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Effect of Thalidomide Treatment of Hens on Embryonic Development and Fertility.* (28443)

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While thalidomide has been associated with an increased number of malformations in human embryos(1), only a few studies have reported malformations in other mammals. Malformations have been reported in rabbits (2,3,4) and in rats(5). Lack of malformations or a significant increase in resorption of embryos has been noted by Sellers(4), Christie(6) and Felisati(3) in thalidomide-treated rats, mice and rabbits. Dalderup(7) suggested abnormalities of dentition might occur. Lucey(8) observed no live births in 44 treated monkeys while there were 11 live births in 57 untreated monkeys. Kemper(9) found 20% gross malformations in embryos from hen's eggs injected with thalidomide and delayed development of the germ in eggs injected before incubation. Kemper and Berger(10) also noted blood changes in 10-week-old chicks given thalidomide daily by stomach tube for 28 to 35 days. A pronounced anemia, with blockage of granulopoiesis believed to be connected with folic acid metabolism, was noted from the 10th day on. Verrett and McLaughlin(11) injected eggs with 5 mg/egg of a thalidomide suspension in alcohol and found 80% hatched with no gross limb malformations. Some embryos that failed to hatch were malformed. Thalidomide in dioxane gave increased incidence of embryonic limb malformation, although no malformations were caused by the same dose of dioxane alone. No report has been found as to the effect on embryos when hens were fed thalidomide. In daily feeding of hens, the drug might be expected to accumulate in the preformed yolk and thus affect the embryo. Experiments to test this supposition have been carried out.

Experimental. Pre-drug treatment of hens. Thirty Heisdorf and Nelson strain, single-comb White Leghorn hens, hatched in March, 1962, were range reared until 7½ months of age when they were housed in laying cages. They had been treated for worms with piperazine citrate 9 days before the experimental period began. These hens were inseminated with pooled semen from 6 Cornell, random-bred White Leghorn males, 7½ months of age, 2 days before the start of the 10-day pre-drug egg collection period, and every 7th or 8th day thereafter. Enough semen was used to give good fertility for 14 days. Eggs collected daily for 10 days before drug treatment began were incubated and examined for infertile eggs and malformed embryos as described below. Three hens had low egg production and were discarded.

Drug treatment of hens. In all experiments, thalidomide was fed in gelatin capsules, by mouth, in the late afternoon. Eggs were collected daily from the start of the drug treatment. All eggs were stored at 8°C and incubated at 5-day intervals. Eggs were candled after 5 days of incubation. All eggs appearing to be infertile were opened and

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examined. Fertile eggs from the drug-treatment periods were opened on the 10th or 12th day of incubation, examined and saved for histological examination. Fertile eggs from periods between drug treatment were carried to hatching and chicks or dead embryos examined for abnormalities. In a preliminary trial to test drug dosage, 3 hens were fed a single dose of 15, 25, or 50 mg of thalidomide and watched for any hypnotic or narcotic effect. The only symptom noted was that the hens were easier to handle. Four days later a larger single dose was given to the same hens, 100, 200 and 500 mg, respectively. The same effect was noted.

The remaining 27 hens were distributed into 3 groups, containing 9 hens each, each group having about equal egg production. The sequence of treatment, amount of drug given daily, total dose and rest periods for groups II and III are shown in Table I. Group I served as the control group throughout.

Results. Table I summarizes the results of treatment for each group of hens as to normal embryos or chicks, deaths at 3 periods of incubation, malformations and infertile eggs. Fertile eggs showing no development past stage 2(12), embryos dying from stage 3 through 12 days of incubation and chicks dead after 12 days of incubation which failed to hatch, have been tabulated separately under "deaths."

Hens in all groups during the pre-drug treatment period had the same fertility (65.6-67.7%, average 66.9%). This is not unusually low for the first insemination period. Hens in Group I, the control group, had the highest fertility, *i.e.*, the greatest number of normal embryos in all test periods (75-89.3%).

There was a reduction in number of normal embryos in Group II fed 2.5 mg thalidomide/kg/day (22.8% lower than controls) and in Group III fed 5 mg/kg/day (19.0% lower). There were also fewer normal embryos after the second drug dose of 25 mg/kg/day (Group II, 12.8% fewer) and the 85 mg/kg/day treatment (Group III, 9.8% lower). It would appear that a tolerance might be built up to the higher drug doses, since this same effect carried over into both post-drug treatment periods when the number of normal chicks hatching was lower than the controls by 35.2% (2.5 mg daily dose); 13.8% (5 mg dose); 13.7% (25 mg dose) and 4.7% for the 85 mg daily dose. Daily records (data not given) showed that in the first drug period of Group II, the number of embryos decreased as the days of drug feeding increased up to the 8th day of feeding. The lowest number of normal embryos in Group III occurred on the first, 8th and 9th days of the first drug period. In the second drug period, the lowest number of normal embryos occurred on the first day of drug treatment.

With the decrease in numbers of normal embryos on drug treatment, there was an increase in numbers of either infertile eggs, fertile eggs with no development, or early death of embryos. There was no increase in grossly malformed embryos with drug; but, on the other hand, more malformed embryos occurred in the control group. The malformations were usually exposed heart and viscera. One embryo from the control group had shortened limbs. Other early-dead embryos had incomplete formation of the vascular system. All embryos were saved for histological studies. The results of this incompleted work will be published separately. However, gross malformation of limbs, as reported for human embryos, did not result from the drug treatment of hens. The greatest effect of the drug was on the number of infertile eggs, or on the number of fertile eggs which failed to develop beyond stage 2.

Thalidomide is stored in the yolk of the ovary. That storage lasts for at least 30 days was apparent in a preliminary experiment on feeding thalidomide to males. The same hens used in the experiments reported here (which were proved infertile after 30 days without drug and reinsemination) were inseminated with sperm from males fed thalidomide for 4 days. Too few eggs were collected and the males were on drug for too short a time to have any significance in male fertility. However, the previous treatment of hens influenced fertility, and the effect lasted over 30 days after drug treatment was stopped. This

TABLE I. Distribution of Normal Embroys or Chicks, Early Deaths, Deaths Before Hatching, Malformed Embryos, and Infertile Eggs from Hens Fed Thalidomide.

Hen group No.	Sequence of treatment	Days	Amt of thalidomide Daily (mg/kg)	Amt of thalidomide Total (mg)	No. of eggs incubated	Normal embryos* (%)	Normal chicks hatched (%)	Deaths Stage 2† (%)	Deaths Stage 3 to 12 days of incubation (%)	Deaths Before hatch (%)	Grossly malformed embryos (%)	Infertile eggs (%)
I	Pre-drug	10	None	None	52	67.3		3.8	1.9		0	26.9
II			"	"	62	67.7		1.6	1.6		1.6	27.4
III			"	"	64	65.6		1.6	0		0	32.8
I	1st	14	"	"	94	88.3		1.1	1.1		0	9.6
II	drug		2.5	70	113	65.5		10.6	1.8		0	22.1
III	treatment		5.0	140	114	69.3		5.3	1.8		0	23.7
I	1st	7	None	None	54		75.9	13.0	3.7	3.7	0	3.7
II	post-drug		"	"	54		40.7	20.4	14.8	16.7	0	7.4
III	treatment		"	"	58		62.1	12.1	5.2	10.3	0	10.3
	Rest period	15										
I	2nd	10	None	None	56	89.3			1.8		1.8	7.1
II	drug		25	500	68	76.5			1.5		1.5	20.6
III	treatment		85	1700	78	80.8			3.8		1.3	14.1
I	2nd	5	None	None	24		75.0			16.7	0	8.4
II	post-drug		"	"	31		61.3			19.4	0	19.4
III	treatment		"	"	37		70.3			21.6	0	5.4

* Examined at 10-12 days of incubation.

† Fertile eggs, no development beyond stage 2(12).

TABLE II. Effect of Thalidomide on Production.

Treatment period	Hen-day egg production		
	Group I (control)	Group II (low drug dose)	Group III (high drug dose)
Pre-drug period	.66	.71	.81
1st drug treatment	.88	.91	.93
1st post-drug treatment	.89	.86	.92
Rest period	.76	.67	.90
2nd drug treatment	.64	.67	.90
2nd post-drug period	.53	.69	.82

was evident in the fact that eggs from hens of Group I (controls) produced 95.5% normal chicks; eggs from Group II hens had 91.2% normal chicks and eggs from Group III, given the highest thalidomide doses, had 81.3% normal chicks. Of the last group, 12.5% of the embryos died at various stages and 6.3% of the eggs were infertile.

The drug had no effert on the hen-day egg production (Table II). The seeming stimulatory effect of the drug on Groups II and III was the result of a greater proportion of hens in the control group going into molt.

Discussion. More work should be done on the length of storage of thalidomide in eggs. If no drug were removed from the yolk, eggs in the ovary might be affected for a long period. In respect to storage of thalidomide, the hen may be somewhat different from mammals. MacKenzie and McGrath(13) observed rapid metabolism and excretion of thalidomide in rats, and Pliess(14) found no abnormal young from female rats receiving large doses of thalidomide. However, malformations can be induced in rats under some conditions(5) and in hen's eggs when they are injected(9,11). Rabbit embryos, mentioned previously, are sensitive to thalidomide (2). Sperm or the ovum in monkeys must also be sensitive to thalidomide since Lucey (8) observed no live births in the treated monkeys, and advanced the hypothesis that thalidomide killed the embryo prior to implantation. This hypothesis is supported by the work reported here.

The increase in number of infertile eggs and of fertile eggs which failed to develop suggests that the drug may affect the ability of the sperm to penetrate the egg or of the germ cell to divide, and that the effect may be on the total cell metabolism. That the cells are generally affected is also apparent in the number of chicks which failed to get out of shell and the general unthrifty appearance of the hatched chicks from the drug-treated hens. Unfortunately no record was made of the weights of the hatched chicks. These observations are in line with the observations of Frank *et al.*(15) that growth of some protozoa was inhibited by thalidomide, and reversed by nicotinic acid, nicotinamide, nicotinamide adenine dinucleotide and vitamin K_1. They suggest the mechanism of toxicity was interference of cellular oxidation. However, the validity of their work might be questioned, since the method used in solubilizing thalidomide may have changed the compound.

Summary. Thalidomide fed in total doses of 70, 140, 500 or 1700 mg per hen (daily dose of 2.5 mg, 5.0, 25 and 85 mg/kg/day) increased the number of infertile eggs, fertile eggs which failed to develop and deaths in early and late stages of development. Egg production was not affected. Thalidomide treatment did not lead to an increase in gross malformations of the embryo. The effect of the drug was apparent on embryological development in the hen for as long as 30 days after discontinuing the drug.

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