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Effect of Maternal Administration of Dimethyl Sulfoxide on the Development of Rat Fetuses. (32148)

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Dimethyl sulfoxide (DMSO) has been widely used for inherent medicinal properties (1,2,3), and for enhancement of drug penetration through the skin(4); however, its embryocidal and teratologic properties have not been fully explored. Caujolle *et al*(5) did not provide data but reported that oral administration of DMSO to rats of both sexes at 5 g/kg/day for 4 days before coitus and then continued in the pregnant females throughout gestation did not noticeably interfere with fertility or development of the young. Abortions were subsequently obtained following administration of undefined doses of DMSO throughout pregnancy(6). Single intravenous injections of up to 12 g/kg to mice on undefined days of gestation significantly increased numbers of resorptions. Subcutaneous administration of 2 and 4 g/kg/day to the rabbit throughout gestation did not alter normal reproductive processes. Malformations were not observed in any of the above studies.

To test a possible teratogen in a given species an agent should be administered during the period of organogenesis at doses which do not affect the maternal organism but which would ideally result in partial loss of young (*e.g.*, fetal LD 50). If under these conditions no malformations result among the surviving offspring the agent is not likely to be teratogenic in the species tested(7).

The doses used to date in the rat were not large enough to be embryocidal and since the mammal may be able to adapt to the continued presence of a teratogen if administered in low dosage prior to the period of organogenesis(8) this study was initiated to determine whether malformations would result following an embryocidal dose of DMSO administered to the rat only during the period of organogenesis.

Methods. Forty mature Sprague-Dawley (C-D) female rats obtained from Charles River Laboratories were mated to males of the same strain. The controls received subcutaneous injections of distilled water (10 ml/kg/day) on Days 8, 9 and 10 of gestation (Day 1 being the day sperm were observed in the vaginal lavage). The remaining rats were administered DMSO† as a 90% aqueous solution on Day 8, Days 8 and 9, or on Days 8, 9 and 10 at 10.25 g/kg/day; one-half of the maternal LD 50 per injection as determined previously for the subcutaneous route(9). Body weights were recorded on Days 1, 8 and 19 of gestation. All female rats were killed on Day 19 by cervical dislocation and the reproductive status was determined. All live, dead and resorbed fetuses were weighed and the young examined for gross developmental abnormalities. The fetuses were then fixed in 70% ethanol prior to clearing to allow skeletal examination(10). A multiple range test(11) was applied for group comparisons.

Results. DMSO did not significantly alter maternal body weight gain, nor the weight of the live fetuses obtained on Day 19. No

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† As obtained from Fisher Scientific Co., Fair Lawn, N. J.

TABLE I. Effect of Maternal DMSO Administration During Pregnancy upon Fetal Development.

Group	Total dose (g/kg)	Live young day 19: Rats with live young (No./gp)	Live young day 19: Avg No. per litter (mean ± SE)	Live young day 19: Avg body wt (g ± SE)	Resorptions day 19: Rats with resorptions (No./gp)	Resorptions day 19: No. resorptions/gp
Control	—	9/9	13.8 ± .7	1.59 ± .1	0/9	0
DMSO (day 8)	10.25	8/8	13.2 ± .6	1.62 ± .1	6/8	6
" (days 8 & 9)	20.50	8/8	13.1 ± .8	1.56 ± .05	5/8	7
" (days 8, 9 & 10)	30.75	13/15*	9.8 ± 1.1†	1.53 ± .07	11/15	58

* Remaining two had resorptions only.
† Significantly different from remaining groups ($P < 0.05$).

maternal deaths occurred and their health seemed unimpaired.

After 3 injections of DMSO (Table I) litter size was significantly decreased ($P<0.05$). Even though 2 females had only resorptions, and the remaining females an average of 5.3 ± 1.4 resorptions each, only 2 of the 127 live young obtained were grossly malformed (slight umbilical hernias) and none exhibited skeletal alterations upon being cleared. A single dead and macerated fetus, which was small and only partially developed, was obtained from the group that received two injections. No skeletal defects or malformations were observed among the remaining fetuses in the study.

The incidence of resorptions among females in the experimental groups appeared to be positively related to DMSO dosage but only in the group injected three times was the average number of resorptions per female significantly different ($P<0.05$) from that obtained in the controls (Table I).

Discussion. Ferm(12,13) reported on the teratogeny of DMSO in the hamster. A significant teratogenic response was obtained following a single intraperitoneal injection of 0.5 ml of 100% DMSO on Day 8 of gestation. The embryos recovered 1, 2 and 3 days later revealed gross cranial defects. After intravenous injection of 50 to 8250 mg/kg on Day 8(13) cranial and other assorted malformations were obtained with doses of 2500 mg/kg and above. Maternal tremors were observed among females receiving 5500 mg/kg or more.

In the current study no maternal effects were noted after subcutaneous administration of 10.25 g DMSO/kg/day for up to 3 days, and even though about one-third of the embryos were resorbed no gross or skeletal cranial defects were observed among the living young when observed on Day 19 of gestation. The dead and macerated fetus obtained did have an abnormally shaped head but interpretation of this finding was impossible in view of the condition of the specimen. Ferm(12,13) removed the hamster young in a more immature state than was done in in this study; it would be interesting to know whether the malformed hamster young seen would survive to term and/or to know whether the resorptions obtained in the rat were live, malformed young earlier in gestation.

Caujolle *et al*(6) obtained no malformations in rats but did note abortions after use of DMSO. In the current study, no abortions were obtained prior to Day 19 in rats after administration of doses higher than those reported by Caujolle, but considerable resorptions were obtained. The 2 cases of slight umbilical hernias noted in the intermediate DMSO dose group could not be attributed solely to the effects of the drug.

Summary. Subcutaneous injection of 31 Sprague-Dawley rats with 10.25 g DMSO/kg body weight/day on Day 8, Days 8 and 9, or Days 8, 9 and 10 of gestation did not significantly influence the body weight gain of the mothers during pregnancy or of the live young obtained on Day 19. A significant decrease in the average number of fetuses per litter and a corresponding increase in the number of resorptions was obtained from mothers given 3 injections of DMSO. With the exception of 2 slight umbilical hernias in the middle dose group, no gross or skeletal malformations were obtained among the 338

live fetuses recovered from the mothers that received DMSO.

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Biosynthesis of Fatty Acids in Blood and Bone Marrow of Normal and Anemic Rabbits. (32149)

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Studies involving incorporation and biosynthesis of fatty acids by blood have yielded data indicating that mixed cells of whole blood and erythrocytes(1-10), erythrocytic ghosts(10,11), platelets(12), reticulocytes(2), and leukocytes(2,5,13-16) can utilize (^{14}C)-labeled fatty acids. Most of the activity has been found in leukocytic lipids and it has also been reported that erythrocytes can synthesize a variety of long-chain saturated and polyenoic acids(1,7,10,13,15,16). Plasma lipids have been found to be labeled in experiments employing plasma as suspending medium for blood cells(1,3,5,6).

This report describes studies which were conducted on the biosynthesis of fatty acids by cellular elements of blood and bone marrow tissue from normal and phenylhydrazine-treated rabbits. In addition, data are given on the activity found in the lipids of plasma which served as suspending medium.

Materials and methods. Precursor fatty acid. Sodium (2-^{14}C) acetate was obtained from Radiochemical Centre, Amersham, England. It was made up to a concentration of 10 μc/0.1 ml with 0.85% NaCl solution and dispensed at the rate of 1-2 μc/ml of blood and bone marrow cell suspension.

Preparation of blood. Blood was collected in heparin by exsanguination of 2- to 3-month-old New Zealand White rabbits. Packed cell volumes averaged 42% and reticulocytes remained below 0.5% in blood from normal rabbits.

In order to produce an artificial anemia, rabbits were injected subcutaneously with an aqueous solution of phenylhydrazine hydrochloride at the rate of 4 mg/kg body weight/day for 7-9 days and bled 2 days after the last injection. Percentage of reticulocytes ranged from 69-93% and packed cell volumes from 22-34%.

Fifty ml of blood were used for incubation with sodium (2-^{14}C) acetate.

Bone marrow preparations. Bone marrow was collected from rabbits that were used to provide material for the blood studies. Tissues obtained from 2-8 femurs were shaken in plasma at 38°C for 15 min to separate and suspend the cells. The suspension was recovered by centrifugal sedimentation, discarding the floating fatty tissue and the sedi-

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