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*).

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10 December 1962

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.

ovum and its implantation as a blastocyst in the uterine wall varies from 6 to 10 days in the rabbit, rat, mouse, monkey, and man. The response of the embryo to damaging agents before implantation differs markedly from its response after implantation. During this period in mice, a teratogenic agent such as radiation is more likely to kill the embryo than it is to cause a congenital anomaly (*1*)

Previous research on the teratogenic properties of thalidomide in animals has been concerned primarily with its effects after implantation and during organogenesis. Several workers have found high resorption rates when the drug was administered before the implantation stage (*2, 3*). Usually much larger amounts of the drug (on a milligram per kilogram basis) were required to produce resorptions and anomalies than appears to be the case in the human (*4, 5*). We chose a dose of thalidomide close to the estimated amount required to produce human anomalies. This dose had no detectable toxic effects in the monkey, and this fact encourages us to infer that sensitivity to thalidomide teratogenicity is similar in monkeys and man.

Others have observed directly the rabbit blastocyst before implantation; thalidomide (about 125 mg/kg), when given to the pregnant rabbit, caused degeneration of the embryonic disk of the blastocyst (*3*). When the early chick embryo was treated with thalidomide "the development of the germ per se ceased or was delayed" (*4*).

No previous study of this nature has been carried out in primates. If one intends to use the rhesus monkey as a test animal for the teratogenic effects of thalidomide, a different dosage and time schedule of drug administration will be required (*6*).

Further investigation of this property of thalidomide and perhaps of related compounds should be undertaken with a more sophisticated approach designed to explore mechanisms by which pregnancy may be prevented.

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27 February 1963

Mutagenic Action of Ethyl Methanesulfonate in Maize

Abstract. *Pollen of corn plants carrying three closely linked genes (α β Sh₂) on chromosome 3 were treated by ethyl methanesulfonate in order to determine the nature of genetic changes produced. In this genetic material the loss of the β gene alone represents a discrete genetic change, possibly a point mutation, while the loss of two or more markers represents chromosome aberrations. Ethyl methanesulfonate, x-rays, and ultraviolet light all induced numerous chromosome aberrations, but only ultraviolet light and probably ethyl methanesulfonate induced discrete genetic changes.*

In barley, the frequency of mutations (as opposed to chromosome aberrations) induced by the alkylating agent diethyl sulfate is higher than that obtained by optimum doses of gamma radiation (*1*). Because of this property of diethyl sulfate, an experiment was undertaken with another alkylating agent, ethyl methanesulfonate (EMS), which is reported to induce mutations and reversions in lower organisms (*2, 3*) and mutations in barley (*4*). This alkylating agent can ethylate the guanine moiety in position 7, thereby causing guanine to pair with thymine rather than cytosine during DNA duplications (*5*). The low toxicity of EMS permits use of concentrations that may produce a relatively high frequency of genetic changes (*2*). This experiment was similar to an earlier one in which closely linked markers were used to compare the nature of genetic changes induced by x-rays and ultraviolet light in maize (*6*).

The compound locus A^b and the closely linked gene Sh_2 were used. The components of the A^b locus, α and β, and the Sh_2 locus extend across less than 0.3 map unit in the long arm of chromosome 3. The diagram below illustrates this segment of chromosome 3 with the dominant genes of the treated male parent and their recessive counterparts on the homologous segment of the chromosome of the egg parent.

♂	*α*	*β*	*Sh*
♀	a_m		*sh*

The simultaneous loss of *α*, *β*, and *Sh* is taken as evidence of chromosomal breakage. The absence of the *β* phenotype alone could be due either to the loss of *β* because of chromosomal breakage or to a mutation of the gene.

In preliminary experiments several methods for introducing EMS into pollen were tried. They included immersing cut leaves in a dilute solution, injecting the solution into young tassel shoots, or introducing a solution by means of cotton wick through a hole drilled into the stem directly below the tassel. These methods failed to produce detectible genetic changes. However, the following treatment was successful: the leaves surrounding the shoot were slit open with a razor blade 3 to 5 days before pollen shedding, the tassel branches individually were imbedded tightly in cotton, and the

Table 1. The frequency of gene losses detected as whole and fractional endosperm changes per 10^4 seeds from a^m *sh* by treated *α β Sh*.

Treatment	Population	*α β Sh* (Colorless, shrunken)	*Sh* (Colored, shrunken)	*α β* (Colorless, normal)	*β* (Dilute, normal)
		Whole endosperm loss			
Control*	11499	7	0	0	0
UV*	8888	267	10	0	11
X-ray *	8739	611	7	0	0
EMS†	12131	41	5	0	1
		Fractional endosperm loss			
Control*	11499	16	1	0	0
UV*	8888	397	91	0	12
X-ray*	8739	90	8	0	0
EMS†	12131	216	16	2	0

*Includes data from earlier experiment (*6*); ultra-violet, 30 seconds; x-ray, 1200 r. † EMS, 0.05*M*.