months and when rechallenged with a similar tumor tissue suspension on the opposite hip were resistant to the second implant.

OPDA has been submitted to the SKI tumor spectrum(11) where it was tested against a variety of 24 mouse, rat, and hamster tumors. A number of tumors showed inhibition within this test system and the results will be detailed elsewhere.

Discussion. The phenylenediamines are physiologically active compounds(12,13,14), and it is therefore a complex experimental problem to establish the mechanism of action of their antitumor properties. Their ability to combine in oxygen consuming reactions with certain catechol amines such as dopa and related products present in some pigmented tumors, is provocative but inconclusive evidence of their mode of action. This class of compounds has also been reported to have inhibitory effects against DPN-dependent enzyme systems(14). In testing the effect of ortho-, meta-, and paraphenylenediamine on respiration and glycolysis of Ehrlich ascites cells in manometric experiments, inhibition by all 3 isomers was observed in respiration but not in glycolysis under conditions of testing (unpublished). This suggests that DPN enzymes are not critically involved in the inhibition of Ehrlich carcinoma cells. It also indicates that aerobic respiration of the cell is more sensitive to these compounds than the anaerobic pathways. From the chemotherapeutic standpoint the structural simplicity of OPDA is of special interest and lends itself to simple chemical substitution and radioactive labeling for a systematic study of its action.

Summary. The antitumor effects of OPDA (orthophenylenediamine) on mouse and hamster melanomas and the Ehrlich solid carcinoma are reported. Complete inhibition of growth of the Ehrlich solid carcinoma was obtained as well as complete regression of some established and measurable tumors.

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Effect of Growth Hormone and Oxytocin Upon Milk Yield in the Lactating Rat.* (24558)

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It has been demonstrated on basis of milk yields that growth hormone (GH) causes an increase in milk secretion in cows(1,2), sheep, (3), and goats(4); while litter growth data suggested that it may be ineffective in rats (5). It is well known, however, that amount of milk removed during nursing or milking is related to quantity of oxytocin discharged from the neurohypophysis(6,7). It seemed of interest, therefore, to reinvestigate the role of

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TABLE I. Effect of GH and Oxytocin upon Milk Removal by Litters of 6 Young on Day 14 Postpartum.

Treatment	No. of lactating rats	Avg wt of: Milk (g)	Litter (g)	Milk / Litter (%)	Mother (g)	Pituitary (mg/100 g)	Adrenal (mg/100 g)	Thyroid (mg/100 g)
Control	30	6.20	163.6	3.8 ± .29[1]	303.5	3.39	23.41	4.06
Control: 3-6 USP units /kg oxytocin IV on day 14	30	9.20	164.2	5.6 ± .14	308.9	3.38	23.57	4.04
Control: .1 USP unit/ kg oxytocin IV on day 14	25	9.81	172.6	5.7 ± .22[2]	299.1	3.42	22.96	3.74
GH: 1 mg/day from days 7-14	28	8.80	163.3	5.4 ± .32[3]	300.7	3.44	23.48	4.08
Idem + .1 USP unit/kg oxytocin IV on day 14	25	7.90	171.7	4.6 ± .10[4]	312.5	3.33	24.16	3.99
Idem, but .3 unit	25	10.80	176.9	6.1 ± .12[5]	316.2	3.29	22.18	4.11

Student's "t"	Probability
1-2,3,5	.005
1-4	.02
2-4	.005
2-5	.3
3-4	.01
3-5	.1
4-5	.005

GH in lactation in rats in terms of milk yields obtained during nursing with and without aid of oxytocin.

Materials and methods. Method of obtaining milk: Mature primiparous lactating rats of the Sprague-Dawley-Rolfsmeyer strain which had reached growth stasis were used. They were housed in individual cages under conditions of uniform temperature (78-80°F) in a room artificially illuminated during normal daylight hours and fed Purina Lab Chow and water *ad libitum*. Shortly after birth each litter was adjusted to 6 young and, whenever possible, were equally distributed as to sex. When 14 days of age, each litter was isolated from their mother for 10 hours. During this interval any milk present in stomachs of young is digested(8) and the mammary glands of mothers become turgid with milk. At the end of isolation, mother and young were reunited and young permitted to nurse 30 minutes. Seventy-eight lactating rats were injected subc. with growth hormone (GH)‡ at a dose of 1 mg/rat/day from days 7-13 of lactation. Twenty-eight GH-treated and 30 control lactating rats, half of which were similarly injected with saline, were assayed for milk yield in this manner. Fifty-five control and 50 GH-treated lactating rats were anaesthetized with Nembutal (45 mg/kg i.p.) 10-20 minutes prior to end of isolation. When completely anaesthetized, each mother was laid on her side and her litter replaced. Oxytocin‡ was then injected rapidly in a volume not exceeding .06 ml into the superior epigastric vein a few minutes after the young had been replaced and while they were actively sucking. After each litter had nursed for 30 minutes, they were removed, weighed, killed by decapitation and stomach contents removed and weighed to nearest 0.1 g. Weight of milk obtained is then expressed as percent litter body weight. Each mother also was killed shortly after nursing and her thyroid, adrenals, and pituitary glands rapidly removed, blotted on filter paper to remove surface moisture and weighed to nearest 0.1 mg. Daily body weights of each mother and her litter were recorded.

Results. Weight of milk, expressed as per cent litter body weight, obtained by litters of 30 untreated rats averaged 3.8% (Table I) with normal distribution. A significantly greater yield (5.7%), which represents an in-

‡ Kindly supplied by Dr. Irby Bunding, Armour Labs., Chicago.

TABLE II. Effect of GH upon Body Weight Gain of Lactating Rats and Their Litters from 6th-14th Days Postpartum. Litter weights at day 14 corrected for weight of milk obtained during 30 min. nursing.

Treatment	No. of rats	Avg wt (g) of Litter Day 6	Litter Day 14	Litter Gain	Litter % gain	Mother Day 6	Mother Day 14	Mother Gain	Mother % gain
Control	45	78.7	166.5	87.8	111.6 ± 2.5[1]	297.0	300.3	3.3	1.1 ± .36[3]
GH, subcut.	63	74.5	159.3	84.8	113.8 ± 2.9[2]	294.4	316.4	22.0	7.5 ± .12[4]

	Student's "t"	Probability
1-2		>.2
3-4		.001

crease of 50%, was obtained when .1 USP unit/kg oxytocin was injected i.v. into Nembutal anaesthetized mothers while the young were nursing. Variability in milk yield was reduced. No further increase in milk yield resulted when 3-6 USP units/kg oxytocin were administered (Table I). GH caused a significant increase in average yield of 42.1% but individual values showed more variability than controls. Milk yield obtained in GH-treated rats with aid of .1 USP unit/kg oxytocin was significantly less than obtained in control rats with the same dose of oxytocin. An increase to .3 USP unit/kg was necessary to elicit near maximum withdrawal of milk (6.1%) which represents a 60.5% increase over untreated controls and a 7% increase over oxytocin-injected controls. This was accompanied by further reduction in variability in milk yields. Percent weight gain of litters of GH-treated lactating rats from days 6-14 postpartum was insignificantly greater than for control litters (Table II). GH-treated mothers gained an average of 2.7 g/day during period of treatment which represents a significant percentage weight increase over .5 g/day gain for controls. Correlation of litter weight with milk yield of controls on day 14 indicated litter weight increased with increasing milk yield to where 7-8 g milk was obtained during 30 minutes nursing (Fig. 1). Greater yields were not reflected in a further increase in litter weight. GH did not cause a significant increase in the wet weight/100 g body weight of maternal pituitary, thyroid, or adrenal glands (Table I).

Discussion. A serious difficulty in lactational studies on rats has been the lack of satisfactory means of milking them and thereby evaluating milk secretion or removal. Lactational performance in rats generally has been inferred by litter growth and survival data whereby an alteration in growth is thought to mean that an alteration in milk secretion has occurred(9-13). This method has considerable merit in evaluating milk secretion in nutritionally or hormonally deficient animals. However, in a normal intact lactating animal, the young have a genetically inherent capacity for growth which cannot be exceeded. We question whether more intense milk secretion can produce litter weight gain in excess of this capacity since, in the present investigation milk yield and litter were correlated only to a point beyond which, though milk yield was greater, no parallel increase in litter weight was observed. Thus, in healthy animals, an increase in amount of milk obtained under standardized conditions, is suggested to mean an increase in milk secretion has occurred. On this basis, in the present study, GH evoked an increase in milk secretion of a similar magnitude as reported for other species(1-4) which, however, was not accompanied by a significant increase in litter weight. We had found previously that amount of oxytocin released due to 30 minute nursing stimuli on day 14 varied from less than .02 to .1 USP unit/kg (7). In the present study administration of .1 USP unit/kg oxytocin resulted in 50% higher yields with greater uniformity of individual values which suggests milk removal and, indirectly, milk secretion, of lactating rats is limited by amount of oxytocin discharged due to the stimulus of nursing. This level may be considered optimal since larger doses of oxytocin failed to evoke greater milk withdrawal. It is suggested GH at level em-

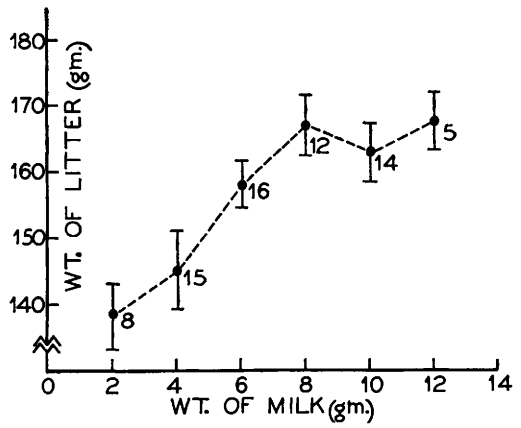

FIG. 1. Correlation of amount of milk obtained by litters of 6 young during 30 min. nursing following isolation of mother and young for 10 hr with weights of litters after nursing. Data obtained on 14th day postpartum. Numbers beside each point represent number of litters. Vertical bars represent stand. error.

ployed may, in addition to being galactopoetic, also be a limiting factor in milk secretion of many rats since milk yields obtained in GH-treated lactating rats with aid of oxytocin were greater than with oxytocin alone. What is perhaps more significant is that greater reduction in variability of individual yields resulted from the combined treatment. It was interesting to observe that GH-treated rats required 3x more oxytocin to obtain maximum yields of milk than did controls. Increased requirement of oxytocin to evacuate milk in such animals might mean a larger secretory area, thus providing more loci for oxytocin action, or an increased threshhold for myoepithelial contraction. Research in progress may clarify this point.

Summary. (1) We have investigated the effect of growth hormone (GH) and oxytocin alone, and in combination, upon lactation in the rat. Amount of milk obtained by a litter of 6 during 30 minutes nursing on day 14 postpartum, expressed as percent litter body weight, was used as the index of response. (2) Control yields averaged 3.8% with normal distribution. Removal of milk with aid of .1 USP unit/kg oxytocin i.v. resulted in 50% higher yield with more uniformity among individual values. (3) GH injected subc., from days 7-13 at dose of 1 mg/rat/day evoked a 41.1% increase in milk yield. Yields obtained in GH-treated lactating rats with aid of oxytocin were greater and more uniform than with GH or oxytocin alone. The significance of this is discussed. (4) GH had no effect upon pituitary, thyroid or adrenal weight/100 g body weight. GH caused a significant increase in percent weight gain of mothers but not their litters. Litter growth rate as an index of lactation in the rat is discussed.

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