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THE EFFECT OF TRYPAN BLUE ON THE POSTNATAL DEVELOPMENT OF SERUM PROTEINS[1]

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The disazo dye trypan blue is known to cause alterations in the serum proteins of the male rat [1,2,3,4] and the pregnant rat [5]. Common to all reports is a decrease in total proteins and albumin and an increase in the alpha globulins. At first the increase in alpha globulins was postulated to be due to the formation of abnormal protein [1,3] but subsequently was shown to be due to a quantitative increase in alpha globulins normally present in rat serum [4,5]. Serum from pregnant rats treated with trypan blue exhibited, in addition to the above changes, a rise in the beta globulins. A similar elevation in serum beta globulins has been reported in the pregnant rabbit following trypan blue injection [7,8,9]. Alterations also have been demonstrated in the serum of fetal rats from trypan blue treated mothers; the fractions altered were albumin and the alpha and beta globulins [6].

The present investigation was undertaken to follow the postnatal development of the serum proteins in rats born to normal mothers and in rats born to mothers treated with trypan blue. It was of prime interest to determine whether or not the

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abnormal serum protein patterns seen in fetuses of treated mothers persisted into adult life.

Methods

Virgin females of Wistar Albino rats (Albino Farms, Red Bank, New Jersey) were used in this study. The animals were fed Rockland Complete Rat Diet ad libitum, with supplemental feedings of lettuce. Day 0 of pregnancy was considered to begin on the morning sperm was found in the vaginal smear. Pregnant rats were given a single intraperitoneal injection of a 2 per cent aqueous solution of trypan blue during the 8th day of gestation (14 mg of dye per 100 gm maternal body weight). Following normal parturition, during the 21st or 22nd day, the newborn rats were examined for malformations and the animals judged to have the best chance for survival were selected for the experiment. Blood was obtained from newborn rats by cutting the major vessels in the neck and collecting the blood in a shallow depression in a piece of lucite. The blood was transferred to centrifuge tubes, allowed to clot, and the serum recovered. Cardiac puncture was used to obtain blood from rats three weeks old and older. Thus, it was possible to draw blood from the same rat at weekly intervals from the third postnatal week until the end of the experiment. Each blood sample was run separately. The total protein content of the serum was measured with a Bausch and Lomb Serum Protein Meter. Paper electrophoresis was carried out in a Spinco cell using Spinco B-2 buffer (Veronal at pH 8.6; 0.075 ionic strength). The paper strips were run for 17 hours at 3.5 ma. The strips were subsequently stained with bromphenol blue and their density was measured in a Spinco Analytrol (recording densitometer) to determine the relative concentration of each

component. Control animals were run concurrently with the experimentals.

Results

Table 1 summarizes the results of this experiment. The total protein concentration and the concentration of the alpha-1 globulins and albumin was significantly less in serum of newborn rats from trypan blue treated mothers than in serum of newborn rats from untreated mothers. During the first two weeks of postnatal life these differences vanished. Serum samples taken from both experimental and control animals on the 14th day after birth had essentially the same concentration in all fractions and in total protein. The only congenitally malformed rats to survive for the duration of the experiment were those having either microphthalmia or apparent anophthalmia.

Conclusions

The results of this experiment show that the normal postnatal development of rat serum protein fractions is accomplished by a gradual increase in the concentration of each fraction from birth until sometime after the eighth week of life, at which time the adult values are attained. Fetal rats (20th day) from trypan blue treated mothers have a significant deficiency in total protein, alpha-1 and beta globulins, and albumin [6]. At the time of delivery, as shown in the present study, the deficiency in beta globulins no longer exists and by the 14th day of postnatal life there is no difference between serum from fetuses of normal mothers and serum from fetuses of dye treated mothers.

There is an apparent correlation between serum protein deficiency and the presence of a malformation at birth; however,

TABLE 1
Total Protein and Protein Fraction Concentration in Serum of Newborn Rats

Treatment	Number of animals	Total protein[1]	Globulins[1] gamma	beta	alpha-3	alpha-2	alpha-1	Albumin[1]
Day 0								
control	10	2.75±.43[2]	0.12±.04	0.43±.07	0.19±.03	0.27±.04	0.54±.07[2]	1.20±.08[2]
trypan blue	7	2.12±.10[2]	0.09±.03	0.36±.01	0.19±.01	0.28±.02	0.38±.02[2]	0.82±.06[2]
Day 4								
control	7	3.13±.05[2]	0.23±.08	0.51±.05	0.18±.02	0.25±.08	0.69±.07[2]	1.27±.10
trypan blue	6	2.92±.07[2]	0.20±.03	0.49±.04	0.17±.02	0.22±.04	0.58±.08[2]	1.26±.07
Day 7								
control	7	3.45±.31	0.21±.04	0.50±.01	0.21±.01	0.23±.02	0.68±.10[2]	1.62±.24
trypan blue	6	3.42±.20	0.33±.05	0.63±.10	0.20±.05	0.27±.04	0.46±.04[2]	1.53±.24
Day 14								
control	5	4.17±.10	0.46±.06	0.73±.07	0.17±.01	0.17±.01	0.34±.08	2.30±.06
trypan blue	6	4.23±.37	0.34±.06	0.82±.20	0.15±.03	0.24±.06	0.44±.09	2.24±.37
Day 21								
control	5	4.93±.24	0.39±.07	0.84±.05	0.16±.08	0.23±.04	0.46±.14	2.85±.24
trypan blue	6	4.77±.38	0.31±.04	0.92±.09	0.23±.04	0.33±.09	0.43±.08	2.55±.17
Day 42								
control	5	5.25±.09	0.27±.06	0.82±.10	0.32±.06	0.28±.07	0.70±.03	2.86±.21
Day 56								
control	5	5.95±.22	0.44±.10	0.86±.08	0.37±.07	0.26±.02	0.86±.01	3.16±.19
trypan blue	6	5.45±.83	0.40±.04	0.71±.08	0.29±.01	0.25±.04	0.66±.14	3.14±.47
7th month								
adult female	5	6.63±.18	0.62±.15	0.96±.11	0.29±.04	0.22±.03	0.84±.14	3.70±.27

[1] Average concentration values expressed as grams per 100 ml with standard deviation
[2] $P = < .01$

the serum protein deficiency does not continue into the juvenile period of the abnormal animals. This observation suggests that the serum protein alterations are not the result of the fetus being malformed but rather the result of some metabolic malfunction in the production of the serum proteins. It may be postulated that the proposed malfunction is under control of the maternal system since the newborn animal can synthesize normal serum proteins after birth.

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