a fly larva which is about to pupate fails to bring about tanning when injected into the thorax of a newly emerged fly with the head ligatured off. It is well known that larval blood at this stage is loaded with ecdyson. (ii) In a converse experiment, blood from a 10- to 20-minute-old fly, injected into the ligatured hind part of fly larva where the anterior part alone had pupated within 24 hours after ligaturing, fails to cause tanning. This preparation is commonly used in the "*Calliphora* test" for ecdyson (*2*) where tanning of the hind part demonstrates the presence of ecdyson. This experiment rules out the possibility that the larva does not contain sufficient ecdyson for adult tanning, since in that event adult blood would have caused tanning in the larva. (iii) A highly concentrated preparation of ecdyson (obtained from P. Karlson-Munich) containing 100 *Calliphora* units per milligram was injected in doses of 5 and 15 *Calliphora* units per fly and proved entirely inactive. These experiments, therefore, definitely rule out a decisive role for ecdyson in the tanning of the adult.

The active factor in blood proved entirely unspecific. Active blood from one fly caused tanning in the two other species, and vice versa, in all combinations, while the same did not happen with inactive blood. Blood from freshly molted nymphs or adults of the cockroach, *Periplaneta americana*, but not from fully darkened ones, induced tanning in the fly. Similarly, blood from newly emerged adults of the beetle, *Tenebrio molitor*, was active, but not blood from fully tanned larvae or adults.

Since ecdyson has been excluded as the active factor in adult fly blood which induces tanning in head-ligatured flies, what are the remaining possibilities? It would theoretically seem that there are three alternatives, namely, a hormone, or an enzyme (for example, a polyphenol oxidase), or a substrate necessary for tanning (for example, a particular polyphenol). No extensive isolation studies have yet been performed with this material, but some insight into the nature of the active material was obtained in the following way.

Active blood from about 30 flies was pooled under mineral oil. The activity was the same before and after this material was centrifuged. A drop of such blood, placed on filter paper, darkened within about 10 minutes. Darkening was somewhat more intense and faster upon addition of *l*-dihydroxyphenylalanine solution. When active blood was heated at 80° or 100°C for 5 minutes and the ensuing precipitate was removed by filtration, the supernatant still retained its activity, but no longer darkened on filter paper, not even upon addition of *l*-dihydroxyphenylalanine. Active blood could be diluted 20 to 30 times without losing its activity. The experiments suggest that the active factor is not a polyphenol oxidase, and that it is not likely to be a simple substrate. The chances therefore are that we are dealing with a hormone. Since this can be neither ecdyson, nor the prothoracotrope hormone of the brain, nor a corpus allatum hormone (since tanning can occur in the absence of the corpus allatum), we may be dealing with a new type of insect hormone.

All the work so far discussed was based on the premise that tanning in the fly is the result of some *activating* mechanism. There still remains the possibility that a delay or failure in the tanning of the newly emerged fly is not so much due to a lack of activation as to the presence of an *inhibitory* mechanism. The following observations indeed suggest such a mechanism. When ligatures are placed between head and thorax, and thorax and abdomen, of a newly emerged fly, the isolated thorax never tans, while tanning occurs in the isolated abdomen in an appreciable number of cases. An injury to an isolated untanned abdomen, even as small as the prick of a pin, frequently causes it to tan, while no such effect can be produced in the isolated thorax. This would suggest that an inhibitory mechanism located in the thorax prevents immediate tanning in the newly emerged fly, and that normal tanning is initiated by removal of this inhibition. If this were true, it should be possible to initiate tanning in the head-ligatured fly by removing the source of this inhibition. All attempts to localize such a tissue have so far failed (*5*).

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Discrimination and Generalization on a Dimension of Stimulus Difference

Abstract. Four pigeons were trained to peck at one key when two identical stimuli were displayed, and at another key when the stimuli were displayed with a wavelength difference of 40 mμ. With stimulus combinations ranging in difference from 0 to 70 mμ in a generalization test, key preference was a function of the degree of difference between the stimuli.

The study of stimulus generalization has usually been confined to specific physical dimensions, such as intensity, size, wavelength, auditory frequency, or position of a tactile or visual stimulus. Generalization along derived dimensions defined by the relationship between stimuli has not been studied in animals. One such relationship is the degree of difference between stimuli. It is clear that animals can discriminate on the basis of identity versus difference of stimuli in solving the oddity problem (*1*) and matching to sample (*2*); the transfer of such a discrimination has been reported for chimpanzees by Robinson (*3*). But a quantitive analysis of this relationship as a stimulus dimension has not been undertaken, even though Ekman (*4*) and others have developed methods for psychological similarity scaling with human subjects.

In our study, monochromatic stimuli illuminating two response keys in an operant behavior apparatus were used to establish the difference dimension (*5*). Four pigeons were trained with the following problem: When the wavelength difference was zero, one key was correct (the "identity key"); when the difference was 40 mμ, the other key was correct (the "difference key"). Sixteen training combinations were used with eight stimulus values ranging from 500 to 570 mμ in 10-mμ steps. For the eight "identity" pairs, one of these eight wavelengths was identically displayed on the two keys. For the eight "difference" pairs, appropriate combinations were used; thus, 500 and 540 mμ were presented, respectively, on the left and right keys, or the reverse; 510 and 550 mμ were presented together, and so forth. For two subjects, the left key was the identity key and the right key was the difference key; this system was reversed for the other two subjects. The stimulus values were produced by projecting collimated white light through Bausch & Lomb interference filters.

The sixteen training conditions were presented in randomized order three or

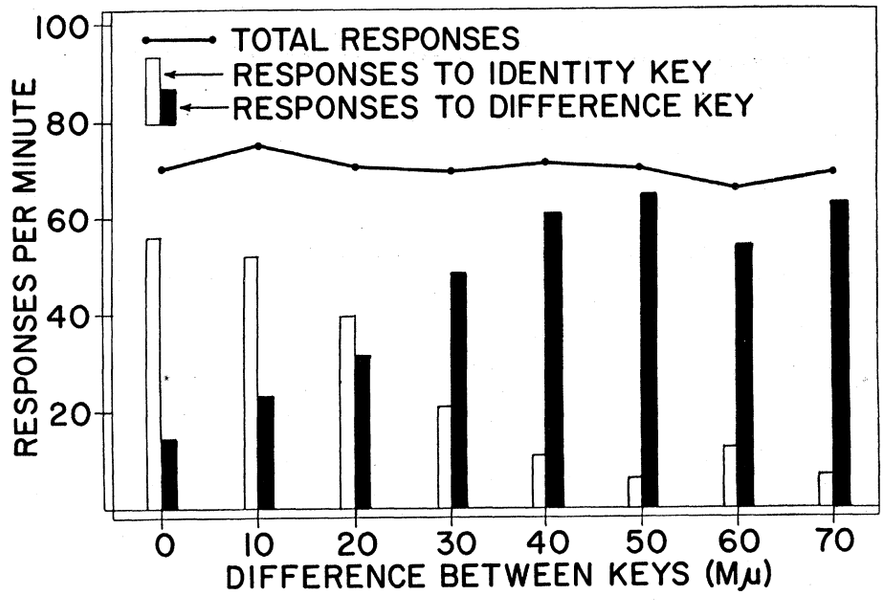

Fig. 1. Results from generalization test in terms of the mean response rates to the identity key and to the difference key; all the stimulus combinations representing a given degree of stimulus difference were combined.

four times each during training sessions. Each trial ended with a reinforcement consisting of presentation of grain for 4 seconds. At the beginning of training, each correct response was reinforced (unless it followed an incorrect response by less than 4 seconds), but after 12 sessions a variable interval reinforcement schedule was introduced. The terminal schedule used in training provided a mean trial period of 37.5 seconds. A 15-second blackout period followed each trial, thus enabling us to record the responses and change the stimuli.

After 32 sessions, all animals were responding between 70 percent and 90 percent correctly on both identity and difference discriminations. Two sessions of generalization testing were then given. All test trials lasted 30 seconds. Following a "warm-up" block of 16 training trials for each session, test trials were randomly introduced in two further blocks of training trials. Each possible stimulus pair drawn from the 500- to 570-mμ range was presented in order to provide a variety of stimulus differences ranging from 0 to 70 mμ. Each degree of difference was presented at least eight times, thus necessitating the repetition of some of the combinations with large differences. Reinforcements were not available during test trials but were maintained during training trials.

The results from the generalization test are presented in Fig. 1 in terms of the mean response rates to the identity key and to the difference key; all the stimulus combinations representing a given degree of stimulus difference were combined. Results from the four individual subjects are shown in Fig. 2 in terms of the percentage of responses to the identity key. From the group results it is clear that while the total response rate to the two keys is almost equal for all the different degrees of stimulus difference, there is a marked, orderly, and appropriate shift between 0 and 40 mμ difference in the distribution of responses. Each subject responded similarly, both for the shift in key preference and for the transfer of the difference discrimination from the 40-mμ difference to 50-, 60-, and 70-mμ differences. In fact, the difference key was chosen by all birds more often with these combinations than with a 40-mμ difference, except for one inversion at 60-mμ difference by subject No. 82.

Reasonably accurate performance during training indicates the achievement of a discrimination based on stimulus differences. Since the same spectral values were used for the identity and difference pairs, and only the combinations of the values differed, this was the only available cue. The test results show, furthermore, that the learning was not restricted to the 16 specific configurations employed in training. New combinations with 50-, 60-, and 70-mμ differences show complete transfer from the 40-mμ difference. The close correspondence between different degrees of stimulus difference and key preference indicates that this continuum can function as a stimulus dimension in a manner similar to the physical continua usually used

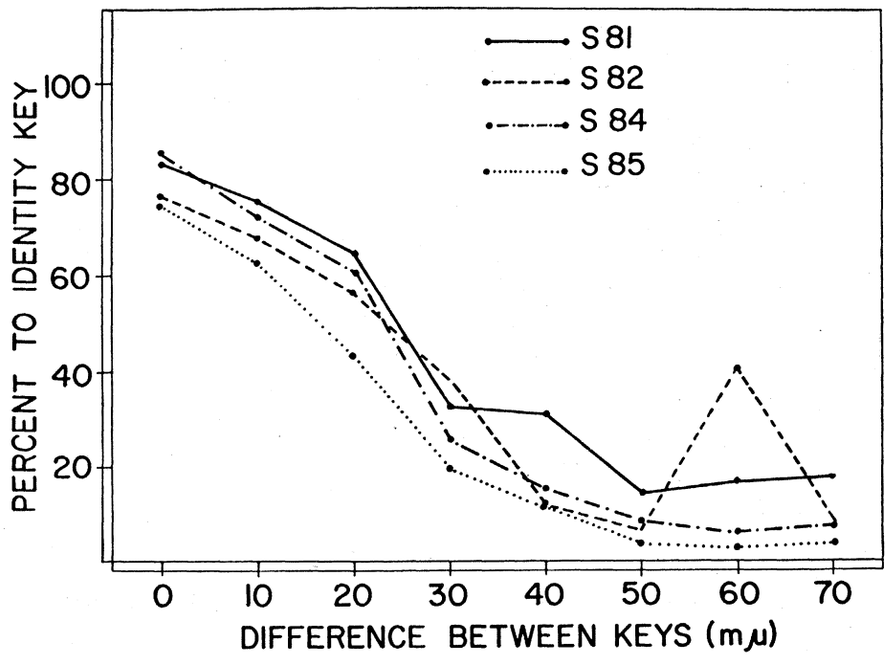

Fig. 2. Results from the four individual subjects in terms of the percentage of responses to the identity key.

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*).

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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 *p*H 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.

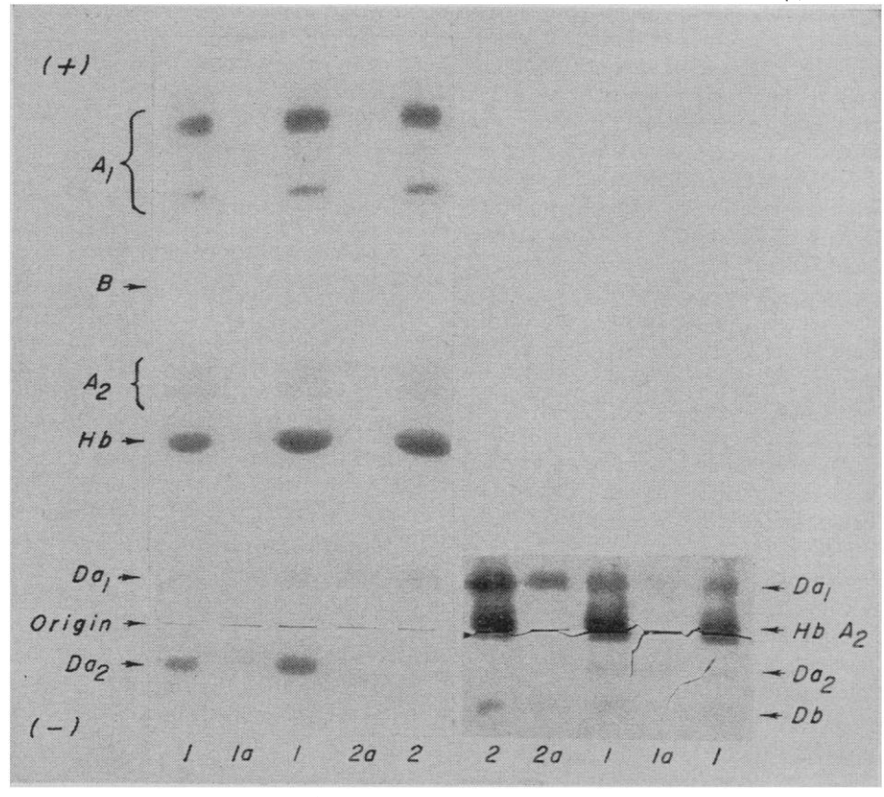

Fig. 1. (Left) Normal and variant erythrocyte esterase patterns after vertical electrophoresis (9 volt/cm) in starch gel (borate buffer, *p*H 8.7) for 18 hours at 3° to 5°C. Incubation with α-naphthyl acetate as substrate and Blue RR salt as dye-coupler. *Db* esterase not visible here. (Right) Protein 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 (0.001*M* phosphate buffer, *p*H 7.0).