TABLE II. Blood Radioactivity Levels in Dogs.

Triolein emulsion			Oleic acid capsule		
4 hr	5 hr	6 hr	4 hr	5 hr	6 hr
(%)					
Pancreatitis—vinylite					
1.5	1.3	1.5	1.1	6.5	7.8
1.5	3.4	4.4	6.5	7.0	8.0
Pancreatectomy					
—	5.3	1.2	10.6	10.1	7.6
0	.3	0	11.3	14.7	4.5
1.6	2.0	2.2	11.7	12.0	9.0

neutral fat and fatty acid test might differentiate between abnormalities of digestion and absorption.

This assumption was tested in the 4 dogs in which pancreatic insufficiency was produced. As seen in Table II post operative neutral fat blood radioactivity levels were markedly depressed. These results attest to the pancreatic insufficiency as demonstrated previously(5). In contrast, blood radioactivity levels following oleic acid test were within the range of normal. The 2 patients with known pancreatic disease (Table I) gave results similar to those obtained in the pancreatic deficient animals. In the few patients with known small bowel disease tested, both triolein and oleic acid curves were depressed (Table I).

The results obtained in the experimental animals and the small number of patients with known gastrointestinal abnormalities suggest the probability that neutral fat and fatty acid will prove helpful in differentiating between abnormalities of digestion and absorption. Similar conclusions have been recently reported by others(4,6).

Summary and conclusion. 1. Constant and reproducible curves of blood radioactivity levels are found in normal humans and dogs after an oleic acid test. 2. Following pancreatectomy and induced pancreatitis in dogs, the curves obtained with oleic acid test were within normal range while those obtained with triolein were depressed.

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Hormonal Influences on Growth and Somatotropic Actions of Autonomous Mammotropes.* (23096)

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Estrogens stimulate secretion and proliferation of mammotropes (Mt) of the anterior pituitary gland(1-3). Mammotropic tumors (MtT) can be induced in rats by prolonged estrogen treatment; they are readily transplantable to estrogen-treated and, occasionally, to normal rats. Upon successive transplantation, the former, that is, dependent MtT, invariably gives rise to autonomous variants. Extracts of such tumors contain mammotropin (MtH; synonym: prolactin)(4). All transplanted mammotropic tumors thus far studied have had a pronounced somatotropic effect and were markedly responsive to estrogens. The present study was undertaken to investigate more precisely the role of hypo-

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TABLE I. Design of Experiments on Grafting of MtT in Variously Treated Rats.

Passage	Strain and age in days at grafting	Sex	No. of animals: Tumor-bearing	No. of animals: Controls	Treatment: Gonadex	Treatment: Adrex	Treatment: Hypex	Treatment: Stilbestrol, mg	Duration of exp. in days*
III a (Fig. 1,5; Table II)	WF 35–40	♀	4†	2				0	140
		♀	4	3				5–6	100
		♀	3	1			×	5–6	85
		♂	3	2	×			0	166
		♂	3	2	×			5–6	86
		♂	2	1			×	5–6	85
III b (Fig. 2)	F 35–40	♀	4	2				0	78
		♀	4	3	×			0	90
		♂	4	1				0	89
		♂	4	2	×			0	89
		♂	4	2	×			6–7	69
III d	WF 44–46	♀	7	7				0	176
		♀	4	3			×	3–4	212
		♂	5	5	×			0	174
		♂	7	7	×	×		0	176
IV a (Fig. 3, 4)	F 58–75	♂	3	4	×	×‡		1.0	57
		♂	4	—	×	×‡		.1	61
		♂	5	4	×	×§		.01	61
		♂	4	—	×	×‡		.001	61
		♂	4	4	×	×‖		.0	62

* From grafting of animals until sacrifice.
† Tumor grafted in both thighs throughout passage.
‡ One animal in each group had regenerated adrenal cortex tissue.
§ Two animals in MtT-bearing group and one control had regenerated adrenal cortex tissue.
‖ One animal in MtT-bearing group had regenerated adrenal cortex tissue.

physeal, gonadal and adrenal hormones on growth of autonomous MtT (Strain 4)(3), and on its somatotropic manifestations.

Materials and methods. Rats of the Fischer strain (subline of the strain of Dr. Wilhelmina Dunning designated F) or F_1 hybrids of an inbred Wistar strain and the F strain (designated WF) were raised in our laboratories and fed Purina chow supplemented with greens. Animals differing in age by more than 5 days were evenly distributed in the experimental groups. Tumor particles suspended in saline were injected into the thigh muscles. Operations were performed and hormone treatments were started before grafting (passage IVa) or within 8 days thereafter (passages IIIa, b, and d). For chronic estrogen treatment, fused cholesterol-diethylstilbestrol (DES) pellets were implanted subcutaneously in the neck. The DES:cholesterol ratio was 1:3 in pellets used in passages IIIa, b, and d. In passage IVa, the pellets weighed 16-18 mg and contained 0.0 to 1.0 mg DES. Adrenalectomized animals were given 2.5 mg desoxycorticosterone trimethylacetate† and 2.5 mg cortisone acetate (Cortone Acetate, Sharp and Dohme) every 2 weeks. In passage IIId this treatment was discontinued after 3 months and the animals were given physiological saline as drinking water. Experimental animals were killed and autopsied in groups with their controls. For histological studies, tissues were fixed in Zenker-formol and stained with hematoxylin and eosin. The design of 4 experiments is shown in Table I. Small numbers of animals were used because of limited breeding facilities.

Results. I. *Effects of hormones on growth of MtT.* Pituitary hormones are not required for proliferation of these mammotropic tumor cells; in fact, MtT grew somewhat faster in hypophysectomized than in normal females in one passage (Fig. 1) and but slightly slower in another (passage IIId). In the latter,

† This preparation (Percorten trimethylacetate) was generously supplied by Dr. Robert Gaunt, Ciba Pharmaceutical Products, Summit, N. J.

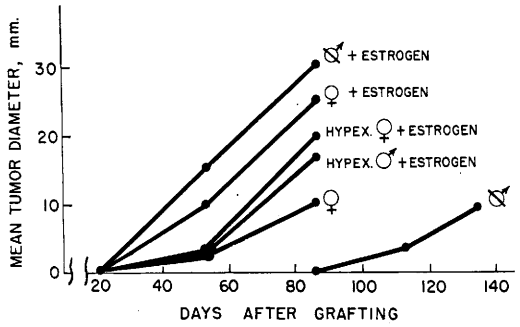

FIG. 1. Effect of diethylstilbestrol, hypophysectomy and castration on growth of autonomous MtT (passage IIIa).

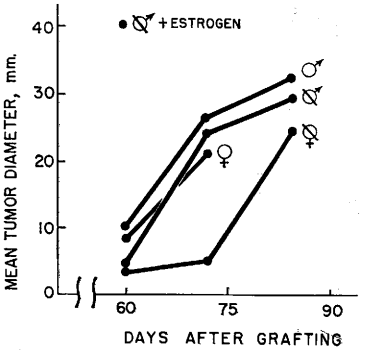

FIG. 2. Effect of gonadectomy and diethylstilbestrol on growth of autonomous MtT (passage IIIb).

mean latency of tumor growth was 122 days (range: 77-167 days) in hypophysectomized animals as compared to 102 days (range: 77-132 days) in intact untreated female hosts. In these experiments, all hypophysectomized animals were DES-treated. Earlier experiments with MtT in mice(2) suggested that without estrogen treatment, tumor growth would have been even slower than in castrated non-hypophysectomized males.

Fig. 1 shows a very long latent period and slow progression of tumor growth in castrated male animals and an extraordinary hastening of tumor growth in such rats treated with DES. Stimulation by DES was less marked in intact females. When the experiment recorded in Fig. 2 was performed, the tumor strain was less responsive to gonadal hormones. The contrast between gonadectomized and normal females was marked but there was little difference between gonadectomized and normal males. These data indicate that the effect of gonadectomy on MtT growth is due to removal of a source of estrogens.

The effect of varying quantities of DES on the growth of autonomous mammotropes is shown in Fig. 3. The rate of Mt proliferation was increased slightly by 10 μg of estrogen, moderately by 100 μg and markedly by 1 mg. In this experiment, the hosts were both adrenalectomized and gonadectomized. Adrenalectomy proved incomplete in several animals. There was, however, no difference in the rate of tumor or body growth between incompletely and completely adrenalectomized animals.

In passage IIId, MtT was grafted on castrated males and on adrenalectomized castrated males. The mean latency of tumor growth was 99 days in the former and 116 days in the latter (range: 54 to 148 days in both groups). The data suggest that adren-

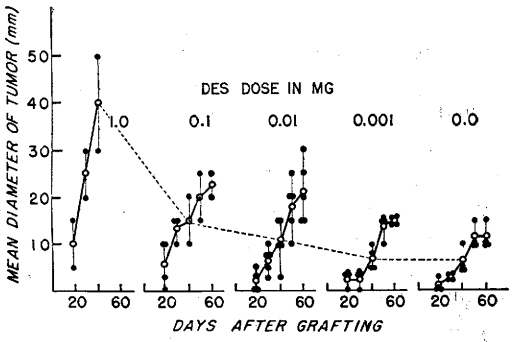

FIG. 3. Stimulation of autonomous MtT by graded doses of diethylstilbestrol (passage IVa). For key, see Fig. 4. Light dotted line connects observations on fortieth day.

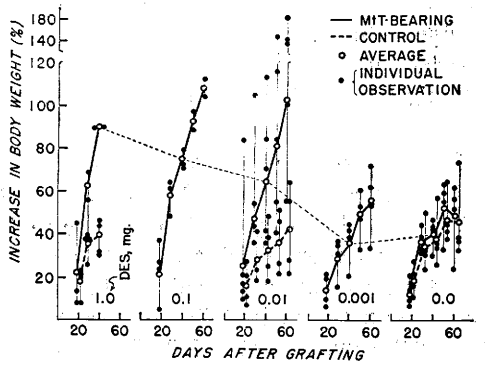

FIG. 4. Effects of different quantities of diethylstilbestrol on body weight of MtT-bearing and control rats (passage IVa). Light dotted line connects observations on fortieth day.

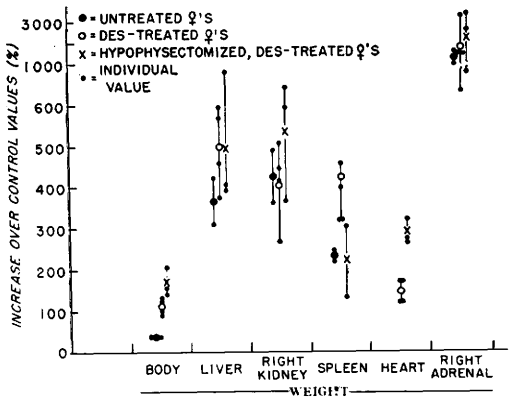

FIG. 5. Somatotropic effects of MtT. Percent increase of body and organ weights of tumor-bearing female rats over those of control animals (passage IIIa).

alectomy does not profoundly affect growth of this autonomous MtT.

II. *Somatotropic effects of MtT.* Gain in body weight (Fig. 4) paralleled that of tumor size (Fig. 3) both increasing with the quantity of estrogen administered and, presumably, with the quantity of hormone secreted by MtT. In order to indicate the somatotropic action of MtT, increases in body and organ weights of tumor-bearing female rats are expressed as percentages of the respective weights of matched controls (Fig. 5). The average weights of the mammary glands of these MtT-bearing rats (not shown in Fig. 5) were: intact females 34 g; DES-treated rats 36 g; hypophysectomized, DES-treated rats 21 g. In Table II, the somatotropic effects in variously treated males are indicated by body weight, body length, and by weights of organs expressed in percent of body weight. Considering time and tumor weight, the greatest gains occurred in hypophysectomized animals.

The data show a marked increase in overall skeletal size of most MtT-bearing animals. The liver, kidneys, spleen and heart were disproportionately enlarged, the greatest relative increase occurring in adrenal weights. These figures do not directly indicate the magnitude of somatotropic effects of MtT. Livers of MtT hosts usually had some fatty degeneration but enlargement appeared to be due mainly to increase in size of liver cells(3).

TABLE II. Effect of MtT on Body Size and Relative Organ Weights in Male Rats (Passage IIIa).

No. of rats	Treatment	Days after graft	Body		Tumor wt, g	% of body wt				
			Wt, g	Length, cm		Liver	Right kidney	Spleen	Heart	Right adrenal
2	DES + MtT	86	376 (365–388)	24.2 (24.2–24.2)	22.5 (19.2–25.8)	9.5 (9.3– 9.6)	.93 (.93– .93)	.54 (.53–.54)	.38 (.36–.39)	.067 (.052–.082)
3	DES	86	178 (166–187)	20.4 (19.7–21.0)	—	3.8 (3.4– 4.2)	.37 (.37– .37)	.26 (.21–.33)	.39 (.38–.41)	.017 (.014–.019)
3	Castrate + MtT	166	300 (289–305)	24.0 (23.5–24.5)	16.1 (13.7–21.7)	9.6 (8.5–11.1)	1.26 (.80–1.56)	.36 (.27–.48)	.45 (.33–.62)	.110 (.068–.166)
2	Castrate	166	308 (304–312)	23.0 (22.5–23.5)	—	3.2 (3.2– 3.3)	.32 (.32– .33)	.25 (.24–.26)	.29 (.29–.29)	.007 (.007–.007)
3	Hypex + DES + MtT	85	153 (143–163)	19.1 (18.4–19.7)	5.3 (1.6–9.0)	7.1 (6.1– 8.0)	.69 (.61– .77)	.38 (.27–.48)	.63 (.59–.66)	.054 (.032–.077)
1	Hypex + DES	85	73	15.5	—	3.4	.29	.22	.42	.006

The kidneys often underwent a marked tubular degeneration. The numbers in Fig. 5 do not indicate as marked a relative increase in body weights in intact as in DES-treated female tumor hosts. This difference may, in part, be accounted for by the greater length of time required for growth of MtT in normal rats and corresponding longer growth periods in their controls. In this experiment (passage IIIa) the intact female rats were killed 140 days after grafting when the tumor weight averaged 17.4 g; the DES-treated females were killed after 100 days when the tumor weight averaged 23.9 g; the hypophysectomized DES-treated females were killed after 85 days when the tumors weighed 4.6 g. Weights of the mammary gland represent the sum of secretions and increase in tissue mass. A detailed analysis of the character of mammary gland changes will be published.

The enormous hypertrophy of the adrenals remains to be explained. Increase in size was accompanied by extensive fatty degeneration of the cortex, often with hemorrhage and necrosis. The enlargement did not appear to be due primarily to adrenotropic effect by the tumor; the thymus of rats with medium-sized tumors was often normal. The thymus is known to undergo atrophy in animals bearing large tumors of all kinds. Grafted adrenotropes (in mice) cause atrophy of the thymus while the tumor is minute(2).

In passage IIIb, of 7 animals bearing tumors measuring 15-20 mm in diameter, the thymus was normal in 4, (average right adrenal weight 57 mg; in 9 controls 14-29 mg, mean, 19 mg), moderately atrophic in 1 (right adrenal weight, 108 mg) and atrophic in 2 (right adrenal weights, 210 and 215 mg). Of 7 animals with tumors 25-30 mm in diameter, the thymus was moderately atrophic in 1 (right adrenal weight, 130 mg) and atrophic in 6 (average right adrenal weight, 203 mg). Of 2 animals bearing tumors measuring 60 mm in diameter, the thymus of 1 was moderately atrophic (right adrenal weight, 123 mg) and completely atrophic in the other (right adrenal weight, 295 mg). There was no clear-cut relationship between size of the thymus and treatment of the host.

Discussion. The present studies indicate that hormones of MtT greatly stimulate growth of the body and viscera, and that the fully autonomous Mt retains a marked responsiveness to estrogen and is not dependent on pituitary hormones for proliferation and function.

Mammotropic and somatotropic effects occurred in parallel and in rough proportion to tumor size. It is possible(2,3) that MtH has intrinsic somatotropic action in rats and mice as has been previously reported in birds(5). Similar overlaps in function are well known; *e.g.*, in oxytoxic and vasopressor principles of the posterior pituitary and among adrenocorticoids. It is also possible that MtT (Mt) produces 2 distinct hormones but the parallel rise of somatotropic effect with mammotropic effects following administration of estrogen is better explained by postulating secretion of one hormone with these two potencies. Contopoulos and Simpson‡ described a body growth-promoting factor in the blood of pregnant rats. It seems likely that this effect was, in part at least, due to MtH. An adrenal growth factor in pituitary extracts distinct from AtH (ACTH) has also been suggested (6).

The autonomous mammotropic tumor studied by us was markedly reactive to the agents which initiated it. Responsiveness of malignant cells may yield clues to the factors involved in their genesis. Thus, autonomy is not synonymous with full release from responsiveness to conditioning factors, and study of responsiveness may help in determining the conditions leading to development of neoplasms as well as the factors controlling them.

Summary and conclusions. 1. The role of gonadal, adrenal, and hypophyseal hormones on growth and somatotropic actions of a transplantable, functional mammotropic pituitary tumor (MtT) was studied in rats. 2. Although this tumor is autonomous (in that it grows in normal rats of both sexes) it is highly responsive to estrogen, the rate of tumor growth and body growth of the host being roughly proportional to the quantity of estro-

‡ Reported in *Fed. Proc.*, 1956, v15, 39.

gen administered. Hormones of mammotropes stimulate body growth and growth of many organs. Somatotropic and mammotropic effects are marked in hypophysectomized rats bearing mammotropic tumors; thus they are independent of other pituitary factors.

Mrs. Lee Zompetti skillfully assisted in this work.

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Binding of Novobiocin with Plasma Proteins. (23097)

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The antibiotic activity of novobiocin *in vitro* is inhibited by the presence of serum (1).* In *in vivo* experiments novobiocin is still present in the blood stream 24 hours after an oral dose(2).* These findings together with evidence from dialysis* have suggested that novobiocin must bind to plasma proteins.

The purpose of this work was to determine the nature and extent of this binding. Fractionation studies with plasma from a dosed dog showed that novobiocin is extensively bound to plasma albumin, and *in vitro* experiments with crystalline bovine albumin showed that the binding is a reversible equilibrium process.

Plasma fractionation. An adult female Beagle dog was given by mouth 100 mg of novobiocin (CATHOMYCIN sodium, Merck) per kg of body weight. After 3 hours blood was withdrawn from the jugular vein, mixed with .15 volume of sodium citrate-citric acid-dextrose (ACD) solution(3), and centrifuged. The clear plasma, amounting to 38 ml, was fractionated by Cohn's Method 10(3). A second dog, not dosed, supplied a like amount of plasma as a control. The plasma fractions were assayed for novobiocin by the microbiological procedure of Frost and Valiant(4).

* To be published.

TABLE I. Novobiocin Concentrations in Plasma Fractions.

Fraction	Novobiocin content as γ/ml of original plasma: Dosed dog	Control dog
Original plasma	164	0
Fractions I, II, & III	<2	0
" IV & V	62	0
" VI*	++++	++++
" IV	4.2	0
" V	35	0

* Fraction VI contained Zn^{++} which interfered with assay.

The results are shown in Table I. The first precipitate, consisting of fractions I, II, and III, did not contain appreciable amounts of novobiocin. Fractions IV and V, precipitated together by the addition of Zn^{++} reagent, contained 38% of total plasma novobiocin. The excess Zn^{++} reagent present with fraction VI interfered with microbiological assay of this fraction. On separation of fractions IV and V it was found that almost all of the novobiocin was bound to fraction V, which consists of the plasma albumin. Quantitative recovery cannot be expected in this fractionation when binding is reversible, first because not all of the novobiocin originally present in the plasma is bound, and second because of additional dissociation brought about by volume increases on reagent addition and washing of precipitates.