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^{++} (dithizone 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 β- than α-naphthyl acetate, and in their demonstration by a protein stain (nigrosin).

The variant band (Da_2) is accompanied by a decrease in both enzyme activity and protein stain intensity of the Da_1 band (Fig. 1). These findings suggest that the variant Da_2 and the normal Da_1 are allelic products. A comparison of chemical responses between Da_1 and Da_2 shows them to be essentially similar, with the exception of two marked differences: (i) the enzyme activity of Da_2 toward both α- and β-naphthyl acetate is greatly increased even though the protein stain associated with Da_1 appears equal in intensity to Da_2, and (ii) Da_2 is almost completely inactivated by $0.5M$ iodoacetamide whereas Da_1 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 diploid 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*).

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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

fed on a cow which had a serum agglutinin titer of $10^{-4.6}$ against a strain of the *T. brucei* subgroup: *Anopheles (Anopheles) implexus* Theo., *Mansonia (Coquillettidia) fuscopennata* Theo., *Aedes (Neomelanoconion)* sp., *Eretmapodites* sp., *Culex (Culex) annulioris* Theo. (Culicidae), *Tabanus taeniola* P. de Beauv., *Chrysops distinctipennis* Aust. (Tabanidae), *Stomoxys* (?) *nigra* Macq., *S.* (?) *calcitrans* L. (Muscidae). Twenty-four hours after the feedings, the abdominal contents were smeared onto separate pieces of filter paper which were dried and stored in a $CaCl_2$ desiccator at 25°C. Each blood smear was cut out of the filter paper and divided into two pieces. One piece from each smear was placed in 0.1 ml of physiological saline on a glass slide. The saline was rinsed through the smear, by use of a small Pasteur pipette, for approximately 30 seconds. Each extract was tested without further dilution, and in every case strong agglutination was observed.

In these experiments agglutinating antibodies against trypanosomes were readily detected in the stomach contents of hematophagous Diptera which had fed upon infected animals. It is evident that the collection of blood meals from such Diptera in the field offers a method of obtaining blood samples from the wild animals on which they feed and that the samples may be tested for the presence of antibodies against any parasitic organism for which a sensitive serological test is available. At least for the larger hematophagous Diptera it is probable that the identification of the host animal (*1*) and the detection of antibodies in that host can be made from the same blood meal. This technique therefore appears to provide a means for determining the presence of antibodies in populations of wild animals with minimum disturbance of the environment.

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Nictitating Membrane: Classical Conditioning and Extinction in the Albino Rabbit

Abstract. The distribution of response latencies and the percentage performance curve of a classical conditioning group, by comparison with a control group, indicated that the extension of the nictitating membrane elicited by a puff of air to the cornea was successfully conditioned to a previously neutral stimulus.

The nictitating membrane (plica semilunaris) in the albino rabbit consists of a curved plate of cartilage covered with glandular epithelium. It is drawn from the inner canthus of the eye laterally across the cornea by a sheet of smooth muscle, but the mechanism of its action is not clearly understood (*1*).

Though there have been no reported attempts to condition this membrane in the rabbit, incidental observations in our laboratory have indicated that an extension of the membrane is reliably elicited by a puff of air to the cornea. We have observed that when the membrane is activated it rarely extends past the midline of the pupil and always leaves a portion of the cornea exposed, and that even highly conditioned rabbits do not appear to be capable of a sustained extension of the membrane. These two properties would appear to provide the experimenter with an even greater degree of control over the sensory consequences of the unconditioned stimulus than that existing in our previously reported study of conditioning of the rabbits outer lid (*2*).

The conditioning apparatus and manner in which the rabbit was restrained within a Plexiglas box has been described (*2, 3*). To permit the recording of movement of the membrane and to insure continual exposure of the cornea, the upper and lower eyelids of the rabbit's right eye were taped open. When the restraining box was positioned in a nonactivated refrigeration unit, a 6-inch speaker and a rod supporting an air jet and gravity-return potentiometer were positioned about 5 inches in front of the rabbit. A silk thread was attached to a rod which was mechanically coupled to the shaft of the potentiometer. A small metal hook connected to the other end of the silk thread was attached to a nylon loop which was sutured in the nictitating membrane of the rabbit's right eye. The signal from the potentiometer was amplified and graphically recorded. The orifice of the air jet was adjusted to deliver an 80-mm puff of compressed nitrogen of 100 msec duration from a position about ½ inch from the dorsal region of the right cornea. The conditioned stimulus (CS)

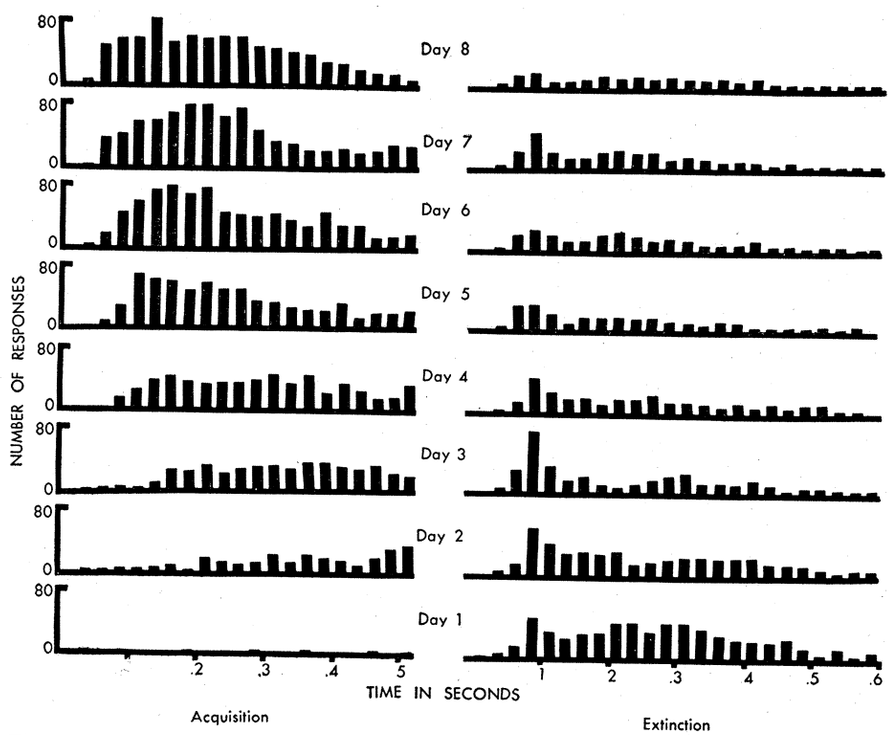

Fig. 1. Latency distributions of all membrane responses of group E in acquisition and extinction.