[12 percent hydrolyzed starch by weight to volume (Connaught)]. The gels were placed between metal cooling plates in which water was circulated at about 15°C, and a gradient of 2 volt/cm along the gel was applied for 16 to 18 hours. After electrophoresis, the horizontally sliced gels were incubated for 1 hour at 37°C with a reaction mixture containing 0.1 ml of 70 percent, weight to volume, sodium lactate, 10.0 mg of nicotinamide adenine dinucleotide, 10.0 mg of tetrazolium salt MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide], and 10.0 mg of phenazine methosulfate per 100 ml of 0.1 tris-HCl buffer, *p*H 8.2.

The pattern of the New Guinea variant LDH was compared with that of the Memphis-4 variant. Under the conditions of electrophoresis the two variants had identical mobilities (Fig. 1); in both cases the banding pattern is consistent with a mutation in the LDH-A subunit. The slower bands of isozymes 2 and 3 in the New Guinea variant are weaker than the corresponding bands of the Memphis-4 variant. This may be due to differential loss of activity in the mutant subunit, which has affected the New Guinea samples to a greater extent than in the case of the Memphis-4 sample because of the unavoidable delay in examining the material from the Highlands. An alternate explanation is that the New Guinea mutant, though having identical electrophoretic properties with Memphis-4, is intrinsically less stable. Study of fresh samples may distinguish between these alternatives.

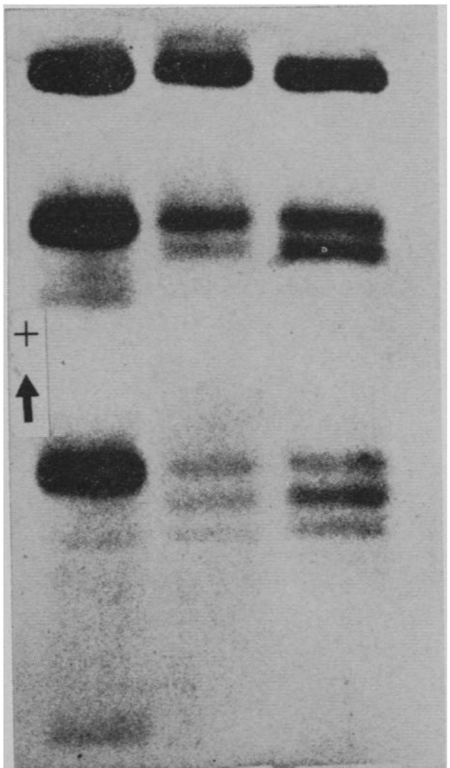

Fig. 1. Starch-gel electrophoretic variants of LDH. (a) Normal, (b) New Guinea variant, and (c) Memphis-4 variant. Bridge buffer: 0.2*M* phosphate–critic acid, *p*H 7.0; gel buffer 1:20 dilution of bridge buffer.

The original Memphis-4 variant (*3*) was detected in three generations of a Caucasian family in the United States. A similar variant, possibly identical, has also been described in two families in Lancashire, England (*4*). There was a total of 15 sibships containing at least one affected member in the two English families and the ratio of affected to total sibs did not differ significantly from 0.5, indicating no measurable selective effect against either the normal or affected phenotype. Although these families resided in the same geographical area a search of parish records covering five generations revealed no relationship between them. The possibility of a more remote connection, however, still exists.

The New Guinea individuals showing the LDH variant are members of three exogamous clans which, in turn, are part of a single clan cluster of Enga-speaking people living in the Lagaip subdistrict of the Western Highlands of New Guinea, 160 km northwest of Mt. Hagen. The clans are traditionally believed to have a common ancestry and although our records do not permit the construction of a genealogical tree relating all the affected persons to one another, it is likely that they are derived from a common source. Moreover, the variant LDH types have probably persisted in this area for several generations.

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Punishment by Response-Contingent Withdrawal of an Imprinted Stimulus

Abstract. *Newly hatched ducklings were exposed to a moving, imprinted stimulus; if they followed it, the stimulus was withdrawn briefly. The tendency to follow gradually declined during punishment periods, but it returned to prepunishment amounts when punishment terminated. This finding attests to the efficacy of withdrawal of reinforcement as a technique for behavioral control.*

Previous research indicates that, in the immature duckling, presentation of an imprinted stimulus can serve as an effective reinforcement for the development and maintenance of a variety of responses. If, for example, presentation of the stimulus is contingent upon a key peck, the tendency to emit this behavior undergoes a marked increase in probability (*1*); similarly, if stimulus presentation is contingent upon a distress call, the frequency of these calls increases (*2*). Both findings imply that, like the presentation of other stimuli that serve as reinforcement (food, water, warmth, and so forth), presentation of an imprinted stimulus can increase the probability of the overt behavior at the moment of stimulus presentation.

In the present investigation we raised the corollary: Can an imprinted stimulus also function in a complementary fashion to reduce the probability of the behavior at the moment of stimulus withdrawal? Since immature ducklings are frequently observed to follow a moving imprinted stimulus, we arranged to withdraw the stimulus briefly whenever the duckling began to follow it.

Specification of the effects of this procedure is important for several reasons. (i) Punishment is often defined as either response-contingent presentation of an aversive stimulus or response-contingent withdrawal of a reinforcing stimulus; and although the former procedure has been studied extensively (*3*), the latter has rarely been investigated (*4–6*). The procedure used here provided an opportunity for examining further punishment via withdrawal of reinforcement. (ii) The accumulated evidence (*7*) indicates that the immature duckling's reactions to an imprinted stimulus are largely examples of filial-type behavior. By investigating punishment via withdrawal of an imprinted stimulus, the present research provided an opportunity to examine an important but seldom explored source of behavioral control in the filial relationship. (iii) In experimentation on imprinting, the tendency for the subject to follow the stimulus is often used as the major index of the strength of a given imprinting procedure (7). Specification of the factors that influence following behavior is relevant to the interpretation of these experiments.

We used three ducklings out of an initial group of six Peking ducklings (*Anas platyrhynchos*) hatched in isolation from eggs from the C & R Duck Farm, Long Island, New York. When hatching was completed, the ducklings were housed in individual, visually isolated cages. The imprinting apparatus was located in a sound-insulated temperature-controlled room and consisted of a box (183 by 76 by 76 cm) divided lengthwise by a fine-mesh brass screen into two approximately equal compartments, one for the duckling and the other for the imprinting stimulus. The imprinting stimulus consisted of a white plastic milk bottle mounted over the superstructure of a model train engine which ran the length of its compartment on HO-gauge track. The lighting was such that, when the subject's runway was illuminated and the stimulus compartment was darkened, reflections from the screen prevented the subject from viewing the stimulus. When, however, the stimulus compartment was illuminated, the imprinting stimulus became visible. Stimulus presentation consisted of illuminating the stimulus compartment and moving the stimulus at approximately 30 cm/sec. A set of infrared photocells was placed across the subject's compartment in the center of the apparatus. With this arrangement, the photocell was activated each time the duckling moved past the center of the compartment as it followed the stimulus. Food and water were available in dishes on one side of the subject's compartment.

All subjects were given four imprinting sessions during the first 48 hours after hatching. Each session lasted 45 minutes. The duckling was placed in the runway, the stimulus compartment was illuminated, and the stimulus was moved back and forth along its track for the entire session. On day 5, the three ducklings which had exhibited the most consistent following behavior were placed individually in the apparatus with the stimulus visible and moving; a baseline measure of following was obtained as the duckling walked back and forth next to the screen. Each time the bird passed the center of the screen, interrupting the photocell beam, one following response was registered. Once the duckling had followed consistently for at least 5 minutes, the first punishment period was introduced. During this period, any following responses (passing the center of the screen) automatically produced an 8-second removal of the stimulus. After 8 seconds, the stimulus reappeared and remained present as long as no following responses were made. Once the following was reduced to a low level, the punishment contin-

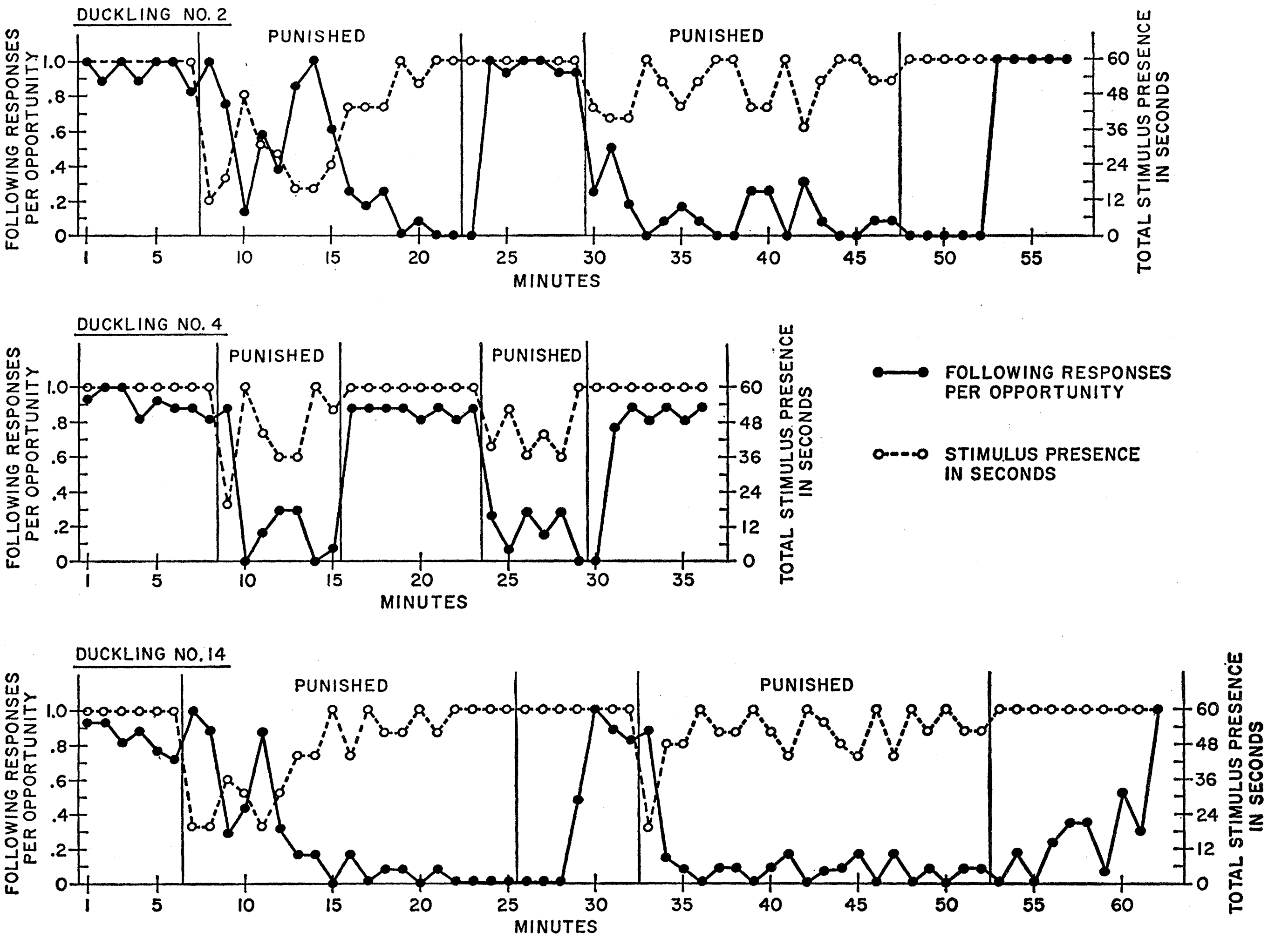

Fig. 1. Following response per opportunity and duration of stimulus presence on a minute-by-minute basis for each duckling. During periods labeled "punished," the imprinted stimulus was withdrawn for 8 seconds each time the duckling interrupted the photocell beam.

gency was removed. At no time was an exteroceptive stimulus used to signal the presence of either a punishment or punishment-free period. When the unpunished following had once again reached relatively high amounts, the punishment contingency was reinstated. Finally, the punishment contingency was again removed when following had been reduced.

The following behavior of each duckling throughout the experiment is summarized in Fig. 1. Since the stimulus was present for varying durations during punishment periods (depending upon how many punished following responses occurred), direct comparisons of response frequency during the several periods are inappropriate. Accordingly, a relative measure, following responses per opportunity, was calculated by dividing the sessions into units of 1 minute each. The total number of seconds of stimulus presence was determined for each unit. (During the punishment-free periods, of course, the stimulus was always present.) This total divided by the number of seconds required for the stimulus to make a single excursion approximates the number of opportunities for the duckling to pass through the photocell beam, given perfect following. In a given unit the number of following responses per opportunity is the number of times the duckling interrupted the photocell beam divided by the number of opportunities to do so, given perfect following, a ratio having the value of 1.0 for perfect following and 0.0 for no following responses. With this procedure it is possible to have a high ratio with only a few seconds' duration of stimulus presence, if the duckling began to follow immediately when the stimulus reappeared after each 8-second removal. Also shown in Fig. 1 is the total duration of stimulus presence in each unit.

In general, the relation between following responses per opportunity and duration of stimulus presence during punishment is approximately inverse. The lack of a perfect inverse relation is due to the variability of the relative positions of the duckling and the stimulus when the latter reappeared after an 8-second withdrawal.

For all subjects, the overall effect of the punishment contingency was to reduce gradually the tendency to follow, so that by the end of a given punishment period the duckling seldom followed, and the stimulus was almost continuously present (Fig. 1). The tendency to follow, however, never disappeared altogether. Thus, once a given punishment period terminated, and following no longer led to stimulus withdrawal, the ducklings again resumed following. All ducklings were observed throughout the session. Many distress calls were heard during stimulus withdrawal when the ducklings were first exposed to the punishment contingency. Later, however, there were relatively few calls during stimulus withdrawal, and none during stimulus presence even when the ducklings did not follow. No duckling ate or drank during the session, and during punishment periods they usually sat and observed the stimulus from the side of the compartment away from food and water.

For two of the ducklings (Nos. 2 and 4), the transitions to high rates of following during punishment-free periods were abrupt and occurred with the first unpunished following response. This may mean that the events during punishment (that is, stimulus withdrawal) were serving in a discriminatory capacity and the following was only suppressed when the response had recently led to stimulus withdrawal. For the third duckling (No. 14), the effects of the prior punishment contingencies were more lasting, and the tendency to follow increased gradually (rather than abruptly) during punishment-free periods. However, duckling 14 was exposed to the punishment contingency longer than the other two ducklings, and this factor may be responsible for the difference. Whether it is or not, however, the present data make it clear that response-contingent withdrawal of an imprinted stimulus provides an effective procedure for reducing the probability of following behavior.

Of the few previous investigations of punishment by withdrawal of a positive reinforcing stimulus, the study by Baer (*5*) is most similar to our work. Baer used the withdrawal of opportunity to watch filmed cartoons to reduce the occurrences of a concurrent food-reinforced lever response in children. Although his procedure yielded reliable response decrements, the lever response was fairly weak to begin with, and during the test it was being extinguished. In the present study the punished response was initially quite strong, and no extinction procedures were used. Even so, our findings revealed substantial response decrements attesting to the efficacy of this form of punishment.

Finally, it can be noted that in many studies of imprinting, the subject's manifest tendency to follow an imprinting stimulus is used as the major index of the degree to which the subject is imprinted (*7*). Since, however, such behavior is influenced by its consequences (that is, affected by the punishment procedure) it seems possible that when following is used as an index of imprinting, instances in which subjects failed to follow the stimulus may reflect factors other than inadequate imprinting.

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Mosaic Numbers

Moore (*1*) finds that the relation

$$y = 1.197 \times \left[\frac{1}{\sqrt{5}} \left(\frac{1+\sqrt{5}}{2} \right)^x - \frac{1}{\sqrt{5}} \left(\frac{1-\sqrt{5}}{2} \right)^x \right] \text{cm}$$

". . . yields a set of values for y which fit the observed mid-interval modal values. . . ." He lists the first eleven mosaic units y in sequence: 1.2, 2.4, 3.6, 6.0, 9.6, 15.6, 21.6, 25.1, 40.7, 65.8, and 106.5.

The theoretical implications of Moore's relation should be elucidated. Since each mosaic unit, with the exception of 21.6, can be obtained by adding the two units preceding it, the recurrence relation $M_{x+2} = M_{x+1} + M_x$ must hold. The best-known numbers which obey this relation are the Fibonacci numbers; these numbers are members of the sequence . . . , 0, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, . . . and are identified by the symbol F_x with initial conditions $F_0 = 0$, $F_1 = 1$. But there are other such sequences which obey the same recurrence relation; the most prominent of these is the Lucas sequence (*2*): . . . , 2, 1, 3, 4, 7, 11, 18, 29, 47, . . . identified by the symbol L_x with initial conditions $L_0 = 2$, $L_1 =$