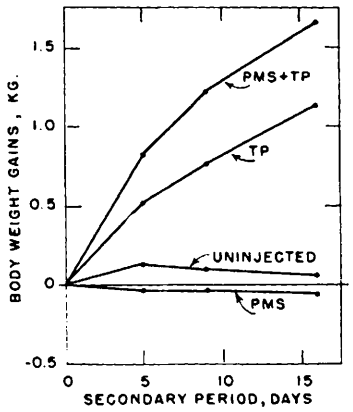

FIG. 2. Body wt gains of mature but sexually inactive male turkeys following administration of testosterone propionate (TP), and of pregnant mares' serum with the androgen (PMS + TP); also, body wt changes of uninjected and PMS injected control birds.

ment until the outcome of these tests is known, attractive though some possible implications of a gonadotrophically conditioned augmentation effect may appear to be.

Summary. Following preliminary treatment with pregnant mares' serum, body weight responses of immature and mature, but sexually inactive, male turkeys to testosterone propionate were considerably augmented when the androgen was administered concurrently with pregnant mares' serum. The serum alone was without appreciable effect on body weight. Whether the observed augmentation is an effect of gonadotrophin or of some other constituent of the serum is not known.

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Sustained Enhanced Growth of Carcinoma EO771 in C57 Black Mice. (18779)

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Injection of lyophilized or long frozen Eo-771 tumor tissue 10-15 days prior to the transplantation of the same tumor renders heterologous(1,2) and homologous(3) hosts more susceptible to the growth of the homologous tumor transplants. This lyophilization(1,2) or XYZ factor(4) has been shown to be specific(3). Previous reports have not considered whether the tendency to enhanced growth induced by the XYZ factor might persist through successive transplant passages from such a treated animal. The present paper reports 17 experiments with 2-6 animal passages of the tumor from an XYZ injected animal, and compares the growth of the secondary passage tumors with a series of control and a series of first passage XYZ tumors.

Materials and methods. The animals employed were 529 C57 Black mice received from Carworth Farms† in batches of 30 and 60 over an 18-month period. Each batch was of same age and sex and were divided immediately into groups of 10, numbered and housed in standard mouse boxes for 1-4 months before use. The animal quarters were

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1. Snell, George D., Cloudman, Arthur M., Failor, E., and Douglass, P., *J. Nat. Cancer Inst.*, 1946, v6, 303.

2. Snell, George D., Cloudman, Arthur M., Woodworth, Eliz., *Cancer Research*, 1948, v8, 429.

3. Casey, Albert E., Ross, Gordon L., and Langston, R. Revae, PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 83.

4. Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1932, v29, 816; *ibid.*; *Am. J. Cancer*, 1934, v21, 760; *ibid., Am. J. Cancer*, 1934, v21, 776; *ibid., Cancer Research*, 1941, v1, 134.

† Carworth Farms, New City, N. Y.

air conditioned and maintained at constant temperature, humidity and lighting and a standard food pellet was given. The control and experimental animals were always from the same batch. Four strains of C57 Black mammary carcinoma Eo771, designated A1, A2, A3 and A4, were obtained from 4 mice, 2549, 2550, 3749, and 3750, the first 2 received in one shipment in 1949 and the latter 2 in 1950 through the courtesy of Dr. George Snell of the Jackson Memorial Laboratory of Bar Harbor, Maine. Transplantation was effected by removing tumor tissue (15-27-day-old growth) aseptically from a subcutaneous growth which had not ulcerated. The tumor tissue was divided into 3 portions: (1) for histopathologic study, (2) for placing in the deep-freeze at 0°F in a sealed sterile container 10-90 days for XYZ material, and (3) for immediate transplantation. The latter was emulsified using a scissors, mortar and pestle and (1) sterile normal saline or (2) usually penicillin buffered with sodium citrate. About 0.1 cc of a 25% emulsion was injected subcutaneously into the left groin. (In some instances most of the tumor emulsion leaked out the needle hole in the skin.) There were usually 2 boxes (10 mice to a box) of control and 2 boxes of experimental mice in each experiment. The tuberculin syringes (one for each set) were filled at the same time. The order of inoculation was (1) control, (2) experimental, (3) experimental, (4) control box or (1) experimental, (2) control, (3) control, (4) experimental box. The XYZ material was frozen tumor tissue which had been kept in the deep freeze at 0°F in a sterile container for 10 days or longer, and was prepared and injected in an entirely similar manner. The injections of frozen tumor tissue were usually into the opposite groin 7-34 days before the tumor transplantation. No tumor growths or palpable inflammatory reactions appeared. The XYZ material was prepared from Eo771 tissue, and XYZ control material was similarly prepared and injected into 73 mice: (1) from Barrett mammary carcinoma(3) of C3H origin adapted to C57 blacks (10 mice), (2) from mammary carcinoma 241-5 (22 mice; carcinogen-induced tumor from Dr. Andervont's(3) laboratory), or (3) mammary carcinoma 755(3) (41 mice), the latter 2 being of C57 black origin (Table I).

TABLE I. Mammary Carcinoma EO771 in C57 Black Mice: Controls, First and Multiple Passage XYZ Series.

Exp. #	St.	Inoc. #	Pos. #	M. day	Σx	Σx²
Control mice						
231	A1	10	10	20	27	102
245	A1	10	6	19		
246	A1	8	8	19	53	302
263	A1	10	6	19		
264	A1	10	10	19	75	459
268	A1	10	8	21	17	58
273	A1	8	8	15	26	110
288	A1	9	8	14	4	4
300	A1	10	7	18	21	102
318	A1	8	7	18	29	153
322	A1	10	9	21	25	90
324	A1	6	6	19	15	47
Subt.		109	93		292	
Mean		109			2.68	
356	A3	10	8	20	13	37
357	A4	8	7	21	37	352
360	A3	10	10	20	19	64
368	A3	10	10	20	45	267
349	A3	9	8	21	15	48
376	A3	11	6	20	12	36
378	A3	9	9	20	40	282
380	A3	10	7	21	12	34
382	A3	9	6	21	20	109
383	A3	9	7	21	25	140
384	A3	11	10	21	26	96
Subt.		106	88		264	
Mean		106			2.49	
225	A1[8]	10	9	20	24	108
261	A1[8]	14	13	19	65	452
266	A1[8]	8	6	21	11	24
297	A1[7]	9	5	18	4	4
Subt.		41	33		104	3480
Mean		41			2.54	
Total		256	214		660	
Control			Mean	256	2.58 ± .17	

Exp. #	XYZ	Int. day	St.	Inoc. #	Pos. #	M. day	Σv	Σv²
Experimental mice								
241	A2	25	A1	10	10	19		
243	A2	25	A1	9	9	19	135	1352
274	A1	18	A1	10	10	15	63	472
290	A1	19	A1	10	9	14	5	4
323	A1	13	A1	10	9	21	13	26
325	A1	15	A1	7	7	19	22	118
354	A1	8	A3	11	10	20	41	224
355	A1	14	A4	9	4	21	7	13
366	A1	8	A3	11	10	20	47	253
379	A3	8	A3	10	6	21	25	122
381	A3	8	A3	10	5	21	7	15
385	A3	8	A3	10	10	21	32	173
386	A3	8	A3	12	10	21	63	612
Total				129	109		460	3384
XYZ		First passages		Mean		129	3.57 ± .32	
337			A1X3	10	9	21	59	547
338			A1X3	7	7	19	11	32
334	755	7	A1X3	9	9	21	69	722
339	755	7	A1X3	10	10	19	44	288
Subt.				36	35		183	
			Mean	36			5.1	

TABLE I (continued)

335	A1	7	A1X3	7	7	21	58	1078
336	A1	7	A1X3	9	8	21	52	606
340	A1	9	A1X3	10	10	19	62	424
341	A1	9	A1X3	10	7	19	56	665
367	A1	8	A1X6					
			A1X4	10	8	20	16	54
Subt.				46	40		244	
			Mean	46			5.30	
328			A1X2	7	6	18	21	77
			A1X4					
345			A1X2	9	9	18	55	389
			A1X4					
352			A1X3	7	7	21	79	1015
			A1X5					
361			A1X4	8	8	20	46	490
			A1X6					
369			A1X4	8	7	20	38	275
			A1X6					
333			A1X3	10	10	22	55	384
			A1X5					
348	755	7	A1X3	8	7	18	9	19
			A1X5					
350	755	7	A1X3	5	5	21	30	177
			A1X5					
Subt.				62	59		333	
			Mean	62			5.37	
Total				144	134		760	
XYZ	Multiple passages			Mean			144	5.28 ± .37

Exp.—experiments; St.—strain; Inoc.—inoculated; Pos.—grew tumors; M.—tumors measured; x—sum of tumor volumes; x^2—sum of squares of tumor volumes; Subt.—subtotal; XYZ—strains source for XYZ material; Int.—interval between XYZ injection and tumor transplantation; A1, A2, A3, A4—Bar Harbor strains of Eo771; $A1^B$, $A1^S$, $A1^7$—mice preinjected with Barrett ca. XYZ, 241-5 XYZ, and 755 XYZ respectively; A1X3, 4, 5, 6—tumor strain A1X 2, 3, 4, 5, or 6 mouse passages removed from an XYZ injected animal.

All experiments with Eo771 during 1949-50 are included in the study except certain stock transfers in which the only tumors measured were those used for transplantation or for XYZ material. There were 256 control mice, 129 mice in the first passage XYZ series, and 144 mice in the multiple passage XYZ series (Table I).

The 256 *control* mice were from 3 groups of experiments: (1) 109 mice in 12 experiments inoculated with the A1 strain of Eo771 mammary carcinoma over a 7-month period, (2) 41 mice in 4 experiments inoculated with the A1 strain of Eo771 several weeks after preliminary treatment with XYZ control material from the Barrett 241-5 or 755 mammary carcinomata, and (3) 106 mice in 11 experiments inoculated with the A3 and A4 strains of Eo771 beginning about one year after the start of the A1 strain experiments.

The 273 *experimental* mice were in 2 categories: (1) 129 mice in 13 experiments injected with Eo771 XYZ material 8-25 days before tumor transplantation (Table I); (2) 144 mice in 17 experiments in the multiple passage XYZ series (Table I). The Eo771 tumor strain used in these 144 mice was a variety of the A1 strain called A1X because of its having originated in an A1-Eo771 XYZ injected A1-Eo771 tumor animal 2-6 mouse passages earlier. This is the only instance in which secondary animal passages have been made from an XYZ injected mouse. (The rule has been to make transfers from control mice only.) The original mouse,‡ No. 3253, from Exp. 325 (Table I) had at 20 days after transplantation (35 days after XYZ injection) a primary tumor in the groin which measured 3.45 cc (2 other animals in the group had larger tumors and 2 almost as large), and at 27 days when transfer was made the primary tumor measured 13.5 cc. (Volume of a tumor in our experiments is artificial, being the product of measurements in cm in 3 dimensions with calipers; measurements after tumor transplantation are routinely made at 19-20 days for Eo771 because deaths from tumor growth or ulcerations begin to occur in some animals at 21 days and thereafter.) Thus the tumor cells injected into each of the 144 mice in the 17 experiments traced back to tumor cells which grew in XYZ injected Mouse 3253. In addition the mice in Experiments 335, 336, 340, 341 and 367 also had an injection of A1-Eo771 XYZ material, and the mice of Exp. 334, 339, 348, and 350 had an injection of XYZ control material

‡ From the tumor tissue in Mouse 3253 a stock transfer was made into mice 3202 and 3203 and 8 others (Exp. 320); from tumor growth in 3203 the mice in Exps. 338, 339, and 340 were injected; from the tumor growth in Mouse 3202, animals in Exps. 334, 335, 336, 337, and 341 were injected; from Mouse 3369 in Exp. 336, animals in Exp. 328 were transplanted; from Mouse 3286 in Exp. 328 were transplanted the animals in Exp. 345, 348 and from Mouse 3287 the animals in Exps. 353, 350, and in stock transfer Exp. 353 were transplanted; from Mouse 3531 of the stock transfer the mice in Exp. 333 were transplanted and from the tumor in Mouse 3339 of Exp. 333 the mice in Exps. 361, 369, and 367 were transplanted.

from 755 mammary carcinoma tissue (Table I). Among the 144 mice 36 represented the 3rd mouse passage from the original XYZ animal and were otherwise entirely like the controls; 46 of the 144 mice in 5 experiments (Exps. 355, 336, 340, 341, 367) were injected with the A1X tumor strain, and had, in addition, a preliminary injection of A1 XYZ material; the remaining 62 in 8 experiments injected with the A1X tumor strain had in addition a second injection of A1 XYZ two or three animal passages back.

Results. The average volume of the primary tumor at 19-20 days among the entire 256 control mice was 2.58 ± 0.17 cc (variance 6.741; variance of the mean 0.0272). In 12 experiments 109 control mice inoculated with the A1 tumor strain had at 19-20 days an average primary tumor of 2.68 cc; in 11 experiments with the A3 and A4 strains, 106 control mice had an average primary tumor size at 20 days of 2.49 cc; and in 4 experiments in which 41 control mice, although inoculated with A1 strain, had preliminary injections of XYZ material from the Barrett, 241-5 or 755 mammary carcinoma strains, the primary tumor at 19-20 days averaged 2.54 cc. There was, therefore, no statistically significant difference between the growth of the controls for the A1 strain, treated or untreated with heterologous XYZ material, and the A3 and A4 strains. In fact, the average size of the tumors was almost identical in the 3 groups.

In the 13 experiments in which 129 mice were inoculated with the A1, A3, and A4 strains, but had received preliminary injections of Eo771 XYZ, the average volume of the primary tumor per animal inoculated was 3.57 cc at 19-20 days as compared with 2.58 cc among the 256 control mice. This difference of 0.99 ± 0.28 cc ($t = 3.5$, $n = 383$, $P = 0.01-$) was statistically significant and indicated that the preliminary treatment with the homologous XYZ material was effective in increasing the average size of the tumor growths by about 40%. There was no statistically significant difference in the incidence of tumors at 19-20 days between the controls (214 or 83.6%) and the first passage XYZ series (109 or 84.5%) (Table I). The mean size of the tumors among the 214 controls was 3.1 cc and among the 109 first passage XYZ series 4.2 cc. The difference 1.14 ± 0.23 cc is highly significant ($t = 5.0$, $n = 321$, $P. = 0.01-$) and even more striking than when calculated for animals inoculated.

The 144 mice in the 3 groups (17 experiments) transplanted with the A1X strain had uniform mean tumor volumes at 19-20 days. The tumor mean for the 36 animals after the 3rd mouse passage was 5.1 cc, for the 46 given another XYZ treatment, 5.3 cc, and for the 62 serially transferred after a second and third XYZ treatment of the tumor cell strain, 5.37 cc; the differences were statistically insignificant between the groups of the A1X strain variously treated. The mean value for the entire 144 animals inoculated was 5.28 cc or twice that for the 256 controls (statistically significant difference) and some 50% more than the mice receiving the preliminary injection of the XYZ material in the first animal passage (statistically significant difference).

Among the 144 multiple passage XYZ mice 134 had primary tumors at 19-20 days (94%) as compared with 214 among the 256 control mice (84%), a difference which was statistically significant ($\chi^2 = 7.2$, $n = 1$, $P. = .01-$). The mean volume of the primary tumors among the 134 positive multiple passage XYZ animals was 5.7 cc at 19-20 days as compared with 3.08 cc among the 214 positive controls. The difference, 2.59 ± 0.36 cc, was statistically significant ($t = 7.2$, $n = 346$, $P. = 0.01-$).

The incidence of primary tumors was significantly less ($\chi^2 = 5.1$, $n = 1$, $P. = 0.023$; probably significant) among the first passage XYZ series (189 or 85%) than among the multiple passage XYZ animals (134 or 94%). The mean size of the tumors in the 109 positive first passage animals was 4.2 cc and among the 134 positive multiple passage animals was 5.7 cc. The difference, $1.45 \pm .47$ cc, was statistically significant ($t = 3.1$, $n = 241$, $P. = 0.01-$).

Discussion. The average size of the primary tumor was constant and uniform at 19-20 days in the Carworth Farms C57 black control mice with 2 different Eo771 strains A1 and A3 studied almost one year apart. This indicates a high degree of uniformity in the animal stock, experimental technic, tumor be-

havior and tumor strains in the control mice. The enhanced growth in the XYZ series is, therefore, all the more significant, and the results amplify and confirm those already published on the effect of the XYZ material in the homologous strain(3). Snell and his group have reported(1,2,5) an enhancing effect of lyophilized (or XYZ) material from Eo771 and 15091a on heterologous strains, which is even more striking than that obtained in this series. In fact, Dr. Snell might explain that the enhanced effect of the homologous material with the Carworth Farms C57 black strain was due to the fact that this strain was genetically somewhat different from the Bar Harbor C57 black strain in which the tumor strains A1, A2, A3, and A4 had been carried previous to use in our laboratory.

The most remarkable result was the apparently permanent enhanced growth of the A1X strain in the 17 experiments herein reported. This might indicate that the second and later passages of a tumor cell strain from an XYZ treated animal might give rise to larger average growths than would occur in the first passage strain. This might seem almost conclusive were it not that the 17 experimental groups of the A1X strain traced back to the same XYZ treated transfer animal (3253). No other experiments with secondary mouse passages are available. Whether this was a mutation—and some form of mutation or adaptation it seemed to be—or a regular occurrence in XYZ injected animals, only further experiments can answer. Attempts to reverse the change are in progress.

5. Kaliss, Nathan, and Jay, George E., Jr., *Cancer Research,* 1950, v10, 227-8; Kaliss, Nathan, Jonas, Gordon and Avnet, Norman L., *Cancer Research,* 1950, v10, 228; Kaliss, Nathan, and Snell, G. D., *Cancer Research,* 1951, v11, 122-6.

Summary and conclusions. 1. Mammary carcinoma Eo771 was inoculated into 529 C-57 black mice in three series of experiments. Tumor growth in 109 control mice (12 experiments) injected with one Bar Harbor Eo771 mammary carcinoma strain averaged 2.7 cc at 19-20 days, and 41 control mice of the same strain (4 experiments) previously injected with heterologous XYZ material averaged 2.5 cc at 19-20 days. An additional 106 control mice in 11 experiments injected with a second Eo771 strain received from Bar Harbor about 1 year later averaged 2.5 cc at 20 days, indicating great uniformity in 2 different Bar Harbor strains of this tumor in Carworth Farms C57 black mice, there being no statistically significant differences. 2. In contrast, 129 C57 black mice in 13 experiments having a preliminary injection of Eo771 XYZ material 8-25 days before tumor transplantation had a 40% increased tumor volume at 19-20 days, or 3.6 cc. This difference of 0.99 $\pm$ 0.28 cc was statistically significant. 3. In an additional 17 experiments during the same period involving 144 mice of the same strain, mean tumor growth in 2-6 animal passages of tumor from an XYZ injected mouse averaged 5.3 cc or more than double the growth of Eo771 in control mice and some 40-50% greater than occurred in the first passage XYZ strains. Whether this greatly enhanced growth was a mutation or a variation induced by the XYZ treatment remains to be determined.

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