

Observations on the Antiproteolytic Reaction of the Serum of Mice: Strain Variations*

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Variations in the antiproteolytic reaction of the serum have been observed in animals and in human patients. Variations among species have been recorded (1, 2) but within a given species there is little variation in the reaction of normal animals except for the guinea pig in which moderate differences have been noted. So far as we know, no previous studies have been made in mice probably because of their small size and blood volume. That this reaction is of some importance in the response of the animal or patient to disease or other stimuli has been recognized. The relationship of the antiproteolytic reaction of human serum to the presence of malignant tumors (3, 4), make it desirable to extend this study to mice because of the importance of pure strains of mice in cancer research. A preliminary study using blood drawn from the heart and testing the antiproteolytic titre by a method previously reported (4), indicated definite variations among different strains of mice. A modification of the method using small amounts of blood from the tail of the mouse was developed in order to be able to preserve the animal essentially unchanged for repeat examination and to determine the effect of tumors and other agents on the titre.

METHOD

The animal is gently placed and held in a small cage with the tail protruding, and a section just large enough to produce bleeding is cut from the tip of the tail. Blood is expressed in droplets by milking with gentle pressure applied over the tail veins beginning at the proximal end. The blood is collected in a capillary tube 10 to 14 cm. in length with a 2 mm. bore.¹ Approximately 0.08 to

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¹ Tubes available commercially as melting point capillary tubes.

0.1 cc. may be collected before clotting occurs. The end away from the column of blood is then flame-sealed so as not to heat the blood. Serum is obtained by centrifuging the capillary tube held by a cork in a test tube at 1500 r.p.m. for 20 minutes.

The capillary tube is snapped in two at the point of separation of serum and cells and the serum is aspirated into a pipette² calibrated to deliver a constant weight of serum, the equivalent volume of which has been determined (5). A pipette which delivers about 35 cu. mm. of serum is used. The serum is delivered at once from the calibrated pipette into a volume of normal saline sufficient to give a $\frac{1}{10}$ dilution. Three-tenth cc. aliquots of this dilution are taken for further dilutions, in increments of 10, to be used in determining the titre of the antiproteolytic reaction. Six such aliquots can be obtained from the amount of serum used, and the dilution must be made in a range in which an end point will be reached, which will vary with the strain tested. The dilutions are then treated as in the routine antiproteolytic test (4). Reconstituted pooled human plasma (PK) is used as a control for daily variations.

Duplicate runs on the same serum gave identical results nine out of ten times, and the maximum variation was one dilution. The titre obtained by this tail blood method was correlated with the titre obtained from heart blood, and the results by the two methods varied no more than one dilution (Table 1). Animals were then tested at intervals of one week and the titres were identical in 66 per cent of tests, with a maximum variation of two dilutions occurring in only 5 per cent of tests. Several animals showed minimal or no variations over a period of three months (Table 2).

RESULTS

Preliminary tests using the heart puncture method were made on over 200 mice of which 62 were normal males of the A, C3H, C57, F, BC, and JK strains.³ All animals were sacrificed and autopsied, and only those free of recognizable disease are included. These tests revealed definite strain differences with the BC and JK strains having high titres, the F's having low titres and the A, C3H and C57 being intermediate (Fig. 1).

² LinderStrom-Lange Pipette.

³ We are deeply grateful to Drs. L. C. Strong and W. U. Gardner who supplied us with large numbers of valuable animals.

Using the tail blood method, 184 animals of the A, C3H, C57, F, L, C₁₂I, JK, BC and the BL, LCS and BRS sublines of the NHO strain were tested. The results of the first tests on each group are outlined in Figure 2. Repeated tests confirmed the results. That the mice fall into these same three groups with high, intermediate, and low titres is apparent. The JK and BC strains and the NHO sublines are in the group with high titres, the A, C3H, C57 and C₁₂I are in the intermediate group and the F's have low titres. The results with the L strain are only preliminary since only one titre was done on each animal and the number of animals is very small. In every strain studied and also in hybrids the titre of the male is definitely higher than that of the female (Fig. 2, A and B).

DISCUSSION

The distinct differences in the serum antiproteolytic titre of mice of different strains are difficult to explain in view of the slight variations found in heterozygous species. In apparently normal dogs and rabbits, under conditions in this laboratory, the greatest variation in actual titres has been from 40 to 80, and over 80 per cent of the animals have titres of 50 or 60 (unpublished data). Guinea pigs on the other hand vary more, but there are no differences as great as that between the low 50 of the female F mouse and the high 140 of the male BRS mouse. These variations are another indication of the basic strain differences in mice. Similar variations in the serum coagulability, cellular components, and hemoglobin of the blood in several strains have also been reported and correlated with tumor susceptibility (6, 7, 8),

as have the strain differences in bio-electric properties (9).

It is tempting to correlate the tumor susceptibility or resistance of these strains of mice with variations in the antiproteolytic titre. A division into high titre (BC and JK strains, and sublines

TABLE 1
SERIES OF TESTS SHOWING CORRELATION BETWEEN TITRES OF HEART BLOOD AND TAIL BLOOD, WHICH REVEALS SIMILARITY OF RESULTS

Strain	Sex	Animal number	Titre: Heart blood	Titre: blood with pipette	Titre: Tail blood with pipette
JK	M	1	130	130	130
	M	2	130	120	120
	F	3	110	100	100
LCS	F	4	100	100	90
	M	5	120	110	120
	M	6	100	100	110
	F	7	80	80	80
PBR	F	8	90	90	100
	M	9	130	130	130
	M	10	130	130	130
C57	F	11	90	80	90
	F	12	90	90	90
	M	13	110	120	120
C3H	M	14	120	110	120
	F	15	90	90	100
	F	16	100	90	90
	M	17	90	90	100
A	M	18	90	100	100
	F	19	70	70	70
	F	20	80	70	80
BC	M	21	100	90	100
	M	22	90	90	100
	F	23	60	60	70
	F	24	70	70	70
BC	M	25	140	140	140
	M	26	130	130	130
	F	27	100	100	100
	F	28	90	90	100

TABLE 2

ANIMALS IN WHICH REPEATED TESTS BY TAIL BLOOD METHOD WERE DONE AT INTERVALS OF 1 WEEK OR MORE (ALL ANIMALS IN EACH GROUP ARE RECORDED; OTHER GROUPS GAVE SIMILAR RESULTS)

STRAIN	SEX	ANIMAL NUMBER	Original	1st week	TITRE: ACTUAL OVER % PK		3d month
					2d week	3d week	
BRS	male	1	120/240	130/260			
		2	110/220	110/220			
		3	110/220	110/220			
		4	130/260	130/260			
		5	140/280	140/280			
		6	110/220	110/220			
		7	110/220	110/220			
		8	100/200	120/240			
		9	110/220	110/220			
		10	130/260	110/220			
		11	100/200	100/200			
		12	110/220	120/240			
C57	male	1	100/183	90/180	90/180	90/180	other treatment
		2	100/183	110/220	110/220	110/200	90/180
		3	100/200	100/200	90/180	90/180	90/180
		34	100/200	100/200	100/200	100/200	other treatment
A	female	31	70/140	70/140	70/140	70/140	70/140
		34	70/140	70/140	70/140	70/140	60/120

BRS, B1 and LCS of the Strong NHO strain), intermediate titre (A, C3H, C57, and C12I) and low titre (F) strains can be made. It is of note that in general it is the intermediate group which is most susceptible to methylcholanthrene-induced⁴ and spontaneous tumors, while the high titre strains are more resistant to MC induced tumors and rarely develop spontaneous tumors except for

been found and indeed little is known about the basic nature or method of formation of this material. Since many of these strains of mice are being studied exhaustively from all points of view, they should be excellent animals for detailed study of this reaction. Further study of their response to various drugs, trauma, and tumor growth is in progress.

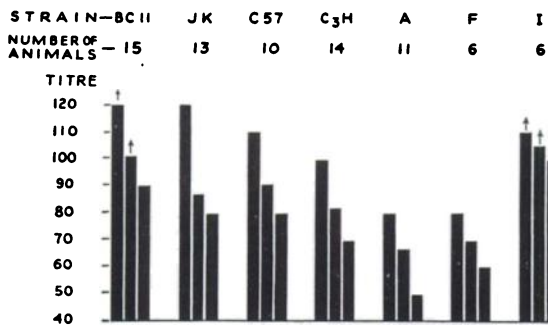


FIG. 1.—Columnar graphs indicating the highest, lowest, and mean titres in male mice of 7 strains. Blood obtained by heart puncture. Strain and number of animals in each group appear at top of figure.

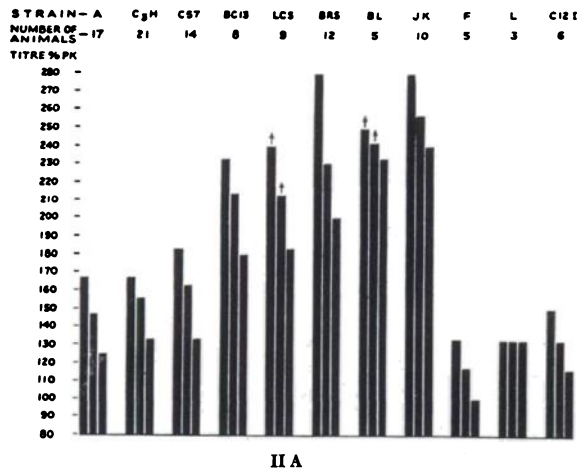


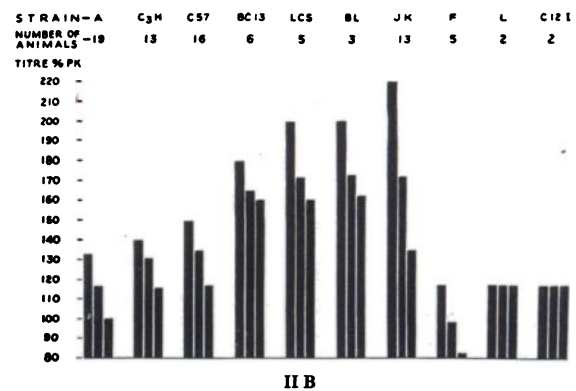
FIG. 2.—Columnar graphs indicating ratio of titre of male and female mice of various strains to plasma control titre (PK). Blood obtained from tail of mice. Strain and number of

SUMMARY

1. A method for determining the antiproteolytic activity of serum of mice is described.
2. Variations have been found in the antiproteolytic activity of the serum in several strains of mice.
3. The possible correlation between these variations and susceptibility to tumor formation is discussed.

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animals in each group appear at tops of figures. Figure 2A—males, Figure 2B—females.

the NHO strain. The F strain with a very low titre is the most resistant to methylcholanthrene-induced tumors. This strain (F) has a high incidence of leukemia (10) and is unusual in many respects, being difficult to breed, subject to many diseases and extremely sensitive to physical trauma.

No satisfactory explanation for the variations of antiproteolytic titre is different strains of mice has

⁴ Unpublished data of Dr. L. C. Strong.

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