Influence of Severe Hemorrhagic Anemia During Pregnancy on Development of the Offspring in the Rat.* (20543)

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Several extrinsic agents are now known to cause maldevelopment in the mammalian embryo if properly applied during the gestation period. These include: maternal dietary deficiency of vit. A(1,2,3), riboflavin(4,5), pantothenic acid(6), folic acid(7,8), and possibly vit. B_{12}(9); at least 2 viral diseases, namely, German measles(10) and hog cholera(11); injections of such chemicals and drugs as nitrogen mustard(12), trypan blue(13) and cortisone(14); such additional maternal conditions as hypoxia(15) and hypervitaminosis A(16); and finally, exposure to ionizing radiations(17,18). In no case is the mechanism of action on the embryo known, but all of these agents with the possible exception of the last must act either on or through the maternal organism. Several of them are known to cause more or less drastic physiologic alterations in the mother, and the question arises as to what extent this maternal reaction may affect the embryo. As part of a series of studies on this subject, the following investigation of hemorrhagic anemia during pregnancy in the rat was undertaken.

Methods. Anemia was induced by allowing free bleeding from the freshly severed tail on each of 3 successive days. By snipping off a segment of the tail from an animal lightly anesthetized with ether, it was usually possible to collect 4 to 6 cc of blood within a few minutes. The procedure was repeated on the 2 succeeding days, with the result that frequently total quantities equivalent to 6, 7 or more cc/100 g body weight were withdrawn over the 3-day period. Each time blood was removed a 5-drop sample was heparinized for hematocrit reading, and for the same purpose a few drops were taken the day after the last bleeding and at irregular intervals thereafter by opening a caudal vessel. The periods of bleeding were begun at various times during pregnancy so that the height of maternal anemia would coincide with known stages in embryonic development. Accordingly 31 pregnant females were bled on the 7th, 8th and 9th days to cause severe anemia on the 9th day when the mesoderm is forming and on the 10th day when organogenesis is beginning. Forty females were bled on the 9th, 10th and 11th days and 37 on the 11th, 12th and 13th days to produce anemia during the period of most active organogenesis between the 11th and 14th days of gestation. Twenty-five females were bled on the 13th, 14th and 15th days so that severe anemia would occur during the period of late organogenesis and early histogenesis on the 15th and 16th days. Pregnancy was dated from 9 AM of the morning on which sperm were found in the vaginal smear, the embryos being considered *one day of age* 24 hr later. To verify pregnancy and count the number of embryos the females were laparotomized immediately following the first bleeding. Mothers surviving the 3 bleedings were allowed to live until the 20th day of gestation when they were killed and the young again counted, then removed, weighed, examined under a magnifying lens for malformations, and fixed. Two young from each litter were fixed in 95% alcohol for clearing by the Schultz-Dawson method in order to evaluate skeletal development. The remainder were fixed in Bouin's fluid and later dissected or sectioned.

Results. The removal of total quantities of blood equivalent to 5 cc or more/100 g body weight caused a precipitous drop in hematocrit values (Table I). In general the extent of drop was proportional to the total quantity of blood removed, but this was subject to considerable variation. The effects of anemia on survival of the mother and her offspring were more closely correlated with quantity of blood removed than with hematocrit reading, for which reason the following

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TABLE I. Hematocrit Reading before and after Induced Hemorrhage on 3 Successive Days, Beginning at Different Times in Pregnancy.

Days bled	Avg hematocrit readings (%) Before first bleeding	24 hr after 3rd bleeding (amt removed—cc/100 g body wt) 5–5.9 cc	6–6.9 cc	7 or more cc
7, 8 and 9	43.3 ± 2.8	21.0 ± 3.2	15.8 ± 1.3	16.0 ± 2.6
9, 10 and 11	42.2 ± 2.9	19.3 ± 1.7	17.4 ± 2.3	14.9 ± 2.1
11, 12 and 13	39.2 ± 4.9	18.1 ± 1.7	16.5 ± 2.8	14.8 ± 1.9
13, 14 and 15	39.7 ± 2.6	17.8 ± 3.0	16.6 ± 1.8	14.4 ± 1.4

± = Stand. dev.

TABLE II. Effects on Gestation and on Offspring of Removing Various Quantities of Blood from Female Rats at Different Times in Pregnancy.

Total blood withdrawn (cc/100 g body wt)	No. of mothers	Pregnancies terminating before 20th day: % maternal death	% complete resorption	Pregnancies going to 20th day: %	% young resorbed*	Avg wt young (g) †	Condition of young
		Bled on 7, 8 and 9th days pregnancy					
4–4.9	7	0	0	100	9	3.57	All normal
5–5.9	9	0	33	67	7	3.56	"
6–6.9	7	43	57	0	—	—	—
7–	8	62	13	25	4	3.38	All normal
		Bled on 9, 10 and 11th days pregnancy					
5–5.9	12	8	25	67	6	3.39	1 litter retarded
6–6.9	15	20	27	53	18	3.20	3 young malformed; 1 litter retarded
7–	13	23	38	39	12	3.18	"
		Bled on 11, 12 and 13th days pregnancy					
5–5.9	7	0	0	100	13	3.41	All normal
6–6.9	21	38	0	62	10	3.38	"
7–	9	44	0	56	5	3.11	1 litter retarded
		Bled on 13, 14 and 15th days pregnancy					
5–5.9	5	20	0	80	11	3.35	All normal
6–6.9	13	54	0	46	0	3.30	"
7–	7	71	0	29	0	3.10	1 litter retarded

* May reach 14% in litters from non-anemic mothers.
† Mean wt of young from non-anemic mothers = 3.34 ± .34 g.

data are presented in relation to quantity of blood removed. Ten non-pregnant adult females bled amounts equivalent to 6 cc or more/100 g body weight showed lowering of hematocrits comparable to those in Table I. Six died within 2 days following the last bleeding, indicating that the treatment was as deleterious to non-pregnant as to pregnant females.

Bleeding on 7-9th days. Removal of less than 5 cc of blood/100 g body weight was without effect on the duration or outcome of pregnancy (Table II). All mothers survived and continued their pregnancies to the 20th day. Nine per cent of offspring underwent intrauterine resorption after laparotomy on the 7th day, but this is within normal range, which may reach 14% in the offspring of non-anemic, laparotomized females. The mean weight of surviving young was normal, and no developmental defects were observed in dissected, sectioned or cleared specimens. Removal of 5 to 5.9 cc of blood/100 g body weight also did not kill any mothers but did

result in early termination of pregnancy in one-third of the cases due to resorption of the entire products of conception (Table II). In pregnancies which continued to the 20th day, however, the offspring were normal in all respects. Hemorrhage in excess of 6 cc/100 g body weight caused early termination of pregnancy in all but 2 of 15 cases, due either to maternal death or complete resorption of young. It is particularly noteworthy that the 2 pregnancies which survived this drastic treatment yielded litters of normal offspring.

Bleeding on 9-11th days. Again removal of quantities of blood between 5 and 5.9 cc/100 g body weight caused premature termination of approximately one-third of pregnancies, but those litters which reached the 20th day were essentially normal (Table II). Young in one of 8 surviving litters were retarded in growth or skeletal development but none was malformed. When 6 to 6.9 cc of blood/100 g body weight were removed, a more pronounced effect was observed in a few cases. Although 8 of 15 pregnancies continued to the 20th day, the mean rate of prenatal death in the surviving litters was increased, one litter contained young that were retarded in growth, and 2 others contained a total of 3 grossly malformed young (Table II). No one of these deviations is in itself convincing evidence that maternal anemia interfered with development, but in the aggregate they indicate that anemia of sufficient severity at this time (11th day and subsequently) has some tendency to affect the offspring. That this tendency is slight and variable is demonstrated by results obtained from mothers bled 7 or more cc/100 g body weight. Although only 5 of 13 such pregnancies continued to the 20th day, there was no significant abnormality in the young. When surviving young from all mothers bled in excess of 6 cc/100 g body weight are combined, it is found that only 3 of 117 or 2.6% were malformed. Thus, it is concluded that although an occasional abnormality may be produced at this time, maternal anemia usually causes either early termination of pregnancy or else permits continuation of pregnancy without detriment to the young. The 3 malformed young showed various skeletal dysplasias and distortions, such as shortening of the snout and extremities. Two exhibited combined polydactyly and syndactyly. The eyes were malformed in 2 instances, the optic cup in one and the lens in another. Aside from developmental defects, all were noted to be edematous at delivery and on sectioning to have foci of degeneration in the central nervous system. Basal ganglia, certain regions of cerebral cortex, and the anterior horns of the spinal cord were the commonest sites of degeneration, and often in these regions signs of localized hemorrhage were evident. Such a pattern of abnormality has not been encountered in several hundred young rats from nonanemic mothers examined in this and other experiments. This, together with the fact that all 3 animals were similarly affected, suggests that the abnormalities were a consequence, however rare, of the maternal anemia.

Bleeding on 11-13th days and on 13-15th days. The effects of hemorrhage during these 2 periods were essentially similar except that bleeding in the later period caused a higher rate of maternal death (Table II). In neither instance was there a significant effect on the young in pregnancies continuing to the 20th day, although one litter from each group of mothers bled more than 7 cc/100 g body weight contained young moderately retarded in growth. Unlike some bled at earlier periods none of these mothers was recorded as having resorbed her entire litter. There was, nevertheless, evidence that death of all the fetuses sometimes preceded that of the mother. Several of the mothers were autopsied soon after spontaneous death, between the 14th and 16th day of gestation; and in at least 4 of these the fetuses were found to have been in process of resorption at the time of maternal death. Whether the fetal death contributed to the maternal death in such cases is uncertain, but it is noteworthy that at the time the mothers died the fetuses, not including membranes and decidua, each weighed 0.2 to 0.5 g. The resorption of several g of necrotic fetal and accessory tissue certainly placed an added burden on an already impaired maternal organism. Maternal death occasionally attributable to death and resorption of the fetuses

seems likely.

Comment. These results indicated an all-or-none mechanism concerning the welfare of the embryo under conditions of maternal hemorrhagic anemia. Either the anemia had little or no effect on the offspring, or its effects were such as to cause maternal death or complete resorption of the conceptus. There was virtually no intermediate condition in which the offspring survived, but under duress or deprivation sufficient to cause maldevelopment. Evidently the physiologic stresses resulting from hemorrhagic anemia are of a nature that affect the viability of the mother or interfere with continuation of her pregnancy before they affect the processes of development. Only bleeding of the mothers on the 9th, 10th and 11th days showed any tendency to interfere with development in surviving embryos, and this was so limited that hemorrhagic anemia at this or any other time can hardly be regarded as a real hazard to normal embryonic development in the rat.

This is in contrast to the action of the known teratogenic agents with which it is usually possible to demonstrate three dose-response relationships, that is, three degrees of effect of the agent upon the offspring. The agent in mild or moderate dose may have no effect, or at somewhat higher doses it may prove universally fatal to all embryos or to the mother. Between these limits exists what can be called the teratogenic zone, a more or less narrow zone in which embryonic life is able to continue but frequently at the expense of impairment or aberration in normal development. A significant teratogenic zone was not clearly demonstrated for hemorrhagic anemia in the rat. This implies that the mere placing of severe physiologic stress upon the pregnant mother is not enough to cause maldevelopment among the offspring. To act as a teratogenic agent it is probably necessary that such stress bear a specific relation to the needs or welfare of the embryo at the time the agent is operative.

Summary. Pregnant rats were rendered severely anemic by permitting free bleeding from the freshly severed tail on 3 successive days. This frequently caused early termination of pregnancy as a result of maternal death or resorption of the entire litter, but litters from mothers surviving this treatment were essentially normal. Only when bleeding was begun on the 9th day was there a minimal effect on the offspring, manifested by malformations in 2.6% of the young, a mild degree of retardation of growth in 4 of 13 litters, and a slight increase above the normal rate of intrauterine resorption in litters reaching term. Since even these minimal effects were not observed after hemorrhage at other times during gestation, it is apparent that maternal hemorrhagic anemia represents no particular hazard to the offspring in surviving litters. This suggests that severe physiologic stresses during pregnancy, however severe, are not always capable of causing abnormality in the offspring.

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