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*).

H. JAMES*
CAROLYN BINKS
Department of Psychology,
Queen's University, Kingston, Ontario

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* Present address: Department of Psychology, Dalhousie University, Halifax, Nova Scotia.

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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

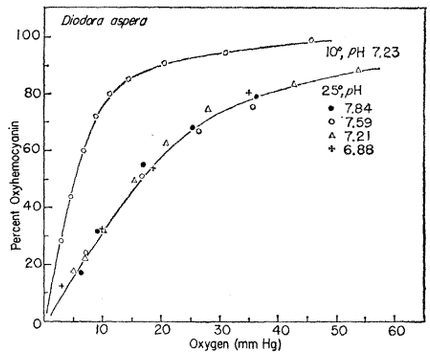

Fig. 1. Effect of *p*H and temperature upon the oxygen equilibrium curve of *Diodora* hemocyanin.

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

the oxygen-combining sites of the hemocyanin molecule. For the curves obtained at 10° and 25°C, n is 1.70 and 1.65 respectively, indicating a moderate positive facilitation occurring among the oxygen-combining sites as the molecule becomes oxygenated.

The absence of a Bohr effect in *Diodora* hemocyanin provides an opportunity to calculate the heat of oxygenation of this pigment uncomplicated by the effects of *p*H. Substituting the reciprocals of the half-saturation pressures (p_{50}) at 10° and 25°C for the equilibrium constants, K_1 and K_2, in the van't Hoff equation (*8*)

$$\Delta H^\circ = \frac{RT_1T_2}{T_1 - T_2} \ln \frac{K_1}{K_2}$$

gives a value of $\Delta H^\circ = -12.6$ kcal/mole of oxygen. This falls within the reported range of values for hemocyanins (*9*) and is very close to the −13 kcal/mole of oxygen found by Manwell (*8*) for the hemocyanin of *Octopus dofleini* (formerly *O. hongkongensis*). However, very few species have been investigated in this respect, so the actual extent of the variation in heats of oxygenation between species is still very uncertain.

In addition to the absence of the Bohr effect, the blood of *Diodora* is unusual in another respect. Samples of blood were taken from six specimens by inserting hypodermic syringes into the base of the gills; the *p*H was 7.13, 7.10, 7.13, 7.10, and 7.09, respectively. All specimens were maintained in tanks of well-oxygenated, running sea water until the moment of sampling. Only samples obtained quickly and easily were used. The average *p*H of 7.08 is appreciably lower than that of the blood of most marine molluscs. The *p*H of the blood of the latter usually is in the range 7.3 to 7.8. Additional studies may reveal the significance of the low *p*H of *Diodora* blood (*10*).

JAMES R. REDMOND
Department of Zoology and Entomology, Iowa State University, Ames

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Thalidomide: Effect upon Pregnancy in the Rhesus Monkey

Abstract. *Thalidomide was administered to 44 female rhesus monkeys immediately after they had mated. There were no live births from these animals, whereas there were 11 live births in 57 untreated monkeys. The results are statistically significant. The hypothesis is advanced that thalidomide killed the embryo prior to implantation.*

The thalidomide disaster stimulated interest in testing drugs on pregnant animals for teratogenic properties. There is close similarity between the reproductive physiology of monkeys and man. Therefore we conducted a pilot study to determine whether the pregnant monkey is a reliable test animal for detection of thalidomide teratogenicity. The effect upon the embryo, reported here, was not anticipated.

The monkeys (*Macaca mulatta*), weighing 5 to 13 kg, belonged to a breeding colony in which each was separately caged. Females were mated for 48 to 72 hours on the 10th to 12th days after the onset of the menstrual cycle. Fertility of the males had been demonstrated by repeated successful conceptions. Each time a female was paired with a male was counted as a single mating.

A technician made a daily list of females to be mated. During the 1-month period of this investigation 101 females were listed and mated. The first two or three on the daily list were arbitrarily started on thalidomide immediately after separation from the male. There were 44 animals in this group. Thalidomide was usually given for 33 to 45 days, but three animals received it for only 6, 7, and 8 days after their second mating. Fifty-seven females from the list constituted the control group. If a female in the treated group menstruated she was continued on the drug and remated on the 10th to 12th day after menses. Similarly, females in the control group were remated.

Each test female drank from a metal tube extending into the cage from an individual water bottle containing thalidomide. The water bottle was not rinsed during the test period, but fresh water and subsequent doses were added to the residual solution. Some refused water on the first day, but their water intakes returned to normal within a few days. Alternate test animals received 50 (20 animals) or 200 mg (24 animals) of thalidomide daily. Regular observations of water intake, general health, and vaginal bleeding were made. The cages were inspected during the day for products of conception.

Results are summarized in Table 1. Past experience had shown some variation from month to month in number of successful matings. The month chosen for this study was one that had been favorable. Nevertheless, the thalidomide-treated monkeys that were mated during this month failed to show signs of pregnancy. One abortion occurred among the untreated animals, but none in the treated ones.

Twelve treated and five untreated monkeys were mated twice and were excluded from statistical consideration, leaving 32 and 52, respectively. If the chi square test is applied to the number of animals in each group, the p value is $< .001$. (If one applies Yates's correction factor to these data, a p value of < 0.2 and $> .01$ is obtained.)

No monkey treated with thalidomide showed behavioral effects, such as excessive sleeping or decreased activity during the test period. Within 6 weeks after this study, three monkeys were remated and became pregnant.

We believe that thalidomide, under these conditions, killed the embryo prior to implantation. The interval between fertilization of the mammalian

Table 1. Effect of thalidomide on pregnancy in *Macaca mulatta*.

Group	Animals mated*	Live births
Colony experience during 11 mo	1003	100
Test period (1 mo)		
Control	57 (52)†	**11**
Treated	44 (32)†	**0**

* All matings were for 48 to 72 hours during the ovulatory period. †These values were used for statistical treatment and they exclude five animals in the control group and 12 in the treated group which were mated twice.