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Mammary Development in the Thyroprived Bovine by Stilbestrol and Thyroprotein Administration.

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The role of the thyroid in mammary gland development and lactation is still controversial. In rats Nelson[1] and Nelson and Tobin[2] report no observable effect while Folley[3] and Karnofsky[4] observed a marked diminution in lactation after thyroidectomy. Leonard and Reece[5] reported thyroidectomy to enhance mammary growth from estrogen therapy in female rats whereas Smithcors and Leonard[6] reported the opposite for male mice and Mixner and Turner[7] report improved mammary growth in thyroprived rats from estrogen and progesterone administration.

Reports of mammary development in thyroidectomized cows and goats are all based upon observations on lactation following gestation. While most workers report a varying depressing effect on lactation following thyroidectomy all observed mammary development as based upon subsequent lactation. In all of these reports, however, the presence of thyroxine from fetal secretions was not ruled out. Spielman, Petersen, and Fitch[8] observed that myxedematous symptoms of thyroidectomized cows began to disappear about midterm due presumably to secretions from the developing fetus or fetal membranes. Although the mammary glands were not as well developed nor lactation as great in the thyroprives as in the normals because of the unknown part fetal secretions might play, observations in these experiments can be interpreted as only indicating the thyroid is concerned with full mammary development.

Since it is established that diethylstilbestrol administration to the non-pregnant cow mediates both mammary growth and lactation somewhat comparable to that following a normal gestation, the use of this drug on thyroprived cows suggests itself as a logical approach to a solution of the problem insofar as this species is concerned. Accordingly 2 thyroidectomized cows, A26 and 823, with marked clinical myxedema, were subjected to diethylstilbestrol administration first alone and then together with thyroid replacement therapy.

A26 was a unipara thyroidectomized when 13 months of age, that conceived by artificial insemination at 21 months of age when markedly myxedematous and carried a normal male fetus to term. About midpregnancy myxedematous symptoms began to disappear and the blood cholesterol became normal. On the basis of comparisons with normal sibs her mammary glands appeared to be about half normal in size at term and initial milk production about one-half that expected. Lactation rapidly declined with cessation at 190 days and an apparently completely involuted mammary gland.

Over a period of 2 years A26 was subjected to a series of 10 experiments. Following experiments in which mammary development occurred or recovery from the myxedematous symptoms sufficient rest periods were allowed for complete involution of the mammary gland and return to the marked myxedematous state. Numbered chronologically, in experiments 1, 3, 6, and 7, she was treated on alternate days with 30 mg diethylstilbestrol. The length of treatment was 31 and 21 days respectively for the first and second 2 of these experiments.

In experiment 2 four ounces of fresh raw

1 Nelson, W. D., *Am. J. Physiol.*, 1939, **126**, 592.

2 Nelson, W. D., and Tobin, C. E., *Anat. Rec. Suppl.*, 1937, **67**, 111.

3 Folley, S. J., *J. Physiol.*, 1938, **93**, 401.

4 Karnofsky, D., *Endocrinology*, 1942, **30**, 234.

5 Leonard, S. L., and Reece, R. P., *Endocrinology*, 1941, **28**, 65.

6 Smithcors, J. F., and Leonard, S. L., *Endocrinology*, 1942, **31**, 554.

7 Mixner, J. P., and Turner, C. W., *Endocrinology*, 1942, **31**, 345.

8 Spielman, A. A., Petersen, W. E., and Fitch, J. B., *J. Dairy Science*, 1944, **27**, 441. Also unpublished data.

thyroid was given daily and 30 mg diethylstilbestrol on alternate days for a period of 29 days. In experiment 3 covering 21 days, 18 g thyroprotein was given daily with 30 mg diethylstilbestrol on alternate days.

In experiments 4 and 9 10 g of thyroprotein were given daily without diethylstilbestrol.

Experiments 5 and 10 were continuations respectively of 4 and 9 with 30 mg of stilbestrol administered on alternate days for 16 days in the former and 29 days in the latter. Thyroprotein administration continued for several months after withdrawal of the diethylstilbestrol treatment.

The effect of diethylstilbestrol alone in the myxedematous state and together with thyroprotein after the myxedematous symptoms had been relieved were also studied in 823, a Holstein female that had been thyroidectomized at 17 months of age. Marked myxedematous symptoms developed in 145 days, post-operatively, before 20 mg diethylstilbestrol were administered on alternate days for 29 days. Next 10 g thyroprotein were administered orally per day for 30 days followed by 20 mg stilbestrol on alternate days for 19 days. The smaller dosage of diethylstilbestrol is occasioned by 823's smaller size.

In the 5 experiments in which diethylstilbestrol was administered to thyroidectomized myxedemic cows there was no evidence of any effect upon mammary development that could be detected by careful palpation. The doses of diethylstilbestrol used were larger and the period of time over which it was administered longer than has been shown necessary to obtain marked mammary development and lactation in normal nonlactating cows.

In the 3 periods when either raw thyroid or thyroprotein were administered there was no discernible mammary development. There were, however, other marked physiological changes. Gradually activity and excitability increased to that of the normal animal. Heart and respiration rates also attained normalcy by the end of 30 days of thyroid therapy. Although appetite increased there was a loss in weight thought to be due largely to the disappearance of edema.

In the 3 experiments in which fresh thyroid or thyroprotein were administered to pronounced myxedematous subjects no mammary development was observable until there was a marked recovery from clinical myxedema and then the development was slow.

In the 3 experiments in which 30 mg of diethylstilbestrol was administered together with thyroprotein, after recovery from clinical myxedema had been effected, mammary development was rapid being distinctly noticeable in 5 to 7 days. Milk secretion was first observed on the 13th day in experiments 5 and 10 and on the 18th day for 823 following the beginning of diethylstilbestrol administration. At first the secretion amounted to but a few ml per day but increased rapidly. The milk production for each of the 3 experiments is given in Table I. The second lactation of A26 and the one for 823 is still in progress. The first lactation of A26 was deliberately terminated in order to prepare her for subsequent experiments.

Although the lactation level is not nearly that which would be expected following normal parturition of normal animals, it is substantial. Although no evidence of mammary development could be detected from administration of diethylstilbestrol in the myxedematous state and substantial development and lactation followed administration of this drug together with thyroprotein subsequent to 30 days of previous thyroid therapy it can not be concluded that thyroxine and stilbestrol act synergistically to promote mammary development. Complete failure of mammary development when these 2 substances were administered simultaneously to myxedematous animals until there was marked clinical improvement, must be considered as *a priori* evidence against such a synergistic relationship.

Since the mammary response to diethylstilbestrol treatment began when relief from myxedema became apparent, it appears that some phase of the myxedema syndrome is responsible for the failure of mammary growth. The lowered metabolism and arrest of growth which follows thyroidectomy could well account for the failure of mammary response to stilbestrol as mammary development is a growth phenomenon and has been shown by Astwood, Geschickter, and Rausch[9] to fail in

[9] Astwood, E. B., Geschickter, C. F., and Rausch, E. O., *Am. J. Anat.*, 1937, **61**, 373.

TABLE I.
Record of 3 Lactations Induced by the Simultaneous Administration of Diethylstilbestrol and Thyroprotein to Thyroidectomized Cows Following 30 Days Previous Treatment with Thyroprotein.

A26 Experiment 5		A26 Experiment 10		823	
1943	Milk (lbs.)	1944	Milk (lbs.)	1944	Milk (lbs)
Feb. 19 da.	36.4	May 13 da.	51.1	June	9.1
Mar.	255.9	June	129.2	July	163.4
April	304.3	July	197.8	Sept.	222.8
May	286.0	Aug.	370.0		
June	189.0	Sept.	432.1		
July	206.0				
Aug. 3 da.	16.1				
	1294.0				

inanition in the rat.

Summary. In the thyroprived cow with pronounced myxedema no mammary development was obtained by diethylstilbestrol or thyroprotein administration. Negative results were also obtained with the simultaneous administration of these two substances as long as myxedema persisted. Substantial mammary development and lactation resulted from diethylstilbestrol administration following recovery from myxedematous symptoms by thyroprotein therapy. It is suggested that the failure of mammary development in the myxedematous animal is due to some phase of the myxedema and that the response obtained following thyroid therapy is not due to a synergistic action of thyroxine and diethylstilbestrol.

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Urethane Administration and Potassium Content of Bronchial Secretions in the Cat.

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During the course of studies upon the chemical composition of respiratory tract fluid (R.T.F.), part of which has been published,[1,2] it has been found that the potassium content of these bronchial secretions is higher in cats under urethane anesthesia than in decerebrate cats. This observation is of coincidental interest because (a) potassium has been found generally[3,4,5,6] though not always[7] related in some obscure manner to sympathomimetic activity; (b) urethane has been reported

1 Perry, W. F., and Boyd, E. M., *J. Pharm. and Exp. Therap.*, 1941, **73**, 65.

2 Boyd, E. M., Jackson, S., MacLachlan, M., Palmer, B., Stevens, M., and Whittaker, J., *J. Biol. Chem.*, 1944, **153**, 435.

3 Camp, W. J. R., and Higgins, J. A., *J. Pharm. and Exp. Therap.*, 1936, **57**, 376.

4 Brewer, G., Larson, P. S., and Schoeder, A. R., *Am. J. Physiol.*, 1939, **126**, 708.

5 Larson, P. S., and Brewer, G., *J. Pharm. and Exp. Therap.*, 1937, **61**, 213.

6 Brewer, G., and Larson, P. S., *J. Pharm. and Exp. Therap.*, 1938, **63**, 272.

7 Seager, L. D., *J. Pharm. and Exp. Therap.*, 1939, **66**, 202.