INHERITANCE OF A SERUM PRE-ALBUMIN VARIANT IN THE MOUSE

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TWO genetically determined differences in serum pre-albumin components have been detected by starch gel electrophoresis. In swine, two electrophoretically different types have been observed (KRISTJANSSON 1963). In mice, the presence or absence of a pre-albumin component having esterase activity has been shown to be under genetic control (PETRAS 1963). The present paper documents another inherited difference in a pre-albumin component of mouse serum, unrelated to that reported by PETRAS. Starch gel electrophoresis reveals that this pre-albumin is present in the sera of some inbred strains of mice, but not of others.

MATERIALS AND METHODS

Animals: The following inbred strains were employed: A/HeJ, A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL/6J, C57BL/10J, DBA/1J, DBA/2J, RF/J, WB/Re, WC/Re.

Serum samples: Blood specimens were obtained from the ventral tail artery. Serum was used fresh, or after storage for up to three years at -20° . Stored sera showed no evident changes in the pre-albumin component.

Starch gel electrophoresis: A slight modification of SMITHIES' (1955) horizontal method was employed. The pH of the gel buffer was 9.0 instead of 8.6, and its molarity was decreased to approximately 75 percent of that recommended by the manufacturer (Connaught Medical Laboratories, Toronto) for the particular lot of hydrolyzed starch. After electrophoresis the gels were sliced horizontally and stained with Amido Black 10B, or, for special studies, with certain specific stains for enzymatic activities (LAWRENCE, MELNICK and WEIMER 1960), or binding properties (GIBLETT, HICKMAN and SMITHIES 1959; BLUMBERG and ROBBINS 1960).

RESULTS

Figure 1 shows the three pre-albumin phenotypes detected on starch gel electrophoresis with a protein stain. These are designated Pre-a, indicating the presence of the pre-albumin component in greatest concentration, Pre-o, indicating absence of any detectable protein at this position, and Pre-ao, indicating an intermediate concentration of the component. The concentrations of the pre-albumin component in sera from inbred and hybrid females are consistently lower than in sera from the corresponding males. The Pre-ao type of serum from the F_1 hybrid male is indistinguishable from that of a mixture of equal amounts of sera from males of the two parental strains. The Pre-ao type of the F_1 hybrid female serum is identical to that of a mixture of sera from females of the parental strains. These

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FIGURE 1.—Pre-albumin phenotypes. (1) Female C57BL/10J (Pre-o); (2) Female (C57BL/10J $\times$ DBA/2J) F_1 (Pre-ao); (3) Female DBA/2J (Pre-a); (4) Male C57BL/10J (Pre-o); (5) Male (C57BL/10J $\times$ DBA/2J) F_1 (Pre-o); (6) Male DBA/2J (Pre-a). The Pre-a band is indicated by an arrow where present.

three phenotypes, and only these three, can be discriminated in segregating generations. The phenotypes are usually discrete and repeatable; however, the distinction between Pre-a and Pre-ao is sometimes difficult, and occasionally the Pre-ao type may be misclassified as Pre-o, because the concentration of the Pre component is too weak to be detected. This is especially true in females. In the genetic studies to be presented, in any instance of doubt a new specimen was taken from the animal in question and tested.

Thirteen inbred strains were surveyed and classified as follows: Pre-a—AKR/J, BALB/cJ, CBA/J, DBA/1J, DBA/2J, RF/J and WB/Re; Pre-o—A/HeJ, A/J, C57BL/6J, C57BL/10J, C3H/HeJ and WC/Re.

The results of studies of the inheritance of this trait are presented in Table 1. The first five crosses of Table 1 are first and second generation crosses involving CBA/J (Pre-a) and C57BL/6J (Pre-o). No differences were detected between male and female F_1 progeny from reciprocal crosses, so a sex-linked trait could be ruled out. The F_1 hybrids were crossed *inter se* and reciprocally with both parental strains. Since no significant differences occurred between the reciprocal backcrosses of either type, these data have been pooled in Table 1. In each cross, only those phenotypes expected on the hypothesis that the trait is controlled by a pair of alleles at an autosomal locus were observed. Progeny from the several crosses

TABLE 1

Distribution of pre-albumin phenotypes among F₁ hybrids and second generation segregants

Cross	Sex	Number of type:			χ^2	P
		Pre-a	Pre-ao	Pre-o		
CBA/J $\times$ C57BL/6J (Pre-a $\times$ Pre-o)	Male	0	6	0	...	..
	Female	0	6	0	...	..
	Total	0	12	0	...	..
C57BL/6J $\times$ CBA/J (Pre-o $\times$ Pre-a)	Male	0	6	0	...	..
	Female	0	6	0	...	..
	Total	0	12	0	...	..
F ₁ $\times$ C57BL/6J* (Pre-ao $\times$ Pre-o)	Male	0	35	39	0.21	0.5
	Female	0	30	45	1.40	0.2
	Total	0	65	84	2.41	0.1
F ₁ $\times$ F ₁ * (Pre-ao $\times$ Pre-ao)	Male	20	28	20	1.96	0.3
	Female	9	40	21	4.83	0.1
	Total	29	68	41	2.11	0.3
F ₁ $\times$ CBA/J* (Pre-ao $\times$ Pre-a)	Male	15	17	0	0.13	0.7
	Female	12	28	0	6.40	0.01
	Total	27	45	0	4.50	0.04
Pre-ao $\times$ Pre-a*	Male	37	33	0	0.23	0.5
	Female	24	34	0	1.72	0.2
	Total	61	67	0	0.28	0.5

* Reciprocal crosses pooled.

were all tested at the same time, and were not identified as to cross until after classification. Consequently, the fact that no unpredicted classifications occurred in any cross is of some significance. However, particularly among females the segregation ratios are rather aberrant. Although the deviation from expected is significant only in the backcross to CBA/J, it is consistently in the same direction. This is presumed to be due to the low concentration of the Pre component among females, resulting in misclassification of some Pre-a individuals as Pre-ao, and some Pre-ao as Pre-o.

Further data on the inheritance of this trait were obtained from another cross produced by mating noninbred Pre-a animals to C57BL/6J (Pre-o), with the F₁ offspring then crossed to DBA/2J (Pre-a). In this study, the progeny were carefully tested and retested and compared with controls for each sex. These data, presented in the last row of Table 1, fit somewhat better with expectations, although an excess of Pre-ao females was again observed.

Table 2 contains data on the progeny of matings between selected F₂ and backcross offspring from the CBA/J $\times$ C57BL/6J crosses. These parental animals were repeatedly tested to establish with certainty the correct classification for each. In each of these progeny tests, all of the expected phenotypes and only the expected phenotypes were observed, confirming the typings of the 16 parental animals.

The data from these three sources are reasonably consistent with genetic control by a pair of alleles, which have been designated *Pre^a*, occurring in those

TABLE 2
Results of CBA/J × C57BL/10J progeny tests

Cross*	Number of parental pairs	Number of type:		
		Pre-a	Pre-ao	Pre-o
Pre-o × Pre-a	1	0	6	0
Pre-o × Pre-ao	2	0	12	9
Pre-ao × Pre-ao	3	7	18	3
Pre-ao × Pre-a	2	7	10	0

* Reciprocal crosses pooled.

inbred strains of the Pre-a phenotype, and *Pre^o*, occurring in the Pre-o strains, at an autosomal locus, *Pre*, denoting serum pre-albumin type.

Markers in a number of linkage groups were also segregating in the crosses studied. Most of the animals classified for pre-albumin type were also classified for the other traits. The data are summarized in Table 3. In all cases, males and

TABLE 3
Segregation of Pre relative to other loci

Cross*	Numbers of progeny				χ^2	P
	Diffuse Hb		Single Hb			
	Pre-ao	Pre-o	Pre-ao	Pre-o		
$\frac{Pre^o Hb^1}{Pre^a Hb^2} \times \frac{Pre^o Hb^1}{Pre^o Hb^1}$	16	21	14	19	0.01	0.9
	Trf-ab		Trf-b			
	Pre-ao	Pre-a	Pre-ao	Pre-a		
$\frac{Pre^o Trf^b}{Pre^a Trf^a} \times \frac{Pre^a Trf^a}{Pre^a Trf^a}$	24	21	24	24	0.10	0.7
	Intense		Dilute			
	Pre-ao	Pre-a	Pre-ao	Pre-a		
$\frac{Pre^o D}{Pre^a d} \times \frac{Pre^a d}{Pre^a d}$	29	25	19	20	0.23	0.6
	Agouti		Non-agouti			
	Pre-ao	Pre-a	Pre-ao	Pre-o		
$\frac{Pre^o a}{Pre^a A} \times \frac{Pre^o a}{Pre^o a}$	13	17	17	23	0.01	0.9
	Black		Brown			
	Pre-ao	Pre-a	Pre-ao	Pre-a		
$\frac{Pre^o B}{Pre^a b} \times \frac{Pre^a b}{Pre^a b}$	22	23	26	22	0.26	0.6
	Ss-HL		Ss-L			
	Pre-ao	Pre-a	Pre-ao	Pre-a		
$\frac{Pre^o Ss^h}{Pre^a Ss^l} \times \frac{Pre^a Ss^l}{Pre^a Ss^l}$	17	6	22	16	1.58	0.2
	Es Type I-II		Es Type I			
	Pre-ao	Pre-o	Pre-ao	Pre-o		
$\frac{Pre^o Es^1}{Pre^a Es^2} \times \frac{Pre^o Es^1}{Pre^o Es^1}$	18	17	22	22	0.02	0.8

* Gene symbols: *Hb*—Hemoglobin, *Trf*—Transferrin, *d*—dilution, *a*—agouti, *b*—brown, *Ss*—Serological serum protein variant, *Es*—Serum esterase.

females from reciprocal crosses were analyzed separately, but no differences were noted, so the data have been pooled to conserve space. The data indicate that the *Pre* locus is not closely linked to *Hb* (linkage group I), *Trf* (II) (SHREFFLER 1963), *a* (V), *b* (VIII), or *Ss* (IX) (SHREFFLER and OWEN 1963), or to the *Es* locus (POPP 1962) which has not yet been assigned to a linkage group.

Nothing positive is yet known about physiological function of the *Pre* component. It has no esterase, oxidase, acid or alkaline phosphatase or leucyl-amino-peptidase activity detectable by specific stains of the starch gels. The lack of esterase activity of the *Pre* component, as well as a difference in distribution among inbred strains, excludes any direct relationship between the *Pre* variant and the pre-albumin esterase variant reported by PETRAS (1963). Autoradiography revealed no iron-binding, tested by addition of $\text{Fe}^{59}\text{Cl}_3$ to the serum, and no thyroxine-binding, tested with I^{131} -thyroxine. The latter was tested because it has been shown that in some species a thyroxine-binding globulin is present in the serum which migrates as a pre-albumin on starch gel electrophoresis (BLUMBERG and ROBBINS 1960). The protein has no lipid content detectable by staining with Sudan Black. Because it is such a minor component, it has not thus far been possible to detect the component by two-dimensional starch gel-paper electrophoresis, or by immunoelectrophoresis.

DISCUSSION

Owing to the difficulties in classification, the evidence presented for genetic control by a pair of alleles at a single autosomal locus is not as conclusive as might be desired. The deviations in the data could reflect a selective advantage of the *Pre-ao* and perhaps the *Pre-o* types, but are probably due to a few misclassifications of female progeny. The results of the progeny tests indicate that dependable classification can be achieved, however.

The precise nature of the difference between the *Pre-a* and *Pre-o* types is unknown. The *Pre-o* serum may contain a homologous protein of lower mobility which is buried beneath the major pre-albumin, or the albumin, or it may completely lack the protein. The trait may not be particularly useful for genetic or biochemical studies with the present starch gel technique for detection and classification. A more sensitive method would be desirable. Attempts to characterize the protein further are continuing, and an effort to develop an immunoelectrophoretic method for classification is in progress.

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SUMMARY

A difference among inbred strains of mice in the presence or absence of a minor serum pre-albumin component has been detected by starch gel electrophoresis. Genetic studies indicate that the difference is determined by an autosomal locus, *Pre*, with a pair of alleles, *Pre*^a and *Pre*^o, controlling respectively the presence or apparent absence of the component. The *Pre* locus does not appear to be in linkage groups I, II, V, VIII, or IX.

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