Effect of Ovariectomy, Adrenalectomy and Hypophysectomy on Growth of Spontaneous Mammary Tumors in Mice.* (21018)

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It is well known that alterations of hormonal stimulations may change the incidence of mammary tumors in susceptible mice. However, the effects of the surgical removal of some endocrine glands (ovaries, adrenals, hypophysis) upon the growth of established spontaneous mammary tumors, as well as on the survival time of mice bearing them and submitted to the above mentioned operations, have not been conclusively established. Baatz(1) reported that ovariectomy does not alter the growth of established spontaneous mammary tumors in mice. Gardner(2) found in mice bearing spontaneous breast tumors, that hypophysectomy performed during pregnancy or immediately after delivery did not change the growth of the tumors and also did not cause regression of the hyperplastic nodules which are considered as precancerous lesions. In humans, however, with carcinoma of the breast, favorable results have been reported following the surgical removal or X-radiation of the ovaries, bilateral adrenalectomy or both combined operations (see review by Nathanson and Kelley(3)). Recently Luft (4) has reported the results obtained in 30 patients with carcinoma of the breast treated by the removal of the pituitary gland. In 10 of those patients hypophysectomy did not change the course of the disease and they died early after the operation. In 10 cases the tumor development was only retarded and in 10 cases there was a dramatic subjective and objective improvement in the disease, namely disappearance of pain, regression of metastic lesions and remarkable effects on the general condition of the patients.

The present experiments deal with the effect of bilateral ovariectomy alone, or combined with bilateral adrenalectomy, and the effect of hypophysectomy alone and combined with ovariectomy and adrenalectomy upon the growth of spontaneous mammary tumors in mice. Also the survival time after the various types of operations was recorded.

Methods. Mice of different strains and hybrids bearing spontaneous mammary carcinomas of a size ranging between 5 and 14 mm were used. Bilateral ovariectomy as well as adrenalectomy was performed through a lumbar incision of the skin and muscular wall. Hypophysectomy was done following the technic described by Thomas(5). In all the groups in which adrenalectomy was done, 1% NaCl solution was given in the drinking water and cortisone acetate was injected subcutaneously once every other day in a dose of one to 5 γ per mouse. Once or twice a week adrenal cortical extract in a dose of 0.1† cc per mouse was injected subcutaneously. Body weight and tumor size were recorded 2 or 3 times a week. Tumor size was measured with calipers, taking the 2 largest diameters of the tumor and the size was arbitrarily calculated according to the formula $S = \sqrt{axb}$, a and b being the 2 major diameters.

Results. The results are summarized in Table I. It can be seen that there was no tumor regression either in the untreated or in the ovariectomized group. In fact all tumors in both groups continued to grow and killed the mice in 53 and 52 days after the operation, respectively.

Bilateral adrenalectomy alone produced in one out of 30 mice a complete regression of the tumor; 3 showed a temporary regression; 6 showed neither increase nor decrease of the tumor size and in 23 the tumor grew pro-

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† Equivalent to 5 dog units (Eschatin, Parke, Davis & Co.)

TABLE I. Effect of Ovariectomy, Adrenalectomy and Hypophysectomy upon the Growth of Spontaneous Mammary Tumors.

Operation*	No. of mice	Complete regression‡	%	Partial regression	%	No change	%	Increase	%
0	22	0		0		0		22	100
†								53	
Ov.	20	0		0		0		20	100
†								52	
Adr.	30	1	3.3	3	10	6	20	20	66.6
†		23		10		13		18	
Adr. & Ov.	27	1	3.7	2	7.4	0		24	88.8
†		28		16				17	
Hyp.	26	3	11.5	5	19.2	3	11.5	15	57.7
†		34		14		12		22	
Hyp., Ov. & Adr.	5	0		1	20	1	20	3	60
†				10		10		8	

* Ov. = Ovariectomy; Adr. = Adrenalectomy; Hyp. = Hypophysectomy.
† Avg survival time in days.
‡ Complete regression of tumor was confirmed histologically.

gressively. The survival time after the operation was as follows: 23 days for the mouse showing complete regression of the tumor; 10 days for the group showing a temporary regression; 13 days for those in which the tumors did not change in size and 18 days for those in which the tumors grew progressively.

Simultaneous removal of both adrenals and ovaries produced 1 complete regression out of 27 mice; 2 temporary regressions and 24 in which the tumors grew progressively. The survival time was 28 days for the mouse in which the tumor regressed completely; 16 days for those showing a temporary regression and 17 days for those in which the tumors grew after the operation.

In the group of hypophysectomized mice, 3 out of 26 animals showed complete regression of the tumor; 5 showed temporary regression; in 3 the tumor did not change in size and in 15 the tumors grew progressively.

The survival time after the operation was 34 days for those in which the tumor regressed; 14 days for those showing temporary regression; 12 days for those in which there was no change in the tumor size and 22 days for those showing progressive growth of the tumor.

Finally, in the small group of mice in which the 3 operations (ovariectomy, adrenalectomy and hypophysectomy) were performed, only 1 mouse out of 5 showed a temporary regression of the tumor; in 1 the tumor did not change in size and in 3 there was an increase in tumor size. The survival time in this group ranged between 8 and 10 days.

Fig. 1 shows the mean body weight changes in different groups of mice after the operation. As can be seen, the hypophysectomized as well as the adrenalectomized mice showed a considerable decrease in body weight. However, in the group of adrenalectomized and gonadectomized animals there was practically no change in the mean body weight after the operation.

Discussion. The above mentioned results indicate that as far as the growth of already established spontaneous mammary tumors is concerned, the removal of both ovaries did not produce any appreciable change in the rate of growth. In fact, all tumors grew after the operation and killed the host in a time comparable to the unoperated controls.

Bilateral adrenalectomy alone and combined with ovariectomy produced complete regression of one tumor in each group (3.3% and 3.7% respectively), few tumors showed either a temporary regression or no change in size, but most of the tumors in both groups (66.6% and 88.8% respectively) grew progressively in spite of the absence of the adrenals or of both adrenals and ovaries.

The group in which hypophysectomy alone

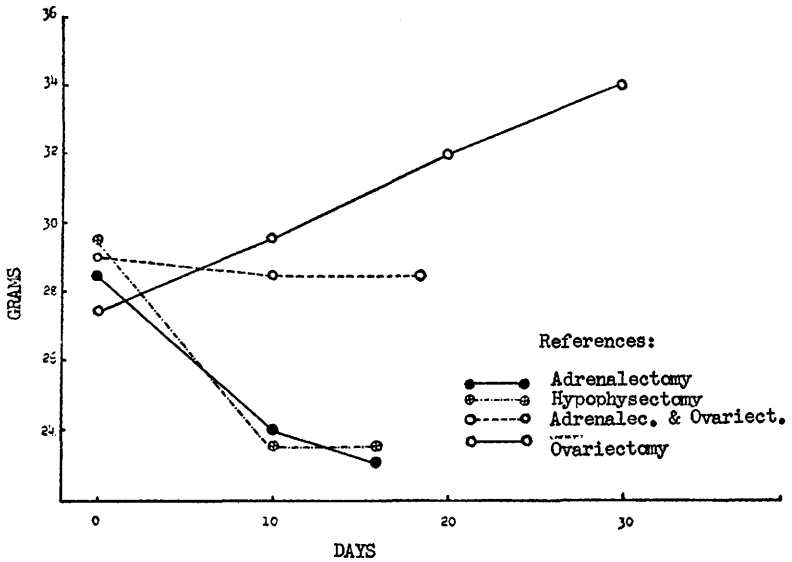

FIG. 1. Mean body weight changes in mice bearing spontaneous mammary tumors submitted to ovariectomy, adrenalectomy, ovariectomy-adrenalectomy and hypophysectomy. Operation at time 0.

was performed showed a higher incidence of complete tumor regressions (11.5%), few tumors showed either a temporary regression or no change, but again most of them (57.7%) continued to grow after the operation, in spite of the absence of the pituitary gland.

The number of mice in the group in which the three operations were performed (ovariectomy, adrenalectomy and hypophysectomy) was too small to permit definite conclusions. In fact, the removal of the 3 glands produced a very high mortality incidence at 12 to 24 hours after the operation.

Experiments are already being undertaken to find out whether tumors showing a temporary regression or no change, would show any alteration in their behavior when transplanted into a new intact, adrenalectomized or hypophysectomized hosts.

Since most of the adrenalectomized or hypophysectomized mice showed a significant decrease in body weight after the operation, it is rather difficult to decide whether the tumor changes obtained are due to the loss of body weight *per se* or to the absence of the glands. It is well known that a reduction in food intake may change the incidence and growth of spontaneous and transplanted mammary tumors, but the fact that most of the tumors grew in spite of the loss in the body weight might be an indication that perhaps inanition was not the only factor responsible for the changes in tumor size obtained.

As far as the survival time after the operation is concerned it is quite clear that in the groups in which either adrenalectomy or hypophysectomy was performed, it was significantly shorter than in the control or ovariectomized groups. The old age of the animals, the presence of the tumors and the difficulty in maintaining the mice upon a properly adjusted replacement therapy might account for the shorter survival time found even in those mice in which there was a complete regression of the tumor.

Summary. 1. The effect of ovariectomy, adrenalectomy, and hypophysectomy upon growth of spontaneous tumors in mice has been studied. 2. Bilateral adrenalectomy alone or combined with ovariectomy produced a complete regression of the tumor in 3.3% and 3.7% of the mice respectively. Hypophysectomy was followed by regression in 11.5% of mice. 3. All groups in which either adrenalectomy or hypophysectomy was performed, showed a shorter survival time than unoperated controls, even in those mice showing a complete regression of their tumors.

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Level of C-Reactive Protein as a Measure of Acute Myocardial Infarction.* (21019)

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The C-reactive protein (CRP) is an abnormal constituent of human serum(1). It is in all likelihood an alpha globulin which is formed by the body in response to an inflammatory reaction(2-4). It is called C-reactive protein because it forms a precipitate with the somatic C-polysaccharide of the pneumococcus(5). Small amounts of this protein may be detected in human serum by a precipitin test using a specific antiserum from rabbits hyperimmunized with purified C-reactive protein(6,7).

Previous studies have shown that a positive test for CRP is a non-specific but sensitive indicator of inflammation of infectious or non-infectious origin(3,4). Anderson and McCarty(8), Stollerman and coworkers(9), and the authors(10) have employed the CRP test for the detection of low-grade inflammation in rheumatic fever. Since myocardial infarction in coronary artery disease is also associated with an inflammatory response, it was felt that the CRP test might be useful in determining the presence of inflammation associated with myocardial infarction.

Material and methods. The CRP precipitin test was performed according to the method of Anderson and McCarty(8). 1.5 cm each of CRP antiserum and the patient's serum were drawn up into a capillary tube (external diameter about 1 mm) and incubated for 2 hours at 37°C. The degree of precipitation (0 to 4+) was read after overnight refrigeration. Each millimeter of precipitate was considered 1+. Serial CRP tests were done on the sera of 5 of the 7 patients with coronary occlusion and myocardial infarction, and on the serum of one patient with acute coronary insufficiency and myocardial necrosis. Single specimens were tested in the other 2 patients with coronary occlusion. All these patients had a typical clinical course and showed the manifestations of necrosis, *i.e.*, fever, leucocytosis, elevated sedimentation rate, and elevated fibrinogen(11-13). These

TABLE I. CRP Tests in Patients with and without Myocardial Necrosis.

Sex	Age (yr)	Day of illness before first (+) test	Sedimentation rate (Wintrobe) mm/hr	CRP test
		Coronary occlusion; myocardial infarction		
♀	55	7	32	2+
♂	58	16	34	2+
♀	61	9	21	2+
♀	55	1½	42	1+
♀	53	3	30	3+
♂	52	2	20	3+
♂	54	2	30	2+
		Coronary insufficiency; myocardial necrosis		
♂	73	5	22	3+
		Coronary insufficiency without necrosis		
♂	59	2	8	Neg.
♂	58	7	5	"
♀	73	14	15	"
♂	50	7	30	"
♀	52	13	15	"
♂	41	7	20	"

*The CRP antiserum was kindly supplied by Schieffelin & Co. through the courtesy of Dr. E. W. Blanchard.