difference in net charge as measured by these electrophoretic methods and would be evidenced by changes in the location of CF activity due to the different electrophoretic mobilities.

Summary. Fractionation of acute phase anti-*Anaplasma* sera and subsequent study of the fractions by CF showed that serologic activity was associated with the *alpha-* and *beta*-globulins of lower mobilities, and the *gamma*-globulins of highest mobility. Similar studies with convalescent sera showed a complete absence of CF activity in the *alpha*-globulins. CF activity in the *beta*-globulin fractions was distributed essentially similar to that found in acute phase sera although the titers of the individual fractions were diminished. The CF activity in the *gamma*-globulins during the convalescent stages was associated with fractions of high and intermediate mobilities. It was confirmed that a relationship existed between the concentrations of globulin components and CF antibody titers.

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Identification of the Hormones Secreted by an Autonomous Mammotropic Pituitary Tumor in Rats. (27518)

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A very useful laboratory tool for studies on pituitary hormones has been provided by Furth who has developed several strains of hormone-secreting pituitary tumors in rats and mice(1,2,3). The purpose of this study is to identify the hormones elaborated by one of Furth's autonomous mammotropic tumors, MtT-F4. On the basis of organ examination and hormone assay, Furth *et al.*(2,4,5) had concluded that the tumor elaborated a single pituitary hormone, mammotropin, which was responsible for body growth, growth and secretion of mammary glands and hypertrophy of the adrenals.

Methods. The primary tumor, MtT-F4, had been induced by Furth by implantation

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of a single pellet of stilbestrol (7-8 mg) into a rat of the Fischer strain. Such treatment induced tumors in 170-432 days after transplantation of the stilbestrol. Furth had succeeded by a system of conditioning in causing the tumor to become autonomous so that it could be carried on by subpassage into normal Fischer rats. *Transplantation.* Male and female Fischer rats were implanted with tumor tissue suspended in saline and injected intramuscularly. Sterile instruments were used and the procedure was kept as clean as possible. The hind legs were the most satisfactory site of inoculation, since large tumors on the neck or back interfered with the operative procedures later. The tumors became palpable in about 3 weeks after inoculation; they were 2-4 cm in diameter by 4-6 weeks. *Operative procedures.* Hypophysectomy was done at various times, in some cases before implantation of the tumor and in other cases after the tumor had become palpable.

Collection of adrenal venous blood. A laparotomy was done under ether anaesthesia. The left adrenal vein was exposed and all of its tributary veins from the body wall were cauterized. Following this, 0.2 ml heparin solution (200 U) was injected into a tail vein. A cannula of fine polyethylene tubing with a metal tip from a 27-gauge hypodermic needle was inserted through the left renal vein and carried into the adrenal vein. The cannula was made secure by a tight ligature around the adrenal vein at its entrance into the renal vein. Adrenal venous blood was collected into a centrifuge tube packed in chipped ice.

Estimation of corticosterone in plasma. Corticosterone (compound B) is the major steroid constituent of rat adrenal vein blood and it was measured by Peterson's method(6) for assay of corticosterone which was modified for use as a micromethod. 0.02 to 0.10 ml plasma is pipetted into a 15-ml ground glass-stoppered centrifuge tube. 1 to 2 ml of water and 4 to 8 ml of redistilled dichloromethane are added. The mixture is shaken for 10-15 seconds, then centrifuged for 10 minutes at high speed. The plasma layer between the water phase and the dichloromethane is removed by aspiration. An aliquot (2 to 4 ml) of the dichloromethane extract is transferred to a ground glass-stoppered tube and 1 to 2 ml of an acid-alcohol reagent added. The acid-ethanol reagent was made up of 65 parts of sulfuric acid (Mallinckrodt, reagent grade) and 35 parts of absolute ethyl alcohol (reagent grade). The steroid is extracted from the dichloromethane by vigorous shaking and the dichloromethane is then removed by aspiration and discarded. The sulfuric acid-alcohol extract is allowed to stand for 3 hours, during which time a fluorescence develops; this is measured in a spectrophotofluorometer (Aminco-Bowman), using a slit arrangement #3, sensitivity 0.03-0.001, activation 470 mμ, fluorescence 540 mμ. The standard solutions in the range of 0.01 to 0.8 μg corticosterone and the unknowns are read against a dichloromethane reagent blank.

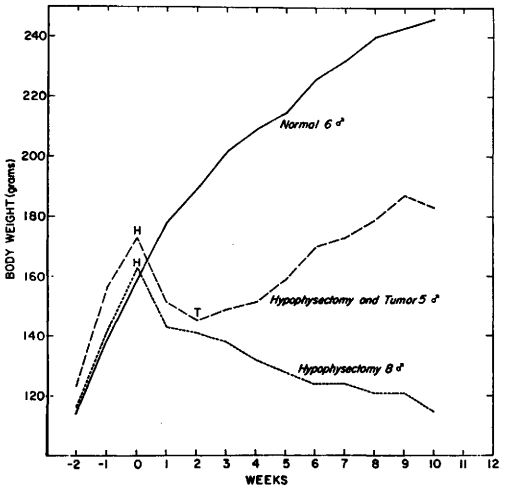

FIG. 1. Growth curves in hypophysectomized male rats bearing MtT-F4 tumors, and in normal and hypophysectomized controls. H–time of hypophysectomy. T–time of tumor implantation.

Results. Evidence of growth hormone secretion. The resumption of a normal rate of growth in the hypophysectomized rats after grafting of the pituitary tumor, MtT-F4, is very good evidence that the tumor elaborates growth hormone (Fig. 1). The body weight curve of the hypophysectomized tumor-bearing rats continued upward for at least 7 weeks.

Body weights and autopsy data on intact and hypophysectomized tumor-bearing rats are given in Table I. Not recorded here are the food and water intakes of the tumor-bear-

TABLE I. Influence of Pituitary Tumor, MtT-F4, on Growth and Adrenal Function.

	No. rats	Age of rats in mo: 2	2.5	3	3.5	4	5	6	7	Corticosterone adr. vein plas., μg/100 ml	Tumor, g	Autopsy, organ weights: Adr., mg	Liver, g	Kidney, g	Gonads, mg
		Body wt, g													
Males															
No tumor															
Intact	6		104	159		209	240*			1687	—	36.7	7.82	1.57	2330
Hypophysectomized	8		106	163*H*	141	132	121			34	—	10.5	3.67	.63	323
Tumor-bearing															
Intact	3	111		168*T*		180	230			1741	20.5	344.0	17.50	3.78	1570
Hypophysectomized	5		123	173*H*	145*T*	151	179			1221	2.5	38.5	7.13	1.38	304
Females															
No tumor															
Intact	3	115	125							1580	—	41.4	6.41	1.18	47.0
Hypophysectomized	12	97	122*H*	106		107	107	105		28	—	10.1	3.00	.50	14.5
Tumor-bearing															
Intact	12	95		138*T*		152	190	240		1800	29.1	353.0	14.5	2.58	51.9
Hypophysectomized	7		124	138*H*	121*T*	123	142	169	188	748	24.8	364.0	10.8	1.44	12.9
Hypophysectomized	9	105*T*	117*H*	135	157					1321	14.8	267.0	12.16	2.00	33.8

* Final body wt taken on day adrenal vein blood collected and autopsy performed. *H*—hypophysectomy *T*—tumor implantation

ing rats, which were increased at least twice the normal amounts. When the tumors had reached a size of 20-30 g, the general health of the rats began to decline and total body weight decreased. At autopsy, the very striking findings were: almost total lack of carcass fat and more than doubling of the kidney and liver weights.

Evidence for ACTH secretion. In Table I are given the mean values for corticosterone in adrenal vein plasma and mean adrenal weights in hypophysectomized and intact tumor-bearing rats and in their controls. Adrenal weights were increased in some cases almost 10 times over that of their controls. The corticosterone level in adrenal vein plasma was the same in the intact rats without tumors as in those with tumors, although the latter had adrenals 10 times normal size. It may be that the stress of the operation of collecting adrenal vein blood in the normal animals stimulated the pituitary in the normal animals to a maximum degree. It is also possible that the adrenals weighing 10 times normal in the tumor animals do not have a functional capacity 10 times normal. A study of the ACTH content and function of the pituitaries of rats with tumors may give some information in this regard. On the other hand, the hypophysectomized tumor-bearing rats had corticosterone levels in the adrenal blood 50 times that of the hypophysectomized controls.

There appeared to be no correlation between tumor size and functional capacity and weight of the adrenal. This was studied in hypophysectomized tumor-bearing rats. The data are shown in Table II. The only striking finding in this study is the great difference between tumor-bearing and non-tumor hypophysectomized animals, both in adrenal size and corticosterone levels in adrenal vein plasma.

Evidence for secretion of mammotropin. The tumor was designated mammotropic tumor by Furth because it stimulated growth of the mammary gland as well as milk production(2). Hypophysectomized rats, both males and females, showed very marked proliferative and secretory changes in the mammary glands. The female rats shown in Ta-

TABLE II. Corticosterone Secretion in Hypophysectomized Female Rats with Pituitary Tumor MtT-F4.*

Days post-hypox.	No. rats	Body wt, g	Tumor, g	Adrenal, mg	Corticosterone adr. vein plas., μg/100 ml
1	9	242	29.1 (11.3-46.4) †	354 (248 -512)	610 (320-1000)
5	2	146	30.3 (25.1-35.5)	234 (206 -264)	800 (400-1200)
12	2	166	40.1 (35.2-45.1)	290 (288 -292)	900 (840- 960)
13	4	148	23.8 (19.1-29.7)	350 (310 -384)	870 (680-1000)
42	3	157	14.8 (10.0-18.0)	267 (265 -272)	1321 (141-2500)
105	5	180	24.8 (19.5-27.5)	366 (228 -452)	748 (480-1000)
14	4	112	No tumor	19.4 (16.0- 21.3)	13 (0- 25)
70	3	104	" "	9.3 (8.6- 10.0)	28 (20- 37)

* Tumors implanted 3-6 wk before hypophysectomy except in case of last group carried for 105 days in which rats were hypophysectomized 2 wk before tumor implanted.
† Mean value and range.

ble I, which were first hypophysectomized and 2 weeks later implanted with tumor, had very marked mammary gland stimulation 3 months after hypophysectomy. Bates has assayed tumor tissue for mammotropin (prolactin) using the pigeon crop sac assay method and has found it to be present but in amounts only 1/200 that found in beef pituitaries(7). There was no evidence of gonad stimulation in the hypophysectomized tumor-bearing rats, except in some of the female rats which had been grafted with the tumor first and then hypophysectomized, and which had enlarged corpora lutea due to the luteotrophic influence of the mammotropin.

Discussion. These studies show quite conclusively that the pituitary tumor, MtT-F4, elaborates growth hormone, ACTH, and mammotropin (prolactin). The hyperpituitary state of the tumor-bearing animals offers many interesting problems for future study. For instance, it is of interest that the tumor-bearing animals with an abundance of growth hormone do not appear to have an accelerated growth rate over normal (non-tumor) animals. Perhaps the body is already responding maximally to growth stimulation; or, the excessive amounts of ACTH may interfere with body growth, although the competition between growth hormone and ACTH does not appear to be a factor in the growth rate of the hypophysectomized tumor-bearing rat. The striking hypertrophy of kidney and liver in the tumor animals presents interesting problems. A condition which was observed but not studied was a hemorrhagic tendency which was partly overcome by injections of vit. K. These tumor-bearing animals have been used for a study of the ACTH-releasing neurohumors which is being reported by the authors(8).

Summary. The autonomous mammotropic tumor of Furth (MtT-F4) has been shown to secrete growth hormone and ACTH. The previous observation of mammotropin production has been confirmed.

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