

to obtain generalization gradients. Some degree of abstraction from specific stimulus values is implied by the use of a relationship between stimuli to produce the dimension.

The shift in preference was not accompanied by a decrement in total response rate to stimulus differences intermediate to, or greater than, those used in training. This differs from parallel studies where cues for a left-right discrimination were specific spectral values displayed identically on both keys (6). In such cases, the presentation of intermediate spectral values in testing was accompanied by a marked decline in total response rate as well as by an appropriate shift in preference. Such test values were not presented during training. In our case, the specific spectral values used in training and testing were of course the same, and all values were presented equally often in training. The absolute response rate therefore appears to be governed by the specific values and not by the relationship between them; only preference is governed by the dimension used as the basis for the trained discrimination.

The use of a derived dimension in this work suggests that the technique can be applied to the investigation of transfer, abstraction, and psychological scaling in animals. While it is meaningless to ask whether a discrimination between specific stimulus values will transfer from one stimulus dimension to another, it is quite reasonable to ask the same question of a discrimination based on stimulus differences. The investigation of such transfer between sets of values, either on a given dimension or on different dimensions, would provide a basis for work on the scaling of similarity in animals (7).

WERNER K. HONIG

ROBERTA W. DAY

Department of Psychology,  
Denison University, Granville, Ohio

#### References and Notes

1. N. Pastore, *Psychol. Repts.* 1, 307 (1955).
2. D. Blough, *J. Exptl. Analysis Behav.* 2, 151 (1959).
3. J. Robinson, *J. Comp. Physiol. Psychol.* 48, 195 (1955).
4. G. Ekman, in *Sensory Communication*, W. A. Rosenblith, Ed. (Technology Press, Boston, and Wiley, New York, 1961), p. 35.
5. For a more detailed description of the apparatus, see W. Honig, *J. Exptl. Psychol.*, in press.
6. W. Honig and J. Shaw, paper read at meetings of the Eastern Psychol. Assoc., April 1962.
7. This research was supported by grant M-2414 from the U.S. Public Health Service.

18 July 1962

5 OCTOBER 1962

## New Genetically Determined Molecular Form of Erythrocyte Esterase in Man

**Abstract.** An altered molecular form of erythrocyte esterase was discovered in hemolysates from two males and one female in three generations of the same family. This indicates that the alternate enzyme form is under the control of a single autosomal gene. Evidence suggests that the atypical esterase is a variant form of erythrocyte carbonic anhydrase.

The enzymes of human erythrocytes which hydrolyze synthetic aromatic esters consist of five basic types currently designated as:  $A_1$ ,  $A_2$ ,  $B$ ,  $C$ , and  $D$  esterases (1). When demonstrated by starch gel electrophoresis with dye-coupling procedures (1, 2), all of these esterases with the exception of the  $B$  esterase are present in two or more electrophoretically distinct forms (isozymes). The characteristic esterase pattern is quite constant among individuals, although some acquired qualitative and quantitative alterations have

been observed (2, 3). Additionally, a genetically determined variation in the  $A_1$  group of isozymes has been described which results in what appears to be an alteration of each of the four  $A_1$  forms (1).

In a survey of erythrocyte esterase patterns in a population of mentally retarded children, an unusual variant was found consisting of a prominent band which migrates cathodally at pH 8.7 (Fig. 1). The subject was a 4-year-old male mongoloid with 47 chromosomes (trisomy-21 karyotype). The same variant was subsequently found in the father and paternal grandmother, establishing that it is under the control of a single autosomal gene. The grandmother is of English descent. Her karyotype was examined and found normal. This variant was not seen in 16 other mongols, and is thus not associated with mongolism. On the basis of evidence cited below, the new enzyme form appears to be an alteration of one of the  $D$  esterases.

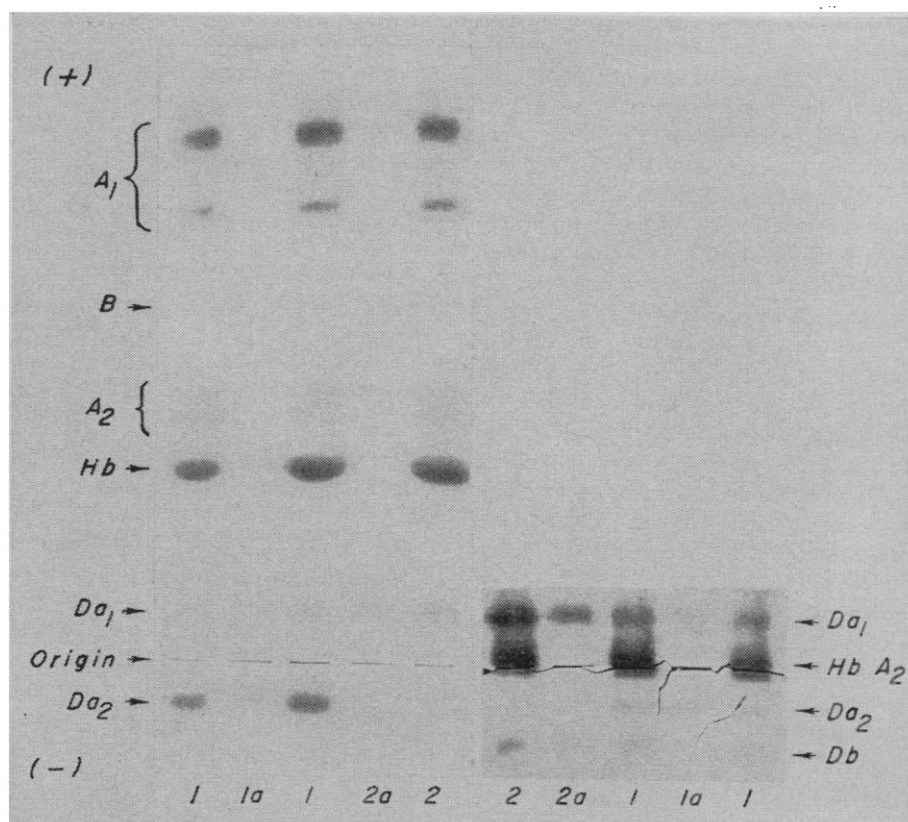


Fig. 1. (Left) Normal and variant erythrocyte esterase patterns after vertical electrophoresis (9 volt/cm) in starch gel (borate buffer, pH 8.7) for 18 hours at 3° to 5°C. Incubation with  $\alpha$ -naphthyl acetate as substrate and Blue RR salt as dye-coupler.  $Db$  esterase not visible here. (Right) Protein stain (nigrosin) of other half of gel shown at left. Abscissa: 1, Variant pattern (paternal grandmother of propositus). 2, Normal pattern (paternal grandfather of propositus). 1a and 2a, Variant and normal  $D$  esterase patterns, respectively, after ethanolchloroform extraction and purification on diethylaminoethanol cellulose (0.001M phosphate buffer, pH 7.0).

While the physiological roles of the red cell esterases are not established, the *D* isozymes, including the new variant, may represent different molecular forms of erythrocyte carbonic anhydrase. This assumption is based on the demonstration of a number of chemical properties common to both carbonic anhydrase and to the isolated *D* forms (4). These properties include: (i) complete selective inhibition by  $10^{-5}M$  acetazolamide, (ii) presence of  $Zn^{++}$  (dithione test), and (iii) dehydration of  $H_2CO_3$  (manometric measurement). The *D* esterases also differ from the other erythrocyte esterases in exhibiting greater activity toward  $\beta$ - than  $\alpha$ -naphthyl acetate, and in their demonstration by a protein stain (nigrosin).

The variant band (*Da<sub>2</sub>*) is accompanied by a decrease in both enzyme activity and protein stain intensity of the *Da<sub>1</sub>* band (Fig. 1). These findings suggest that the variant *Da<sub>2</sub>* and the normal *Da<sub>1</sub>* are allelic products. A comparison of chemical responses between *Da<sub>1</sub>* and *Da<sub>2</sub>* shows them to be essentially similar, with the exception of two marked differences: (i) the enzyme activity of *Da<sub>2</sub>* toward both  $\alpha$ - and  $\beta$ -naphthyl acetate is greatly increased even though the protein stain associated with *Da<sub>1</sub>* appears equal in intensity to *Da<sub>2</sub>*, and (ii) *Da<sub>2</sub>* is almost completely inactivated by 0.5*M* iodoacetamide whereas *Da<sub>1</sub>* is only slightly inhibited at this concentration. A more detailed chemical characterization of the *D* esterases is in preparation.

Whether the esterase variant described here imparts any selective advantage or disadvantage is not apparent; the two adult subjects are grossly normal and fertile. It is possible that the variant enzyme would show an altered response to some unusual environmental stress, as in the case of a genetically determined, atypical serum cholinesterase which inefficiently inactivates the drug succinylcholine (5). This atypical cholinesterase and the red cell esterase variant both represent alternate enzyme forms which retain activity but show changes in specificity.

Knowledge as to the frequency of alternate but active enzyme forms must await study of other enzymes and large populations. However, it seems likely that the phenomenon may be fairly general, that it may provide the basis for much of intraspecies variation, and that it may operate as a mechanism of "forward" mutation in the evolution of dip-

loid organisms. Its occurrence would represent a unique advantage over the haploid state, permitting the organism to "experiment" with new enzyme forms while retaining function of the normal allele (6).

CHARLES R. SHAW

FRANK N. SYNER

Hawthorn Center, Northville, Michigan

RICHARD E. TASHIAN

Department of Human Genetics,  
University of Michigan Medical  
School, Ann Arbor

#### References and Notes

1. R. E. Tashian and M. W. Shaw, *Am. J. Human Genetics*, in press.
2. R. E. Tashian, *Proc. Soc. Exptl. Biol. Med.* **108**, 364 (1961).
3. Z. Hart, C. R. Shaw, F. N. Syner, in preparation.
4. R. E. Tashian, unpublished data.
5. W. Kalow, in *Biochemistry of Human Genetics*, G. E. W. Wolstenholme and C. M. O'Connor, Eds. (Churchill, London, 1959), pp. 39-56.
6. We wish to thank Dr. Zwi Hart of the Plymouth State Home and Training School for obtaining blood samples from the propositus. We are also indebted to Dr. Margery W. Shaw for karyotype analysis, and to Lucille Setter and Dorothy P. Douglas for technical assistance. This study was supported in part by a grant from the Scottish Rite Foundation for Research in Schizophrenia and a grant (RG-6892) from the U.S. Public Health Service.

16 April 1962

### Detection of Antibodies in Blood Meals of Hematophagous Diptera

**Abstract.** Antibodies are detectable by an agglutination technique in the blood meals of hematophagous Diptera that are fed on laboratory and domestic animals infected with organisms of the *Trypanosoma brucei* subgroup. It is likely that both the antibody status and the identity of the animal fed on can be determined from the same blood meal from wild Diptera. The technique may be applicable to other infections for which sensitive serological tests are available.

Identification of the vertebrate hosts of hematophagous Diptera by serological tests on the blood meal is a widely used technique (1). As far as is known, however, no attempts have been made to examine the blood samples obtained from such Diptera for antibodies circulating in the host's blood at the time of feeding.

In order to determine whether, and for what period after feeding, agglutinins against a strain of the *Trypanosoma brucei* subgroup are detectable in the blood meals of *Glossina*, 107 two-day-old *Glossina morsitans* Westw., hatched in the laboratory from puparia

collected near Singida, Tanganyika (lat. 4°49'S, long. 34°45'E), were fed on a rat which had a serum agglutinin titer of  $10^{-4.9}$  against a strain of the *T. brucei* subgroup. Groups of flies, maintained singly in gauze-ended 3- by 1-inch glass tubes at about 24°C, were killed at intervals after feeding, and the abdominal contents were smeared on filter paper. The smears were dried and stored in a desiccator over  $CaCl_2$  at 25°C and tested for the presence of agglutinins after 1 day and after 25 days. Groups of smears were extracted in physiological saline (0.2 ml per smear) at 0°C for 4 hours. The extract was filtered by centrifugation in a Hemming filter with Whatman No. 1 filter paper and, since *G. morsitans* take approximately 20 mg of blood at their first feed, the serum dilution of the extract from each group of smears was considered to be  $10^{-1}$ . Serial twofold dilutions of the extract were made, and the agglutination test was carried out (2). The results showed that antibodies against *T. brucei* subgroup organisms were detectable in the blood meals of laboratory-maintained *G. morsitans* for at least 96 hours after feeding, and that the agglutinin titer was undiminished up to 24 hours after feeding. Furthermore, no diminution in titer took place when the blood smears were stored in the desiccator for 25 days. No agglutinins were found in extracts from smears from flies fed on a control rat.

To test the accuracy and sensitivity of the technique, the following experiment was carried out. Two 2-day-old *G. morsitans* were fed, one on a cow with a serum agglutinin titer of  $10^{-4.6}$  against a strain of the *T. brucei* subgroup, and one on a control cow without serum agglutinins to that strain. After 24 hours at 24°C the flies were killed, and their abdominal contents were smeared onto filter paper. After 1 hour in the desiccator, each smear was cut into 50 pieces which were placed on separate 3- by 1-inch glass slides and, after addition of 0.05 ml of physiological saline, the extracts were randomly distributed in a series 1 to 100. After the extracts had stood for 10 minutes at room temperature they were tested for agglutinins by an operator unacquainted with their individual identity. Within 30 minutes each extract was correctly identified.

To determine whether the method was applicable to blood meals of other species of Diptera, the following were