Comparison of Two Different Transplantable Mammotropic Pituitary Tumors. Hormone Content and Effect on Host. (31404)

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Extensive studies have been made of the hormone content of the mammotropic tumor (MtT) line F4 established by Dr. Jacob Furth and of the hormonal effects in the host (1-8). The primary tumor was induced in the pituitary of Fischer rats by stilbestrol pellets. In the course of studies on chemical carcinogenesis and the induction and properties of hepatomas in Buffalo-strain rats, Dr. Morris observed a huge hemorrhagic pituitary in a rat from a group of rats receiving a diet containing 2,4,6-trimethyl-aniline (TMA). Portions of this pituitary were transplanted as tumor line 7315. Some of the host rats after 6 transfers developed large mammary glands, adrenals and splanchomegaly. These effects on the host were similar to those in rats bearing the F4 line of tumor. Hence the following studies were made as a basis for comparison of the properties of the two tumor lines.

Methods. The primary tumor used to establish line 7315 was found in the pituitary of a rat which had ingested an average of 1.6 mg of TMA daily in its diet for a period of 18 months. No apparent hormonal activity was noted until the 6th transfer. At that time some animals bearing one tumor line developed hypertrophied mammary glands containing a milk-like fluid and very large adrenals. This subline was designated 7315a (active) contrasted to another subline 7315i (or inactive) which produced no such effects on mammary glands or adrenals. The tumor transplants were placed subcutaneously. Female rats of the Buffalo-strain bearing tumors of the eighth and twelfth transfer generation were used. Corticosterone was determined by the fluorescence method of Peterson(9). Liver fat was measured by weighing the lipids in a washed CH_3Cl-CH_3OH extract of liver. Liver glycogen was measured using an anthrone method on a solution of the ethanol precipitate from a 30% KOH digest. Ascorbic acid was determined by the method of Mindlin and Butler(10). Prolactin concentrations were estimated by the systemic cropsac assay method of Bates (11). Growth hormone assays were based on the weight increase of hypophysectomized rats during a period of 10 daily subcutaneous injections(12). ACTH was assayed by the method of Munson(13) by Dr. Herman Cohen.

Results. Hormone concentration in the tumor: MtT 7315a contains and produces the same 3 hormones at MtT F4(2). The concentration of prolactin is the same in both tumors but the concentrations of ACTH and growth hormone in MtT 7315a were less by a factor of at least 10 (Table I). The tumors from adrenalectomized rats had increased con-

TABLE I. Hormone Concentrations of MtT 7315a and Effects on Adrenals and Liver.

		Hormone concentration in lyophilized tumor			Liver		Adrenal		
	Generation	Prolactin, IU/g	GH, IU/g	ACTH, IU/g	Glycogen, %	Fat, %	Ascorbic acid, mg/100 g	Corticosterone, μg/100 g	Blood corticosterone, μg/100 ml
Controls	12	(100)*	(30)*	(200)*	5.1 ± .3	4.9 ± .6	336 ± 18	6	8 ± 2
7315a	8	2.5	<.5	no data	3.9 ± .3	5.4 ± .5	no data	no data	no data
7315a	12	2.0	<.5	<.04	2.6 ± .5	4.9 ± .5	92 ± 8	4.6 ± 1.5	63 ± 3
7315aĀ†	12	2.0	.5	.38	.4 ± .2	4.5 ± .6	—	—	7 ± 1
F4‡	Pool	2.6	4.0	2.0	3.2 ± .5	6.3 ± .5	180	no data	65

* Potencies of lyophilized pituitaries.
† Adrenalectomized 37 days and maintained with a pellet of DOCA and given 0.9% NaCl to drink.
‡ Data from Fischer rats(3,4).

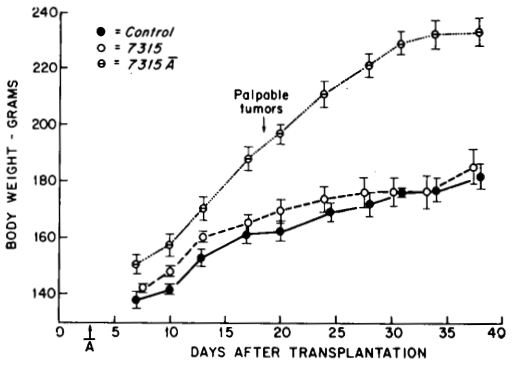

FIG. 1. Showing lack of effect of growth hormone from tumor 7315a on body weight unless the host rats were adrenalectomized (Ā).

centrations of growth hormone and ACTH. On the basis of the weights of the thyroids and ovaries of the host rats, the tumors did not produce any TSH or gonadotropins.

Effect of MtT 7315a on body weight: Twenty rats were transplanted with MtT 7315a. Ten of these were adrenalectomized 3 days later. The body weight curves for these 2 groups and a group of 10 control rats are shown in Fig. 1. The tumors became palpable on about day 18. The intact rats with the tumor grew at the same rate as the controls which indicates a rough balance between increased production of the growth hormone and of ACTH by the tumor. The adrenalectomized rats grew rapidly after the tumor appeared because the anabolic effects of growth hormone from the tumor were not counterbalanced by the catabolic effects of adrenal corticoids. The results are similar to those obtained with the MtT F4 tumor(3). When the tumors were larger than 25 g (Table II), the weight of adrenal glands of rats bearing MtT 7315a or MtT F4 were increased to about the same extent, although the ACTH concentration in the tumors was greatly different. The weights of the kidney and liver were less in the rats with MtT 7315a which would correlate with less GH and ACTH. Thymi were essentially absent. The white fat depots were minimal in both cases due to lipolysis by the excess pituitary hormones. Mammary glands were appreciably larger in rats with MtT 7315a perhaps as a result of a hormonal balance with prolactin predominating. Preputial glands were enlarged but

TABLE II. Effect of MtT 7315a on Organ Weights of Intact and Adrenalectomized Buffalo Rats.

	Generation	No.	Tumor age, days	Tumor wt, g	Body wt, g	Mammary, g*	Adrenal, mg*	Pituitary, mg*	Thymus, mg*	Pancreas, mg*	Preputial gland, mg*	Liver, g*	Kidney, mg*	Heart, mg*	Fat: Empty gut, g*	Fat: Brown, mg*	Fat: White, g*
Controls	12	9	—	—	193 ±8	<.1	22.9 ±.6	5.9 ±.6	119 ±7	475 ±36	36	3.83 ±.1	790 ±10	347 ±7	2.5 ±.1	216 ±19	1.65 ±.1
7315a	8	7	47	7.2 ±.7	203 ±14	3.7 ±.2	135 ±13	4.2 ±.2	0	307 ±30	35 ±4	5.6 ±.3	970 ±70	424 ±21	3.0 ±.15	246 ±22	1.00 ±.14
7315a	12	8	41	27.7 ±2.4	187 ±6	2.4 ±.25	197 ±10	4.6 ±.2	0	349 ±20	80 ±7	7.33 ±.41	1320 ±50	489 ±18	3.1 ±.2	204 ±22	.33 ±.09
7315aĀ†	12	5	40	31.1 ±1.7	223 ±9	.55 ±.06	0	3.4 ±.4	186 ±22	329 ±29	45 ±2	4.24 ±.4	1080 ±100	422 ±23	4.3 ±.2	72 ±15	.1 ±.02
F4‡	Pool	14	90	28 ±4	277 ±10	.7 ±.1	195 ±25	3.5 ±.2	0	425 ±19	168 ±22	11.0 ±.5	2020 ±220	400 ±11	4.3 ±.2	127 ±9	.11 ±.2

* Weights are per 100 g body wt.
† Adrenalectomized 37 days and maintained with a pellet of DOCA and given 0.9% NaCl to drink.
‡ Data from Fischer rats(3).

were definitely smaller in the rats with MtT 7315a than with MtT F4.

Effect of adrenalectomy on organ weights: The extent of hypertrophy of the liver, kidney and mammary and preputial glands was greatly reduced by adrenalectomy (Table II). Only the thymus gland was increased to or above normal size. These changes after adrenalectomy are similar to the changes found in rats with MtT F4 after adrenalectomy(3), and confirm the necessity of adrenal hormones for the hypertrophy observed in the liver, kidney and heart.

Adrenal functions The ascorbic acid concentration (AAA) of the large adrenals from rats with MtT 7315a was 92 mg/100 g, which is about as low as ever found with maximally stimulated adrenals (Table I). This is one clear cut difference from rats with MtT F4 whose adrenals contained twice as much ascorbic acid. The AAA level is thought to be a matter of hormonal balance(14) and suggests that MtT F4 rats have a higher ratio of GH/ACTH in their blood than MtT 7315a rats do.

At the same time the concentration of corticosterone in the plasma was the same in rats with either tumor. The concentration in the adrenals was the same as in normal rats (15). One might expect the blood level of corticosterone to be higher in rats with MtT 7315a when the AAA is lower. Apparently the GH/ACTH ratio to prevent maximal response to ACTH is larger for AAA depletion than for corticosterone levels in the blood.

Induction of diabetes: Eighty percent of the pancreas was removed from 2 rats 14 days after transplantation. Eight days later and 4 days after the tumor was palpable both rats were excreting more than 3 g of glucose daily in their urine. This hormonal induction of diabetes is similar to the findings in MtT F4 rats(16).

Discussion. The extent of growth in rats is partially controlled by a hormonal balance between GH and adrenal corticoids. Intact rats with MtT 7315a did not grow, suggesting that GH/corticoid ratio is at the balance point whereas rats with MtT F4 have been shown to grow, indicating a slight excess of GH and a larger GH/corticoid ratio. After adrenalectomy, which reduces the corticoid value to 0, rats with either tumor grow at a rapid near maximal rate. Hormonal levels in the blood could not be measured but radioimmumoassay procedures should produce valuable objective data eventually.

While the prolactin concentrations were the same in both tumors the GH and ACTH concentrations were less by a factor of 10 or more (Table I). It is thought that the GH/corticoid ratio was less in MtT 7315a because (1) body weight gain was less, (2) hypertrophy of liver, kidney and preputial glands was less. Since the actual blood level of corticosterone was the same in rats with MtT 7315a and F4 this means that the major changes were in the GH level. (3) The minimal level of AAA in rats with MtT 7315a indicates that there was not enough GH to prevent maximal depletion by ACTH. In MtT F4 the maximal depletion is prevented by excess GH.

Summary. A pituitary tumor (7315a) from a rat treated with 2,4,6-trimethylaniline was transplantable. Studies during the eighth and twelfth transfer generation of 7315a showed the tumor to be similar to the mammotropic tumor MtT F4 established by Furth in that it contained the same 3 pituitary hormones, which produced similar changes in the organs of the host. MtT 7315a and MtT F4 contained the same concentration of prolactin (2 IU/g dry weight) but MtT F4 contained more than 10 times the concentration of growth hormone and ACTH found in MtT 7315a. Correspondingly rats with MtT 7315a grew less rapidly and had less hypertrophy of the liver, kidney and preputial gland than rats with MtT F4, but the mammary glands were larger. Both tumor lines induced equally large adrenals (9 × normal) and blood levels of corticosterone (8 × normal) but the ascorbic acid concentration of the adrenal was different (7315a: 94 *vs* F4: 180 mg/100g) due to difference in growth hormone levels.

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Effect of Oxytocin Upon Litter Weight Gain in Rats.* (31405)

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In a series of studies from this laboratory, the influence of one or more hormones upon the yield of milk of rats on days 14 to 20 has been reported(1,2,3,4,5). The method used was to separate the mother from the young for a period of 10 hours on each test day. The young and mothers were then weighed and placed with the mothers for a nursing period of 30 minutes. In the initial experiment, it was observed that the endogenous oxytocin discharged was inadequate to permit complete milk removal since the injection of oxytocin during the nursing period increased the mean milk yield 50%(3). In subsequent studies, oxytocin was injected when the young were returned to the mother and a second injection was given after 15 minutes. The milk yield was then determined either by the increase in weight of the young or by the decrease in weight of the mother or both.

In these studies, it was shown that individual hormones and combinations of hormones injected from day 7 to 20 of lactation stimulated marked increases in milk yield on the test days. However, the litter weights of the experimental groups in most experiments were not significantly greater than the control groups. If, as is claimed, the milk secretion of the experimental groups is markedly increased, why then do not the litters take advantage of the increased available milk and grow faster than the controls?

To seek an answer to this problem, it is necessary to understand the nursing habits of the rat. Normally, the young nurse their mothers for approximately 1 to 5 minutes at about hourly intervals. At the nursing period, the neural stimulus causes the release of oxytocin and permits the removal of milk. If the amount of milk present in the gland is limited, it may be completely removed before the oxytocin is inactivated‡. If the amount of milk present increases, then increasing amounts of milk present in the glands may not be removed by this short nursing period and the growth rate of the litter would not be increased accordingly even though increased amounts of milk were being secreted

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‡ Study of the biological half-life ($t_{1/2}$) of oxytocin in the rat indicates that it is very short. One earlier estimate indicated a $t_{1/2}$ of 1 minute and 40 seconds(6). In a recent study of Aroskar *et al*(7), it was suggested that the rapid disappearance of oxytocin from the blood of the rat was not due to its inactivation by the blood, but rather to the uptake of the hormone by the kidneys and liver. That the $t_{1/2}$ of oxytocin in the rat is shorter than indicated above is suggested by the report of Folley and Knaggs (8), that the $t_{1/2}$ is only 1 minute and 22 seconds in the goat.