A second possibility for the observed suppression of IgM antibody in the presence of the tumor may relate to the production of the abnormal gamma globulin by some type of feedback mechanism. Myelomas occurring in inbred strains of mice produce a range of gamma ss and gamma 1A globulins (11). The gamma ss and gamma 1A are both IgG globulins. Suppression of IgM globulin production through passive transfer of IgG globulin has been reported (5) as an indication of a probable feedback control on IgM production. If such a mechanism were operating in the experiments reported in the present study it could be concluded that the abnormal IgG globulin produced by the neoplastic plasma cells caused an inhibition of IgM production. The inhibition by the cells of the tumor would correspond to the feedback control of the IgG globulin on normal IgM antibody and in this way help to account for the lower circulating antibody titers in the tumorous mice of group I, Table I.

Summary. The plasma cell tumor, X5563, grown in C_3H/eb mice has been shown to reduce significantly the numbers of hemolytic plaques demonstrable among spleen cells of tumorous animals as compared to their controls 5 days after a primary intraperitoneal immunization with sheep erythrocytes. At the same time amounts of circulating hemagglutinin are also greatly reduced in the presence of this tumor during the primary response. Few plaques were found among spleen cells of mice receiving a second similar immunization whether the mice were tumorous or not although reasonably high circulating hemagglutinin titers were observed in these mice. From these results it was concluded that the X5563 tumor affects the production of IgM globulin to a more marked degree than it does IgG globulin production. Possible competitive processes are suggested to account for the observed suppressive effect of the tumor on antibody production.

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Teratogenic Activity of Six Disazo Dyes in the Wistar Albino Rat* (32659)

ALLAN R. BEAUDOIN (Introduced by B. L. Baker)
Department of Anatomy, The University of Michigan Medical School, Ann Arbor, Michigan

The first disazo dye reported to be teratogenic was trypan blue (1). Since that time, additional structurally related dyes also have been found to possess teratogenic activity in rats (2–4). The mechanism of teratogenic action of the disazo dyes is not known. Possible mechanisms of teratogenic action have been reviewed by Beck and Lloyd (5).

This report presents observations of similarities and dissimilarities in the teratogenic action of six disazo dyes: trypan blue (TB), Evans blue (EB), Niagara blue 4B (NB4B), Niagara sky blue 6B (NSB6B), Congo red (CR), and Niagara blue 2B (NB2B).

Materials and Methods. Virgin females

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TABLE I. Teratogenic Activity of Six Disazo Dyes in Wistar Rats.

Treatment (dye : mg/100 gm)	No. maternal deaths	No. mothers surviving	No. implantation sites	Fetuses resorbed (%)	Survivors malformed (%)	Implantation sites affected by treatment (%)
Control	0	15	187	3.2	0.5	3.7
Distilled water	0	5	68	0	0	0
Trypan blue						
5	0	16	216	31.0	7.4	36.1
7	0	16	192	54.2	18.2	62.5
14	9	50	623	53.3	55.8	79.5
20	1	10	153	54.0	62.0	82.5
Evans blue						
7	5	15	173	23.1	17.3	36.4
14	12	2	22	41.0	24.1	54.6
20	10	0	—	—	—	—
Niagara blue 4B						
7	0	10	114	13.5	3.8	15.8
14	0	9	104	38.5	14.1	47.1
20	10	25	290	57.0	44.2	62.8
Niagara sky blue 6B						
7	0	5	58	3.4	0	3.4
14	0	10	113	27.4	8.6	33.6
20	5	18	176	50.6	48.3	74.4
Congo red						
14	1	9	103	9.7	3.2	12.6
20	6	22	267	7.5	15.4	21.7
Niagara blue 2B						
7	0	12	159	5.1	0	5.1
14	4	17	221	5.8	2.4	8.1
20	3	19	216	23.2	10.8	31.5

of Wistar albino rats (Albino Farms, Red Bank, New Jersey) were used in this study. The animals were maintained on Rockland Complete Rat Diet *ad libitum*, with supplemental feedings of lettuce. Day 0 of pregnancy was considered to begin on the morning sperm were found in the vaginal smear. Pregnant rats were given a single intraperitoneal injection of a 2% aqueous solution of the dye to be tested during the eighth day of gestation. The dosages employed ranged from 5 mg of dye per 100 gm of maternal body weight to 20 mg/100 gm. Pregnancy was terminated on the day 20; the placentas were weighed, and the fetuses were recovered, weighed, and fixed in either Bouin's fluid or 95% alcohol. Fetuses fixed in 95% alcohol were subsequently stained for visualization of bone by the alizarin red technique. The Bouin fixed fetuses were free-hand sectioned with a razor blade into 2-mm slices and each section was examined for malformations. All dyes used in this study were purchased from Matheson, Coleman & Bell Co., Norwood, Ohio. The identity of the dyes was verified by the method of Lloyd and Beck (6).

Results. Table I presents the activity of six teratogenic disazo dyes in pregnant Wistar rats. Evans blue was the only dye poorly tolerated by the pregnant rat. Injection of 14 mg of this dye per 120 gm of maternal body weight caused the death of 86% of the mothers treated. Higher dosages were lethal to all mothers injected. Each dye caused an increase in the number of fetuses malformed as the amount of dye injected was increased. All dyes except CR caused a greater number of resorptions with increased

TABLE II. Incidence (%) of Malformations Following a Teratogenic Dose of Each Dye.

Malformation	Trypan blue (14 mg/100 gm)	Evans blue (7 mg/100 gm)	Niagara blue 4B (20 mg/100 gm)	Niagara sky blue 6B (20 mg/100 gm)	Niagara blue 2B (20 mg/100 gm)	Congo red (20 mg/100 gm)
Apparent anophthalmia	29.8	37.5	15.1	58.5	42.2	18.2
Hydrocephalus	15.4	15.6	33.4	15.1	31.6	33.2
Exencephaly	15.1	6.3	6.5	3.8	—	—
Microphthalmia	5.7	9.4	6.5	11.3	—	21.2
Vertebral	5.7	6.3	4.3	1.9	—	—
Encephalomeningocele	5.4	3.1	6.5	—	—	—
Megalophthalmia	3.9	—	1.7	1.9	—	—
Tailless	3.6	—	10.8	—	—	—
Other	15.4	21.8	15.2	7.5	26.2	27.3[a]
Mean number of malformations per fetus	1.7	1.2	1.6	1.2	1.1	1.1

[a] Of which three fourths were hydronephroisis.

dosage. Trypan blue proved to be the most potent teratogen of the dyes tested followed by Evans blue, Niagara blue 4B, Niagara sky blue 6B, Congo red, and Niagara blue 2B.

The principal defects observed in offspring of all dye-treated rats were ocular malformations and hydrocephalus (Table II). Niagara blue 4B produced a preponderance of hydrocephaly while all other dyes produced ocular defects as the principal malformation. The more potent dyes (TB, EB, and NB4B) caused a greater number and diversity of abnormalities than did the less potent dyes (NSB6B, CR, and NB2B). The category "Other" in Table II includes defects that occurred in small numbers, i.e., heart abnormalities, gastroschisis, hydronephrosis, hydroureter, fused kidney, absence of kidney, absence of eyelids, sirenomelia, imperforate anus, umbilical hernia, phocomelia, short tail, short trunk, spina bifida, meningocele, and anencephaly. The greatest array of defects were found in offspring of the TB treated animals.

The effect of all injected dyes was to reduce the weight gained during pregnancy (Table III). The most marked retardation of weight gain occurred between the day 8 (day of injection) and day 13. After the day 13, the weight gain approached normal, except in animals treated with NSB6B.

A comparison of fetal and placental weights between control and dye-treated animals showed a significant decrease in fetal weight following administration of all dyes except NB4B and CR (Table IV). The dyes had no effect on placental weight.

Ten multiparous mothers (4–5 prior litters) from 12 to 15 months in age were injected

TABLE III. The Effect of Disazo Dyes on Maternal Weight Gain during Pregnancy.

Treatment[a]	No. animals	Av wt. (gm)		
		Days 0-8	Days 8-13	Days 13-20
Control	10	18.9	25.6	66.0
Trypan blue	20	25.4	11.1	49.6
Evans blue	10	24.8	3.3	51.2
Niagara blue 4B	13	23.2	5.5	43.4
Niagara sky blue 6B	11	17.1	2.5	19.3
Niagara blue 2B	10	24.0	2.4	69.3
Congo red	10	21.6	4.9	74.3

[a] All dye injections were made intraperitoneally during day 8.

TABLE IV. Fetal and Placental Weights in Treated and Control Rats.

Treatment (dye: mg/100 gm)	No. of fetuses	Av fetal wt.	Av placental wt.
Control	135	3.69 ± 0.28	0.53 ± 0.05
Trypan blue 14	231	3.17 ± 0.42[a]	0.52 ± 0.08
Evans blue 7	123	3.27 ± 0.36[a]	0.66 ± 0.17
Niagara blue 4B 20	124	3.47 ± 0.17	0.53 ± 0.10
Niagara sky blue 6B 20	101	3.18 ± 0.28[a]	0.66 ± 0.15
Niagara blue 2B 20	89	3.12 ± 0.51[a]	0.56 ± 0.10
Congo red 20	281	3.62 ± 0.44	0.52 ± 0.05

[a] $p = .01$.

with a teratogenic dose of trypan blue in order to compare the effect of age on teratogenic activity. The only significant change observed was in the number of offspring per litter, a reduction occurring with age and parity (12.3 sites in primipara; 6.7 sites in multipara). The same types of malformations were produced by the treatment and with the same frequency as observed in the young mothers in their first pregnancy.

Discussion. The results of this investigation show that selected dosages of six disazo dyes can be teratogenic to the developing embryo of the Wistar albino rat when injected into the mother during the eighth day of gestation. Attempts to correlate disazo dye structure with teratogenic action have met with little success (7). Beck and Lloyd have shown the efficacy of teratogenic action to be markedly influenced by the purity of the dye (8, 9). Variation in the incidence of resorptions and malformations has been reported in rats of different "strains" and "substrains" when injected with identical doses of the same sample of trypan blue (10). Niagara blue 2B has been reported to be nonteratogenic in the Sherman rat (11) but teratogenic in the Wistar rat (3). These results have been interpreted as indicating a modifying effect of the animal's genetic constitution on the teratogenic action of the dye. This may explain, in part, the variability in teratogenic action of the disazo dyes as reported in the literature.

The diversity in types of abnormalities produced by disazo dye treatment appears to vary with the teratogenic potency of the dye injected. Trypan blue caused the greatest number of different types of malformations and NB2B the least. This observation is probably due to differing levels of susceptibility to the teratogens by the developing organ systems. The most potent dyes are able to affect the development of the less susceptible organs whereas the least potent dyes can only affect the development of the more susceptible organs. The developing eye appears to be the most susceptible organ to the teratogenic effect of the disazo dyes.

Short periods of fasting, (24–36 hours) during days 7–10 of pregnancy, have been shown to produce congenital malformations in mice (13–15). A similarly conducted experiment did not produce any malformations in the Wistar rats used in this experiment, although some resorptions were found (unpublished data). An extended period of fasting (89 hours) begun during day 5 of gestation produced resorptions and malformations in Sprague-Dawley and Silver-Hooded rats (16). The administration of trypan blue in conjunction with this fasting increased the teratogenic and lethal effects of the dye and increased the number of types of defects produced. How much the weight loss exhibited by the animals in the present experiment was due to decreased food intake is not known, however, it is unlikely that prolonged fasting occurred. The results of the present experiment show no correlation between failure to gain weight and production of malformations. Rats treated with a weak teratogen (NSB6B) gained only 30% of the weight gained by control animals during pregnancy whereas animals treated with the most potent teratogen (TB) gained 85% during the same period.

Maternal and fetal weight have been shown to affect the incidence of cortisone induced cleft palate in mice (17). Maternal age and

parity have been implicated in human teratogenesis (review by Fraser, 18). In the present experiment maternal weight (at the onset of treatment), maternal age, and parity had no significant effect on the teratogenic outcome of dye treatment. Fetuses from dye-treated mothers usually weighed less than fetuses from control mothers but there were exceptions, namely fetuses from mothers treated with CR and NB4B. It has been suggested that in mice there is a causal dependence of fetal weight on placental size (19). A reduction in placental weight did not accompany a reduction in fetal weight in the present investigation. Fetal weight at autopsy can be fairly well correlated with the retardation in weight gain during pregnancy.

The disazo dyes can be used to produce malformations in rats and other animals. However, no causal relation has been demonstrated between the chemical and biological properties of the dyes and their teratogenic action. The various effects studied do not reveal a common denominator in action or effect of the dyes. There is no agreement as to the site or sites of action of the disazo dyes, be it maternal, placental, or fetal. It is certainly possible that the site of dye action in mammals will be found to be different from the site of action in the nonplacental animals and that dye action *in vivo* will differ from dye action *in vitro*.

Summary. The teratogenic action of six disazo dyes was investigated in the Wistar albino rat. Appropriate dosages administered during day 8 of gestation showed the most potent teratogenic dye to be trypan blue followed by Evans blue, Niagara blue 4B, Niagara sky blue 6B, Congo red, and Niagara blue 2B. The diversity in types of malformations produced in the offspring of dye-treated mothers varied with the potency of the dye injected. Trypan blue caused the greatest number of different types of malformations and Niagara blue 2B the least. Ocular defects and hydrocephalus were the two most frequent abnormalities following treatment with each dye. There was no consistent correlation between dye action and maternal weight at the onset of treatment, maternal weight gained during pregnancy, maternal age, parity, fetal weight at autopsy, or placental weight.

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