TABLE I. Effect of Hyperthyroidism Produced by Thyroxin on Adenosine Phosphates and Glycogen of Liver in Guinea Pigs.

	AMP	ADP	ATP	TCA-extractable glycogen, mg/g	Non-extractable glycogen, mg/g
	(μM/g)				
Normal (7 exp.)	.945 ± .25	1.49 ± .17	.99 ± .09	31.4 ± 1.7	3.6 ± .6
Hyperthyroid (7 exp.)	.77 ± .19	1.82 ± .10	.86 ± .11	1.9 ± .3	3.4 ± .6

Values as mean and stand. error of mean.

Model DU spectrophotometer. The residue from the TCA extraction was digested with 30% KOH solution and the glycogen isolated by precipitation with ethanol. The material was dissolved in water, proteinaceous material precipitated by addition of TCA, and the glycogen re-precipitated by ethanol. Glycogen determinations were made by the anthrone method(4) using purified glycogen isolated from rabbit liver as a standard.

Results obtained are given in Table I. It is evident that ATP comprises less than one-third of total adenosine phosphates present in liver in water-soluble forms, and that ADP is the form present in highest concentration. This distribution makes it possible to ascertain whether there is any shift in the balance in the thyrotoxic state. Neither with respect to total adenosine phosphates nor to distribution between ATP, ADP and AMP, was any significant difference found between normal and thyrotoxic animals. These data, therefore, lend no support to the theory that thyroid hormone produces a dissociation of phosphorylation from oxidation. However, there is still the possibility that such a shift in the balance between ATP and the dephosphorylation products might be obtained acutely, as a result of a single injection of a sufficiently large dose of tri-iodothyronine.

The decrease in glycogen content of the liver, which is well known as an effect of the hyperthyroid state, is clearly seen to be limited to that fraction which is extractable by TCA. This finding strengthens the hypothesis of Bloom *et al.*(2) that TCA-extractable glycogen is more active metabolically than the fraction which cannot be extracted from the liver by this means.

Summary. Chronic hyperthyroidism induced in guinea pigs by daily injection of thyroxin has no effect on concentrations of ATP, ADP, and AMP in the liver. This finding gives no support to the postulate that thyroxine produces its metabolic effects by dissociating phosphorylation from oxidation. Reduction in glycogen content of liver that results from chronic hyperthyroidism is entirely in the fraction extractable by TCA.

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Effect of Reserpine on Oxytocin and Lactogen Discharge in Lactating Rats.* (24929)

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It is generally accepted that oxytocin as well as lactogen are reflexly released from the hypophysis in lactating animals and that both hormones are important factors in determin-

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ing amount of milk obtained during nursing or milking. Although lactogen is essential for initiation and maintenance of lactation(1), oxytocin causes contraction of the myoepithelial elements(2) surrounding mammary alveoli and small ducts thus forcing milk into larger ducts where it is available for withdrawal. Recent investigations have indicated reserpine may stimulate production and release of lactogenic hormone(3-5) but may depress that of thyrotropin(6), gonadotropin (7) and, under certain conditions, adrenocorticotropin(8). Little is known, however, of the effect of the drug upon oxytocin release. In the present study we have determined the effect of reserpine upon oxytocin discharge as evaluated by the milk let-down method of Grosvenor and Turner(9). We have also determined lactogenic hormone content of the pituitary of 14-day postpartum lactating rats immediately prior to and following 30 minutes nursing.

Material and method. Seventy-two adult primiparous lactating rats of the Sprague-Dawley-Rolfsmeyer strain weighing 260-320 g were housed in individual cages and allowed free access to food and water. Shortly after parturition, each litter was reduced to 6 young and when 14 days old was isolated from their mother for 10 hours. During the isolation period, the mothers were treated as follows: (a) 39 received single subcutaneous injections of reserpine‡ in dose of 10 μg/100 g either 10 or 120 minutes prior to replacement of litters. (b) 8 received single subcutaneous injections of reserpine (10 μg/100 g) 120 minutes prior to replacement of litters followed by .2 USP/kg oxytocin 2 minutes prior to replacement of litters. (c) 25 served as controls and were uninjected. At end of isolation period, 15 reserpine-treated and 10 control mothers were killed with ether and their pituitaries rapidly removed, weighed individually and frozen until assayed for lactogenic hormone. The remainder were reunited with their litters and allowed to nurse for exactly 30 minutes. Immediately after nursing the mothers were killed, their pituitaries removed and prepared as above. The litters were then weighed, killed by decapitation and stomach contents removed and weighed. Quantity of milk obtained by litters of lactating rats in 30 minutes, expressed as percent litter body weight, was used as index of milk let-down activity. Four or 5 pituitary glands were pooled for each lactogenic hormone assay. These were crushed in an agate mortar, suspended in a measured amount of distilled water and assayed in adult common pigeons by the micromethod of Grosvenor and Turner(10). Solutions of standard lactogenic hormone§ were run simultaneously in some assays. Average crop gland responses were converted to mg lactogenic hormone by use of the regression equation from the assay procedure(10) since the average response to the standard was 5-10% of that produced with the corresponding dose of the standard in the original assay. Gross observations were also made on the effect of reserpine on behavior of the animals.

Results. Rats treated with reserpine did not exhibit ptosis or any external signs of sedation. Nursing behavior appeared normal for upon replacement of the litters the mothers would retrieve their young and begin nursing within 1-4 minutes. Amount of milk obtained by litters of lactating rats injected with reserpine 120 minutes prior to litter replacement was significantly less than that obtained by control offspring ($P<.001$). Although the quantity of milk obtained by young of mothers treated 10 minutes before nursing was significantly less ($P<.05$) than that obtained by control young, the range of values was greater than that of animals treated 120 minutes before litter replacement as evidenced by the larger standard error (Table I). When oxytocin was injected after reserpine, significantly larger ($P<.03$) quantities of milk were obtained by offspring in comparison with control group. Pituitary lactogenic hormone concentration of control mothers killed at end of 10 hour isolation period was 122.1 I.U./g pituitary (prenursing level). Following 30 minutes nursing, the hormone content had decreased approximately

‡ Kindly supplied by Ciba Pharmaceutical Products and Eli Lilly and Co.

§ Highly purified sheep preparation estimated to contain 20 I.U./mg.

TABLE I. Effect of Reserpine on Quantity of Milk Obtained in 30 Minutes by Litters of 14-Day Postpartum Lactating Rats following 10 Hours Isolation of Mother and Litter.

Treatment	No. of rats	Time of treatment prior to nursing (min.)	Avg wt (g) of: Litters	Milk	Avg % Milk wt / Litter wt
Control	15		186.4	7.87	4.25 ± .31*
Reserpine, 10 μg/100 g	10	10	169.5	5.26	3.09 ± .41
Idem	14	120	174.6	4.02	2.28 ± .22
" , then oxytocin, .2 USP/kg	8	2	161.3	9.84	5.51 ± .32

* Stand. error of mean.

87% to a postnursing level of 16.4 I.U./g. Administration of reserpine 120 minutes prior to litter replacement decreased the prenursing level 48% (64.1 I.U./g) when compared with the control level (Fig. 1). The postnursing level of reserpine-treated animals was 23% (12.7 I.U./g) lower than the control value.

Discussion. Reserpine has been shown to exert an inhibitory effect upon the ovary, thyroid and adrenal gland(6-8) presumably through suppression of neural centers within the hypothalamus and/or reticular formation with subsequent inhibition of tropic hormone discharge. Results of the present study indicate that reserpine inhibits oxytocin release centrally rather than peripherally since above normal milk yields were obtained in reserpine-treated animals following injection of oxytocin. These observations would appear to rule out competition between oxytocin and reserpine at the myoepithelial level, at least, to any significant degree. Milk yield greater than normal is probably a result of the dosage of oxytocin employed. Although comparison of responses resulting from hormone administration by different routes of injection is inadvisable, it is of interest that a single intravenous injection of .1 USP/kg oxytocin results in milk yields comparable to those obtained with .2 USP/kg subcutaneously, whereas .05 USP/kg intravenously produced yields comparable to that of controls of the present study(11). Data obtained from animals injected with reserpine 10 minutes prior to litter replacement suggests a certain level of the drug must be present in general circulation before maximum inhibition of milk let-down is assured. It is possible that reserpine may be acting at any of the higher nerve centers involved in the let-down reflex or at centers within the spinal cord. However, it has been shown(12) that reserpine has no effect upon spinal reflex arcs at doses which normally produce symptoms characteristic of reserpine activity. Since ptosis was not evident in rats treated with the drug, it appears that inhibition of milk let-down reflex by reserpine occurs at higher centers. Pharmacological(13, 14) and hormone secretion(6-8) studies indicate the hypothalamus and reticulum as the principal sites of action of reserpine, particularly when low doses of the drug are used.

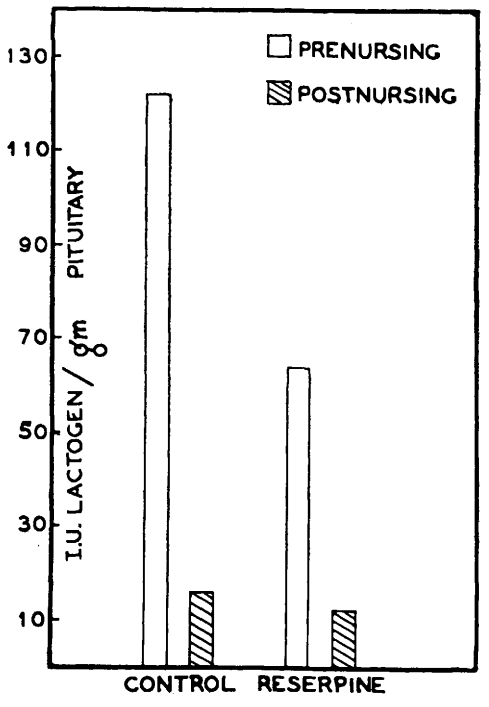

FIG. 1. Effect of reserpine on pituitary lactogenic hormone concentration following 10 hr isolation of mother and young (prenursing level) followed by 30 min. nursing (postnursing level). Reserpine administered 120 min. prior to end of isolation period.

Recent investigations have shown that atropine and dibenamine inhibit reflex release of both oxytocin and lactogenic hormone(15). These observations suggest that both adrenergic and cholinergic components are involved in the reflex arc. Reserpine has been shown to exert a central sympathetic depressant effect(13). It is probable, therefore, that the drug inhibits oxytocin release through suppression of sympathetic components in the region of the hypothalamus which would result in a reduction in the circulating level of the hormone with subsequent decrease in let-down of milk.

Several investigators(3-5) have suggested reserpine may stimulate production and discharge of lactogenic hormone. The lower prenursing level of animals treated with the drug 120 minutes before end of isolation period appears to substantiate this view. The lower prenursing level of this group may not reflect the actual lactogen releasing activity of reserpine since pituitary restoration occurs at a very rapid rate. Grosvenor and Turner(16) have shown that approximately 50% of the prenursing level is restored within 2-3 hours following 30 minutes nursing. The observation that reserpine may initiate lactogen discharge while at the same time inhibit oxytocin release suggests that the drug may exert a direct action upon the adenohypophysis. On the other hand, the lactogen releasing activity of reserpine may be mediated by suppression of an inhibitory hypothalamic center such as that which has been postulated for control of follicle-stimulating-hormone release(17).

Summary. 1) The effect of reserpine upon release of oxytocin and lactogenic hormone from the hypophysis has been studied in 14-day postpartum lactating rats. Oxytocin discharge, evaluated by milk let-down yield per timed nursing, was significantly inhibited. Results indicate the drug inhibits oxytocin discharge by suppression of central sympathetic centers involved in the let-down reflex. 2) Decrease of pituitary lactogen content (prenursing level) by approximately 48% indicates reserpine stimulates release of lactogenic hormone. It is possible that the effect is mediated by suppression of an inhibitory hypothalamic center controlling lactogen discharge or by direct action of drug upon adenohypophysis.

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