were computed by the methods described above. The means of the differences between those of irradiated and control mice are plotted (as *whole* logs) in Fig. 2. The slope shows how much greater was the increase in susceptibility to the challenge inoculations produced by each increment of 100 r acute X-radiation, approximately .54 log of inoculum per 100 r.

Discussion. The differences in LD_{50}s of *Ps. aeruginosa* for irradiated and control mice in each challenge inoculation indicate a slight but progressive increase in susceptibility to this bacterial infection from the 9th through the 15th weeks of exposure to 15 r/day. The change was too small to have been demonstrable by use of 10-fold gradations of inocula as used in our previous experiments on mice exposed to 34 r/day(1). At 9 weeks, the shortest exposure time, the effect on susceptibility was so slight as to raise the question whether any change at all could have been detected earlier.

After 15 weeks' irradiation (total accumulation 1350 r) the increase in susceptibility, while still small, was clearly demonstrable. It is unfortunate that exposures could not have been prolonged beyond that time. Comparison of this effect of chronic γ irradiation with that produced by a single exposure shows that an accumulation of 1350 r (at 15 r a day) increased susceptibility to the challenge inoculations much less than did an acute exposure to 300 r X-radiation.

The effect on total leucocyte counts was about the same. The geometric mean of the white counts on 5th day following exposure to 300 r was 2400(1). After accumulating 1350 r (at 15 r a day) it was 2200.

Summary. 1) CF-1 female mice were exposed to approximately 15 r a day radiation for 9-15 weeks and challenged at intervals by intraperitoneal inoculation with graded doses of *Pseudomonas aeruginosa.* Comparison of LD_{50} of irradiated and control mice in each challenge showed that between 9th and 15th weeks a slight but demonstrable increase in susceptibility to experimental infection had occurred. 2) The increase caused by accumulation of 1350 r during 15 weeks exposure was, however, very much less than that resulting from a single acute exposure to 300 r X-radiation.

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Induction of Lactation and Mammary Growth by Pituitary Grafts in Intact and Hypophysectomized Rats.*† (25801)

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Recent investigations suggest that the hypothalamus normally inhibits prolactin secretion by the AP, and that severance of the pituitary from its cranial connections results in increased production of this hormone. Desclin(1-2) and Everett(3) reported that autotransplantation of the pituitary to the kidney capsule of the rat maintained functional corpora lutea for prolonged periods of time. Eckles *et al.*(4) noted lactation in several

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women with metastic breast cancer after pituitary stalk-section, and Loeb and Kirtz(5) and Muhlbock and Boot(6) reported that subcutaneous isografts of pituitaries into intact mice induced mammary growth. It was of interest to see whether pituitaries grafted to the kidney capsule of intact or hypophysectomized rats, after estrogen priming, could produce sufficient prolactin to induce secretion and maintain or increase mammary growth.

Methods. Sixty mature virgin, female Carworth rats, weighing 180-210 g each, were injected subcutaneously with 10 μg estradiol in corn oil once daily for 10 days to partially develop their mammary glands. In all except 10 controls, pituitaries from similar donor rats were implanted underneath the kidney capsule on days 1, 5 or 7 of estradiol treatment. Twenty-four of these rats were hypophysectomized on day 11 by the parapharyngeal approach, and these and all other rats were given no further treatment for the next 5 days. On the 16th day the rats were killed, and the right inguinal mammary glands, pituitary graft, adrenals, thymus, ovaries and uterus were removed and prepared for microscopic examination by standard histological technics. The latter 4 organs were also weighed. Ratings of 0-3 were assigned for mammary development and lactation in each case, depending on extent of mammary lobulo-alveolar development and distention of alveoli with secretion.

Results. In previous experiments it was established that rats at the end of 10 days' injection with 10 μg estradiol daily developed considerable lobulo-alveolar tissue (avg rating = 1.5), and that absence of further treatment for the following 5 days resulted in regression of the mammary glands to essentially a bare duct system(7). This was also evident in the present controls. By contrast, in 24 intact rats with pituitary grafts, lactation was initiated in 20 and mammary lobulo-alveolar structure was maintained or increased in 23 rats. Pituitary implants made on day 1 induced lactation in all 7 rats and produced greater mammary growth than in the 17 rats implanted on day 7 (Table I). In 26 hypophysectomized rats with pituitary grafts, mammary secretion was initiated in only 8 and lobulo-alveolar tissue was maintained in 12 of 26 animals. Mammary glands of the remaining hypophysectomized rats were atrophied and contained mainly ducts, as in the

TABLE I. Effects of Pituitary Implants in Intact and Hypophysectomized Rats on Mammary Glands and Several Organ Weights.

Treatment	No. of rats	No. with secretion	No. with LA*	Avg body wt (g)		Avg organ wt (mg)/100 g body wt			
				Initial	Final	Adrenals	Thymus	Ovaries	Uterus
Intact rats									
Controls, saline	10	0	0	210	218	27.5 ± 1.5‡	99.9 ± 8.8	26.1 ± 1.8	207.1 ± 19.0
Pit. implant on day 1	7	7 (1.0)†	7 (2.5)†	202	224	30.8 ± 2.8	38.7 ± 17.8	25.6 ± 5.2	191.2 ± 19.3
Idem 7	17	13 (0.9)	16 (1.8)	195	205	30.2 ± 2.4	76.1 ± 14.6	21.5 ± 2.8	168.7 ± 19.8
Hypophysectomized rats									
" 1	12	2 (0.1)	4 (0.7)	187	174	20.7 ± 2.2	70.0 ± 11.6	22.4 ± 2.0	156.0 ± 10.4
" 5	14	6 (0.5)	8 (1.6)	189	174	16.1 ± 1.1	62.1 ± 7.2	17.2 ± 1.6	156.0 ± 9.7

* LA = lobulo-alveolar development.

† Avg rating = $\frac{\text{Sum of individual ratings (0-3)}}{\text{Total No. of rats in group}}$.

‡ Stand. error of mean.

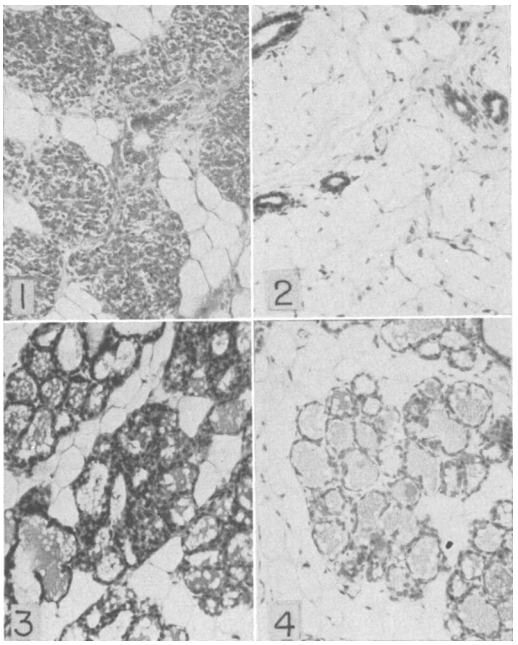

FIG. 1-4. Histological sections of mammary tissues from estrogen-primed rats with and without pituitary grafts: (1) From non-grafted rat on 10th day of estradiol treatment. Note development of lobulo-alveolar system but no secretion. (2) From non-grafted rat on day 16. Note regression to bare duct system. (3) From intact rat grafted with pituitary on day 1, showing well-developed alveoli extended with secretion. (4) From hypophysectomized rat grafted with pituitary on day 5, also showing secretion and development of alveoli. ×72.

controls. Representative microphotographs of mammary gland sections are shown in Fig. 1-4.

The pituitary grafts appeared healthy and well vascularized in most intact and hypophysectomized rats. In a few hypophysectomized rats, grafts could not be recovered from the kidney capsule and had apparently slipped out. However, the presence of heavily luteinized ovaries in these rats suggested that the pituitaries were functional. Everett(3) has similarly described pituitary transplants to the kidney capsule of rats which slipped out and became functional elsewhere. The ovaries of both intact and hypophysectomized rats with grafts showed a predominance of healthy corpora lutea with few or no small follicles. The corpora lutea were usually larger in the intact than in the hypophysectomized rats. Ovaries of 2 intact rats implanted on day 7 had large follicles and only small corpora lutea, and these showed no mammary secretion. Adrenal weights were significantly lower in hypophysectomized rats than in intact rats, and appeared to be atrophied upon microscopic examination.

Discussion. These data show that pituitary isografts to the kidney capsule of rats can initiate secretion and maintain or extend mammary growth developed by prior estradiol treatment. Preliminary experiments also indicate that in intact postpartum rats after litter removal, pituitary isografts to the kidney capsule can inhibit mammary involution (Nicoll and Meites, unpublished). Mammary growth and secretion were much more pronounced in grafted intact than in grafted hypophysectomized rats, and might have been due to a relative deficiency of ACTH, TSH and STH secretion by the latter animals. Secretion of these 3 hormones as well as FSH and LH are markedly reduced when the pituitary is removed from its normal cranial site (8). Adrenal cortical hormones as well as prolactin are required to initiate lactation in hypophysectomized rats(9), and adrenals of the hypophysectomized rats with pituitary grafts in the present experiment were notably reduced in size.

The present results, in agreement with others, indicate that the transplanted pituitary does not need CNS stimulus to secrete prolactin, and actually produces more prolactin when its connection to the hypothalamus is severed. We have recently reported that many agents can induce mammary growth and secretion in estrogen-primed rats, including adrenergic and cholinergic drugs(7,10), CNS stimulators and inhibitors(11), various tranquilizing drugs(11,12), electrical stimulation of uterine cervix(13), nonspecific stresses(14) and rat hypothalamic extract‡(15). Some of these, such as reserpine, chlorpromazine or electrical stimulation of the uterine cervix may act by removing CNS inhibition to prolactin secretion. Others may act directly on the anterior pituitary to remove CNS inhibition or by some other mechanism. It remains

‡ Positive results with hypothalamic extracts suggest that the CNS may contain a prolactin-releasing center.

to be established that a prolactin-inhibitory neuro-hormone is actually present in the CNS.

Summary. 1. Sixty mature female rats were injected with 10 μg estradiol daily for 10 days to develop their mammary glands. On days 1, 5 or 7, all except 10 control rats were each implanted underneath the kidney capsule with a pituitary from a similar untreated donor rat; on day 11, 26 implanted rats were hypophysectomized. All rats were killed on day 16. 2. Control mammary glands regressed to a bare duct system and showed no secretion, whereas 23 of 24 intact rats with pituitary grafts and 12 of 26 hypophysectomized rats with grafts showed mammary secretion and/or lobule-alveolar development. Most rats with pituitary implants had ovaries with large corpora lutea and few or no follicles. These results demonstrate that grafted pituitaries in the rat secrete greater than normal amounts of prolactin.

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Chemotherapy of Sarcoma 180 by Combinations of DL-Glyceraldehyde with 6-Thioguanine or with Azaserine and 6-Chloropurine.* (25802)

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Glyceraldehyde has been reported in a number of instances to be a potent glycolytic inhibitor; this was recently reviewed briefly by Gold(1). The mechanism of inhibition was shown by Lardy *et al.*(2) to be due to condensation of L-glyceraldehyde with dihydroxyacetone phosphate to yield L-sorbose-l-phosphate, which subsequently interfered with the hexokinase reaction. In view of this inhibition and of the knowledge that nucleotide formation requires carbohydrate as well as purine or pyrimidine moieties, the present study was designed to test anti-neoplastic properties of combinations of inhibitors of these systems.

Methods. Experiments were performed on 9 to 11-week-old female Ha/ICR Swiss mice (A. R. Schmidt Co.). Tumor transplantation was carried out by withdrawing ascites fluid from a donor mouse bearing a 7-day tumor growth. The fluid was centrifuged for 2 minutes in a clinical centrifuge (1600 x g), supernatant peritoneal fluid was decanted, cells were diluted 1:10 with isotonic saline, and 0.1 ml (approx. 2 x 10^6 ascites cells) of cell suspension was inoculated into each animal. Mice were distributed into groups of comparable weight and maintained during experi-

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