in general less reactive; but against virus No. 52102 it was definitely more active than was the GD VII antiserum.

All the antisera had a lower titer against strain No. 47218. This strain differs from the others in that it did not adapt to egg or tissue culture while strains Nos. 4727, 4771, and 52102 readily did so.

Summary. Satisfactory antisera to various Theiler strains were prepared in hamsters. Using Theiler-free suckling mice in neutralization tests, reciprocal cross reactions between GD VII and TO strains were demonstrated. Serologic differences within the TO group were also noted, the titer of a serum being in each case higher for the homologous than the heterologous strains.

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Occurrence of Melanocyte Stimulating Hormone (MSH) in a Transplantable Pituitary Tumor. (22488)

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Transplantable adrenocorticotropin (ACTH) secreting tumors have been produced in LAF_1 mice by Furth *et al.*(1,2,3) by ionizing irradiation. There was some indication that they released ACTH. To date, there has been no report on the ACTH content of the tumors. In the case of thyroid stimulating hormone (TSH) secreting tumors (3) an extremely high content of TSH has been reported. In view of many reports on the relationship of melanocyte stimulating hormone (MSH) and ACTH, it was of interest to assay the ACTH tumors for MSH, ACTH and vasopressin. It was found that the concentration of MSH was extremely high while the ACTH and vasopressin were relatively low.

Methods. Tumor Transplants. Several LAF_1 mice bearing Strain 2 adrenotropic tumor were sacrificed, the tumors were removed aseptically and homogenized with 5 volumes of isotonic saline. One-tenth ml of this suspension was injected intramuscularly into male LAF_1 mice weighing 22 to 28 g. Three weeks after implantation they were bilaterally adrenalectomized and injected subcutaneously with one mg each of desoxycorticosterone acetate (DOCA). The tumors were palpable about 8 weeks following implantation. Once palpable, they reached the size of 1-6 g within a few weeks. All the reported experiments were carried out with tumors which had been transplanted twice following receipt in our laboratory. *Assays.* ACTH assays were conducted by the intravenous technic described by Munson, Barry and Koch(4). Vasopressin determinations were by the standard U.S.P. XV method. All samples for ACTH and vasopressin were prepared by homogenization with 0.1N hydrochloric acid followed by dilution with isotonic saline. MSH determinations were made using the *in vitro* method of Shizume and Lerner(5). Samples were extracted with 10

TABLE I. MSH, ACTH and Vasopressin Content of 12 Adrenotropic Tumors.

Tumor wt (g)	MSH (u/g)	ACTH (USP u/mg)	Vasopressin (I.U./mg)
3.05	1.0×10^7	.0016	<.0005
4.96	7.5×10^6	<.0005	"
1.60	2.1 "		
.28	4.5 "		
2.75	3.5 "		
5.05	1.0×10^7	.0003	<.0015
2.15	1.5×10^6		
.40	1.0×10^7		
.97	1.6 "	<.0005	"
1.98	5.7×10^6		
.41	3.3 "		
.30	5.7 "		
Mean and S.E.	$6.7 \pm 1.3 \times 10^6$		

volumes of approximately 0.1N acetic acid and diluted with frog Ringer solution. The final pH was approximately that of the diluting solution. An MSH preparation (CDF-G456) containing 4.0 x 10^9 units/g was used as a standard.

Results. Table I summarizes the assay data on 12 adrenotropic tumors removed from LAF_1 mice after 2 transplantations. All were transplanted and sacrificed at the same interval. The MSH concentration, expressed in terms of fresh tissue equivalents, was 6.7 $\pm$ 1.3 x 10^6 units/g which was approximately that found in normal mouse pituitaries (Table II). On the contrary, the ACTH concentration was less than 1/20 that of the normal pituitary. Vasopressin determinations confirmed that there was no posterior pituitary contamination. Histological structure of the tumors and pituitaries of adrenalectomized mice indicated that the tumors were of anterior lobe origin and not from the intermediate or posterior lobes.

Samples of skeletal muscle from the hind leg not implanted with tumor cells and from adrenalectomized mice were also assayed. Both contained less than 1/1000th the concentration of MSH found in the tumors. There was a suggestion that the concentration in the muscle of tumor bearing animals was higher than the normals but both were so low that the results were of doubtful significance.

Discussion. The melanocyte stimulating hormone, sometimes referred to as intermedin, was shown by Zondek and Krohn(6) to be derived from the intermediate lobe of the pituitary. Many workers have pointed out that MSH and ACTH possess many of the same chemical and physical properties but are distinct substances. Lerner and Lee(7) isolated swine MSH which possessed very little if any ACTH activity. However, pure swine ACTH has about 1% inherent MSH activity(8). Shizume and Lerner(9) have shown that blood and urinary MSH levels can be lowered by cortisone and hydrocortisone. The above observations indicate that there may be a common pathway for the synthesis of ACTH and MSH. Judging from the data of Furth *et al.*(3) on adrenal responses it appears that the original tumors contained more ACTH than that reported in this study. It is well known that tumors tend to lose their functional identity as a result of repeated transplantation. In this case, the capacity for producing ACTH must be less than for MSH.

Of particular interest was the MSH content of the tumors as compared to that of the normal mouse pituitary. Within limits of error of the assay, the concentrations were the same yet the tumors were in some cases 2 to 3,000 times the size of the pituitary. If the tumors were releasing the hormone at the same rate as the pituitary, the blood and urine values for MSH should be markedly elevated. Therefore, the LAF_1 mouse with the Strain 2 adrenotropic tumor should be an excellent animal to study the physiology and pharmacology of MSH in the mammal.

Summary. Strain 2 adrenotropic tumors of LAF_1 mice were assayed for MSH, ACTH and vasopressin. The concentration of MSH was approximately that of the normal mouse pituitary. ACTH and vasopressin concentrations were very low. The possible relation-

TABLE II. MSH and ACTH Content of Pooled Mouse Pituitaries.

	No. animals	MSH (u/g)	ACTH (USP u/mg)
Tumor bearing	11	9.0×10^6	.022
Normal	6	7.0 "	.043
Adrenalectomized	6	$1\text{-}2 \times 10^7$	.039

ship of MSH and ACTH was discussed. The adrenotropic tumor-bearing animal should be advantageous in the study of the action of MSH in the mammal.

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Effect of Magnesium Ion on Glycogen Fraction Synthesis in Rat Tissues.* (22489)

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Magnesium is intimately associated with carbohydrate metabolism through its function as an activator in enzymatic processes involving phosphatase(1). However, opposing views exist regarding the promotion of glycogen synthesis by magnesium. Injection of Mg salts was claimed by Franke(2) to promote hepatic glycogenesis, but according to Peters and Van Slyke(3) the data have not been confirmed and are not convincing. Reid(4) on the other hand demonstrated a 6-fold increase in liver glycogen of cats after injecting Mg as chloride or gluconate. No reference to the effect of Mg on the synthesis of the glycogen fractions of Bloom(5) has been found by the writers.

This report presents the results of glycogen fraction studies in heart, liver and skeletal muscle of the rat in an attempt to elucidate the relationship of the TCA soluble and TCA insoluble glycogen fractions after Mg salt injections.

Methods. White rats weighing between 200 and 400 g were fasted 18 hours prior to injection I.P. with 0.2M Mg sulfate. Each rat received approximately 1.6 mg of Mg ion per 100 g of body weight. The sexes were equally mixed. Control rats were injected with equivalent molar concentrations of sodium chloride. The animals were then sacrificed by decapitation at one or 4 hours. The entire heart and samples of liver and gastrocnemius muscle were taken for analysis for TCA soluble and TCA insoluble glycogen fractions using the procedure outlined by Bloom, *et al.*(5). After hydrolysis of the glycogen in sulfuric acid, the isolated glycogen samples were measured quantitatively by the anthrone procedure of Seifter, Dayton, Novic and Muntwyler(6). A separate series of 12 fasted rats was injected with Mg sulfate at the same dose level as the rats in the glycogen series for the serum Mg data. These were sacrificed after one hour. Another 15 rats similarly treated were sacrificed after 4 hours. An additional 12 untreated rats were used as controls. Blood was withdrawn by direct heart stab and analyzed for serum Mg using the method of Orange and Rhein(7) as modified by Platner and Hosko(8).

The results of the glycogen and serum Mg determinations are presented in Table I.

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