

Quantitative Studies on Relationship Between Estrogen and Mammary Gland Carcinoma in Strain C3H Mice.

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The first experimental proof of a qualitative and quantitative relationship between the internal secretions of the ovary and carcinoma of the mammary gland in mice was presented by Leo Loeb^{1, 2} and more recent investigations³ have amply confirmed the qualitative phase of this relationship. The following experiments concern the quantitative aspects of the problem.

Materials and Methods. The data were obtained from 206 strain C3H mice* of which 122 were experimental and 84 were control animals. Their diet consisted of fresh Purina dog chow, to which lettuce was added at irregular intervals.

All the experimental animals were males and all were at least 5 months old when they died or were sacrificed. They were divided into 7 groups (Table I) and were injected with estradiol benzoate in a solution of sesame oil (Progynon-B) from the age of 2 weeks onward as follows:

Group I received 3000 rat units in 2 doses over a period of 3 days.

Groups II-VI inclusive received 100 rat units weekly for 4, 8, 12, 16, and 20 weeks respectively.

Group VII received 100 rat units weekly throughout the duration of life.

The control groups consisted of 38 untreated breeding male (Group VIII) and 46 untreated breeding female mice (Group IX),

¹ Lathrop, A. E. C., and Loeb, Leo, *J. Cancer Res.*, 1916, **1**, 1.

² Loeb, Leo, *J. M. Research*, 1919, **40**, 477.

³ Cori, C. F., *J. Exp. Med.*, 1927, **45**, 983; Murray, W. S., *J. Cancer Res.*, 1928, **12**, 18; Lacassagne, A., *Compt. rend. Acad. d. sc.*, 1932, **195**, 630; Bonser, G. M., *J. Path. and Bact.*, 1935, **41**, 33; Burrows, H., *Am. J. Cancer*, 1935, **24**, 613; Cramer, W., and Horning, E. S., *Lancet*, 1936, **1**, 247; Suntzeff, V., Burns, E. L., Moskop, M., and Loeb, Leo, *Am. J. Cancer*, 1936, **27**, 229.

* The parent animals were obtained in 1938 from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine. In New Orleans the strain has been propagated only by brother to sister matings. A high percentage of spontaneous carcinoma of the mammary gland occurs among the breeding females of this strain.

TABLE I.

Group	Experimental mice									Control mice	
	I	II	III	IV	V	VI	VII	VIII	IX		
Incidence of Tumors in Relation to Dosage of Estrogen.											
No. of animals	20	11	20	14	19	19	19	38	46		
No. of tumors	0	0	3	1	11	9	10	0	32		
Incidence tumors (%)	0	0	15.0	7.1	57.8	47.3	52.6	0	69.5		
Total dosage rat units	3000	400	800	1200	1600	2000	Avg 3270	0	0		
No. of doses	2	4	8	12	16	20	Avg 32.7	0	0		
Mode of dosage	Alternate days at age 2 weeks weekly weekly weekly weekly weekly weekly weekly weekly weekly weekly										
Incidence of Tumors in Relation to Age in Months.											
Age range	6.30- 19.83	8.86- 18.80	5.13- 19.46	6.20- 18.30	6.00- 13.36	5.45- 15.53	5.66- 12.46	6.00- 18.66	6.93- 18.86		
Avg age at which injections were ended	0.53	1.06	2.10	3.00	4.04	4.94	7.90	—	—		
Avg interval between last injection and death											
With tumors	—	—	4.83	3.10	5.93	4.20	0	—	—		
Without tumors	13.50	10.00	7.73	7.76	3.10	5.53	0	—	—		
Entire group	13.50	10.00	7.30	7.33	4.76	4.86	0	—	—		
Avg age at death											
With tumors	—	—	6.90	6.60	9.86	9.06	8.60	—	12.96		
Without tumors	14.03	11.06	9.83	10.60	7.40	11.56	7.06	14.03	9.86		
Entire group	14.03	11.06	9.40	10.33	8.80	9.80	7.90	14.03	12.03		

all of which were over 6 months of age at the time of necropsy (Table I).

It is important to note that after the specified course of injections the mice in Groups I-VI inclusive received no further treatment.

The presence or absence of tumors was determined by gross inspection at necropsy, a sufficient number of tumors having been examined microscopically to establish the accuracy of gross diagnosis. Small masses of questionable identity were not regarded as neoplasms.

Results. No tumors developed in the animals of Group I or Group II. A low incidence was observed in the animals of Groups III and IV, and the tumors developed at an earlier age in the mice of these groups than in the mice of any other experimental group. Moreover, all 3 animals which developed tumors in Group III were from the same litter. A relatively high and essentially equal percentage of tumors developed in mice of Groups V, VI and VII and the neoplasms occurred at approximately the same average age in each group. The female controls (Group IX) developed a higher percentage of tumors than any of the experimental groups, and the tumors appeared at an older age. No tumors were noted in the male controls (Group VIII). The complete data are shown in Table I.

Summary. 1. Beginning at the age of 2 weeks, 122 non-breeding male strain C3H mice were treated with estrogen for varying periods of time. At the end of the specified course of injections the mice received no further treatment until they died or were sacrificed. 2. Four weekly doses of 100 rat units of estrogen produced no mammary gland carcinomas; 8 and 12 weekly doses of 100 rat units produced a low incidence of these tumors. 3. After 16 weekly doses of 100 rat units of estrogen had been given a relatively high percentage of carcinoma of the mammary gland developed; the administration of 20 weekly doses or an average of 32.7 weekly doses of 100 rat units did not raise the incidence of tumors above this level. 4. Three thousand rat units of estrogen administered over a period of 3 days at the age of 2 weeks produced no tumors.