there are specific entities or genes underlying genetic susceptibilities to specific types of neoplasia induced by methylcholanthrene. This interpretation seems to be favored over the alternative concept that in cancer susceptibility, there is a general cancer "gene" that underlies all types of cancer. That is, that there is a gene that determines the difference between the biological states of cancer and not cancer.

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Mucolytic Enzyme Systems. IV. Relationship of Hyaluronidase Inhibition by Blood Serum to Incidence of Mammary Cancer in Mice.*

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From previous investigations in this series, it was concluded that the level of hyaluronidase inhibitor in blood serum is elevated in a wide variety of both virus and bacterial infections[1-3] as well as in malignant processes.[4] While these investigations were still in progress Friou and Wenner[5] reported a similar elevation in rheumatic fever as determined by the mucin clot test, and Thompson and Moses[6] also found this effect in pneumonia by the clot test. Fulton, Marcus and Robinson[7] used a method based on decapsulation of a group A hemolytic streptococcus to measure the inhibition by serum and reported no significant differences between normals, and patients with rheumatoid arthritis.

The inhibiting factor which undergoes change is distinct from the specific antibody inhibitors which are elicited in response to hyaluronidase acting as an antigen. By electrophoresis at pH 8.6 it was found that the non-antibody inhibitor in the serum migrated chiefly with the albumin.[8] The antibody inhibitor would be expected to be found in the gamma globulin fraction. The factor in question appears to be a non-specific inhibitor since it is capable of inhibiting the enzyme from diverse sources, and furthermore its elevation seems to be a non-specific response to both infection and malignancy. While it is still premature to advance a theory, one might postulate that this response is a defense mechanism designed to counteract the invasiveness potentiated by hyaluronidase. While many organisms do not in themselves possess hyaluronidase, their invasiveness appears to be enhanced, nevertheless, by the presence of the enzyme,[9] and, accordingly, it is conceivable that a general response against hyaluronidase activity might be employed as a general defense mechanism.

The possibility that hyaluronidase may be involved in the invasive processes of cancer,[10-16] and the great elevation of the serum

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TABLE I.
Hyaluronidase Inhibitor in Blood Serum of Cancer Mice.

Group	Strain	No. mice	Presence of milk agent	Presence of tumor	Mean inhibitor conc. (A) per cc serum	Difference of means	t*	P
1	Breeders	13	—	—	19.2	0.8	.20	>.55
	Ax	11	+	+	18.4			
	Aa							
2	Virgins	22	—	—	16.1	0.2	.05	>.55
	Ax	19	+	—	15.9			
	Aa							
3	Zb	19	—	—	12.8	1.2	.60	>.55
	Zz	14	+	+	11.6			
4	$ZbAxF_1$	19	—	—	10.1	3.6	1.45	.15
	$ZzAaF_1$	3	+	+	6.5			

* Statistical quantity used for less than 30 observations to calculate probability (P) that the differences could have resulted from chance alone.

inhibitor in metastatic carcinoma,[4] led to the present investigation of the level of the inhibitor in the blood serum of various strains of mice in relation to the genesis of mammary cancer. It was hoped that some light might be shed on the question of whether the hyaluronidase inhibitor has any significance in resistance to mammary cancer in mice.

Materials and methods. The mice employed were of the following strains used by Bittner and Huseby.[7]

Aa stock—*A* stock cancer mice nursed by mothers with *"a"* milk agent.

Breeders—high cancer line, with cancer when used.

Virgins—low cancer line, without cancer when used.

Ax stock—Decended from 1 animal of the *A* stock that had been nursed by a female of *X* or *CBA* strain. Without cancer when studied. No cancer without milk agent.

Zz stock—*Z* or *C3H* stock cancer mice possessing *"z"* milk agent with cancer when used.

Zb stock—Fostered *Z* stock mice without milk agent. Descendents from females fostered by mice without milk agent. Without cancer when used.

Zb Ax F_1—First hybrid generation of *Zb*♀ × *Ax*♂. Without cancer when used. Devoid of milk agent.

Zz Aa F_1—First hybrid generation of *Zz*♀ × *Aa*♂. High cancer incidence, both *"a"* and *"z"* milk agents present. With cancer when used.

The mice were anesthetized with ether, and 0.5–1.0 cc of blood was taken from the jugular vein of each animal. After clotting, the serum was withdrawn and stored at −25° until used for the determination of hyaluronidase-inhibitor content. The details of the viscosimetric method used, which employed hyaluronidase from bull testes and hylauronic acid from human umbilical cords, have been given in an earlier paper.[1] The value (A) expressing degree of inhibition is defined as $\left(\frac{R-Ro}{Ro}\right)$, where (Ro) equals the time in seconds for the viscosity of the reaction mixture without serum to fall to half its initial value, and (R) equals the corresponding time in seconds for the viscosity to fall to half the initial value in the presence of serum. Only 0.02 cc of serum is required in the 6 cc of total reaction mixture prepared for each measurement. The concentration of inhibitor was calculated as

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TABLE II.
Relation of Cancer Incidence in Strains of Mice to Hyaluronidase Inhibitor in Blood Serum.

Group	Strain	No. mice	% Cancer[17]	Mean inhibitor conc. (A) per cc serum	Groups compared	Difference of means	K*	P
1	Aa breeders	13	86.7	15.3	1 and 2	0.5	0.1	>.55
					1 and 3	3.5	1.2	.230
2	Aa virgins	40	3.9	14.8	1 and 4	5.8	2.1	.036
3	Zz breeders	30	95.1	11.8	2 and 4	5.3	2.8	.005
4	ZzAa breeders	21	97.6	9.5	3 and 4	2.3	1.5	.134

* Statistical quantity used for 30 or more observations to calculate probability (P) that the differences could have resulted from chance alone.

(A) per cc serum.

Results and discussion. From the data in Table I it is apparent that within *A* and *Z* strains no significant difference was found between the inhibitor levels in the serums of mice with or without the milk agent, and with or without tumors. The differences in the incidence of cancer between the virgin *A* and *Z* strains appears to result from an "inherited hormonal factor" in the *Z* strain.[17] Bittner and Huseby[17] emphasized the possibility that the inherited susceptibility to mammary cancer may be the same in both strains, while other genes control the "hormonal factor." From the present data it would appear that there is a tendency toward an inverse relationship between the level of the hyaluronidase inhibitor in the serum and the presence of the "inherited hormonal factor". A direct relationship between the porphyrin level and the latter factor was indicated by the work of Bittner and Watson.[18]

Since the presence of milk agent or tumor had no appreciable effect on the inhibitor level in the mouse serum, the comparisons in Table II deal only with cancer incidence and the inhibitor. None of the data in Table I could be compared directly with those in Table II because different batches of enzyme and substrate were used to obtain the data for each table. Variations in the preparations do not justify direct comparisons of results obtained with different lots. A comparison of *A, Z,* and their hybrid strains reveals a tendency toward lower inhibitor levels in strains capable of higher cancer incidence as virgins and which bear the "inherited hormonal factor."

Significant differences in the inhibitor levels of Group 2 and 4, and 1 and 4 may be noted in Table II. These differences accompany, in inverse relation, the differences in cancer incidence. Within the *A* strain, large differences in the cancer incidence in breeders and virgins is not associated with a significant difference in the inhibitor level. Furthermore no important differences in the inhibitor are seen between *Aa* and *Zz* strains or the *Zz* and *ZzAa* strains.

Summary. 1. Within the *A, Z,* and AZF_1 strains of mice, no significant differences in the hyaluronidase-inhibitor levels in serum were found between individuals with and without the milk agent which determines the incidence of mammary cancer. Neither was a significant difference observed between virgins and breeders of the same strain or between individuals with and without tumors. 2. There appeared to be a tendency for the inhibitor titer to vary inversely with the strain incidence of mammary cancer in the *A, Z,* and AZF_1 mice. 3. A tendency toward an inverse relationship between the inhibitor level and the presence of the "inherited hormonal factor" follows.

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