leukemia virus isolated by Moloney(9). We have observed cylindrical structures in megakaryocytes of bone marrow and spleen of both rats and mice with passage A virus-induced leukemia.

The leukemic virus isolated by Moloney (9) may not be different from that originally (1) recovered from spontaneous mouse leukemia by Gross(10). In our study, the size of passage A (Gross) virus particles, based on measurement of 270 particles, was 990 Å, which is about the same as that reported by Dalton(8) for the leukemic virus isolated by Moloney.

Summary. 1. Ultrathin sections of bone marrow, spleen, lymph nodes, and mediastinal tumors from 11 rats with leukemia induced by mouse leukemia virus (Gross), were examined in the electron microscope. 2. In organs of all 11 leukemic rats, 2 types of particles could be observed. One type, described as virus particles (990 Å average diameter), with an electron-dense center (630 Å average diameter), surrounded by a less dense zone, and limited by another membrane, not always clearly defined. 3. The other type, described as vesicular or doughnut-type particles, with an electron-lucent center, surrounded by an inner membrane or ring staining strongly with uranyl acetate (490 Å average diameter) and an outer ring or membrane (840 Å) reacting less with the uranyl acetate stain. Frequently, but not always, another membrane (580 Å diameter) could be seen between the inner and outer membranes. 4. Both types of particles were found in megakaryocytes of bone marrow and spleen. 5. Virus particles were also observed in intercellular spaces of lymph nodes and mediastinal tumors, and occasionally within cytoplasmic inclusions of cells present in these tissues. 6. Characteristic cylindrical structures were observed in megakaryocytes of bone marrow and spleen of leukemic rats. 7. Neither virus nor doughnut-type particles could be found in either bone marrow, spleen, thymus or lymph nodes of 12 normal rats.

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Effect of Ethionine in Castrated Male Rats, with and without Testosterone.* (27565)

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The methionine analogue, ethionine, has been shown to induce fatty livers in female but not in male rats(1,2). It has been demonstrated that ethionine inhibits incorporation of methionine and glycine into liver protein(3) and inhibits synthesis of liver enzymes(4-9) in mature female or immature male rats. Farber and Corban(10) reported that ethionine inhibited protein synthesis in the liver of female but not of male rats and suggested that the sex difference in the effects of ethionine was mediated by androgens. We have observed that ethionine is a more effective tumor-growth inhibitor, and is more toxic, in female than in male rats(11), and

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TABLE I. Effect of Castration and of Testosterone on Tumor Growth and Carcass Weight in Male Wistar Rats.

Exp.	Group	No. rats	Condition	Treatment	Initial body wt (g)	Final body wt (g)	Tumor wt (g)	Change in carcass wt* (g)	% tumor inhibition
I	A	11	Normal	Water	290† (229-343) ‡	294 (228-333)	5.4 ±.31§ (.54-11)	- 1	0
	B	15	Castrated	"	259 (200-307)	263 (204-315)	5.5 ±.38 (.37-13)	- 2	0
	C	12	Normal	Ethionine‖	280 (236-344)	249 (211-298)	4.2 ±.23 (.60- 9.0)	-35	22
	D	14	Castrated	" ‖	250 (186-303)	201 (152-261)	2.3 ±.13 (.38- 4.6)	-51	58
II	A	4	Normal	Water & peanut oil	352 (330-400)	349 (325-390)	2.5 ±.47 (.50- 5.2)	- 5	0
	B	5	Castrated	*Idem*	261 (225-273)	261 (225-278)	2.6 ±.22 (.71- 4.5)	- 3	0
	C	5	"	Water & testosterone¶	278 (225-327)	278 (221-318)	2.7 ±.21 (1.1 - 4.0)	- 3	0
	D	7	"	Ethionine** & peanut oil	320 (287-373)	294 (247-343)	1.6 ±.18 (.23- 3.2)	-28	38
	E	5	"	Ethionine** & testosterone¶	279 (255-357)	264 (214-350)	2.3 ±.24 (.82- 3.9)	-17	12

* Difference between initial wt and final wt minus tumor wt.
† Avg value. ‡ Range of values. § Probable error of mean.
‖ 100 mg ethionine/100 g body wt daily for 4 days; 50 mg/100 g body wt daily for 3 days: avg, 79 mg/100 g body wt.
¶ 1 mg of testosterone propionate per rat per day, in 0.1 ml of peanut oil.
** 50 mg/100 g body wt daily for 7 days.

that the nonprotein methionine in the liver of ethionine-treated male rats was all in the free form(12) while a large part of that in the liver of similarly treated female rats was apparently in a microbiologically-inactive conjugate(13). It was of interest, therefore, to determine whether the effect of ethionine administration on tumor growth, host-toxicity, and liver methionine was influenced by the presence of androgens.

Materials and methods. Experiment I. Male Wistar rats, maintained on Purina Laboratory Chow, were castrated at about 8 weeks of age. Normal male litter mates were used as controls. Approximately 8 weeks later each of the rats was inoculated subcutaneously by trocar, at 2 lateral axillary sites, with the Walker carcinosarcoma 256. Beginning on the 7th day following tumor implantation animals bearing palpable tumors were grouped as shown in Table I and the experimental animals (groups C and D) received daily intraperitoneal injections of an aqueous solution of DL-ethionine (20 mg/ml) at a level of 100 mg of ethionine/100 g body weight. At the end of 4 days the mortality in experimental group D had reached 40% so the dosage of groups C and D was reduced to 50 mg/100 g body weight for the last 3 of the 7-day treatment period. Control rats (groups A and B) received comparable injections of distilled water. The animals were fasted 4 hours before and 8 hours following the last injection and then sacrificed. Livers and tumors were removed and treated as described previously(13,14). The tungstic acid-deproteinized liver samples were used for preliminary studies of the use of the method of Moore and Stein(15,16) for determination of methionine.

Experiment II. A second group of Wistar male rats, maintained on Purina Laboratory Chow, were castrated at 4 weeks of age. Normal male and female litter mates were used as controls. At about 14 weeks of age the animals were grouped as shown in Tables I and II and given daily intraperitoneal injections of 0.1 ml of peanut oil or 0.1 ml of peanut oil containing 1 mg of testosterone propionate, per rat. At the end of the second week of in-

jections all of the animals were inoculated with the Walker carcinosarcoma 256, as described above, and on the 5th day following tumor inoculation rats without palpable tumors were discarded. Groups D and E, as well as one group of female controls (Table II), were given 7 daily intraperitoneal injections of an aqueous solution of DL-ethionine (20 mg/ml) at a level of 50 mg/100 g body weight. The daily injections of testosterone or peanut oil were maintained throughout the experimental period. Animals not receiving ethionine were given comparable injections of distilled water. The animals were fasted 4 hours before and 8 hours following the seventh injection of water or ethionine and then sacrificed. Livers and tumors were removed and treated as stated above with the exception that the liver samples were deproteinized with 5% trichloroacetic instead of tungstic acid. The excess trichloroacetic acid was removed by washing with ether (17) before evaporation to dryness under reduced pressure. An aliquot of the final liver samples was hydrolyzed by refluxing in 6 N HCl for 24 hours. The hydrolysates were used for determination of total methionine while the remaining aliquot of the liver samples was used for determination of free methionine, using the column chromatographic method described above.

Results and discussion. Castration markedly enhances the effects of ethionine in male rats, both as to its toxicity (as measured by carcass weight loss) and its ability to inhibit tumor growth (Table I). Castration, *per se*, had no effect on carcass weight loss or tumor growth. When testosterone was administered to castrated male rats the reaction to ethionine approached that of normal male rats. It may be seen in Fig. 1 that the inhibition of tumor growth by ethionine in castrated male rats resembled that observed in female rats while the inhibition in castrated male rats receiving testosterone was similar to that in normal male rats. It may be concluded from these results that the sex difference observed in this laboratory (11) in ethionine-treated rats is, at least in large part, related to the androgens.

In the preliminary determinations of free

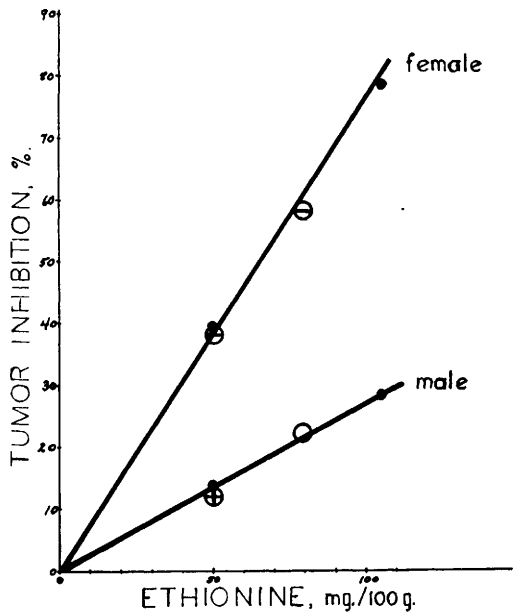

FIG. 1. Relationship between per cent tumor inhibition and daily dose of ethionine in normal and castrated rats, with and without testosterone. —●— Curves drawn from averages found in this laboratory (11,14) for normal male and female rats. ⊖ Castrated male rats. ⊕ Castrated male rats receiving testosterone propionate. ○ Normal male control rats.

methionine in the rat livers from Exp. I, using the Dowex-50 column, the excess tungstic acid from the deproteinization was troublesome and greater ease (and probably accuracy) could be obtained using a deproteinizing agent more easily removed. In Exp. II, therefore, the samples were deproteinized with trichloroacetic acid. The data in Table II indicate that a) concentration of nonprotein methionine in the liver of male rats is higher than that in female rats; b) concentration of nonprotein methionine in the liver of castrated male rats resembles that in female rats while the concentration in castrated male rats receiving testosterone is the same as that in normal males; c) injection of ethionine into castrated male rats receiving testosterone increases the concentration of nonprotein methionine in the liver and all of the methionine is in the free form; d) injection of ethionine into castrated male rats (and normal female rats) decreases the concentration of free methionine but tends to increase the total nonprotein methionine, indicating the

TABLE II. Concentration of Nonprotein Methionine in the Liver of Tumorous Wistar Rats.

Group*	No. rats	Sex	Condition	Treatment	Nonprotein methionine† Free	Total
A	4	♂	Normal	Water & peanut oil	17	18
C	5	♂	Castrated	" " testosterone‡	17	18
B	5	♂	"	" " peanut oil	12	12
—	7	♀	Normal	" " " "	12	14
E	5	♂	Castrated	Ethionine§ & testosterone	20	21
D	7	♂	"	Ethionine & peanut oil	8.9	16
—	7	♀	Normal	" " " "	9.4	15

* Group designation in Table I, Exp. II.
† γ/g of liver, wet wt.
‡ 1 mg of testosterone propionate per rat per day, in 0.1 ml of peanut oil.
§ 50 mg/100 g body wt daily for 7 days, in aqueous solution (20 mg/ml).

presence of a methionine conjugate. These observations are in agreement with those obtained previously(12,13) using microbiological assay methods.

It would appear possible, therefore, that a difference exists in the pattern of methionine metabolism in male and female animals and that this difference is mediated through the androgens. It is interesting to note that the dietary methionine requirement of women(18, 19) was lower than that reported for men (20,21).

Summary. 1. The per cent tumor inhibition, change in carcass weight, and concentration of nonprotein methionine in the liver, resulting from administration of ethionine to normal, castrated, and testosterone-treated castrated male rats, has been determined. 2. Tumor inhibition by ethionine, and the toxicity of this substance, was greater in castrated than in normal or testosterone-treated castrated male rats and closely resembled that observed in female rats. 3. The concentration of free nonprotein methionine in the liver of castrated male rats was similar to that found in female rats and differed from that observed in normal male or testosterone-treated castrated male rats. The presence of a methionine conjugate was indicated in the liver of castrated male and normal female rats following ethionine administration while such a conjugate was lacking in the liver of normal male or testosterone-treated castrated male rats. 4. It was concluded that the sex difference observed in tumor inhibition, toxicity, and concentration of nonprotein methionine in the liver of ethionine-treated rats was mediated by the androgens.

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