GOATS

Goat No.	Trial No.	Hormone	Length of Treatment days	Daily Milk Yield		Rating of Potency
				% Change	Av. % Change of All Trials	
438	1	50 mgs. DMEDES	10	+ 9)		
"	2	" " "	10	- 4)	+3	
304	1	" " "	10	0)		
"	2	" " "	10	+ 7)		
156	1	100 mgs. DMEDES	10	- 52)		
"	2	" " "	10	- 26)		
880	1	" " "	10	- 52)	-43	
"	2	" " "	10	- 42)		
279	1	200 mgs. DMEDES	10	- 31)		
"	2	" " "	10	- 47)	-54	
108	1	" " "	10	- 62)		2
108	2	" " "	10	- 77)		
279	5	400 mgs. DMEDES	5	- 57	-73	
108	5	" " "	5	- 90		
156	3	200 mgs. Hexestrol	10	-100)	-100	
880	3	" " "	10	-100)		1
438	5	" " "	5	- 50)	- 53	
304	5	" " "	5	- 56)		
438	3	200 mgs. Monomestrol	10	+ 10)	+ 8	6
304	3	" " "	10	+ 7)		
279	3	200 mgs. DPDES	10	0	- 14	4
108	3	" " "	10	- 29		
279	4	200 mgs. Benzestrol	5	- 25	- 29	3
108	4	" " "	5	- 33		
438	4	400 mgs. DES	5	- 39	- 30	5
304	4	" " "	5	- 22		

No depression of milk occurred, and the level was increased to 100 mgs. daily for the next five days. Surprisingly, there was still no lowering of the milk yield. The dosage was increased to 200 mgs. daily for the following five days. Still there was no reduction in milk yield, although in the previous experiment this amount of hexestrol in these same goats caused a 50 and 56 per cent drop in milk production. An increase to 300 mgs. daily for five days had no effect on lactation.

These two goats had been given hexestrol at increasing levels for 20 successive days with no effect on their lactation. Since the only important difference in their treatment on the metabolism cages was the change in diet, this was suspected to be responsible for the ineffectiveness of the hexestrol. After the two goats had been on the regular ration for approximately three weeks, they were again given 200 mg. capsules of hexestrol daily for five days. A prompt depression of approximately 50 per cent in daily milk yield occurred.

Ten days after the ration was changed again. The regular grain mixed with one-half part of "cerograss" and the regular dry hay was fed. Hexestrol was given at the 200 mg. level for five days, and a 22 and 29 per cent reduction in milk yield was observed.

Whether the ineffectiveness of the hexestrol on lactation was due to a greater vitamin content in the "cerograss" and fresh green grass, or to the possibility that the goats ate more, is not definitely known. Many studies have shown that the natural estrogens are inactivated in the liver, and that the B vitamins are essential for this purpose (Biskind, 1946). Apparently, the synthetic estrogens are also inactivated in the liver, although to a lesser degree than the natural estrogens (Zondek et al., 1943; Lipschutz et al., 1944; and Zimmerberg, 1946).

It is believed that ruminants manufacture sufficient B vitamins for their own ordinary needs (see Brody, 1945). However, it is possible that a large amount of a foreign substance, such as hexestrol, represents an extraordinary need for B vitamins which the goat is not able to supply. Hence the effectiveness of an additional source of B vitamins from the "cerogras" and freshly cut green grass.

It is possible that these goats may have eaten more because they found the mixture of grain and "cerogras", and fresh cut grass, more palatable than the regular ration. An increased feed intake might serve to offset some of the inhibitory effects of estrogen on lactation. Although the feed intake of these goats was not measured, they showed no loss in body weight during the 20 day trial with hexestrol.

4. EFFECT OF ESTROGEN ADMINISTRATION ON THE METABOLISM OF LACTATING GOATS. - When the two goats in the previous experiment were placed on the metabolism cages and injected subcutaneously with four milligrams of diethylstilbestrol daily, this not only resulted in a drastic reduction in milk yield, but also in a loss of appetite and a reduction in feces and urine output. Although the food intake was not measured, it was obvious by the third day of injection, that they refused to eat or drink. They both lost seven pounds each during the three day period. It therefore seemed possible that one of the mechanisms through which large dosages of estrogen exert their inhibitory effects on lactation might be by way of loss in appetite. A reduced food intake is known to cause a quick decline in daily milk production in rats, goats, and cows (Brody, 1945).

Two goats, 348 and 123, which kidded in the same week of March, 1947, and were producing about the same quantity of milk, were put on metabolism cages about a month later. The feeding troughs in front of the cages were so arranged that it was possible to keep the grain and hay in separate compartments. After determining the optimal amounts necessary to maintain production, the goats were regularly given 3 pounds of mixed grain and 3 pounds of cut hay per day. Separate buckets for each goat were filled with 10 liters of water daily. At the end of each 24 hour period the unused portions of the grain, hay, and water were measured, as well as the feces and urine output.

Diethylstilbestrol was injected at a dosage level of 0.25 mgs. daily for five days, and was then increased to 0.50 mgs. daily for the following five days. These levels of diethylstilbestrol had no significant effect either on milk yield or the metabolism of the goats (Fig. 4). Mixner et al. (1944) also found these dosages too small to depress lactation in goats.

When the injection level of diethylstilbestrol was raised to one milligram daily, the effects became noticeable within 24 to 48 hours. The rapid decline in milk yield was paralleled by a similar decline in feed and water intake, and feces and urine output (Fig. 4). Goat 123 was much more severely affected by the estrogen treatment than goat 348. Although it was intended to inject these animals for a 10 day period, the drastic effects of the treatment on goat 123 made it seem advisable to stop injections after 8 days. This goat had ceased to eat and drink, and had lost 21 pounds in body weight.

After the injections were stopped, the food intake and milk yield gradually increased. After a week, the goats regained their appetites completely, but the milk production did not quite regain its original level.

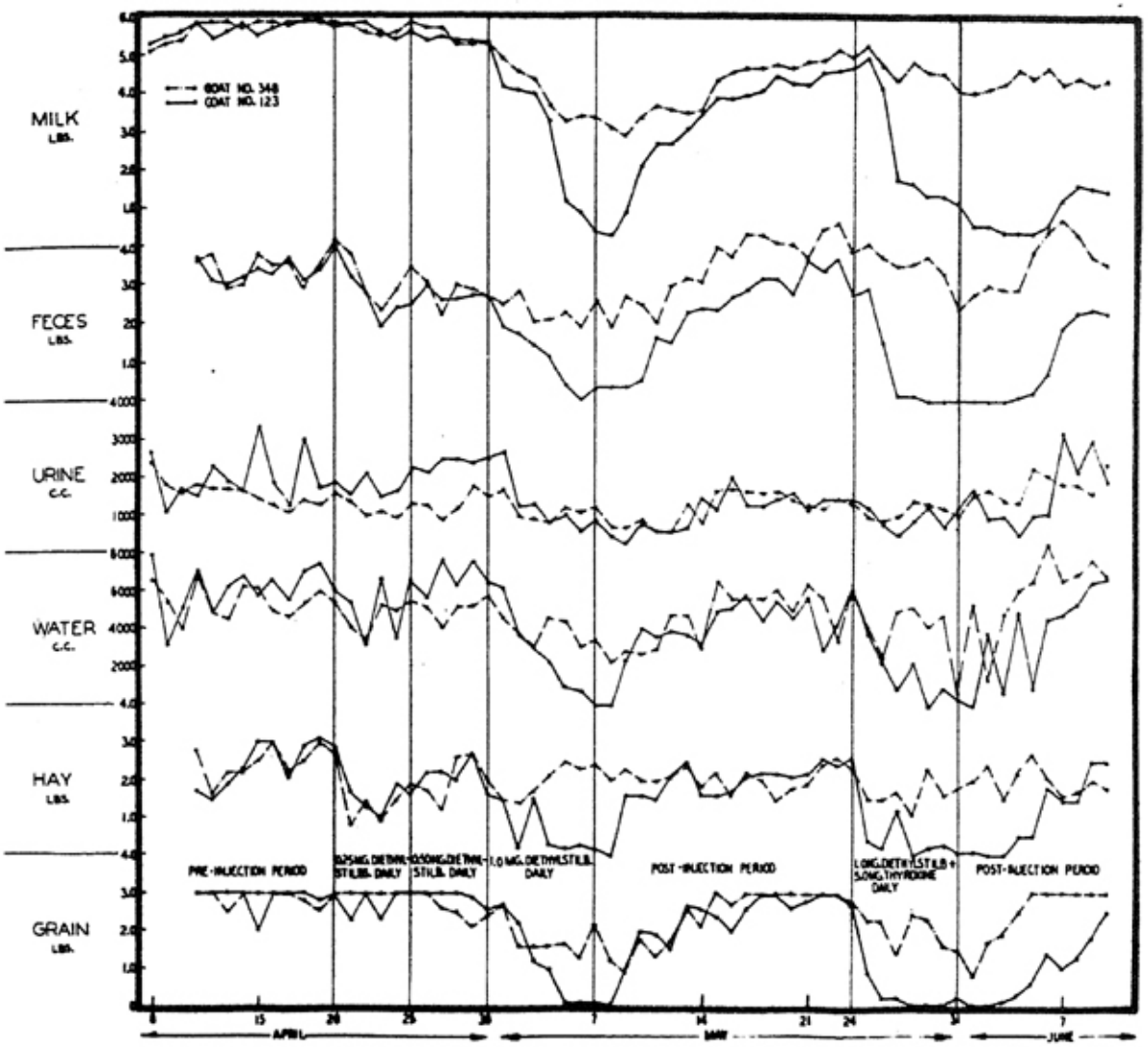

Fig. 4. Effect of diethylstilbestrol, and of diethylstilbestrol plus thyroxine, on lactation and metabolism of two parous goats. Note the quick depression in milk yield and food and water intake which occurred following the administration of 1.0 mg. of diethylstilbestrol daily, starting on April 30. In both trials the effects were more drastic in goat 123 than in goat 348. Note the differences in responses of the two goats caused by the combination of diethylstilbestrol and thyroxine.

TABLE 7. EFFECT OF DIETHYLSTILBESTROL ON METABOLISM OF LACTATING GOATS
(One mg. diethylstilbestrol daily for eight days)

Daily Values	Milk lbs.		Feces lbs.		Hay lbs.		Grain lbs.		Urine per 100 ml.		Water Intake per 100 ml.		Body Weight lbs.	
	348	123	348	123	348	123	348	123	348	123	348	123	348	123
Before	5.4	5.4	2.8	2.8	2.0	1.6	2.4	2.6	15.7	25.0	58.0	65.4	94	99
After	3.0	0.4	2.0	0.1	No significant change	0.0	1.0	0.0	6.2	3.0	29.4	0.0	88	78
Decrease	2.4	5.0	0.8	2.7		1.6	1.4	2.6	9.5	22.0	28.6	65.4	6	21
(One mg. diethylstilbestrol plus five mgs. thyroxine daily for eight days)														
Before	5.1	4.8	4.0	2.9	2.3	2.6	2.8	2.7	14.6	14.6	60.6	62.6	95	97
After	4.2	0.5	2.5	0.1	1.1	0.0	0.8	0.0	No significant change		7.3	0.0	82	69
Decrease	0.9	4.3	1.5	2.8	1.2	2.6	2.0	2.7			53.3	62.6	13	28

Some of the significant values of each component of the energy intake and outgo are presented in Table 7. These represent the values which were taken just before the first injection of diethylstilbestrol was begun and the lowest values which were reached during or soon after the last injection.

After a 16 day recuperation period, the two goats were injected with five milligrams of thyroxine daily in addition to one milligram of diethylstilbestrol. It was considered possible that this dosage of thyroxine might overcome some of the inhibitory effects of estrogen on milk production and food intake.

In goat 123 the effects of this combination of hormones on metabolism appeared to be more drastic in most respects than when diethylstilbestrol alone was given. Each goat lost seven pounds more in body weight at the end of the experiment. The urine output of both goats was not appreciably reduced during this treatment, probably due to the diuretic action of thyroxine. The milk yield of goat 348 was reduced by only 0.9 pounds compared to 2.4 pounds when diethylstilbestrol alone was given. Apparently she was able to maintain milk production at the expense of body weight, since she lost 13 pounds compared to only 6 in the previous trial. It will be shown that 10 milligrams of thyroxine daily can completely override the inhibitory effects of one milligram of diethylstilbestrol on lactation in goats.

A lactation-inhibiting dose of estrogen usually results in a loss of body weight in goats. For example, goats 437 and 826 were given 200 mgs. each of the dimethyl ether of diethylstilbestrol daily by capsule for 10 days. The milk yield in goat 437 was reduced from 7 pounds daily to 5 pounds, and she lost 9 pounds in body weight. The milk yield of goat 826 was reduced from 5.1 pounds daily to 1.2 pounds, and she lost 26 pounds in body weight.

There are two items worthy of mention in connection with this experiment. One is that these two goats came into heat a few days after the injection of the 0.5 milligram level of diethylstilbestrol. The other is that the loss in appetite may be selective in nature and not extend equally to all types of food. For example, when a handful of fresh green grass was occasionally offered to these goats when their appetites were most severely depressed by the estrogen treatment, they ate the grass with relish.

A large part of the ability of estrogens to depress lactation, in goats at least, is mediated through a loss of appetite. The relation of food and water intake to milk production is of obvious importance. Although administration of estrogens can increase the lactogen secretion of the pituitary to some degree even in starving animals (Meites and Turner, 1947a), this is of little value when there is a deficiency of nutrients with which to manufacture milk.

5. CONCLUSIONS. - The inhibitory action which large dosages of estrogen are capable of exerting on body growth, gonad function, and lactation have been appreciated generally. The influence which estrogens may exert on appetite has been largely overlooked in considering these inhibitory effects. There are only a few reports in which reduction in food intake were noted following estrogen administration.

Korenchevsky and Dennison (1934) injected either 20, 60 or 180 I.U. of estrogen daily to rats, and observed that the latter two dosages caused a decrease in food consumption of 11 per cent. However, on a body weight basis, the food intake was unchanged. Blanchard et al. (1945) reported that the implantation of 60 milligram tablets of benzestrol into rats reduced the food intake to an average of only 9 grams daily compared to 12-14 grams consumed by the control rats. Cameron et al. (1946) likewise observed that rats fed estradiol appeared to eat less food. Noble (1939b) noted that estrogen causes a decrease in the fluid intake of rats.

Through what channels large dosages of estrogen may induce a decrease in appetite is not definitely known. A reduction in the secretion of certain metabolic hormones by the pituitary, a direct inhibitory effect of important enzyme systems, an unfavorable influence on the nervous system--all could effect a reduction in appetite. In connection with the latter possibility (Brobeck et al., 1947) studied the energy exchange in rats during the normal estrous cycle, and observed that food intake and body weight were reduced during estrus, while the opposite was true during diestrus and pseudo-pregnancy. They stated that these effects were probably mediated through the hypothalamus, which is believed to exert a regulative effect on metabolic functions in the body.

Although a decrease in appetite appears to be an important part of the lactation-inhibiting action of large dosages of estrogen in goats, and probably other animals as well, it is not believed to be the sole cause of the reduction in milk secretion. It does not explain the greater effectiveness of estrogen in intact as compared to ovariectomized rats. It also may not apply to the lactation-inhibiting action reported for certain androgens, although McEuen et al. (1937) and Selye (1941) have demonstrated that large dosages of testosterone can reduce growth in rats.

III. THE EXPERIMENTAL AUGMENTATION OF LACTATION

A. Review

The declining phase of the lactation curve is associated with a gradual involution of mammary secretory tissue and a decrease in secretion of lactogenic hormone by the pituitary. Thyroid secretion rate also declines during the course of lactation (Schultze and Turner, 1945). Apparently the suckling stimulus alone is insufficient to maintain lactation at a high level.

In attempting to augment or prolong milk secretion, it seems important that the original stimuli present during pregnancy and at parturition be restored. During pregnancy optimal growth of the mammary glands occurs under the influence of estrogen and progestin, and at about the time of parturition sufficient estrogen is available to enhance greatly the secretion of pituitary lactogen. Other favorable influences in preparation for lactation may be exerted. Can these stimuli be duplicated as lactation proceeds?

Various pituitary extracts have been used successfully for increasing lactation in cows and goats (Turner, 1939); Folley, Malpress and Young, 1945). Whole pituitary extracts contain thyrotrophin, which stimulates the thyroids. They also contain gonadotrophic and adrenotrophic hormones, which stimulate the gonads and adrenals to secrete various steroid hormones, which in turn may stimulate the growth of the mammary tissue. Not least important, whole pituitary extracts contain lactogenic hormone--the specific factor which stimulates milk secretion in the mammary gland.

Although whole pituitary extracts would seem ideal from the above viewpoints, there are valid objections to their use. The extracts must be injected, must be preserved at a low temperature, and are expensive.

Thyroprotein, a thyroid like synthetic substance, offers greater practical possibilities for increasing lactation, since it can be fed to animals. Its favorable effect on lactation is generally attributed to its stimulation of certain metabolic activities, i.e. increase in food consumption, assimilation, blood circulation, and in the availability of milk precursors, etc. (Ralston et al. 1940). It does not appear to induce mammary growth or stimulate the secretion of lactogenic hormone by the pituitary.

Estrogen has been used successfully for initiating lactation, but has generally been considered inhibitory or ineffective for use in established lactation. It stimulates the secretion of lactogenic hormone by the pituitary and induces a variable degree of mammary growth. Potent preparations can be given orally to animals. The feasibility of administering small amounts of estrogen throughout the lactation cycle has not yet been investigated. Since estrogen can increase the secretion of lactogenic hormone by the pituitary and induce some growth of the mammary gland, while thyroid preparations can speed up metabolic processes favorable to lactation, the two together should be superior to either alone.

Weichert and Boyd (1934) observed that in pregnant rats, experimental hyperthyroidism induced earlier mammary development and secretion than in control rats. Mixner and Turner, (1943) observed that thyroxine in suitable amounts increased the efficiency of progesterone and estrone in stimulating lobule-alveolar mammary growth in mice. It is not known whether thyroid and estrogen can act synergistically in increasing pituitary lactogen secretion.

B. Experimental

1. EFFECT OF A SMALL DOSAGE OF DIETHYLSTILBESTROL ON THE PITUITARY LACTOGEN CONTENT AND BODY WEIGHT OF PARTURIENT RATS. - Injection of large dosages of diethylstilbestrol to rats for the first six postpartum days induced a small increase, rather

than a decrease in pituitary lactogen content. Would small amounts of estrogen, injected daily during the normal span of lactation in the rat, maintain a high pituitary lactogen content without depressing lactation? If this were possible, it might afford a means of augmenting and maintaining established lactation.

Fifty-four parturient rats divided into four pairs of control and injected animals, were killed on the 3rd, 7th, 14th, and 21st day after parturition. On the third postpartum day the litter of each was reduced to six. A total dosage of 0.2 mg. of diethylstilbestrol by daily injection was given every mother rat during its respective postpartum period before sacrifice. The rats to be killed on the third postpartum day received the hormone in a single injection on the day of parturition.

Results can be seen in Table 8 and Figure 5. In the group sacrificed on the third postpartum day, the pituitary lactogen content was increased 36 per cent on a body weight basis. In the rats killed on the 7th, 14th and 21st days after parturition, the pituitary lactogen content was increased 52, 25 and 68 per cent, respectively. On the 14th and 21st days after parturition, the injected groups contained as much pituitary lactogen as in the control rats on the 3rd day, when the pituitary lactogen content was at its peak level.

Evaluation of the effects of the diethylstilbestrol on lactation is more difficult. Insofar as could be judged by macroscopic appearance, the mammary glands of the injected rats were as full of milk as the control rats. There was no loss of maternal instinct, as the mother rats were seen to be with their litters in the nests on almost all occasions.

The young of the injected groups, with the exception of those killed on the third postpartum day, made smaller weight gains than the control litters. The ingestion of estrogen in the milk may have accounted in part for this growth difference. Also it is possible that the dosage of diethylstilbestrol (0.2 mg.) was above an optimal level, since most of the injected mother rats lost slightly in body weight whereas the control mother rats showed small gains.

This experiment demonstrates the possibility of maintaining a level of pituitary lactogen after parturition by the administration of relatively small dosages of estrogen.

2. EFFECT OF THYROXINE AND ESTROGEN-THYROXINE COMBINATIONS ON ESTABLISHED LACTATION IN GOATS. - When one milligram of diethylstilbestrol was injected daily into lactating goats, milk yield decreased. Addition of 5 milligrams of thyroxine to the estrogen was found to counteract partially the effect of the latter on the lactation of one of two goats. Would 10 milligrams of thyroxine completely overcome the effect of the estrogen on lactation?

Three goats, numbers 447, 304 and 438, were used. Having freshened in the spring of 1946, they were approaching the end of their lactation period, when injections were begun December 13. Milk production was between 1.1 and 2.2 pounds daily at the start of the experiments (Table 9).

The goats were injected for 10 days and then left a month without treatment. This schedule was repeated four times, starting on December 13, 1946, and ending May 16, 1947.

In the first trial they were injected as follows: number 447 received 1 mg. of diethylstilbestrol daily, and numbers 304 and 438 were given 1 mg. of diethylstilbestrol plus 10 mgs. of thyroxine daily. The trend of the results can be seen in Figure 6(a).

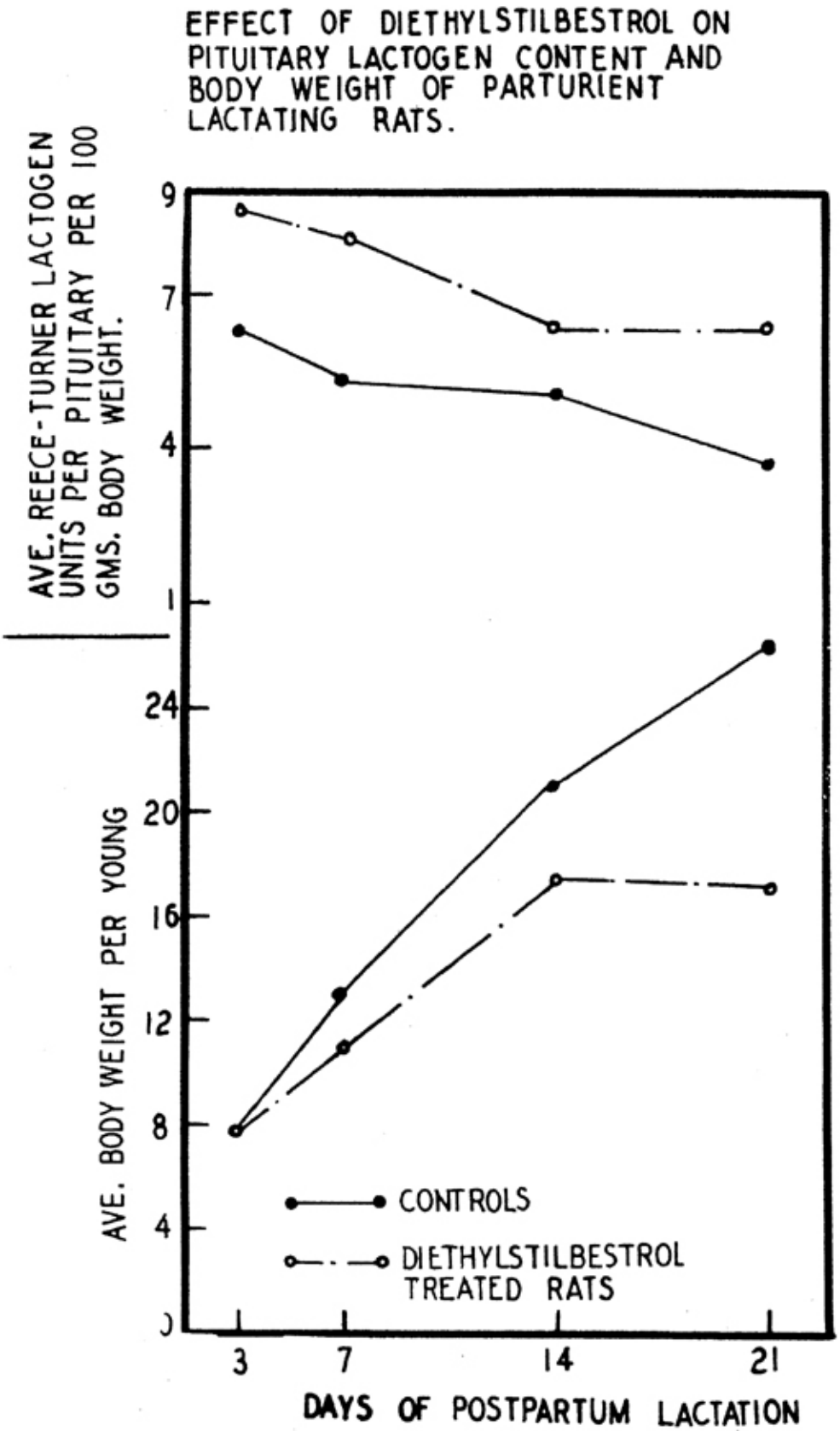

Fig. 5. Effect of a small dosage of diethylstilbestrol injected during the lactation cycle of rats. Note that the pituitary lactogen content of the injected rats was always greater than in the controls. However, the litters of the controls grew more rapidly than those of the injected rats.

As expected, the daily milk yield of goat 447 was depressed during the 10 day injection period, from 2.2 to 1.2 pounds. The two goats receiving both hormones began to show a remarkable and unexpected rise in milk yield, after four days treatment. Ten mgs. of thyroxine was more than sufficient to offset the depressing effect of one mg. of diethylstilbestrol.

After the injections were stopped, the daily milk yield continued to rise for four days. It had increased from 1.1 to 2.7 pounds daily in goat 304, or by 145 per cent; and from 1.8 to 4.4 pounds daily in goat 438, or by 144 per cent. Milk production from goat 447 returned to the normal level after the diethylstilbestrol injections were stopped. After a month, the daily milk production of goats 304 and 438 still was more than 60 per cent above the pre-injection levels.

The effect of the injections on the body weights of these three goats is shown in Table 9. Number 447, which received diethylstilbestrol only, lost 7 pounds in body weight, whereas the two goats which received both hormones lost 18 pounds each. Previously, when two goats were given 1 milligram of diethylstilbestrol plus 5 mgs. of thyroxine, the loss in body weight also was greater than when the goats were given the estrogen alone.

TABLE 8. EFFECT OF A SMALL DOSAGE OF DIETHYLSTILBESTROL ON PITUITARY LACTOGEN CONTENT AND BODY WEIGHT OF PARTURIENT LACTATING RATS

No. of rats	Treatment	Day of Sacrifice Post-partum	Average Body Weight				Av. Pit. Wt.	Av. R.T. Lactogen			Average % increase in lactogen of injected groups
			On 3rd Day		On Day of Sacrifice			per pit.	per mg. pit.	per 100 gms. body wt.	
			Mother	Young	Mother	Young					
			gms.	gms.	gms.	gms.	mgs.				
8	Controls	3	194	6.9	194	6.4	9.87	12.2	1.3	6.3	
8	0.2 mgs. Diethylstil.	3	196	6.8	196	6.8	11.37	16.8	1.5	8.6	36
6	Controls	7	203	6.7	206	13.0	10.00	11.0	1.1	5.3	
6	0.2 mgs. Diethylstil.	7	186	6.3	183	11.0	11.61	14.8	1.3	8.1	52
6	Controls	14	199	7.6	203	21.0	9.69	10.3	1.1	5.1	
6	0.2 mgs. Diethylstil.	14	221	8.1	219	17.4	14.74	14.1	1.0	6.4	25
7	Controls	21	185	7.3	196	26.2	9.42	7.4	0.8	3.8	
7	0.2 mgs. Diethylstil.	21	184	7.4	179	17.0	10.61	11.6	1.1	6.4	68

TABLE 9. EFFECT OF ESTROGEN, THYROXINE, AND ESTROGEN-THYROXINE COMBINATIONS ON LACTATION IN GOATS

Goat No.	Trial No.	Daily Treatment (10 day period)	Body Weight lbs.			Milk Yield (lbs.)			
								Diff.	
			Before	After	Diff.	Before	After	lbs.	%
447	1	1 mg. Stilb.	114	107	- 7	2.2	1.2	-1.0	- 45
438	1	1 mg. Stilb. + 10 mgs. Thyrox.	113	95	-18	1.8	4.4	+2.6	+144
304	1	1 mg. Stilb. + 10 mgs. Thyrox.	110	92	-18	1.1	2.7	+1.6	+145
438	2	10 mgs. Thyrox.	107	97	-10	2.9	4.5	+1.6	+ 55
304	2	10 mgs. Thyrox. + 1 mg. Stilb.	105	96	- 9	2.0	3.4	+1.4	+ 70
438	3	10 mgs. Thyrox. + 1 mg. Stilb.	107	103	- 4	2.7	4.7	+2.0	+ 74
304	3	10 mgs. Thyrox.	107	105	- 2	1.9	3.7	+1.8	+ 99
438	4	3 mgs. Thyrox.	115	116	+ 1	3.9	4.6	+0.7	+ 18
304	4	3 mgs. Thyrox. + 0.25 mgs. Stilb.	113	116	+ 3	2.7	4.2	+1.5	+ 55

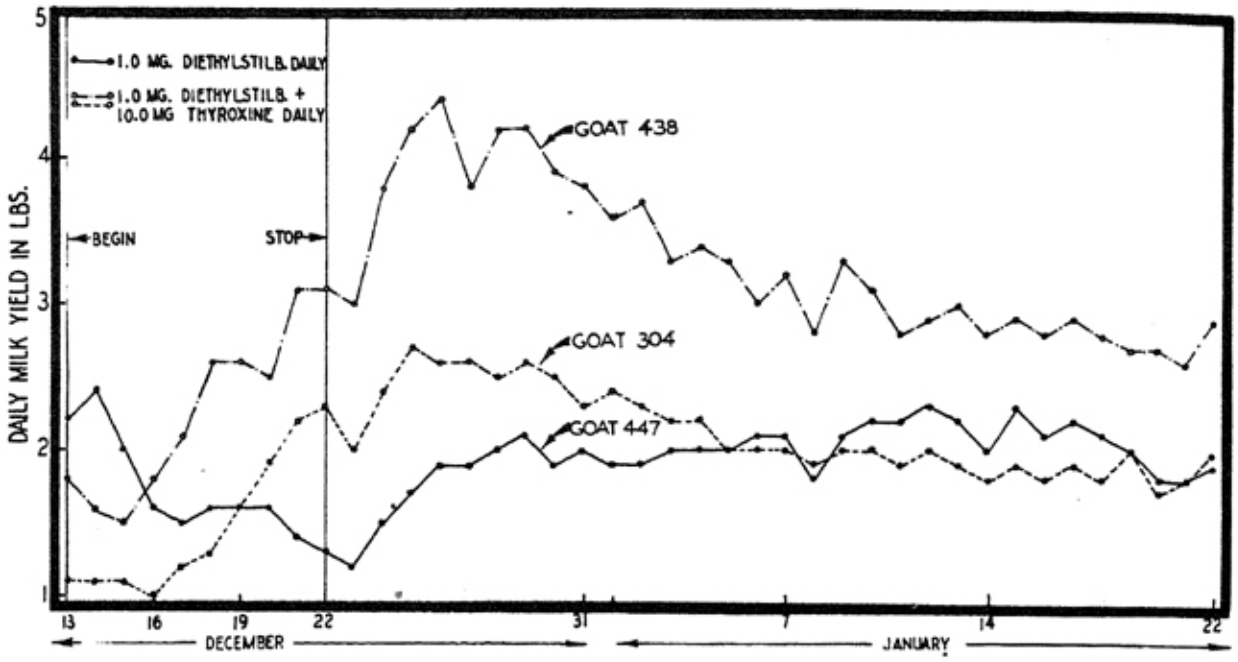

Fig. 6 (Section a). - Effect of thyroxine, and of estrogen-thyroxine injections, on established lactation in goats.

One mg. daily of diethylstilbestrol reduced the daily milk yield of goat 477 by 45 per cent. The same amount of estrogen plus ten mgs. of thyroxine daily increased the daily milk production in goats 438 and 304 by 144 and 145 per cent, respectively. Note that milk production continued to rise in these two goats for several days after the injections were stopped, and then gradually fell. On January 22, the milk yield of these two goats still was more than 60 per cent above the original level.

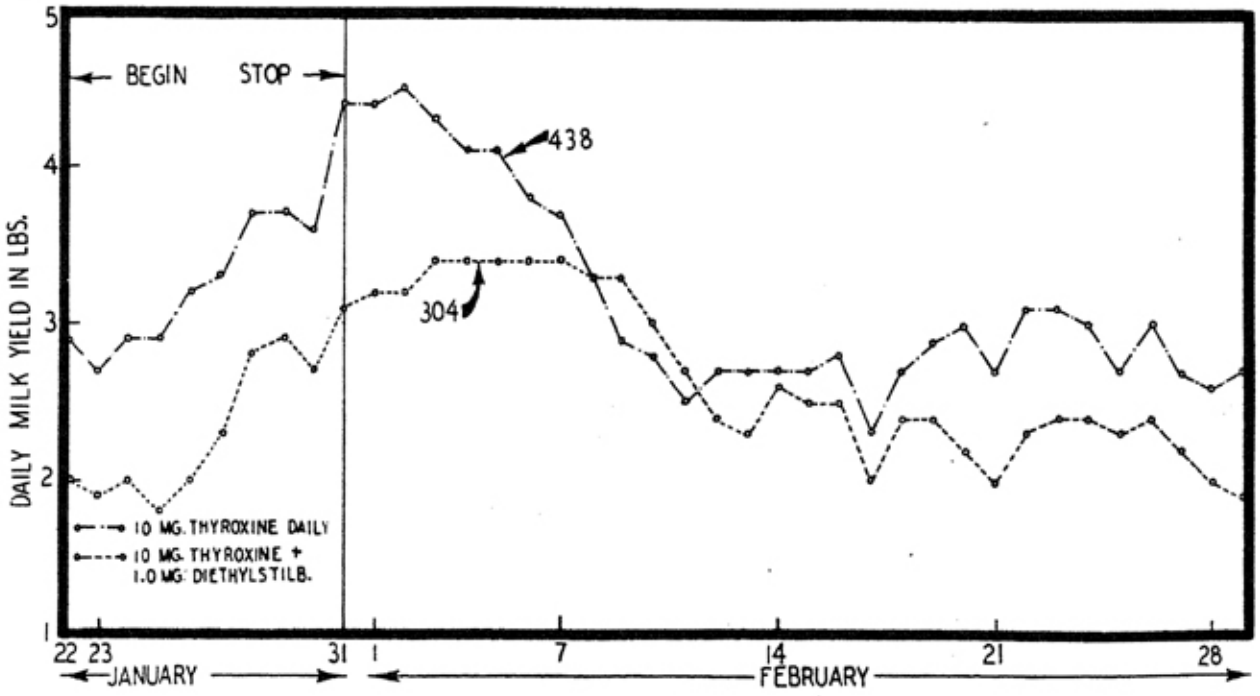

Fig. 6 (Section b). - Effect of thyroxine, and of estrogen-thyroxine injections, on established lactation in goats.

Goat 438 was given thyroxine only, and goat 304 received both hormones. Daily production in goat 438 rose to within 0.1 pound of the peak in the previous trial, whereas in goat 304 it rose 0.7 pounds higher than in the previous trial. Note the better persistence in goat 304.

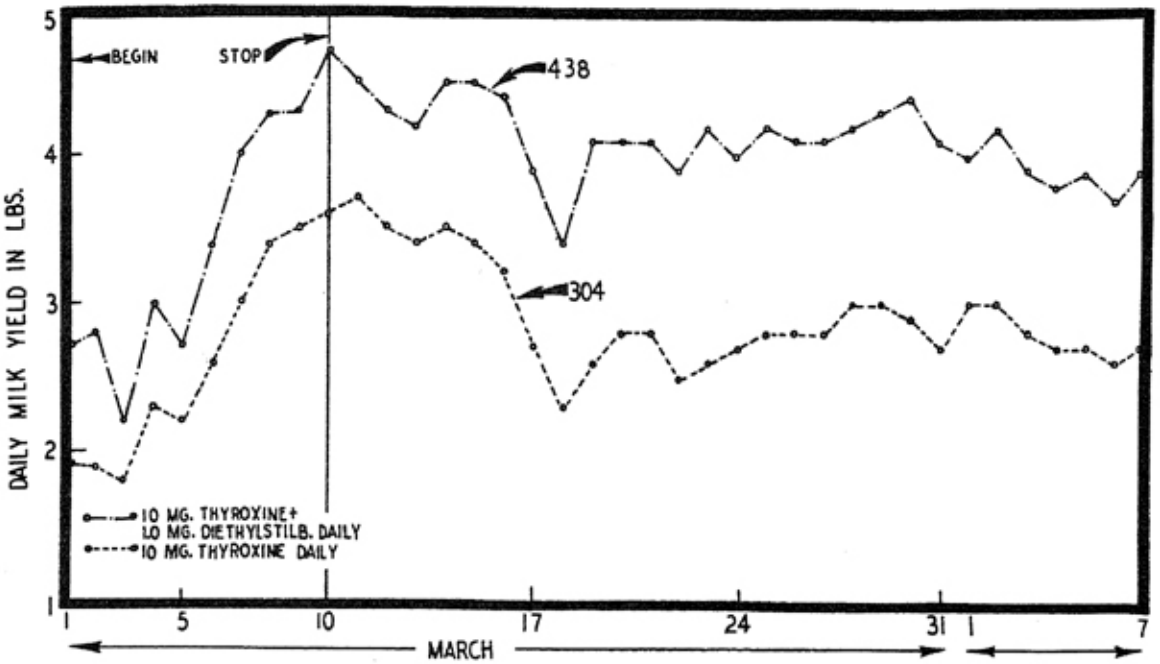

Fig. 6 (Section c). - Effect of thyroxine, and of estrogen-thyroxine injections, on established lactation in goats.

Treatment of the two goats was reversed. On a percentage basis, milk production rose somewhat more in goat 304, which received thyroxine only, than in goat 438 but this difference does not seem significant in the chart. Note the higher level of persistence in goat 438.

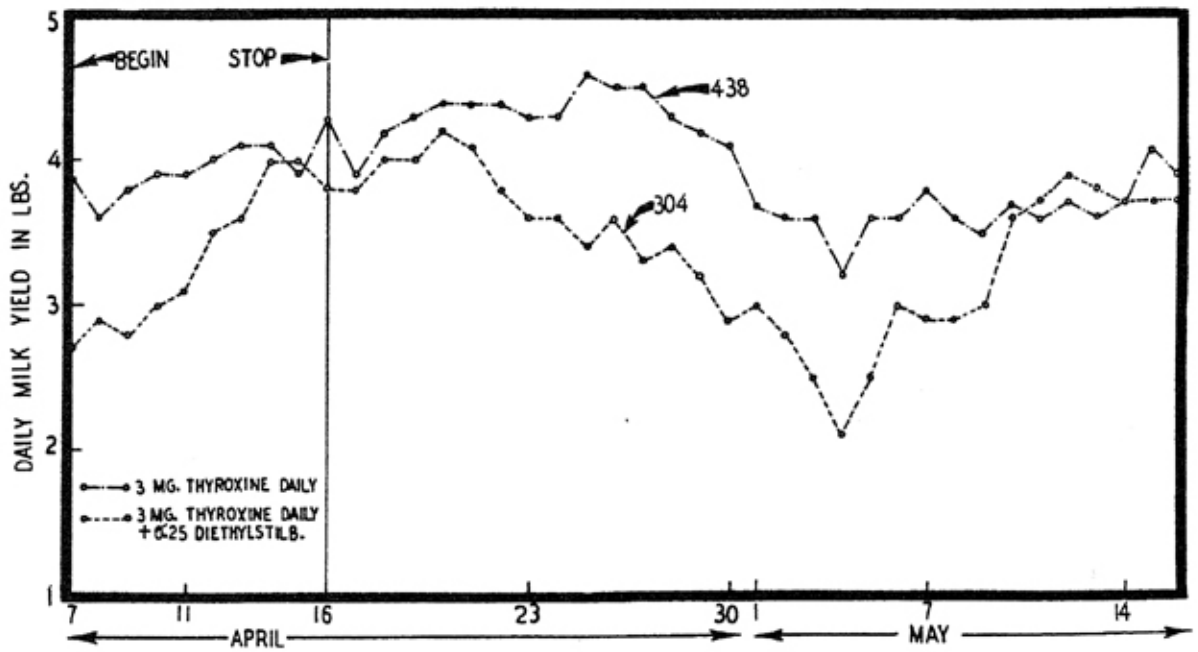

Fig. 6 (Section d). - Effect of thyroxine, and of estrogen-thyroxine injections, on established lactation in goats.

Smaller amounts of the two hormones were used. The superiority of the two hormones over estrogen alone is clearly indicated in goat 304 during the injection period. The goats were turned out to pasture May 3. Compare the present milk production with that at the beginning of the experiment.

To determine how much of the increase in milk yield of the two goats was due to thyroxine alone, and how much to the estrogen-thyroxine combination, number 438 was given 10 mgs. of thyroxine daily and number 304 was given 10 mgs. of thyroxine plus 1 mg. of diethylstilbestrol, beginning Jan. 22, 1947.

The daily milk yield of goat 438 rose from 2.9 to 4.5 pounds (Figure 6(b)) or to the same peak as in the previous trial, representing a 55 per cent increase in milk production. In goat 304, the daily milk yield rose from 2.0 to 3.4 pounds, which is 0.7 pounds higher than the peak in the previous trial, representing an increase of 70 per cent.

A week after the injections were stopped the daily milk production of goat 438 returned to the pre-injection level. The milk production of goat 304 fell more gradually, and did not reach its pre-injection level until the end of the month. The two goats lost only 9 to 10 pounds in body weight during this trial compared to 18 pounds before. However, they weighed 5 to 6 pounds less at the beginning of this trial. This experiment suggests that the estrogen-thyroxine combination is superior to thyroxine alone.

In the third trial, the hormone treatment of the two goats was reversed. This time, goat 304 was given 10 mgs. of thyroxine daily, and goat 438 was given the same amount of thyroxine plus 1 mg. of diethylstilbestrol. As seen in Figure 6(c), the daily milk yield rose again in both goats, but on a percentage basis the increase was greater in 304.

After the injections were stopped, the milk yield of 438 persisted at the high level of approximately four pounds daily for the entire month. The daily milk yield of number 304 also persisted better in this trial. It appears that number 438, which received both hormones, made the better record.

These goats lost only a few pounds body weight each in this third trial, although they started at about the same weight as in the previous trial. Either the goats had developed a certain resistance to the weight depressing effects of either or both hormones, or else they ate more. The latter seems more probable, since they were fed about 3 pounds of mixed grain daily with an unlimited supply of lespedeza and alfalfa hay.

On the fourth trial, smaller amounts of the two hormones were administered. Goat 438 was injected with 3 mgs. of thyroxine daily, and goat 304 with the same amount of thyroxine plus 0.25 mg. of diethylstilbestrol. This amount of diethylstilbestrol previously had been found to be optimal for initiating lactation in virgin or non-parous goats, but had no effect in parturient lactating goats (Mixner et al. 1944).

Figure 6(d) shows the rise in daily milk production was much more marked in number 304, which received both hormones. In this goat, the daily production rose from 2.7 to 4.2 pounds, representing an increase of 55 per cent, while in 438 it rose from 3.9 to 4.6 pounds, an increase of only 18 per cent. Neither lost in body weight as a result of these injections.

After the injections milk production dropped more drastically in goat 304 than in 438. This probably is due to the fact that number 304, which had been infested with demodectic mange for several weeks previous, became much worse after the injections. She was treated on May 3 for this condition and turned out to pasture. Number 438 was also turned out and the milk yields increased to about 4 pounds each.

As a result of the four 10-day treatments, these two goats were giving as much milk in June 1947 (approximately 4 pounds each) as in

June 1946. This represents over 100 per cent more milk than these goats were producing on December 13, 1946, when the experiment was begun.

3. THE EXPERIMENTAL INDUCTION AND MAINTENANCE OF LACTATION IN GOATS BY THE INCLUSION OF ESTROGEN AND THYROPROTEIN IN THE FEED. - DeFremery (1936) reported that the induction of the udders of virgin female goats with estradiol benzoate elicited mammary growth. An abundant lactation was then induced by injecting lactogenic hormone. Folley, Scott-Watson and Bottomley (1940) and Lewis and Turner (1940, 1942) demonstrated that the administration of estrogen alone was capable of initiating lactation in goats. Walker and Stanley (1941), Reece (1943), Hammond and Day (1944), Folley, Malpress and Young (1945) and others, extended these observations to cattle.

In view of the promising results obtained with estrogen and thyroxine in augmenting and prolonging lactation in the two goats reported previously, it seemed worthwhile to try these two hormones for the induction of lactation. These hormones were available in potent form for oral use, and could easily be put into the ration of goats.

Two grams of the dimethyl ether of diethylstilbestrol (DMEDES) were mixed with 100 pounds of grain. These goats were fed between 2.0 to 2.5 pounds of grain daily, and therefore consumed approximately 40 to 50 mgs. of DMEDES per day. This was about half of the amount (100 mgs.) shown to depress lactation in goats.

The feeding of the DMEDES to three mature, multiparous, nonpregnant goats was begun on December 28, 1946. These three goats weighed approximately 120 pounds each and were in good health. On February 24, 1947, 50 grams of thyroprotein were added per 100 pounds of grain. This meant they would consume a little more than 1 gram of this substance daily.

In two of the goats, numbers 880 and 156, the udders became visibly enlarged by the middle of March, while in goat 279 the udder remained small, loose, and apparently unstimulated. Milking was first begun on April 7. After a week, only 100 to 200 milliliters could be expressed from goats 880 and 156, and none from goat 279.

Milking was then suspended for a two-week period and resumed on April 26. The udders of 880 and 156 were swollen with accumulated milk, and more than a pound was removed from each goat. These two goats started an upward trend in milk production for more than 8 weeks (Figure 7), and production persisted at peak levels for an additional 6 weeks.

After a week of failure to obtain milk from goat 279, she was dropped from the experiment. Her udder remained small and unstimulated. A great variability in the lactational response of goats and cows to estrogen stimulation had been noted previously by Lewis and Turner (1942), Hammond and Day (1944) and Folley, Malpress and Young (1945).

The DMEDES was increased from 2 to 3 grams per 100 pounds of grain on April 30, and on May 24 the thyroprotein was increased from 50 to 75 grams. This provided these goats with approximately 60 to 75 milligrams of DMEDES, and 1.5 to 1.75 grams of thyroprotein daily. The goats continued to increase in production and maintained their body weights.

On June 18 the thyroprotein was increased further, to 100 grams per 100 pounds of grain. This proved detrimental to body weight, as the goats lost 11 and 14 pounds respectively, in 10 days. The daily milk

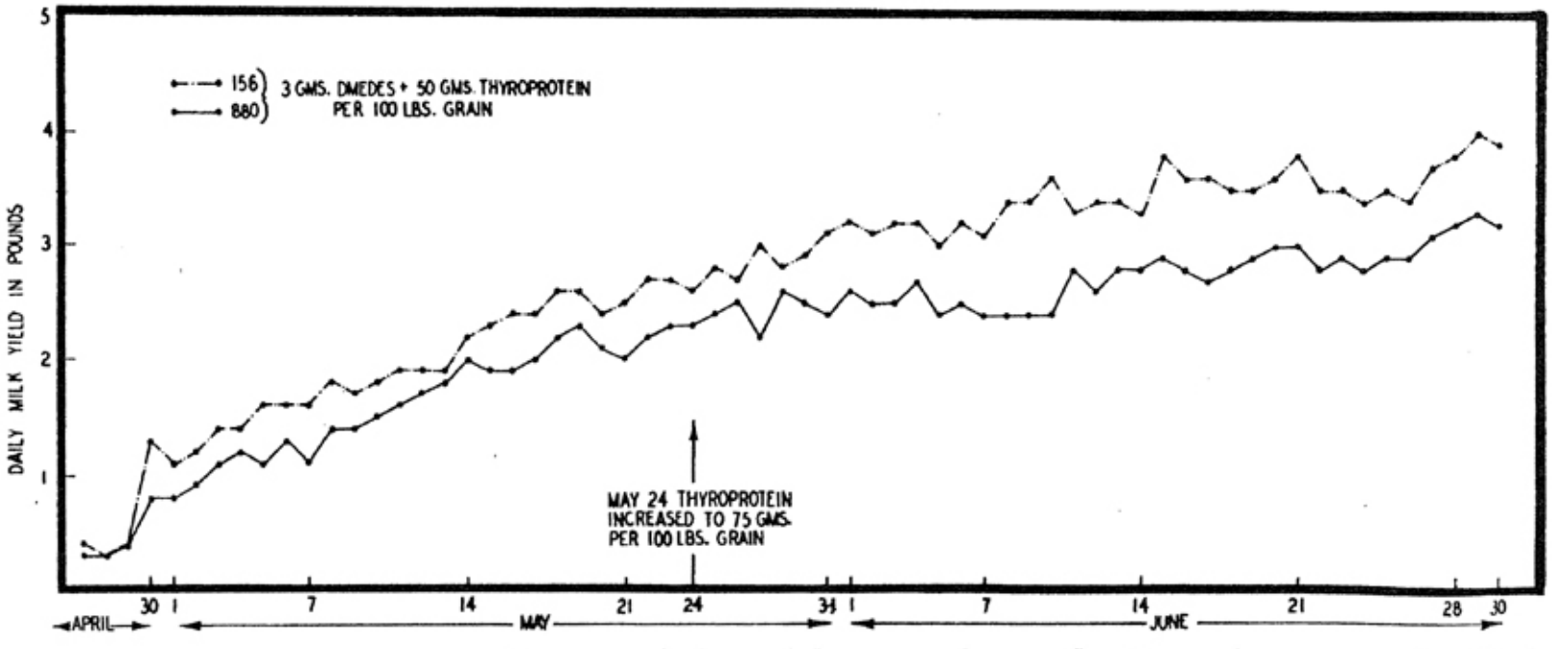

Fig. 7. Experimental lactation induced in two mature, multiparous goats by feeding thyroprotein and the dimethyl ether of diethylstilbestrol (DMEDES). Production attained after eight weeks is about equal to that previously produced by these goats as a result of pregnancy. Peak production was maintained for about six more weeks after the above chart was made.

yield continued to increase. On June 28 the thyroprotein was reduced to the 75 gram level, and the goats quickly regained their original body weight.

Apparently, 3 grams of DMEDES and 75 grams of thyroprotein per 100 pounds of grain are optimal for lactation in mature goats. These amounts have been fed to five other lactating goats in an effort to maintain milk production, and no detrimental effects have been noted on body weight after seven weeks.

DISCUSSION

Estrogen-thyroid combinations in goats may be superior to estrogen alone in initiating lactation, and to thyroid alone in augmenting lactation. Certain considerations, however, indicate that the relative dosages of the two hormones administered may be very important in determining their combined effectiveness.

Some dosages of estrogen may decrease the thyrotrophic hormone of the pituitary, (Meites and Turner, 1947) thereby reducing the output of thyroid hormone. Danforth, Greene and Ivy (1937) and Mahaux (1944) claimed that large dosages of estrogen may counteract in part the metabolic effect of thyroid when the two hormones were given simultaneously.

Thyroid hormone is capable likewise of counteracting certain effects of estrogen. Van Horn (1933) reported that castrate rats, made hyperthyroid by the feeding of desiccated thyroid, required three times as much estrogen to eliminate castration changes in the vagina compared to rats which were not fed thyroid. Meyer and Wertz (1938) also stated that the demand for estrogen rises when thyroid hormone is administered, while Smelser (1939) came to similar conclusions regarding the effect of thyroxine on androgen requirements.

Fleischmann (1946) noted that thyroxine, when administered simultaneously with estradiol dipropionate in immature chicks, neutralized the ability of the estrogen to effect certain typical changes in the blood chemistry, but did not inhibit growth of the oviduct. He concluded that the inhibitory effect of thyroxine on estrogen was confined to metabolic and not to structural changes.

Korenchevsky and Hall (1941) showed that either estrogen or thyroxine can cause hypertrophy of the adrenal cortex in castrate rats. When both hormones were given, a greater hypertrophy of the adrenal cortex occurred than produced by either alone. Similarly, both hormones produced a greater loss of body weight than when either was given alone.

Estrogen and thyroid preparations apparently produce opposite effects on appetite. Administration of estrogen causes a decrease in food and water intake of goats. Koger, Hurst and Turner (1942) and Koger and Turner (1943) found that thyroid substances caused an increase in the food consumption of mice.

From the reports cited and from the results obtained in the goats, the following conclusions relative to lactation may be drawn: Thyroid preparations should be administered in amounts adequate to overcome any unfavorable effect of estrogen on metabolism, such as reduction in food intake, while permitting estrogen to exert its stimulating effects on pituitary lactogen secretion and mammary growth. The amount of estrogen administered should not counteract the effects of thyroid preparations on metabolic process favorable to lactation. Optimal levels of the two hormones should be established for different species.

SUMMARY

1. When rabbit litters were uniformly reduced to two each on the fifth postpartum day and the mothers killed 15 days later, the pituitary lactogen content of the mothers was the same as that of rabbits with their entire litters. The extent and intensity of nursing did not influence the lactogen content of the pituitaries of postpartum rabbits.

2. In rats, the absence of suckling for the first week after parturition resulted in a decrease of pituitary lactogen to the estrous level, and the disappearance of mammary secretion.

3. Rabbits suckled for the first 20 days after parturition contained more pituitary lactogen and more mammary secretion than non-suckled rabbits. Maximum pituitary lactogen content and maximum mammary secretion possible in a parturient rabbit were not attained in non-suckled animals.

4. The induction of hypothyroidism in lactating rats, by the administration of thiouracil for the first 14 days after parturition, resulted in a moderate decrease in pituitary lactogen content and mammary secretion. The young also weighed less than those of the control rats.

5. Starvation of mother rats from the 10th to the 14th days after parturition resulted in a marked decrease in pituitary lactogen and cessation of milk secretion. The mother rats lost one-fourth of their body weight, and the body weight of the young was also reduced.

6. Administration of large doses of diethylstilbestrol and testosterone propionate to parturient rats for the first six postpartum days did not reduce the lactogen content of the pituitary. Only a small decrease in mammary secretion was evident, although 38 and 11 per cent of the young died, respectively.

7. Six synthetic estrogens were administered orally and compared for their ability to depress established lactation in goats. They were rated in the following order of potency; hexestrol, dimethyl ether of diethylstilbestrol, benzestrol, dipropionate of diethylstilbestrol, diethylstilbestrol, and menomestrol.

8. Diet apparently influences the lactation-inhibiting action of estrogen in goats. When "cerogras" (a dehydrated and finely ground cereal grass rich in vitamins) and freshly cut green grass were added to the regular ration of two goats, large doses of hexestrol were completely ineffective in depressing lactation.

9. The lactation-depressing effect of large dosages of estrogen was shown to be associated with a loss of appetite in goats. In two goats, the reduction in food and water intake and loss in body weight incident to the injection of 1 mg. of diethylstilbestrol daily, paralleled the decrease in milk yield. This may explain in part how large doses of estrogen can depress lactation without decreasing pituitary lactogen secretion.

10. Daily injections of small doses of diethylstilbestrol for the first 21 days postpartum in rats was found to maintain a higher pituitary lactogen content than in untreated rats. However, the young of the estrogen treated rats weighed less than those of the untreated rats.

11. The lactation-depressing effect produced by injecting 1 mg. of diethylstilbestrol daily into two goats was completely counteracted by the simultaneous injection of 10 mgs. of thyroxine. The two hormones increased the milk yield of the goats about 145 per cent. As a result of four 10-day injections with the two hormones, milk production after six

months was still over 100 per cent above that at the beginning of the experiment.

12. Two dry, mature, multiparous goats were fed the dimethyl ether of diethylstilbestrol and thyroprotein in the grain ration. After four months, milking was begun. Daily milk production rose steadily for over eight weeks to peaks of 3 to 4 pounds, and persisted at these levels for six more weeks. It is believed that the treatment with the two hormones may be superior to estrogen alone in initiating lactation, and to thyroprotein alone in augmenting lactation.

BIBLIOGRAPHY

Anselmino, K. J., und Hoffman, F. 1936. "Studien zur Physiologie der Milchbildung. IV. Mitteilung ueber die Laktionshemmung durch Follikelhormon." Zentralbl. f. Gynak. 60:501.

Barsantini, J. C., Masson, G., et Selye, H. 1936. "Effects des Steroides sur la Lactation." Rev. Canad. Biologie 5:407.

Biskind, M. S. 1946. Nutritional Therapy of Endocrine Disturbances in Vitamins and Hormones 4:147.

Blanchard, E. W., and Stebbins, R. B. 1945. "The Effects of Oral and Parenteral Administration of a New Synthetic Estrogen, Benzestrol, in the Rat." Endocrinology 36:297.

Brobeck, J. R., Wheatland, M., and Strominger, J. L. 1947. "Variations in Regulation of Energy Exchange Associated with Estrus, Diestrus and Pseudopregnancy in Rats." Endocrinology 40:65.

Brody, S. 1945. "Bioenergetics and Growth." Reinhold Publishing Corp. New York.

Cameron, A. T., Guthrie, J. S., and Carmichael, J. 1946. "The Effects of Oestrogens and of Mild Chronic Starvation on the White Rat." Canad. J. Research 24:105.

Cole, H. A. 1933. "The Mammary Gland of the Mouse, During the Oestrous Cycle, Pregnancy, and Lactation." Proc. Roy. Soc. B. 114: 136.

Danforth, D. N., Green, R. R., and Ivy, A. C. 1937. "The Effect of Female Sex Hormones upon the Oxygen Consumption Rate of Normal Rats and upon Tolerance to Desiccated Thyroid." Endocrinology 21:361.

DeFremey, P. 1936. "On the Influence of Different Hormones on Lactation." Proc. Physiol. Soc. 87:10.

Desclin, L. 1945. "Contribution a L'Etude de la Structure et du Fonctionnement de l'Hypophyse Pendent le post Partum." Extrait Arch. Biologie (Liege) 56:261.

Edelmann, A., and Guant, R. 1941. "Inhibition of Lactation in the Rat." Physiol. Zoology 14:373.

Fleischmann, W. 1946. "Effect of Thyroxin on Estrogen-induced Changes in Fowl." Fed. Proc. (Part II) 5:28.

Folley, S. J. 1938. "Experiments on the Relation between the Thyroid Gland and Lactation in the Rat." J. Physiol. 93:401.

Folley, S. J. 1941. "Effects of Oestrogens on Lactation." Lancet 1:40.

Folley, S. J., and Kon, S. K. 1938. "The Effect of Sex Hormones on Lactation in the Rat." Proc. Roy. Soc. B. 124:476.

Folley, S. J., Malpress, F. H., and Young, F. G. 1945. "Induction of Lactation in Goats and Cows with Synthetic Oestrogens and Anterior Pituitary Extracts." J. Endocrinology 4:181.

Folley, S. J., Scott-Watson, H., and Bottomley, A. C. 1940. "Induction of Lactation in Goats with Diethylstilbestrol Dipropionate." J. Physiol. 98:15.

Folley, S. J., Scott-Watson, H. M., and Amoroso, E. C. 1943. "Further Experiments on Lactation in Thyroidectomized Rats: The Role of the Parathyroids." J. Endocrinology 3:178.

Gessler, C. 1936. "Influence of Folliculin on the Basal Metabolic Rate." Arch. Internat. de Pharmacodyn. et de Therap. 54:263.

Hain, A. M. 1935. "The Effect (a) of Litter-size on Growth and (b) of Oestrone Administered During Lactation (Rat)." Quart. J. Exper. Physiol. 25:303.

Hammond, J. 1925. "Reproduction in the Rabbit." Oliver and Boyd, Edinburgh.

Hammond, J. Jr., and Day, F. T. 1944. "Oestrogen Treatment of Cattle: Induced Lactation and other Effects." J. Endocrinology 4:53.

Hesselberg, C., and Loeb, L. 1937. "The Structure of the Secreting and Retrogressing Mammary Gland in the Guinea Pig." Anat. Rec. 68:103.

Holst, S., and Turner, C. W. 1939. "Lactogen Content of Pituitary of Pregnant and Lactating Rabbits and Guinea Pigs." Proc. Soc. Exper. Biol. and Med. 42:479.

Hooker, C. W., and Williams, W. L. 1942. "Retardation of Mammary Involution in Mice by Injection of Lactogenic Hormone." Endocrinology 28:42.

Hughes, A. M. 1944. "Cretinism in Rats Induced by Thiouracil." Endocrinology 34:69.

Ingelbrecht, P. 1935. "Influence du Systeme Nerveux Central sur la Mamelle Lactente chez le Rat Blanc." Compt. Rend. Soc. de Biol. 120:1369.

Karnofsky, D. 1942. "The Effect of Thyroidectomy on Lactation in the Albino Rat." Endocrinology 30:234.

Koger, M., Hurst, V., and Turner, C. W. 1942. '"Relation of Thyroid to Growth. I. Effects of Crystalline Thyroxine upon Rate of Growth, Food Intake and Body Composition of Female Albino Mice." Endocrinology 31:237.

Koger, M. and Turner, C. W. 1943. The Effects of Mild Hyperthyroidism on Growing Animals of Four Species." Mo. Agr. Exper. Sta. Res. Bul. 377.

Korenchevsky, V., and Dennison, M. 1934. "The Effect of Oestrone on Normal and Castrated Male Rats." Biochem. J. 28:1474.

Korenchevsky, V., and Hall, K. 1941. "Correlation between Sex Hormones, Thyroid Hormones and Desoxycorticosterone as Judged by Their Effects on the Weights of Organs of Gonadectomized Rats." Biochem. J. 35:726.

Kuramitsu, C., and Loeb, L. 1921. "The Effect of Suckling and Castration on the Lactating Mammary Gland in Rat and Guinea Pig." Am. J. Physiol. 56:40.

Lewis, A. A., and Turner, C. W. 1940. "Effect of Stilbestrol on the Mammary Gland." Amer. Soc. Animal Prod. Proc. p. 63.

Lewis, A. A., and Turner, C. W. 1942. "The Effect of Stilbestrol and Anterior Pituitary Extract upon Lactation in Goats." J. Dairy Science 25:895.

Lipschutz, A., Quintana, U., and Bruzzone, S. 1944. "Differential Behavior of the Liver towards Natural and Artificial Estrogens." Proc. Soc. Exper. Biol. and Med. 55:43.

Mahaux, J. 1944. "Influence of Variations in Estrogen Administration on Basal Metabolism and Thyroid Activity." Acta. Med. Scand. 119:277.

Meites, J., Bergman, A. J., and Turner, C. W. 1941. "Relation of Litter to AP Lactogen Content of Nursing Rabbits." Proc. Soc. Exper. Biol. and Med. 46:670.

Meites, J. and Turner, C. W. 1942a. "Studies Concerning the Mechanism Controlling the Initiation of Lactation at Parturition. I. Can Estrogen Suppress the Lactogenic Hormone of the Pituitary?" Endocrinology 30:711.

II. Why Lactation is not Initiated during Pregnancy." Endocrinology 30:719.

III. Can Estrogen Account for the Precipitous Increase in the Lactogenic Content of the Pituitary Following Parturition?" Endocrinology 30:726.

Meites, J., and Turner, C. W. 1942b. "Studies Concerning Mechanism Controlling Initiation of Lactation at Parturition. IV. Influence of Suckling on Lactogen Content of Pituitary of Postpartum Rabbits." Endocrinology 31:340.

Meites, J., and Turner, C. W. 1947a. "Studies Concerning the Induction and Maintenance of Lactation. I. The Mechanism Controlling the Initiation of Lactation at Parturition." Mo. Agr. Exper. Sta. Res. Bul. 415.

Meites, J., and Turner, C. W. 1947b. "Effect of Thiouracil and Estrogen on Lactogenic Hormone and Weight of Pituitaries of Rats." Proc. Soc. Exper. Biol. and Med. 64:388.

Meyer, A. E., and Wertz, A. 1938. "Influence of Thyroid Hormone on Estrin Action." Proc. Soc. Exper. Biol. and Med. 38:843.

Mixner, J. P., Meites, J., and Turner, C. W. 1944. "The Stimulation and Inhibition of Milk Secretion in Goats with Diethylstilbestrol." J. Dairy Science 27:957.

Mixner, J. P., and Turner, C. W. 1943. "The Mammogenic Hormones of the Anterior Pituitary. II. The Lobule-alveolar Growth Factor." Mo. Agr. Exper. Sta. Res. Bul. 378.

Monroe, R. A., and Turner, C. W. 1946. "Thyroid Secretion Rate of Albino Rats during Growth, Pregnancy and Lactation." Mo. Agr. Exper. Sta. Res. Bul. 403.

Mulinos, M. G., and Pomerantz, L. 1940. "Pseudo-hypophysectomy. A Condition Resembling Hypophysectomy Produced by Malnutrition." J. Nutrition 19:493.

McEuen, C. S., Selye, H., and Collip, J. B. 1937. "Effect of Testosterone on Somatic Growth." Proc. Soc. Exper. Biol. and Med. 36: 390.

McShan, W. H., and Meyer, R. K. 1946. "The Effect of Estrogens on the Succinoxidase System of Liver and Pituitary Tissue." Arch. Biochem. 9:165.

Nelson, W. O. and Tobin, C. E. 1937. "Studies on the Physiology of Lactation. VII. Lactation in Thyroidectomized Rats and Guinea Pigs." Endocrinology 21:670.

Nelson, W. O. 1939. "The Effect of Thyroidectomy upon Lactation." Proc. Am. Physiol. Soc. p. 176.

Noble, R. L. 1939a. "Effect of Continuous Oral Administration of Aqueous Diethylstilbestrol Solutions to Rats." J. Endocrinology 1:128.

Noble, R. L. 1939b. "Effects of Synthetic Oestrogens and Carcinogens When Administered to Rats by Subcutaneous Implantation of Crystals or Tablets." J. Endocrinology 1:216.

Preheim, D. V. 1940. "Studies on Thyroidectomized Rats with Special Reference to Lactation and Growth." Endocrinology 27:494.

Ralston, N. P., Cowsert, W. C., Ragsdale, A. C., Herman, H. A., and Turner, C. W. 1940. "The Yield and Composition of the Milk of Dairy Cows and Goats as Influenced by Thyroxine." Mo. Agr. Exper. Sta. Res. Bul. 317.

Reece, R. P. 1943. "Initiation and Maintenance of Lactation in Dairy Heifers by Hormone Administration." Proc. Soc. Exper. Biol. and Med. 52:145.

Reece, R. P., and Leonard, S. L. 1941. "Effect of Estrogens, Gonadotropins and Growth Hormone on Mammary Glands of Hypophysectomized Rats." Endocrinology 29:297.

Reece, R. P., and Turner, C. W. 1937. "The Lactogenic and Thyrotropic Hormone Content of the Anterior Lobe of the Pituitary Gland." Mo. Agr. Exper. Sta. Res. Bul. 266.

Schultze, A. B., and Turner, C. W. 1945. "The Determination of the Rate of Thyroxine Secretion by Certain Domestic Animals." Mo. Agr. Exper. Sta. Res. Bul. 392.

Selye, H. 1941. "Effect of Dosage on the Morphogenetic Actions of Testosterone." Proc. Soc. Exper. Biol. and Med. 46:142.

Selye, H., Collip, J. B., and Thomson, D. O. 1934. "Nervous and Hormonal Factors in Lactation." Endocrinology 18:237.

Selye, H., and McKeown, T. 1934a. "The Effect of Mechanical Stimulation of the Nipples on the Ovary and the Sexual Cycle." Surgery, Gynec. and Obstet. 59:836.

Selye, H., and McKeown, T. 1934b. "Further Studies on the Influence of Suckling." Anat. Rec. 60:323.

Sherwood, T. C. 1938. "The Relation of Estrogenic Substance to Thyroid Function and Respiratory Metabolism." Am. J. Physiol. 124:114.

Smelser, G. K. 1939. "Testicular Function and the Action of Gonadotropic and Male Hormones in Hyperthyroid Male Rats." Anat. Rec. 73:273.

Turner, C. W. 1933. "The Physiology and Biochemistry of Milk Secretion." University of Missouri, Columbia, Missouri.

Turner, C. W. 1939. "The Mammary Glands." In Allen, E. Sex and Internal Secretions. The Williams and Wilkins Co., Baltimore (2nd Ed.)

Turner, C. W., and Gomez, E. T. 1936. "The Effect of Ovariectomy upon Lactation in the Albino Rat." Mo. Agr. Exp. Sta. Res. Bul. 370.

Turner, C. W., and Reineke, E. P. 1936. "A Study of the Involution of the Mammary Gland of the Goat." Mo. Agr. Exp. Sta. Res. Bul. 235.

Van Horn, H. H. 1933. "The Relation of the Thyroid to the Hypophysis and Ovary." Endocrinology 17:152.

Walker, S. M., and Stanley, A. J. 1941. "Effect of Diethylstilbestrol Dipropionate on Mammary Development and Lactation." Proc. Soc. Exper. Biol. and Med. 48:50.

Weichert, C. K., and Boyd, R. W. 1934. "Stimulation of Mammary Gland Development in the Pregnant Rat under Conditions of Experimental Hyperthyroidism." Anat. Rec. 59:157.

Weichert, C. K., and Kerrigan, S. 1942. "Effects of Estrogens upon the Young of Injected Lactating Rats." Endocrinology 30:751.

Williams, W. L. 1941. "The Effect of Non-suckling and the Non-removal of Milk upon Individual Mammary Glands in the Lactating Mouse." Yale J. Biol. and Med. 14:201.

Zimmerburg, H. 1946. "The Inhibition of Diethylstilbestrol in vitro." J. Biol. Chem. 166:97.

Zondek, B., Sulman, F., and Sklow, J. 1943. "Inactivation of Stilbestrol by Liver in vitro." Endocrinology 33:333.