fasting insulin levels in the diabetic subjects. Although the 35-50 minute insulin levels were similar in the 2 groups, the initial fasting insulin level was lower in the diabetics (Fig. 1). The prolonged lowering of blood sugar in diabetics (Fig. 1) following exogenous insulin is similar to that observed following I.V. tolbutamide. In the latter instance, endogenous insulin levels reach fasting levels though the blood sugar levels remain lower than fasting.

Discussion. Within the framework of these experiments, several points can be made. First, the t½ of exogenous insulin measured by immunoassay appears quite short and the values confirm the observations of Samols and Orskov. Secondly, there is no obvious significant difference between normal subjects and untreated mild adult diabetic patients, though the number of subjects studied is small. It should be noted that these patients were not obese and that their blood sugar response to the injected insulin was of the same order of magnitude as that seen in the normal subjects. One normal subject who was moderately obese manifested a markedly decreased blood glucose response although the level of circulating exogenous insulin was identical to that noted in other subjects. His insulin t½ (9.0) was not different from that of a thin, normal control with a good sugar response to insulin (8.5).

No correlation between insulin t½ and fasting insulin levels was observed. It would be of interest to extend these observations to a larger series of diabetics and to study the endogenous insulin decay rates.

Conclusions. The data provide no evidence for a significant alteration either in the decay rate of exogenous insulin or in the response to insulin in non-obese newly diagnosed, mild diabetics. A short t½ for insulin is confirmed (6.5-9.0 minutes).

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Effects of Transplanted Pituitary Tumors on Host Pituitary Prolactin Secretion.* (32435)

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Implantation of anterior pituitary (AP) tissue into the infundibular recess of the rat results in a depression of adrenal function(1). Implantation of ACTH into the median eminence reduces ACTH content in the pituitary and abolishes the increase in hypothalamic CRF induced by adrenalectomy(2). Gonadotropin secretion is also depressed by implantation of gonadotropins into the hypothalamus(3,4,5,6).

Recently, MacLeod *et al*(7) reported that prolactin and growth hormone (GH) were reduced in the host AP of rats bearing a transplanted pituitary tumor ($MtTW_5$) which secretes large amounts of prolactin and GH. Prolactin and GH were detected by disc electrophoresis and were not assayed biologically. The present study was undertaken to determine whether pituitary tumor ($MtTW_5$ and $MtTW_{15}$) transplants could alter the content of hypothalamic prolactin-inhibiting factor (PIF) and pituitary prolactin concentration in the host rat.

Materials and methods. Eight-week-old in-

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bred Wistar rats were given transplants (12th passage) of "mammosomatotropic" pituitary tumors ($MtTW_5$ or $MtTW_{15}$) subcutaneously in the dorsal neck region. The rats were maintained in a temperature-controlled (75 ± 1°F) and artificially illuminated room (14 hours daily). Tumors from 2 rats were removed by sterile technique and cut into fine pieces with iris scissors in medium 199 (pH 7.4). Host rats were each injected subcutaneously below the neck with 0.1 ml volume of this mixture. Rats were examined once every week, and tumors became palpable at about 4-5 weeks after transplantation. When the tumor attained about 1.5-2.0 cm in diameter, 12 tumor-bearing rats were ovariectomized and adrenalectomized to remove possible feedback from ovarian and adrenal cortical hormones. A group of 12 control animals were also ovariectomized and adrenalectomized at the same time. The animals were maintained on a diet supplemented with physiological saline and sugar cubes for 2 weeks. At the end of 2 weeks, the rats were sacrificed. Tumor size was measured and the pituitary glands were removed and frozen immediately. Hypothalami from tumor-bearing and control rats were also removed, placed in cold 0.1 N HCl and frozen until ready for assay.

The length of the tumor bearing period was 66 days in the first experiment. In a second experiment, the tumor bearing period was 104 days. The animals were killed by guillotine and the AP's were assayed for prolactin concentration and the hypothalami for PIF content. Another group of 5 rats bearing $MtTW_5$ pituitary tumors was sacrificed at the end of 6 months, when tumor size was about 5 cm in diameter. AP prolactin and hypothalamic PIF were also assayed in these rats.

Hypothalamic PIF was assayed by incubating neutralized hypothalamic acid extract with male rat AP, according to the method of Kragt and Meites(8). Two hypothalamic equivalents per AP were used in the first and second experiment. In the third experiment one hypothalamic equivalent per AP was used. In each incubation, the AP from a mature male rat was separated from the posterior lobe, halved, weighed and each half was placed in a separate 25 ml Erlenmeyer flask. Three pairs of flasks were used. Each flask contained 2 hypothalamic equivalents from tumor-bearing rats in 3 ml of medium 199, or a similar amount of hypothalamic equivalents from control animals. All incubations were carried out in a Dubnoff metabolic shaker (60 cycles/min) under constant gassing with humidified 95% O_2-5% CO_2 at 37.5 ± 0.5°C. At the end of incubation, the medium was separated from the AP tissue and assayed for prolactin.

Prolactin activity was determined by the sensitive pigeon crop assay of Lyons(9) as modified by Reece and Turner(10). The paired comparison method was employed for all experiments. A total of one fourth of an AP or 0.8 ml of incubated medium from tumor-bearing rats was injected once daily over one side of the crop sac, and material from control rats was injected over the other side of the crop sac of each pigeon.

Results. The results are summarized in Table I. The host AP weight of both $MtTW_{15}$- and $MtTW_5$-bearing rats was significantly decreased ($P<0.05$) as compared with the controls. Intense stimulation of mammary lobulo-alveolar growth was observed in the tumor-bearing rats even though ovaries and adrenal glands were removed. Control animals showed only duct elements in the mammary tissue.

The AP prolactin concentration showed a significant decrease ($P<0.05$) in the tumor-bearing rats in Experiments 2 and 3 when compared with the control rats. Prolactin concentration did not show a significant difference ($P>0.05$) between tumor-bearing and control rats in the first experiment. In Experiment 1, the experimental animals had smaller tumors (1-2 cm in diameter) and had a shorter period of transplantation than in the 2nd and 3rd experiments. In the latter 2 experiments, the tumors were also much larger (2-5 cm) and the animals bore the tumors for a longer period of time. In these rats, the pituitary tumor decreased prolactin concentration to about 1/4 of that of the controls. In all 3 experiments, the PIF content of the hypothalamus was significantly increased ($P<0.05$) in the tumor-bearing rats,

TABLE I. Effect of "Mammosomatotropic" Pituitary Tumors ($MtTW_5$ and $MtTW_{15}$) on Pituitary Prolactin and Hypothalamic PIF.

Exp No.	Treatment & avg size of tumor at autopsy	No. of rats	AP wt, mg	AP prolactin			Hypothalamic PIF		
				No. of assay pigeons	IU/AP	IU/100 mg AP	HE/AP‡	No. of assay pigeons	Prolactin released into medium, IU/100 mg AP
1	Controls	12	8.71 ± .23	7	.21 ± .14	2.41	2	9	1.34 ± .23†
	$MtTW_{15}$ (1-2 cm)	12	6.24 ± .30	7	.17 ± .07	2.72	2	9	.80 ± .11†
2	Controls	12	10.67 ± .29	4	.42 ± .09*	3.91	2	9	1.06 ± .21†
	$MtTW_{15}$ (2-3 cm)	12	6.76 ± .96	4	.11 ± .04*	1.61	2	9	.49 ± .11†
3	Controls	5	8.38 ± .55	8	.43 ± .03†	5.07	1	6	4.01 ± 2.0*
	$MtTW_5$ (5 cm)	5	6.22 ± .45	8	.11 ± .01†	1.77	1	6	1.27 ± .34*

* Significant difference ($P < .05$).
† Significant difference ($P < .001$).
‡ No. of hypothalamic equivalent per incubated AP.

as indicated by the smaller amount of prolactin released by the AP into the incubation media when compared with prolactin released by AP incubated with control hypothalamic extract.

Discussion. The results of the present study indicate that the $MtTW_5$ and $MtTW_{15}$ pituitary tumors decrease pituitary prolactin content and increase hypothalamic PIF. The pituitary "mammosomatotropic" tumors secrete large amounts of prolactin into the blood(11) and stimulate marked mammary development even in the absence of ovaries and adrenals(12). This was observed in these rats. The high content of prolactin in the blood apparently acts on the hypothalamus to increase PIF content and presumably thereby reduces AP prolactin concentration and weight of the host pituitary. This constitutes the first evidence that hypothalamic PIF content can be increased as well as decreased. Many agents such as the suckling stimulus, estradiol, reserpine, epinephrine, acetylcholine and perphenazine reduce hypothalamic PIF content(cf. 11), and presumably thereby increase secretion of prolactin as indicated by mammary growth and secretion in a variety of animals.

The reduction in pituitary content of prolactin in the tumor bearing rats agrees with the similar finding of MacLeod *et al*(7), who used disc electrophoresis as a semi-quantitative test for pituitary prolactin. Investigations are continuing in this laboratory on the effects of these pituitary tumors on hypothalamic content of GHRF and other releasing factors, as well as on pituitary concentrations of GH and other hormones.

Summary. The effects of transplanted "mammosomatotropic" pituitary tumors ($MtTW_5$, or $MtTW_{15}$) were determined on hypothalamic PIF content and pituitary prolactin concentration in host adrenalectomized-ovariectomized rats. The high concentration of prolactin in the blood of the tumor bearing rats significantly increased hypothalamic PIF content and reduced pituitary prolactin concentration and pituitary weight. This constitutes the first evidence that high prolactin in the blood can increase production

of hypothalamic PIF, and presumably thereby depress pituitary prolactin secretion.

Addendum. In agreement with the data presented here, we (Clemens, J. A., Meites, J., *The Physiologist,* 1967, v10, 144) found that implantation of prolactin into the *median eminence* of intact and castrate female rats reduced significantly pituitary weight and pituitary prolactin concentration, and increased hypothalamic PIF content. Profound involution of the mammary glands was also observed.

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In vitro Studies with Thyrotropin Releasing Factor.* (32436)

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The presence of a thyrotropic hormone (TSH)-releasing factor (TRF) in hypothalamic extracts of several species has now been well established(1-7). The isolation in our laboratory of what we think is essentially pure thyrotropin releasing factor(8) provided material for various *in vivo* and *in vitro* investigations. *In vivo* studies have shown that 1 nanog of porcine TRF will stimulate the release of TSH in codeine-thyroxine treated mice and that a dose of 4 nanog will deplete pituitary TSH content in the same animals (8-11). TRF has no effect in hypophysectomized mice(10). The present report describes *in vitro* studies carried out with porcine TRF and the effect of thyroxine, triiodothyronine, Actinomycin D as well as α- and β-melanocyte-stimulating hormone (MSH) on TSH release *in vitro* from isolated rat pituitaries in response to TRF. Preliminary reports of some of this work were briefly presented at recent meetings(12,13).

Materials and methods. Highly purified preparations of TRF were obtained from lyophilized defatted pig hypothalami by extraction with 2 N acetic acid, followed by heating and lyophilization, reextraction with glacial acetic acid and gel filtration on Sephadex(8). Further purification was effected by phenol extraction, chromatography on carboxymethylcellulose (CMC), countercurrent distribution (CCD) and free flow electrophoresis (FFE). The final purification step consisted of partition chromatography on Sephadex(8) and the TRF thus obtained was homogeneous in 6 thin-layer chromatography and electrophoresis systems(8,11). The doses of TRF are given in terms of dry weight.

Actinomycin D (Act D, Merck and Co.) was administered to rats in doses of 200 μg/100 g body weight, 3 hours before removal of pituitaries or added to preincubation and incubation media at a concentration of 1-10 μg/ml. The sodium salts of triiodothyronine (T_3) and thyroxine (T_4) were dissolved in 0.1 N sodium hydroxide, diluted with saline and added to preincubation and incubation media

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