represent mean values and vertical lines a single standard error of mean. Fractions were heated without prior $Al(OH)_3$ adsorption and pH of each was maintained at 6.5 with citrate buffer(14).

FIG. 4. Effect of a variety of pH's was determined on AHF activity of 2 normal and 3 transfused v.W.d. plasmas. Each sample was run 3 times independently. After having been thawed and adsorbed with $Al(OH)_3$, pH of each plasma was brought to the desired point with 0.5 N HCl or NaOH. After having been maintained at designated pH for 30 min at 37°C, each sample was returned to pH 7.2 with the appropriate acid or base. Final concentrations were adjusted with saline and samples were centrifuged at 4°C and 3,000 rpm to remove precipitated proteins, then stored at 0°C until assayed for AHF activity.

FIG. 5. Thromboplastin generation tests (TGT) performed on $Al(OH)_3$ adsorbed plasmas. Topmost curve is one performed on v.W.d. #3 before transfusion. Other curves were produced by using a single normal and 4 transfused v.W.d. plasmas.

1. Graham, J. B., *J. Med. Ed.*, 1959, v34, 385.
2. Jacob, F., Monod, J., *J. Mol. Biol.*, 1961, v3, 318.
3. Cornu, P., Larrieu, M. J., Caen, J., Bernard, J., *Brit. J. Haem.*, 1963, v9, 189.
4. Nilsson, I. M., Blombäck, M., Blombäck, B., *Acta Med. Scand.*, 1959, v164, 263.
5. Cornu, P., Larrieu, M. J., Caen, J., Bernard, J., *Nouv. Rev. franc. Hemat.*, 1961, v1, 231.
6. Biggs, R. Matthews, J. M., *Brit. J. Haem.*, 1963, v9, 203.
7. McLester, W. D., Graham, J. B., *Nature*, 1963, v197, 708.
8. Graham, J. B., *Thromb. Diath. Haem. Suppl.*, 1963, v9, 119.
9. Langdell, R. D., Wagner, R. H., Brinkhous, K. M., *J. Lab. Clin. Med.*, 1953, v41, 637.
10. McLendon, W. W., Barrow, E. M., Hurt, J. P., Bryan, F. T., Wagner, R. H., *Fed. Proc.*, 1961, v20, 57.
11. Margolis, J., *Brit. J. Haem.*, 1961, v7, 21.
12. Wagner, R. H., personal communication.
13. Biggs, R., Douglas, A. S., *J. Clin. Path.*, 1953, v6, 23.
14. Blombäck, M., *Arkiv. für Kemi.*, 1958, v12, 387.
15. Mertz, E. T., Owen, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, v43, 204.

Received December 16, 1963. P.S.E.B.M., 1964, v115.

Replacement Therapy with Calciferol in Thyroparathyroidectomized Lactating Rats.* (29031)

S. DJOJOSOEBAGIO† AND C. W. TURNER
(With technical assistance of Mary E. Powell and James E. Willard)
Department of Dairy Husbandry, University of Missouri, Columbia

Successful replacement therapy was achieved in thyroparathyroidectomized (TPEX) lactating rats using 60, 80 and 100 μg Hytakerol (A.T.10)/100 g body weight (BW)/day and 3 μg L-thyroxine (T_4)/100 g BW/day administered from day 7 to 13 of lactation(1). The effectivenes of A.T.10 in increasing milk secretion in normal rats was reported also(2).

It was claimed that Hytakerol contained 1.25 mg crystalline dihydrotachysterol (DHT) per ml whereas the new product is labeled to contain only 0.25 mg DHT per ml. It was reported, however, that Hytakerol does not contain DHT but is dihydrovitamin-D2 II, a chemical compound that can be obtained either by reduction of tachysterol or Vit. D2(3).

It is desirable, therefore, to test the effectiveness of the parent compound (Vit. D2 or calciferol) on lactation intensity in experimental animals. When 0.2 mg calciferol/100 g BW was given to normal lactating rats, an increase in milk yield of 39% above control on day 14 of lactation was observed (unpub.).

Materials and methods. Lactating rats of the Sprague-Dawley-Rolfsmeyer strain were

* Contribution from Mo. Agr. Exp. Sta., Journal Series No. 2670. Approved by Director.

† Kentucky Research Foundation Fellow from Fac. of Vet. Med. and An. Husb., Univ. of Indonesia, Bogor.

housed in individual cages, fed Purina Lab. Chow‡ and water *ad libitum*. The animal room was standardized at a temperature of 78±1°F. On day 4 post-partum litters of each dam were reduced to 6.

Thyroparathyroidectomy (TPEX) of the experiment group was done on day 4 of lactation. The operation was conducted under Nembutal Sodium anesthesia with a dose of 30 mg/kg BW injected intraperitoneally. The control group were sham-operated in the same way as the experimental animals, the parathyroid and thyroid glands were exposed but not removed. One hour before operation, blood samples were collected by heart puncture under ether anesthesia. The blood was immediately centrifuged and the serum was stored in deep freeze until used for calcium determination by the chloranilate method(4).

Eight hours after operation *a second blood sample was taken from rats of both groups* for serum calcium determination as criteria for completeness of gland removal. If the second serum calcium determination showed a drop of less than 3 mg%, the animal was excluded. Immediately after collection of the *second blood samples, the TPEX*-group were injected (s.c.) with 0.2 mg calciferol§/100 g BW plus 3 μg T4/100 g BW and the sham-operated group were injected (s.c.) with 0.05 ml sesame oil/100 g BW, and 0.1 ml alkaline 0.9% NaCl/100 g BW. The sesame oil served as vehicle for the calciferol (0.2 mg/0.05 ml) and the alkaline saline solution for T4 (3 μg/0.1 ml). Injections were made daily to day 19 of lactation.

Mothers and young were weighed daily. On day 14, 16, 18, and 20 milk yields were estimated by the differences in pre- and post-nursing litter weight after 10 hours' isolation from the dams. One USP unit of Oxytocin (OXT) was injected into the dam (s.c.) immediately before nursing to aid in complete milk removal.

On day 20 mothers were killed with ether, 6 posterior mammary glands were removed to determine the deoxyribonucleic acid (DNA) content by the method described previously (5). The DNA content of mammary glands in mg/100 g BW was used as index of mammary gland growth or mammary gland involution.

Third and fourth blood samples were collected for serum calcium determination on day 14 and 20 of lactation, 24 hours after the previous administration of the materials.

Results. Calciferol at the level of 0.2 mg/100 g BW in conjunction with 3 μg T4/100 g BW injected daily from day 4 to 19 was able to support lactation in TPEX-rats. The milk yields on days 14, 16, 18 and 20 were 91% ($.30 > p > .25$), 90% ($.25 > p > .20$), 88% ($.10 > p > .05$) and 105% ($.30 > p > .25$) respectively of the sham-operated group (Table I). The p values indicate that the differences in milk yield of sham-operated and experimental animals were not statistically significant.

The litters, however, were smaller than controls. The weight of the litters of the TPEX-group on day 7 and day 20 were 66.6 g and 156 g and of the sham-operated were 79 g and 197 g. No differences were observed in the DNA content of the mammary glands on day 20 of lactation between the two groups (TPEX: 9.6 ± 0.5 mg; sham-operated: 9.1 ± 0.4 mg; $.25 > p > .20$).

The milk yield of the sham-operated group was higher than the normal-non-sham-operated animals (Table I) but there was no difference in the content of the DNA of the mammary glands (normal-non-sham-operated: 9.3 ± 0.5 mg).

With administration of calciferol the serum calcium level of the TPEX animals was brought up to the control level (Table I). It is worthwhile to mention here that the serum calcium of the TPEX-group 8 hours after removal of the glands was higher than the value reported by Munson(6). The animals used in our experiments were fed *ad libitum* with food containing adequate calcium and Vit. D, while Munson's rats had been maintained on a diet low in calcium for 10 to 20 days before the operation.

‡ Purina Lab. Chow is reported to contain 1.42% Ca, 0.96% P and 5 USP units/g Vit. D.

§ Kindly supplied by Sterling-Winthrop Res. Inst., Rensselaer, N. Y.

TABLE I. Effect of Calciferol on Milk Yield and Serum Calcium in Thyroparathyroidectomized Lactating Rats.

		Serum calcium on day:				Milk yield on day:*			
		4		14	20	14	16	18	20
No. of rats	Treatment	Pre-operative Mean ± S.E. (mg %)	8 hr post-operative Mean ± S.E. (mg %)	Mean ± S.E. (mg %)	Mean ± S.E. (mg %)	Mean ± S.E. (g)	Mean ± S.E. (g)	Mean ± S.E. (g)	Mean ± S.E. (g)
27†	Normal control; .05 ml sesame oil/100 g BW	—	—	8.3 ± .3	9.5 ± .4	6.9 ± .9	9.0 ± .5	9.8 ± .8	8.2 ± .6
13	Sham-operated control; .05 ml sesame oil/100 g BW; .1 ml saline/ 100 g BW	10.8 ± .4‡	10.2 ± .8	8.7 ± .8	9.7 ± .3	8.7 ± 1.3	11.2 ± 1.4	12.7 ± 1.0	12.0 ± .7
21	TPEX; .2 mg calciferol/ 100 g BW; 3 μg T4/100 g BW	‡	6.4 ± .3	9.2 ± .8	9.6 ± .5	7.9 ± .7[1]	10.1 ± .7[2]	11.2 ± 1.5[3]	12.6 ± .6[1]

S.E. = Standard error.
TPEX = Thyroparathyroidectomy.
T4 = L-thyroxine.
* Litters of each dam were reduced to 6.
† Data from Djojosoebagio and Turner (unpbl.) 27 animals for milk determination, only 10 animals for serum calcium determination.
‡ 64 animals divided into: 13 for sham-operated control, 21 for TPEX, 23 for other purposes and 7 animals discarded, considered as "failure" in the operation.

Level of significance of differences from sham-operated control:
[1,4] $.30 > p > .25$
[2] $.25 > p > .20$
[3] $.10 > p > .05$

Discussion. It is generally believed that Vit. D facilitates intestinal absorption of calcium(7,8). It has been suggested that Vit. D_2, similar to parathyroid hormone, causes mobilization of calcium from bones to the circulation(9,10). The importance of calcium in milk synthesis by the mammary glands is well recognized. A severe decrease in serum calcium may develop a condition of "milk fever" and it is believed that the cause of this condition is due to parathyroid hormone deficiency(11).

The present experiment showed that crystalline calciferol at the dose given was able to replace parathyroid gland function in TPEX lactating rats. In addition, these data confirmed the earlier report(12) in which it was suggested that the rise of serum calcium by calciferol was not mediated through stimuation of parathyroid glands. It is interesting that the milk secretion of the sham-operated group was higher than the milk secretion in normal non-sham-operated rats, indicating that the operation had no depressing effect upon subsequent lactation. It is apparent that the smaller sham-operated group included rats of somewhat greater normal lactation capacity than the larger control group.

Summary. Thyroparathyroidectomized lactating rats were administered calciferol (Vit. D_2) at a level of 0.2 mg/100 g BW in conjunction with 3 μg L-thyroxine/100 g BW from day 4 to 19. Milk yields determined on day 14, 16, 18, and 20 were not significantly different from the milk yield of a group of sham-operated rats but were higher than in a larger group of control animals. The litter weight of the experimental group was less than the sham-operated controls. Eight hours after operation serum Ca declined from 10.2 to 6.4 mg% but was restored to normal levels by calciferol on day 14 and 20. DNA content of the mammary glands of the two groups was similar. These data suggest that calciferol can take the place of the parathyroid hormone in mobilizing serum calcium adequate for the requirements of lactation in rats.

1. v. Berswordt-Wallrabe, R., Turner, C. W., *J. Dairy Sci.*, 1960, v43, 1838.

2. Djojosoebagio, S., Turner, C. W., *J. Animal Sci.*, 1962, v21, 1020 (Abstract).

3. Terepka, A. R., Chen, P. S., Jr., Jorgensen, B., *Endocrinology*, 1961, v68, 996.

4. Chiamori, N., Henry, R. J., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 817.

5. Griffith, D. R., Turner, C. W., *ibid.*, 1962, v110, 862.

6. Munson, P. L., *N. Y. Acad. Sci.*, 1955, v60, 776.

7. Haavaldsen, R., Nicolaysen, R., *Acta Physiol. Scandinav.*, 1956, v36, 102.

8. Nicolaysen, R., Eeg-Larsen, N., *Viatamins and Hormones*, 1953, v11, 29.

9. Carlsson, A., *Acta Paediat.*, (Stockh.), 1955, v44, 548.

10. Linquist, B., *ibid.*, (Uppsala), 1952, v41, Suppl., 86.

11. Dryerre, H., Greig, J. R., *Vet. Record*, 1925, v5, 225.

12. Hibbs, J. W., Pounden, W. D., *J. Dairy Sci.*, 1955, v38, 65.

Received December 18, 1963. P.S.E.B.M., 1964, v115.

Hemodynamic and Renal Effects of Octapressin.* (29032)

L. D. LONGO, J. A. MORRIS, R. W. SMITH, R. BECK AND N. S. ASSALI
Departments of Obstetrics and Gynecology, and Physiology, University of California School of Medicine, Los Angeles

Vasopressin possesses 3 main pharmacological properties: inhibition of diuresis, vasoconstriction, and contraction of intestinal smooth muscle. Recent studies(1,2) have indicated that a synthetic analogue octapressin† (2-phenylalanine-8-lysine-vasopressin) has marked vasoconstrictor but minimal antidiuretic activities.

* These studies were supported by grants from Nat. Heart Inst., Nat. Inst. Health, U.S.P.H.S. and Am. Heart Assn.

† Furnished as PLV-2 by Sandoz Pharmaceuticals, Hanover, U.S.A.