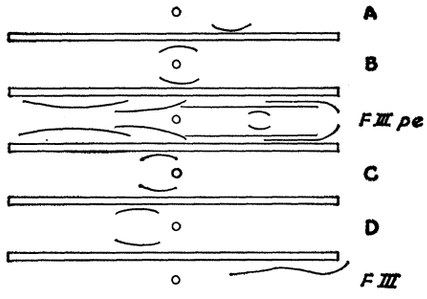

Fig. 3. Immunoelectrophoretic patterns of fraction III (*FIII*) and its digestion products. *FIIIpe,* the nondialyzable material from peptic digestion of fraction III; *A, B, C, D,* the four protein bands from starch-gel electrophoresis of *FIIIpe,* in sequence with *A* the slowest moving and *D* the fastest. The troughs contain goat anti-rabbit γ-globulin serum.

on Sephadex G-50, and two peaks were obtained by elution with 0.01 *M* NaCl. Neither produced specific precipitation with the goat antiserum either in agar (immunoelectrophoresis) or in saline. Both, however, inhibited the precipitation of this antiserum with fraction III, as determined by delay of flocculation time; this demonstrated that the fractions combined with antibody. Neither produced detectable inhibition of the specific precipitin reaction when bovine γ-globulin was added to homologous rabbit antiserum.

It seems likely, in view of the pattern given by starch-gel electrophoresis, that the dialysate consists of a more complex mixture of peptides than the recovery of two peaks from chromatography on Sephadex G-50 would indicate (*12*).

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Escape and Avoidance Learning in Newly Hatched Domestic Chicks

Abstract. *Under the conditions specified, chicks fail to learn either to escape or to avoid shock on the day of hatching. Chicks trained for the first time on the day after hatching quickly learn to escape but do not learn to avoid shock. Avoidance learning first appears on the third day of life, and from that time the number of chicks learning to avoid increases with age, so that by the fifth day of life the majority are able to do so.*

In the course of some experiments on the effects of noxious stimuli on imprinting (*1*), it was noticed that very young domestic chicks seemed to show no concern as they were being harnessed to an apparatus in which they had received a very powerful electric shock some 12 hours previously. The experiment described here was made in order to obtain some systematic evidence on the development of escape and avoidance learning in these animals.

The apparatus consisted of a two-compartment box of a kind commonly used for classroom demonstrations of instrumental avoidance conditioning in rats. One half of the box was painted white and contained a grid floor to which the scrambled shock source (Grason-Stadler model E6070B) supplied a short circuit current of 4 ma at 350 volts, 60 cy a-c. The other half of the box was painted black and had a solid floor. One side of the shock compartment was made of clear Plexiglass through which the conditioned stimulus (CS), the light from a 60-watt lamp placed 4 inches away, could be seen when it was on.

The procedure was as follows: After the chick had been placed in the center of the grid, the CS was turned on. Ten seconds later the shock (UCS) was automatically supplied to the grid in the white compartment, and both CS and UCS remained on for a further 30 seconds or until the chick had crossed to the black half of the box, whichever occurred earlier. The chick was then removed from the apparatus and returned to its cage. There was a 1-minute interval between the start of one trial and the start of the next. Each chick was run for 100 trials, or until it had reached a criterion of five successive avoidances or eight avoidances in ten consecutive trials, whichever occurred first. A thin coating of electrode paste was smeared on the chick's feet every ten trials. Control chicks were run at the same time in a dummy apparatus; they were treated in exactly the same way except that they were never given an electric shock.

Fifty-five New Hampshire × Barred Rock chicks were hatched in the laboratory and reared in individual fiber-glass cages in a constantly illuminated room kept at 90°F. They were divided into five independent groups; seven chicks were trained when they were less than 15 hours old (day 1), ten when they were between 25 and 43 hours old (day 2), nine when they were between 49 and 67 hours old (day 3), nine when they were between 73 and 91 hours old (day 4), and ten when they were between 97 and 115 hours old (day 5). Two additional chicks were run as controls (no shock) at corresponding ages.

The results were as follows: None of the day-1 group learned either to escape or to avoid the shock; all except one of the day-2 group learned to escape but none learned to avoid; from day-3 the proportion of chicks learning to avoid increases with age. The mean escape latencies of the chicks in the different groups which failed to learn to avoid shock are given in Fig. 1, from

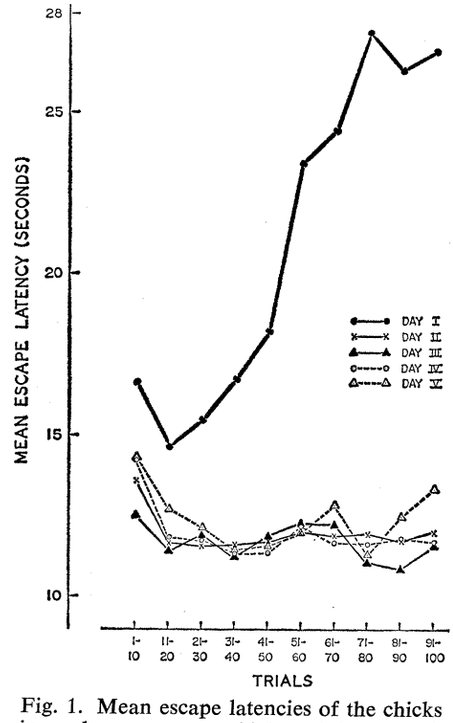

Fig. 1. Mean escape latencies of the chicks in each age group which failed to reach the criterion of avoidance learning.

Table 1. Performance measures of those chicks which learned to avoid shock.

Measure	Day of age		
	3	4	5
Percentage reaching avoidance criterion	33	44	70
Trials to criterion			
Median	22	28	35
Range	17 to 22	11 to 43	15 to 52
Trial of first avoidance			
Median	10	8.5	11
Range	9 to 10	4 to 20	7 to 24

which it can be seen that, after an insignificant ($t = < 1$) improvement from the first to the second block of ten trials, the performance of the day-1 birds deteriorated with practice. The escape latencies of those older birds which failed to learn to avoid rapidly decrease to an asymptotic value of less than 2 seconds from the onset of shock; the combined latencies of these chicks in the day-2 to day-5 groups are significantly shorter on trials 91 to 100 than on trials 1 to 10 ($t = 3.4$; df = 23; $p = 0.01$). The overall increase with age in the proportion of chicks in each group which did reach the criterion of avoidance learning is significant ($\chi^2 = 15.5$; df = 4; $p < 0.01$); the performance measures for these chicks are given in Table 1. None of the control chicks crossed from the white to the black compartment.

Much more evidence is needed, about both early learning and avoidance conditioning, before the developmental changes reported here can be explained. Although no doubt exists that chicks can acquire a simple habit on day 1 (*2*), and by day 2 can learn to respond to a conditioned stimulus in the absence of the unconditioned stimulus with which it was previously paired (*3*), very little is known about the relation of this form of learning to the conventional varieties of conditioning which have been extensively studied in adult animals. Similarly, whereas the failure of the day-2 chicks to learn to avoid despite their ability to learn to escape is consistent with other evidence (*4*) that avoidance does not arise simply as a result of the progressive shortening of escape latencies with practice, no alternative explanation for the emergence of anticipatory avoidance is presently available (*5*).

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Bohr Effect: Absence in a Molluscan Hemocyanin

Abstract. *The hemocyanin of the keyhole limpet,* Diodora aspera, *shows no Bohr effect within the* pH *range 6.88 to 7.84. At 10°C the pressure of half-saturation is 5 mm-Hg of oxygen pressure. A moderately positive interaction occurs among the oxygen-combining sites during oxygenation. The heat of oxygenation is calculated to be approximately −12.6 kcal/mole of oxygen. The* pH *of normal blood of* Diodora *is lower than that of many other marine molluscs.*

Comparative studies of the hemocyanins of the arthropods and molluscs have shown that the functional characteristics of these blood respiratory pigments vary considerably among the different species (*1, 2*). One such characteristic of interest is the degree to which the oxygen equilibrium curve is shifted by changing *p*H. All crustacean hemocyanins examined show, like most hemoglobins, a normal Bohr effect; the oxygen affinity is reduced by increased acidity. Among the molluscs, however, this situation is much more variable, ranging from the extreme normal Bohr effect shown by the hemocyanin of the squid (*3*) to the strong inverse Bohr effect exhibited by the hemocyanins of certain marine snails (*2, 4*). Midway in this range lie the hemocyanins of several chitons where the Bohr effect is less striking (*3*). Although this great variation in response to *p*H exists, there is only one hemocyanin known in which, within physiological limits, the combination with oxygen is not affected by changing *p*H. Manwell (*5*) reported this for the hemocyanin of the giant

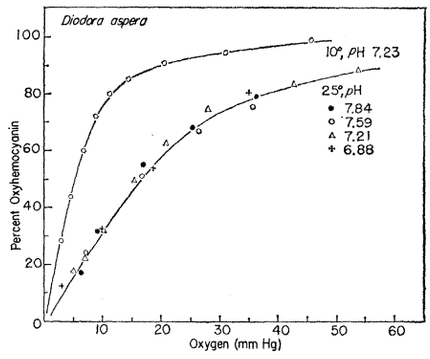

Fig. 1. Effect of *p*H and temperature upon the oxygen equilibrium curve of *Diodora* hemocyanin.

gumboot chiton, *Amicula stelleri* (formerly *Cryptochiton*). I now report a similar finding for the hemocyanin of the keyhole limpet, *Diodora aspera* Eschscholtz.

Blood was obtained from recently collected specimens by slitting the body wall just medial to the base of the gills and allowing the blood to run into a test tube. Large specimens yielded enough blood for a single determination; otherwise the blood of two or three specimens was pooled. The whole blood was centrifuged and used immediately. The *p*H of 1.5 ml of blood was adjusted to desired values by the addition of a small quantity of tris buffer. Oxygen-equilibrium curves were determined by the vacuum-pump spectrophotometric method (*6*). Optical-density measurements were made at 580 mμ. Spectral-absorption curves showed this to be the major absorption peak, in the visible range, of the oxygenated pigment.

Figure 1 illustrates oxygen equilibrium curves obtained at 25°C with *p*H varying from 6.88 to 7.84. The oxygen equilibrium curve does not appear to shift within this range of *p*H. The second curve (Fig. 1) illustrates the physiological position of the oxygen equilibrium curve. This study was conducted on animals taken from Puget Sound (*7*) where the average water temperature is about 10°C. At this temperature the hemocyanin of *Diodora* becomes half saturated with oxygen when the oxygen pressure is approximately 5 mm-Hg. If these two oxygen equilibrium curves are plotted in the form of log p against log $y/(100\text{-}y)$, where p is the partial pressure of oxygen and y is the corresponding percentage saturation, the slope n is an approximation of the degree of interaction of