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SERUM PROTEINS AND TERATOGENESIS[1]

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Observations have been reported which suggest that altered electrophoretic patterns of the serum proteins may accompany pregnancies that terminate in the birth of abnormal offspring. The teratogenic disazo dye, trypan blue, has been shown to cause alterations in the proteins of the maternal serum of pregnant rabbits and in the protein composition of the 9-day rabbit embryo yolk sac fluid (1,2,3). These papers report a significant increase in the alpha and beta globulins. An "abnormal" protein band has been reported in male rats treated with heavy doses of trypan blue (4,5). This "abnormal" protein band was subsequently shown to represent an alteration in a normally occurring alpha globulin component of rat serum (6,7). Evidence has been presented that serum proteins of the fetal rat are altered when the mother is treated with a teratogenic dose of trypan blue (6). Significant differences are reported between control and experimental fetuses in beta globulins, alpha-1 globulins and albumin.

Abnormal electrophoretic patterns of serum proteins have been reported in human pregnancies (8). An abnormal outcome was observed in 78% of those women exhibiting an abnormal electrophoretic pattern of serum proteins at some time

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during pregnancy. On the other hand, of the pregnancies showing normal serum proteins, only 13% terminated abnormally.

The present experiment was undertaken in an effort to determine if alpha and beta serum globulins can affect the outcome of pregnancy.

Methods

Virgin females of Wistar Albino rats (Albino Farms, Red Bank, N.J.) were used in this study. The animals were maintained on a Rockland Complete Rat Diet ad libitum. Day 0 of pregnancy was considered to begin on the morning sperm was found in the vaginal smear. Rats received a teratogenic dose of trypan blue during the 8th day of pregnancy (14 mg of dye per 100 gm body wt). Forty-eight hours later the rats were exsanguinated and the serum pooled for subsequent fractionation by a commercial laboratory. Samples for control serum were obtained from non-treated 10-day pregnant rats.

A sample of each alpha and beta fraction was subjected to paper electrophoresis to test its purity. Alpha globulin fractions were about 75% pure and beta globulin fractions about 90% pure. Colorimetric determinations of the samples from trypan blue treated pregnant rats revealed the alpha globulin fraction contained 0.06 mg of trypan blue per 100 gm sample and the beta globulin fraction 0.04 mg per 100 gm sample. The globulin fractions were prepared in saline for injection into rats during the 8th day of gestation. The dose administered (150 mg of alpha globulin; 100 mg of beta globulin) was calculated to approximately double the normal physiological amount of globulin present in a 250 gm rat during the 8th day of pregnancy (142 mg of alpha globulins; 110 mg of beta globulins).

At the time of intracardiac injection a volume of blood was withdrawn from the recipient rat equal to the volume of globulin solution to be injected (1.5 ml). Forty-eight hours after injection blood was withdrawn and serum obtained. Blood was again drawn on the 20th day just prior to autopsy. The serum was analyzed by the Spinco paper electrophoresis system. The relative

concentration of each component was determined in the Spinco Analytrol. Total protein content of the serum was measured with a Bausch and Lomb Serum Protein Meter. Fetuses obtained at autopsy were examined for the presence of malformations.

Results

Table I summarizes the fate of rats receiving injections of alpha and beta serum globulins. Globulins from untreated rats were well tolerated. On the other hand, globulins from pregnant rats treated with trypan blue caused a high maternal mortality. Embryonic resorption occurred in all treated groups, although no fetal malformations were observed.

TABLE I

Effect of intracardiac injection of alpha and beta serum globulins into female rats on the 8th day of gestation

Treatment	Number of mothers	Number of maternal deaths	Number of implantations	Number resorbed	Number malformed
control alpha globulin	9	2	73	4	0
trypan blue alpha globulin	12	3	80	3	0
control beta globulin	7	1	78	1	0
trypan blue beta globulin	9	6	42	3	0
untreated	13	0	162	2	0

TABLE II

Total protein and protein fraction concentration in rats treated with alpha and beta serum globulins

Treatment	Number of mothers	Day of gestation	Total protein*	globulins* gamma	beta	alpha-3	alpha-2	alpha-1	albumin*
control beta globulin	6	10	6.04±.22	0.69±.14	1.20±.14	0.39±.03	0.34±.09	0.82±.09	2.60±.30
		20	6.16±.46	0.37±.07	0.95±.14	0.41±.10	0.45±.20	1.45±.14	2.53±.41
trypan blue beta globulins	3	8	6.70±.10	0.79±.07	1.14±.21	0.24±.01	0.24±.01	0.78±.17	3.51±.05
		10	5.15±.09	0.62±.10	1.05±.10	0.32±.07	0.23±.02	0.71±.06	2.22±.50
		20	5.90±.41	0.38±.10	0.98±.18	0.34±.12	0.42±.03	1.44±.28	2.34±.05
untreated	13	8	6.30±.43	1.09±.33	1.09±.10	0.32±.04	0.35±.02	0.78±.02	2.65±.36
		10	6.25±.54	0.87±.26	1.08±.12	0.32±.04	0.30±.06	0.76±.14	2.86±.36
		20	6.40±.52	0.49±.14	0.93±.13	0.36±.06	0.40±.10	1.54±.22	2.65±.30
control alpha globulins	7	10	5.83±.30	0.59±.14	0.96±.15	0.28±.05	0.25±.05	0.70±.09	3.05±.34
		20	6.25±.54	0.33±.08	1.01±.11	0.38±.09	0.44±.22	1.55±.13	2.50±.31
trypan blue alpha globulins	4	8	6.56±.54	0.77±.10	1.00±.04	0.27±.03	0.27±.03	0.70±.17	3.58±.48
		10	6.11±.59	0.70±.03	1.06±.06	0.27±.03	0.27±.01	0.75±.01	3.06±.57
		20	6.30±.30	0.32±.03	0.90±.10	0.39±.01	0.39±.01	1.49±.31	2.81±.53

*concentration expressed as grams per 100 ml with standard deviation

Table II presents the values for the concentration of serum globulins and albumin, as well as, total protein concentration in untreated pregnant rats and in pregnant rats treated with serum globulins. Inspection of the results suggest a reduction in total protein and albumin following administration of beta globulins derived from trypan blue treated pregnant rats. A statistical analysis was not done because of the low number surviving the treatment. Gamma, alpha, and beta globulins were unaffected. Injections of beta globulins from untreated pregnant rats or alpha globulins from treated or untreated rats, did not alter total proteins or serum components beyond the expected normal range.

Conclusions

Treatment of pregnant rats with serum alpha and beta globulins derived from trypan blue treated pregnant rats did not cause malformations in the offspring. The number of resorptions was only slightly increased by this treatment. Since electrophoresis showed the samples to be relatively free from contamination we can assume that the altered alpha and beta globulins cannot, in themselves, be responsible for the induction of malformations in developing rat fetuses. Information currently available suggests that the altered serum proteins are a secondary manifestation of the effects of trypan blue in the rat, perhaps resulting from interference with normal hepatic function. The lowering of total proteins observed following injection of beta globulins from trypan blue treated pregnant rats is also known to occur following administration of the teratogenic disazo dyes as well as following such varied insults as whole body x-irradiation (9) and vitamin deficiency (10). The results of this paper do not lend support to the theses that a causal relation exists between altered serum proteins and abnormal development.

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