THE EFFECT OF TRYPAN BLUE ON THE POSTNATAL DEVELOPMENT
OF SERUM PROTEINS¹

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(Received 17 January 1966)

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

¹Supported by Public Health Service Research Grant HD 00400 from
the National Institute of Child Health and Human Development
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>
<thead>
<tr>
<th>Treatment</th>
<th>Total Protein (mg/dl)</th>
<th>Serum Globulin</th>
<th>Serum Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1.23 ± 0.04</td>
<td>0.23 ± 0.04</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.52 ± 0.04</td>
<td>0.25 ± 0.03</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.32 ± 0.03</td>
<td>0.24 ± 0.02</td>
<td>0.90 ± 0.01</td>
</tr>
</tbody>
</table>

*Note: Values are means ± standard deviation.*
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.

References