ent tissues may reflect minor differences in the chemical groupings or conformation about the active centers of the enzymes; alternately, the properties of essentially identical enzymes may be differently influenced in different cell types by components of the particulate mitochondrial matrix with which the monoamine oxidases are associated. The differential destruction by heat of activity toward various substrates may appear to suggest the presence of separate enzymes for the different substrates, but the results could, alternately, mean that conformational or gross structural changes induced by heat denaturation have quantitatively different effects upon either combination or reactivity of the enzyme with different substrates. The present conclusions do not extend to and are not altered by the possible presence in the tissues of other enzymes with quantitatively minor amine oxidase-like activities.

Summary. Fractionation of rat liver homogenates suggests a dual intracellular localization of monoamine oxidase, with the major portion in the mitochondria and a smaller but significant portion sedimenting with the microsomal fraction. Comparative studies on substrate specificities and Michaelis constants of monoamine oxidase preparations from liver, kidney and heart of rat and guinea pig indicate marked tissue differences in monoamine oxidases. Multiple substrate studies and other data support the view that each tissue contains a single, distinctive major monoamine oxidase.

1. Blaschko, H., Richter, D., Schlossmann, H., *Biochem. J.,* 1937, v31, 2187.
2. Kohn, H. I., *ibid.,* 1937, v31, 1693.
3. Zeller, E. A., *Pharmacol. Rev.,* 1959, v11, 387.
4. Cotzias, G. C., Dole, V. P., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 157.
5. deDuve, C., Beaufay, H., Jacques, P., Rahman-Li, Y., Sellinger, O. Z., Wattiaux, R., de Coninck, S., *Biochim. Biophys. Acta,* 1960, v40, 186.
6. Hawkins, J., *Biochem., J.,* 1952, v50, 577.
7. Zile, M., Lardy, H. A., *Arch. Biochem. Biophys.,* 1959, v82, 411.
8. Weiner, N., *J. Neurochem.,* 1960, v6, 79.
9. Weissbach, H., Redfield, B. G., Udenfriend, S., *J. Biol. Chem.,* 1957, v229, 953.
10. Sarkar, S., Zeller, E. A., *Fed. Proc.,* 1961, v20, 238.
11. Weiner, N., *Arch. Biochem. Biophys.,* 1960, v91, 182.
12. Kobayaski, Y., Schayer, R. W., *ibid.,* 1955, v58, 181.
13. Hardegg, W., Heilbronn, E., *Biochim. Biophys. Acta,* 1961, v51, 553.
14. Werle, E., Röewer, F., *Biochem. Z.,* 1952, v322, 320.
15. deDuve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., Appelmans, F., *Biochem. J.,* 1955, v60, 604.
16. Strittmatter, C. F., Ball, E. G., *J. Cell. and Comp. Physiol.,* 1954, v43, 57.
17. Lowry, O., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.,* 1951, v193, 265.
18. deDuve, C., Wattiaux, R., Baudhuin, P., *Adv. in Enzymol.,* 1962, v24, 291.
19. Dixon, M., Webb, E. C., *Enzymes,* New York, Academic Press, 1958, 20.

Received September 3, 1963. P.S.E.B.M., 1963, v114.

Genetic Control of Albumin Phenotypes in Horses.* (28766)

CLYDE STORMONT AND YOSHIKO SUZUKI
Department of Veterinary Microbiology, School of Veterinary Medicine, University of California, Davis

Evidence that a minimum of 6 codominant autosomal alleles control transferrin phenotypes in horses has recently been reported from this laboratory(1). During further studies, using a starch-gel procedure recommended by Kristjansson(2), we have observed individual variation in the patterns of proteins which migrate in the albumin and post-albumin regions of the gels. Three

* Supported by grants from California Thoroughbred Breeders Assn., American Shetland Pony Club and Jockey Club.

phenotypes named A, AB and B have been observed. This report is concerned with a description of those phenotypes and presentation of data on their genetic control.

Materials and methods. Plasma samples (blood collected in a solution of 2% sodium citrate plus 0.5% NaCl) were obtained from known families of Thoroughbred horses and Shetland ponies.

The technique of starch-gel electrophoresis was the same as that described by Kristjansson(2) except that the amount of Connaught's starch used in preparing the gels ranged from 58 to 60 g depending upon the resolution obtained with different lots of starch. The interior dimensions of our gel-forms were 21.7 cm × 12 cm × 0.6 cm. The points of insertion of the filter-paper strips used for the loading of plasma samples was 7 cm from one end of the gel, and usually 10 samples were run per gel. The runs were stopped when the borate boundary had migrated 10.5 cm beyond the insert line. In all other respects, the procedure was precisely the same as that described by Kristjansson.

Results. Fig. 1 illustrates the appearance of the 3 albumin phenotypes. The principal diagnostic bands in each of the 3 phenotypes are shown in solid lines in the diagram in Fig. 1. The stippled lines represent extremely faint bands which were not always seen.

Phenotype A was characterized by a single thick, densely staining mass of material (zone 1 in the region labelled Alb. in Fig. 1) followed by 2 bands (positions 3 and 4 in the region labelled Post-Alb.) which usually but not invariably stained with the same intensity.

In phenotype AB each of the zones labelled 1 and 2 in the albumin region (Fig. 1) were occupied by a mass of densely staining material and these two masses were usually followed by 3 relatively thin bands (positions 3, 4 and 5 in Fig. 1) which stained with about equal intensity.

Phenotype B was characterized in part by a mass of densely staining material in zone 2 which seemed to blend gradually into less densely staining material in zone 1, as indicated by the cross-hatched lines in the diagram in Fig. 1. The material in zone 2 was followed by a thin band in zone 3 and a somewhat thicker and more densely staining band in zone 5. Occasionally a very thin band could be seen in zone 4, as indicated by the stippled line in the diagram of Fig. 1.

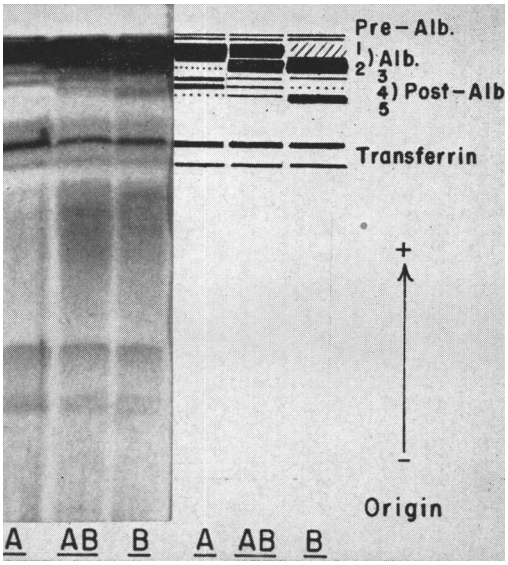

FIG. 1. A starch gel on 3 samples of plasma, all from horses of transferrin phenotype FF, which illustrates the resolution of the 3 albumin phenotypes A, AB and B. Zones 1 and 2 in the albumin (Alb.) region and zones 3, 4 and 5 in the post-albumin (Post-Alb.) region indicate the positions of the diagnostic bands. Stippled lines indicate extremely faint bands which may or may not be seen on individual gels. Cross-hatched lines in zone 1 in phenotype B represent diffuse but rather densely staining material which often obscures the outlines of the thick band in zone 2.

With some experience in reading the gels, rapid diagnosis of the 3 phenotypes could be made by examining either the albumin region (zones 1 and 2) or the post-albumin region (zones 3, 4 and 5). In effect, diagnosis of the phenotypes in one region served as an independent check on the readings in the other.

Data on the inheritance of the 3 phenotypes are summarized in Table I. The observed numbers of offspring from the 6 kinds of matings are consistent with the interpretation that the 3 phenotypes are controlled by a pair of codominant, autosomal alleles, designated here as a^A and a^B. Accordingly, the phenotypes A, AB and B represent, respectively, the genotypes a^Aa^A, a^Aa^B and

a^Ba^B. In the mating A × AB (Table I), there was one offspring of phenotype B not expected on the basis of the genetic theory. Since the possibility of misclassification of the albumin phenotypes of that offspring and its putative parents was excluded, this unexpected type stands either as evidence for illegitimacy, evidence for a mutation or as evidence requiring modification of the theory of two alleles. Although there was no evidence from the transferrin types and blood groups which would confirm the possibility of illegitimacy, we are, nevertheless, inclined to regard the exception as evidence for illegitimacy.

A gene-frequency analysis, based on the distribution of the phenotypes in parental animals in 2 breeds of horses, is shown in Table II. As may be noted, the observed distribution of the 3 phenotypes in each breed did not differ significantly from the expected distributions, thereby lending further support to the intrepretation based on 2 alleles. The frequencies of the alleles in each breed are shown in the footnote to Table II. The two breeds differ significantly with respect to those frequencies.

Data showing that the 2 albumin alleles of horses segregate independently of the alleles controlling transferrins and also of those controlling blood groups in 7 of 8 blood-group systems will be published later.

Discussion. Based on the present evidence for 2 codominant albumin alleles, and on evidence for 6 codominant transferrin alleles published elsewhere(1), the number of serum or plasma phenotypes now demonstrable in horses is 63. But the limit of variability in the albumin and transferrin systems of horses is probably not yet established. Refinements in techniques are almost certain to show subdivisions within the present categories. Then, too, Ashton(3) has observed phenotypic variation in the prealbumins and 2 apparently unrelated systems of alpha-globulins of horses, and these systems are yet to be explored genetically.

TABLE I. Inheritance of Albumin Phenotypes.

Kinds of matings	No. of offspring of phenotypes A	AB	B	P of X^2
A × A	1	0	0	
A × B	0	16	0	
A × AB	9	10	1*	>.90
B × B	0	0	28	
B × AB	0	28	13	>.01
AB × AB	7	23	8	>.20

* Not expected on the basis of the genetic theory.

TABLE II. Gene-Frequency Analysis of Distribution of 3 Albumin Phenotypes in 2 Breeds of Horses.

Breeds*	No. of animals of phenotypes A	AB	B	P of X^2
Obs Thoroughbreds	3	21	39	
Exp "	3	21	39	1.00
Obs Shetlands	22	48	49	
Exp "	18	56	45	>.05

* Frequencies of the two alleles a^A and a^B, as computed from the observed (Obs) phenotypes are, respectively, 0.214 and 0.786 in Thoroughbreds and 0.387 and 0.613 in Shetlands.

The present observations, along with those on transferrins and blood groups in horses, have immediate practical application in the solution of problems of questionable parentage.

Summary. By means of a starch-gel technique suggested by Kristjansson, 3 albumin phenotypes A, AB and B are demonstrable in the serum of horses. As indicated by data on the inheritance of these phenotypes and by a gene-frequency analysis of the distribution of the phenotypes in 2 breeds of horses, the results are consistent with the interpretation that the 3 phenotypes are controlled by a pair of codominant, autosomal alleles. These 3 phenotypes can be diagnosed by examining either the albumin region or the post-albumin region of the gels. Hence, diagnosis in one region serves as an independent check on the other.

We are indebted to Dr. F. K. Kristjansson for calling our attention to the particular method of starch-gel electrophoresis which led to the resolution of the 3 albumin phenotypes.

1. Braend, M., Stormont, C., *Nord. Vet. Med.*, in press.
2. Kristjansson, F. K., *Genetics*, 1963, v48, 1059.
3. Ashton, G. C., *Nature*, 1958, v182, 1029.

Received September 3, 1963. P.S.E.B.M., 1963, v114.