response from 0 to 200 key/sec, Tectronix 122 amplifier with a frequency response of from 1.6 cy to 40 kcy/sec, and a Quan-Tec 210 preamplifier with a frequency response up to 100 kcy/sec. The hydrophone was a highly directional barium titanate unit designed by the U.S. Navy Electronics Laboratory at Point Loma, Calif. It had a resonant frequency of 125 kcy/sec and a nearly flat response below 100 kcy/sec. I used a Tectronix 535 oscilloscope to monitor these signals and the frequency response of the system depended upon the combination of the instruments named above and band-pass filter setting used. I have designed and am having built a special set of portable equipment for continuing this work.

3. W. N. Kellogg, R. Kohler, H. N. Morris, *Science* **117**, 239 (1953); W. N. Kellogg, *Porpoises and Sonar* (Univ. of Chicago Press, Chicago, 1961).

4. I have chosen to call this first very sharp pulse a "precursor" pulse because it so closely resembles in appearance the precursor pulse in certain shock-pulse phenomena with which I have done extensive work.

24 December 1962

Genetic Control of Hemerythrin Specificity in a Marine Worm

Abstract. *A biochemical polymorphism of coelomic hemerythrin has been found in the sipunculid* Golfingia gouldii; *the electrophoretically "fast" and "slow" coelomic hemerythrins differ in their oxygen equilibria and by a single peptide in tryptic and chymotryptic "fingerprints." All individuals of this sipunculid have the same vascular hemerythrin, which is electrophoretically different from any of the coelomic hemerythrins. Vascular and coelomic hemerythrins of another sipunculid,* Dendrostomum cymodoceae, *have quite different "fingerprints." Thus, on the basis of two separate types of evidence the tissue-specific hemerythrins appear to have a distinct genetic basis. The embryological and phylogenetic implications are discussed.*

Vascular (blood) hemoglobin and muscle hemoglobin (myoglobin) have been known for many years to be biochemically distinct proteins. Recently it has been shown that the amino acid sequences are different (*1*); this means that myoglobin and hemoglobin are the product of different structural genes (cistrons). Further evidence for this separate genetic basis has come from the failure to find any difference in the myoglobin from normal human beings (Hb A homozygous) and from individuals with sickle-cell anemia (Hb S homozygous) (*2*). Thus, these tissue-specific hemoglobins are under the control of separate genes. Several researchers have suggested that tissue-specific proteins—for example, the "isozymes"—are significant with regard to the general problem of the genetic control of development (*3, 4*). If this is true, then tissue specificity of certain proteins should be a very widespread phenomenon.

I have found tissue-specific hemoglobins in the annelid worm *Travisia* (*5*); in addition, two species of sipunculid worms were found to have separate vascular (tentacular) and coelomic hemerythrins occurring in morphologically different cells (hemerythrocytes) and possessing different denaturation, solubility, electrophoretic, and oxygen-equilibrium properties (*6*). As the differences between vascular and coelomic hemerythrins are very great, and because they persist in spite of purification and dialysis against common buffer solutions, it has been suggested that the proteins differ in their primary structure (*6*). During my investigation, 50 individuals of two populations of the sipunculid *Dendrostomum zostericolum* were surveyed, by electrophoresis and analysis of oxygen equilibria, to find if any differences existed between individuals; none were found, and thus no genetic speculation was possible.

In a survey of the sipunculid *Golfingia*—erroneously called *Phascolosoma* (*7*)—*gouldii*, variations in hemerythrin type have been found which are proof of separate structural genes for vascular and coelomic hemerythrins (*8*).

Coelomic and vascular hemerythrins were obtained without contamination, as described elsewhere (*6*). In *Golfingia gouldii* the amount of vascular hemerythrin is very small, less than 0.005 ml of cells in a large individual. This quantity is sufficient, however, for a single starch gel electrophoretic analysis. For the initial screening of individuals, unpurified hemerythrins were used. For other chemical studies hemerythrins were purified by ammonium sulfate fractionation; these preparations showed no trace of contamination with other proteins, either when tested in starch gel electrophoresis or in the analytical ultracentrifuge. Hemerythrin can be detected in starch gels by virtue of its reddish-brown color; a more sensitive test involves the Nitroso-R Salt staining procedure for iron (*9*).

In Fig. 1 it may be seen that individual sipunculids have any one of three different coelomic hemerythrin patterns, whereas the vascular hemerythrins are identical. In the experiment reported here, the latter were pooled to obtain comparable concentrations and to emphasize the identity of the vascular hemerythrins in each individual. It is also apparent that the vascular hemerythrin has a more rapid electrophoretic mobility under the conditions of the experiment than any of the coelomic hemerythrins. Studies in other starch gel buffer systems indicate that the vascular hemerythrin has a more acid isoelectric point. Three coelomic hemerythrin electrophoretic types were found in a population of 181 individual sipunculids: 73 individuals were of the "slow" type, 16 were of the "fast" type, and 92 gave the intermediate electrophoretic pattern, which represents the heterozygote (*10*). Since the hemerythrin molecule dissociates in 6*M* urea to an ultracentrifugally and electrophoretically homogeneous subunit of approximately one-eighth the normal molecular size (*11*), the heterozygote, according to hypothesis, should possess a family of molecules containing different proportions of the two kinds of polypeptide chains; this would account for the intermediate electrophoretic pattern and the presence of traces of the "slow" and "fast" hemerythrins in the heterozygote. In addition, an artificial mixture of equal amounts of "fast" and "slow" hemerythrins, after standing at room temperature for a few hours, will give an electrophoretic pattern identical to that of the presumed heterozygote.

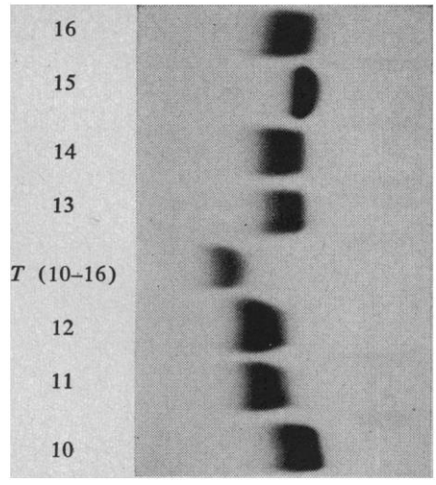

Fig. 1. Starch gel electrophoresis of coelomic and vascular hemerythrins from seven individuals of the sipunculid worm *Golfingia gouldii* (borate buffer; final gel *p*H, 8.3; electrophoresis at 250V for 16 hours at 0°C). The coelomic hemerythrins of sipunculids 11 and 12 are the "fast" type, sipunculid 15 is the "slow" type, and sipunculids 10, 13, 14, and 16 are the heterozygotes. The vascular (tentacular) hemerythrins are identical, and in the experiment were pooled [*T* (10–16)].

Another approach to the problem involves the "fingerprinting" technique, which has been used so successfully in

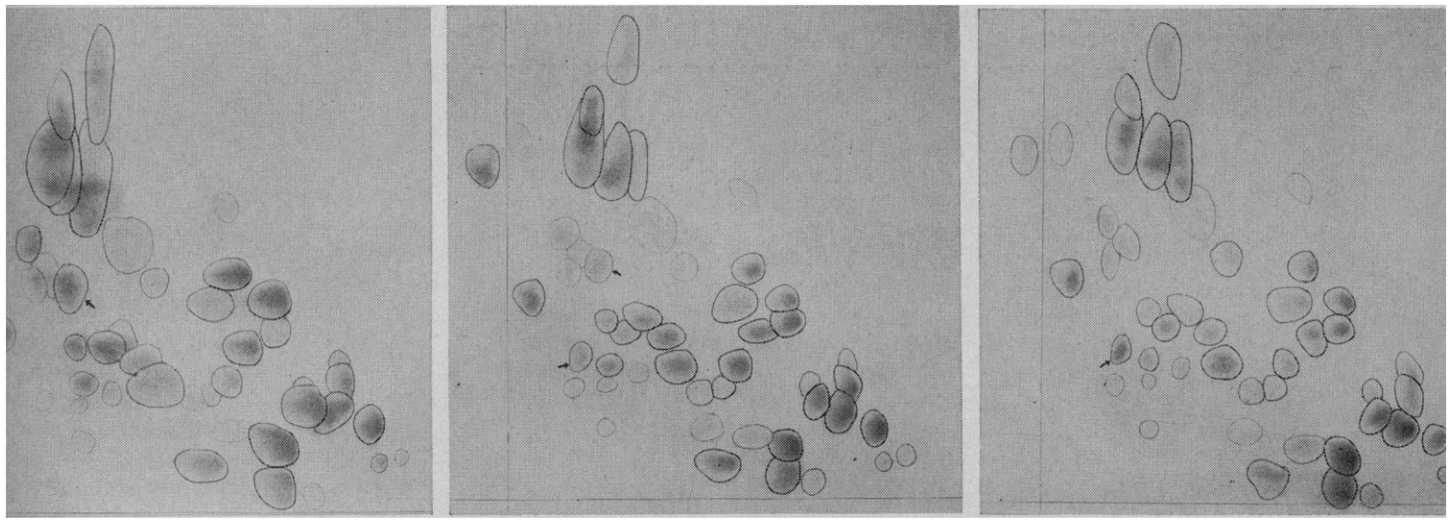

Fig. 2. Peptide patterns ("fingerprints") of chymotryptic digests of (left) "fast," (middle) "slow," and (right) "heterozygous" coelomic hemerythrins of *Golfingia gouldii*. The arrows mark the "fast" and "slow" peptides. The abscissa is the electrophoretic direction; the ordinate is the chromatographic direction.

the analysis of abnormal human hemoglobins (*12*) and in phylogenetic studies (*4, 13*). Using the procedure of Katz, Dreyer, and Anfinsen (*14*), I have performed two-dimensional chromatography and high-voltage electrophoresis of tryptic peptide digests of the various purified coelomic hemerythrins. Twenty-six peptides are resolved clearly with ninhydrin staining; three others can be seen with difficulty, but their presence can be detected by watching the color change during ninhydrin development. The "fast" and "slow" hemerythrins differ by a single peptide, the heterozygote having both peptides. Similar results were obtained with chymotryptic digests (Fig. 2); despite the lower specificity of chymotrypsin, the resolution of the "mutated" peptides is much better with the chymotrypsin digests than with the trypsin digests, for with the latter the "mutated" peptides do not stain intensively with ninhydrin and they overlap with other peptides.

These results were confirmed in six separate sets of digestions and subsequent "fingerprintings." Although not enough vascular hemerythrin of *Golfingia gouldii* could be obtained to allow purification and subsequent "fingerprint" comparison with the coelomic hemerythrin, such a comparison of vascular and coelomic hemerythrin has now been made for the Australian sipunculid *Dendrostomum cymodoceae* (Fig. 3) (*8*). Comparison of results for both the tryptic and the chymotryptic digests indicates that coelomic and vascular hemerythrins are quite different protein molecules, having relatively few peptides in common, although there is a similarity in general number and pattern of peptides. Thus there is structural evidence as well as genetic evidence that vascular and coelomic hemerythrins are the products

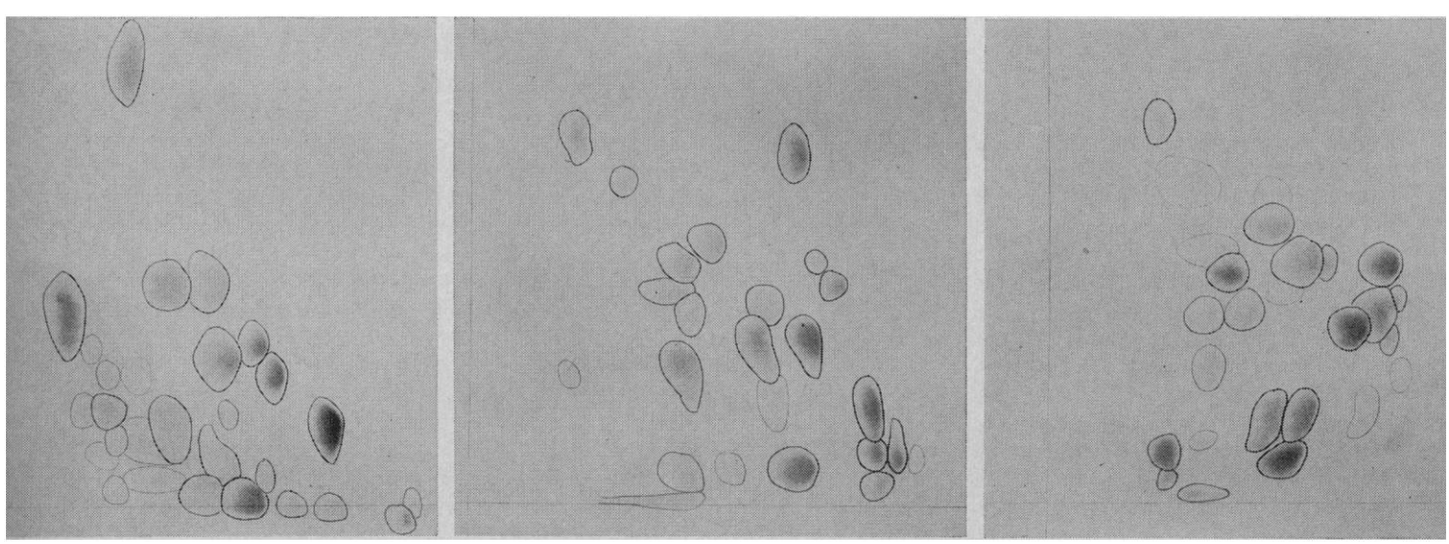

Fig. 3. Peptide patterns ("fingerprints") of tryptic digests of hemerythrins. (Left) Coelomic hemerythrin of *Dendrostomum cymodoceae*; (middle) vascular hemerythrin of *D. cymodoceae*; (right) coelomic hemerythrin of the ecardine brachiopod *Lingula reevei*. Note that there is a considerable difference between the vascular and the coelomic hemerythrins in *Dendrostomum* and that the hemerythrin of the supposedly unrelated brachiopod *Lingula* is no more different in general number and pattern of peptides. The abscissa is the electrophoretic direction; the ordinate is the chromatographic direction.

of separate genes. Coelomic hemerythrins of another sipunculid, *Phascolosoma agassizii,* and of the brachiopod *Lingula reevei* have also been "fingerprinted"; neither form possesses a separate vascular system (*15*). A tryptic "fingerprint" of hemerythrin of the brachiopod *Lingula* is included in Fig. 3; certain similarities to sipunculid hemerythrins—similarities in pattern and in the position of a few peptides—may be seen. In particular, all the hemerythrins examined have two histidine-rich tryptic peptides, both migrating approximately 18 cm electrophoretically (1800 v, 70 minutes), and one migrating 10 to 12 cm and the other 16 to 18 cm chromatographically (solvent front at the end of the paper; time, approximately 16 hours). It is highly probable that brachiopod and sipunculid hemerythrins evolved from a common gene, as has been suggested by Itano (*16*) for myoglobin and the various polypeptide chains of human hemoglobin. The finding of the similarity between brachiopod and sipunculid hemerythrins is of phylogenetic significance, for these two phyla are not usually considered to be closely related (*17*).

Elzinga, in our laboratory, has started an analysis, by both enzymatic and chemical means, of the C- and N-terminal amino acids of the "fast" and "slow" *Golfingia* coelomic hemerythrins (*18*). He has found, by both the dinitrofluorobenzene method and the leucine aminopeptidase technique, that the only N-terminal amino acid is glycine. The C-terminus, determined by carboxypeptidase, is isoleucine. These results give additional support to the previously mentioned evidence that a single type of polypeptide chain makes up the hemerythrin molecule—and thus a single structural cistron codes one type of hemerythrin.

Electrophoresis at various *p*H's in formate, acetate, borate, phosphate, and tris-EDTA-borate gels indicates that the difference in rate of migration of "fast" and "slow" hemerythrins persists until a *p*H of 3 to 4 is reached; at *p*H's below those values the "fast" and "slow" hemertyhrins have identical electrophoretic properties. These results suggest the substitution of a glutamic or aspartic residue in the "fast" hemerythrin for an uncharged amino acid in the "slow" pigment.

It is important to know whether the "abnormal" coelomic hemerythrins differ from the normal in their physiological properties. Oxygen equilibria of purified and dialyzed preparations are shown in Fig. 4 in the linear transformation based on the Hill approximation (*19*). When "fast" and "slow" hemerythrins are compared, it is found that the mutational event has led to a 37-percent decrease in oxygen affinity. The oxygen equilibrium curve for the heterozygote is exactly intermediate in position. Since the "fast" and the "slow" hemerythrin were found to differ by a single peptide in digests with trypsin and digests with chymotrypsin—enzymes with very different specificities as to the intraprotein bonds that will be cleaved—it is highly probable that substitution of a single amino acid residue produces the difference between the hemerythrins. This postulated change would result in a considerable difference in the oxygen-binding properties of the two kinds of hemerythrin and thus would be a change of potential physiological and ecological significance (*19*); however, the fact that the genetic data (*10*) for the population fails to indicate a significant deviation from the Hardy-Weinberg equilibrium indicates that neither type of hemerythrin is of obvious advantage to a sipunculid as compared to the other.

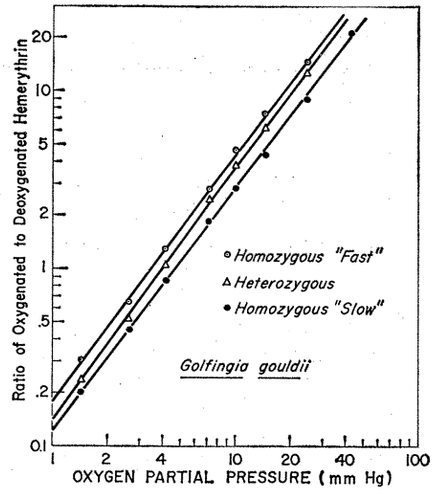

Fig. 4. Oxygen equilibria of purified coelomic hemerythrins from individual sipunculids of the "fast," "slow," and "heterozygous" types. The hemerythrins (2-ml samples) were dialyzed against a common potassium phosphate buffer (*p*H = 7.4, μ = 0.60; oxygen equilibria evaluated at 25°C; hemerythrin concentration 4 percent). There is no Bohr effect.

I have also compared both the "fingerprints" and the oxygen equilibria of several pairs of closely related hemoglobins (*4*). "Bovine A" and "Bovine B" differ by a single peptide but have identical oxygen equilibria inside and outside the cell. "Single" and "diffuse" mouse hemoglobins differ by one peptide and show a slight, probably not significant, difference in oxygen affinity. The lampreys *Ichthyomyzon unicuspis* and *Petromyzon marinus* have hemoglobins having all but two tryptic or two chymotryptic peptides in common; however, the Bohr effect for hemoglobin of *P. marinus* is almost twice that for hemoglobin of *I. unicuspis.* The hemoglobins of smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*M. salmoides*) differ by five peptides, some in each polypeptide chain; the hemoglobins differ enormously in oxygen affinity and in Bohr effect. Riggs and Wells (*20*) have found that Hb S has a lower oxygen affinity than Hb A, though the Bohr effect and the heme-heme interactions for the two are similar; both these hemoglobins of adult human beings have been known for some time to differ by a single amino acid (*12*). Thus it appears that in some instances one or a few amino acid substitutions can lead to a dramatic difference in the physiological properties of the respiratory pigment and in other instances the mutation has no apparent significance in this respect. This set of findings supports my earlier suggestion (*19, 21*), made largely on the basis of chemical modification experiments, that only a certain portion of the respiratory pigment molecule determines its oxygen equilibrium properties. This is probably a rather general property of protein systems, for it is known that several enzymes—for example, papain, chymotrypsin, and enolase—can be chemically modified even to the extent of removal of 20 to 60 percent of the amino acids without changing the kinetic properties (*22*).

Thus it appears that vascular and coelomic hemerythrins are different protein molecules, differing at the level of primary structure. Independently, the population genetics studies on *Golfingia* indicate that vascular and coelomic hemerythrins are the product of different genes. These related studies are the first report of a biochemical polymorphism at the single-allele level in a population of marine animals. The demonstration of the phenomenon of cell-protein specificity and its separate genetic basis in so esoteric a group as the phylum Sipunculida is significant to theories of chemical differentiation which have rested almost entirely on studies of the "isozymes" of a few vertebrates.

Since the different coelomic and vas-

cular hemerythrocytes in sipunculids arise originally from the same mesodermal cell, and since there is no trace of coelomic hemerythrin in vascular hemerythrocytes, and vice versa, there must also be separate "control genes" regulating the activation of the different hemerythrin cistrons. One is tempted to draw analogies between such "control genes" and those that have been found in microorganisms (*23*) and in maize (*24*), and those found to be regulators of the differentiation of human (*25*) and vertebrate (*4*) ontogenetic hemoglobin sequences.

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8. I thank Dr. C. Ladd Prosser and Dr. J. D. Roslansky for sending specimens of the sipunculid *Golfingia gouldii* from the Marine Biological Laboratory, Woods Hole, Mass. Dr. S. J. Edmonds, department of zoology, University of Adelaide, South Australia, generously provided living specimens of the large *Dendrostomum cymodoceae*, thus enabling me to obtain enough vascular hemerythrin for purposes of this study. Nora Banner, department of zoology, University of Hawaii, sent me specimens of the brachiopod *Lingula reevei*. C. M. Ann Baker and Martha Foght helped in some of the electrophoretic and "fingerprinting" studies. Dr. Oliver Smithies and Otto Hiller, department of medical genetics, University of Wisconsin, gave advice on the vertical starch gel electrophoretic technique, which has given such excellent resolution of various protein systems. This research was supported by the U.S. Public Health Service (grant RG-7939) and the National Science Foundation (grant G-18082).
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10. These results give a gene frequency of 0.34 for the "fast" hemerythrin gene and 0.66 for the "slow" hemerythrin gene. Assuming a Hardy-Weinberg equilibrium, one would expect, out of a total of 181 individuals, 78.2 "slow's," 81.5 "heterozygotes," and 21.3 "fast's." Thus, the data given here do not show a significant deviation from genetic equilibrium ($\chi^2 = 2.98$; degrees of freedom, 2. For significance at the 5-percent level, the χ^2 must be greater than 5.99). Selection pressure must be quite strong to show a significant deviation from Hardy-Weinberg equilibrium; in spite of vigorous selection pressures for various economically advantageous traits, no significant departure from the Hardy-Weinberg equilibrium is seen for various genetically based polymorphisms of ovalbumins and ovoglobulins in nine populations of the domestic fowl [C. M. A. Baker and C. Manwell, *Brit. Poultry Sci.* **3**, 161 (1962)].
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7 January 1963

Signal Detection in the Rat

Abstract. *An auditory detection experiment was performed with rats as subjects, and the data were analyzed with a signal detection model. Rats were run at fixed sound pressure levels, and their responses were partitioned so that operating characteristics could be constructed. Measures of detectability, $(d_e)^{1/2}$, were calculated from the operating characteristics, and show that $(d_e)^{1/2}$ is a function of sound pressure levels, rising as these levels rise.*

The theory of signal detectability (*1*) has been used over the past several years as a model for the decision-making processes inherent in psychophysical studies with man. This model allows an observer's responses to be partitioned into two parts, the probability of a *yes* response when a signal is present, $p(y/\mathrm{SN})$, and the probability of a *yes* response when a signal is absent, $p(y/\mathrm{N})$. These two probabilities are determined by the parameters of the testing situation. By sampling from a variety of testing conditions and recording the $p(y/\mathrm{SN})$ as a function of the $p(y/\mathrm{N})$, a set of points emerge which define a response-conditional operating characteristic, from which $(d_e)^{1/2}$, a detectability index, can be calculated. This model provides a theoretical basis of assessing this index, which is largely independent of specific testing parameters that are associated with a correct detection.

In order to determine this model's applicability in the field of infrahuman psychophysics, where the interaction of reward and sensitivity has been generally neglected, the following experiment was performed.

Four male albino rats weighing approximately 350 g were allowed access to water for 1 hour per day for 2 weeks before experimentation was begun. The rat worked in a small cage constructed of stainless-steel rods, mounted in a 1¼-inch plywood enclosure lined with 3 inches of fiber glass. Two levers entered the cage through its floor, one on the left of a rat facing the cage front, lever L, and one on its right, lever R. The auditory signals were presented by an electrostatic speaker facing the front of the cage, by means of an oscillator, electronic switch, attenuator, and amplifier chain.

The naive rat was placed in the cage with only the lever L present, with a 2-kcy tone at a sound pressure level of 65 db (relative to 2×10^{-4} dyne/cm^2) continuously present. The rat was trained to press this lever, for which it received one drop of water per lever press. Gradually the on-time of the tone was reduced to about 5 seconds, and alternated with a 10-second tone-off period. At this time the rat was rewarded only for presses occurring in the presence of the tone. When this behavior became stable, lever R was placed in the cage, and the rat was trained to press this lever on a fixed-interval 7-second schedule (FI 7). Under this schedule the first press after a 7-second interval had elapsed turned on a 2-second tone. During this time a single reward was available if the rat then pressed lever L. A small photoelectric system was mounted over lever R. When its beam was broken by the rat the FI timing circuits were operative. This arrangement tended to stabilize the rat's